NITROGEN REGENERATION DURING AMINO ACID DEGRADATION AND THE ACTIVITY OF BACTERIA IN PLANKTON COMMUNITIES OF HALIFAX HARBOUR, NOVA SCOTIA, CANADA

BY

JAMES T. HOLLIBAUGH

Submitted in partial fulfilment for the Degree of Doctor of Philosophy given by the Department of Oceanography at Dalhousie University, October 1976.

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ABSTRACT

Experiments have been performed to determine the fate of dissolved free amino acids released in natural, summer, coastal plankton communities off Halifax, Nova Scotia, Canada. An increase in *in vivo* chlorophyll fluorescence observed with enrichments of 1.0 μM or greater L-arginine was found to follow the release of ammonia from the added amino acids. Micro-autoradiography of natural populations exposed to ^{14}C-labelled L-arginine and L-glutamate at 0.1 μM revealed that the radio-activity was associated with fecal pellets and detritus, but not with phytoplankton cells, suggesting that bacteria were responsible for amino acid degradation.

Further evidence that amino acid uptake and degradation were bacterially mediated came from experiments with metabolic inhibitors, amino acid analogues, the specificities of substrate induced uptake systems, and from the induction and loss of the ability to take up L-arginine in a population contained in a carboy. These data further suggest that the observed kinetics of amino acid uptake and degradation are related to the metabolic activation of a large fraction of the bacteria from a resting state.

The kinetics were affected by the incubation temperature and the presence of other organic compounds but not by light or 10.0 μM nitrate or ammonia. Nitrogen regeneration varied from 60 to 85% of the theoretical maximum for L-arginine and from 60 to 100% (average 80%) for a variety of other amino acids supplied at 10.0 μM.

LIST OF SYMBOLS AND ABBREVIATIONS

ATP - adenosine triphosphate

C° - Centigrade degrees

°C - temperature measured on the Centigrade scale.

DNA - deoxyribonucleic acid.

DPM - disintegrations (of an unstable atom such as ^{14}C) per minute.

k_s - the substrate concentration at which an enzyme reaction described by Michaelis-Menton kinetics, $v = \frac{V_{max} S}{k_s + S}$, proceeds at one half the maximal velocity. S and V_{max} are the ambient substrate concentration and the maximal velocity, respectively.

ℓ - liters

$Ly°min^{-1}$ - langleys/minute, a measure of incident radiation.

m - meters.

mg - milligrams, 10^{-3} grams.

ml - milliliters, 10^{-3} liters

mm - millimeters, 10^{-3} meters

mM - millimoles/liter, 10^{-3} moles/liter

mRNA - messenger ribonucleic acid

N - normality, moles/liter of hydrogen or hydroxide ion.

nm - nanometers, 10^{-9} meters.

pH - negative $\log_{10}$ of the hydrogen ion activity.

Q_{10} - rate of a process at T + 10 °C divided by the rate of the process at T °C, here rate is replaced by the duration of the lag period preceding the increase in the rate of uptake of a substrate.

r	-	intrinsic rate of increase, here used to denote selection for a high growth rate.
r^2	-	correlation coefficient for a linear regression.
RNA	-	ribonucleic acid
RPM	-	revolutions/minute
v/v	-	volume to volume ratio.
V_{max}	-	velocity of an enzyme reaction described by the Michaelis-Menton equation, (See k_s), at saturating substrate concentrations.
μCi	-	microcuries, 10^{-6} curies = 2.22×10^6 DPM
μg-at N/ℓ	-	10^{-6} gram-atoms of nitrogen/liter.
μM	-	micromoles/liter, 10^{-6} moles/liter.
%	-	percent, parts per hundred.
‰	-	parts per thousand .
>	-	greater than (a>b; a is greater than b).
<	-	less than.

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Heterotrophy in the pelagic environment has only recently been studied quantitatively. Parsons and Strickland (1962) developed a technique for measuring heterotrophic production analogous to Steeman Nielsen's (1952) technique for measuring primary production. Radioactively labeled organic substrates were added to a sample of a pelagic community and heterotrophic production was taken as the rate of incorporation of the substrate into particulate matter. Many of the ambiguities and problems associated with the measurement of primary production have been eliminated so that it is now a standard technique, but problems still plague the assay for heterotrophic activity.

One of the problems is that the choice of substrate is still largely up to the experimenter. If a standard technique is to be developed, a standard substrate must be designated, but it is unlikely that any organic substrate will be taken up as uniformly by heterotrophs as bicarbonate is utilized by phytoplankton.

The next problem encountered is that of substrate concentration. Parsons and Strickland (1962), restricted by analytical techniques (still a problem with some substrates), chose to add the substrate to a level such that the naturally occurring substrate was an insignificant fraction of the total substrate in a given experiment. This would saturate uptake systems, however, and provide an overestimate of the actual uptake rate in situ. This is acceptable for comparative purposes but didn't provide the maximum information on the dynamics of the system. Subsequent authors, (Williams and Askew 1968, Wright and Hobbie 1965), chose to add trace amounts of labelled substrates so that the added

substrate formed an insignificant fraction of the whole. They were then required to determine the natural substrate concentrations to estimate turnover times and substrate fluxes. A technique for the kinetic analysis of the flux of a substrate through a system evolved from this approach, (Wright and Hobbie 1966). This analysis was based on the application of Michaelis-Menton enzyme kinetics to mixed systems, and follows from suggestions put forth in Parsons and Strickland (1962).

One of the primary reasons for choosing to work with amino acids was that they represent a potentially important source of fixed nitrogen for phytoplankton growth. They also represent a potentially important source of energy and reduced carbon for heterotrophic growth, and nitrogen regenerated from dissolved free amino acids could also be important to phytoplankton ecology. Understanding the dynamics and physiology of the utilization of dissolved free amino acids is the first step to understanding the utilization of amino acids bound as polymers in proteins and peptides and as ligands attached to clay particles or metal ion complexes.

In addition, a relatively large body of literature exists on the concentrations and oxidation rates of certain amino acids in seawater. Techniques for the analysis and handling of amino acids are available from the fields of biochemistry and physiology, and the metabolism of these compounds is well known for a variety of representative systems. Most common amino acids are available with a ^{14}C label at any or all of the carbon atoms, and many are available with a ^{15}N label, so that tracer techniques can be readily applied to the study of their utilization in natural communities.

Arginine and glutamic acid in particular represent two of the extremes in this group of compounds. Arginine is basic with a pK_a of 12.5 for the guanidinium group and a C/N ratio by atoms of 6/4. Glutamic acid on the other hand has a pK_a of 4.2 for the gamma carboxyl group and a C/N ratio of 5/1 by atoms. Arginine is rarely found in high concentrations ($>10^{-8}$ M) in seawater while glutamic acid is one of the more abundant amino acids, frequently attaining concentrations $>10^{-7}$ M (Pocklington 1971, Chau and Riley 1966, Riley and Segar 1970, Jeffries 1969, Andrews and Williams 1971). Preliminary experiments indicated that arginine was degraded more rapidly than most common amino acids by natural communities. Glutamate was also degraded fairly rapidly and this pair was chosen for comparison because of the differences in their properties.

The substrate concentrations used were selected to be as close to the natural levels as possible and yet sufficiently high to permit the analysis of both substrates and degradation products. Within these restraints, 10.0 µM was chosen as the usual concentration; however, experiments with lower concentrations were performed to see if any major changes in dynamics were observed at these lower concentrations. Biological variability and analytical sensitivity set the lower limit between 0.5 and 1.0 µM of substrate. These concentrations are from 1 to 3 orders of magnitude higher than the analytically determined concentrations for open ocean water, however, the biologically significant substrate concentrations may not be that of bulk water but rather higher concentrations found in the vicinity of particles (Bell and Mitchell 1972, Bell et al. 1974, Paerl 1974, Wangersky 1974). In

addition, Williams (1973b) and Gocke (1976) found only a slight increase in the respiration ratio of a variety of substrates with increasing concentration, suggesting that the increased concentrations do not greatly affect the metabolic processes and pathways in active cells.

One of the main points raised by Parsons and Strickland (1962) in discussing their method is that it is not possible to determine what organisms are using the organic substrates, only that they are being taken from a dissolved state into a particulate state. This uncertainty has resulted in a discussion by various authors as to the identity of the users of the various fractions of dissolved organic compounds. One aspect of this discussion concerns the bacterial versus algal utilization of organic compounds, particularly the nitrogen-containing amino acids. Until recently it has been difficult to obtain direct information on the fate of these compounds Brock and Brock (1968) developed a micro-autoradiographic technique that allowed direct observation of the organisms using added labelled substrates. A modification by Paerl (1974) has simplified the original procedure making routine work feasible, particularly if only qualitative results are desired.

Section I of this thesis attempts to identify the primary sinks of arginine and glutamate in the Halifax Harbour plankton communities. The interactions of the bacterial and algal components of these communities with respect to the utilization and mineralization of the organically bound nitrogen in arginine and glutamate is also investigated in this section. This pathway for nitrogen regeneration has been discussed by

Wright (1974). It is potentially important to phytoplankton nutrition and may contribute significantly to the nitrogen requirements of primary production, but unfortunately there is little data on the process.

Based on the evidence to be presented in Section I, it seems likely that mineralization of amino acid nitrogen to ammonia by heterotrophs followed by the utilization of the ammonia by phytoplankton is the major pathway for the return of arginine and glutamic acid nitrogen to the phytoplankton in the communities studied. Wright (1974) and Hobbie and Crawford (1969) have found that the respiration rates and ratios of a variety of amino acids vary from compound to compound under identical conditions. The experiments presented in Section II were performed to determine if the kinetics of degradation and the nitrogen regeneration ratios accompanying the degradation of a variety of amino acids similarly varied from compound to compound. This information would be of use in attempting to assess the importance of nitrogen regenerated in this manner to the nitrogen budget of these communities. Comparison of the kinetics and regeneration ratios of a variety of amino acids may also allow one to make inferences concerning the physiology of the heterotrophs.

In the experiments presented in Section II, differences in the kinetics of utilization of various amino acids by a given community, and in the kinetics of utilization of a given amino acid by different communities, were found. A lag period preceding a marked increase in the rates of uptake and degradation was found to be a property of the utilization of amino acids supplied at 10.0 μM to these communities.

Similar lags followed by increased rates of utilization have been observed for a variety of substrates by Williams and Gray (1970), Williams (1970), Vaccaro (1969) and Williams and Askey (1968). These authors conclude that the rate of increases they observed were due to an increase in the bacterial population, although the possibility that the lag and subsequent rate increases were related to enzyme induction required to adapt the population to a new substrate was mentioned. A third possibility, the increases in substrate level lead to the activation of a fraction of the population that has previously been dormant is suggested by Hobbie (personal communication), Jannasch (1974), and Jannasch and Mateles (1974). Section III presents experiments performed to investigate the basis of the increases in the rates of uptake and degradation of amino acids and the physiological and environmental factors affecting the duration of the lag preceding the rate increases.

Section I. Amino Acids and Phytoplankton Nutrition.

INTRODUCTION

The nitrogen nutrition of marine phytoplankton has been the focus of a great deal of research effort. This stems primarily from the supposed role of combined nitrogen as the growth limiting nutrient in the open ocean (Ryther 1963, Thomas 1969, 1970a, b) Organic nitrogen sources have been suggested by various authors as a means of meeting the requirement of a cell when inorganic fixed nitrogen is in short supply. Recent work has shown that urea can be used by many phytoplankters (McCarthy 1972, Carpenter et al. 1972). In addition, phytoplankton have been shown to take up a variety of other nitrogenous organic compounds (Hellebust and Lewin in prep., Hellebust 1970, White 1974, Neilson and Lewin 1974). Of the many possible organic nitrogen sources, amino acids have been suggested by many authors as potentially important to phytoplankton (North 1975, Wheeler et al. 1974, Schell 1974, Thomas 1970a, Degens 1970). This is based on observations that a variety of algae can utilize amino acids at low concentration in axenic culture and that amino acids comprise the largest fraction of the potentially useful organic nitrogen, next to urea, in the sea (Wheeler et al. 1974, Hellebust 1970, Degens 1970, North 1975, Thomas 1966).

Some recent attempts to extrapolate laboratory studies to nature include Schell's (1974) measurements of the uptake and incorporation of ^{15}N and ^{14}C labelled amino acids by particulate matter which he assumed to be phytoplankton. North (1975) demonstrated the utilization of naturally occurring amines by Platymonas sp. and speculated that a similar situation would exist in a natural population of phytoplankton. Such observations provide some evidence that amino acid nitrogen could be used directly by phytoplankton in nature.

A problem that is always encountered in extrapolating from laboratory studies to nature is that natural systems contain a large number of potentially competing organisms or reactions. Although phytoplankton can take up amino acids, photodecomposition, adsorption, and bacterial uptake are among the other ways in which amino nitrogen may be removed from the water column. Photodecomposition (Bada 1971) and bacterial degradation (Wright 1974) offer mechanisms whereby the nitrogen could be returned to the water column as inorganic fixed nitrogen. As photodecomposition requires the presence of free, unchelated transition metal ions, there seems to be some question as to the rate at which it would take place, and hence its importance, in the sea (Bada 1971)

Some authors (Derenbach and Williams 1974, Andrews and Williams 1971, Wright and Hobbie, 1966, Hobbie et al. 1968) have suggested that bacterial uptake of organic substrates proceeds more rapidly and at lower substrate concentrations than algal uptake of these same substrates. They infer that bacteria would "out-compete" the algae unless the substrate was present at unusually high concentrations. This conclusion is based on size fractionation and carbon dioxide production (Derenbach and Williams 1974, Andrews and Williams 1971, Williams 1970), micro-autoradiographic evidence (Paerl 1974, Munro and Brock 1968), and kinetic arguments (Hobbie et al. 1968, Wright and Hobbie 1966). None of these studies offer definitive proof, however, all provide evidence that bacteria would "out-compete" algae for a substrate such as an amino acid present at a low concentration (1 0 μM). The present work is an attempt to assess the

relative importance of the algal versus bacterial pathways of amino acid decomposition and amino nitrogen utilization

MATERIALS AND METHODS

All of the water used in this study was collected from the outer portion of Halifax Harbour, Nova Scotia, Canada (44°36'20"N, 63°33'11" W, fig 1). The experiments were performed during the summer and early fall months of 1974. Fixed nitrogen (nitrate plus nitrite plus ammonia), above the thermocline was low during this period, ranging in concentration from 0.5 µg-at N/ℓ to values of 1 to 4 µg at-N/ℓ in the fall. The water was collected from a small boat in the late morning of the day on which the experiment was begun from 10 cm below the surface by immersing a 20ℓ plastic carboy with the mouth pointing forward while slowly underway. The carboy was soaked in 0.5 M hydrochloric acid between uses.

The water was brought back to the laboratory where it was pre-treated by enriching it with phosphate, silicate, a trace metals-ethylene-diaminetetraacetic acid solution, and a vitamin mixture, to a final concentration equal to 1/40 of the "f" formulation of Guillard and Ryther (1962) unless otherwise noted. The pre-treatment was necessary when measuring chlorophyll production as a function of added nitrogen to avoid difficulties associated with growth limitation by a plant nutrient other than nitrogen. Tris (tris(hydroxymethyl) aminomethane) was omitted from the pre-treatment, as this compound and glycylglycine were found to interfere with subsequent ammonia analysis. The concentration of nutrients added in the pre-treatment as defined above will be refered to hereafter as a fraction of the concentration of Guillard and Ryther's

(1962) formulation minus nitrogen, hence f/40-N was the usual pre-treatment. After pre-treatment the water in the carboy was partitioned into 1 50ℓ aliquots contained in 2ℓ Erlenmeyer flasks by siphoning. The intake end of the siphon tube was covered with a 100 μ pore size nylon mesh to exclude large particles. Nitrogen sources were then added to duplicate sets of flasks as demanded by the format of the experiment.

The flasks were placed in a Conviron plant growth chamber (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) on a 16/8 light/day cycle at a light intensity of 0.03 ly min^{-1} in the photosynthetically active region of the spectrum The incubator was set at a temperature within 2 C° of the <u>in situ</u> water temperature at the sampling station. Between pre-treatment and partitioning the water was stored briefly in a 10°C cold room. Substrates were added to the flasks and the experiment was begun within 4 hours of the time of collection During this time the temperature of the water in the carboy did not change by more than 2 C°.

Chlorophyll fluorescence was measured at the 10x sensitivity setting on a Turner Model 111 Fluorometer in 10x75 mm cuvettes against a distilled water blank with Wratten #47B and #2A excitation wavelength filters and a Wratten #25 emission filter Nitrate plus nitrite was measured according to, Strickland and Parsons (1968) using nitrate values uncorrected for nitrite. Ammonia was determined by the phenol-hypochlorite method (Solórzano 1971). The amino acids used were tested and found not to interfere with the ammonia analysis. All glassware was soaked overnight in 0.5 N hydrochloric acid, rinsed with distilled

water and inverted to drain. Ammonia determinations for each flask were performed in triplicate on unfiltered water removed directly from the flasks. Ammonia values in duplicate experimental flasks were within 0.5 µM when less than 10 µM and within 3 µM at levels above this. The relative fluorescence values of duplicate flasks had a maximum range of ten percent.

Water for micro-autoradiography was collected and pre-treated as above except that enrichment was to f/72-N. Additions of a mixture of unlabelled and ^{14}C uniformly labelled L- arginine or glutamic acid were made to triplicate flasks containing 1.50ℓ water samples to give 0.10 µM and 5.0 µCi/ℓ. The dark flasks were wrapped in aluminum foil and incubated with the illuminated flasks in the growth chamber. Twenty-five milliliter aliquots were filtered onto 25 mm diameter HA Millipore® filters at 730 Torr. Just before the filter was sucked dry the vacuum was shut off and 15 mL of GF/C filtered seawater containing 100 µM each of L- arginine and glutamic acid and 3% v/v of glutaraldehyde was added to the filter. After two minutes the vacuum was turned on and the solution was drawn through the filter. The damp filters were immediately taken through desalting and staining with Erythrosin B (Paerl 1974) after which they were dried, mounted on clean glass sides, dipped in emulsion, exposed, and developed according to Brock and Brock (1968). The slides were then examined and photographed on a Zeiss Photomicroscope II at 350x using transmitted light. Background was determined on control filters treated as above except that no ^{14}C labelled substrate was added to the water.

Substrate uptake was followed during this experiment by filtering 50 ml aliquots from the flasks at 730 Torr through 25 mm diameter HA Millipore® filters. The filters were placed in scintillation vials and counted in 10.0 ml of a fluor composed of 1:1 v/v Triton X-100. toluene fluor (Pugh 1973). Liquid scintillation counting was done in a Packard Tri-Carb counter equipped with an Absolute Activity Analyzer to correct for quenching and counting efficiency.

RESULTS AND DISCUSSION

Figure 2 shows the results of an experiment to determine the lowest substrate addition of arginine that would result in an increase in chlorophyll fluorescence. Additions of as little as 0 10 µM L-arginine (0.40 µg at N/ℓ) were detected while additions of 1.0 µM or more produced significant increases. Chlorophyll fluorescence increased until limitation by a nutrient other than nitrogen stopped plant growth, which in this experiment occurred with additions of greater than 5.0 µM.

It was initially thought that the increases in chlorophyll fluorescence were due to the direct utilization of amino acid nitrogen by the phytoplankton, however, ammonia was detected in the flasks in which chlorophyll fluorescence increased. The release of ammonia from L-arginine is shown in figure 3. In order to determine the optimum pre-treatment and the importance of the uptake of ammonia by phytoplankton on the apparent ammonia production, 10.0 µM (40.0 µg-at N/ℓ) of L-arginine was added to experimental flasks which were enriched with varying concentrations of the pre-treatment nutrients. One set of duplicates was incubated in the dark and one in the light. It can be

seen that the ammonia levels in the flasks varied inversely with the pre-treatment nutrient concentrations; after a nutrient other than nitrogen became limiting to growth the ammonia uptake ceases. This result is clearly shown in the dark flasks where the nitrogen liberated from arginine as ammonia reached 80% of the theoretical value. The production of ammonia in darkened flasks was independent of pre-treatment nutrient level. Ammonia was taken up from an ammonium chloride addition control incubated in the light, but at a reduced rate relative to the flasks with L-arginine additions. This control was pre-treated to f/80-N, and seems to have been inhibited in the production of chlorophyll relative to the flasks with L-arginine additions. This may be due to the low pre-treatment nutrient concentrations.

Figure 4 shows the chlorophyll fluorescence curves for the same experiment. These show a direct relationship between fluorescence and level of nutrients in the pre-treatment as would be expected if phyto-plankton growth was limited by one of the nutrients in the mixture. By comparing figures 3 and 4 it can be seen that, with the exception of the control, ammonia production preceded any increases in chlorophyll fluorescence. This relationship is also seen in figure 5 in which flasks with ammonium chloride additions displayed growth approximately ten hours before flasks supplied with D- or L- arginine or glutamic acid nitrogen.

The difference in the incubation time required before ammonia was released from D- or L- arginine in figure 6 is evidence that the decomposition of arginine was not a physical-chemical process but was biologically mediated. In another experiment, sea water was filtered

through a sterile 0.22μ filter into sterile glassware and handled with aseptic technique. No ammonia was released (figure 7, "filter sterilized"). As the flasks were incubated in light, this observation also provides evidence that the breakdown of arginine is not a photochemical reaction.

A difference in the rates of breakdown of the D- and L-enantiomers was observed in experiments with arginine. The D-enantiomer took longer to begin breaking down, but ammonia was released more rapidly once degradation had commenced This is reflected in the chlorophyll fluorescence curves of figures 5, 8 and 9, and is shown directly by the ammonia release shown in figures 6 and 7 Figures 7 and 8 are from the same experiment and show that the increase in fluorescence is accompanied by the release of ammonia. The rate of uptake of ammonia by the phytoplankton must have been very close to its rate of release in this experiment, as the concentration actually observed in the water was never very high. In the extreme case, the accumulation of ammonia would not be observed in the system unless the rate of uptake of ammonia by phytoplankton was slowed, or the release rate accelerated. The D-enantiomer seemed to upset the balance between the rates of ammonia uptake and release as can be seen in figure 7. The ammonia concentration increased rapidly during the breakdown of the D-enantiomer outstripping phytoplankton uptake and resulting in a spike of ammonia in the water. This was soon taken up, however, by the now rapidly growing phytoplankton population in the microcosm, and if no other nutrient became limiting ammonia would

disappear from the system. Limiting phytoplankton growth by a nutrient other than nitrogen or by performing the experiment in the dark has the same effect (fig. 3).

The last evidence that bacteria were responsible for the mineralization of amino nitrogen comes from micro-autoradiography. As can be seen in figure 10a and b, the radioactive label was not associated with identifiable phytoplankton. Detrital particles, fecal pellets, etc. were seen to be labelled in figure 10c, d and e. Occasionally round particles like that shown in figure 10f were labelled. These particles were heavily stained with Erythrosin B and regular in shape and size, which suggests that they are eukaryotic cells, possibly enclosed in a mucus sheath, but it is not possible to identify them or to determine whether they contain chlorophyll or not. They may be colorless flagellates or some other form of obligate heterotroph, but they are rare in this sample and were probably unimportant in terms of the cycling of organic nitrogen in this system. The sample from which the photographs of figure 10 were taken was exposed to ^{14}C labelled L-arginine for eight hours. No differences were observed in the labelling patterns between L-glutamic acid and L-arginine or between the light and dark flasks. The label was associated with large particles, suggesting that the active bacteria in this system were also associated with particles. This agrees with the results of Paerl (1974, 1975) for Lake Tahoe and Southern California coastal water. Although there was no killed control, the similarity between the labelling patterns for arginine and glutamate, the linearity of the uptake curve (fig. 11) and the fact that the labelling pattern was not dependent on the length of

the incubation, (40 minutes to 12.5 hours), suggests that the association of the label with the particles was not due to simple adsorption, but rather to bacterial uptake.

The uptake of the radioactive amino acids is plotted in figure 11. The light and dark uptake rates were the same for arginine and were barely different for glutamic acid, with the light rate being slightly higher than the dark rate for this compound. L-glutamic acid was taken up at about twice the rate of L-arginine The uptake curves for both compounds are linear (r^2=0.99) for the entire experiment. By the end of the 12 5 hour incubation period, 11.5% of the arginine in the water and 22.5% of the glutamic acid in the water had been removed . There was no noticeable decline in the uptake rate, if the added substrate comprised the bulk of the substrate in the water; the linearity of the uptake curves suggests that the half saturation constant for the process is less than 0 10 µM.

CONCLUSIONS

Based on the evidence presented above, the primary sink for L-arginine and L-glutamate released into the water column during the summer on this station seems to be the bacteria present in the water. Derenbach and Williams (1974), Williams (1970) Hobbie et al. (1968), Crawford et al. (1974), Wright and Hobbie (1966) and Paerl (1974) have obtained similar results with other substrates in other systems. The nitrogen contained in these compounds is rapidly returned to the water column as ammonia, (fig. 3). The amount of ammonia released from L-arginine in this experiment was 80% of the nitrogen contained in the added substrate,

presumably the remaining 20% was retained by the bacteria to meet their nitrogen requirements. It is not known whether or not this ratio is constant for a given substrate, presumably it would be a function of the C.N ratio of the total diet of the bacterial population. Wright (1974) has discussed the release of organically bound nitrogen that should accompany the degradation of amino acids.

A rapid increase in the rate of degradation of D- and L- arginine and glutamic acid took place after lag periods of relatively constant but slow rates of degradation (figs. 3, 6 and 7) The duration of this lag phase seems to be different for each of these compounds, and may be due to the growth of a specific nutritional type of bacteria, or due to some physiological change in the existing population. Williams and Gray (1970) and Vaccaro (1969) obtained similar results with different substrates in their studies.

The ammonia released during the degradation of these compounds was rapidly taken up by the phytoplankton in the communities. This pathway for nitrogen regeneration may contribute significantly to the nitrogen requirements of phytoplankton growth in the communities. present at this station.

Section II. Lag Periods and Ammonia Release During the Degradation of Several Amino Acids.

INTRODUCTION

It has been demonstrated (Hobbie et al. 1968, Hobbie and Crawford 1969, Williams 1970, Section I of this study and others) that amino acids released in coastal plankton communities are rapidly catabolized. For some of these compounds the amount of carbon dioxide released during this process is greater than would be expected from simple decarboxylation reactions and indicates that they are used as energy sources (Hobbie and Crawford 1969, Gocke 1976, Williams 1970, Seki et al. 1972). The twenty protein amino acids have small C·N ratios, the highest being 9:1 by atoms for phenylalanine ranging downward to 1.5·1 for arginine. The C N ratio for plankton is in the range 3.1 to 9·1 (Parsons and Takahashi 1973, Parsons et al. 1961). If 6:1 is taken as an estimate of the C.N ratio in heterotrophs, it follows that a population of heterotrophs metabolizing only amino acids would have to release nitrogen in order to maintain the C.N ratio. The C:N ratio of the total diet of the population should be the actual determinant of nitrogen release. In extreme cases, Thayer (1974) has suggested that hetrotrophs (possibly, but not necessarily, bacteria) will compete with algae for a nitrogen source when metabolizing compounds with large C:N ratios. Wright (1974) has discussed nutrient regeneration during the catabolism of organic substrates and remarks upon the lack of data on this phenomenon which is important in environments with low rates of nutrient input. Data on the release of ammonia during the catabolism of D- and L-arginine and glutamate were presented in Section I.

Plankton communities are currently assumed to function as multi-component steady state systems governed by Michaelis-Menton kinetics with respect to the uptake and metabolism of organic substrates (Wright and Hobbie 1966). Changes in the kinetic parameters are attributed to either an increase in the population size or a change in the taxonomic structure of the population (Jannasch and Mateles 1974, Williams 1973a, Wright 1974, Wright and Hobbie 1965). Contradictory data have been published (Vaccaro and Jannasch 1966, 1967, Vaccaro 1969, Hamilton and Preslan 1970, Stumm-Zollinger and Harris 1971, Williams 1970, Williams and Askew 1968), and the validity of the assumption has been questioned by some authors (Williams 1973a, Thompson and Hamilton 1973, 1974, Jannasch 1974, Jannasch and Mateles 1974) Vaccaro and Jannasch (1967), Vaccaro (1969), Williams and Gray (1970) have demonstrated that heterotrophic populations are subject to conditioning so that uptake rates increase and the variance of rate measurements decreases after exposure to a substrate. They interpreted their results as selective growth in the heterotroph population; however changes in the metabolism of pre-existing organisms could give rise to the same results The sigmoid to step functions they observed for changes in carbon dioxide production and substrate uptake rates were observed for ammonia release in Section I.

The following experiments were performed to investigate the relationship between molecular structure, C.N ratio, the duration of a lag preceding a marked increase in the rates of ammonia release, and the fraction of nitrogen released as ammonia during the degradation of a

variety of amino acids. The release of ammonia was measured because it is of primary interest in nitrogen regeneration. The relative duration of the lag preceding the increase in the rate of ammonia release seemed to be a conservative property of the degradation of these compounds in the experiments performed in Section I (figs. 6 and 7), suggesting a possible physiological rather than taxonomic basis for the lag. One of the objects of the experiments presented here was to see if rate increases after a lag are common to the degradation of all amino acids and if so to determine the relative durations of these lags for amino acids other than D- and L- arginine and glutamate.

MATERIALS AND METHODS

These experiments were performed during late May and early June of 1975. Plankton communities were collected as raw seawater samples at Station A in the outer portion of Halifax Harbour (fig. 1). During this period the water column at this station was weakly stratified, with a temperature of 8 to 10°C at the surface and 5 to 7°C at the bottom in 23m of water. The salinity was 29 °/oo on the surface and 31 °/oo at the bottom. The major spring bloom of phytoplankton had occurred about a month earlier, but large fluctuations in the standing crop are normally observed in this area.

The water was collected, pre-treated, and partitioned as in Section I. Substrates were added to the flasks to a final concentration of 10.0 µM. Each substrate was tested in duplicate; duplicate controls with ammonium chloride or L-arginine additions were run with each experiment. The flasks were incubated in a dark cabinet at 10°C and

samples were taken as indicated in the figures

Stock solutions of substrates were made from reagent grade chemicals dissolved in distilled water passed through a Millipore® Super Q deionizing unit. Stock solutions were 10.0 mM and were prepared for each experiment except for the L-arginine stock solution which was filter sterilized and stored at -20°C in a sterile serum bottle. Analytical methods were as described in Section I

RESULTS

The results are presented in figures 12-14. Descriptive parameters derived from the data presented in the graphs are compiled in Tables I and II The maximum rate of ammonia production was derived from the segment of a given curve having the maximum slope. This parameter is sensitive to the sampling interval and is probably always an underestimate. The lag period is taken as the time from the addition of the substrate to the beginning of the segment of maximum slope. This parameter is much less sensitive to the length of the sampling interval than is the maximum rate of decomposition.

The percentage yield, or regeneration ratio, is calculated from the ratio of nitrogen released as ammonia to that supplied as amino acid nitrogen. This ratio is a function of two variables; the total amount of the amino acid taken up, and the proportion of the nitrogen assimilated by the cells. Amino acid uptake systems have been

observed to operate at quite low substrate concentrations, (<1 0 µM; Jannasch and Mateles 1974, Hobbie and Crawford 1969, Hobbie et al. 1968, Section I, and others), so it is assumed that all of the substrates added in these experiments were taken up and that the amount of ammonia released depends only on nitrogen assimilation by the cells. Yields were calculated from the maximum concentrations of ammonia observed in the plateau region of the curves These plateaus represent an equilibrium condition that was stable for 72 hours in an experiment with L-arginine (fig. 3). The yields are not corrected for changes in the ammonia concentration of the ammonium chloride control as these were in general slight and probably due to atmospheric contamination, or ammonia release accompanying the degradation of organic nitrogen present in the water at the time of collection (figs 13 and 14) or to uptake by the plankton (fig. 12). Tests for nitrate plus nitrite and urea (Strickland and Parsons 1968) in the flasks during decomposition of L-arginine were negative. It is likely that the catabolism of L-lysine and L-tryptophan had not gone to completion when their yield values were calculated.

The values observed for these parameters vary from experiment to experiment for the L-arginine control. These differences may be due to differences in the chemical and biological conditions of the environment when the sample was collected. Because of the temporal variation, direct comparisons between parameters can be made only for substrates in the same experiment. Comparisons of parameters between experiments should only be made between ratios of the parameter

to the respective value for the L-arginine control of the same experiment.

DISCUSSION

Some of the substrates tested were not observed to be degraded during the experiment. They may not have been taken up appreciably by the hetrotrophs during this period. Alternatively these compounds may have had lag periods preceding the increase in the rate of ammonia release which were longer than the duration of the experiment. This is suggested by the long lags shown by L-lysine and L-trytophan degradation (fig 12). In another experiment with a late summer community incubated at 15°C, L-lysine was taken up and degraded after a 72 hour lag and L-leucine was degraded only after a 96 hour lag. The length of the lag period for L-arginine was found to have a "Q_{10}" of approximately 3.0. All of the compounds that were degraded displayed an increase in the rate of ammonia release after a lag period. The length of the lag varied considerably between compounds, as did the initial rates of ammonia release. It is interesting that urea was not degraded to ammonia in these experiments. Mitamura and Saijo (1975), Carpenter et al. (1972) and McCarthy (personal communication) also found that urea was not degraded to any extent by marine heterotrophs. Its low energy yield per molecule transported would make it a poor energy source for heterotrophs. This factor may be important in explaining the high concentrations of this compound observed relative to amino acids in the water column (McCarthy 1972, Carpenter et al. 1972, Pocklington 1971).

With the exception of taurine, the amino acid analogues tested were not degraded. Taurine occurs abundantly in marine plankton (Cowey and Corner 1963a, b, Jeffries 1969). The other analogues do not occur naturally so that heterotrophs would not be expected to have evolved biochemicaly pathways for their utilization except by accident or because of structural resemblance to compounds for which catabolic pathways exist. Until uptake is measured directly, the possibility that the analogues were taken up but not degraded cannot be ruled out.

There seems to be no obvious correlation between the molecular structure of an amino acid and the length of time preceding the increase in the rate of ammonia release. The lag period may be related in a very general way to the ease of entry of the carbon skeleton of a transported molecule into the Krebs cycle, but to attempt to explain the lags exclusively on that basis leads to many contradictions. The duration of the lags for D- and L- arginine retained their relationships to one another even when the actual value for the duration had changed. Variations in the physiological state of the heterotrophs could change the duration of the lag period without affecting the sequence of the lag periods observed when several substrates are compared.

The amount of ammonia released during the degradation of these substrates bears little relation to the C:N ratio of the substrate. This suggests that the fraction of nitrogen released is determined biochemically by the catabolic pathways by which the substrates are degraded A reasonable estimate of the fraction of nitrogen that

would be regenerated as ammonia accompanying the mineralization of a mixture of amino acids is 80%. This ratio will vary as a function of the substrate mixture and with as yet unknown environmental factors (compare the L-arginine curves and yields in figures 12, 13 and 14 which are from experiments performed in consecutive weeks). The C·N ratio of the total diet of the heterotrophs may also be a factor.

The average nitrogen regeneration ratio of 80% is much higher than carbon respiration ratios reported for these same substrates (Gocke 1976, Seki et al. 1972, Williams 1970, Hobbie and Crawford 1969), which averaged around 30%. Gocke (1976), Wright (1974), and Williams (1973b) report a slight increase in carbon respiration ratios with increasing substrate concentrations, but the increases they observe cannot account for the 2.7 fold differences between their carbon and my nitrogen regeneration ratios. Hobbie and Crawford (1969) observed no change in the respiration ratio of aspartic acid supplied at concentrations ranging from 44 to 175 µg/ℓ.

The difference in the carbon respiration and nitrogen regeneration ratios may be due to the fact that the carbon respiration ratios are not equilibrium measurements as are the nitrogen regeneration ratios I have reported. If we assume, for the moment, an average C·N ratio of 4:1 for an amino acid mixture, and if we take 25 and 50% as the range of carbon respiration ratios from Hobbie and Crawford (1969), and 80% as a nitrogen regeneration ratio from my data, then amino

acid carbon and nitrogen would be incorporated into particulate matter by heterotrophs in a final ratio of 10:1 to 15:1 if they are growing on the amino acid mixture alone. These ratios are somewhat higher than the values reported for plankton, (3.1 to 9.1, Parsons and Takahaski 1973, Parsons et al 1961). If the carbon dioxide released during Hobbie and Crawford's (1969) measurements were primarily due to decarboxylation reactions in the initial steps of catabolism which produce little energy, subsequent respiration would increase the fraction of carbon released as carbon dioxide. Changing the respiration ratio to 75 or 80% with an 80% nitrogen regeneration ratio gives C·N incorporation values of 4·1 to 5 1. Changing the nitrogen regeneration ratio to 50% and using Hobbie and Crawford's values for the respiration ratio gives incorporation ratios of 4·1 to 6·1. Clearly, experiments such as those of Hamilton and Austin (1967) and Wang et al (1958), and the concurrent measurement of carbon dioxide and ammonia release accompanying the degradation of amino acids are needed to resolve the apparent contradiction between carbon and nitrogen metabolism in heterotrophic populations.

CONCLUSIONS

This work demonstrates that other amino acids display the same kinetics for degradation as L-arginine. The durations of the lag periods preceding increases in the rate of ammonia release seem to be functions of the way the molecules are metabolized by the

heterotrophs. The fraction of nitrogen released as ammonia during the degradation of the amino acids tested also varied from compound to compound, but was not related to the C:N ratio of the compound. The nitrogen regeneration ratio of L-arginine varied from experiment to experiment, presumably in response to changes in environmental factors, while the duration of the lag remained fairly constant. The regeneration ratio ranged from 60 to 85%. The other amino acids examined in this study had values in this range or higher, 80% is a reasonable mean value for the amino acids studied here. Because of the variability of the nitrogen regeneration ratio compared to the lag time, the duration of the lag to the increase in the rate of degradation can be predicted more accurately than the nitrogen regeneration ratio for a given amino acid.

SECTION III Metabolic Adaptation in Natural Bacterial Populations Supplemented with Selected Amino Acids.

INTRODUCTION

Section I demonstrated that the primary pathway for the recycling of amino acid nitrogen at a station in outer Halifax Harbour was via the bacterial degradation of those compounds resulting in the release of their nitrogen as ammonia. This supplements the findings of many workers (Hobbie, et al. 1968, Andrews and Williams 1971, Derenbach and Williams 1974, Williams 1970, Williams and Askew 1968 and others) by providing direct evidence for nutrient regeneration as discussed in Wright (1974). It differs from the results of Scheil (1974) who reported the direct utilization of amino acid nitrogen by phytoplankton in Auke Bay, Alaska.

The kinetics of ammonia release observed in Section I and II differed from the linear, (or log, if growth were involved), curves that would be expected from a steady state system controlled by Michaelis-Menton kinetics (Wright and Hobbie 1966, Williams 1973a) A lag period was followed by a marked increase in the rate of release of nitrogen from the amino acid supplied. The length of the lag time, rate of release, and percentage yield of ammonia varied with the amino acid supplied and with water conditions, (figs. 3, 6, 12, 13, 14).

Plausible explanations of these observations include the growth of a small, metabolically active population of heterotrophs into a population large enough to account for the observed rate of ammonia

release. The growth may be in response to the added substrate or may be a "wall effect" resulting from enclosure of the population in the experimental vessel, as discussed by Zobell and Anderson (1936) In this interpretation, the lag period and pulse of ammonia would be artifacts of the experimental design. The lag period would be only an apparent lag as the rate of ammonia release during the early stages of population growth would be too low to be detected. Williams and Gray (1970), Williams (1970), Vaccaro (1969), Vaccaro and Jannasch (1967) have treated this problem in some depth.

Another plausible explanation, also treated by the above authors and mentioned by Paerl (1974), Hobbie et al (1972) and others, is based on metabolic adaptation or activation in the population stimulated by the presence of the substrate In this model the bacteria revert to a dormant state in times of nutritional stress. The fraction of the population that is active or dormant would depend on the nutritional history of the population and hence will vary spatially and temporally in a given body of water, (Gardner and Lee 1975, Tanaka et al. 1974, Jannasch 1974, Jannasch and Mateles 1974, Bell et al. 1974, Andrews and Williams 1971, Paerl 1974, 1975, Droop and Elson 1966). The substrate concentrations causing the bacteria to revert from dormancy to activity will determine the spatial and temporal scale of bacterial activity, however, behavioral responses to the presence of a substrate (Bell and Mitchell 1972, Adler 1969, Droop and Elson 1966, Paerl 1974, 1975), could modify a model of bacterial activity based strictly on bulk water substrate

concentrations.

The experiments discussed in this section were performed to gain information on the mechanism giving rise to the observed kinetics of ammonia release It was hoped that additional information would allow one to assess the relative values of the two proposed models or to point out the need for another alternative. Other objectives were to obtain empirical information of predictive value applicable to substrate degradation in mixed natural communities and to investigate the effects of physical factors such as temperature and light on the kinetics of amino acid degradation

MATERIALS AND METHODS

The populations used in this study were collected from Station A during September and October of 1975. The water column during the September experiments was stratified; surface temperatures were in the vicinity of the seasonal high of 16°C. A series of early fall storms eroded the thermocline, the surface temperature dropped rapidly to 10°C during the last week of September and remained at that temperature during the October experiments.

Water samples were collected, pre-treated and partitioned into the experimental vessels as in Section I. Substrates were added to the flasks which were then placed in a dark incubator whose temperature was within 1 C° of the in situ temperature at the time of collection.

Substrate stocks were prepared from reagent grade chemicals as

10.0 mM solutions in deionized distilled water. The solutions were prepared for immediate use or were filter sterilized and stored frozen at -15°C in sterile serum bottles. Substrate additions to the flasks were made by pipet or sterile syringe. Final substrate concentrations were 10.0 µM of each substrate added to a given experimental flask unless otherwise noted.

Lincomycin-HCl and erythromycin gluceptate (Ilotycin gluceptate) were produced by UpJohn Canada Ltd. and Eli Lilly Canada Ltd. respectively and were obtained from a local pharmaceutical distributor as pure compounds. Cycloheximide was obtained from Sigma.

Ammonia was determined as in Section I. Amino acids were determined as primary amines by a technique similar to that of North (1975) developed by R. Zika of this department. Fluorescamine was purchased under the trade name Fluram from Roche Diagnostics. A working solution of 100 mg/500 ml was made up in spectranalyzed grade acetone freshly distilled from anhydrous cupric sulfate. The reaction took place at room temperature at a pH of 9.4. Full details of the technique including a modification that permits the detection of primary amines at .01 µM can be obtained from R. Zika. Fluorescence of the derivative was measured at 25°C on a Aminco SPF 125 spectrofluorometer equipped with a constant temperature jacket for the sample chamber. The excitation and emission wavelengths were adjusted for maximum response at the beginning of each run and were around 395 and 495 nm respectively.

ATP was determined by the method of Holm-Hansen and Booth (1966)

on samples collected on Reeve-Angel 984H glass fiber ultrafilters (nominal pore size 0.1 μ). Samples were counted on a JRB 2000 ATP photometer.

The distribution of the amino acid carbon during degradation was followed in one experiment using ^{14}C uniformly labelled substrates. Fifty milliliter samples were withdrawn from the experimental vessels and filtered through glass fiber ultrafilters. The filtrate was acidified and scrubbed for at least 20 minutes with nitrogen gas and the carbon dioxide collected by passing the gas through 5 ml of 1.00 N sodium hydroxide. After scrubbing, a 1.00 ml aliquot of the filtrate was placed in scintillation vial. 0 50 ml. of the sodium hydroxide solution was placed in a scintillation vial and 0.40 ml of 1.00 N hydrochloric acid added to facilitate solution in the fluor. The filter was also placed in a vial and 10 ml of a fluor composed of 1.1 v/v Triton X-100:toluene fluor was added (Pugh 1973). Samples were capped, shaken, allowed to equilibrate in the sample belt, and counted in a Packard Tri-Carb Liquid Scintillation Counter equipped with an Automatic Activity Analyzer to correct for quenching and counting efficiency. The trapping and counting system for the carbon dioxide was from 80 to 90% efficient as described (see Addendum). Substrate carryover in the damp filters was negligible as determined by a double filter technique. Radiochemicals were purchased as chromatographically pure uniformly ^{14}C labelled L-isomers from New England Nuclear Canada, Ltd.. These were mixed with carrier L- amino

acids (Sigma) to give specific activities of 1.9, 1.3 and 1.0 μCi/μ mole for L-arginine, L-lysine and L-leucine respectively.

The experiments were set up so that each treatment was duplicated. The ammonia concentration was determined in triplicate samples from each of the duplicate flasks; amino acid determinations were in duplicate or triplicate. Usually 1.50 ℓ of raw seawater contained in a 2ℓ flask was used for each treatment, 750 ml of water in 1.0 ℓ flasks were used for the temperature and light experiments. Duplicate flasks were within ± 0.5 μM for ammonia concentrations to 10 μM, and ± 2 μM for ammonia concentrations above this. Arginine concentrations measured in duplicate flasks were within ± 0.2 μM.

RESULTS

Accelerated microbial activity is frequently reported when raw seawater is placed in an enclosure, (Zobell and Anderson 1936). This "bottle effect" is sometimes attributed to the proximity of the walls of the vessel and increased surface area for bacterial growth. It has also been attributed to changes in the fluxes of dissolved gases, solutes, or particulate matter related to the disruption of a steady state flow-through system by placing it in an enclosure. If the effect was important in these experiments and was related to the presence of a glass surface, then changing the amount of the surface area should affect the kinetics of uptake of an added substrate. Specifically, the lag preceding the increase in uptake rates should decrease and the rate of uptake should increase when the

surface/volume ratio increases. Figure 15 shows the results of such an experiment. No significant difference was observed in the kinetics of the disappearance of L-arginine from the medium in flasks of different surface to volume ratios, (0.7, 0.5 and 0.3 cm^{-1} for 500, 1000, and 2000 ml flasks containing 400, 750, and 1500 ml of water respectively and treating the flasks as cones) The 20 ℓ carboy (surface/volume ratio = 0.15 cm^{-1}) had a longer lag-time than the flasks if times to 50% substrate depletion are compared, however the carboy was not removed from the incubator for sampling as were the flasks. In addition, the temperature inside the incubator briefly rose a few degrees when the door was opened for sampling. These facts, and the greater thermal inertia of the large volume of water and glass in the carboy relative to the flasks, meant that the water in the carboy was incubated at a lower temperature, on the average, than the water in the flasks. The duration of the lag for L-arginine was subsequently observed to be strongly temperature dependent (figs. 27, 28).

Increasing the length of time that the water is in the bottle before the substrate is added should increase bacterial activity, numbers of bacteria, and substrate degradation rates if the bottle effect was important in these experiments. The lag preceding the increase in the rates of uptake or degradation of an added substrate should decrease as the length of time in the bottle before the substrate is added increases. Figure 16 shows that the lag for L-arginine decomposition increased slightly, and the maximum rate of

ammonia release decreased slightly, with increasing pre-incubation time in the flasks in the absence of added substrate.

Figure 17a shows the decrease in concentration of L-arginine, L-lysine and L-leucine in experimental flasks with time. These compounds were chosen because they had displayed distinctly and repeatedly different kinetics for ammonia release in earlier experiments. Figure 17b gives the ammonia release curves, figure 17c the ATP concentrations in the flasks, and figures 17d, e and f the ^{14}C distribution curves for this experiment. The slight initial uptake of the substrates is shown most clearly in the ^{14}C uptake curves. Ammonia release, carbon dioxide release and amino acid uptake with L-arginine and L-lysine additions all proceed at an increased rate after an initial lag period. The lag period is of approximately the same length for all of these processes for a given substrate. The addition of L-arginine and possibly L-lysine resulted in a transitory increase in the ATP concentration occurring at the same time as or just following the increase in degradation rates. The decrease in amino acid concentrations proceded at a constant rate until a basal level not different from a "no additions" control or the seawater blank is reached. There is no obvious reason for the variability of the L-leucine filtrate ^{14}C activity curve; the drop in concentration indicated by fluorescamine is not reflected in the other curves because this point was taken after the last points for the other determinations (see Addendum).

Figure 18 presents the results of two experiments on the effect

of inhibitors of protein synthesis on the kinetics of the uptake of L-arginine. Erythromycin is fairly specific for Gram-positive bacteria, Lincomycin affects both Gram-positive and negative bacteria. Cycloheximide affects eukaryotic organisms. All act at the level of translation, they block the elongation of a growing peptide chain by binding irreversibly to the mRNA-ribosome-peptide complex (Caskey 1973). Figure 18 shows that lincomycin at high concentrations somewhat lengthened the lag period and slowed the rate of L-arginine uptake, however, it did not block the increase of the uptake rate as did erythromycin. Cycloheximide had no effect on the uptake of L-arginine. It is possible that the lack of an effect for lincomycin and cycloheximide is due to decomposition of the substrates in the medium, however the fact that the same kinetics were observed for L-arginine uptake in the presence of these compounds at concentrations differing by an order of magnitude suggests that this is not the case.

The results of an investigation into the uptake of D- and L-arginine are enlightening at this point. Table III shows that if the population is adapted to use D-arginine and then presented with the L-enantiomer, the latter compound is taken up rapidly with no lag. If the population is adapted to use L-arginine and then presented with the D-enantiomer, rapid uptake commences only after a lag time of 12-18 hours, equal in duration within experimental error to the differences between the lags preceding the increase in uptake rates for the isomers commencing with unconditioned populations (fig. 19). If erythromycin is added at an inhibiting concentration to the L-induced system, no uptake

of the D-enantiomer is observed; however, the L- form is taken up as usual. If the same experiment is performed with the D- induced system both isomers are removed from the water. These results would be expected if one postulated the induction of a racemase, the activity of which preceded that of an L- specific permease system.

The results of the preceding experiments indicate that protein synthesis is necessary for metabolic adaptation in the populations studied. This adaptation seems to involve both uptake and catabolic enzymes, although the induction of a permease system is sufficient to explain these results. If such an enzyme is induced, then after a period of time in the absence of the inducing compound, the enzyme concentration and hence its activity should decline through normal protein turnover. It should then be possible to re-induce the enzyme by the addition of more substrate. This result was obtained when the appropriate experiment was performed (fig. 20). A bacterial population in a carboy of raw seawater was adapted to use L-arginine and then assayed for the ability to remove L-arginine from the water thereafter. A decline in this activity took place over 6 days. After the activity had disappeared (determined as no uptake of a 10 μM addition of L-arginine after 4 hours) two 1.50 ℓ aliquots were withdrawn from the carboy. One was supplemented with L-arginine while the other served as a control. Activity returned to the supplemented aliquot after 12 hours, a significantly shorter lag than the initial lag, suggesting that a portion of the enzyme system or its precursors were still present.

What concentration of amino acid must be present to stimulate metabolic adaptation? In one experiment L-arginine was added to raw seawater to give initial concentrations of 10.0, 5.00 and 1.00 µM. A marked increase in the uptake rate following a lag was observed at 10.0 and 5.00 µM and is suggested by the shape of the curve for the 1.00 µM flasks (figure 21). In other experiments (Section I) the lag and increase is observed with L-arginine additions of 0.5 and 1.0 µM when increased chlorophyll fluorescence is used to indicate the production of ammonia.

An investigation was made into the utilization pattern when the population is presented with more than one amino acid simultaneously. The results are shown in figure 22. Concurrent uptake of L-arginine and L-orthinine or L-arginine and L-lysine was observed when the pairs of compounds were added to the experimental vessels Diauxic uptake of the compounds was not observed. These results are consistent with the uptake system specificities shown in Table IV for L-arginine + L-orthinine but not for L-lysine + L-arginine.

The decreased lag period for L-lysine uptake in the presence of L-arginine is similar to results obtained with L-arginine in the presence of other organic compounds. Figure 23 shows the effect of additions of D-glucose, glycolate, and glutarate on the degradation of L-arginine. The addition of glucose greatly reduced the lag for the release of ammonia from L-arginine. Glycolate and glutarate had no observable effect on L-arginine catabolism.

Figure 24a shows the effect of some intermediates of major metabolic pathways on the uptake of L-arginine, and Figure 24b shows their effects on L-arginine catabolism. D-glucose, citrate, succinate, and succinate plus acetate all shorten the lag period preceding the increase in the rates of L-arginine uptake and catabolism Acetate alone shortens the lag period slightly. Acetate added with succinate further decreases the already shortened lag for L-arginine uptake in the presence of succinate alone. There is no significant difference between the ammonia yields in flasks with added organic compounds. The experiment was terminated before uptake and catabolism had ceased in the flasks with no additions and with only acetate added. A comparison of the times required for 50% of the L-arginine to be taken up and 50% of the final yield of ammonia to be released provides a good demonstration of the synchrony of these two processes.

An investigation was made into the effect of changing the C.N ratio of a mixture of substrates on the uptake and catabolism of L-arginine. D-glucose was added to flasks containing 10.0 µM L-arginine to give glucose concentrations of 0.0, 10.0 or 50.0 µM. These results are given in Figure 25. Glucose at 10.0 µM decreased the lag preceding the increase in the rate of uptake of L-arginine by 10 hours, however, no further decrease in the lag time was observed with the addition of more glucose. This agrees with the results obtained when substrates with different amounts of carbon and energy per molecule were added to a constant concentration as demonstrated in Figures 23 and 24. A decrease in the total amount

of L-arginine taken up was observed in the presence of 50.0 µM glucose. This repression shows up during the latter stages of uptake and in the equilibrium condition reached after 48 hours. In contrast, L-arginine was taken up to the same extent in the presence of 10 0 µM glucose as when L-arginine alone was supplied The differences are also reflected in the ammonia values at equilibrium, taken at 84 hours (Table V).

The results presented in Table V are calculated on the assumption that a constant fraction of the total carbon taken up is respired. If the fraction of substrate carbon respired increased with increasing substrate concentration as suggested by Wright (1974), the C:N ratio of incorporation could be constant.

An experiment was performed to determine whether the presence of nitrate or ammonia had an effect on the degradation and release of ammonia from L-arginine (fig. 26). The equilibrium concentration of L-arginine nitrogen appearing as ammonia is the same in all treatments, and the duration of the lag preceding the increase in the uptake rate is the same for the control and L-arginine in the presence of ammonia. Due to the more rapid rate of degradation in the period between 32 and 45 hours, the lag time preceding the increase in the rate of degradation of L-arginine in the presence of nitrate appears to be about 4 hours shorter as estimated by the time to 50% ammonia yield; however, this is probably not significant

The experiments presented above have aimed primarily at understanding the physiological basis for the observed curves. The data on decreased

lag periods associated with the simultaneous addition of more than one substrate, and the decrease in the lag following reactivation (figs. 20, 23 and 24) are applicable to an understanding of the ecology of the bacterioplankton. The following experiments provide data which will be useful in any attempt to formulate a predictive model of bacterial activity based upon the results of the experiments on physiology.

The effects of light and temperature on the length of the lag period for L-arginine uptake are shown in figure 27. There is no significant difference between L-arginine uptake in the darkened flasks and those placed in the light (0.01 langley min^{-1} in the photosynthetically active portion of the spectrum). This agrees with results obtained in earlier experiments on ammonia production where it was observed that the only effect of light was to stimulate phytoplankton growth which removed ammonia from the water making it difficult to accurately determine the release kinetics. When limitation by a nutrient other than nitrogen prevented phytoplankton growth, the ammonia release curves were the same in both dark and light flasks (Section I, figs. 3 and 4).

Water temperature exerts a strong effect on the duration of the lag preceding the increase in uptake rate. The slope of the line relating the times for the disappearance of 50% of the substrate (a measure of the lag time) to temperature (fig. 27) is approximately $-5/hr./C^{o-1}$, giving a "Q_{10}" value of 3.0. The maximum uptake rate increases in a similar fashion; however, due to the frequency of

sampling it is impossible to estimate this parameter accurately from this data. A repeat of this experiment over the temperature range 3° to 25°C (see Addendum) gave a slope of -3.9 hr/C° and a "Q_{10}" of 2.4

The experiment which provided the data for figure 27 was performed with water collected from Station A on 25 September. Figure 28 presents data from experiments run under comparable experimental conditions (Station A, dark, f/40-N pre-treatment, 1.50 ℓ of water in 2 ℓ flasks, L-arginine at 10 0 μM), run at the in situ temperature at different times of the year. Most of the experiments were performed in the early summer and early fall, hence the clustering of points at the high and low ends of the temperature scale. The slope of the regression line is -5.7 hr./C° with $r^2 = 0.96$, and agrees well with the temperature dependent regression obtained by incubating sub-samples of one water sample at different temperatures.

These data indicate that at this station the water temperature is probably the primary factor which determines the length of the adaptation lag for the uptake of a given substrate. Over broader geographical areas, temperature is not the only important controlling factor, as is suggested by the experiments with Sargasso Sea water shown in Figure 29 Despite the warm temperature (23° for the experiment shown in Figure 29a and 21° for those in 29b), there was a pronounced lag period in the kinetics of the degradation of L-arginine and L-glutamate. Variation in bacterial activity and the duration of

the lag periods takes place over much shorter distances as well. Figure 30 gives the results of uptake experiments performed with water taken from various areas of Halifax Harbour. Station B was near a major sewer outfall off Herring Cove, Station C was offshore in clean shelf water. The differences in the water sources are reflected in the total primary amines determined as L-arginine by the fluorescamine technique, and salinities given in the figure. There was little difference in the uptake lags for Station A and C, however, Station B had a greatly reduced lag period. This is probably due to a combination of the presence of other organic compounds in the water and recent exposure to substrates.

DISCUSSION

In these experiments a mixed natural population behaved as if it were a pure culture. Stumm-Zollinger and Harris (1971), Stumm-Zollinger (1966), Gaudy and Gaudy (1966) and Gaudy et al. (1963) have also observed mixed populations of bacteria taken from waste waters to act as pure cultures with respect to catabolite repression and allosteric interactions in the utilization of a variety of substrate pairs. Stumm-Zollinger (1968), however, suggests that in some cases this may be in part due to the growth of certain bacterial species, a point of view held by Jannasch and Mateles (1974).

The observations presented in this thesis, especially the results of the experiments on "bottle effect", on the effects of inhibitors of protein synthesis, on the loss and recovery of the ability to take up L-arginine, on the utilization of D- and L-arginine, on the specifities of amino acid uptake, and on the increase in the concentration of ATP

observed during active uptake and catabolism of L-arginine, strongly suggest that the increases in the rates of substrate uptake and catabolism as indicated by ammonia release, are due to metabolic adaptation or enzyme induction in the population. The other experiments may also be interpreted in this way. Except for ATP data, which is susceptible to interference from non-bacterial ATP, there were no direct measurements of population changes. While it is possible that some increase in cell numbers took place during the lag period, this does not seem to be important in view of the data presented above.

Although most of this work was done with L-arginine, the adaptation lag for the increase in the rates of uptake or degradation was observed for most other amino acids examined (figs 12, 13, 14). The factors observed to affect the length of the activation period include temperature, the type of substrate, the presence of other substrates and time since a previous exposure to the substrate. The interaction of these factors may explain many of the differences reported by various authors in the rates of degradation of amino acids, and a similar picture may emerge when other organic compounds are examined.

As only the uptake of L-arginine was measured in experiments where non-nitrogenous carbon sources and L-arginine were added to water samples simultaneously, it is not known if there is a decrease in the lag period for the uptake of the other carbon sources as well. When L-ornithine or L-lysine were presented to a population together with L-arginine, a decreased lag was observed that was reciprocal but

asymmetric (fig. 22). This could be explained for the L-arginine: L-ornithine interaction on the basis of the co-induction of uptake systems; however, the L-arginine:L-lysine interaction is not so readily explained. Although L-lysine, which has an adaptation lag period of about twice that of L-arginine under a given set of conditions, triggered the adaptation of the population to the use of L-arginine, the reciprocal effect was not found. Yet L-lysine was taken up together with L-arginine after a lag that was greatly reduced for L-lysine but only slightly reduced for L-arginine. In this case, as with the non-nitrogenous substrates discussed above, the mechanisms for shortening the lag may be related to a more fundamental phenomenon than the induction of a transport system and may include the activation of a dormant population or an increase in the cellular energy charge which will increase the rate of protein biosynthesis.

The uptake observed initially in these experiments (fig. 17 a,d,e, and f, 32a,b,c) may correspond to the uptake that would be measured in a short term incubation at a saturation substrate concentration which would correspond to the heterotrophic potential measurement of Parsons and Strickland (1962). If so, estimates of this type would underestimate the true potential of a population, as the activity of the unadapted population is only a small fraction of that observed in an adapted population. Within the framework of the kinetic analysis developed by Wright and Hobbie (1966) from Parsons and Strickland's (1962) assay, the substrate adapted or activated population would have a much higher maximum uptake velocity (V_{max}) than the unadapted

population These experiments could correspond to a low substrate level enrichment culture, but only if there is cell multiplication. The curves in figures 17d, e, and f, and 32a, b, and c for the uptake and degradation of ^{14}C labelled substrates indicate that a steady state is reached and this precedes adaptation, as filterable radioactivity increases at a constant rate as does the concentration of labelled carbon dioxide.

It is possible to account for this pattern of uptake in at least three ways. A fraction of the population may have been exposed to the substrate recently enough to have retained some enzyme activity; some cells are active but will become dormant like the rest of the population if deprived of an energy source for a long enough period of time. This is suggested by the increased lag observed with increasing lengths of pre-incubation observed in figure 16 and by the decrease in the L-arginine uptake activity of the population in the carboy in figure 20. Alternatively, the activity in the population as a whole may decline to a low but non-zero value in the prolonged absence of an inducing substrate, all cells in the population are active but at a very low level. Another possibility is that the population of cells is composed of species that adopt two different strategies of survival at low substrate levels: some go into a resting state while others maintain an active metabolism on the low substrate levels by having extremely efficient transport systems; the population is composed of two sub-populations, one of which is always active, the other of which is opportunisticly active. This strategy is discussed by Jannasch (1974).

The length of the lag period preceding the rate increases observed here is more consistent with a metabolic activation model than with a simple enzyme induction model. The time necessary to produce the enzyme complement needed to metabolize the added substrates seems greater than would be expected on the basis of simple enzyme induction in a metabolically active population (Watson 1965). Postgate (1967) and Postgate and Hunter (1962, 1963) document the progressive decline of cell protein and then cell RNA when bacteria are placed in mineral media in the absence of an energy source. Inducible enzymes are the first proteins to be catabolized, followed by structural proteins and constitutive enzymes. It follows that substrates which are intermediates of major metabolic pathways will be able to supply the carbon and energy to reverse this deterioration more rapidly and after a more prolonged starvation period than intermediates of peripheral pathways. A substrate which permits the production of more energy per molecule transported (citrate as opposed to succinate or acetate) should allow more immediate and complete recovery or activation of a population, all other things being equal. Wright (1974) has suggested that the relative mineralization, and hence the relative rates of mineralization of amino acids, is related to the ease of entry of their carbon into the central energy producing pathways This is also suggested by the data on the uptake and catabolism of L-arginine in the presence of the various organic substrates (fig. 24) discussed above.

The limit in the reduction of the activation lag period by the addition of other substrates may be due to metabolic

constraints on the rates of synthesis of enzymes and other metabolic machinery needed for the recovery of activation of cells. Postgate (1967) was not concerned with the formation of a true resting stage as discussed in Ramaley and Bernlohr (1966) or Murrell (1967), the development of active vegetative growth from such a resting stage would be expected to take a finite period of time regardless of the type or quantity of substrate offered. Postgate and Hunter (1962) documented the degradation of cellular RNA as well as protein during starvation Loss of RNA from the ribosomal or transfer fraction will retard the ability of a cell to recover and thus lengthen the lag period before a substrate can be metabolized by a pathway containing inducible enzymes. It follows that substrates catabolized by pathways whose enzymes are resistant to degradation will be more independent of the effects of starvation and will be better suited to supply the cell with the energy and substrates needed to recover from starvation. The uptake and catabolism of a substrate is an autocatalytic event; catabolism of a small amount of substrate permits the synthesis of enzymes to catabolize more substrate. This supplies the energy and reduced carbon necessary for the synthesis of other cellular materials, ultimately resulting in DNA replication if the amount of substrate taken up exceeds some threshold. The production of ATP as a transient peak during the catabolism of L-arginine (fig. 17c, 32d) can be interpreted in this way.

Survival strategies in marine bacteria exposed to a fluctuating substrate resource could take at least two forms. One could depend on retaining a small surplus of energy and precursor molecules and

exist in a resting form during the periods when substrates are unavailable (Zymogenous, r selected: cf. Jannasch 1974). A second could rely on the preservation of some minimal level of activity in metabolic pathways for which the probability of taking up enough of the substrate of those pathways in a given time period to at least meet the energy cost of maintaining the activity is sufficiently high (autochthonous or K_s selected. cf. Jannasch 1974). Failure to maintain the activity and to take up the required quantity of substrate during the time interval, or to maintain the potential for the activity would result in the death of the cell. Energy yield per molecule transported and catabolized, and the facility with which the molecule or its primary degradation products enter the energy producing pathways will be important considerations. The probability of encountering a given type of molecule will be an environmental constraint; however, behavioral modifications will influence this aspect of acceptability (Alder 1969, Bell and Mitchell 1972; Bell et al. 1974). Alder (1969) has shown that bacteria can respond to chemical signals without taking up the substrate. This could allow them to travel up a gradient to reach substrate concentrations at which uptake systems could operate. Alder worked with Escherichia coli, a bacterium adapted to relatively high substrate levels compared to marine bacteria (Jannasch and Mateles 1974). Other systems can respond to much smaller substrate gradients (Rosen 1975) and presumably a nutritionally dilute environment could select for more sensitive chemoreceptors in bacteria adapted for survival in this environment. Selection for long half-lives in the enzymes of peripheral pathways catabolizing molecules that are energy

rich and frequently encountered could lead to the establishment of these pathways and provide a mechanism for niche differentiation in bacterioplankton.

CONCLUSIONS

The lack of a pronounced bottle effect (figs 15, 16), the results of the experiments with inhibitors of protein synthesis, (fig. 18, Table III), the results of the experiment on the loss of L-arginine uptake activity and its reinduction (fig. 20), the specificities of the uptake activity (Table IV), and the transitory increase in ATP observed during active L-arginine uptake and catabolism (fig. 17c, 32d) suggest that the lag observed in amino acid uptake and degradation is related to the activation of a metabolically quiescent population or a switch in substrate utilization triggered by the addition of the amino acid

Various factors have been observed to modify the duration of this lag period in natural populations of marine bacterioplankton. Of the factors discussed in this paper, water temperature and the presence of other readily metabolized substrates seem to be the most important. Enzyme production during the lag may be part of the general reactivation of a metabolically quiescent population. The interaction of those factors may help to explain some of the general trends and variability observed in experiments measuring the kinetics of the uptake of organic substrates by bacterioplankton.

Bacterial activity can be expected to be dependent on the flux of energy (reduced carbon) through a given system. Lag times for

adaptation to a substrate, which should be related to the fraction of the population that is active at a given time, could range from zero in productive areas such as eutrophic estuaries or lakes, to long periods in extremely oligotrophic areas. Patchy distributions of substrates, either spatially or temporally, could lead to a patchy distribution of activity and variability in uptake rates measured in short incubations.

GENERAL CONCLUSIONS

Although it is tempting to speculate that phytoplankton utilize organic nitrogen directly from the environment, the results of this study suggest that if this does occur at this station, it is not an important contribution to the nitrogen required for primary production, nor is it important as a sink for amino acids released into these communities. Schell (1974) concluded that phytoplankton at his station used amino acid nitrogen directly. Derenbach and Williams (1974) and Wright and Hobbie (1966) obtained results similar to those I obtained in this study. The primary sink for amino acids in Halifax Harbour plankton communities seems to be bacterial respiration and assimilation.

Nitrogen regenerated as ammonia during bacterial respiration of amino acids at this station may be important in maintaining primary production in communities occurring here in stratified water columns. The release of ammonia accompanied the degradation of all amino acids tested, and if it is shown that bacteria can use amino acid polymers, it is probable that ammonia will be released during their degradation as well. Nitrogen regeneration seems to be related to the C:N ratio of the diet of the bacteria rather than to the C:N ratio of the amino acid supplied (Sections II and III, figs. 12, 13, 24 and Table V). This is perhaps to be expected, and agrees with Thayer's (1974) work on the competition between algae and bacteria for nitrogen in an environment rich in reduced carbon.

Lags in uptake and nitrogen regeneration were seen with most amino acids tested. The duration of this lag period, which was suggested to

be due to metabolic adaptation, is affected by the types of substrates available to the bacteria, as is nitrogen regeneration. Complex diets (two or more substrates supplied simultaneously) significantly shortened the lag for the increase in the rate of uptake of the amino acid supplied. As bacteria at this station will probably never be using a single substrate at a time, the lags measured in these experiments probably represent the maximum lag periods for these substrates. Vaccaro (1969) and Williams and Gray (1970) found similar lag periods in the utilization of some non-nitrogenous organic compounds, so they may be a general property of bacterial activity that is observable only when substrates are present at higher than bulk water concentrations.

The possibility that bacteria adapt metabolically to changes in substrate types or levels in the environment does not seem to have been seriously considered in the open literature of biological oceanography. These adaptive processes are well known in biochemistry and molecular biology where they were of inestimable value in helping to understand how the information coded in DNA is expressed as a protein molecule (Watson 1965, Beckwith and Zipser 1970). The current model of bacterial activity is that bacteria behave toward substrates as steady state systems governed by the Michaelis-Menton relationship (Wright and Hobbie 1966, Jannasch 1974). Uptake rates vary because the environmental concentrations of a substrate vary, and the Michaelis-Menton kinetic parameters (K_s and V_{max}) for a substrate vary from species to species. Increased activity (V_{max}) with time is usually attributed to increases in biomass (Williams and Gray 1970, Williams 1970, 1973a, Vaccaro 1969).

Bacteria are modeled as metabolically active but growing very slowly at low substrate levels, ready to respond rapidly to increases in substrate levels according to the Michaelis-Menton equation (Parsons and Takahaski 1973, Jannasch and Mateles 1974).

The bacterial activity measured at low substrate levels in oligotrophic areas has been assigned to autochthonous or K_s selected bacteria possessing uptake systems with extremely high affinities for the substrate, but low maximum uptake velocities and growth rates. The more opportunistic zymogenous bacteria (r or V_{max} selected) possess uptake systems with lower substrate affinities but higher maximum velocities and growth rates. They lose out in competitions at low substrate levels but dominate at substrate concentrations above some threshold value (Jannasch 1974).

The question is, does losing the competition for a substrate at low substrate levels mean death for the organism? Do bacteria particularly marine bacteria selected for survival at low substrate concentrations and fluxes (Williams 1970, Andrews and Williams 1971, Riley and Segar 1970), starve to death as Postgate and Hunter (1962) and Jannasch (1968) suggest, or do they go into some sort of metabolically quiescent state to await increases in substrate concentrations? It is tempting to reinterpret bacterial ecology simplictically in terms of alternative states of activity. At low substrate levels, the majority of the population would be in a resting state or metabolically "off". The bacterial activity measured under these circumstances could

be attributed to a small fraction of the population that is still "on". This fraction may be active because they possess uptake systems with higher substrate affinities, because their threshold for the "on-off" transition is lower, or because they have, through a random process, been in contact with higher substrate concentrations recently enough to have retained activity. At higher substrate concentrations a larger fraction of the population would be "on".

If the "off-on" transition involves the synthesis of uptake systems, uptake rates and V_{max} would increase during the transition because there would be more uptake sites per unit biomass than in the same bacteria before the transition. K_s values should be constant unless qualitatively different uptake systems are produced at higher substrate levels. The occurrence of more than one uptake system transporting a given substrate with different kinetic parameters is known for a variety of organisms (Grenson et al. 1966, Hellebust 1970, Kennedy 1972, Wallen 1973), and Jannasch (1968) has studied increases in K_s values for marine bacteria grown in higher than natural substrate levels. The error inherent in determinations of a K_s value increases as that value decreases, particularly when the Lineweaver-Burke transformation is used to linearize the data (Dowd and Riggs 1965). This fact, and the fact that these determinations are frequently highly variable in oligotrophic marine systems (Vaccaro and Jannasch 1966, 1967, Hamilton and Preslan 1970) will make changes in K_s values difficult to document.

In relatively eutrophic ecosystems, a larger fraction of the

population will be "on" and hence the increase in uptake rates will be smaller. I have observed that the duration of the lag is a function of the time since previous exposure to the substrate. Thus it is also likely that the lag period will be shorter in the eutrophic system because the probability of a given bacterium having been exposed to an activating substrate concentration in a given time interval is higher than in the oligotrophic system. In the extreme situation there will be no lag and no increase in activity with time following the addition of a substrate.

Competition among marine bacteria no longer needs to be based on kinetic parameters alone. The magnitudes of the threshold values for the "on-off" and "off-on" transitions for different species of bacteria, and behaviorial differences as discussed by Alder (1969) and Bell and Mitchell (1972) provide other parameters for niche differentiation. It is plausible that a bacterial species adapted to substrate concentrations that are higher than average for bulk water and which would otherwise be eliminated by a species which had a high substrate affinity (low K_s) uptake system in a strictly K_s-V_{max} controlled universe, could survive by reverting to a resting stage until chancing upon or chemotactically seeking out locally higher substrate concentrations such as would be found around a dead plankter. If the hypothetical bacterium were able to use polymeric substrates (starches, proteins, etc.) that either have low molecular diffusion rates or are insoluble, then the substrate actually used for growth would not be lost due to diffusion during the activation period. The free, monomeric sugars

and amino acids may act as chemical signals to alert the bacterium to the presence of a concentration of these polymeric substrates.

SUMMARY

1) The primary mechanism for the removal of dissolved free amino acids from the coastal station studied was heterotrophic utilization by bacteria.

2) Amino acid nitrogen was released as ammonia during the catabolism of these compounds.

3) The released ammonia was used rapidly for phytoplankton growth.

4) Nitrogen regeneration ratios, $\frac{\text{ammonia produced}}{\text{amino acid nitrogen supplied}} \times 100$, varied with the amino acid used and with the C:N ratio of the diet but not with the C.N ratio of the amino acid. These ratios ranged from 60% to 100% and averaged 80%.

5) Nitrogen regeneration ratios for L-arginine varied from 60 to 85% from experiment to experiment.

6) An initial lag period of slow uptake or degradation as indicated by ammonia release was followed by an abrupt and dramatic increase in the rates of uptake and degradation of added amino acids.

7) The duration of the lag period varied with the amino acid used, with incubation temperature, and with the presence of other simple organic substrates. Light and the presence of nitrate and ammonia did not affect either the lag period or the regeneration ratio.

8) The increase in the uptake of D-arginine followed a prolonged lag relative to L-arginine. The data suggest that the prolongation was due to the time needed for the synthesis of a racemase, the enzymatic activity of which preceded that of the L- specific uptake system.

9) The rate of uptake of L-arginine by a population that had been taking up the substrate at a high rate was observed to decrease gradually over a period of days to a negligible rate of uptake. A high rate of uptake was observed to follow a greatly reduced lag period when the population was re-exposed to the substrate

10) The pattern of a lag followed by a rapid increase in the uptake rate or degradation rate of L-arginine was observed when the substrate was added to give concentrations of 1.0 μM or greater.

11) Protein synthesis was required for the increase in rates.

12) The evidence suggests that the lag is due to the time necessary for metabolic activation or adaptation in the population.

13) These results are discussed in light of current steady state models of bacterial activity.

ADDENDUM

The experiment on the temperature dependence of the activation lag presented here was performed to extend the range of temperatures used in the original determination, and to determine whether the observed temperature dependence and "Q_{10}" varied with the population and environmental conditions. The water used in these experiments, was collected as in Section I from Station A on the 6th of July, 1976 for the temperature dependence experiment and on the 20th of July, 1976 for the carbon partitioning experiment. The *in situ* temperatures at the times of collection were 14 and 17°C on the 6th and 20th of July, respectively. The water was pre-treated and partitioned as in Section 1.

L-arginine supplied at 10.0 µM was the substrate for the temperature dependence experiment, which was performed as outlined in Section III. Duplicate flasks were incubated at 3, 5, 10, 15 and 20 and 25°C in the dark and the concentration of L-arginine, determined by the fluorescamine technique described in Section III, was measured at the times indicated. The results presented in figure 31 are similar to those obtained in the previous experiment. The slope of the linear portion of the curve in figure 31b (10 to 25°C temperature range) was - 3.9 hr/C° as compared to a slope of -5.0 hr/C° obtained in the previous experiment. The values of the "Q_{10}" for the activation process estimated from the 10 C° intervals 25°- 15°C, 20° - 10°C and 15° - 5°C are 2.6, 2.0, and 2.6 respectively, which gives an average value of 2.4, compared to a value of 3.0 estimated

from the slope of the curve of the activation time versus temperature graph for the previous experiment. It can be seen that the maximum rate of uptake declines with decreasing temperature, as would be expected and as has been reported previously for North Pacific heterotrophs taking up glucose (Takahashi and Ichimura, 1971).

As was noted in the Materials and Methods of Section III, it proved to be necessary to lower the pH of the sodium hydroxide solution used to trap the carbon dioxide evolved during the degradation of L- arginine, lysine and leucine. This was because a diphasic scintillation cocktail resulted with 1.00 N sodium hydroxide additions exceeding approximately 0.1 ml. It was also noted that this procedure increased the variability of the measurements of ^{14}C-labelled carbon dioxide, as well as lower the efficiency of trapping to approximately 80%. In addition, Iverson et al. (1976) found that radioactivity is lost when aqueous solutions of ^{14}C-labelled bicarbonate ion are counted directly in Aquasol. I have also observed this effect for the Triton X-100:toluene fluor used in this study. As a result the experiment was repeated using phenethylamine to trap the evolved carbon dioxide as in Hobbie and Crawford (1969).

L-arginine, L-glutamate and L-lysine supplied at 10.0 µM were used in the carbon partitioning experiment. Ammonia, ATP, and amino acid (primary amine) concentrations were determined as described in Section I and III and followed in separate duplicated 3.0 ℓ samples of water contained in 4 ℓ Erlenmeyer flasks.

Carbon partitioning was followed in duplicate 1 5ℓ aliquots of water contained in 2ℓ flasks. Unlabelled substrates were added to give 10.0 µM, then 10.0, 10.0 and 13.0 µCi/ℓ of ^{14}C uniformly labelled high specific activity L- arginine, glutamate, and lysine were added to the appropriate flask. All flasks were incubated together in a dark incubator at 17°C.

At the times indicated in figure 32 triplicate 50.0 ml subsamples were taken by pipet and filtered at 730 Torr through Reeve Angel 984 H glass fiber ultrafilters. This filter was placed in a scintillation vial, capped and immediately frozen at -20°C. The filtrate was placed in a 250 ml wide mouth conical flask. A #8 rubber stopper that held a piece of polyvinyl chloride rod bent into a U-shaped clip to hold a scintillation vial vertically inside the flask a few millimeters above the surface of the filtrate was used to close the flask. A 25 mm diameter Gelman A-E glassfiber filter was placed in the mouth of a scintillation vial and saturated (approximately 0.2 ml) with phenethylamine (Packard). The vial was quickly clipped to the stopper, then approximately 0.1 ml of 36 N reagent grade sulfuric acid was added to the filtrate in the flask with minimal mixing and the flask was immediately stoppered. The flasks were placed on a rotary shaker at 200 RPM for at least an hour. At the end of this period the scintillation vial was removed from the flask, the phenethylamine saturated filter was pushed to the bottom of the vial with a clean glass rod, and the vial was capped. One milliliter of the acidified filtrate was then pipetted into another scintillation vial which was also capped. At the end of the experiment 10 ml of the 1:1 Triton X-100:toluene fluor was added to

all vials. The vials were allowed to reach thermal equilibrium in the sample belt and counted in a Packard Tricarb liquid scintillation counter. Quenching was determined by an automatic external standardization mechanism. The counting efficiency for these samples was 85%. Retention of the label in the damp filters was negligible as determined by a separate experiment. The efficiency of the trapping system was 101 ± 2% ($\bar{X}$ ± S.D., n=20) as determined with a 5.0 μCi/ℓ ^{14}C-labelled sodium bicarbonate (New England Nuclear Canada Ltd. ampoules) solution in filtered (Reeve-Angel 984 H glass fiber) seawater treated exactly as if it were a sample, except that the filters were counted immediately. The activities of the bicarbonate solution and of the filters were determined by counting in in the Triton-toluene fluor to which had been added either 0.2 ml of phenethylamine or two drops of 1.00 N sodium hydroxide, and in a fluor composed of 2400 ml toluene, 1512 ml absolute ethanol, 16 gm POP, and 0.060 gm POPOP. Cab - O -Sil and two drops of 1.00 N sodium hydroxide were added to the latter fluor before the sample to be counted was introduced. All three fluor and base combinations compared favorably, however quenching in the Triton-toluene fluor with added phenethylamine lowered the counting efficiency slightly relatively to the other two fluors (84 as opposed to 87%). The presence or absence of Cab-O - Sil had no effect on the counts, which agreed very well with the activity reported by the supplier. When the aqueous bicarbonate solution was counted in the Triton-toluene fluor with no added base, loss of up to 50% of the activity was observed, although this number was generally closer to 30%.

The results of this experiment, shown in figure 32 and Table VI

compare favorably with those shown in figure 17 for the previous experiment. A slight decline in the total activity recovered, (the sum of the DPM per 50 ml in the filtrate, on the filter, and collected as carbon dioxide), was observed. This may be due to the loss of carbon to the atmosphere as a gas, or to carbon assimilated by micro-organisms growing on the walls of the flask. The slow decline in this value, (12, 19, and 8% after 98 hr for L-arginine, glutamate and lysine respectively) suggest that carbon losses to wall growth or the atmosphere are small, and that bacterial activity on the walls of the flask is not important relative to the activity in the water contained in the flask.

An apparent discrepancy between the nitrogen regeneration ratio and the carbon respiration ratio was discussed in Section II. It can be seen from Table VI that the nitrogen regeneration ratio increased with time for these substrates. The fraction of carbon respired similarly increased with time and substrate removal for L-lysine, but this value was fairly constant for L-arginine and L-glutamate. The respiration ratios observed here (69, 61 and 55% for L- arginine, glutamate and lysine measured after substrate depletion) are much higher than the values reported in the literature (8.4, 61.3 and 11.6 for arginine, glutamate and lysine; Hobbie and Crawford 1969, 3-19 for lysine and 21-36% for an amino acids mixture; Gocke 1976, and 22% for an amino acids mixture; Williams 1970) except for glutamate. This is probably due to the difference in the experimental procedure; the above authors used short incubations and low substrate concentrations. Because of the short incubation periods, substrates that do not feed directly into the Krebs cycle may have accumulated in an intracellular pool before they had an

opportunity to be degraded. Various authors (Gocke, 1976, Wright 1974, Williams 1973b, and Hobbie and Crawford 1969) have observed an increase in the respiration ratio of a substrate with increases in the substrate concentration which may be related to the time needed to fill the intracellular pools. While these increases in the respiration ratio are small for amino acids (Gocke 1976, Hobbie and Crawford 1969) they may have contributed to the generally higher values I observed.

A comparison of the radiocarbon data with the fluorescamine data in figure 32 suggests that some of the carbon supplied as amino acid must have been returned to the medium as dissolved organic carbon (DOC). When L-arginine was the carbon source, radiocarbon determinations indicated that only 88% of the carbon had been taken up and assimilated or respired. When the L-arginine concentration was determined by fluorescamine, the substrate was removed to a level not different from the blank for Halifax Harbour seawater, 0.2 to 0.7 µM determined as arginine. Radiocarbon data similarly indicated that 88% of the added L-lysine had been removed from the medium after 118 hours, fluorescamine gave a figure of 100% as for L-arginine. After 94 hours, 62 and 96% of the L-lysine had been removed from the medium as indicated by ^{14}C and fluorescamine measurements respectively, although the agreement between the duplicate flask ^{14}C data at this point was not good.

Finally, with the exception of the 36 hour points for L-arginine, a comparison of the nitrogen regeneration ratio using the radiocarbon data as a measure of the substrate taken up, and the carbon respiration ratio indicates that the nitrogen regeneration ratio for L-arginine and

L-lysine degradation is greater than the carbon respiration ratio for these substrates. This can in part be related to the release of DOC, as the regeneration ratios calculated using the fluorescamine data as a measure of the substrate taken up are 86 and 77% for L-arginine after 57 hours and L-lysine after 118 hours respectively. The nitrogen regeneration ratios for those two substrates are still larger than the respective carbon respiration ratios, however (68 and 65%). For glutamate, where uptake measured by ^{14}C and fluorescamine are in good agreement, (i.e. DOC is not released) the carbon respiration ratio exceeds the nitrogen regeneration ratio (64 and 38% respectively) after all of the substrate has been removed from the medium. The differences between L-glutamate and L-arginine or L-lysine with respect to nitrogen regeneration, carbon respiration, and DOC release may be related to the central role of glutamate in both carbon and nitrogen metabolism as opposed to the more peripheral positions of arginine and especially lysine. (Brown et al. 1974, DeHauwer et al. 1964, Maas 1961).

Finally, the ATP data presented in figure 32d demonstrates in greater detail the transient increase suggested for L-arginine in figure 17c. It is possible that the increase was observed in the samples to which L-lysine had been added due to the long interval between the 70 and 94 hour points. The transient increase in the concentration of ATP may be related to a transient increase in the energy charge of the cells in this population.

LEGENDS FOR FIGURES

Figure 1. Map of Halifax Harbour, Nova Scotia, Canada showing the locations of Stations A, B, and C.

Figure 2. Increase in chlorophyll fluorescence with increasing additions of L-arginine. Pre-treatment was to f/40 minus a nitrogen source and without Tris (f/40-N, see text). Ammonium chloride was added to one set of flasks at 10.0 µM as a control.

Figure 3. Ammonia release for L-arginine under nutrient pre-treatments varying from f/80-N to f/20-N. L-arginine was added to 10 0 µM to all experimental flasks. Figures 3 and 4 are from the same experiment. Ammonium chloride was added to give 40.0 µM in the control.

Figure 4. Chlorophyll fluorescence increases with increasing pre-treatment nutrient concentration from f/80-N to f/20-N. L-arginine was added to 10.0 µM to all experimental flasks. Figures 3 and 4 are from the same experiment. An ammonium chloride addition to give 40.0 µM served as a control.

Figure 5. Chlorophyll fluorescence increases with various substrates, all at 20.0 µM. Pre-treatment was to f/40-N

Figure 6. Ammonia production during the decomposition of D- or L-arginine and L-glutamic acid. Pre-treatment was to f/30-N. Figures 6 and 7 are from the same experiment.

Figure 7. Ammonia release from 5.00 µM additions of various substrates. Pre-treatment was to f/40-N. Figures 9 and 10 are from the same experiment.

Figure 8. Increases in chlorophyll fluorescence with the addition of various substrates, all of 5.00 µM. Pre-treatment was to f/40-N. Figures 9 and 10 are from the same experiment.

Figure 9. Increases in chlorophyll fluorescence with the addition of 10.0 µM of various substrates. Pre-treatment was to f/48-N.

Figure 10. ^{14}C Autoradiographs. The sample was incubated with ^{14}C-labelled L-arginine for 8 hrs. Radioactivity appears as a cloud of black dots surrounding and superimposed on the labelled object. Substrate was added to 0.10 µM and 5.00 µCi/ℓ, pre-treatment was to f/72-N.

Figures 10a, 10b, are unlabelled phytoplankton: 10a contains a clearly recognizable Prorocentrum micans, 10b contains a cell of a Chaeteceros sp. probably C. wighami. Figures 10c, 10d, 10e are labelled detrital particles and fecal pellets. Figure 10f is an unknown labelled particle, possibly an algal cell. It is not known whether or not the particle contained chlorophyll. All photographs were taken from the same slide.

Figure 11. Uptake curves for the autoradiography experiment. Pre-treatment was to f/72-N. Radioactive substrate was uniformly labelled and was mixed with carrier substrate to give 0.10 µM and 5.00 µCi/ℓ. The regression lines are linear with

$r^2 = 0.99$. Uptake rates are:

L-glutamic acid, in light	2.5×10^{-9}	M/hr
" " " dark	2.2 x "	"
L-arginine, " light	1.2 x "	"
" " dark	1.2 x "	"

Figure 12. Ammonia release accompanying the degradation of various amino acids and urea in the dark by a plankton community 29 May 1975. L-arginine, L-lysine, glycine, L-tryptophan, L-glutamate, L-leucine, urea, and L-valine supplied at 10 0 µM were tested.

Figure 13. Ammonia release accompanying the degradation of various amino acids in the dark by a plankton community collected 5 June 1975. L-arginine, L-citrulline, L-histidine, L-ornithine, L-aspartate, L-proline, and L-threonine supplied at 10.0 µM were tested.

Figure 14. Ammonia release accompanying the degradation of various amino acids and analogues in the dark by a plankton community collected 12 June 1975. L-arginine, DL-Nle; DL-norleucine, DL-AIB; DL-α-aminoisobutyrate, DL-α-methyl Glu, DL-α-methyl glutamate supplied at 10.0 µM were tested.

Figure 15. L-arginine uptake and ammonia production in the dark as a function of bottle size. L-arginine was added to give a concentration of 10.0 µM in Erlenmeyer flasks and a 25 liter carboy. The 2000 ml flask contained 1500 ml; the 1000 ml, 750 ml; the 500 ml, 400 ml and the 25ℓ carboy contained 18ℓ of water initially. The water was collected 25 September.

Figure 16. The effect of sample pre-incubation on the length of the lag period for ammonia release in the dark from additions to 10.0 µM of L-arginine. L-arginine was added to replicate 1.50 ℓ aliquots of seawater contained in 2 liter Erlenmeyer flasks after the water had been in the flasks for 0, 20 or 45 hours. The water was collected 11 September.

Figure 17. Uptake and degradation of L-arginine, L-lysine and L-leucine by natural populations of bacteria. a) Uptake as measured by fluorescamine; b) ammonia production accompanying degradation; c) ATP levels in the water, length of bar indicates range of data at a point; d,e, and f) partitioning of ^{14}C into filtrate, particulate and acid volatile (carbon dioxide) fractions during the degradation of L-arginine, L-lysine and L-leucine, respectively. Initial substrate concentrations were 10.0 µM in 1.50 ℓ contained in 2 ℓ Erlenmeyer flasks. See text for details. The water was collected 18 September.

Figure 18. The effect of various inhibitors of protein synthesis on the uptake of L-arginine initially at 10.0 µM. a) Cycloheximide, erythromycin, and lincomycin at various concentrations and combinations of erythromycin and lincomycin. The experiment was performed on water collected 2 October. b) Lincomycin added to water collected 23 October.

Figure 19. Uptake of D- and L- arginine, both initially added to 10.0 µM. Water collected 2 October, experiment run in 1.50 ℓ of water in 2ℓ Erlenmeyer flasks.

Figure 20. Induction and loss of uptake activity in the bacterial flora contained in 18 ℓ of water in a 25 ℓ Pyrex carboy. L-arginine was added to give 10.0 μM initially. After bacterial activity had been induced (60 hr lag), it was assayed in duplicate 100 ml aliquots withdrawn from the carboy. L-arginine was added to the aliquots to give 10.0 μM and the amount remaining after 4.0 hours was determined by fluorescamine. After the uptake activity had ceased, (no uptake after four hours), duplicate 1.50 ℓ aliquots were withdrawn from the carboy. One was left unchanged as a control and L-arginine was added to the other to give 10.0 μM (232 hrs). Uptake and uptake activity were assayed as before using fluorescamine. Water was collected 25 September. Length of bar indicates uptake in an assay.

Figure 21. Lag time until activation as a function of added substrate concentration; threshold concentration required to initiate activation. Additions of L-arginine to give 10.0, 5.00 and 1.00 μM were made to water collected on 25 September.

Figure 22. Uptake of L-arginine, ornithine, lysine and glutamic acid. Individual compounds added to 1.50 ℓ aliquots in 2 ℓ flasks to give 10.0 μM; flasks to which two substrates were added contain 10.0 μM of each substrate. All substrates were added at the same time at the beginning of the experiment. Water was collected 23 October.

Figure 23. Ammonia release accompanying the catabolism of L-arginine by coastal plankton communities in the presence and absence

of other organic compounds. The experiment was begun 11 Sept. 1975.

Figure 24. Uptake and catabolism of L-arginine in the presence and absence of added organic substrates. a) Uptake of L-arginine. b) Ammonia release accompanying the catabolism of L-arginine. Experiment begun 25 September 1975.

Figure 25. Uptake of L-arginine in the presence of 0.0, 10.0, or 50 µM glucose Experiment begun September 25, 1975

Figure 26. Ammonia release accompanying the degradation of L-arginine by coastal plankton communities in the presence or absence of added inorganic fixed nitrogen. Experiment begun 11 September 1975.

Figure 27. L-arginine uptake kinetics as a function of temperature and illumination. The inset gives the concentration of ammonia in the darkened flasks at 89 hours. Experiment begun 25 September 1975.

Figure 28. Length of lag period as determined by the time to 50% completion of the uptake or degradation of L-arginine as a function of temperature using populations collected at the dates indicated and incubated in the dark at the <u>in situ</u> temperature. The slope of the regression line is -5.7 hr./C° and $r^2 = 0.96$.

Figure 29. Ammonia release accompanying the degradation of 20 µM L-arginine or L-glutamate by plankton communities from 10m depth in the Sargasso Sea. a) Experiment performed 6 February 1975 at 26°N, 63°W, water temperature 23°C.

b) Results of two experiments at 33°N, 63°W. Connected points; 9 February; isolated points, 12 February 1975, water temperature 21°C.

Figure 30 Uptake of L-arginine by plankton populations collected from different locations in Halifax Harbour. Station B was near a sewer outfall, Station C was in clean coastal water and Station A is near the main channel leading to the inner harbour. The inset gives some environmental parameters for the water at the time of collection, 25 September 1975. The surface temperature was the same at all stations and they were incubated together.

Figure 31. Temperature dependence of the duration of the activation lag for L-arginine. a) Uptake curves, L-arginine supplied at 10.0 μM to water collected 6 July. b) Lag duration versus incubation temperature (x) and maximum uptake rate observed versus temperature (o). The slope of the line is -3.9 hr/C . See Addendum for details.

Figure 32. Amino acid uptake, carbon partitioning and nitrogen regeneration. L- arginine, glutamate, and lysine were supplied at 10.0 μM and 10, 10, 1and 13 Ci/ℓ, respectively, to water collected 20 July. a) L-arginine, b)L-glutamate, c) L-lysine, d) ATP in flasks. Filled circles: total ^{14}C radioactivity (DPM) recovered from 50.0 ml.

Open circles: activity in 50.0 ml of filtrate after acidification.

Filled squares: activity in 50.0 ml collected on the filter.

Open squares: activity in 50.0 ml trapped in phenethylamine after acidification.

Filled triangles, dotted line: ammonia concentration in the water.

Open triangles, dotted line: primary amine concentration in the water.

See Addendum for details.

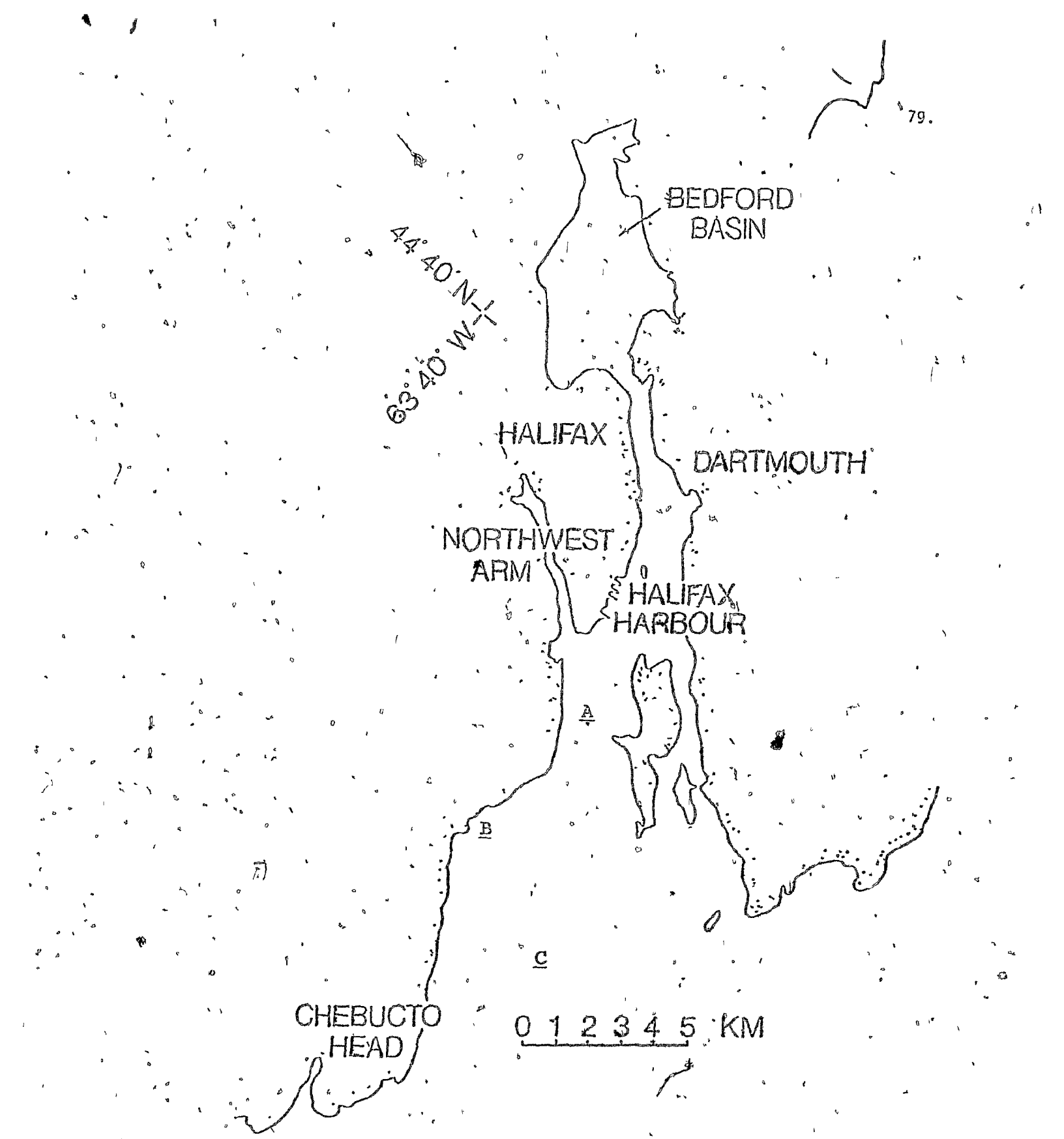

BEDFORD
BASIN
44°40'N
63°40'W
HALIFAX
DARTMOUTH
NORTHWEST
ARM
HALIFAX
HARBOUR
A
B
C
CHEBUCTO
HEAD
0 1 2 3 4 5 KM

Figure 1.

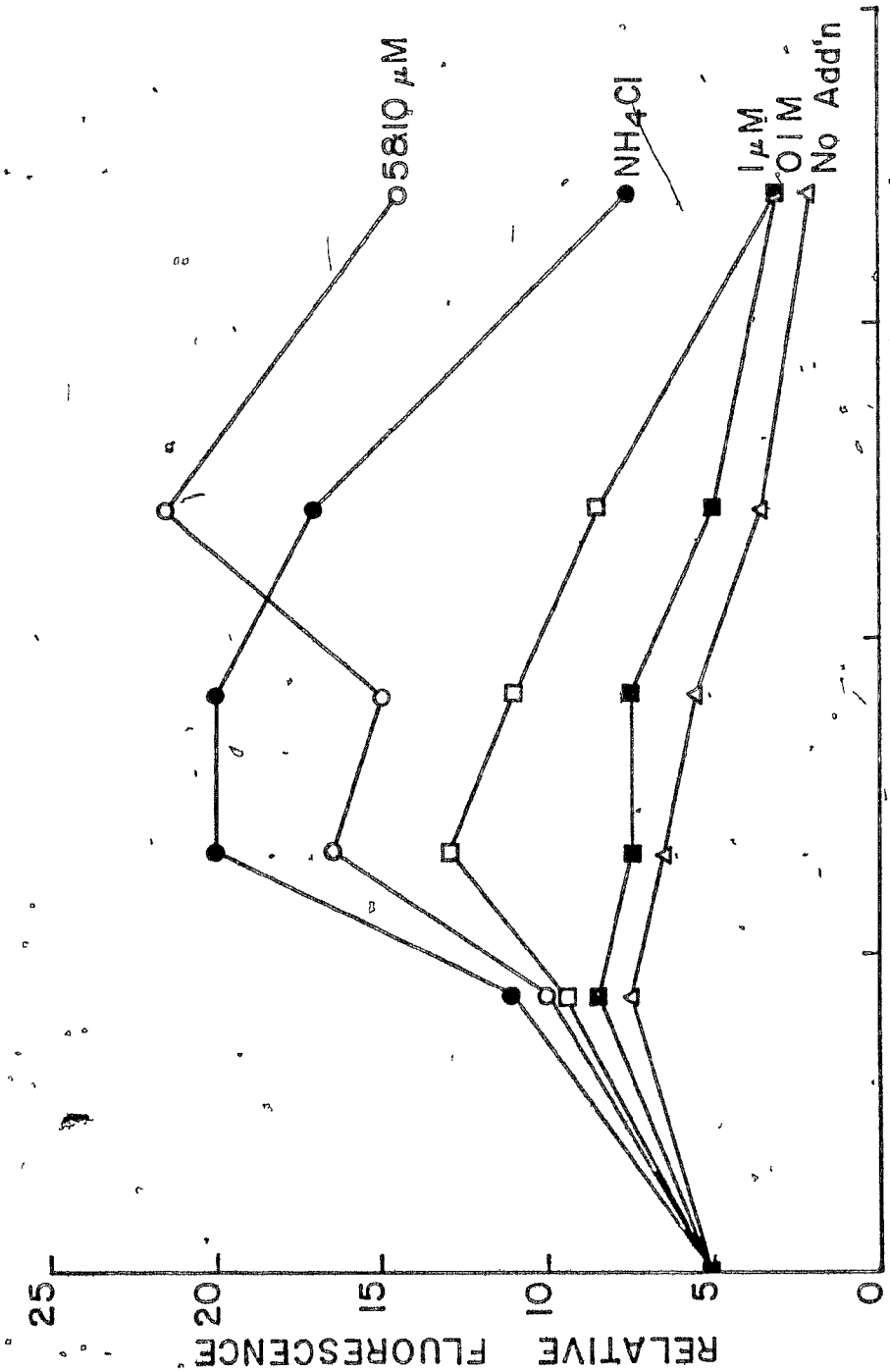

RELATIVE FLUORESCENCE
25
20
15
10
5
0
24
48
72
96
HOURS
5810 μM
NH4Cl
1 μM
0.1 M
No Add'n

Figure 2.

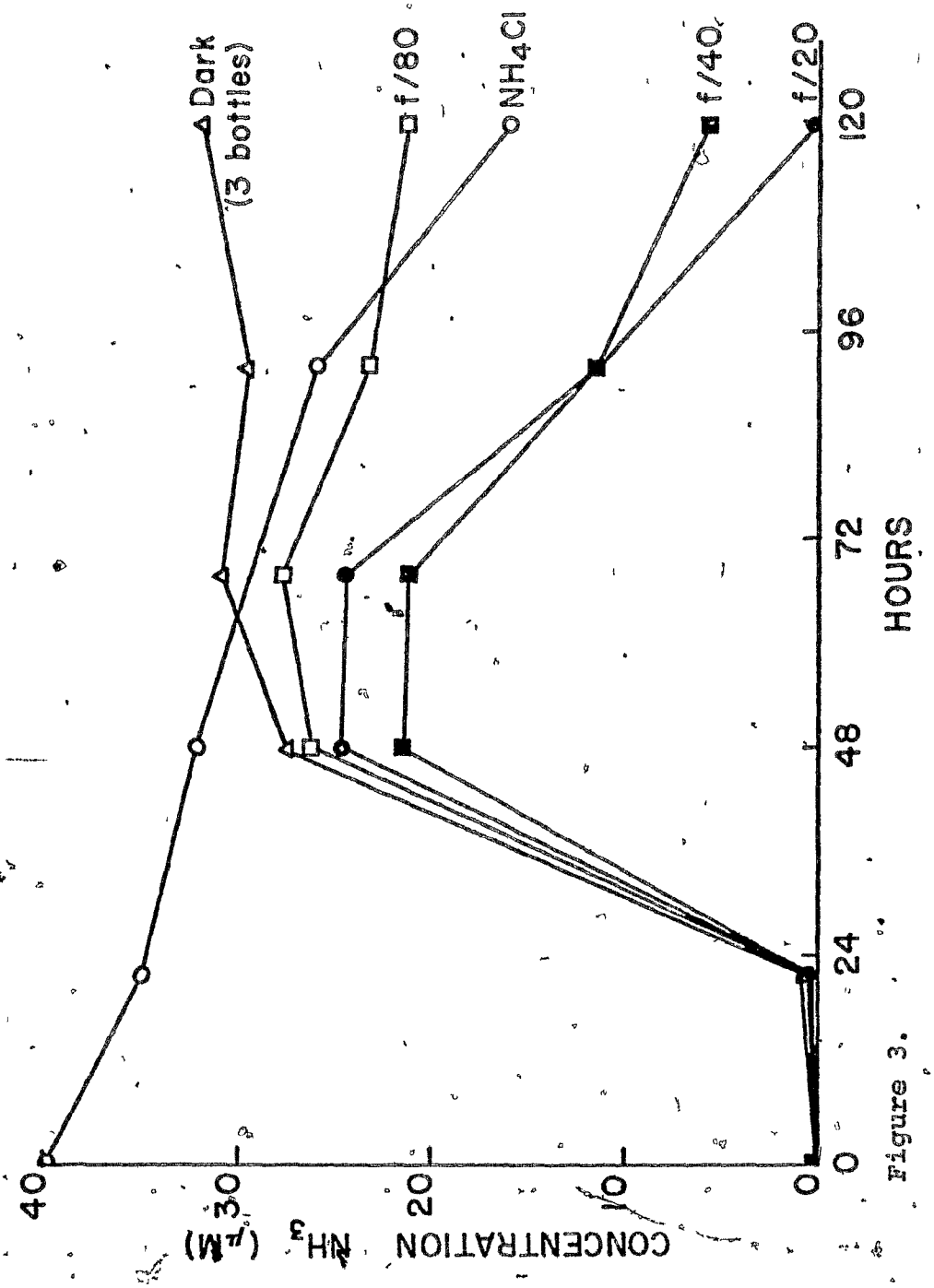

Dark
(3 bottles)
f/80
NH4Cl
f/40
f/20
0
24
48
72
96
120
HOURS
0
10
20
30
40
CONCENTRATION NH3 (μM)

Figure 3.

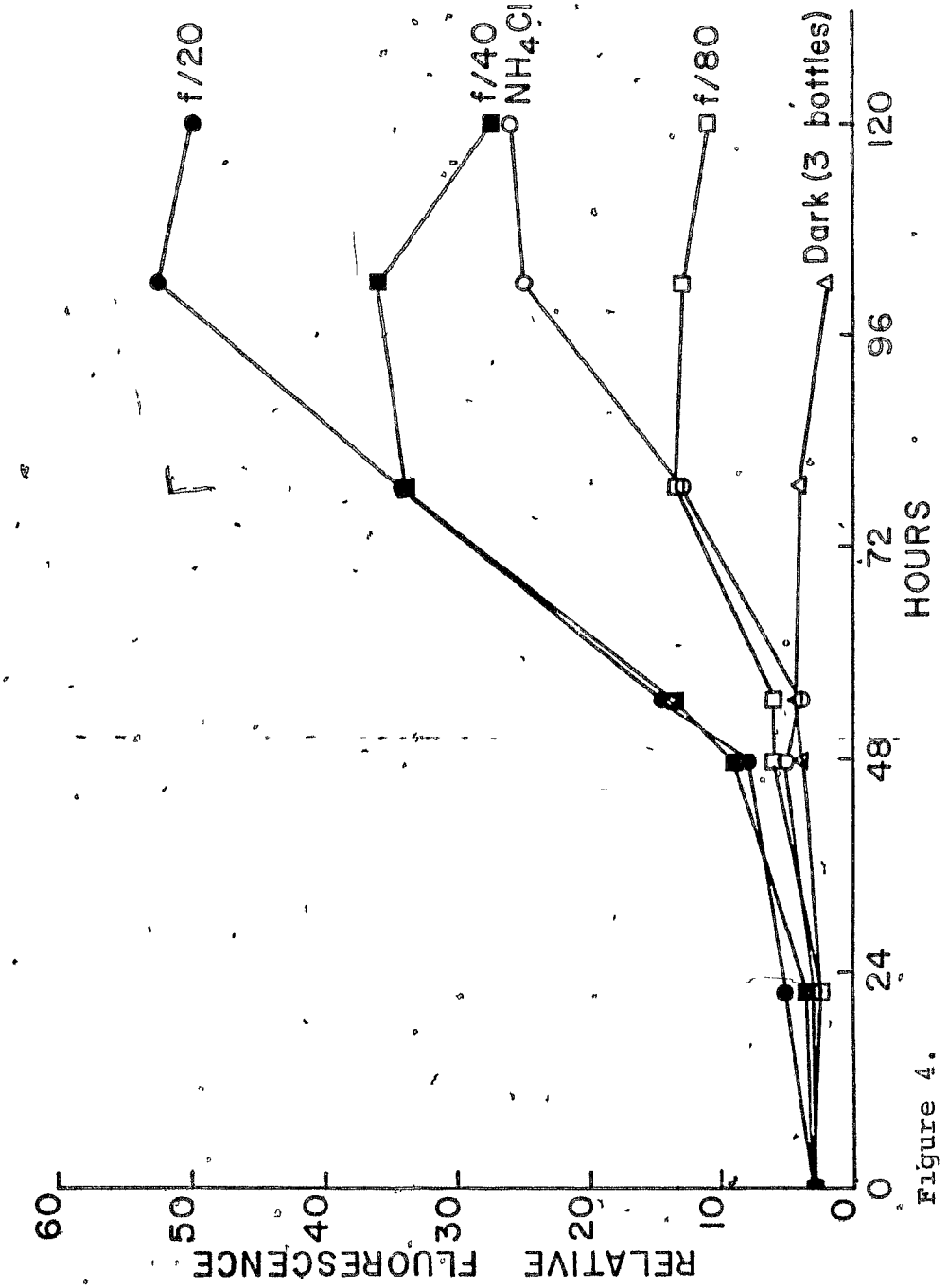

f/20
f/40
NH4Cl
f/80
Dark (3 bottles)
RELATIVE FLUORESCENCE
0
10
20
30
40
50
60
0
24
48
72
96
120
HOURS

Figure 4.

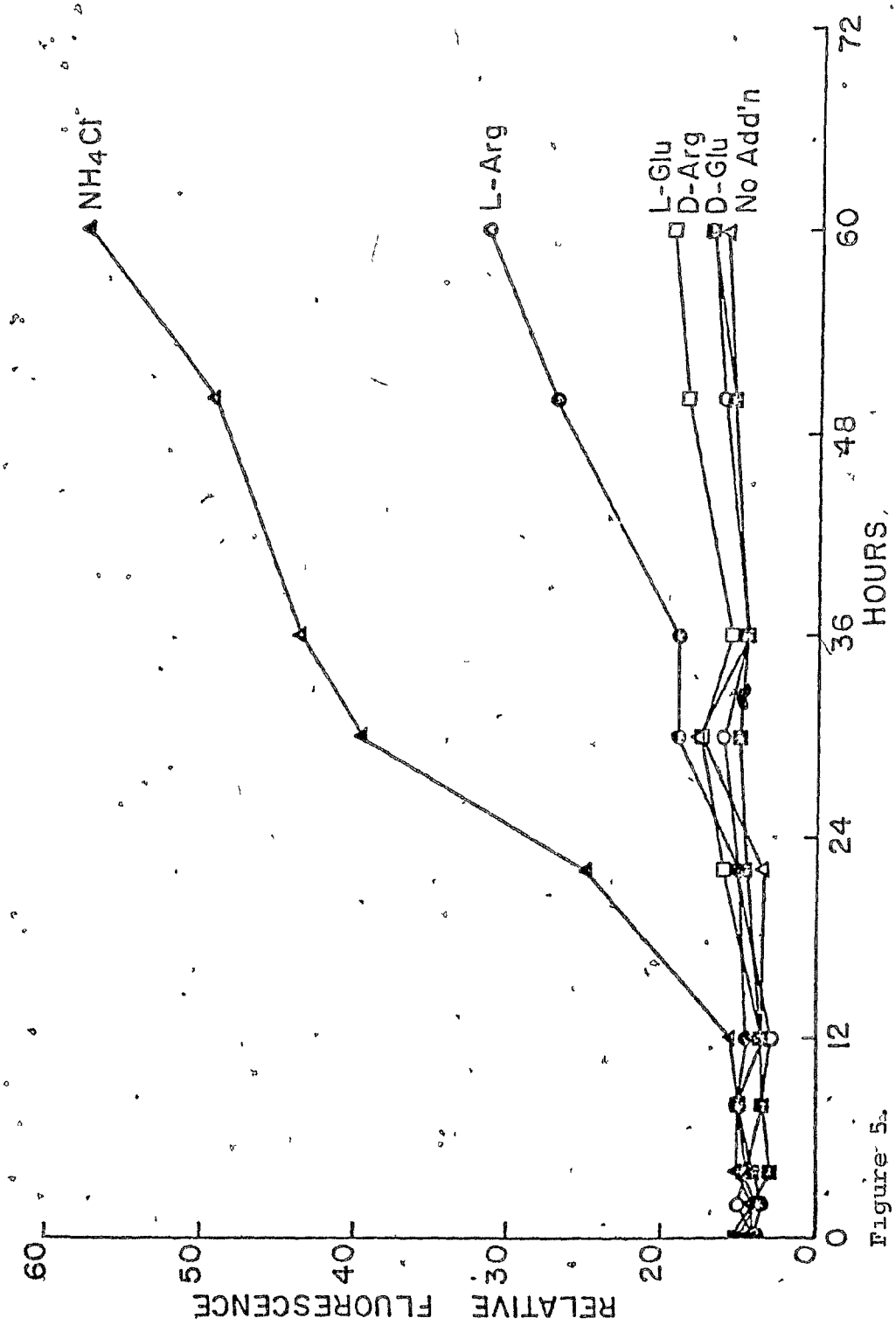

NH4Cl
L-Arg
L-Glu
D-Arg
D-Glu
No Add'n
RELATIVE FLUORESCENCE
HOURS
0
20
30
40
50
60
0
12
24
36
48
60
72

Figure 5.

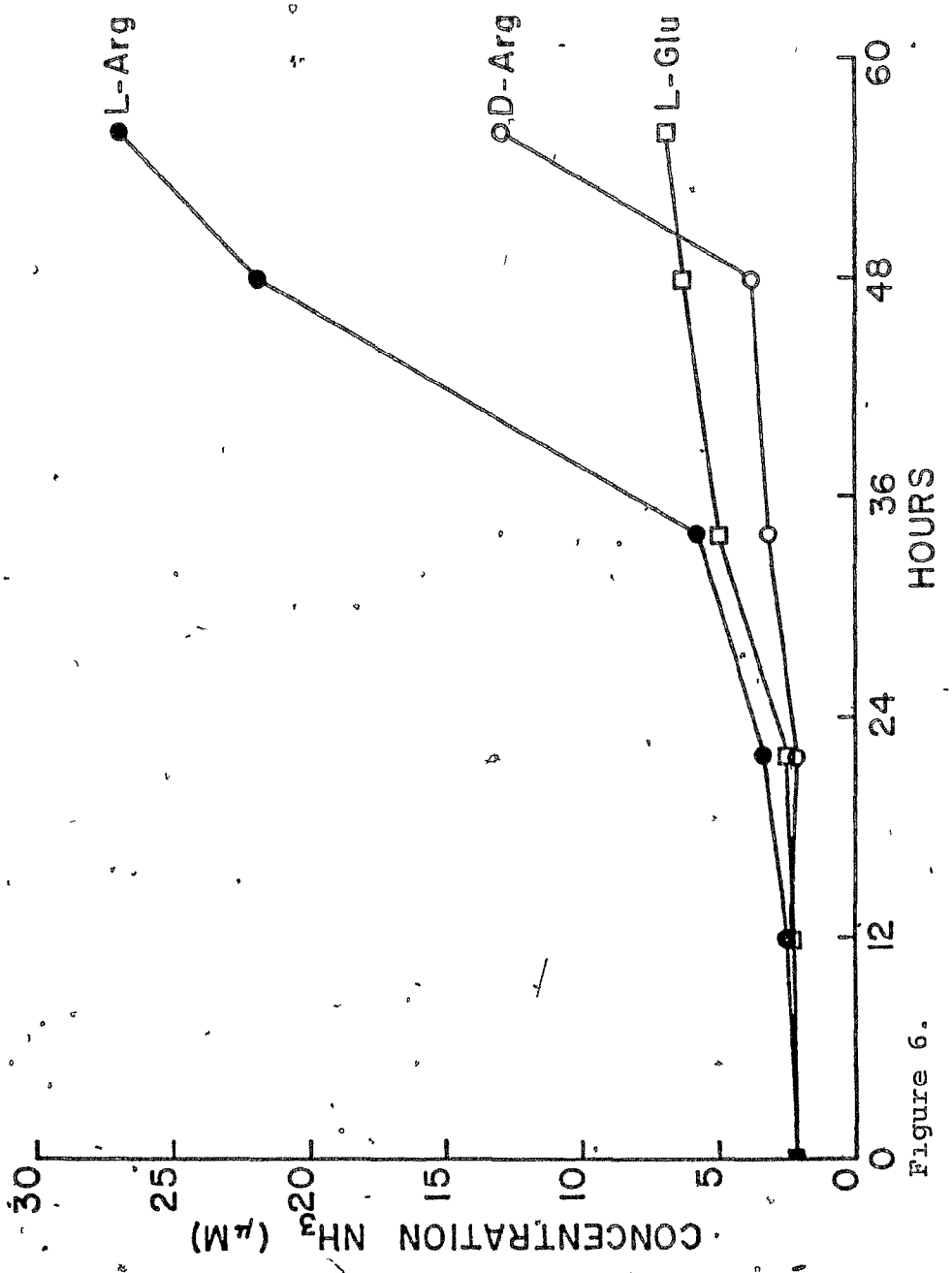

L-Arg
D-Arg
L-Glu
0
12
24
36
48
60
HOURS
0
5
10
15
20
25
30
CONCENTRATION NH3 (μM)

Figure 6.

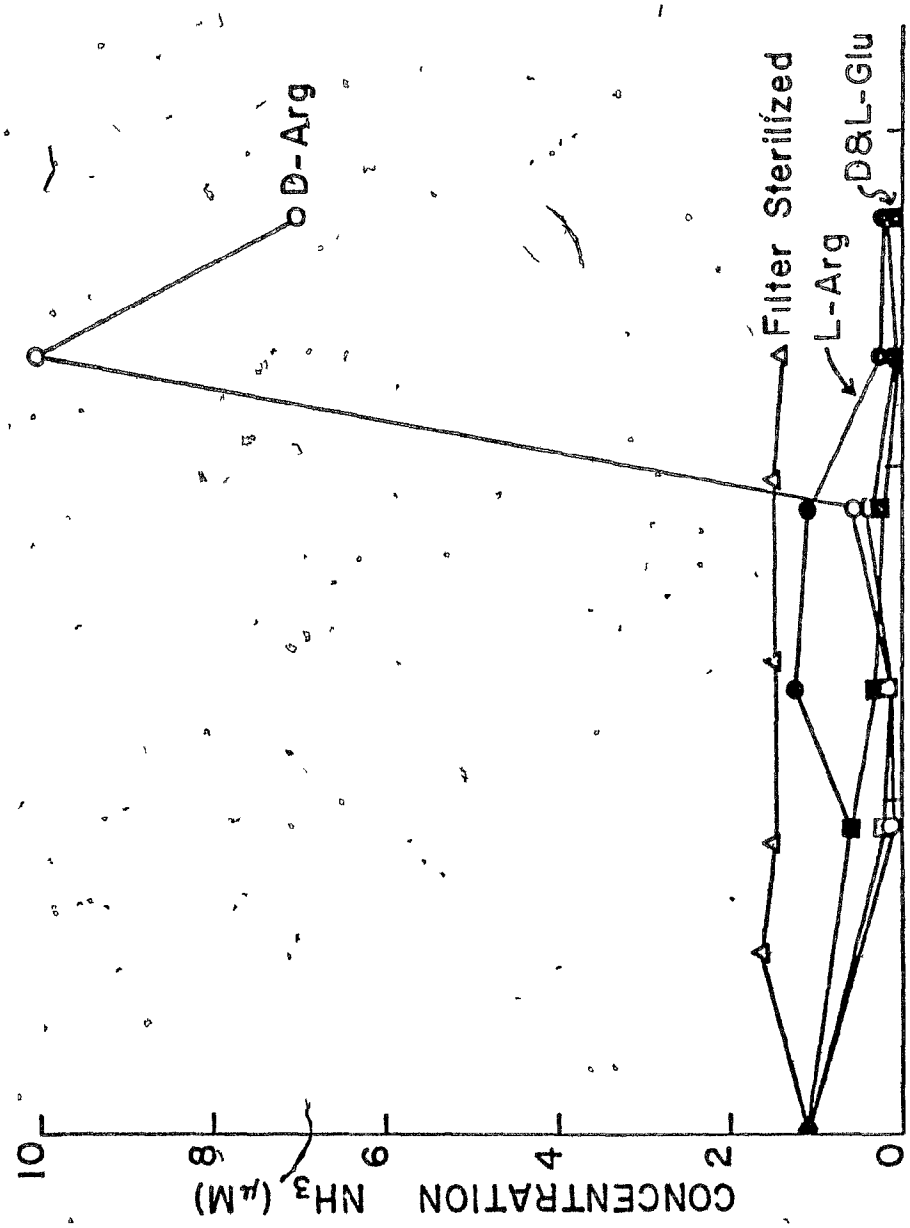

D-Arg
Filter Sterilized
L-Arg
D&L-Glu
72
48
24
0
HOURS
10
8
6
4
2
0
CONCENTRATION NH3 (μM)

Figure 7.

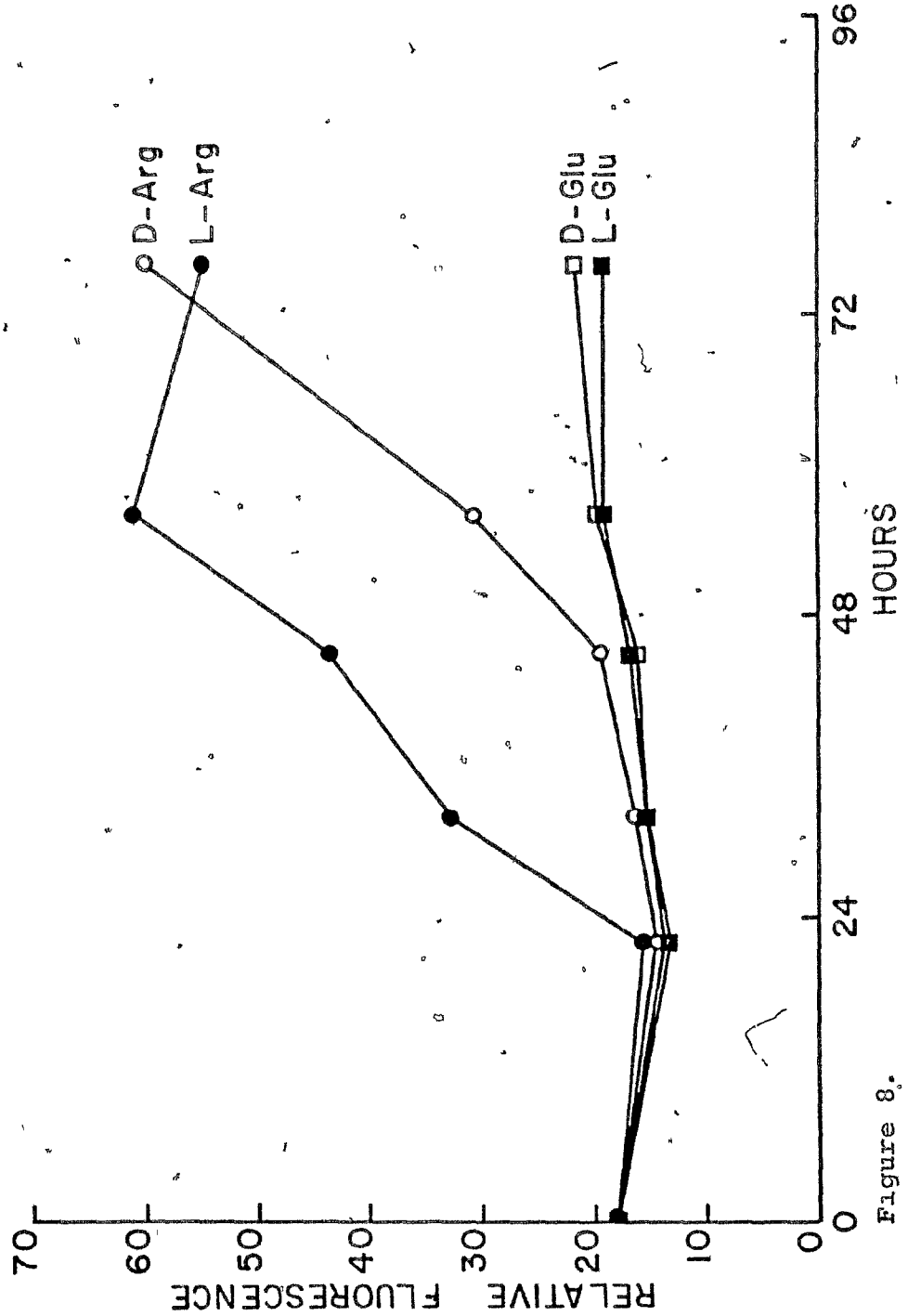

D-Arg
L-Arg
D-Glu
L-Glu
RELATIVE FLUORESCENCE
HOURS
0
10
20
30
40
50
60
70
0
24
48
72
96

Figure 8.

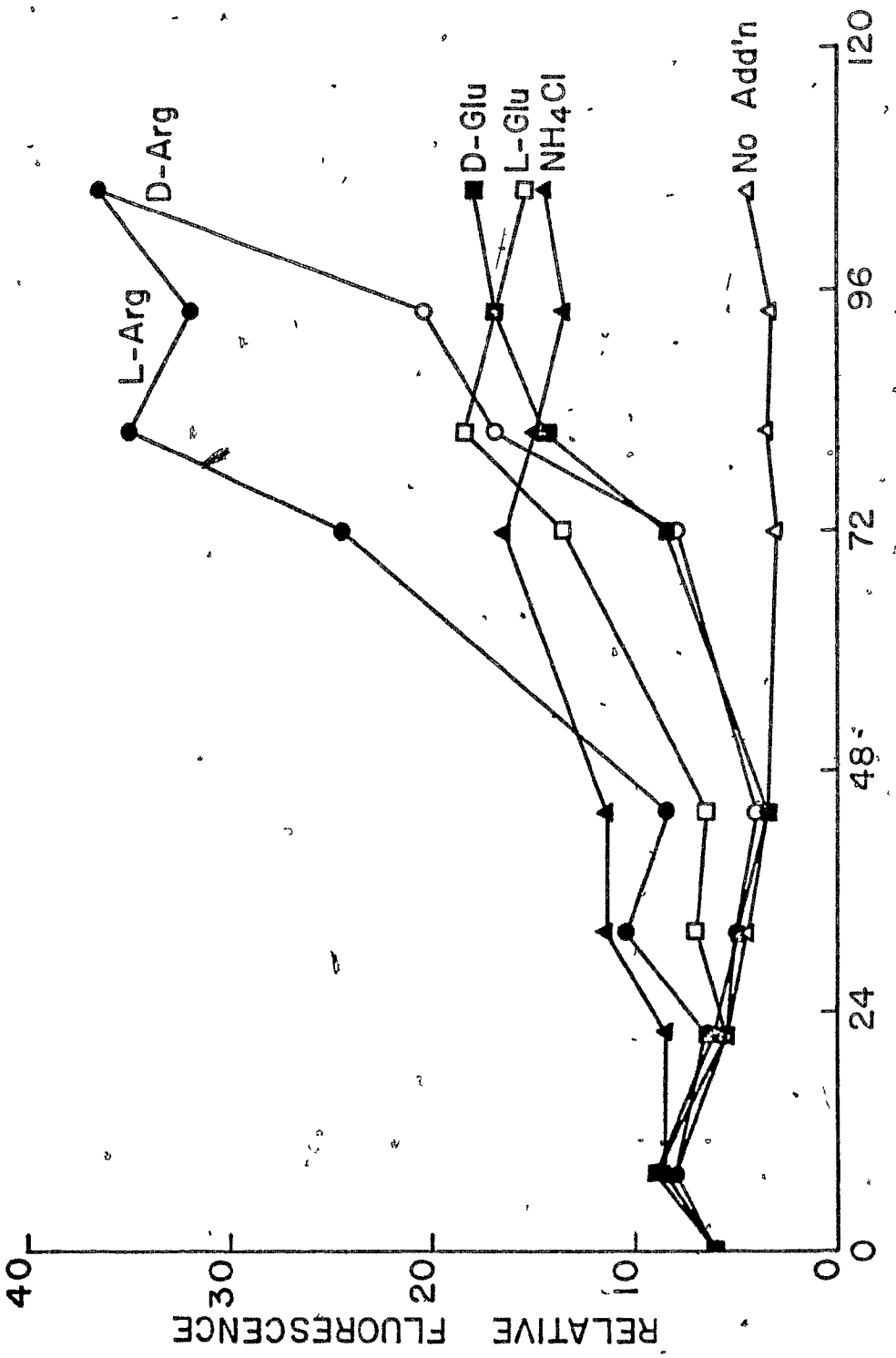

RELATIVE FLUORESCENCE
40
30
20
10
0
0
24
48
72
96
120
HOURS
L-Arg
D-Arg
D-Glu
L-Glu
NH4Cl
No Add'n

Figure 9.

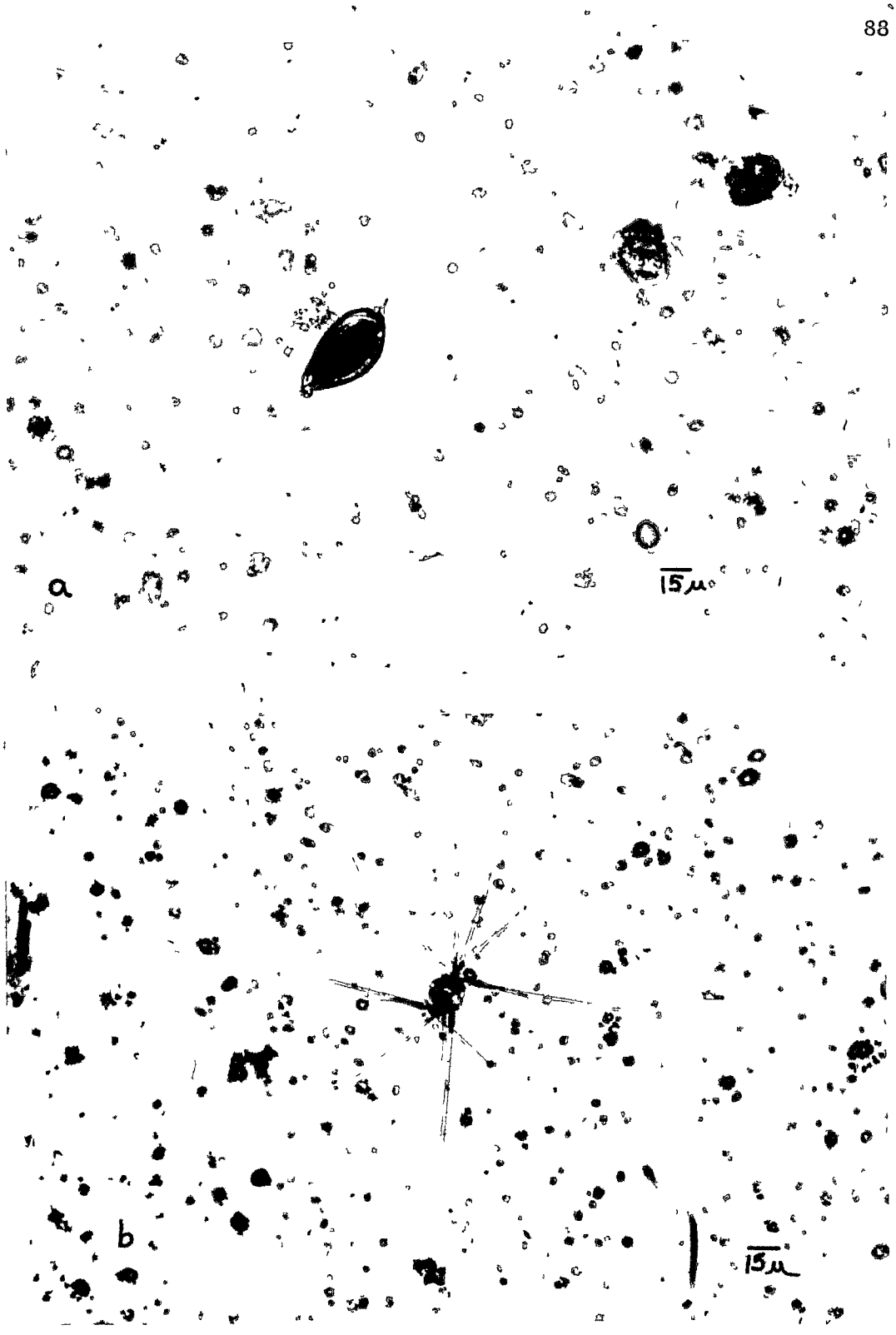


Figure 10.

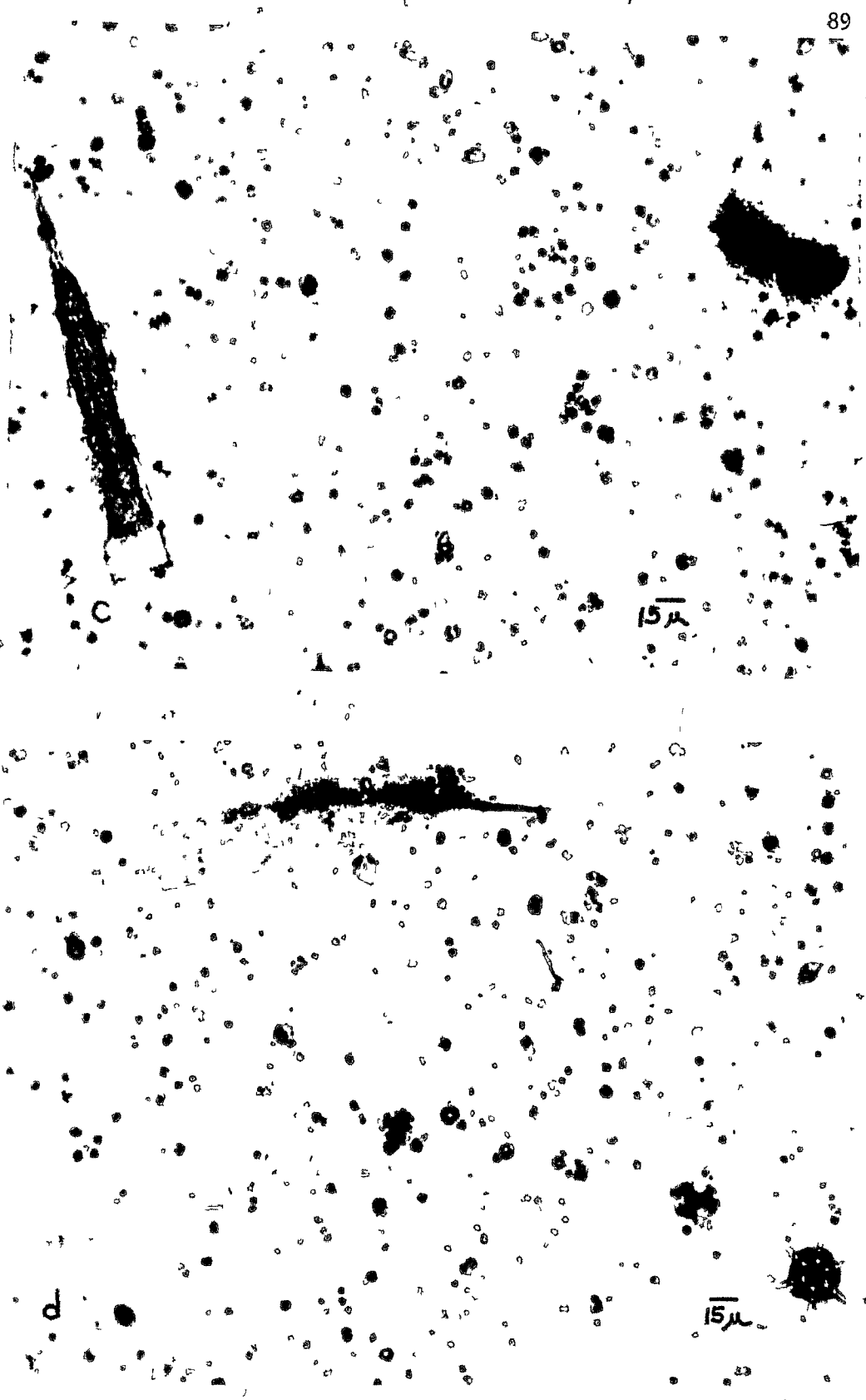


Figure 10.

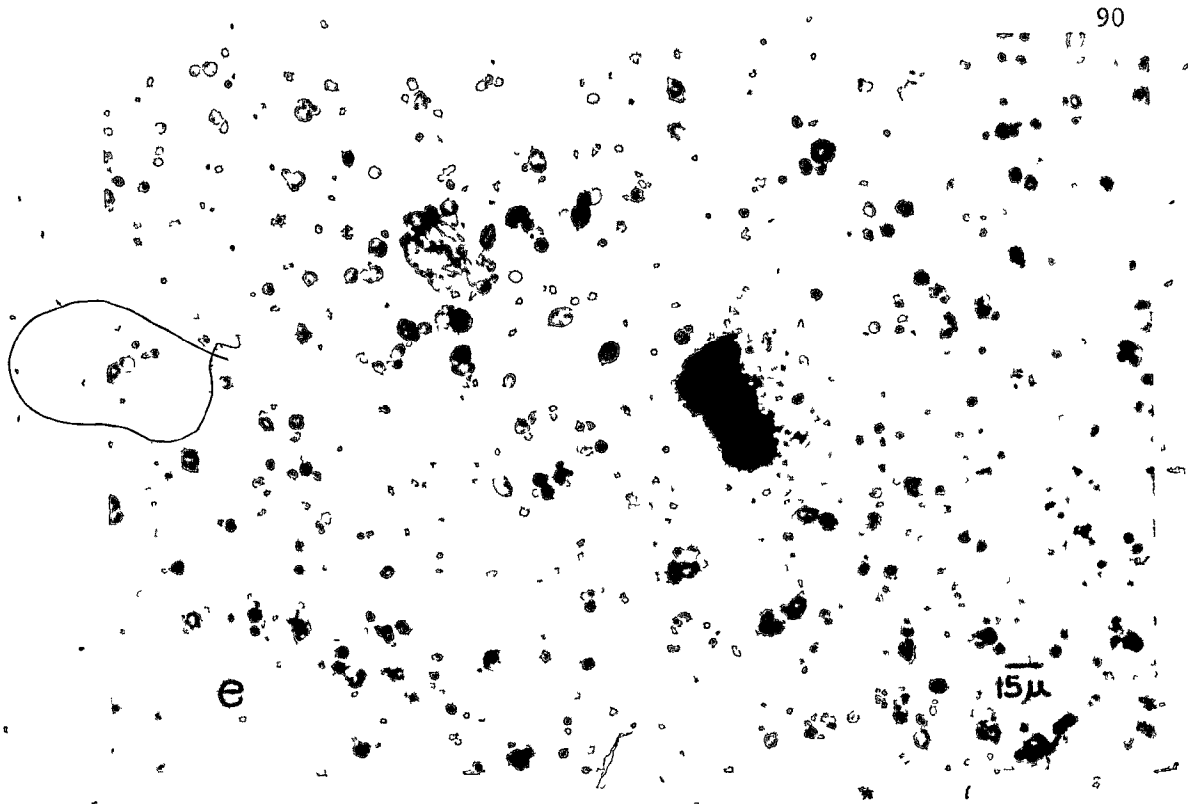


Figure 10.

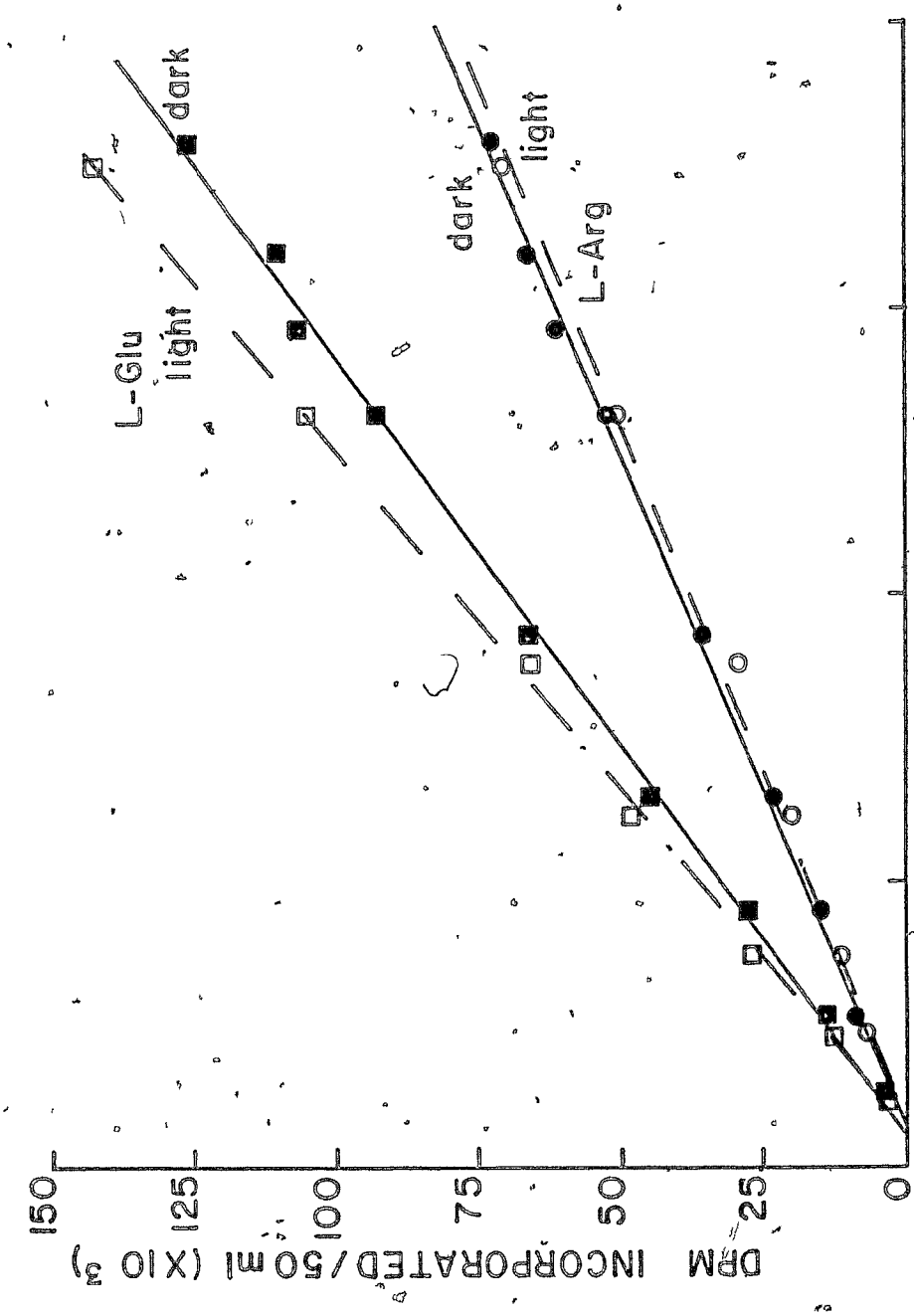

DPM INCORPORATED/50 ml (X10 3)
150
125
100
75
50
25
0
0
3
6
9
12
HOURS
L-Glu
light
dark
L-Arg
light
dark

Figure 11.


L-Arg
L-Lys
Gly
NH4Cl
L-Try
L-Glu
L-Leu
Urea, L-Val
Hours
0
24
48
72
96
120
144
0
5
10
15
20
25
NH3 concentration (μM)

Figure 12.

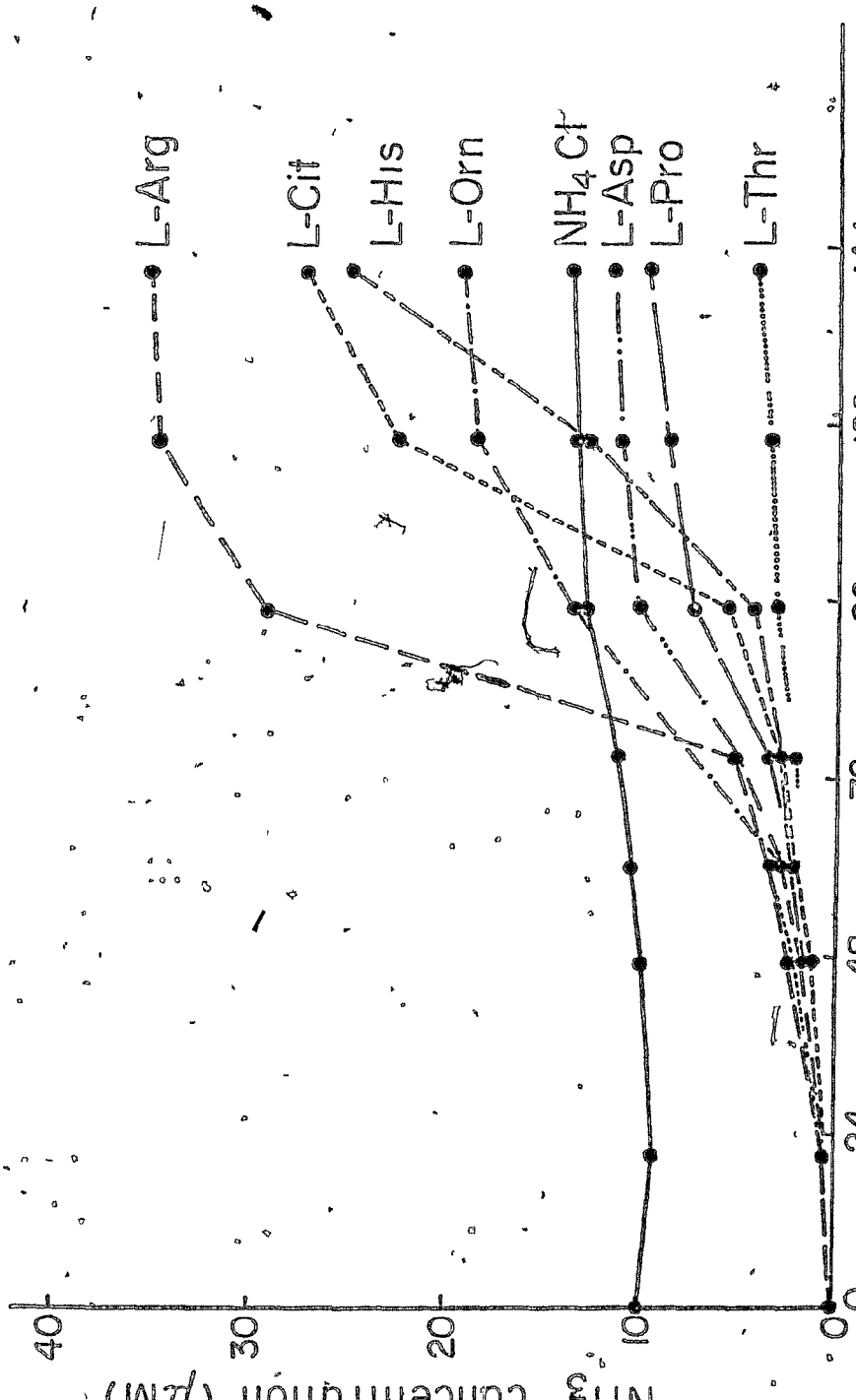

L-Arg
L-Cit
L-His
L-Orn
NH_4Cl
L-Asp
L-Pro
L-Thr
0
10
20
30
40
NH_3 concentration (μM)
0
24
48
72
96
120
144
Hours

Figure 13.

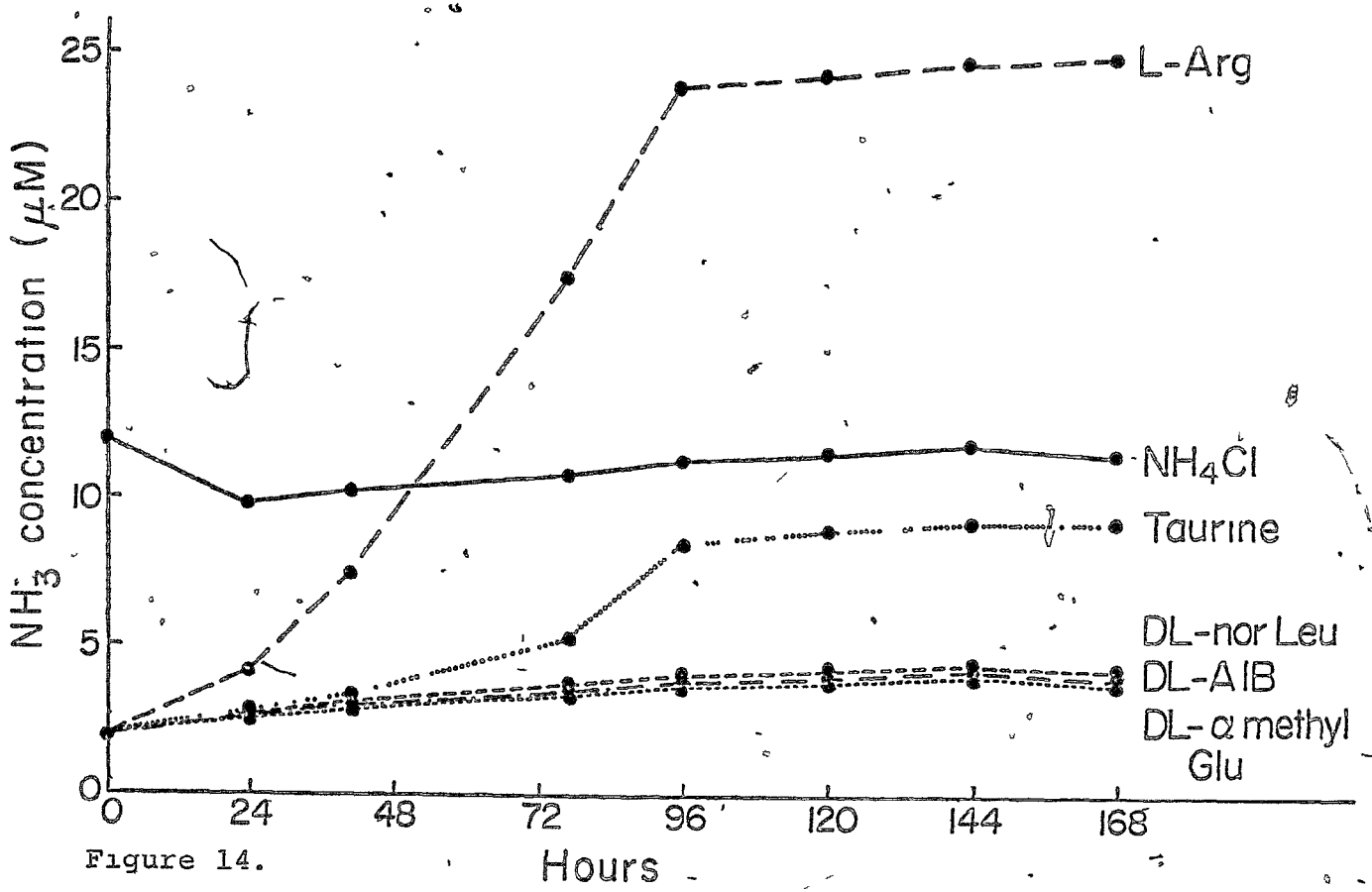

25
20
15
10
5
0
NH_3 concentration (μM)
0
24
48
72
96
120
144
168
Hours
L-Arg
NH_4Cl
Taurine
DL-nor Leu
DL-AIB
DL-α methyl Glu

Figure 14.

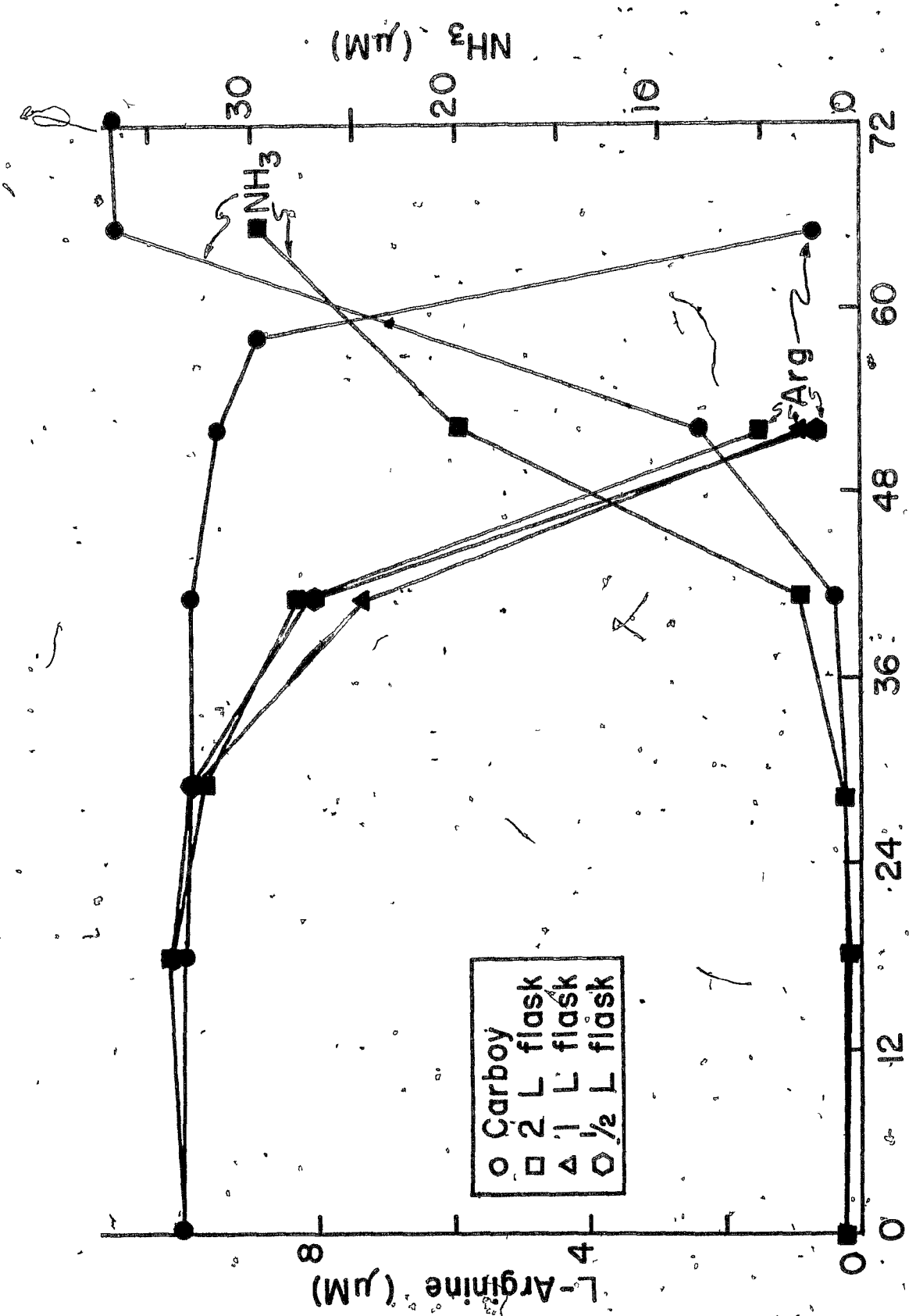

NH3 (μM)
30
20
10
0
72
60
48
36
24
12
0
Hours
NH3
Arg
L-Arginine (μM)
8
4
0
Carboy
2 L flask
1 L flask
½ L flask

Figure 15.

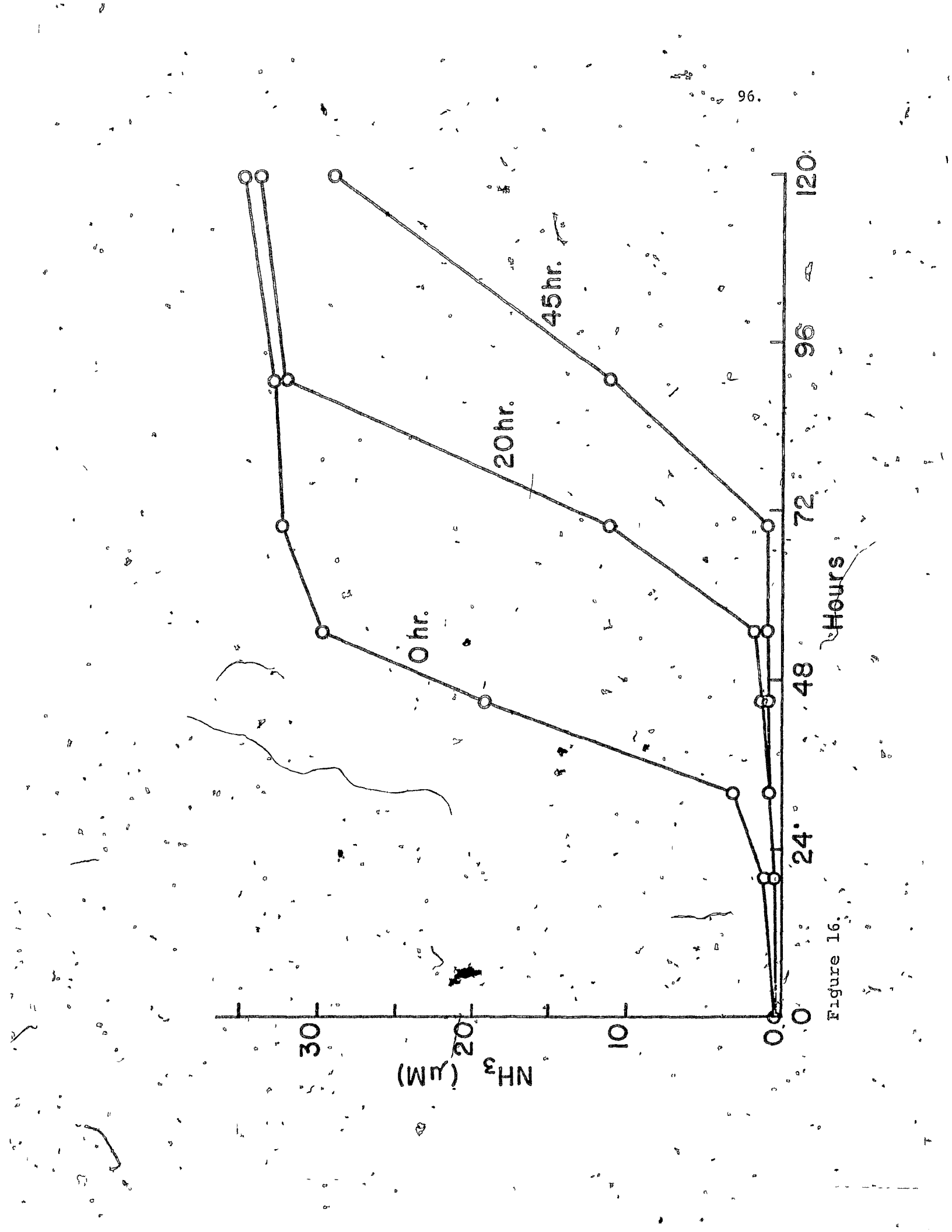


Figure 16.

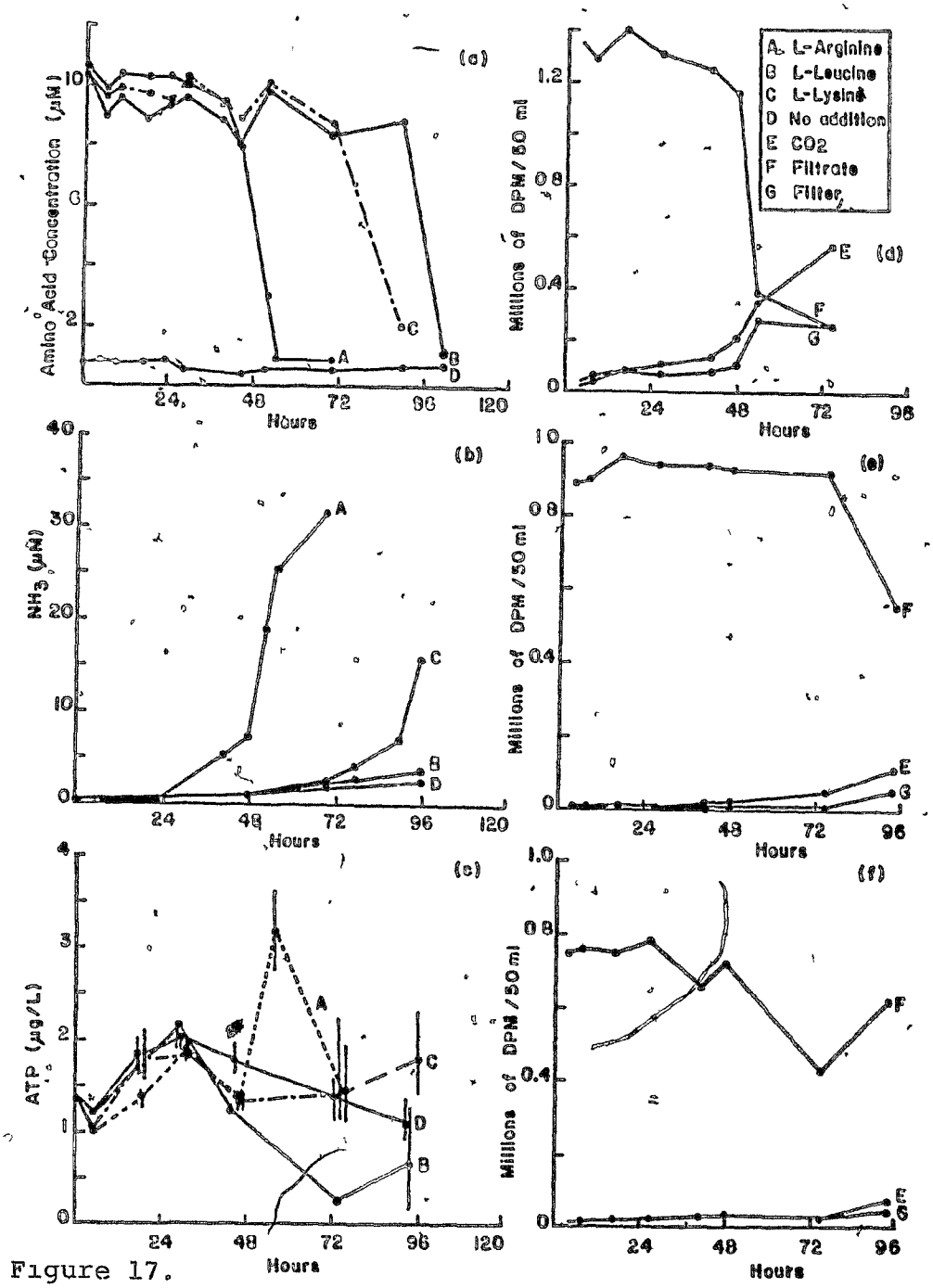
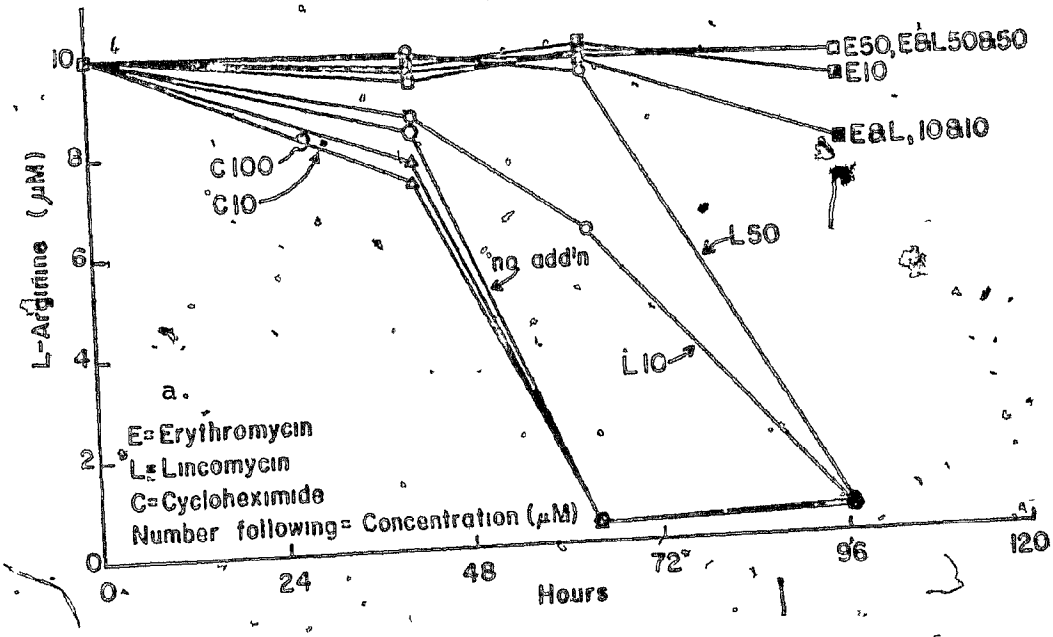

(a)
Amino Acid Concentration (μM)
Hours
(b)
NH3 (μM)
Hours
(c)
ATP (μg/L)
Hours
(d)
Millions of DPM / 50 ml
Hours
A L-Arginine
B L-Leucine
C L-Lysine
D No addition
E CO2
F Filtrate
G Filter
(e)
Millions of DPM / 50 ml
Hours
(f)
Millions of DPM / 50 ml
Hours

Figure 17.


E50,E&L50&50
E10
E&L, 10&10
C100
C10
no add'n
L50
L10
a.
E= Erythromycin
L= Lincomycin
C= Cycloheximide
Number following= Concentration (μM)
L-Arginine (μM)
Hours
0
24
48
72
96
120
2
4
6
8
10

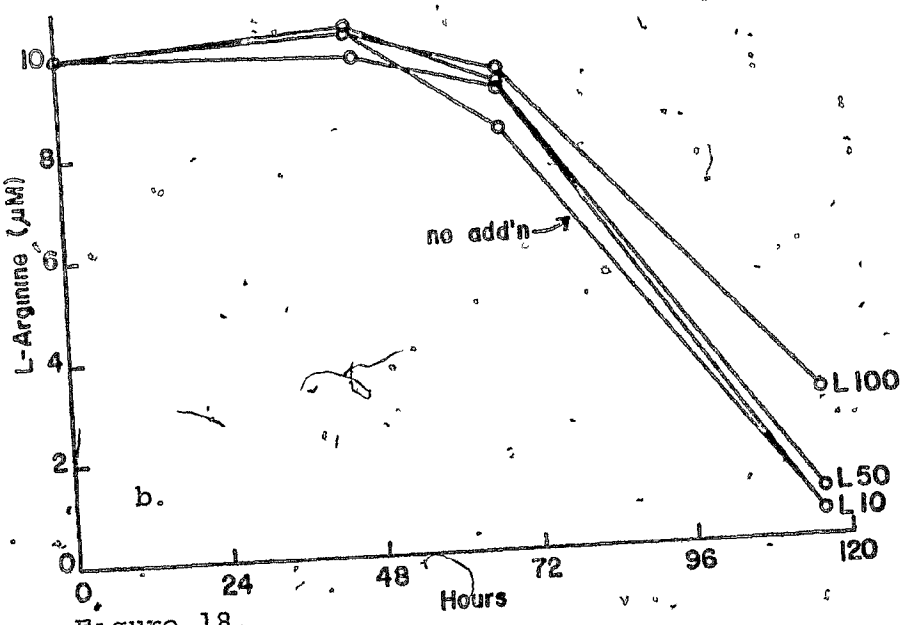

no add'n
L100
L50
L10
b.
L-Arginine (μM)
Hours
0
24
48
72
96
120
2
4
6
8
10

Figure 18.

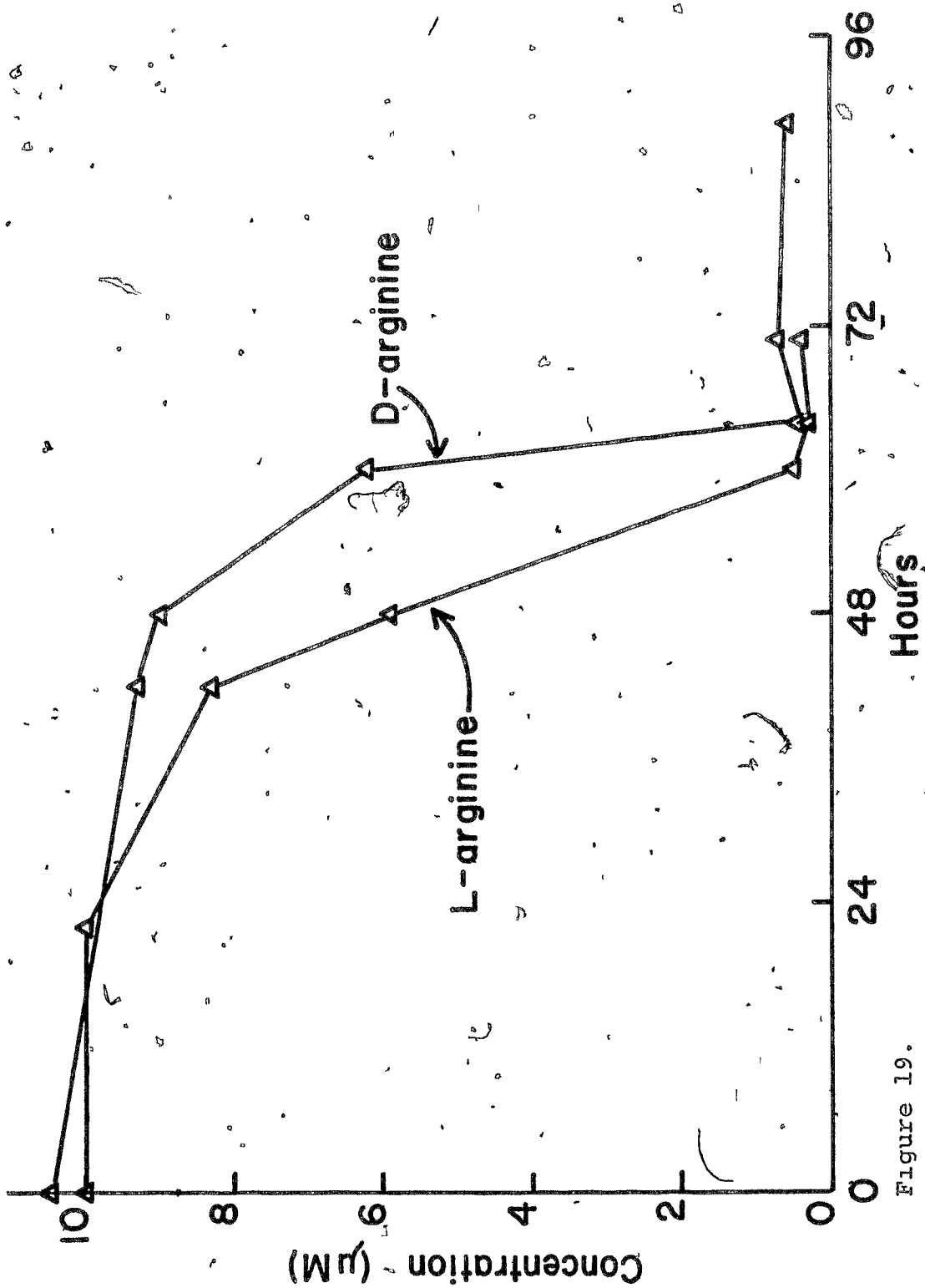

D-arginine
L-arginine
Concentration (μM)
0
2
4
6
8
10
0
24
48
72
96
Hours

Figure 19.

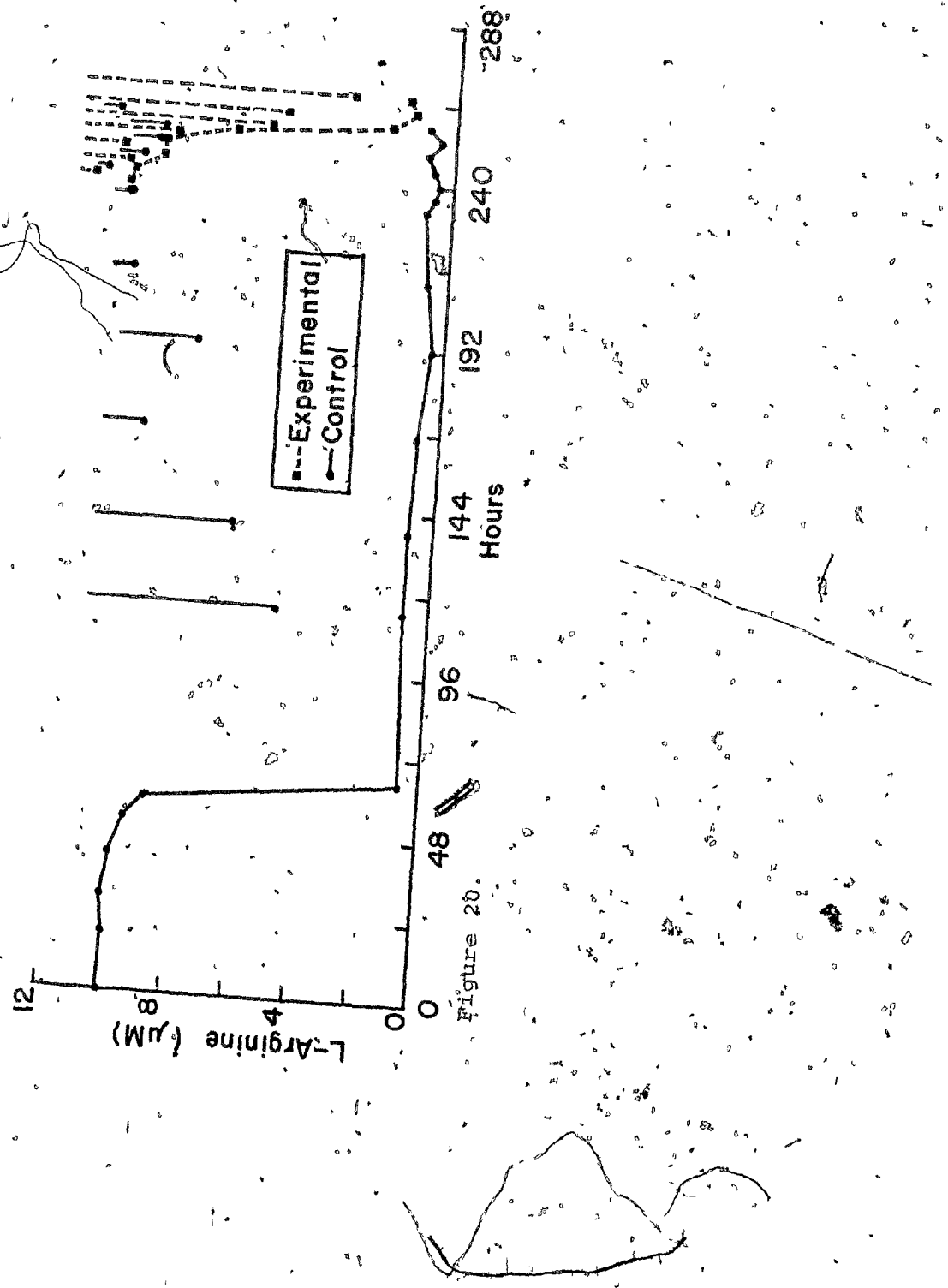

Experimental
Control
288
240
192
144
96
48
0
Hours
12
8
4
0
L-Arginine (μM)

Figure 20.

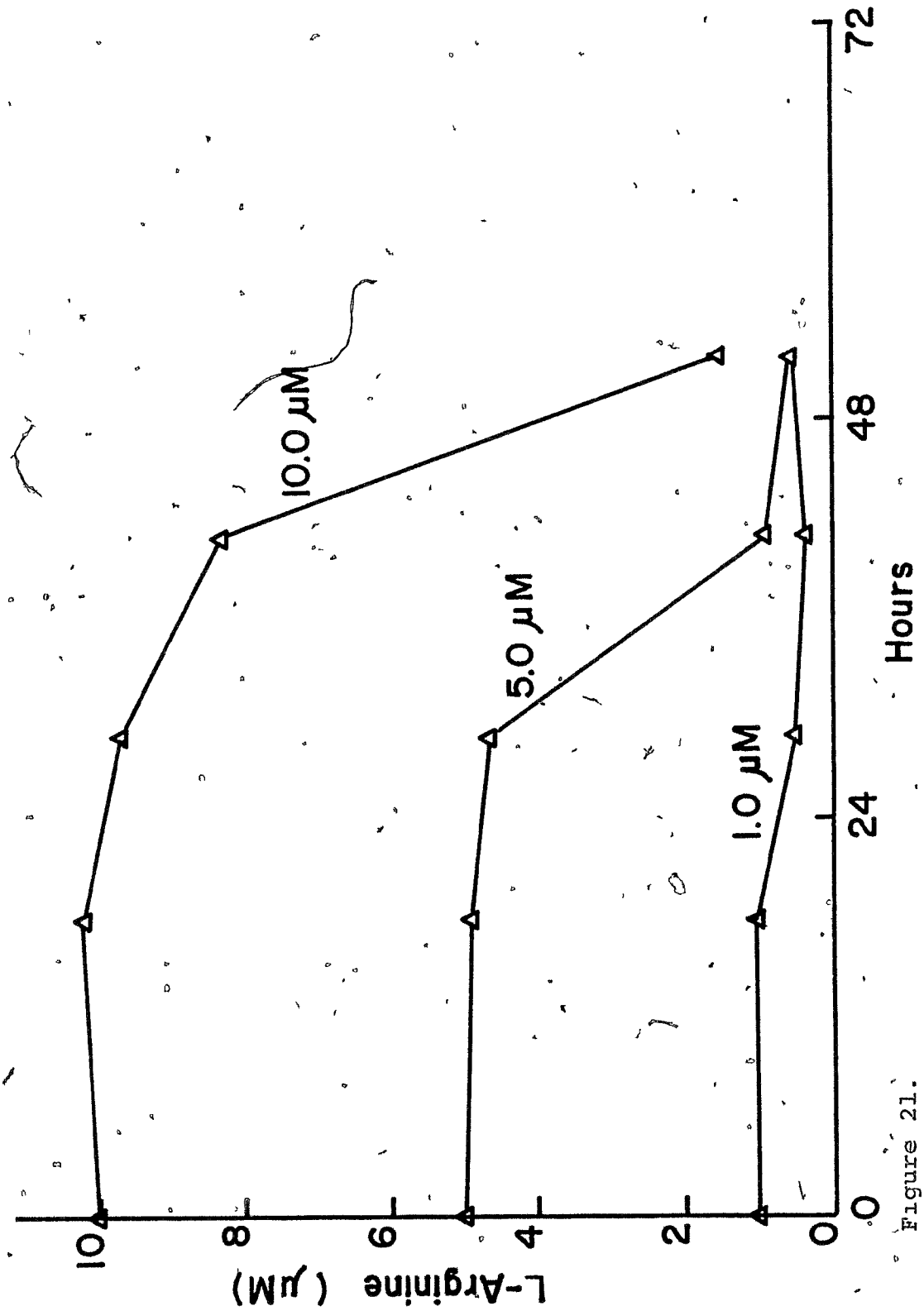


Figure 21.

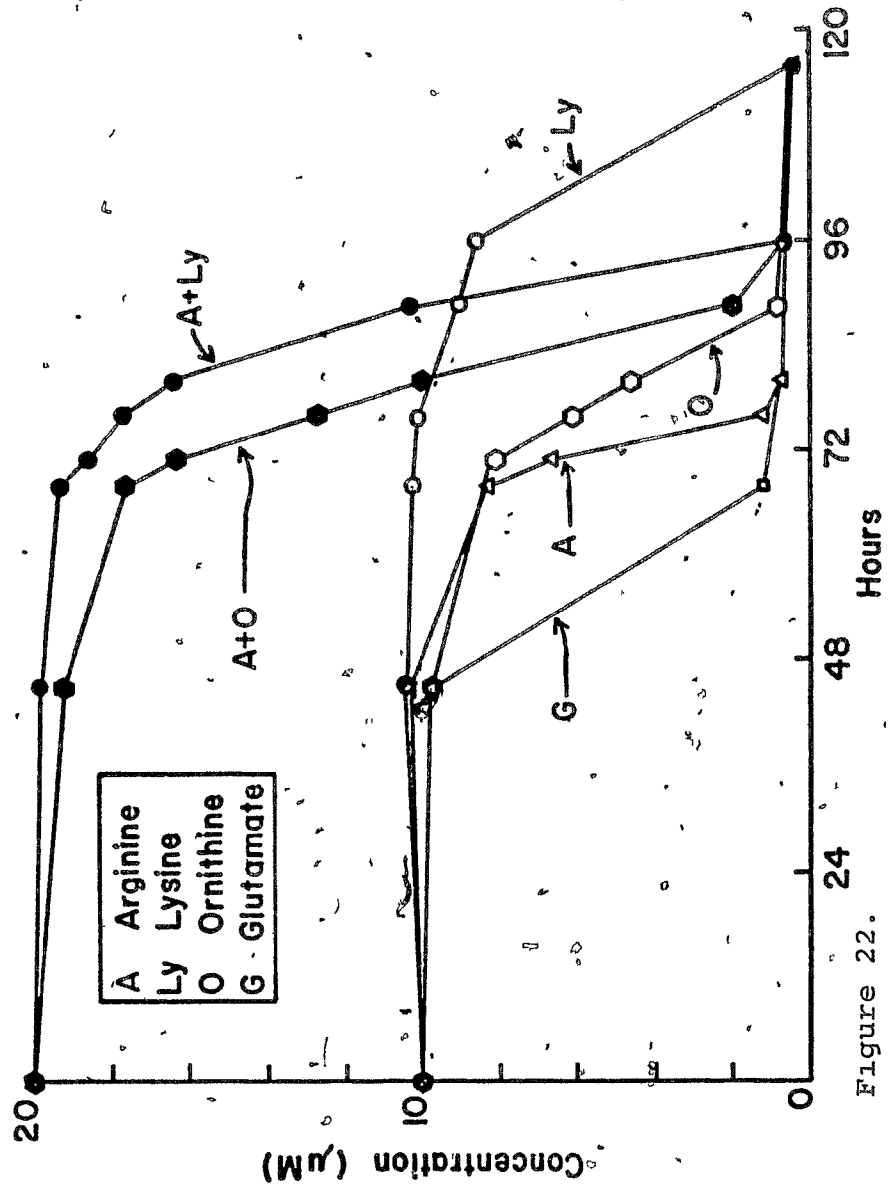

A Arginine
Ly Lysine
O Ornithine
G Glutamate
A+Ly
A+O
Ly
O
A
G
Concentration (μM)
20
10
0
0
24
48
72
96
120
Hours

Figure 22.

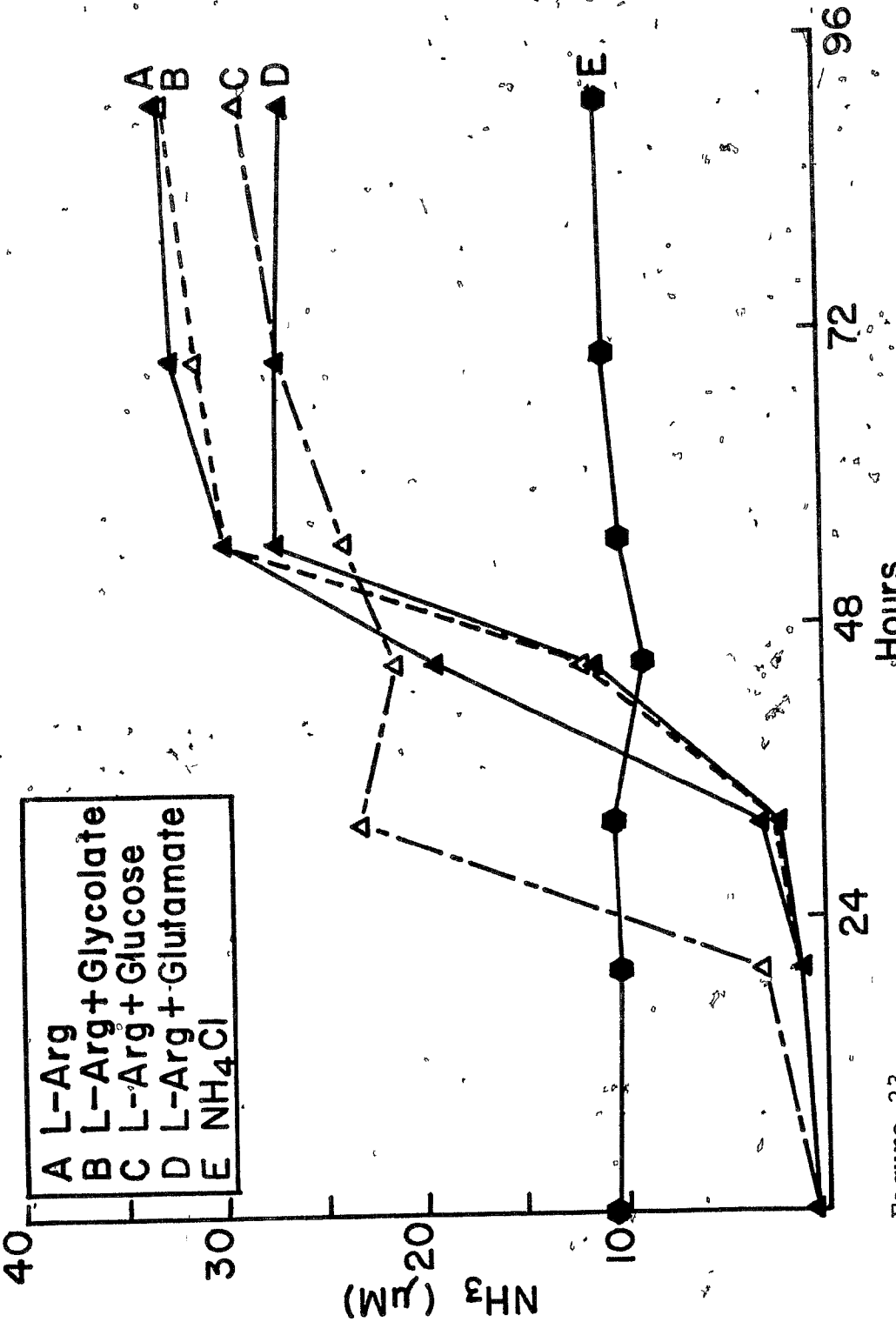

A L-Arg
B L-Arg+Glycolate
C L-Arg+Glucose
D L-Arg+Glutamate
E NH_4Cl
NH_3 (μM)
40
30
20
10
24
48
72
96
Hours
A
B
C
D
E

Figure 23.

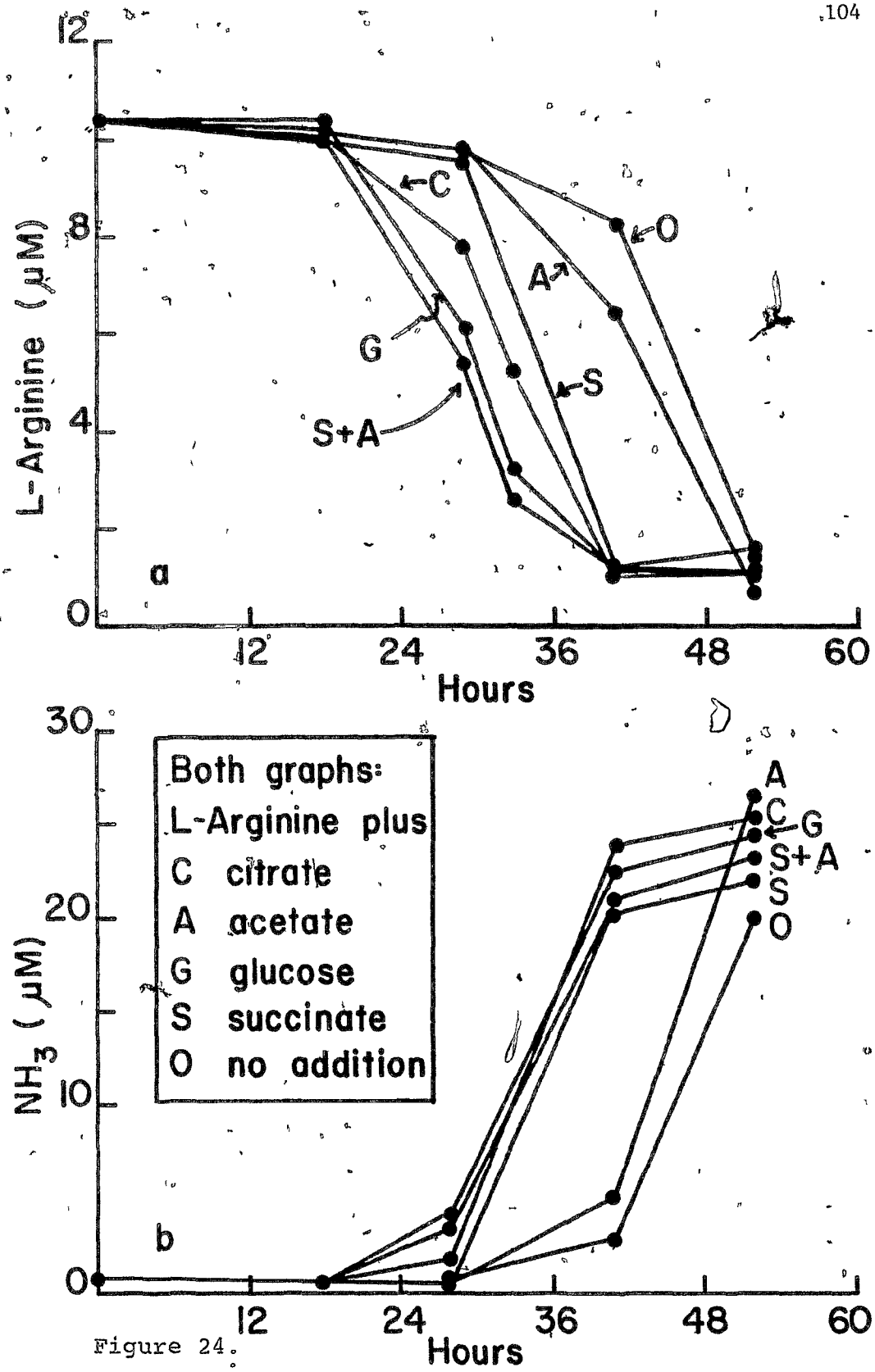

L-Arginine (μM)
12
8
4
0
←C
←O
A
G
S
S+A
a
12
24
36
48
60
Hours
30
20
10
0
NH3 (μM)
Both graphs:
L-Arginine plus
C citrate
A acetate
G glucose
S succinate
O no addition
A
C
G
S+A
S
O
b
12
24
36
48
60
Hours

Figure 24.

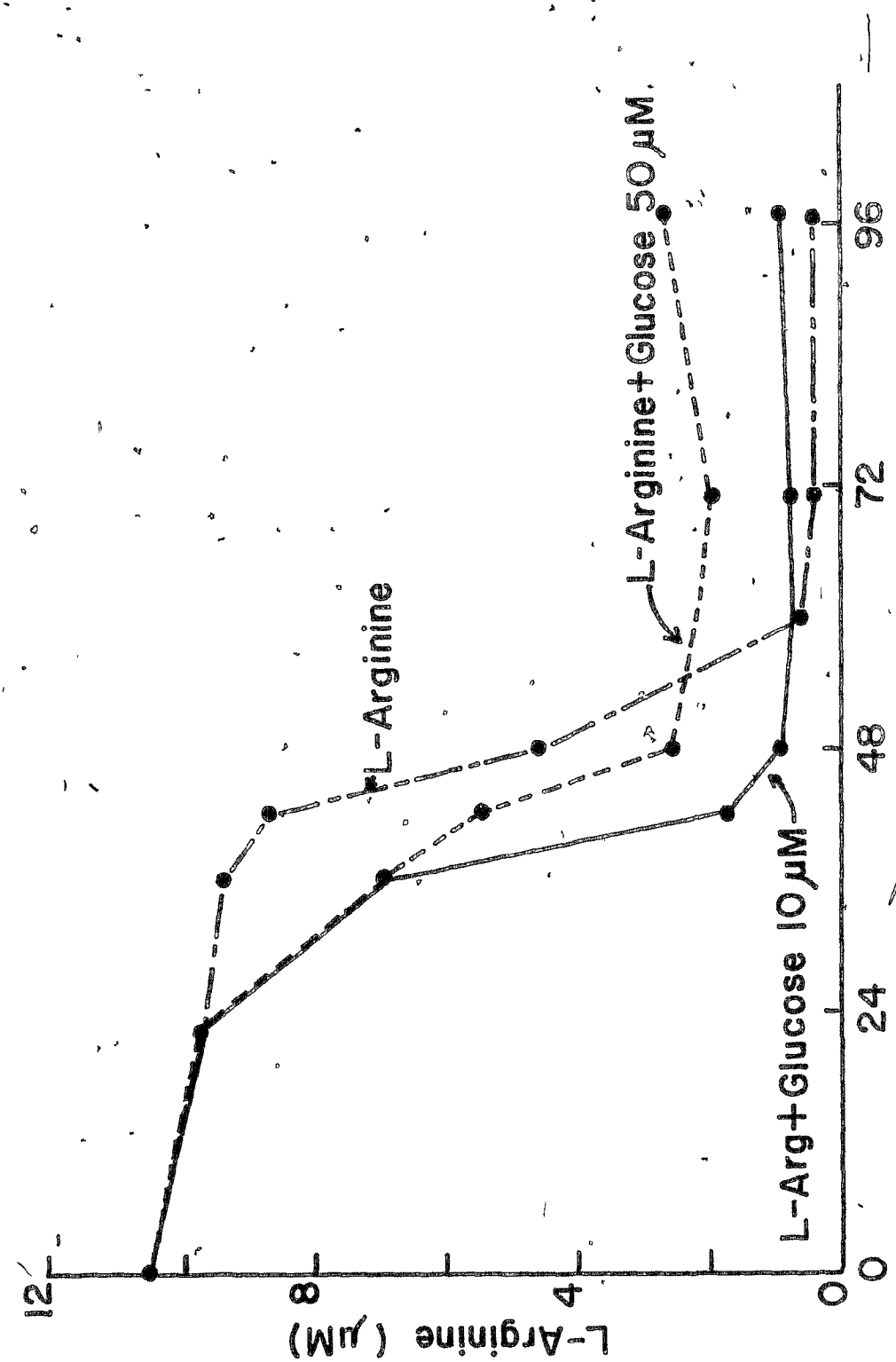

L-Arginine (μM)
12
8
4
0
0
24
48
72
96
Hours
L-Arginine
L-Arginine+Glucose 50 μM
L-Arg+Glucose 10 μM

Figure 25.

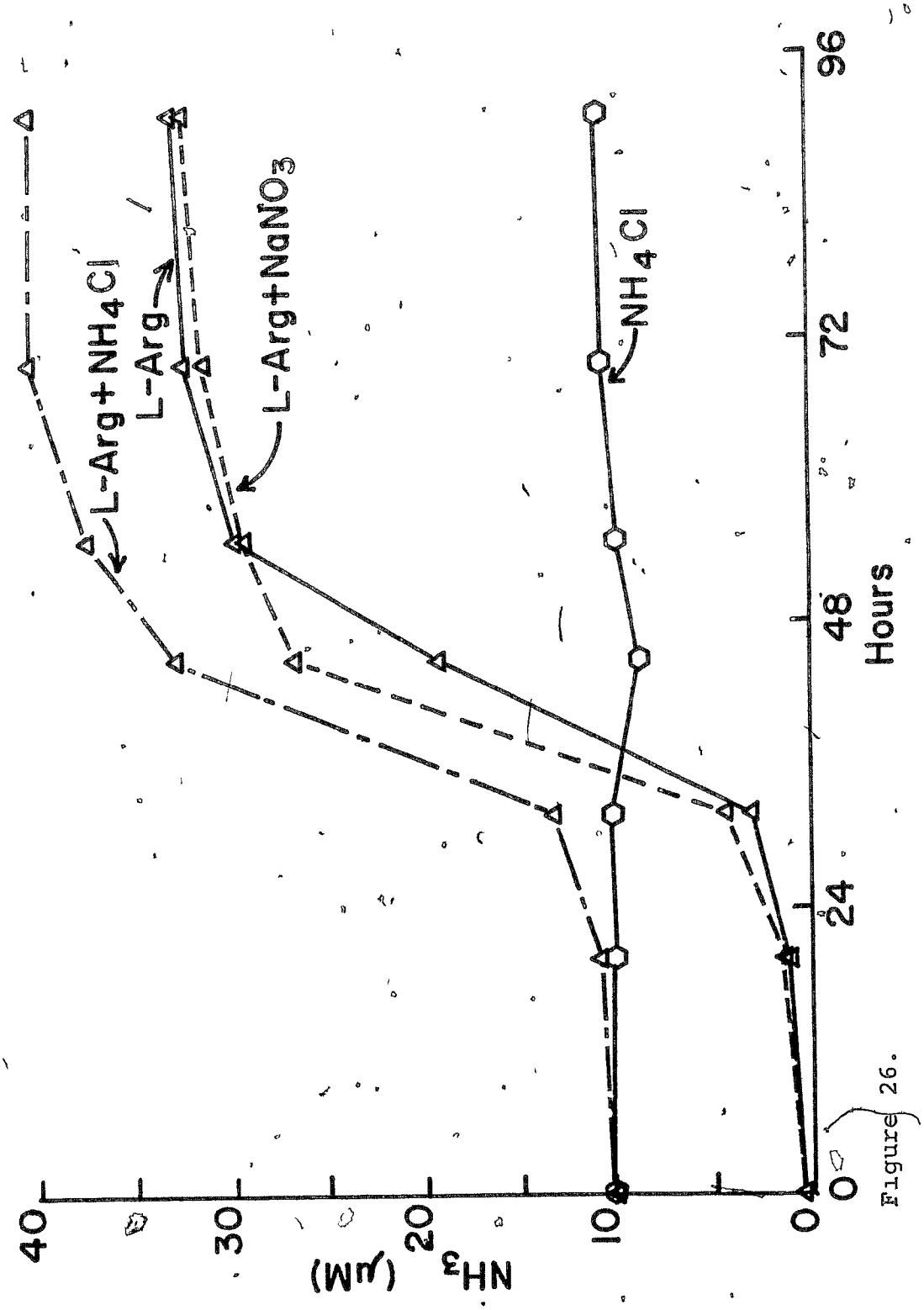

L-Arg+NH4Cl
L-Arg
L-Arg+NaNO3
NH4Cl
NH3 (μM)
Hours
0
10
20
30
40
24
48
72
96

Figure 26.

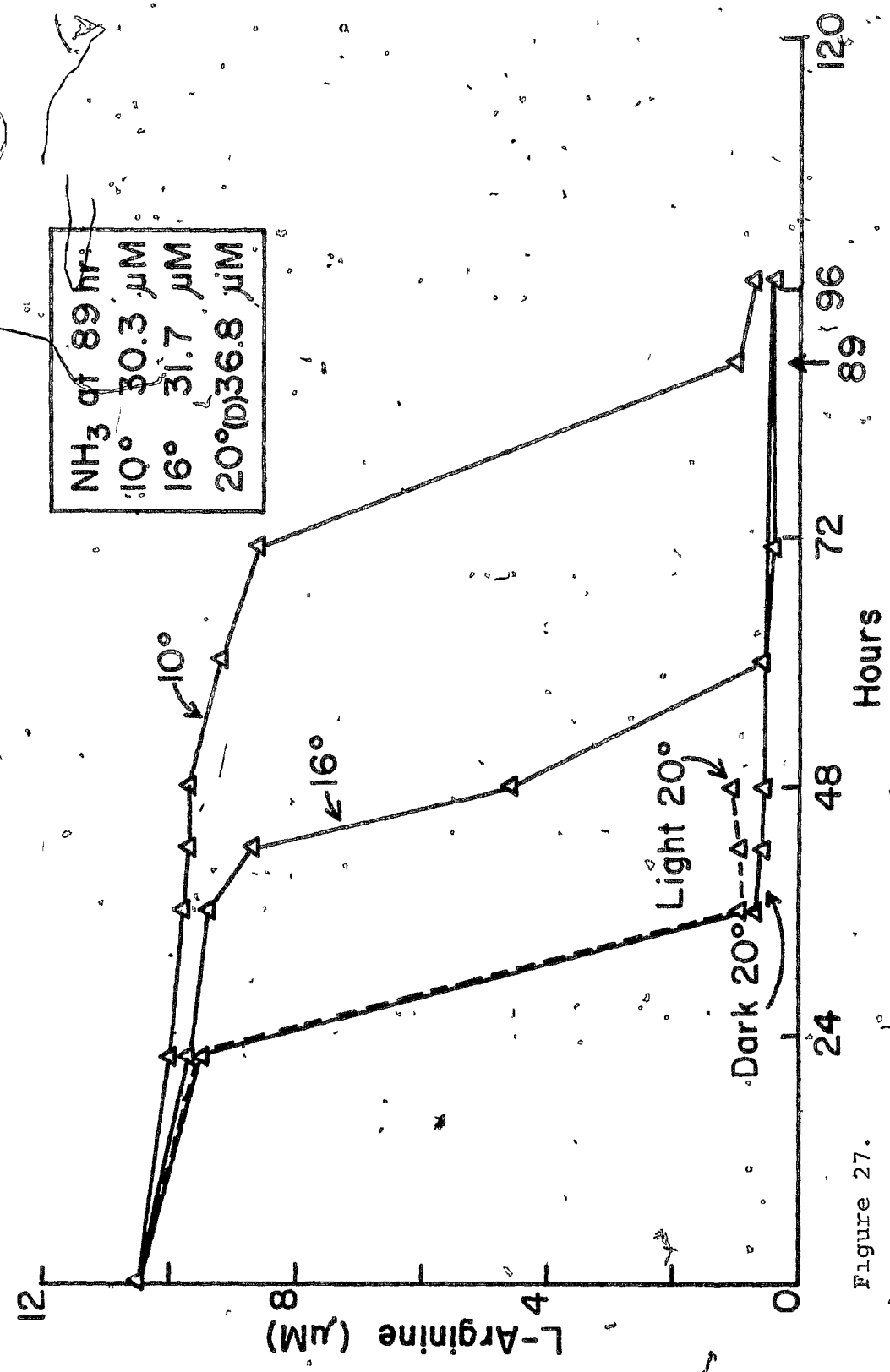

NH3 at 89 hr.
10° 30.3 µM
16° 31.7 µM
20°(D)36.8 µM
10°
16°
Light 20°
Dark 20°
L-Arginine (µM)
Hours
0
4
8
12
24
48
72
89
96
120

Figure 27.

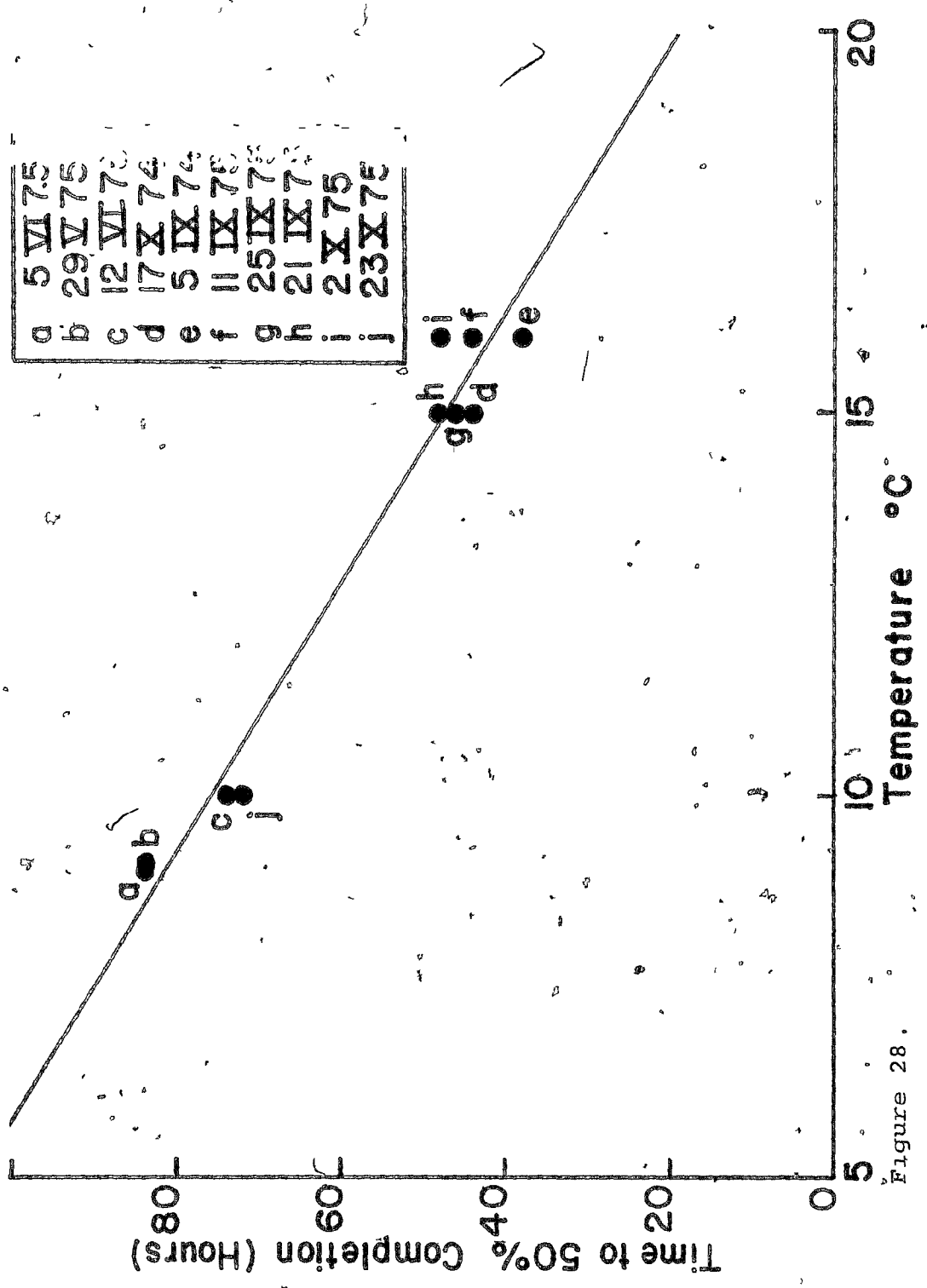
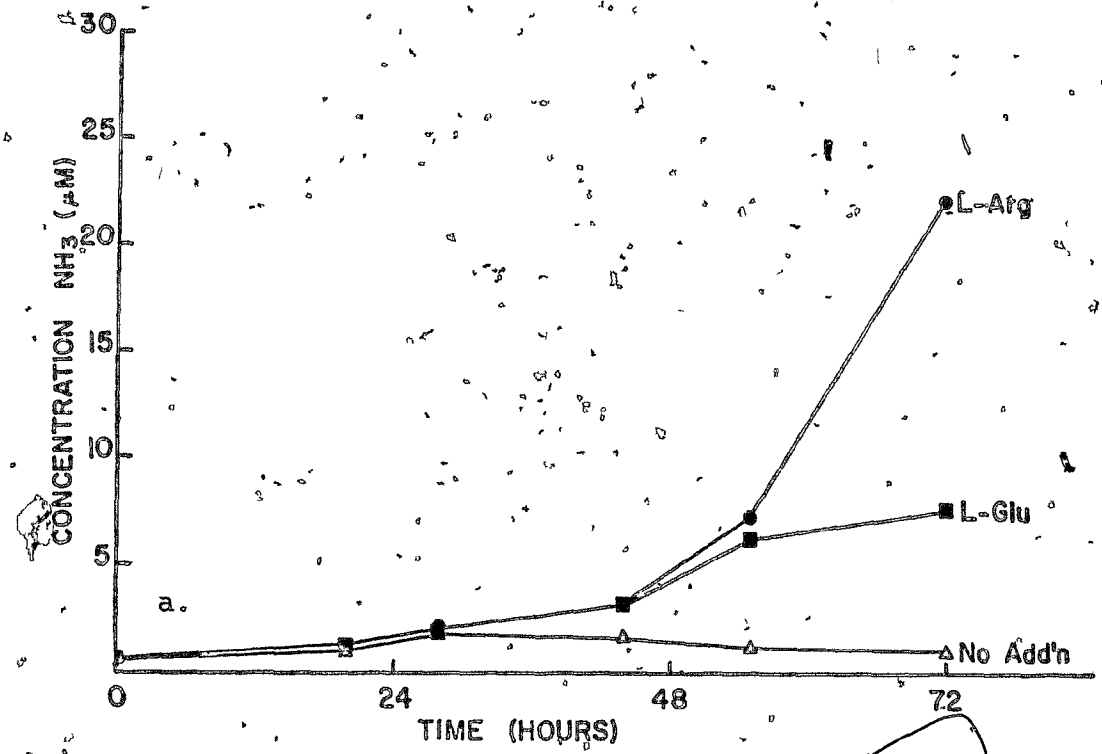

a 5 VII 75
b 29 V 75
c 12 VII 73
d 17 X 74
e 5 IX 74
f 11 IX 73
g 25 IX 73
h 21 IX 73
i 21 X 75
j 23 X 75
Time to 50% Completion (Hours)
0
20
40
60
80
5
10
15
20
Temperature °C

Figure 28.


CONCENTRATION NH3 (μM)
30
25
20
15
10
5
0
24
48
72
TIME (HOURS)
a.
L-Arg
L-Glu
No Add'n

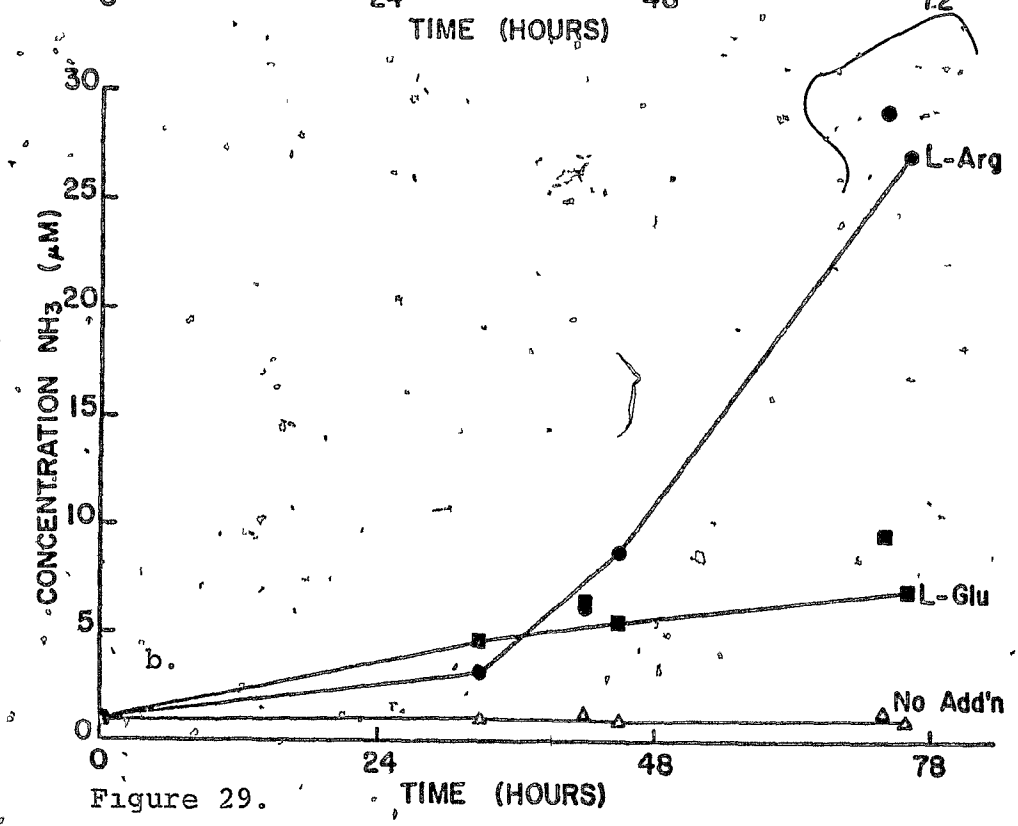

CONCENTRATION NH3 (μM)
30
25
20
15
10
5
0
24
48
78
TIME (HOURS)
b.
L-Arg
L-Glu
No Add'n

Figure 29.

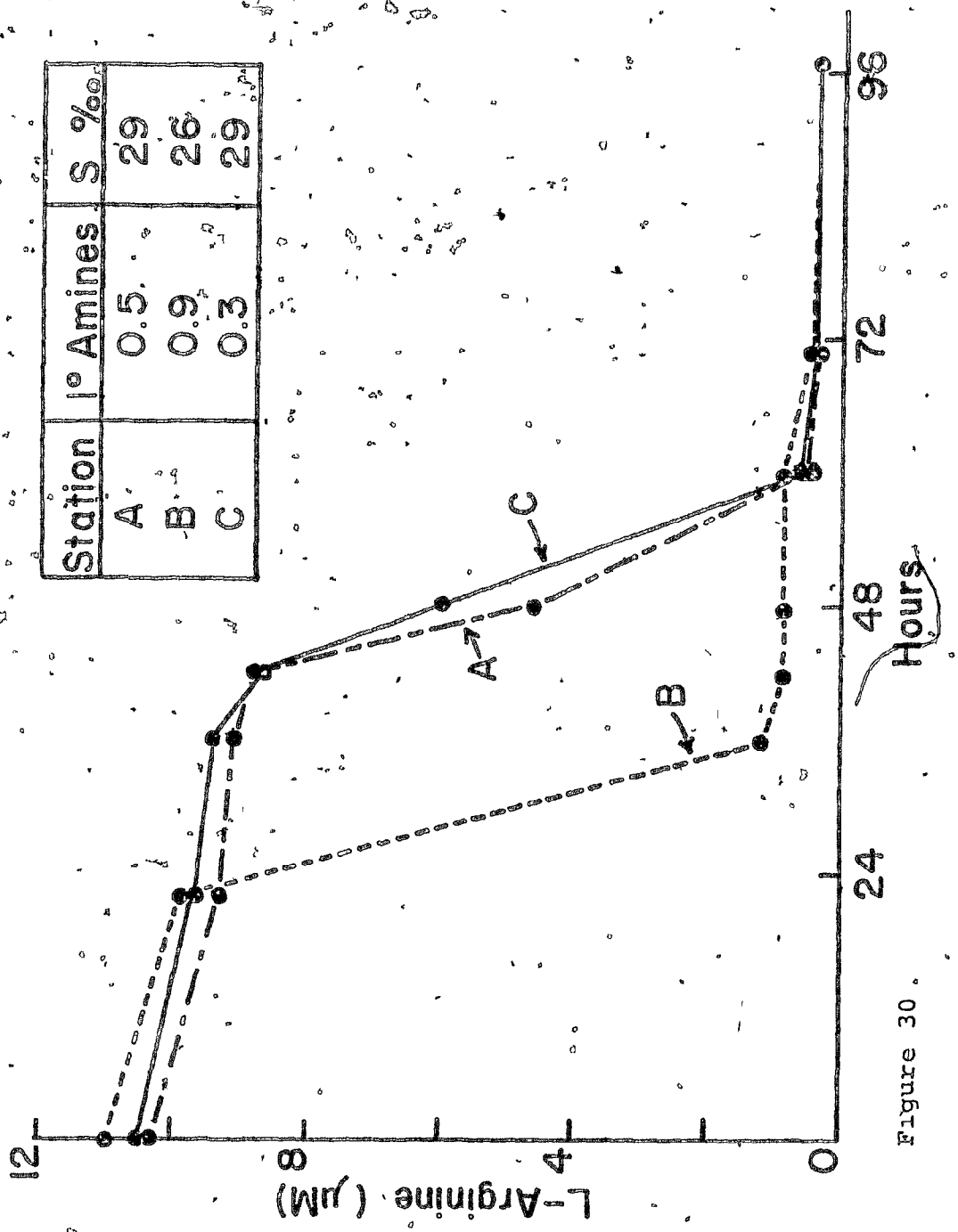

Station
l° Amines
S ‰
A
0.5
29
B
0.9
26
C
0.3
29
L-Arginine (μM)
12
8
4
0
24
48
72
96
Hours
A
B
C

Figure 30.

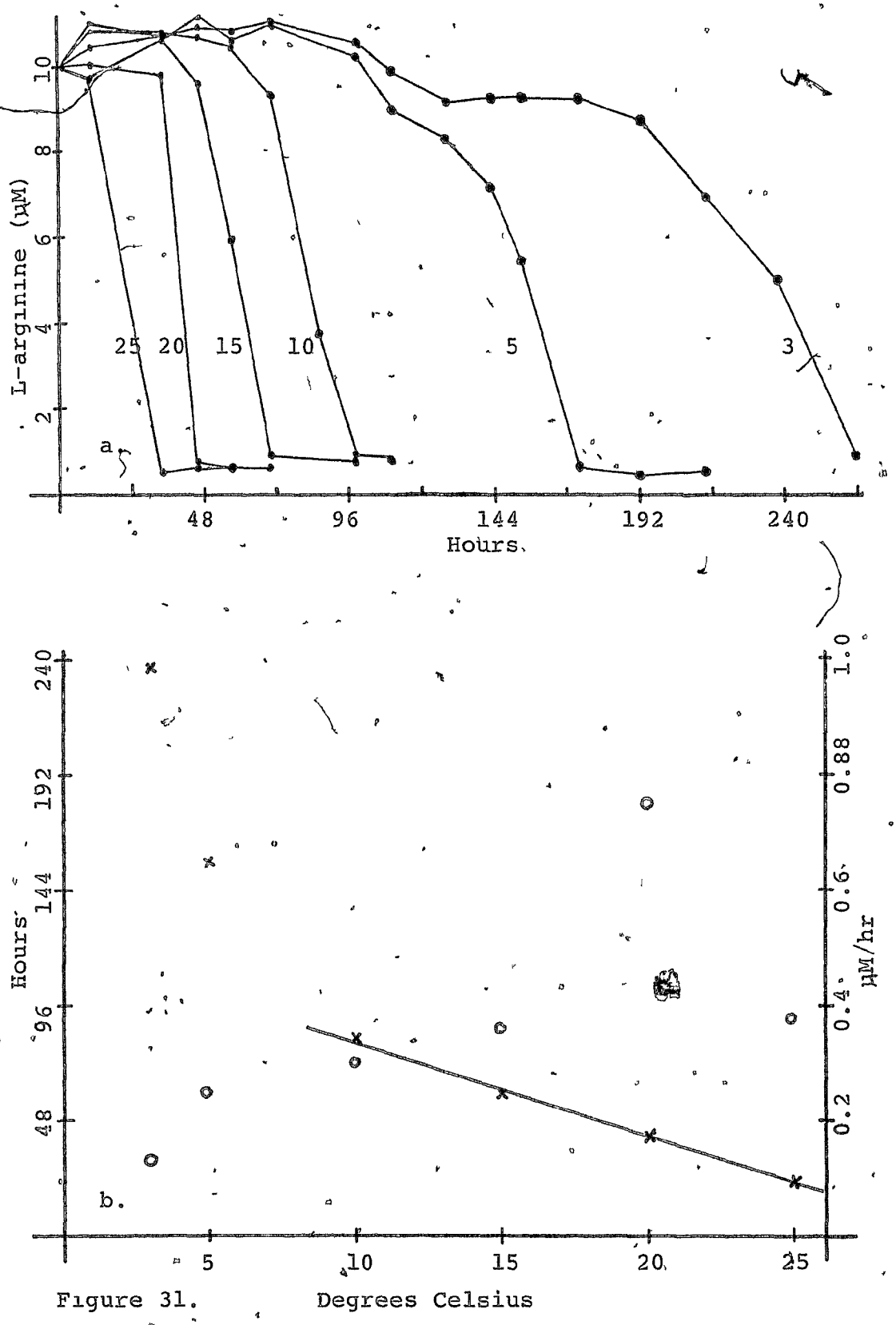

L-arginine (µM)
10
8
6
4
2
25
20
15
10
5
3
a
48
96
144
192
240
Hours.
Hours
240
192
144
96
48
1.0
0.88
0.6
0.4
0.2
µM/hr
b.
5
10
15
20
25
Degrees Celsius

Figure 31.

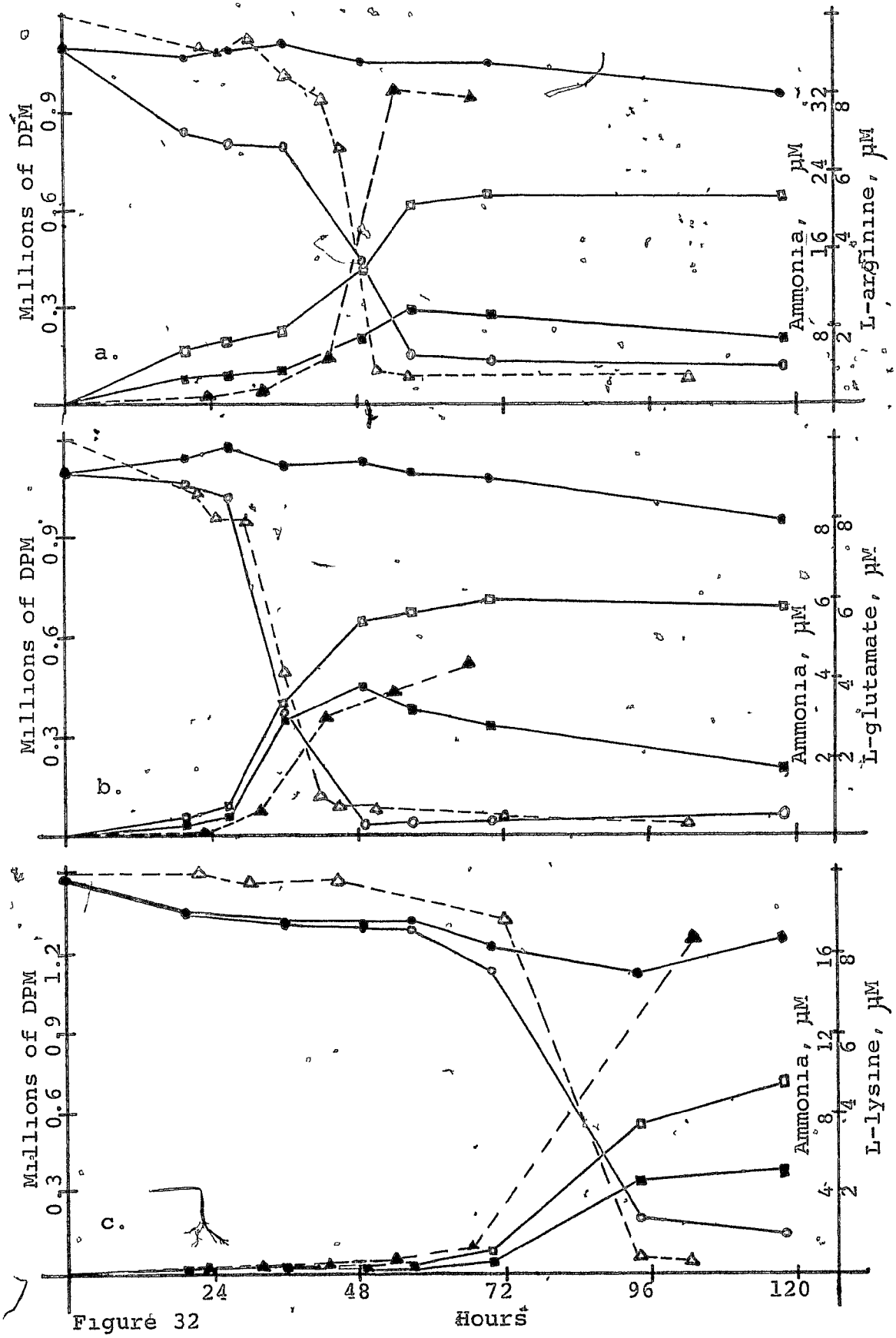

Millions of DPM
0.3
0.6
0.9
a.
Ammonia, µM
L-arginine, µM
b.
L-glutamate, µM
1.2
c.
L-lysine, µM
24
48
72
96
120
Hours

Figure 32

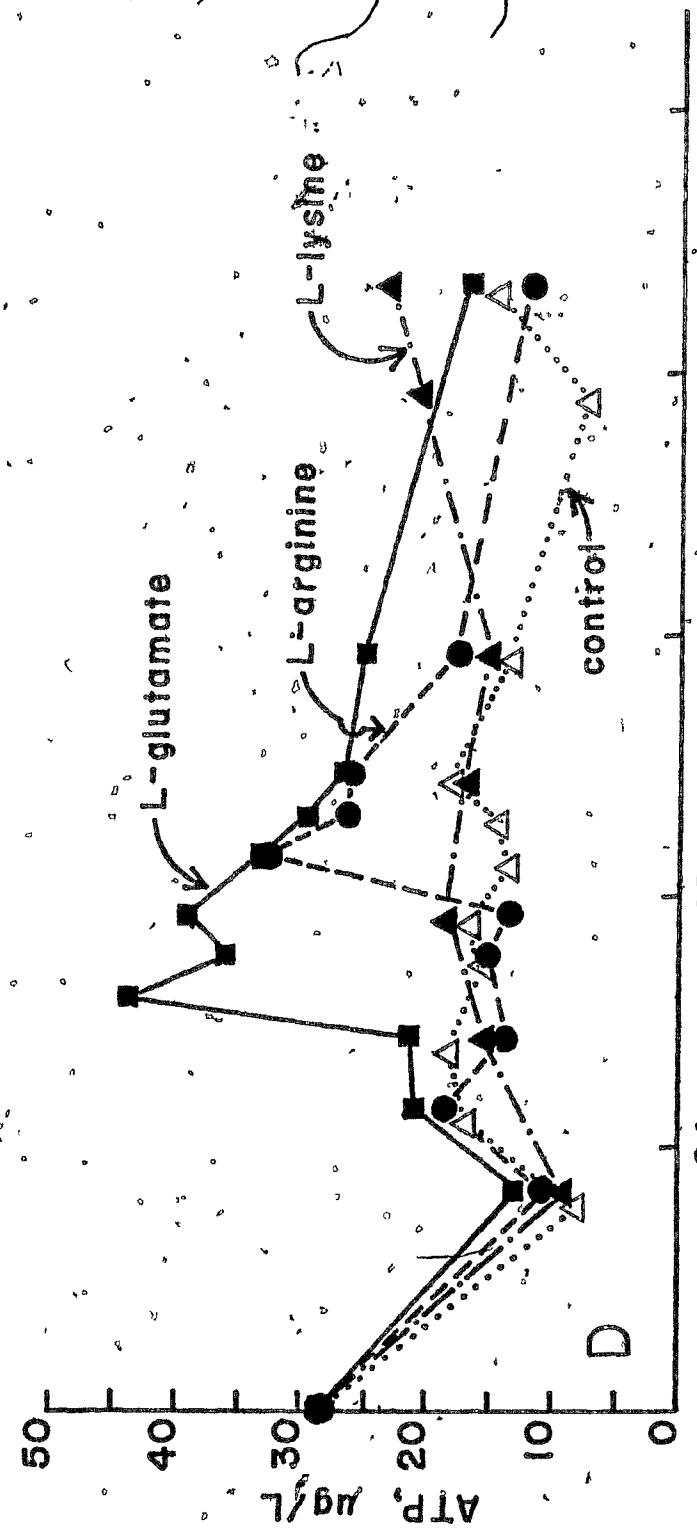

L-lysine
L-arginine
L-glutamate
control
ATP, µg/L
Hours
D
0
10
20
30
40
50
24
48
72
96
120

Figure 32d.

Substrate	C N (by atoms)	% Yield	max rate (μM/hr)	Tmax rate (hr)
Urea	12	0	0	—
L-Arginine	6'4	59	67	74
Glycine	2.1	95	.42	44
L-Lysine	6 2	62	45	116
L-Glutamate	5.1	61	.15	58
L-Valine	5'1	0	0	—
L-Trytophan	11 2	32	20	116
L-Leucine	'6 1	0	0	—

Table 1. Parameters for the degradation of various amino acids and urea, all added to give 10.0 μM. Percentage yield, maximum rate of degradation, and the lag time to maximum rate of degradation, ($T_{max\ rate}$), are all taken from the curves of figure 14.. See text.

Substrate	C N (by atoms)	%Yield	max rate (μM/hr)	T max rate (hr)
L-Arginine	6 4	87	1 11	75
L-Citrulline	6 3	90	7.7	95
L-Histidine	6 3	82	53	118
L-Ornithine	5 2	96	.46	75
L-Aspartate	4 1	114	26	75
L-Threonine	4 1	0	0	
L-Proline	5 1	95	19	75

Table II. Parameters for the degradation of various amino acids added to give 10.0 μM. Percentage yield, maximum rate of degradation, and the lag time to maximum rate of degradation, ($T_{max\ rate}$), are derived from the curves of figure 15. See text for details.

Table III. Enantiomeric specificity of the arginine uptake system and the induction of racemizing activity. The uptake systems were activated with either D- or L-arginine at 10.0 µM, the uptake curves are given in fig. 21. 100 ml aliquots were removed from the flasks and inhibitors were added to the indicated concentrations 2 hours before the test substrate was added to give a final concentration of 10.0 µM. The amount of the substrate remaining in the vessels was determined at the times indicated.

TABLE III. UPTAKE OF D- AND L- ARGININE BY COASTAL PLANKTON COMMUNITIES.

A. SPECIFICITY OF THE UPTAKE SYSTEMS

UPTAKE INDUCED WITH L-ARGININE, GIVEN D-		INDUCED WITH D-, GIVEN L-	
% UPTAKE	AFTER HOURS	% UPTAKE	AFTER HOURS
26	19	95	19
99	30	99	30

B. EFFECTS OF METABOLIC INHIBITORS

UPTAKE SYSTEM:		L- INDUCED				D- INDUCED	
GIVEN:		D-ARGININE		L-ARGININE		D-	L-
% UPTAKE AFTER (HOURS)		7	30	7	19	22	23
INHIBITOR, CONCENTRATION (uM)							
CYCLOHEXIMIDE,	10	0	96	98	98	97	97
	100	0	98	98	98	97	96
ERYTHROMYCIN	10	0	4	62	96	97	95
	50	0	2	56	95	87	97
LINCOMYCIN	10	0	67	97	97	96	97
	50	0	10	70	96	96	96
ERYTHROMYCIN + LINCOMYCIN	10+10	0	6	81	98	97	95
	50+50	0	4	84	99	93	94
NO INHIBITORS		0	97	98	101	102	98

Table IV. Substrate specificity of uptake systems induced by L-arginine, L-lysine, L-ornithine, and L-glutamate. Uptake was induced by substrate added to give 10.0 µM. Uptake curves are given in fig 24. Test substrates were added to 100 ml aliquots to a final concentration of 10.0 µM 18 hours after the depletion of the inducing substrate. Amino acid concentrations in the test vessels were determined 6 hours later. Aspartate and histidine uptake measurements had to be repeated 24 hours later; these values are given as % activity (% substrate taken up) because activity in the controls had dropped to 86%, 53%, and 50% for L-arginine, ornithine, and glutamate, respectively, over this time period. The uptake system for L-lysine, which had a longer activation lag, was fully activated when the measurements were made.

Table IV

Given:	Induced with: L-Arg	L-Orn	L-Glu	L-Lys
L-Arg	7.51 100	4.92 91.4	-.37 -5.61	7.24 104.3
L-Orn	2.14 31.7	5.38 100	-.92 -10.9	8.08 116.4
L-Glu	6.49 86.4	5.82 108.2	6.59 100	7.36 106.1
L-Lys	0.00 0.00	-.31 -5.8	-.53 -8.0	6.94 100
D L-Cit	-1.73 -19.4	1.43 25.6	-.66 -10.02	-.86 -12.4
L-Thr	.65 8.7	0.00 0.00	0.00 0.00	-0.8 -11.5
L-His	-7.3	-2.45	-32.3	-0.7 -1.0
L-Gln	7.69 102.4	7.51 139.6	5.28 80.1	6.51 93.8
L-Asp	20.4	72	-11.2	5.06 72.9

Table V. EFFECTS OF GLUCOSE ADDITIONS ON L-ARGININE UPTAKE AND DEGRADATION.

Glucose added, µM	L-arginine taken up, µM	Ammonia released, µM	Ammonia retained, µM	Carbon taken up, µg-atoms	C:N, by atoms
0.0	9.6	31.7	6.7	60	9.0
10.0	9.6	28.7	9.7	120	12.4
50	8.0	13.7	18.3	348	19.0

* All of the carbon added as L-arginine and D-glucose is assumed to be taken up and incorporated; i.e., no excretion or respiration of the substrates. The general relationship remains the same if a constant fraction of the su substrates are respired, but the absolute values of the C:N ratios will be different. See text.

TABLE VI. Carbon Respiration and Nitrogen Regeneration Ratios Accompanying the Degradation of L- Arginine, Glutamate, and Lysine.

Substrate and hours after its addition	% substrate carbon removed from medium[A]	% carbon assimilated[B]	% carbon respired[C]	% nitrogen released ^{14}C[D]	% nitrogen released fluorescamine[E]
L-arginine					
20	22	32	68	- [F]	- [F]
36	29	30	70	22 [F]	40 [F]
49	58	33	67	85	54 [F]
57	86	32	68	94	86
70	88	30	70	92	86
L-glutamate					
20	7.6	38	62	- [F]	-
36	67	47	53	22 [F]	25 [F]
49	97	41	59	35	36
57	96	36	64	38	39
70	96	32	68	38	38
L-lysine					
20	1.6	66	34	-	-
36	2.6	59	41	-	-
49	3.0	49	51	108[F]	130[F]
57	3.9	45	55	-	-
70	9.9	38	63	-	-
94	62	39	61	- [F]	- [F]
118	88	35	65	85	77

A; $\frac{\text{DPM filter + DPM carbon dioxide}}{\text{total DPM recovered}}$, DPM = disintegrations per minute

B; $\frac{\text{DPM filter}}{\text{DPM filter + DPM carbon dioxide}}$

C; $\frac{\text{DPM carbon dioxide}}{\text{DPM filter + DPM carbon dioxide}}$

D; $\frac{\text{ammonia concentration}}{10 \times n \times (A)}$, where n = µg-at N/µmole of substrate = 4, 1, or 2 for arginine, glutamate or lysine

E; $\frac{\text{ammonia concentration}}{10n(10\text{-concentration of substrate as determined by fluorescamine})}$

F; ammonia concentration determined by linear interpolation or assumed to be the same as at the previous point. Control value, (no additions), has been subtracted.

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