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# Salinity dependence, uptake kinetics, and specificity of amino-acid absorption across the body surface of the oligochaete annelid *Enchytraeus albidus*

D. Siebers & H.-P. Bulnheim

*Biologische Anstalt Helgoland (Zentrale); Hamburg 50, Federal Republic of Germany*

ABSTRACT: *Enchytraeus albidus* is able to absorb dissolved $^{14}$C-labeled neutral amino acids (glycine, L-alanine, L-valine, α-aminoisobutyric acid) and an amino-acid mixture from ambient water across the body surface against considerable concentration gradients. Saturation kinetics and susceptibility of glycine uptake to competitive inhibition by alanine suggest mediated transport. Absorption of neutral amino acids is an active process. Exchange diffusion of preloaded α-aminoisobutyric acid against external glycine or α-aminoisobutyric acid could not be detected. Results on inhibition of glycine uptake by a variety of low-molecular-weight substances indicate that glycine absorption is highly specific for neutral amino acids and somewhat less for basic amino acids; it is unspecific for non-α-amino acids, acidic amino acids, carbohydrates, and organic acids. Rates of transintegumentary net influx of glycine are nearly identical to $^{14}$C-glycine influx, suggesting that only small amounts of amino acids are released, as compared with the capacity for uptake. Thus, $^{14}$C-amino-acid influx data are used for characterization of the uptake system. Glycine uptake is positively correlated to external salinity. In fresh water, absorption is nearly zero; between 10 and 20‰ S, uptake increases markedly reaching maximum values at 30‰ S; these remain almost constant at 40‰ S. Transport constants and maximum uptake rates increase with rising salinities. Since absorption of glycine and L-valine is susceptible to sodium depletion, similar mechanisms presumably underly salinity-dependent uptake of amino acids and sodium-dependent solute transport. Oxygen consumption is not significantly modified by different external salinities. Estimates of nutritional profit gained from absorption of amino acids vary between 4 and 15% of metabolic rate for glycine absorption and between 10 and 39% for uptake of an amino-acid mixture, according to external concentrations (10 and 50 µM) and salinities (20 and 30‰ S).

## INTRODUCTION

To different extents, various marine invertebrates exhibit the ability of absorption, accumulation, and metabolic utilization of organic matter which is dissolved in very low amounts in the ambient water. This capacity seems to be reduced in animals provided with chitinous exoskeletons such as crustaceans. Several authors suggest that these absorption processes, which take place in addition to feeding on particulate food via the digestive system, might be of considerable nutritional significance (for references see Stephens, 1972, 1975; Jørgensen, 1976).

In fresh-water invertebrates, such uptake processes through the body surface have been demonstrated as not occurring or proceeding at much slower rates than those documented for marine animals. By comparing rates of absorption of dissolved organic substances in brackish water of different salinity levels, a decrease in uptake was recorded with declining salinities in *Nereis* species (Stephens, 1964), *Ophiactis arenosa* (Stephens & Virkar, 1966), *Aurelia aurita* polyps (Shick, 1973) and *Mytilus edulis* gills (Bamford & Campbell, 1976). In intact specimens of the bivalve *Rangia cuneata* glycine uptake was suppressed by low salinities, but not in a preparation of gill tissues (Anderson, 1975).

Owing to its broad ecological range, comprising marine, brackish, limnic, and terrestric environments, the oligochaete annelid *Enchytraeus albidus* was investigated as to its ability to absorb several exogenous neutral amino acids and an amino-acid mixture. Special attention was devoted to the dependence of amino-acid uptake on various salinity levels and to some aspects of sodium-dependent amino-acid transport. The latter plays an extraordinary role in the absorption of nutrients across single cells and tissues including intestinal epithelia (Schulz & Curran, 1970; Wiseman, 1974) and the body surfaces of intestinal parasites (Pappas & Read, 1975); on the contrary, little appropriate information is available in regard to the uptake mechanisms present in the integument of free-living invertebrates. Further experiments were directed to studies on the specificity of mediated amino-acid uptake and the significance of this mode of nutrition in relation to metabolic rates.

## MATERIALS AND METHODS

*Enchytraeus albidus* Henle was cultivated in moistened earth and fed with oat flakes. For experimental purposes the worms were transferred to the bottom filter of aquaria (0–40 ‰ S, in steps of 10 ‰) and fed with fish food (TetraMin). Under these conditions we have maintained the oligochaetes for over a year. After acclimation to submersal life for at least two weeks at 15° C they were used for experiments.

Experimental assays (15° C) were performed in scintillation vials, containing 0.1 μCi of uniform $^{14}$C-label (glycine, L-valine, amino-acid mixture) and α-amino [1-$^{14}$C] isobutyric acid (AIB), 0.05–2.4 μmoles of unlabeled respective amino acids, and about 50 mg of worms in a final water volume of 10 ml. Since the worms did not consume more than 10 % of the initially dissolved oxygen, the vials were not aerated. During inhibition experiments, glycine concentrations were kept constant at 50 μM, while the substances to be tested were added at final concentrations of 50, 250, or 500 μM, respectively.

For experiments concerning effects of Na-depletion on amino-acid absorption from 10 μM solutions the incubation media were 20 ‰ sea water, 348 mM NaCl (with a salinity equal to 20 ‰ sea water as measured by aerometry), 87 mM NaCl + 261 mM KCl (LiCl, choline-Cl), equimolar to 348 mM NaCl. These media also served for 4 h preincubations, necessary to observe significant effects.

All uptake experiments lasted 0.5 h (uptake rates as a function of time are linear for at least 2 h) and were terminated by rinsing the worms twice with inactive water

of appropriate salinity and dissolution in 0.5 ml soluene-350 (Packard) for 1 h at 60° C. After addition of 10 ml of counting solution (5 g PPO + 0.3 g POPOP $l^{-1}$ toluol) samples were counted in a Tracerlab liquid scintillation counter with quench corrections by internal standard.

Experimental sea water was ultrafiltered natural North-Sea water, diluted with distilled water or concentrated by addition of a commercial natural sea salt mixture and filtered in a circuit in the dark for several weeks. Obviously due to the activity of filter-inhabiting bacteria, amino-acid concentrations measured with the fluorescamine (Serva) reagent according to North (1975) and Stephens (1975) ranged between undetectable amounts – this water served for experimental use – and about 50 nM.

Analysis of preloaded AIB-washout was performed according to methods described by Ahearn & Townsley (1975) for the apodous sea cucumber *Chiridota rigida*. 100 mg of oligochaetes were preloaded with 10 $\mu$M $^{14}$C-AIB for 1 h (10 ml 20 ‰ sea water contained 100 nmoles of AIB, including 0.5 $\mu$Ci of $^{14}$C-label). Thereafter, the worms were thoroughly rinsed and transferred to 10 ml of washout media, containing 20 ‰ sea water or 50 $\mu$M AIB or glycine, dissolved in 20 ‰ sea water. At appropriate time intervals (0.5, 1–4 h) loss of $^{14}$C-activity in 5 ml of washout medium was counted after addition of 5 ml of Instagel (Packard). The oligochaetes were transferred each time to a new set of washout media. Finally, the activity remaining in the worms was counted.

Net uptake from 50 µM glycine solution was measured by immersion of 100 mg of oligochaetes in a final water volume of 10 ml. After appropriate time intervals 0.5 ml served for fluorometric determination of primary amines with fluorescamine.

Free amino acids in *E. albidus* were determined with ninhydrin (Troll & Cannon, 1953) and free glycine by automated amino-acid analysis (Unichrom, Beckman) after extraction of low molecular weight organic material as described by Siebers et al. (1972) for decapod hemolymph samples.

$O_2$ uptake at 20° C (0, 20, 40 ‰ S) and 16° C (20 ‰) was determined in a flow-through system by use of a polarographic oxygen analyzer (Eschweiler & Co, Kiel, FRG). In conjunction with a recorder $pO_2$ was continuously measured by a Clark-type electrode operating in combination with a magnetic stirrer (cf. Bulnheim, 1974). Measurements were made in air-saturated aqueous solutions at constant flow rates (9 or 12 ml $h^{-1}$, respectively). The worms were inserted in a small respiration chamber where they could wriggle into some filtering wadding in order to avoid undulations of their extruded bodies. Individual $pO_2$ determinations were performed for minimal periods of 3 h.

Uptake rates in different incubation media were compared by means of the t-test. The numbers of replicates varied between 4 and 12 and are given in the text.

## RESULTS

### Absorption of $^{14}C$-labeled amino acids

Integumentary absorption of glycine, L-alanine, L-valine, and AIB as a function of various ambient concentrations is shown in Figure 1. Uptake rates reach saturation levels at higher concentrations, suggesting mediated influx due to a definite number of transport sites. Maximum absorption rates ($v_{max}$) and transport constants ($K_t$) obtained from Lineweaver-Burk plots are presented in Table 1. Within the employed concentration range apparent diffusional entry of neutral amino acids was not detectable. In glycine-uptake experiments the radioactivity of alcoholic extracts of *E. albidus* co-chromatographs with authentic glycine on cellulose thin layer plates developed with phenol : water (3:1), suggesting that unchanged glycine was transported. Absorption of naturally occurring amino acids (glycine, L-alanine, and L-valine) did not

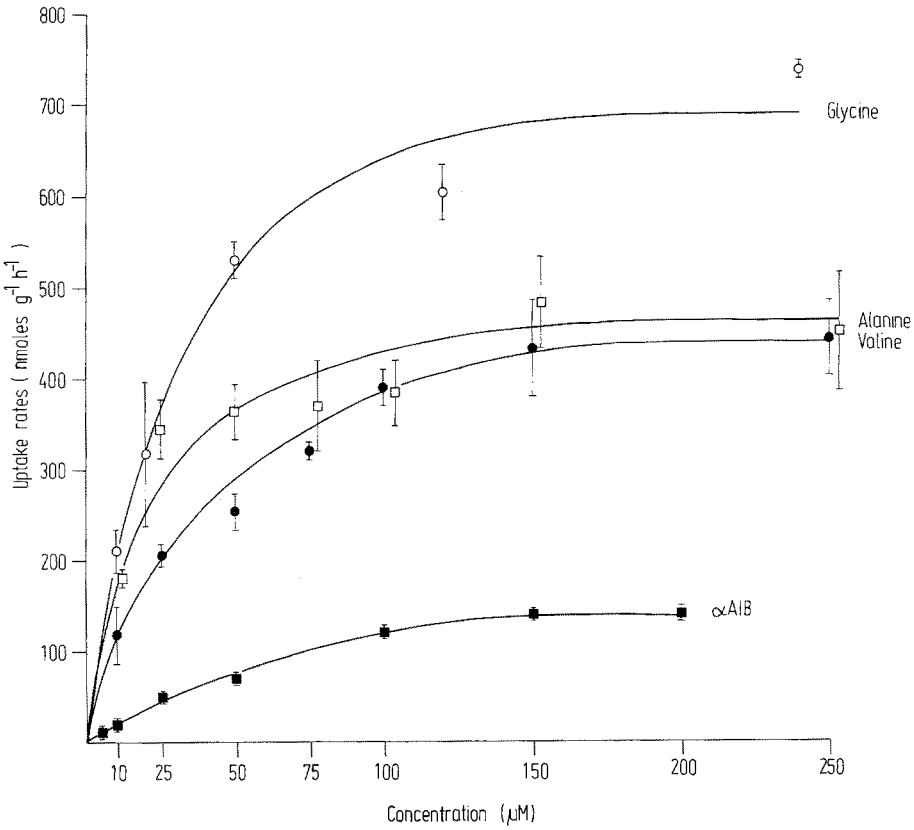


Fig. 1: Uptake rates of dissolved glycine (○), L-alanine (□), L-valine (●), and α-amino-isobutyric acid (■) across the body surface of *Enchytraeus albidus* in relation to external concentrations. Data were obtained from influx of $^{14}C$-labeled amino acids at 20 ‰ S. Vertical bars represent standard deviations from a sample number of n = 5

differ greatly. In contrast, influx of the non-metabolizable analog AIB is reduced considerably. For the sea cucumber, *Chiridota rigida*, Ahearn & Townsley (1975) reported a transport for glycine which proceeds four times faster than that for AIB under similar conditions.

Influx of a $^{14}C$-labeled amino-acid mixture (440 ± 70 nmoles $g^{-1} h^{-1}$) was reduced by 17 % in comparison to glycine uptake (530 ± 20 nmoles $g^{-1} h^{-1}$) at equal external concentrations and salinities (50 µM, 20 ‰ S). Comparable reductions are found with other concentrations and salinity conditions (Table 5). An explanation for lower uptake rates of the amino-acid mixture as compared to glycine absorption may be provided by the finding that basic amino acids (Siebers, 1976) and acidic amino acids (unpublished results) are absorbed by rates amounting to only few percentages of glycine uptake, while neutral amino acids (Fig. 1) do not differ greatly in uptake rates. Charged amino acids amount to 33 % in the mixture applied, and the bulk is represented by neutral amino acids.

Table 1

*Enchytraeus albidus*. Kinetic data of amino-acid uptake, obtained from influx of $^{14}C$-labeled amino acids. $v_{max}$ = maximum uptake rate, $K_t$ = transport constant

| Amino acids | Salinity (‰) | $v_{max}$ (µmoles $g^{-1} h^{-1}$) | $K_t$ (µM) |
|---|---|---|---|
| glycine | 10 | 0.18 | 18 |
| glycine | 20 | 0.69 | 20 |
| glycine | 30 | 2.0 | 37 |
| glycine | 40 | 2.0 | 39 |
| L-alanine | 20 | 0.47 | 17 |
| L-valine | 20 | 0.45 | 30 |
| α-aminoisobutyric acid | 20 | 0.15 | 50 |

Uptake of amino acids occurs against considerable concentration gradients. Free amino acids in whole worms (n = 5; 30 ‰ S) amount to 82 ± 14 µmoles per g fresh weight (13 ± 3 µmoles of glycine $g^{-1}$). Since absorption proceeds from the medium into a membrane cell, calculation of concentration gradients requires intracellular amino-acid concentrations. In marine invertebrates free amino-acid concentrations of body fluids are generally very low in comparison to intracellular concentrations (Florkin, 1969); in most species only 1–3 % of total free amino acids are dissolved in extracellular fluids. With reference to this mode of amino-acid distribution, the amounts of free amino acids measured in whole worms could be attributed to intracellular fluid volume. Estimations of intracellular amino-acid concentrations were derived from a reliable estimation of cell water in the annelid *Glossoscolex giganteus*, which amounted to 44 % of wet weight (cf. Florey, 1970, p. 24). At 30 ‰ S concentrations calculated for intracellular fluid volume amount to 186 mM (free amino acids) and 30 mM (glycine). Concentration gradients at an external concentration of 10 µM are $1 : 2 \cdot 10^4$ (free amino acids) and $1 : 3 \cdot 10^3$ (glycine).

## Measurement of net influx

Results on influx of labeled substrates do not necessarily represent net fluxes, which are composed of influx and efflux. In order to obtain information on net fluxes of glycine in comparison to influx of $^{14}$C-glycine we used the fluorescamine reagent which forms highly fluorescent and stabile derivatives with primary amines including amino acids. It allows rapid and sensitive determinations. From Figure 2 it becomes obvious that *E. albidus* rapidly reduces the glycine concentration of the incubation medium. This finding demonstrates positive net influx. The ambient concentration of 50 µM glycine was reduced by 11.4 µM (100 mg of worms, final volume 10 ml, S = 30 ‰) within the first incubation hour, resulting in a net influx rate of 1140 ± 210 nmoles $g^{-1}$ $h^{-1}$. This rate differs only insignificantly from $^{14}$C-glycine influx at 30 ‰ S (Fig. 3) which amounts to 1220 ± 190 nmoles $g^{-1}$ $h^{-1}$.

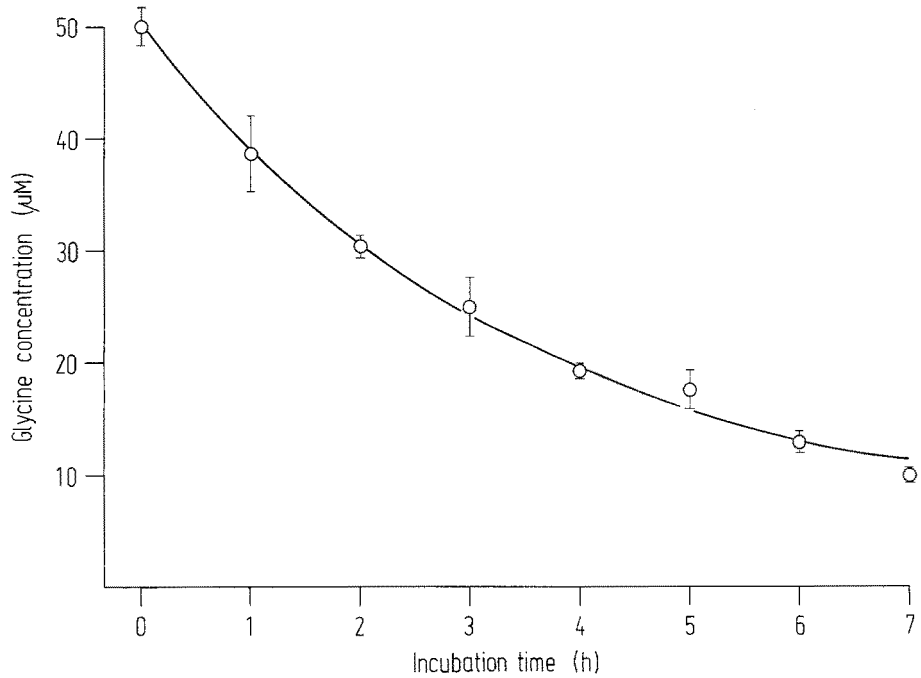


Fig. 2: *Enchytraeus albidus*. Integumentary net influx of glycine in relation to incubation time. (For experimental details see text)

## Salinity dependence of $^{14}$C-glycine absorption

The data measured on absorption of dissolved $^{14}$C-glycine in relation to different external salinity levels and glycine concentrations are presented in Figure 3. Glycine absorption across the body surface of *E. albidus* is positively correlated to increasing

ambient salinities. This is valid for each of the five concentrations tested. Glycine uptake is virtually zero in fresh water, whereas low values were measured at 10 ‰ S. Between 10 and 20 ‰ S a considerable increase occurred; at 30 ‰ S maximum values are attained which remain on almost the same levels at 40 ‰ S. Transport constants ($K_t$) for half maximum uptake rates, as derived from Lineweaver-Burk plots, and maximum rates ($v_{max}$) were found to increase with rising salinities (Table 1).

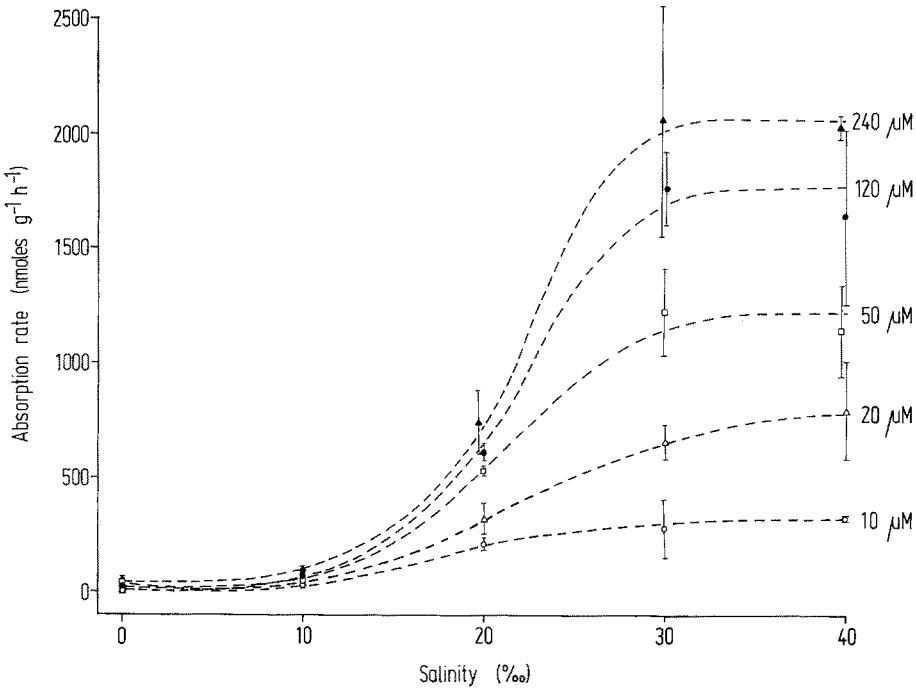


Fig. 3: Absorption rates of dissolved $^{14}$C-glycine across the body surface of *Enchytraeus albidus* in relation to salinity and external glycine concentration. Vertical bars represent standard deviations, obtained from a sample number of n = 4

The dependence of absorption on ambient salinity could also be established, when a complex amino-acid mixture was tested (see Table 5), suggesting that the relationship found between velocity of uptake and salinity is valid for the majority of amino acids contained in the mixture.

## Sodium dependence of amino-acid uptake

When glycine and L-valine are absorbed from 10 µM solution in which brackish water of 20 ‰ S is exchanged against 348 mM NaCl (its salinity level corresponds to 20 ‰) uptake is reduced to 75 % (glycine) and 65 % (L-valine) in comparison to 20 ‰ S controls (Table 2). For these experiments preincubation periods of 4 h were necessary to realize significant effects. The reductions in uptake suggest that absorption

of glycine and L-valine partly depends on one or several still unknown sea-water components. When, however, 75 mole-% of Na are exchanged against K, uptake is reduced to values amounting to 15 % (glycine) and 12 % (L-valine) of controls. Furthermore, considerable reductions are observed, when K is exchanged with Li or choline. These results suggest that a large proportion of the absorption of glycine and L-valine depends on the presence of external Na which cannot be replaced by K, Li, or choline.

## Washout of preloaded α-aminoisobutyric acid

After preloading oligochaetes by incubation in 10 µM $^{14}$C-AIB for 1 h, we tested whether or not addition of 50 µM AIB or glycine to the washout media could have some influence on $^{14}$C-AIB loss. Independent from the composition of the washout media, release of $^{14}$C-AIB is observed (Fig. 4), which does not differ significantly

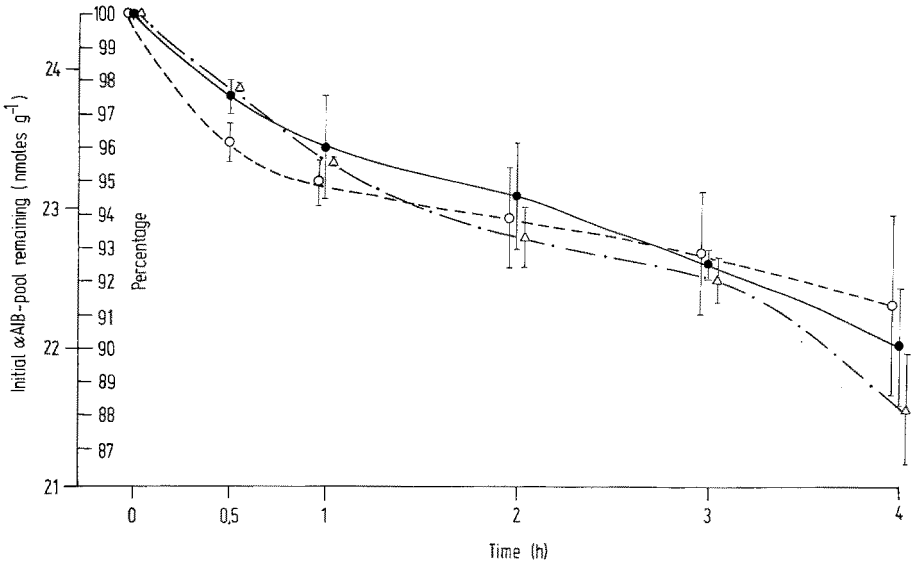


Fig. 4: *Enchytraeus albidus*. Washout of α-amino [1–$^{14}$C] isobutyric acid after preloading with 10 µM AIB for 1 h at a salinity of 20 ‰ in relation to time and composition of washout medium. Washout media consisted of 20 ‰ brackish water in absence (●) and in the presence of 50 µM glycine (△) or 50 µM AIB (○). Vertical bars represent standard deviations, referring to a sample number of n = 5

between the three media employed. This loss amounts to approximately 3 % of the internal AIB-pool established within the first hour of washout analysis. The sea cucumber, *Chiridota rigida*, was shown to exhibit both homo- and heteroexchange diffusion of AIB with exogenous AIB and glycine from a relatively high internal AIB-pool (Ahearn & Townsley, 1975). In *E. albidus* washout characteristics in the

Table 2

*Enchytraeus albidus*. Effect of Na-depletion on uptake rates of glycine and L-valine from 10 µM solutions after preincubation in appropriate incubation media for 4 h (15 °C, 20 ‰ S, n = 5). As compared to controls (20 ‰ S), * indicates statistical significance of differences in uptake rates ($0.05 > p > 0.01$), ** indicates high significance ($p < 0.01$)

| Composition of incubation media | Glycine | | L-Valine | |
|---|---|---|---|---|
| | Uptake rate ± s. d. ($\mu$moles $g^{-1}$ $h^{-1}$) | Percentage | Uptake rate ± s. d. ($\mu$moles $g^{-1}$ $h^{-1}$) | Percentage |
| 20 ‰ S | 0.20 ± 0.03 | 100 | 0.17 ± 0.01 | 100 |
| 348 mM NaCl | 0.15 ± 0.02 * | 75 | 0.11 ± 0.02 ** | 65 |
| 87 mM NaCl + 261 mM KCl | 0.03 ± 0.01 ** | 15 | 0.02 ± 0.004 ** | 12 |
| 87 mM NaCl + 261 mM LiCl | 0.01 ± 0.002 ** | 5 | 0.01 ± 0.001 ** | 6 |
| 87 mM NaCl + 261 mM choline-Cl | 0.01 ± 0.001 ** | 5 | 0.01 ± 0.001 ** | 6 |

absence and in the presence of different external amino acids do not reveal the existence of such exchange diffusion processes.

## Specificity of glycine influx

Specificities of amino-acid transport mechanisms in relation to the charge of the molecule have been known for about three decades (Christensen, 1975). In order to obtain some information on the specificity of glycine transport we tested the influence of different concentrations of possible inhibitory substrates on influx of $^{14}$C-glycine from 50 $\mu$M solution (Tables 3, 4). All of the 18 neutral amino acids tested inhibited glycine absorption (Table 3). With increasing inhibitor concentrations the percentage of inhibition rose from about 30 to about 80 %. The inhibition

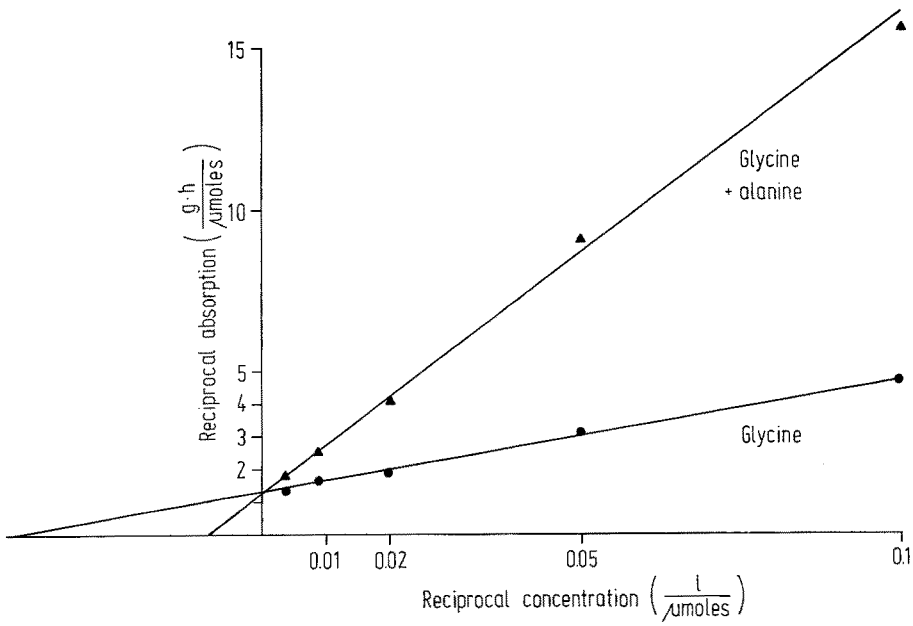


Fig. 5: Concentration dependent uptake of glycine across the body wall of *Enchytraeus albidus* in the presence (▲) and absence (●) of 50 $\mu$M alanine. Reciprocal absorption is plotted against reciprocal concentration (20 ‰ S, 15 °C, n = 4)

of glycine uptake by other neutral amino acids is regarded as the result of competition, as demonstrated for inhibition of glycine uptake from increasing ambient concentrations by a fixed concentration of alanine (50 µM) and by comparison to uptake rates in the absence of alanine. The reciprocal values of uptake rates were plotted against the reciprocal values of glycine concentration (Lineweaver-Burk plot) (Fig. 5). The common intercept of the slopes of the inhibited and non-inhibited plot indicates competitive inhibition.

Table 3

*Enchytraeus albidus*. Effect of various concentrations of neutral amino acids on uptake of $^{14}$C-glycine from 50 μM solution (n = 4). * indicates statistical significance of glycine uptake inhibition according to t-test ($0.05 > p > 0.01$), ** indicates high significance ($p < 0.01$). "+" before the figure of percentage uptake inhibition indicates an increase in uptake

| Neutral amino acids tested for glycine uptake inhibition | Percentage of $^{14}$C-glycine uptake inhibition in the presence of different concentrations of neutral amino acids | | |
|---|---|---|---|
| | 50 μM | 250 μM | 500 μM |
| L-alanine | 45 * | 84 ** | 90 ** |
| L-α-amino butyric acid | 65 ** | 88 ** | 90 ** |
| α-aminoisobutyric acid | 7 | 65 ** | 67 ** |
| L-citrulline | 26 * | 58 ** | 65 ** |
| L-cysteine | + 4 | 78 ** | 87 ** |
| L-hydroxyproline | 71 ** | 91 ** | 94 ** |
| L-iso-leucine | 53 ** | 79 ** | 89 ** |
| L-leucine | 44 * | 74 ** | 87 ** |
| L-methionine | 18 ** | 69 ** | 84 ** |
| L-norleucine | 27 ** | 71 ** | 83 ** |
| L-phenylalanine | 23 ** | 52 ** | 68 ** |
| L-proline | 47 * | 84 ** | 90 ** |
| sarcosine | 54 ** | 81 ** | 84 ** |
| L-serine | 16 | 51 ** | 77 ** |
| L-threonine | + 3 | 26 ** | 52 ** |
| L-tryptophan | 26 * | 68 ** | 77 ** |
| L-tyrosine | 25 ** | 46 ** | 52 ** |
| L-valine | 31 * | 71 ** | 82 ** |

Reduction in glycine uptake rates comparable to those affected by neutral amino acids also occurred in the presence of the amino acid amides asparagine and glutamine (Table 4). This result is interesting in regard to the corresponding acidic amino acids aspartic acid and glutamic acid which did not inhibit glycine uptake (Table 4). Also taurine did not affect integumental translocation of glycine: it carries an $-SO_3^-$-group which is negatively charged. Future experiments shall provide evidence as to whether it shares a common absorption mechanism with acidic amino acids. Inhibition of glycine uptake by other amino acids depends on their charge. The result that inhibitory effects caused by neutral amino acids do not occur in the presence of acidic amino acids is confirmed by the action of serine which strongly inhibits glycine absorption, while phosphoserine with a negative charge from an additional phosphate group does not (Table 4). Non-inhibition of glycine uptake by acidic amino acids suggests that glycine absorption does not interact with acidic amino-acid transport.

Reductions in glycine absorption rates induced by the presence of basic amino acids are generally smaller and much less uniform than those caused by other neutral amino acids. Histidine, which is known to be disregarded as basic by intestinal transport systems (Wiseman, 1974), inhibits glycine absorption by percentages comparable to those of neutral amino acids (Table 4). This study does not deal with DL-specificity; however, the result that L-histidine inhibits glycine uptake considerably more than DL-histidine may be taken as an evidence for the existence of DL-specificity.

Considerable reductions in glycine uptake are observed in the presence of arginine and ornithine; a minor reduction is caused by lysine. The findings mentioned provide some evidence of mutual interactions between transport of neutral and basic amino acids; this evidence is confirmed by results on basic amino-acid transport in *E. albidus* (Siebers, 1976).

β-alanine and γ-amino butyric acid do not reduce glycine absorption rates (Table 4). The prerequisiste for inhibition of glycine transport becomes obvious: a molecule must have the α-amino carbonic acid configuration and a neutral charge. Among all neutral amino acids which fulfilled this prerequisite there was only small selectivity.

The presence of low molecular carbohydrates (glucose, fructose, ribose) and organic acids (lactic acid, malic acid, α-keto glutaric acid, and succinic acid) did not change glycine uptake rates.

Table 4

*Enchytraeus albidus*. Effects of various concentrations of charged amino acids, other related ninhydrin-positive substances, low molecular neutral carbohydrates, and organic acids on uptake of $^{14}$C-glycine (n = 4). * indicates statistical significance of glycine uptake inhibition according to t-test ($0.05 > p > 0.01$), ** indicates high significance ($p < 0.01$). "+" before the figure of percentage uptake inhibition indicates an increase in uptake

| Substances tested for glycine uptake inhibition | Percentage of $^{14}$C-glycine uptake inhibition in the presence of different concentrations of charged amino acids, amino acid-related substances, carbohydrates and organic acids | | |
|---|---|---|---|
| | 50 µM | 250 µM | 500 µM |
| acidic amino acids | | | |
| L-aspartic acid | 11 | 1 | 21 |
| L-glutamic acid | 16 * | 15 * | 12 |
| o-phospho-L-serine | 25 | + 13 | 10 |
| taurine | 3 | + 23 | 4 |
| basic amino acids | | | |
| L-arginine | 19 | 30 * | 62 ** |
| L-histidine | 28 * | 66 ** | 75 ** |
| DL-histidine | + 2 | 31 * | 52 * |
| L-lysine | 13 * | 10 * | 15 ** |
| L-ornithine | 6 | 22 | 38 * |
| amino acid amides | | | |
| L-asparagine | 27 * | 32 * | 45 * |
| L-glutamine | 37 * | 70 ** | 86 ** |
| β- and γ-amino acids | | | |
| β-alanine | + 10 | + 7 | + 8 |
| γ-amino butyric acid | 24 | 9 | 16 |
| carbohydrates | | | |
| D-(+)-glucose | 12 | 18 | + 3 |
| D-(—)-fructose | 6 | + 9 | + 7 |
| D-(—)-ribose | + 13 | 20 | + 5 |
| organic acids | | | |
| L-lactic acid | 9 | 1 | 6 |
| L-malic acid | 9 | 9 | 6 |
| α-keto glutaric acid | + 5 | + 15 | + 13 |
| succinic acid | 2 | 10 | 14 |

## Contribution of accumulated nutrients to metabolic requirements

In the present context it was of interest to obtain some information on the oxygen demand of *Enchytraeus albidus* in order to assess the contribution of accumulated amino acids to the metabolic requirements of this oligochaete. Since the experiments on uptake of glycine were conducted with specimens ranging from 2 to 3 mg wet weight, respiratory rates of about 3 mg individuals were measured and taken as basis for further considerations. These investigations, which initially were performed at 20° C, revealed only slight differences in $O_2$-uptake rates between the various salinities tested. They yielded 1.2 $\mu$l $O_2\,h^{-1}$ (fresh water), 1.1 $\mu$l $O_2\,h^{-1}$ (20 ‰ S), and 1.1 $\mu$l $O_2\,h^{-1}$ (40 ‰ S) (with reference to worms of 3 mg fresh weight). Complementary $O_2$ determinations made at the experimental temperature (16° C) and salinity (20 ‰ S) provided the following data: 0.82 ± 0.15 $\mu$l $O_2\,h^{-1}$ as related to specimens of 3.2 ± 0.2 mg weight (n = 14).

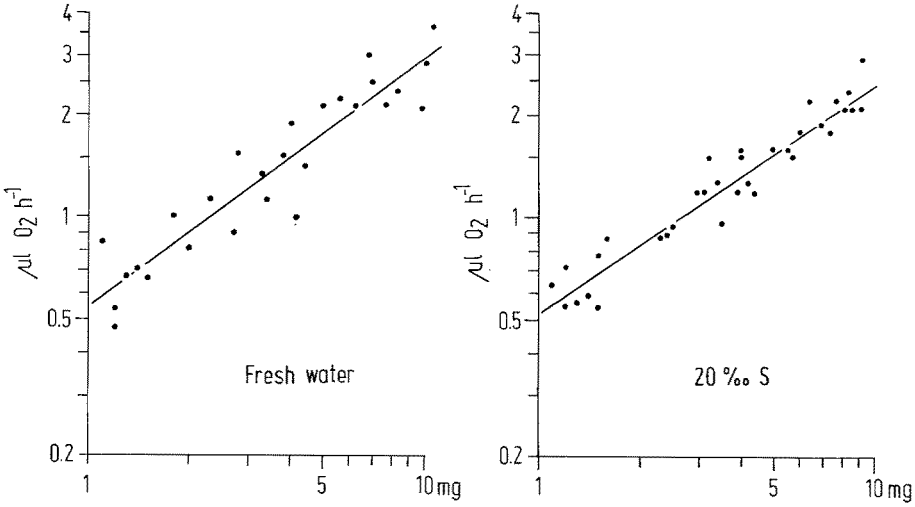


Fig. 6: Relationship between oxygen uptake (standard metabolic rate) and body size (fresh weight) of *Enchytraeus albidus* (20 °C, fresh water and 20 ‰ S)

The data reported refer to standard metabolic rates. $O_2$ consumption of enchytraeids, which displayed continuous undulating movements, could be also measured. Such active rates exceeded standard rates by a factor of about two. Comparable high $O_2$-uptake rates have also been measured in other oligochaetes which lead an interstitial mode of life (Nielsen, 1961).

In addition, the question whether or not respiratory metabolism is weight- or surface-related in *E. albidus* was tested by $O_2$ determinations comprising a weight range between 1 and 10 mg. Oxygen consumption can be expressed as a power function of body size: $\log y = \log a + b \cdot \log x$, where y = $O_2$ uptake, x = weight, b =

regression coefficient reflecting the slope of the logarithmic plot of $O_2$ uptake against body weight, and a = coefficient indicating the intercept on the y-axis ($O_2$ uptake per unit weight).

The results of the measurements made at 20° C (Fig. 6) can be expressed by the following regression equations: $\log y = \log 85.15 + 0.73 \log x$ (fresh water, $n = 27$) and $\log y = \log 55.82 + 0.68 \log x$ (20 ‰ S, $n = 35$). The b values obtained ($0.73 \pm 0.06$ and $0.68 \pm 0.04$) demonstrate that metabolism is not proportional to weight ($b = 1$); it rather appears to be a function of body-surface area ($b = 0.67$). As expressed by the calculated b values there is no significant difference between the slopes of the two regression lines ($p > 0.05$).

With reference to amino-acid concentrations in sea water, oxygen consumption, and absorption rates of glycine and an amino-acid mixture, an attempt was made to estimate the magnitude of the contribution of absorbed dissolved organic substances to the energy requirements of *E. albidus* in two selected external concentrations and salinity regimes (Table 5).

For the following considerations the energetic correspondence between 1.05 mg of protein (roughly 1 mg of amino acids) and 1.0 ml of oxygen necessary for total mineralization was taken as basis for the amino-acid mixture (e.g. Hoar & Randall, 1969, p. 208). Calculations of the energy profit gained from absorbed glycine were derived from the fact that the oxidation of 1 mole of glycine to 2 $CO_2$, 1 $H_2O$, and 1 $NH_3$ requires 1.5 moles of $O_2$.

The nutritional profit from glycine absorption varies between 4 and 16 % of standard metabolic rate and ranges between 10 and 39 % from uptake of an amino-acid mixture according to external concentrations (Table 5). Therefore, the metabolic profit gained from this additional food source in *E. albidus* must be regarded as considerable.

## DISCUSSION

### Absorption of amino acids

Integumentary absorption of neutral amino acids by *Enchytraeus albidus* is considered an active process, which is characterized by saturation kinetics and susceptibility to competitive inhibition. It occurs against a concentration gradient of several orders of magnitude. As reported by Siebers (1976), distribution ratios (radioactivity per 50 mg of worms : radioactivity per 50 µl of ambient water) following a period of 0.5 h of uptake from 10 µM glycine (and L-valine) at 20 ‰ S amount to 10.6 (and 8.9 for L-valine) in *E. albidus*. They exceed the equilibrium distribution ratio of 1 by a factor of approximately 10, suggesting a transport mechanism capable of transferring the substances against the concentration gradient. Absorption of glycine and L-valine from 10 µM solutions at 20 ‰ S was shown to depend on metabolic energy as indicated by uptake inhibition in the presence of 50–250 µM NaCN. Inhibition of glycine and L-valine uptake from 10 µM solutions at 20 ‰ S by 0.1, 0.5, and 1 mM ouabain suggests secondary active transport (Siebers, 1976). Furthermore, glycine and

Table 5

*Enchytraeus albidus.* Nutritional significance of glycine and an amino-acid mixture absorbed from ambient water in relation to external concentration and salinity. Data are calculated for individuals of 3 mg fresh weight and 16 °C

| Components of calculation | Glycine | | | Amino-acid mixture | | |
|---|---|---|---|---|---|---|
| salinity (‰) | 30 | 20 | 30 | 30 | 20 | 30 |
| external concentration (μM) | 10 | 50 | 50 | 10 | 50 | 50 |
| uptake rate (nmoles $h^{-1}$) | 0.9 | 1.6 | 3.7 | 0.7 | 1.3 | 3.1 |
| oxygen consumption (μl $O_2$ $h^{-1}$) | | 0.8 | | | 0.8 | |
| % of metabolic rate equivalent to absorption | 4 | 7 | 16 | 10 | 20 | 39 |

L-valine transport is susceptible to depletion of external $Na^+$ and to reduction in ambient salinity levels as demonstrated above.

Transport constants between 17 and 50 µM (Table 1) correspond to values recorded for other marine invertebrates (Jørgensen, 1976; Sepers, 1977). $K_t$-values of about 30 µM have been reported for uptake of dissolved organic substances in pogonophores (Southward & Southward, 1970), oyster gills (Bamford & Gingles, 1974), and coelenterates (Shick, 1975). They are between 1 and 2 orders of magnitude lower than $K_t$-values calculated for nutrient absorption by intestinal epithelia (Wiseman, 1974) and by the body surface of intestinal parasites (Pappas & Read, 1975). These values demonstrate the adaptation of absorptive processes to the low concentrations of dissolved organic substances in marine environments. $K_t$-values of pelagic heterotrophic bacteria are, however, even much smaller than those of marine invertebrates (Sepers, 1977).

Effects of salinity on kinetic data (Table 1) correspond to temperature effects reported by Shick (1975) for *Aurelia aurita* polyps. When the temperature was raised from 17 to 32° C, $K_t$-values for glycine uptake rose from about 10 to about 40 µM and maximum uptake velocities increased.

## Net influx of glycine

Transintegumentary net influx of glycine (1140 nmoles $g^{-1}\,h^{-1}$) in *E. albidus* is nearly identical to $^{14}$C-glycine influx (1220 nmoles $g^{-1}\,h^{-1}$). This data suggests that only small amounts of amino acids are released, as compared to the capacity for uptake. Jørgensen (1976) has also drawn this conclusion for "undisturbed invertebrates" on the basis of his results obtained from mussels and from findings on polychaetes published by Ahearn & Gomme (1975). Amino acid net losses in *E. albidus* must be small in comparison to the net influx of glycine. This permits to use $^{14}$C-amino-acid influx data for characterization of the uptake system in terms of Michaelis-Menten kinetics and for drawing further conclusions in regard to salinity and sodium dependence.

As reported by Stephens (1975), net influx of glycine from 50 µM solution amounts to 750 nmoles $g^{-1}\,h^{-1}$ in *Capitella capitata* and 840 nmoles $g^{-1}\,h^{-1}$ in *Nereis diversicolor*. These values agree with those found in *E. albidus*.

Investigations concerning amino-acid uptake by drinking or by bacteria associated with the body surface have not been undertaken in *E. albidus*. From unchanged uptake rates of D-glucose from 5 µM solution by *Nereis diversicolor* furnished with thin cotton threads around head and tail ends for preventing connexion between the incubation medium and the intestinal tract, Ahearn & Gomme (1975) concluded that drinking plays a negligible role in uptake of radioactive D-glucose. Taylor (1969) measured L-glutamic-acid absorption by *Nereis virens* in incubation media, containing 50 $\mu g\,ml^{-1}$ of chloromycetin or 50 $\mu g\,ml^{-1}$ of streptomycin. He found no significant differences in uptake rates compared to controls which had been incubated without antibiotics and concluded that bacteria associated with the mucus covering the polychaete did not significantly contribute to the uptake of L-glutamic acid. To the authors'

knowledge results concerning contributions of bacteria or drinking to uptake of dissolved organic matter in soft-bodied marine invertebrates have not been reported to be contradictory to the above cited findings.

## Salinity and sodium dependence

The salinity-dependent absorption of organic solutes from the ambient water, as exemplified by some previously published results (Siebers & Bulnheim, 1976) and the above reported experiments on *E. albidus*, appears to be similar to the sodium dependence of solute transport across a variety of animal membranes (Schultz & Curran, 1970; Schultz et al., 1974; Saier & Stiles, 1975, p. 59). Accumulation and incorporation of glycine by gills of the bivalve, *Rangia cuneata*, were also shown to be inhibited in sodium-free water by Anderson (1975), who suggested a linkage of glycine uptake to a sodium dependent co-transport system.

The energy coupling of active amino-acid and sugar transport across animal membranes is regarded as being performed by an ATP-ase-dependent Na-gradient. However, in bacteria, this energy coupling is Na-independent. It is performed by a $H^+$-gradient for amino-acid transport and by group translocation for glucose transport (Saier & Stiles, 1975, pp. 51–65). These findings may provide an explanation for the results obtained on the decrease in the capacity of absorbing dissolved amino acids with declining salinities. In contrast to bacteria, invertebrates cannot absorb (or only in negligible amounts) dissolved amino acids or carbohydrates from fresh water, since their transport processes are Na-dependent.

With regard to the alterations of uptake rates at decreasing salinity levels, Stephens (1964) demonstrated a striking correlation between the onset of osmotic regulation and the decline of the ability to accumulate glycine from the medium in *Nereis* species. He supposed that the processes of osmoregulation are incompatible with a rapid accumulation of amino acids dissolved in the medium. As demonstrated by Schöne (1971), a hyperosmotic regulation of the body fluids is maintained at salinities below 15 ‰ in *E. albidus*. Compared with the results reported on the increase of uptake rates between 10 and 20 ‰ S there is also evidence of such a threshold at which extracellular regulation of osmotic concentration becomes apparent.

## Availability of dissolved amino acids

For comparisons between $K_t$-values and actual absorption rates in marine environments it is necessary to take into account the concentrations of dissolved amino acids estimated in sea water. Unfortunately, representative and reliable data are not yet available in sufficient amounts. From different localities in the German Bight, Bohling (1970, 1972) reported free amino-acid concentrations between 1 and 3 $\mu$M with maxima of up to 5 $\mu$M including seasonal and annual variations. Among the amino acids dissolved in sea water, glycine was shown to occur in relatively high amounts (about 26 mol-%). As reviewed by Jørgensen (1976), total dissolved amino

acids rarely exceed values of 50 $\mu$g $l^{-1}$ ($\sim$ 0.5 $\mu$M) in unpolluted sea water. According to Sepers (1977) total amino-acid concentrations usually do not go beyond 150 $\mu$g $l^{-1}$. Rather high levels of dissolved amino acids, ranging between 50 and 80 µM, have been found by Stephens (1963, 1975) in interstitial waters from the intertidal zone of sand-mud flats of the Woods Hole area (USA), and about 50 µM in samples of the upper levels of interstitial water in the Danish Limfjordregion. Clark et al. (1972) determined concentrations between 2 and 4 $\mu$M in samples adjacent to the sediment surface from different depths in southern California coastal waters.

At a concentration of about 1 µM of amino acids dissolved in surface sea water, the uptake system of *E. albidus* would operate far below half saturation (20–40 µM). But at about 50–80 µM, corresponding to levels measured by Stephens (1963, 1975), the uptake system of *E. albidus* would be about half-saturated.

## Nutritional aspects

Calculations to evaluate the contribution of transintegumentary absorption of dissolved organic molecules to energy needs have been performed for a variety of marine invertebrates (Taylor, 1969; Southward & Southward, 1972; Stephens, 1972, 1975; Sorokin & Wyshkwarzev, 1973; Schlichter, 1975). These authors concluded that dissolved organic compounds actually may offer a supplemental or substantial nutritional source for soft-bodied marine invertebrates. Evidently, pogonophores totally rely on the pool of dissolved organic matter which they take up across their body wall from the surrounding sea water.

On the other hand, Sepers (1977) calls into question a nutritional profit gained from the transintegumentary uptake of dissolved organic compounds in most species studied, since heterotrophic bacteria exhibiting much lower transport constants are obviously better adapted to the very low substrate concentrations. Therefore, Sepers argues that in natural waters uptake of dissolved organic compounds appears to be primarily a bacterial process. Indeed, at concentrations between 50 and 150 $\mu$g $l^{-1}$ (roughly 0.5–1.5 $\mu$M) amino acids in pelagic areas, uptake rates of marine invertebrates must be regarded as too low to provide more than a few percentages of metabolic energy as estimated from the rates of oxygen consumed. In interstitial waters, however, concentrations of dissolved organic matter are possibly high enough to provide a substantial nutritional source for infaunal soft-bodied invertebrates.

In conclusion, recognizing the limitations of information on the amounts of organic matter present in sea water, the difficulties in determining the total flux of organic compounds into a marine organism (and in opposite direction) as well as the dependence of the uptake process on various external factors, it is conceivable that nothing more than order-of-magnitude estimates can be presented to date.

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First author's address: Dr. D. Siebers
Biologische Anstalt Helgoland (Zentrale)
Palmaille 9
D-2000 Hamburg 50
Federal Republic of Germany