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Use of Dissolved Amino Acids by the Foraminifer *Notodendrodes antarctikos*[1]

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SYNOPSIS. Foraminifera are a ubiquitous and sometimes numerically important component of benthic communities. This paper discusses the role of free amino acids in the nutrition of *Notodendrodes antarctikos*, a large arborescent foraminifer from an oligotrophic embayment of the Ross Sea, Antarctica. The effects of temperature and substrate concentrations suggest a carrier mediated transport system which facilitates the accumulation of a wide variety of free amino acids at concentrations found in the interstitial waters of its sedimentary habitat. Involvement of isotopically labeled amino acids in the metabolism of this organism is discussed.

INTRODUCTION

The order Foraminiferida comprises an extremely diverse and abundant group of marine protozoa which, although little understood, may constitute an important component of most marine communities. This order is taxonomically diverse with approximately 4,800 species (Boltovskoy and Wright, 1976). However, the biology or ecology of only a few species has been studied in detail. Knowledge of metabolic rates and nutrient sources is important as these organisms are an abundant component of many benthic communities. This study describes some physiological and ecological aspects of a large agglutinated foraminiferal species.

Investigations of foraminifera have been numerous (see Boltovskoy and Wright, 1976, for review). The vast majority of published information about living foraminifera is zoogeographic and systematic. These studies show that foraminifera occur in all oceans and at all depths. Foraminifera are abundant and diverse constituents of most marine communities (Wolff, 1960; Wigley and McIntyre, 1964; Thiel, 1975; Coull *et al.*, 1977; Bernstein and Meador, 1979). They are possibly the most abundant eukaryotic organisms in the deep sea (Hessler, 1974; Tendal and Hessler, 1977; Bernstein *et al.*, 1978; Smith *et al.*, 1978). Even though they represent a large standing crop and biomass, and are probably influential in the metabolism of benthic communities (Smith *et al.*, 1978), knowledge of their biology or their ecological positions is limited.

The literature suggests that foraminifera are important predators, mineralizers, agents of disturbance, and prey for larger animals. Lipps and Valentine (1970) suggested that foraminifera occupy an important intermediate position between bacteria, detritus and other small organisms which provide their nourishment and larger organisms which consume them. Foraminifera are considered to be omnivorous (Boltovskoy and Wright, 1976) and have been shown to consume bacteria, cyanobacteria, yeast, dinoflagellates, diatoms and other unicellular algae with some degree of selectivity (Lee *et al.*, 1966; Lee, 1974) as well as other small organisms and detritus. DeLaca *et al.* (1981) described the uptake of dissolved organic material including amino acids and carbohydrates by benthic foraminifera.

Notodendrodes antarctikos

The foraminifer used in this study is *Notodendrodes antarctikos* (DeLaca *et al.*, 1980) (Fig. 1). This species is characterized by a large (up to 38 mm, $\bar{x}$ = 20 mm), agglutinated, arborescent test. The complex test includes a subcentral double-walled bulb, a dentritic root system, and a stem which bifurcates into a crown of branches. The

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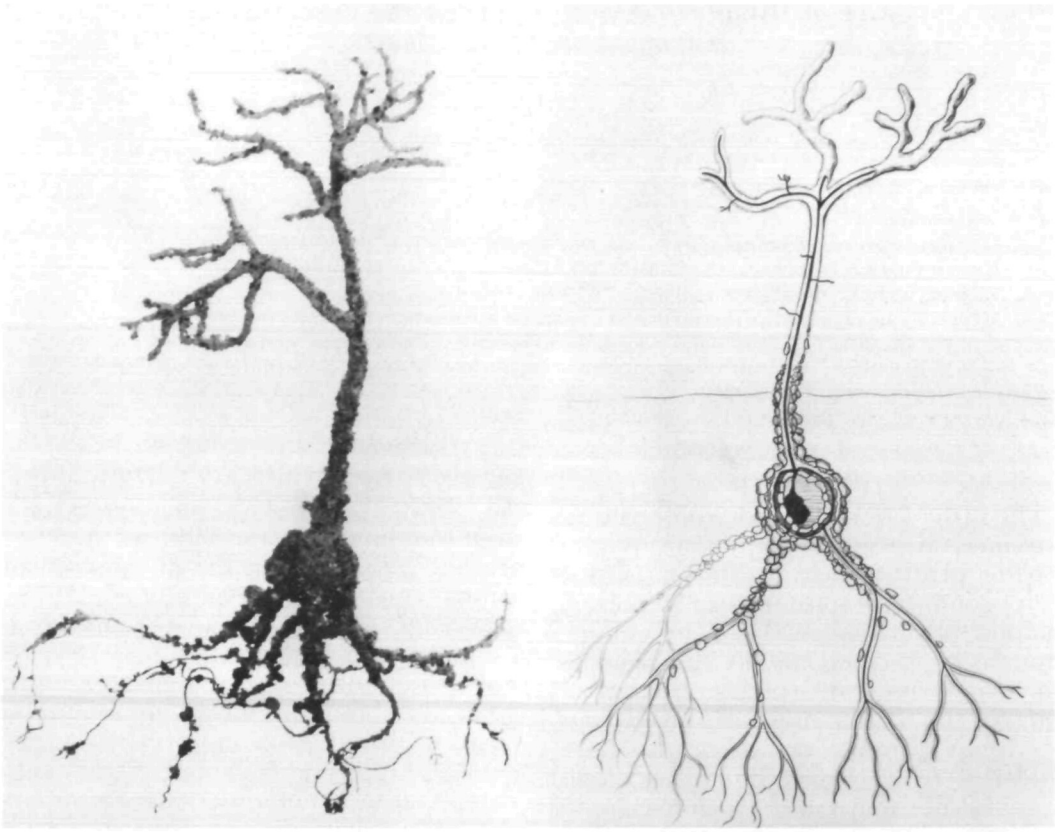

FIG. 1. A. A photograph of *Notodendrodes antarctikos*. Average size = 38 mm ($\bar{x}$ = 20 mm, n = 32). The complex root system and bulb reside in the sediment. The stem, arising from the bulb, passes through from 1 to 5 mm of sediment into the water column and terminates in a crown of branches. B. A cutaway drawing showing the distribution of protoplasm within the test. The cytoplasm extending into the roots is extremely thin and offers a large amount of absorptive surface area. The upper portions of the animal were found to capture particulate material.

bulb and the finely branching root system reside in the sediment. The stem extends from the top of the bulb through 1–5 mm of sediment into the overlying water, where it terminates in branches.

Light and scanning electron microscopic (SEM) studies have shown that feeding (in *in situ* fixed specimens) on particulate organic material occurs only infrequently. Cytoplasm penetrates through the walls of the branches and stem and forms small pseudopodial clusters which extend into the water. Particulate material including bacteria, diatoms and detritus, probably resuspended by active metazoa, occasionally adheres to these pseudopodia. Liquid scintillation, autoradiographic and light microscopic studies of foraminifera which were exposed to tritium-labeled marine bacteria demonstrated that bacteria can be captured by pseudopodia and that digestion takes place outside of the test. The labeled cytoplasmic material was transferred to the cytoplasm residing in the bulb.

The root system appears (through SEM) to be composed of hollow fibrous organic tubes. These organic tubes, which radiate into the surrounding sediment, have high tensile strength and undoubtedly act as a hold fast. All specimens which were fixed *in situ* were found to have cytoplasm extending into the roots. This cytoplasm has

not been found to pass outside of the root sheath. Although the root system was not implicated in the consumption of labeled bacteria, autoradiography of specimens exposed to labeled amino acids indicated that the root system is an important site for uptake of dissolved organic material.

Notodendrodes antarctikos was found to have a clumped dispersion with abundances of approximately 100/m². The sediments at the study site were predominately silt, mud and fine sand, and were well oxygenated. The most conspicuous components of the associated macro-fauna include the bivalve *Adamussium colbecki,* large rosselid sponges, crinoids, ophuroids and irregular echinoids.

Study site

The study site, New Harbor, is a shallow-water embayment on the western side of McMurdo Sound, Antarctica (Fig. 2). Although the Sound is a cul-de-sac to ship movements because of an impenetrable southern ice shelf, water currents are not restricted. Biological studies (Dayton and Oliver, 1977) conclude that the two sides of the Sound differ in water movement and nutrient levels. Organic input into the east side results from sedimentation of organics originating in northerly ice-free areas as well as late summer *in situ* productivity (as the annual ice cover thins, breaks up and moves north). These southward-moving currents then disappear under the Ross Ice Shelf and have been detected at White Island. Similar conditions exist on the western side of the Sound as far south as Marble Point where southerly currents cease. South of Marble Point the western side is bathed by northward-moving currents which originate from below the extensive Ross Ice Shelf, where they may have had a long residence time (Littlepage, 1965). Biological investigations of the water column there characterize it as oligotrophic (low in plankton and particulate organic material) (Dayton and Oliver, 1977; Holm-Hansen *et al.,* 1977; Hodson *et al.,* 1981). Dayton and Oliver (1977) concluded from studies of faunal affinities and the relative abundances of macro-faunal invertebrates that New Harbor was more similar to the bathyal deep sea than the more productive eastern side of the Sound.

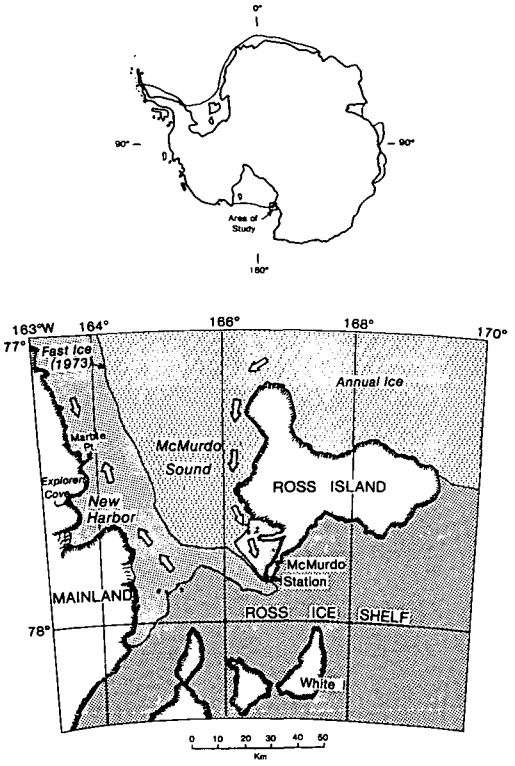

FIG. 2. Antarctica, showing the location of the Ross Ice Shelf with Ross Island at its northwestern corner. The species used in this study was found to exist only on the oligotrophic western side of McMurdo Sound at Explorers Cove, New Harbor. The arrows indicate the probable direction of current flow.

METHODS AND MATERIALS

I collected the specimens used in this study from Explorers Cove, the northern embayment of New Harbor, at depths from 18 to 34 m and placed them individually in plastic tubes 6.4 cm in diameter. In the laboratory the foraminifera were washed free of sediment with a fine stream of water, and maintained free of sediment at −1.5°C until needed. Experiments were usually conducted within a few hours to two days of specimen collection.

Prior to experimentation the foraminifera were separated and washed in 10 serial baths of filter-sterilized sea water (FSSW) (0.22 μm Millipore Corp.). Light, epiflu-

orescent and scanning electron microscopical examination indicated that the tests were freed of bacteria by this procedure.

I collected interstitial water samples using dialysis tubing (3,500 mwco, Spectrapor) that I filled with distilled water containing 35 g/liter NaCl, fashioned into bags, and autoclaved. The bags were buried in the sediment next to *N. antarctikos* by removing a 2.5 × 10 cm rectangular block of sediment, depositing the bags at 1–2 cm depth, and replacing the sediment block. Other dialysis tube-bags were placed at the sediment-water interface or suspended on poles at various distances from the bottom. They were left in the environment for 2–7 days before retrieval. The collected water was filtered through 0.22 μm filters, sealed in ampuoles and frozen for later analyses. The filters were checked for evidence of bacterial contamination. Analyses of free primary amines were conducted using fluorescamine (North, 1975). While the data from these analyses are presented in glycine equivalents, the results are probably an overestimate of amino acid concentration since fluorescamine reacts with other amines which may be present. However, free amino acids were separated and identified in selected samples using high pressure liquid chromatography (HPLC) after fluorescence derivitization with orthopthaldialdehyde (see Stephens, 1982). The preliminary data substantiate the approximate amino acid concentrations obtained by the fluorescamine technique.

Influx rates were determined by measuring the amount of radioactivity accumulated in the test animals. The experimental medium consisted of 10 ml of FSSW to which (depending on the experiment) various amounts of labeled and unlabeled compounds were added. Unless stated otherwise, the amount of amino acids in the FSSW used in an experiment has been analyzed using the fluorescamine technique. This value was assumed to be free amino acids, recorded in glycine equivalents and used in computations. "Time zero" and killed (100 μl of 0.1 μM NaCN) controls were used.

Labeled specimens were washed in two baths of FSSW to remove labeled water and were digested in Protosol (New England Nuclear, NEN). The resulting slurry of Protosol and inorganic debris was suspended in Aquasol (NEN) and distilled water (10:3) and counted. This count with corrections for controls and counting efficiency was considered to represent total sample radioactivity.

The experiments that compare bound *versus* total amino acid uptake were accomplished by washing each specimen twice in cold 10% trichloroacetic acid (TCA), hot (90°C) 10% TCA, 3% $HClO_4$, 95% ethanol, and ether. The remaining inorganic and organic residue was suspended in Aquasol/water gel for counting.

Biomass of *N. antarctikos* was estimated by measurement of protoplasm volume and test chamber volume.

Respiration rates were measured with a coulometric microrespirometer that functions by the release of counted, precise volumes of oxygen (generated through electrolysis) to replace that used by an organism residing in a small respiratory chamber filled with sea water (see Heusner, 1970). The production of oxygen within the respiratory chamber permits long-term observations. Specimens of *N. antarctikos* were monitored this way for up to 14 days. The device can detect consumptions of as little as 0.02 nl with 1% accuracy. The system was adjusted to replace oxygen in 1-nl pulses.

RESULTS

The fluorescamine technique was used to determine the concentration of naturally occurring primary amines in interstitial and overlying waters. Values of samples from 1 to 2 cm in the sediment, a depth at which the root system of the foraminifera would be deployed, varied from 22 to 60 μM with a mean value of 35 μM. Sea water at the sediment-water interface showed average concentrations of 6.8 μM and sea water samples from 10 cm above the interface and higher contained from 0.5 to 1 μM primary amines. Analyses of individual amino acids from sea water using the HPLC showed similar trends. The relative proportion of free amino acids in interstitial water samples is presented in Table 1.

TABLE 1. *Uptake rates of specific amino acids and their concentrations in interstitial water and experimental media.*

Amino acid	$\bar{x}$ uptake velocity (μmoles g^{-1} hr^{-1})	Conc. of AA in experimental medium (μM)	Percent free AA in interstitial water	Percent AA in protein hydrolysate*
Alanine	8.43×10^{-2}	6.7	4.6	9.3
Arginine	2.21×10^{-2}	3.3	7.3	6.3
Asparagine	5.83×10^{-2}	5.0	1.8	0
Aspartic acid	1.07×10^{-1}	5.0	4.6	9.0
Glycine	9.86×10^{-2}	10.0	21.0	4.6
Histidine	2.74×10^{-2}	3.3	0	4.0
Phenylalanine	2.21×10^{-2}	2.2	1.8	6.7
Proline	6.31×10^{-2}	4.0	0	5.6
Serine	9.77×10^{-2}	6.7	36.2	4.8
Tyrosine	1.73×10^{-2}	2.2	6.3	3.6
Glutamic acid	1.63×10^{-2}	3.0	8.2	11.8
Valine	0		4.6	6.8
Isoleucine	0		1.8	4.8
Leucine	0		1.8	11.8
Lysine	0		0	5.1

* An analysis given by Amersham Corporation for its product CFB.25 ([U-^{14}C]algal protein hydrolysate).

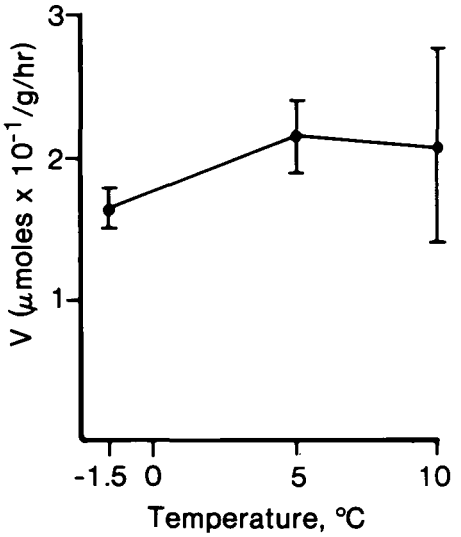

FIG. 3. The effect of temperature on the uptake velocity of algal protein hydrolysate (at 1.68 μM concentration). The duration of these experiments varied from 35 min to 1 hr. The bars represent the standard deviation (n = 5).

Two different estimates of biomass give approximate values of 1 mg wet weight. An analysis of the amount of stained protoplasm after removal from the test indicates that the average size of the sarcode is 1 μl. This volume is approximately the same as that of the innermost bulb chamber. Assuming a density of 1.0 g/cm^3, the average individual has a mass of approximately 1 mg. The data presented in this paper for amino acid uptake and oxygen consumption are given per gram per hour.

Amino acids with neutral, polar and charged R groups and proline were presented to *N. antarctikos*. All were actively taken up. The uptake rates of 11 amino acids are presented in Table 1. Individual (uniformly labeled) ^{14}C amino acids (Amersham CFB.103) were used at 5 μCi in 10 ml of FSSW. The final concentrations of the labeled amino acids used in these experiments is presented in Table 1 (the FSSW were considered to be free of amino acids). Only the labeled compounds were used to calculate these concentrations. They are similar to probable environmental concentrations experienced by *N. antarctikos* under natural conditions.

A more realistic estimation of the amounts of amino acids taken from sea water was achieved by measuring the uptake rate of a mixture of amino acids at 25 μM total concentration. This experiment used 5 μCi of uniformly labeled [^{14}C]algal protein hydrolysate (see Table 1 for composition) in FSSW (0.5 μM naturally occurring primary amines) to which unlabeled amino acids were added, in the same proportions as the hydrolysate to achieve the final concentration. The mean value of 6.14×10^{-1} μmoles g^{-1} hr^{-1} (SD = 8.7×10^{-2}, n = 8) at −1.0°C was found by the experiment.

Figure 3 presents the results of an experiment which examined the effect of temperature on uptake velocity. I used five animals with controls for each temperature. The experimental medium consisted of 5 μCi protein hydrolysate in 10 ml FSSW (1.84 μM final concentration). The organisms were incubated at −1.5°, 5° and 10°C for 1 hr. As indicated in Figure 3, uptake rate increased with increasing temperature between −1.5° and 5°C and then seemed to decrease slightly between 5° and 10°C. The apparent decrease in mean uptake velocity and the increase in standard devia-

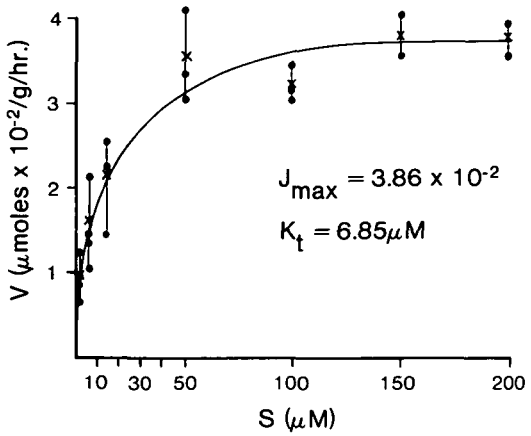

FIG. 4. Velocity of uptake as a function of the concentration of glutamic acid in sea water. The values for J_{max} (μmoles/g-hr) and K_t were obtained from a Hanes-Wolff linear transformation of the data.

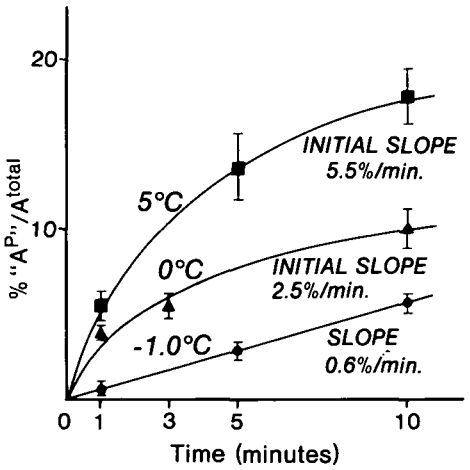

FIG. 5. Incorporation of the amino acids of algal protein hydrolysate at three temperatures into cold and hot TCA insoluble material. Data are presented as a ratio of the fraction of radioactivity recovered to total intracellular radioactivity.

tion for the 10° experiment probably reflects the stress of higher temperature. Observations indicate that this species is relatively tolerant of temperature change; it survived exposures of 10°C for several hours.

Figure 4 presents the uptake of uniformly labeled [^{14}C]glutamic acid at various concentrations. This curve is clearly hyperbolic and suggests that the transport system for glutamic acid in *N. antarctikos* can be described by the Michaelis-Menten equation. The data were therefore analyzed by a Hanes-Woolf Plot (where substrate concentration divided by rate of influx is plotted against substrate concentration). As shown in Figure 4, J_{max} (maximal rate of influx) for glutamic acid is 3.86×10^{-2} μmoles g^{-1} hr^{-1} and the K_t (substrate concentration at which the rate of uptake = $J_{max}/2$) is 6.12 μM.

Figure 5 presents data on the incorporation of radiolabeled amino acids by *N. antarctikos* into TCA insoluble material at three temperatures, −1°, 0° and 5°C. In this study 1.8 μCi of protein hydrolysate was added to 10 ml of FSSW in three flasks (final amino acid concentration = 1.66 μM). I exposed the foraminifera to this medium for varying times. The extraction procedure used on the foraminifera solubilized unbound amino acids, short chain polypeptides, aminoacyl and t-RNA. The radioactive material remaining in the precipitate is considered in this study to be protein-bound. Further differentiation was not possible with the small sample size. At −1.0° the incorporation rates were consistent with 0.6%/min and represent rapid synthesis at ambient temperatures. Data presented for higher temperatures show marked increases in rates of synthesis. However, these results are difficult to interpret, as the effect of temperature would affect both the kinetics of uptake carriers and the enzymes responsible for binding the amino acids. All rates are relatively high when compared to values reported for vertebrate tissues.

Examination of efflux of labeled material from the foraminifera, as seen by ^{14}C appearing in the medium, indicated that over a period of 30 min the loss was negligible. After initial labeling of 10 foraminifera in 1.8 μCi of protein hydrolysate at 1.66 μM final amino acid concentration for 10 min, the animals were washed briefly and placed in 5 ml of FSSW (0.8 mM, by fluorescamine). The time series for the first 15 min showed a slow increase in radioactivity to approximately 14% of the total taken up by the organism. Examination of

sea water at 5-min intervals for an additional 15 min showed no increase in radioactivity.

Oxygen is consumed by *N. antarctikos* at the rate of 93.6 μl g^{-1} hr^{-1} (SD = 28.3 μl g^{-1} hr^{-1}, n = 192) at −1°C. I monitored the respiration of single individuals which were placed with 1 ml of FSSW (25 μM) in sterilized glass vials for up to 200 hr. I am currently investigating factors that influence oxygen consumption in this species. Variations in respiratory rate have been observed and show a positive correlation with substrate concentration.

DISCUSSION

Notodendrodes antarctikos is an abundant benthic foraminifer. It is large and represents an obvious component of the benthos of an unusually oligotrophic antarctic environment. This species does consume particulate organic material (POM); however, water column studies have shown POM concentrations to be conspicuously low, and studies of individual foraminifera found them to have POM attached to pseudopodia only rarely (3 of 30 specimens) (DeLaca *et al.*, 1980).

While the amount of POM and DOC in the water column is low and presumably seasonal, concentration of dissolved amino acids in the interstitial water is relatively high and possibly more stable. This study presents evidence that *N. antarctikos* is capable of accumulation of dissolved amino acids which are naturally present in its sedimentary habitat. Uptake studies using 11 amino acids of varying characteristics demonstrate that this species is generalized in its ability to take up amino acids. The effects of both temperature and substrate concentration on the uptake velocity of amino acids suggest a carrier-mediated transport system rather than simple diffusion as a mechanism in accumulation of these compounds.

Dissolved radiolabeled amino acids taken up by this species enter metabolic pathways. They are both rapidly bound to TCA insoluble material (probably protein) and are given off as $^{14}CO_2$ (DeLaca *et al.*, 1981). The significance of the rapid incorporation of dissolved amino acids into insoluble material is not known. A possible advantage may be to reduce the endogenous free amino acid pool thereby facilitating transport.

The nutritional significance of dissolved amino acids to several marine invertebrates has been discussed by other workers (Southward and Southward, 1972; Stephens, 1972, 1981). Those studies have demonstrated that the influx of dissolved amino acids is capable of providing a significant portion of the cost of oxidative metabolism for the organisms investigated. The relative importance of absorbed amino acids to the energy metabolism of *N. antarctikos* may similarly be compared using the average uptake rate of protein hydrolysate (6.14×10^{-1} μmoles g^{-1} hr^{-1} at 25 μM total amino acids in sea water) with the average rate of oxygen consumption (93.6 μl g^{-1} hr^{-1} at approximately 25 μM total amino acids in sea water) at the near ambient temperature of −1°C. Using an approximate conversion factor of 1 μg of amino acid to 1 μl of oxygen consumed and an average molecular weight for the amino acids included in the hydrolysate of 131.43 g, the metabolic cost of respiration by this species would be offset by 93.6 μg of amino acid. The uptake velocities found during this study would supply 80.7 μg g^{-1} hr^{-1} and therefore supply 86.2% of the average respiratory cost. During the progress of long-term respiratory studies I have maintained this species with only dissolved organic material present in the medium. The presence of lipid-rich inclusions in the cytoplasm (possible storage products) is discussed in DeLaca *et al.* (1980). It is possible that these inclusions were being utilized for energy during those studies.

Morphological and physiological adaptations of this complex organism appear well suited for survival under this seemingly unpredictable and nutrient-poor water column. Similarities in morphology and environment between *N. antarctikos* and certain abundant species of the deep-sea foraminifera (*e.g.*, the Komikiacea [Tendal and Hessler, 1977]) are striking. The results of these and further shallow-water in-

vestigations underway at New Harbor may provide some insight into the ecological roles of foraminifera in the deep sea.

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