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Uptake of Amino Acids by Three Species of *Nereis* (Annelida: Polychaeta). I. Transport Kinetics and Net Uptake from Natural Concentrations

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ABSTRACT: Epidermal uptake of alanine, serine and glutamic acid was investigated in salinities of 18 and 32 ‰ S in the polychaetes *Nereis succinea, N. virens* and *N. diversicolor*. The three amino acids were chosen because they were the most abundant free, interstitial amino acids in sediment from the sampling locality. Glutamic acid was absorbed at low, but similar rates in the three species in both salinities. In 18 ‰ S, serine was absorbed at equal rates by *N. succinea* and *N. virens*, but a lower uptake occurred in *N. diversicolor*. In 32 ‰ S, serine uptake increased in all species; the greatest increase was found in *N. succinea*. Alanine was absorbed at almost similar rates in both salinities. The highest uptake occurred in *N. succinea*, followed by *N. virens* and *N. diversicolor*. Uptake kinetics generally demonstrated higher affinities (low K_m) and lower potential uptake rates (V_{max}) in 18 than in 32 ‰ S. The uptake capacity in the three *Nereis* spp. did not appear to be related to natural interstitial concentrations of amino acids. The most abundant free, intracellular amino acids were taurine, alanine and glycine. In 32 ‰ S, the proportion of taurine was reduced, compared with 18 ‰ S. The free amino acid spectrum in the worms was not reflected in capacity or affinity of amino acid uptake. When the *Nereis* spp. were exposed to natural concentrations of interstitial amino acids, a significant net uptake occurred. Simultaneously, a small quantity of amino acids was released. The magnitude of the uptake from natural concentration levels of amino acids suggests that these nereids obtain a considerable energetic benefit under in-situ conditions.

INTRODUCTION

Marine sediment-inhabiting invertebrates are usually considered to feed exclusively on particles, e.g. bacteria, algae, or larger organisms (e.g. Yonge, 1928). However, a large potential source of food is contained in low-molecular-weight dissolved organic matter in marine sediments (Krom and Sholkovitz, 1977). This dissolved organic matter (DOM) may be important to animals living in the sediment, as most soft-bodied invertebrates are able to absorb small organic molecules through an active, epidermal uptake mechanism (Stephens, 1972; Jørgensen, 1976). Marine polychaetes are thus reported to absorb several species of DOM: Simple sugars (Ahearn and Gomme, 1975); amino acids (Stephens, 1972; Jørgensen, 1979); fatty acids (Testerman, 1972); and hydrocarbons (Lyes, 1979). Other sediment-dwelling organisms like nematodes (Lopez et al., 1979) and pogonophorans (Southward and Southward, 1972, 1979) have also been found to possess significant uptake capacities for DOM. Mechanisms of ion and osmoregulation have been reviewed comprehensively in 'Marine Ecology', Volume II, by Gilles (1975).

Some authors claim, however, that uptake of DOM from natural concentrations by invertebrates is insignificant as the invertebrates simultaneously release DOM, e.g. as amino acids (Johannes and Webb, 1970), and that bacteria due to their abundance and high substrate affinity, are much more efficient in reducing natural levels of dissolved organics (Hobbie and Webb, 1968; Siebers, 1979). Considering only free, dissolved amino acids in the water, these objections are probably true. However, considering the concentration of free amino acids in interstitial water in shallow coastal sediments (Stephens, 1975; Jørgensen et al., 1980a, b), the impact of uptake by invertebrates may be of considerable energetic and nutritional importance (Jørgensen, 1980). Yet only a few studies have demonstrated directly a net uptake of interstitial amino acids by invertebrates. Stephens (1975) and Stephens et al. (1978) report that the polychaete *Nereis*

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diversicolor and the sand dollar *Dendraster excentricus* are both able to reduce natural sediment concentrations of free amino acids, measured as total primary amines. Reports of uptake of individual amino acids occurring in the interstitial water have not been published, probably due to analytical difficulties.

In the present paper, we demonstrate absorption of specific, interstitial amino acids by three species of *Nereis: N. succinea* Leuckart, *N. virens* (Sars), and *N. diversicolor* (O. F. Müller) – using high-performance liquid chromatography for assay of the amino acids. Among the species investigated, *N. succinea* appears to prefer organic-rich sediments. A high total content of organic matter in marine sediments is generally reflected in high concentrations of dissolved organics (Krom and Sholkovitz, 1977). Therefore, it is expected that *N. succinea* encounters higher concentrations of dissolved organic matter than *N. virens* and *N. diversicolor* and might have evolved high capacities for DOM uptake. This was investigated using the three most abundant interstitial amino acids at the sampling locality (alanine, serine, glutamic acid). The observed uptake rates are compared with concentrations of free interstitial amino acids in the sediment and with the intracellular pools of amino acids in the nereids.

MATERIALS AND METHODS

Individuals of *Nereis succinea, N. virens*, and *N. diversicolor* were collected from a small, shallow estuary, Kysing Fjord, on the east coast of Jutland, Denmark. The fjord has a length of 3 km and an area of 1.86 km^2. The salinity in the main part of the fjord ranges from 4 to 22 ‰ S, depending mainly on wind direction (see Muus, 1967, for further details). Animals for experiments were obtained by sieving sediment, while population densities were determined from 3 to 5 cores (area 0.0143 m^2, depth 25 cm). The distribution of the three *Nereis* species was investigated along a 450 m transect beginning at the mouth and heading into Kysing Fjord. In the laboratory, worms for experiments were kept in salinities of 18 or 32 ‰ S in aerated aquaria with 5 to 6 cm of sediment at 15 °C.

Organic matter in the upper 0–5 cm sediment was determined as loss on combustion at 500 °C of sediment dried at 105 °C. Undisturbed sediment cores were obtained using acrylic tubes of 4.6 cm internal diameter and with a length of 25 cm. Interstitial water for experiments and amino acid analysis was extracted from the sediment by centrifugation in small containers (Blackburn, 1979) which were supplied with thick, precombusted glass fiber filters (Gelman Instrument Corp., Michigan, USA). The samples were centrifuged at 500 rpm for 10 min to minimize disturbance of the sediment (Jørgensen et al., 1980b).

Amino acid uptake experiments with the three *Nereis* species were carried out with natural seawater which had been stored for 4 to 6 weeks in the dark at 6 °C prior to use. For the experiments, the salinity was adjusted to 18 or 32 ‰ S using a commercial sea salt mixture (Wimex Meeressalz, Krefeld, FRG) and finally the sea water was filtered through 0.45 µm pore size filters. Uptake kinetics of alanine, serine, and glutamic acid which are the most common free amino acid in Kysing Fjord sediment (Jørgensen et al., 1980a) were investigated using L-(U-^{14}C)amino acids (The Radiochemical Centre, Amersham, England) as tracers, and sufficient L-^{12}C-amino acids (Sigma Chemical Corp., Missouri, USA) to give final concentrations of 1, 5, 10, 20, 30, 40, and 60 µM. At each concentration, 20 worms (wet weights between 20 and 300 mg) of each species absorbed amino acids in 5 ml medium in 20 separate glass vials. The test temperature was 15 °C. Uptake was measured as change in radioactivity of the solution during the experiment. One-ml samples were taken and 20 µl of 1 N HCl was added to drive off CO_2 1 h before counting. The samples were then added to 2.5 ml of Lumagel SB (Lumac Systems, Basel, Switzerland) and counted in a liquid scintillation counter. Quenching was corrected for with internal (^{14}C-toluene) and external standards.

Uptake rates were calculated according to Jørgensen (1979) using a regression which describes the uptake rate (Y) as a function of the weight (X) : $Y = a \cdot X^b$ where a is the y-intercept, and b the slope of the uptake-weight correlation when plotted in a log-log plot. The use of such regressions are necessary as the relative surface area of the worms decreases with increasing weight. Uptake rates were calculated from individual uptake of the 20 worms of different weights, and rates standardized to 100 mg individuals are given to facilitate direct comparisons of various uptake experiments. Kinetic calculations of the maximum uptake rate, V_{max}, and the half-saturation constant, K_m, of the amino acids were determined from Eadie-Hofstee plots (Lehninger, 1972).

Natural interstitial amino acids were extracted from sediment samples collected from Kysing Fjord on 16 February 1980. After extraction, the interstitial water was filtered through 0.22 µm pore size filters and stored at − 20 °C until use. Three individuals of each *Nereis* species were exposed to 6 ml of this interstitial water in separate vials at 15 °C. The concentration of specific amino acids was then followed at regular intervals, by taking samples of 0.2 ml.

Release of amino acids by the nereids was investigated in sea water made from the commercial sea salt mixture dissolved in redistilled water. The artificial sea water initially contained 0.514 µM of amino acids due to impurities in the sea salt. Three worms of each

species were rinsed in this water twice, blotted for a few seconds, and then placed in separate glass vials containing 6 ml of the aritificial sea water. Samples of 0.2 ml were taken from these vials at regular intervals for analysis of amino acids. The test temperature was 15 °C.

The free intracellular pools of amino acids in the worms were extracted in 80 % reagent grade ethanol (Merck, Darmstadt, FRG) according to Anderson and Bedford (1973). Three individuals of each species were extracted for 24 h. Initially, a gentle ultrasonic treatment was applied. At the end of the extraction, the extracts were centrifuged to precipitate particulate matter and 4 or 8 µl of the extracts were used for analysis of amino acids.

In one set of experiments, a Micron (Micron, USA) flow-meter supplied with an electromagnetic flow-probe was used for measurements of ventilation rates. A 210 mg individual of *Nereis virens* was placed in the flow cell (a plastic tube with an internal diameter slightly larger than the width of the worm, connected to an electromagnetic flow-probe) in 1 µM solutions of alanine, serine, or glutamic acid at 15 °C and 18 ‰ S. ^{14}C-amino acids were used as tracers. When the worm was actively ventilating, the amino acid concentration in water which had passed the body of the worm, was measured.

The oxygen consumption of the polychaetes was measured using either Winkler procedure (Strickland and Parsons, 1972), or an oxygen electrode (Radiometer E 5046; Radiometer, Copenhagen, Denmark).

Individual amino acids were measured as o-phthaldialdehyde derivatives by reversed phase high-performance liquid chromatography (HPLC) using methanol-buffer gradients (Lindroth and Mopper, 1979). The derivatives were quantified fluorometrically in an Aminco Fluorocolorimeter (American Instrument Company, Maryland, USA) supplied with a self-constructed 5 µl flow cell of Pyrex glass, and with a primary filter of 340–390 nm and a secondary cut-off filter of 418 nm. O-phthaldialdehyde (Merck, Darmstadt, FRG) does not react with proline or hydroxyproline, but reacts with ammonium. A Sigma calibration amino acid solution was used as a standard.

In the following, international three-letter symbols for amino acids are used as abbreviations. In addition, these symbols are used: ABA (amino-butyric acid), cit

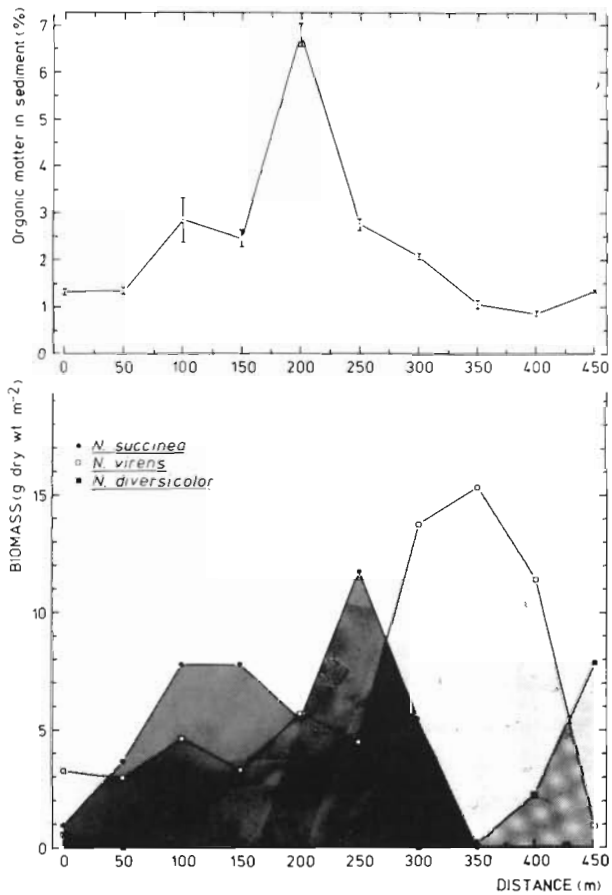

Fig. 1. *Nereis succinea, N. virens, N. diversicolor.* Distribution along a transect from the mouth and 450 m into Kysing Fjord. Organic matter values expressed as means ± S. D. of 3 samples. Material collected June, 1979

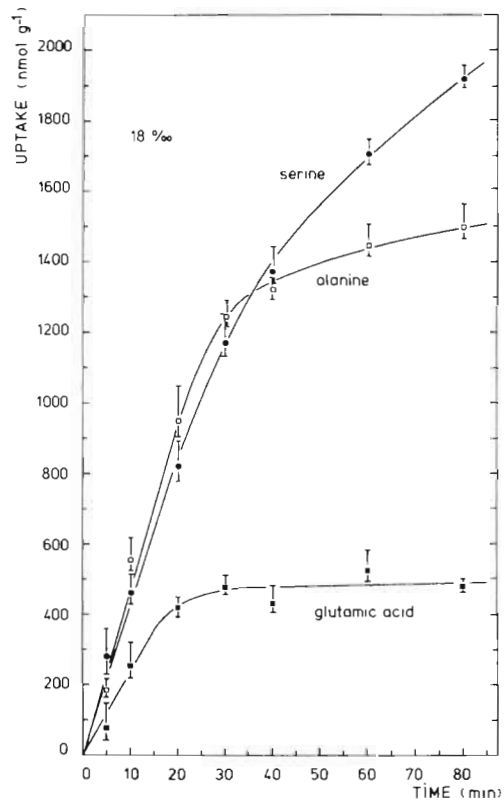

Fig. 2. *Nereis diversicolor.* Uptake of 10 µM serine, alanine, and glutamic acid as a function of time. Data expressed as means ± S. D.

(citrulline), DAPA (diaminopimelic acid), orn (ornithine), and tau (taurine). All weights of the animals are given as wet weights, exept in Figure 1, where dry weight of the biomass is used.

RESULTS

Natural Distribution of *Nereis* spp. in Kysing Fjord

The occurrence of *Nereis succinea* coincides with that of a *Mytilus edulis* flat characterized by a high organic content in the sediment, while *N. diversicolor* is the only *Nereis* species in the main part of the estuary; outside these areas, *N. virens* prevails (Fig. 1).

Uptake Kinetics of Serine, Alanine, and Glutamic Acid in *Nereis* spp.

In preliminary studies, each *Nereis* species absorbed serine, alanine, and glutamic acid for increasing periods of time from 10 μM solutions. The uptake of glutamic acid by *N. diversicolor* suggests that this species has a limited uptake capacity for glutamic acid, as the uptake stabilized after 20 min (Fig. 2); the absorption of serine and alanine was considerably larger and no stabilization occurred. Similar results were found in *N. virens* and *N. succinea*. In order to facilitate a comparison of alanine, serine, and glutamic acid uptake by the three species, an incubation time of 15 min was chosen as the uptake was linear within this period.

Uptake rates of alanine, serine and glutamic acid demonstrated that the *Nereis* spp. tested have larger capacities for uptake of alanine and serine than of glutamic acid. In a salinity of 18 ‰, alanine was absorbed fastest by *N. succinea*, followed by *N. virens* and *N. diversicolor* (Fig. 3). Serine was absorbed most slowly by *N. diversicolor*; faster, but similar rates occurred in *N. virens* and *N. succinea*. Uptake of glutamic acid was similar for all 3 species. In 32 ‰ S (Fig. 4), uptake rates were comparable to those found in 18 ‰ S, except that uptake of serine had increased in all species. The most significant increase was observed in *N. succinea* in which uptake increased from 550 nmol g^{-1} h^{-1} in 60 μM and 18 ‰ S, to 1200 nmol g^{-1} h^{-1} in 60 μM and 32 ‰ S. Similarly, the

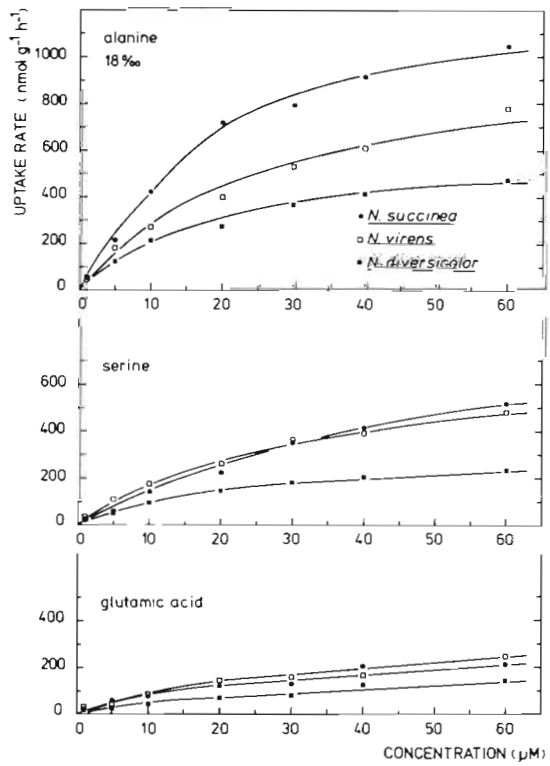

Fig. 3. *Nereis succinea, N. virens, N. diversicolor.* Uptake of alanine, serine, and glutamic acid as a function of concentration in 18 ‰ S. Average coefficient of correlation of linear regressions: 0.87 ± 0.08 (Mean ± S. D.; n = 63)

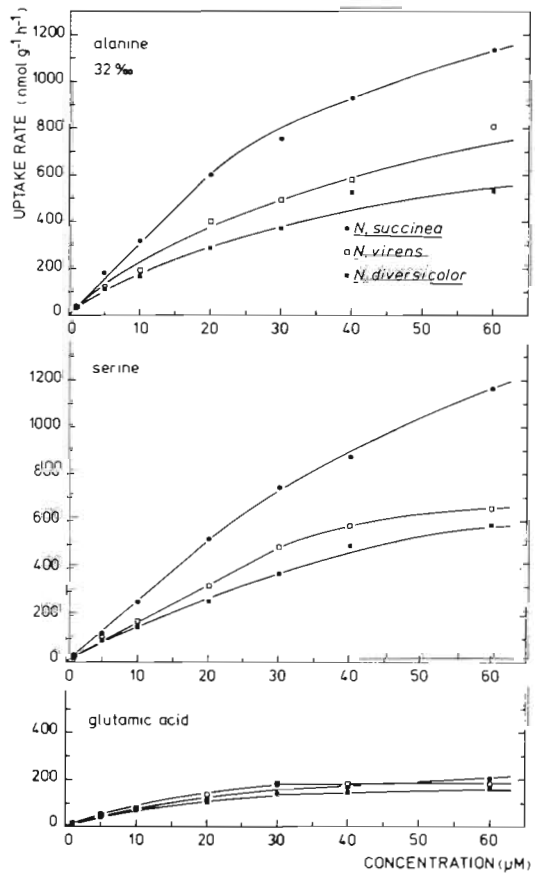

Fig. 4. *Nereis succinea, N. virens, N. diversicolor.* Uptake of alanine, serine, and glutamic acid as a function of concentration in 32 ‰ S. Average coefficient of correlation of linear regressions: 0.84 ± 0.11. (Mean ± S. D.; n = 63)

Table 1. *Nereis succinea, N. virens, N. diversicolor*. Maximum uptake rate, V_{max}, and half-saturation constant, K_m, of alanine, serine, and glutamic acid at 18 and 32 ‰ S. Calculated from uptake rates in Figures 3 and 4. Coefficients of correlation of the Eadie-Hofstee plots were: 0.93 ± 0.07 (18 ‰ S) and 0.92 ± 0.06 (32 ‰ S) (Means ± s.d.)

Salinity (‰)	Amino acid	*N. succinea*		*N. virens*		*N. diversicolor*	
		V_{max} nmol g^{-1} h^{-1}	K_m µM	V_{max} nmol g^{-1} h^{-1}	K_m µM	V_{max} nmol g^{-1} h^{-1}	K_m µM
18	Alanine	1504	26	900	20	604	20
	Serine	1090	67	655	25	344	28
	Glutamic acid	370	37	267	18	200	35
32	Alanine	2320	61	1631	68	743	30
	Serine	2765	84	1535	72	1458	87
	Glutamic acid	273	24	317	32	243	22

uptake rates of *N. diversicolor* increased from 220 to 600 nmol g^{-1} h^{-1} while a minor increase occurred in *N. virens*. Uptake of glutamic acid did not differ from that in 18 ‰ S.

The uptake kinetics generally demonstrated higher substrate affinities in 18 than in 32 ‰ S. In 18 ‰ S, the transport constants (K_m) were similar, except that the serine affinity is low in *N. succinea* and that *N. virens* has a high affinity for glutamic acid (Table 1). In 32 ‰ S, the K_m of alanine and especially those of serine have increased, in contrast to the K_m of glutamic acid which were slightly reduced. The transport constants of serine demonstrate that a high uptake rate need not be reflected in a high affinity.

Free Amino Acids in the Sediment Inhabited by Nereids

The concentration of free amino acids in the 0–4 and 4–8 cm sediment from typical *Nereis succinea* and *N. virens* localities did not differ significantly, though

Table 2. Concentration of free amino acids in sediment from typical *Nereis succinea* and *N. virens* localities. Concentrations in nM. Sediment cores sampled at Jan. 18, 1980. 1.3 °C. Results expressed as means of 6 cores. Standard deviations given for total concentrations. – not detected. Amino acids presented in elution order

Amino acid	*N. succinea* sediment		*N. virens* sediment	
	0–4 cm	4–8 cm	0–4 cm	4–8 cm
Aspartic acid	252	831	883	1176
Glutamic acid	222	1054	789	3532
Aspartate	112	239	493	2679
Serine	621	799	755	524
Glutamate	53	287	245	478
Histidine	116	208	222	665
Citrulline	40	83	86	80
Threonine	110	358	193	263
Glycine	381	2571	660	735
Arginine	44	354	248	601
Taurine	–	160	–	165
β-alanine	23	536	106	254
Tyrosine	50	323	107	169
Alanine	203	2011	383	1904
γ-ABA	30	77	112	164
α-ε-DAPA	–	–	108	198
α-ABA	–	56	42	28
Tryptophane	51	86	341	269
Methionine	231	230	87	139
Valine	98	486	123	204
Phenylalanine	43	151	66	105
Isoleucine	57	236	107	139
Leucine	1294	1483	174	477
Ornithine	897	1141	420	647
Lysine	288	367	101	120
Total	5198 ± 636	14092 ± 4352	6727 ± 2006	15545 ± 5407

slightly higher mean concentrations occurred in the *N. virens* sediment (Table 2). The concentration of glutamic acid was somewhat higher in the *N. virens* sediment, but the concentrations of serine and alanine were similar. The data do not indicate that *N. succinea* is exposed to higher concentrations of amino acids.

Free Extractable Amino Acids (FEAA) in the Nereids

Free amino acids are important osmotic effectors in tissues of most marine invertebrates (Gilles, 1975). The concentration of FEAA in the *Nereis* spp. may therefore be reflected in the uptake capacity of specific amino acids since absorbed amino acids act as FEAA (Jørgensen, 1979).

The total amounts of FEAA increased in all species when the salinity changed from 18 to 32 ‰ (Fig. 5). The amino acids appear to be quantitatively more important in 32 ‰ S than in 18 ‰ S, since the pools increased an average of 216 % in the three species while the salinity increased only 78 %. The highest amounts of FEAA were found in *Nereis succinea* in both salinities. In all species glycine, taurine, and alanine made up the main portion of the FEAA. Interestingly, in 18 ‰ S the percentage of taurine exceeded that of alanine, but in 32 ‰ S alanine was more important than taurine.

The FEAA pools are not directly related to uptake kinetics: (1) The increased importance of alanine as a FEAA in 32 ‰ S is not reflected in a higher uptake rate or in a low K_m-value. (2) The increased uptake of serine in all three species in 32 ‰ S is not explained by a larger requirement for this amino acid as a free intracellular amino acid. (3) In 18 ‰ S glutamic acid constituted 9 % of the FEAA in *Nereis diversicolor*, but the uptake kinetics show that this species has an extremely low uptake of glutamic acid in 18 ‰ S. The higher uptake rate of alanine (in 18 and 32 ‰ S) and of serine (in 32 ‰ S) in *N. succinea* may be found in the twofold larger pools of FEAA in this species, compared with *N. virens* and *N. diversicolor*.

Uptake of Natural Interstitial Amino Acids

When individuals of *Nereis succinea*, *N. virens*, and *N. diversicolor* were placed in Kysing-sediment pore water, a fast initial reduction in the total concentration of amino acids was observed; then the reduction declined (Figs 6, 7, 8). After 20 h, however, the total concentration had increased, compared with the 8-h concentrations. The concentration of individual amino acids (Table 3, Figs 6, 7, 8) reveals that both uptake and release of amino acids determined the total concentrations. In the first 8 h, the most abundant amino acids were typically absorbed, though an increase of some of these amino acids occurred after 2 h in *N. virens*. After 20 h, release had increased the concentration of some amino acids, e.g. ornithine, while others were still reduced.

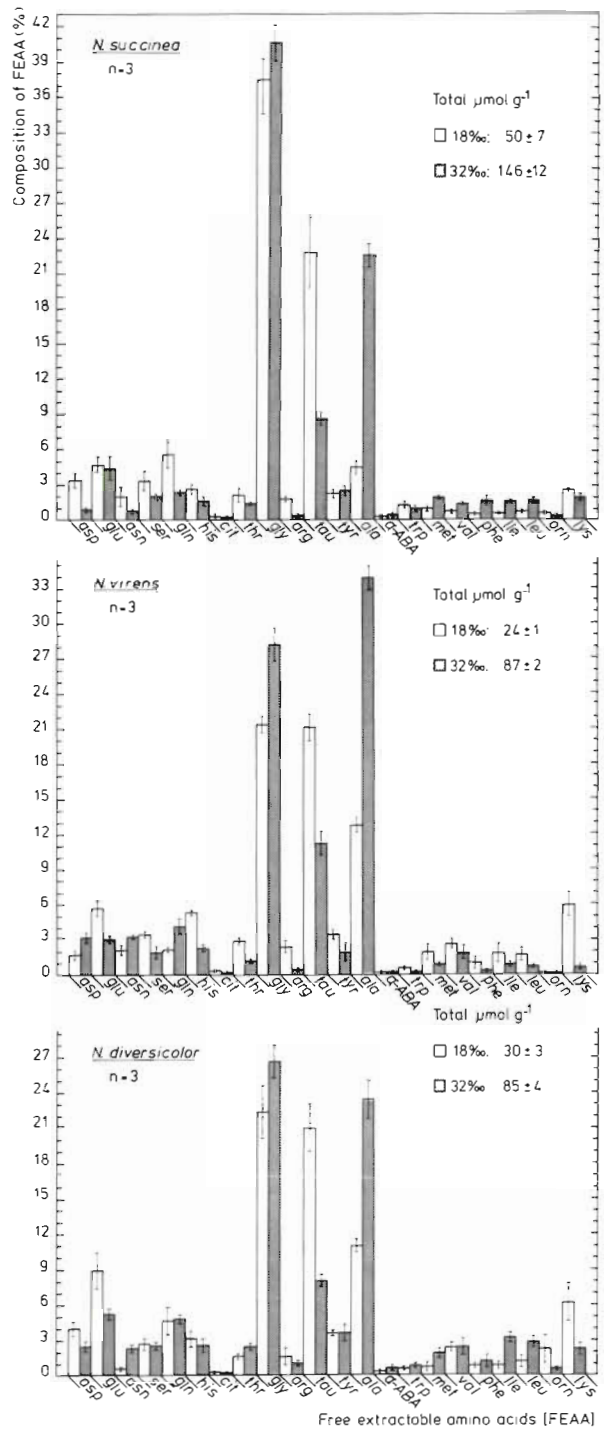

Fig. 5. *Nereis succinea*, *N. virens*, *N. diversicolor* Composition of free extractable amino acids (FEAA) in 18 and 32 ‰ S. Data expressed as means ± S. D., indicated by the error bars. Amino acids presented in elution order

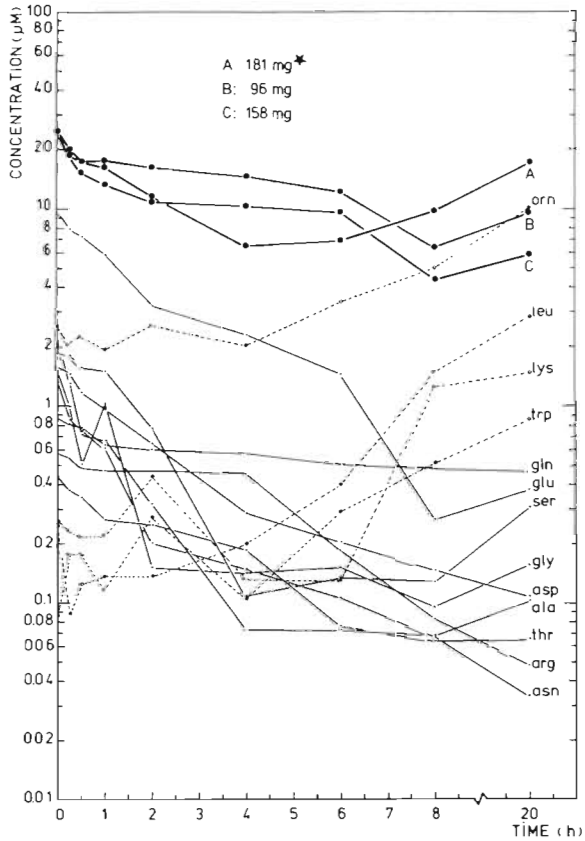

Fig. 6. *Nereis succinea*. Uptake of natural interstitial amino acids. Heavy lines: total concentrations. Concentration of individual amino acids shown for individual marked with star. Thin lines: amino acids mainly absorbed; dotted lines: amino acids mostly released

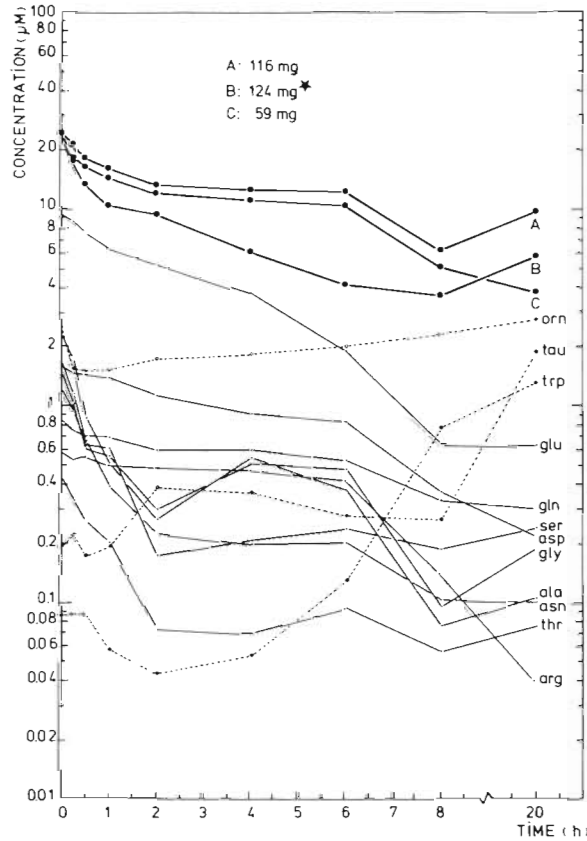

Fig. 7. *Nereis virens*. Otherwise as legend to Figure 6

The uptake rates of alanine and serine by the three species of *Nereis* were similar to rates predicted from the Michaelis-Menten equation ($V = V_{max} \cdot S / K_m + S$, where V is the actual uptake rate and S amino acid concentration), using values for K_m and V_{max} from Table 1, but the actual rate of glutamic acid was higher than the calculated rate. Thus, the 124 mg individual of *N. virens* (Fig. 7) had an actual uptake rate of 340 nmol g^{-1} h^{-1} from the initial concentration of 9.554 µM while the calculated uptake rate predicts a rate of 93 nmol g^{-1} h^{-1}.

Glutamate and arginine were absorbed to minor extents, but after 4–6 h, the concentrations decreased abruptly (Figs 6, 7, 8). This sudden decrease was probably caused by bacterial assimilation. A similar decline occurred in several other amino acids after 4–6 h, also indicating that bacteria may have proliferated.

Simultaneously with the uptake, amino acids were released from the polychaetes, as the concentrations of some amino acids mainly increased during the experiments. Individual variations in released amino acids occurred, but ornithine was generally the most common. Lysine, leucine, tryptophane, or valine accumulated in other experiments. Other amino acids may have been released but absorbed by the worms or by bacteria.

Release of Free Amino Acids from the Nereids

When the worms were placed in artificial sea water, there was little change in the total concentrations of amino acids at first, but after 1 h a slow increase began which continued throughout the rest of the experiment (Fig. 9). This was caused by uptake and release of various amino acids as indicated in the amino acid spectrum in the *Nereis succinea* experiment. The total concentrations slowly increased to about 1 µM at 4 h, and after 21 h concentrations from 2.3 µM *(N. diversicolor)* to 8.5 µM *(N. virens)* were found in the vials. Ornithine dominated in most of the samples, but lysine and leucine were also abundantly released amino acids. Figure 9 suggests that release of amino acids is merely related to the weight of the worms rather than to the species. The importance of bacteria was not determined.

The low concentrations of most amino acids in Figure 9 might be due to fast uptake of released amino

acids by the worms, thus indicating dynamic steady-state concentrations and not actual release. This was investigated in *Nereis virens* using 0.100 μM concentrations of alanine, serine, and glutamic acid, respectively, in artificial sea water. ^{14}C-amino acids were used as tracers. Assuming unchanged specific activity, the average concentration of the added amino acids was 0.059 μM after 4 h, while the total concentration of alanine, serine, and glutamic acid, respectively, was 0.087 μM (determined with HPLC). This shows that amino acids simultaneously were absorbed and released. It should be noted, however, that the two processes probably proceed independently.

Energetic Importance of Uptake of Natural Interstitial Amino Acids

As amino acids absorbed by *Nereis* spp. participate in catabolic pathways of the worm (Jørgensen, 1979), it is reasonable to compare to energy obtained from absorption of amino acids with the respiratory expenditure of the worms. Accumulation of interstitial amino acids was calculated as difference in total concentration, initially and after 30 min of uptake, from Figures 6, 7, and 8. Respiration rates of both resting and actively ventilating worms were used (E. Kristensen, in preparation). Energy obtained from absorbed amino acids was related to the respiration assuming that the complete oxidation of a mixture of amino acids requires 1.24 μg O_2 μg^{-1} amino acid (Stephens, 1975). The calculations (Table 4) show that resting worms are provided with sufficient energy to cover the total respiratory energy requirement (perhaps not totally in *N. succinea*) by absorption of interstitial amino acids. When the worms are ventilating, the energetic benefit decreases, but a substantial energetic gain is still achieved.

Table 3. Concentration of free interstitial amino acids in sediment from Kysing Fjord. Pore water from the upper 10 cm of sediment was extracted from cores collected at Febr. 7, 1980. 0.7 °C. Concentrations in nM. Amino acids given in elution order

Amino acid	Concentration (nM)
Aspartic acid	1592
Glutamic acid	9554
Aspartate	1242
Serine	1658
Glutamate	866
Histidine	351
Citrulline	116
Threonine	448
Glycine	1522
Arginine	567
Taurine	195
β-alanine	175
Tyrosine	406
Alanine	2351
γ-ABA	184
α-ABA	30
Tryptophane	86
Methionine	92
Valine	323
Phenylalanine	176
Isoleucine	178
Leucine	249
Ornithine	2153
Lysine	263
Total	24777

Uptake of Amino Acids by *Nereis virens* in a Flow Cell

When a 210 mg individual of *Nereis virens* was placed in 1.0 μM solutions of alanine, serine, and glutamic acid, respectively, in a flow cell, the amino acids were reduced to 0.816 μM (alanine), 0.855 μM (serine), and 0.923 μM (glutamic acid) at a ventilation rate of 1.0 ml min^{-1}. The experimental setup is comparable to *in-situ* conditions of *Nereis* species (Muus, 1967) and demonstrates that ventilation may supply the worms with both oxygen and dissolved organic matter.

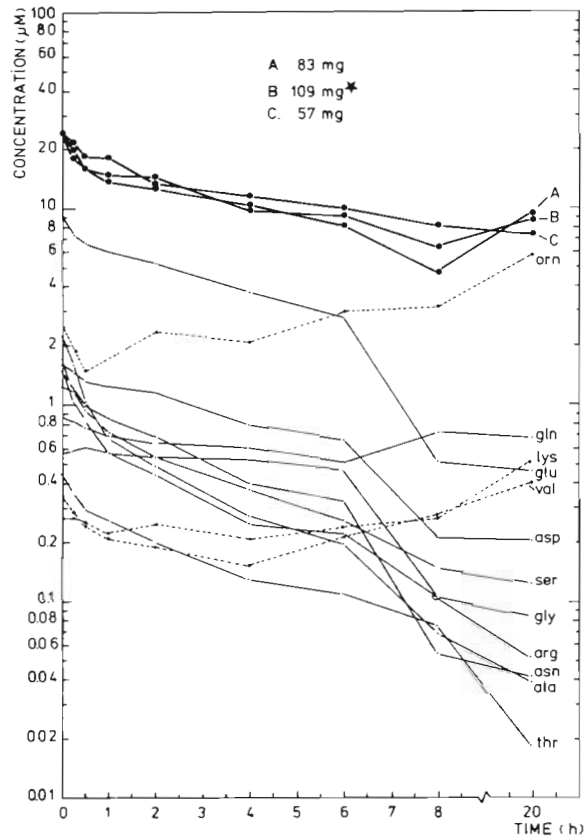

Fig. 8. *Nereis diversicolor*. Otherwise as legend to Figure 6

Table 4. *Nereis succinea, N. virens,* and *N. diversicolor.* Energetic significance of uptake of natural interstitial amino acids as shown in Figures 6, 7, and 8. Absorbed amino acids determined during initial 30 min of uptake. Respiration rates of resting worms measured individually in 50 ml bottles while respiration rates of ventilating individuals were determined in tubes connected to a flowmeter Respiration of each ventilating worm based on 5 replicates. 18 ‰ S; 15 °C

Species	Weight	Absorbed amino acids[1]	Oxidation of absorbed amino acids[2]	Respiration rates Resting	Ventilating	Sustenance of metabolic energy requirement Resting	Ventilating
	mg	µg (30 min)$^{-1}$	µg O_2 (30 min)$^{-1}$	µg O_2 (30 min)$^{-1}$		%	
N. succinea [a,b]	96	5.7	7.0	6.0	31.0	116	23
	181	5.4	6.7	8.9	47.1	75	14
	158	6.8	8.5	8.2	43.0	103	20
N. virens [c,d]	124	8.1	10.5	5.2	13.3	195	76
	116	4.9	6.1	5.0	25.1	122	49
	59	6.0	7.4	3.7	7.0	200	105
N. diversicolor [e,f]	109	6.4	7.9	6.4	16.8	123	47
	57	4.5	5.6	4.3	9.8	132	57
	83	6.3	7.8	5.4	13.4	144	58

[1]Calculated from an average molecular weight of the actual amino acids of 115
[2]Oxidation of a mixture of amino acids requires 1.24 µg O_2 µg^{-1} amino acid (Stephens, 1975)
Oxygen consumption (µg O_2 h^{-1}) as a function of weight (W):
[a] 0.742 $W^{0.611}$, $r = 0.87$; $n = 14$ [c] 1.251 $W^{0.437}$, $r = 0.89$; $n = 13$ [e] 0.694 $W^{0.621}$, $r = 0.92$; $n = 13$
[b] 3.047 $W^{0.660}$, $r = 0.89$; $n = 6$ [d] 0.425 · $W^{0.858}$, $r = 0.99$; $n = 7$ [f] 0.652 $W^{0.0840}$, $r = 0.99$; $n = 6$
a, c, e resting worms; b, d, f ventilating worms

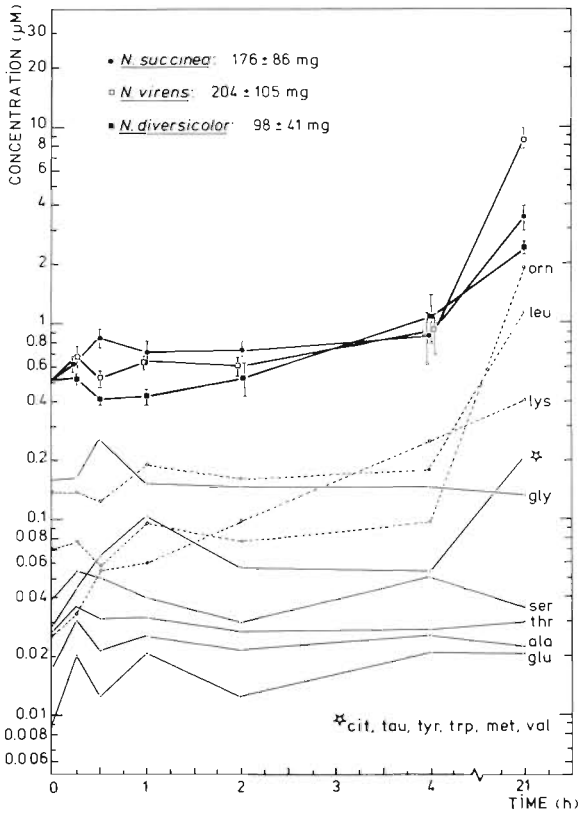

Fig. 9. *Nereis succinea, N. virens, N. diversicolor.* Release of free amino acids. Heavy lines: total concentrations. Data expressed as means ± S. D. Concentration of individual amino acids shown for a 160 mg *N. succinea.* Dotted lines: most abundant released amino acids; thin lines: concentration of remaining amino acids

DISCUSSION

The natural distribution of the *Nereis* species in Kysing Fjord (Fig. 1) can probably be explained by an increased tolerance to changes in salinity, including extremely low salinities, in *N. diversicolor* (Smith, 1955; Neuhoff, 1979), and by an increased tolerance to low oxygen tensions and presence of H_2S in the rich sediments, in *N. succinea* (Theede et al., 1973). In more stable salinities and in more sandy sediments with a low organic content, *N. virens* appears to possess competitive advantages. The present observations are in agreement with Theede et al. (1973) who studied *Nereis* spp. in Kiel Bay.

The 3 *Nereis* species are typical marine invertebrates in terms of qualitative amino acid uptake. In kinetic studies, higher uptake rates of neutral than of acidic amino acids, especially of glutamic acid, are generally reported (Stephens, 1964; Taylor, 1969; Stephens, 1975; Stephens et al., 1978; Southward and Southward, 1979). However, differences in both capacity and affinity of amino acid uptake were observed in *N. succinea, N. virens,* and *N. diversicolor. N. diversicolor* had low uptake rates but high affinities of alanine, serine and glutamic acid, in contrast to *N. succinea* which demonstrated high uptake rates and low affinities. Intermediate uptake rates occurred in *N. virens* while the amino acid affinity was higher in 18 ‰ S than in 32 ‰ S.

The absorption rate of alanine and glutamic acid in all 3 species was slightly influenced by salinity

whereas the serine uptake increased when the salinity was changed from 18 to 32 ‰ S. The affinity, however, of both serine, alanine and glutamic acid declined in 32 ‰ S. The *Nereis* spp. apparently compensate for this reduced affinity by increasing the absorption potential (V_{max}). A similar mechanism has been reported to occur in *Enchytraeus albidus* (Siebers and Bulnheim, 1977). As the amino acid uptake probably is interlinked with Na^+ transport across the epidermis (Anderson, 1975), the observed changes in affinity may not exclusively be found in amino acid transport mechanisms, but also in transport characteristics of Na^+.

Dynamics of the present amino acid uptake are difficult to relate to natural concentrations of amino acids or to intracellular pools of free amino acids. Thus, the high affinity to glutamic acid appears to be superfluous as natural glutamic acid concentrations often are relatively high (Jørgensen et al., 1980a, b); furthermore, glutamic acid only accounts for a few percent of the internal pool of free amino acids in the worms. In contrast, both affinity and natural concentration of alanine were low, while alanine was an important free amino acid in the *Nereis* spp. In addition, the increased uptake of serine in 32 ‰ S was not caused by an increased requirement for this amino acid as a free internal amino acid.

According to Gilles (1975), the composition of free amino acids in tissues of marine invertebrates demonstrates considerable changes in different salinities. In the *Nereis* spp., alanine, glycine and taurine were the most abundant free amino acids in both salinities. Lange (1963) found a linearity in salinity and in total content of free amino acids and taurine in the mussel *Mytilus edulis*. Contrary to this, our observations indicate an increasing, nonlinear significance of free amino acids in increasing salinities. This discrepancy may be due to changes in the concentration of proline which, according to Jeuniaux et al. (1961), is abundant in *N. diversicolor*. Proline was not determined in the present study. The importance of taurine in the *Nereis* spp. was less in 32 ‰ S than in 18 ‰ S; correspondingly, the proportion of alanine was greater. We are uncertain if this observation is an experimental artifact. Some amino acids are essential to marine invertebrates, e. g. lysine (Gilles, 1975); if taurine is also essential to *Nereis* spp., its reduced percentage in 32‰ S may be due to an insufficient supply of taurine in the worms diet, as they were adapted to 32 ‰ S (from 18 ‰ S) during 3 weeks in sediment aquaria. We have not been able to find evidence for this in the literature.

The higher uptake rate of serine and partly of alanine in *Nereis succinea*, compared with *N. virens* and *N. diversicolor*, must probably be sought in the generally higher pools of free amino acids in this species. The present investigation does not demonstrate a correlation between natural concentrations of amino acids and uptake capacity in the *Nereis* spp. The observed variations in the free pools may rather be explained on the basis of different osmoregulatory strategies of the worms (Jeuniaux et al., 1961).

The extracted, free interstitial amino acids from Kysing Fjord sediment were sufficient to provide the *Nereis* spp. with a significant net influx of amino acids. As expected, the neutral amino acids glycine, alanine, and serine were predominantly reduced, but an unusually large uptake of glutamic acid also occurred. Uptake of glutamate and arginine was negligible in all species within the first hours, but after 4–6 h both were reduced, indicating that new amino acid-absorbing organisms (e.g. bacteria) may have appeared in the medium. These two amino acids may therefore be used as indicators of bacterial activity.

Simultaneously with amino acid absorption, a release took place. Experiments by Ahearn and Gomme (1975) showed that previously absorbed dissolved organics (D-glucose) are partly released in *Nereis diversicolor*. Concerning amino acids, these two processes probably occur independently, since released amino acids seem to originate in the excretion of nitrogeneous waste from the animals (Pandian, 1975). The present release experiments demonstrated that the concentration of some amino acids remained unchanged in the medium, while others accumulated. The ^{14}C-experiments demonstrated that alanine, serine, and glutamic acid were simultaneously absorbed and released. The rather constant concentrations of some amino acids shown in Figure 9, e.g. alanine, threonine and glutamic acid, indicate equal release and uptake rates, whereas the increasing concentrations, e.g. of ornithine and lysine, might be caused by low uptake or fast release. If high bacteria concentrations were present at 21 h, they were unable to reduce the ornithine. Environmental conditions, e.g. long periods of oxygen depletion, influence the release of amino acids in *Nereis* spp. (Jørgensen and Kristensen, 1980).

The flow-cell experiment suggests that ventilation in *Nereis* burrows also may have a nutritional aspect. If the interstitial water surrounding the worms is frequently replaced, these are continuously exposed to high interstitial amino acid concentrations. However, if the renewed water is transported from above the sediment, lower concentrations of amino acids are available to the worms. Actual concentrations of amino acids in worm burrows are presently being studied.

In the natural environment, *Nereis* spp. influence the prevalent concentrations of amino acids by (1) reducing ambient amino acids; (2) adding amino acids to the

surrounding sea water. Flow experiments suggest that both processes probably occur continuously, at least for 48 h (Jørgensen, 1980). Most released amino acids are presumably immediately absorbed by organisms in the sediment. Others, like ornithine, may be of lower assimilative value to both bacteria and invertebrates, since this amino acid frequently occurs in high concentrations, compared to other amino acids, in marine sediments (Jørgensen et al., 1980a, b).

The extracted interstitial amino acids were of considerable quantitative importance to the *Nereis* spp. as their total metabolic energy expenditure was sustained from the uptake. Actively ventilating worms also obtained a significant benefit from absorption. Since *Nereis* spp. also obtain food by other modes of feeding, amino acids may be considered a valuable nutritive supplement. Similar observations of sediment-inhabiting animals were reported by Stephens (1975) and Stephens et al. (1978) in *N. diversicolor* and in the sand dollar *Dendraster excentricus*; both absorbed naturally occurring primary amines (mainly amino acids).

The concentrations of individual amino acids administered to the interstitial water in this study may not be identical to *in-situ* concentrations. During extraction, absorption of amino acids to sediment particles increases with increasing content of organic matter (Jørgensen et al., 1980b). The actual concentrations of free amino acids in the *Nereis succinea* sediment may, therefore, have been underestimated. Furthermore, our sediment cores were collected in winter, and need not represent concentrations of amino acids in periods with higher biological activities. Since a gentle extraction procedure was applied, the observed amino acids undoubtly represent the most easily available amino acids, i.e. amino acids being accessible to the *Nereis* species.

The dynamics of dissolved organic matter in marine environments are generally considered to be determined by autotrophic and heterotrophic microorganisms (Fenchel and Blackburn, 1979). The present observations emphasize, however, that invertebrates should be included when assimilation and turnover of dissolved organic matter are calculated.

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