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Direct use of dissolved organic carbon by agglutinated benthic foraminifera

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Foraminifera are known to obtain nutrients in a variety of ways: they are omnivores, carnivores or herbivores[1-3] and some species are known to use the extracellular metabolites of their photosynthetic endosymbionts[1,2,4]. None, however, has previously been proven to utilize exogenous dissolved organic carbon directly, although this is well known in other marine species[5,6]. Knowledge of foraminiferal trophic positions is important because foraminifera are common in most marine communities[1] and may be the most abundant eukaryotic organism in the extensive deep-sea benthos[7-9]. Our studies of benthic foraminifera from an unusual Antarctic shallow water embayment now show that certain species utilize both particulate and dissolved organic material in their nutrition.

Our studies were conducted at McMurdo Sound, Antarctica, located in the southwest corner of the Ross Sea at latitude 77 °S and longitude 166°E (Fig. 1). The Sound is approximately 50 km wide; however, its eastern and western sides differ greatly in water column productivity and benthic biomass due to local oceanographic conditions[10] (see Fig. 1). The eastern side of McMurdo Sound is extremely productive during the austral summer, whereas the western side at New Harbor has very low productivity. The western side is rich in nitrate, phosphate and silica and low in plankton and particulate organic material (ref. 11 and D.M.K., unpublished data). Based on water analyses, species diversity and faunal affinities, New Harbor is more similar to the deep sea than to other shallow-water areas around Antarctica[11]. The organisms we discuss here were found only at New Harbor. Our investigation covered an area of 15–40 m depth, with a temperature of −1.8 °C. The sediments were rich in organic material. Analyses of dissolved amino acids, as shown by fluorometric analyses[12] of water collected in dialysis bags, demonstrated maximum concentrations in the sediment (20–30 μM at 1 cm) and smaller concentrations in the water column (5 μM at the sediment water interface, 1 μM at 10 cm from the bottom). This trend in the distribution of amino acids is similar to that of total dissolved organic carbon in the deep sea[13].

Our studies focused on *Notodendrodes antarctikos*[14] and an as yet undescribed species. Both are arborescent agglutinated foraminifera in the superfamily Ammodiscacea (Fig. 2). These large foraminifera are conspicuous components of the bottom assemblages at New Harbor, with abundances of >200 individuals per m^2 (ref. 14). Our initial studies on the physiology and trophic positions of these organisms revealed that they use captured suspended particulate organic material and dissolved organic materials in their nutrition. As seen by scanning electron microscopy of specimens which were fixed *in situ* and later dried using a critical-point technique, suspended particles are trapped in sticky cytoplasmic masses which extend from the branches of the tests. The foreign material caught in pseudopodia consists of benthic diatoms, bacteria, amorphous flocculent organic and inorganic material (such as silt, sand, broken diatom frustules and sponge spicules) with adhering bacteria. This material is probably suspended by the activities of larger invertebrates. Marine bacteria, labelled with ^{3}H-adenine (in the laboratory) were presented to foraminifera *in situ*, and these foraminifera were later fixed *in situ*. Microautoradiography revealed that the labelled bacteria are captured, digested and incorporated into the protoplasm of the organisms. However, the capture of particulate matter, in natural conditions, seems to occur infrequently in the population studied (3 of 32 specimens).

The foraminifera were tested for their ability to take up dissolved organic carbon by exposing them to several radiolabelled substrates, including amino acids (algal protein hydrolysate, Amersham), glucose and adenine (Table 1). We found that they took up the first two but did not absorb adenine. As bacterial contamination in our experiments could have produced erroneous results, we used ^{3}H-adenine (rapidly taken up by most bacteria[13]) in some of our experiments as a control in dual-label experiments with ^{14}C-labelled substrates. When tritium was detected in liquid scintillation analyses, those samples were assumed to be contaminated and were eliminated. 'Time zero' and NaCN- killed controls were used in all experiments.

Experiments measuring the uptake of dissolved organic carbon were conducted in the laboratory. Foraminifera cleaned in filter-sterilized sea water (0.22 μm) were placed in 10 ml of filtered (0.22 μm) sea water (1.0 μm free amino acids in solution) containing 2.5 μCi of ^{14}C-UL-protein hydrolysate

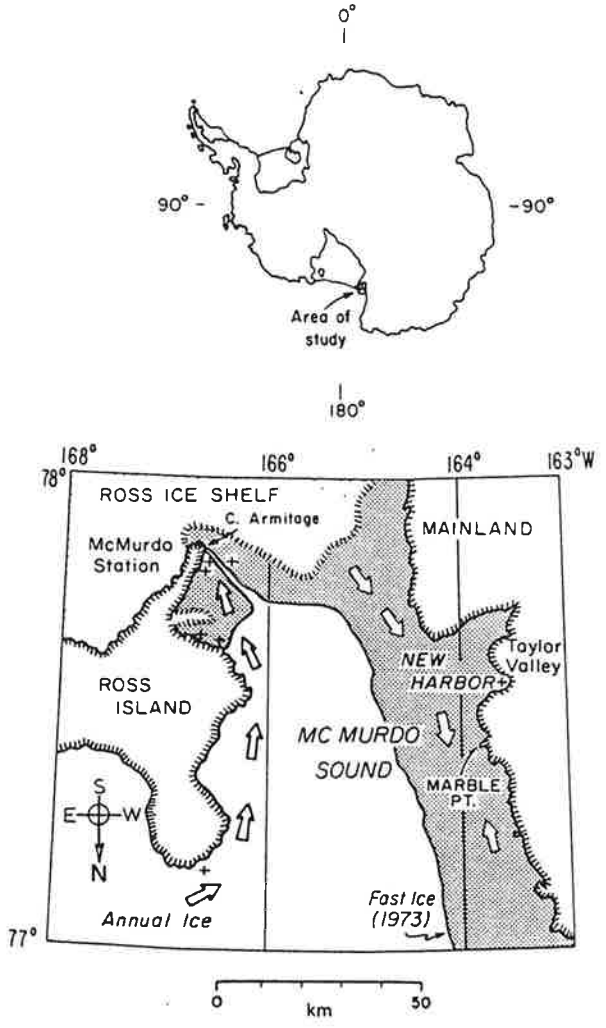


Fig. 1 Antarctica, showing the location of the study area. [During past investigations in McMurdo Sound we have surveyed several localities (+).] The two new species of foraminifera discussed in the present report were found only at New Harbor. Arrows indicate the probable direction of water movement. In the eastern and central parts of the Sound currents move south, advecting large standing crops of plankton from more northerly waters. The annual break up of sea ice on the eastern side allows local productivity to increase dramatically. In contrast to the highly productive eastern side, the western side of the Sound is bathed by northward moving currents[12] which probably originate under the Ross Ice Shelf and are poor in organic nutrients.

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(0.0165 μmol free amino acids). The final concentration of free amino acids in solution was 2.65 μM, and incubation times of 0.58, 1.08, 2.25, 3.25 and 4.17 h were used, all experiments being conducted at −1.0 °C. Following incubation, the foraminifera were washed and: (1) solubilized in hot and cold 10% trichloroacetic acid (TCA), (2) placed in sealed reaction flasks with 10 ml of filtered sea water for measurement of $^{14}CO_2$ evolution, or (3) fixed for autoradiography.

Cellular contents of solubilized foraminifera were analysed using liquid scintillation. Uptake rates of 6.14×10^{-4} μmol h^{-1} (s.d. $= 4.26 \times 10^{-4}$, $n = 20$) were found. The tests of individual organisms were subsequently removed, washed three times in hot and cold 10% TCA to remove any remaining soluble material and re-analysed. Up to 41.3% of the label was incorporated into non-soluble material that remained in the test.

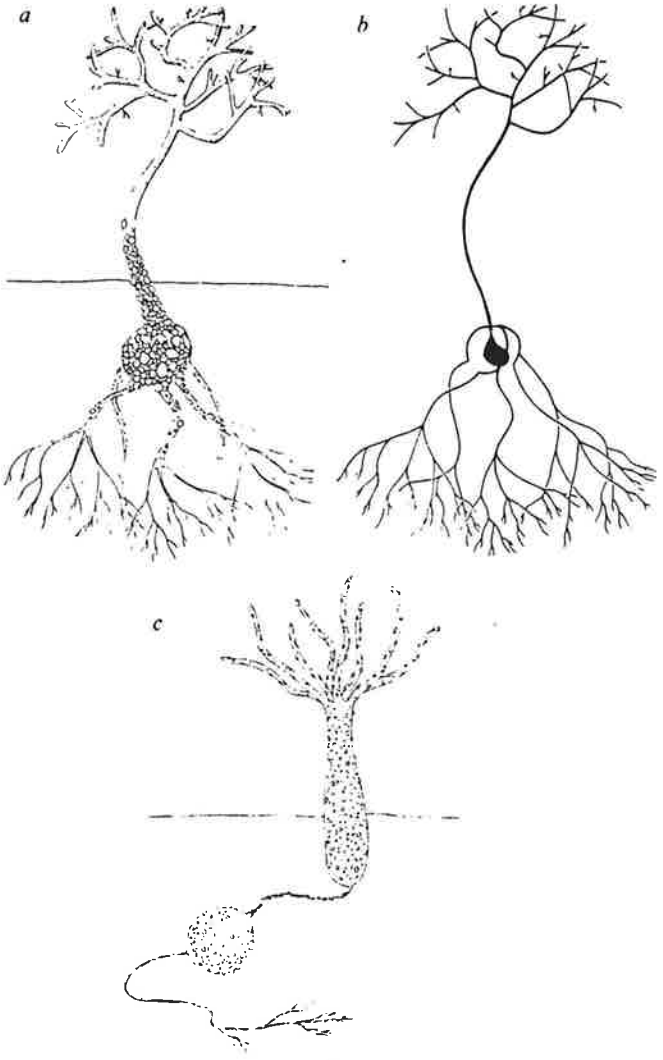


Fig. 2 *a*, *Notodendrodes antarctikos*[14] drawn in life position. The branching upper half extends above the sediment-water interface and the bulb and root system are buried in the sediment (average length 20 mm). *b*, The protoplasm of this species as would be seen if the test was removed. Cytological studies have shown that approximately one-third of the protoplasm (containing the nucleus) resides within the bulb. About one-third of the cytoplasm extends up the stem and occupies the branching system. Small pseudopods extend from the branches and upper portions of the stem. Particulate organic material is entrapped in these web-like projections. The remaining one-third of the cytoplasm resides in the extensive root system. Autoradiographic studies have shown that although dissolved organic material is taken up on all exposed cytoplasmic surfaces, the highest uptake occurs in the root system where the surface area is greatest. *c*, An unnamed agglutinated foraminifer (Notodendrodidae gen. et sp. n.) drawn in life position (average size 18 mm).

Table 1 Summary of uptake

Protein hydrolysate	
Total uptake	6.14 μmol h^{-1}
Incorporation into TCA-insoluble form	41.3% h^{-1}.
$^{14}CO_2$ liberated in 12 h	10%
Glucose	
Total uptake ^{14}C-glucose	6.1×10^{-2} μmol h^{-1}
$^{14}CO_2$ liberated in 12 h	46%
Adenine	
Total uptake	Undetectable

Experiments were conducted at −1 °C. Final concentration of free amino acid in laboratory experiments was 2.65 μM. Concentration of free amino acid in the sediment where the organisms are found was found to be 20–30 μM. See text for further explanation. Concentrations of glucose in the environment and laboratory experiments were not adequately measured.

The labelled foraminifera placed in reaction vials were incubated for 12 h at −1°C. The incubation was terminated with the addition of 0.2 ml of ethanolamine (placed on filter paper in the reaction vessel) and 1 ml H_2SO_4 (0.5 M) was injected into the seawater. After 2 h at room temperature (23 °C), the ethanolamine and filter paper were analysed by liquid scintillation. The results demonstrated that 10.0% of the total ^{14}C taken up was respired.

The results of autoradiography showed that the foraminifera took up dissolved organic carbon through any cell surface; consequently, uptake was a function of surface area. The 'root system', with its large surface area, was the principal site of uptake in the organisms analysed.

These two species of foraminifera seem to have ecological and physiological adaptations which are well suited to a highly seasonal oligotrophic environment. At New Harbor, primary productivity is very low and limited to a period of 1 or 2 months during the year, during which time the growth of a diatom flora on the undersurface of the 3–4-m thick sea ice or on the sediments of very shallow water (< 10 m) provides sparse organic input. The under-ice flora rains down to the bottom, where a portion is directly utilized while the remainder enters the detritus food web. Decomposition of this sedimented organic material generates relatively high concentrations of dissolved organic carbon in the sediment and supports a large and metabolically active microbial population (D. M. K., unpublished data). These foraminifera can capture and digest this particulate material either as it settles to the bottom or as it is resuspended by deposit-feeding invertebrates, although capture of particles seems to occur only infrequently. Dissolved organic carbon may contribute significantly to the nutrition of these foraminifera. During periods of no primary productivity, ~10 months of the year, they may sustain themselves on the high dissolved organic carbon reserves which are continually generated by the activities of heterotrophic microorganisms in the sediment.

Our results demonstrate that foraminifera are able to use both saprozoic and holozoic (phagotrophic) modes of nutrition. The amount and type of organic material available to foraminifera are therefore both much greater and more diverse than was previously thought. Re-evaluation of current ideas of the trophic positions of these extremely abundant organisms in benthic ecosystems is necessary, especially in deep-sea communities where they have been found to represent the highest test-free biomass of all eukaryotic organisms[9].

Present studies are analysing the metabolic rates of these organisms and will determine the proportion of their total energy requirements supplied by dissolved organic carbon.

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