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DISTRIBUTION OF AMINO ACIDS TO INTERNAL TISSUES AFTER EPIDERMAL UPTAKE IN THE ANNELID *GLYCERA DIBRANCHIATA*

BY MICHAEL QAFAITI AND GROVER C. STEPHENS

Department of Developmental and Cell Biology, University of California at Irvine, Irvine, CA 92717, USA

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Summary

Net uptake rates by *Glycera dibranchiata* Ehlers of 18 amino acids, each present in artificial sea water at an initial concentration of 1 μmol l^{-1}, were estimated using high-performance liquid chromatography (HPLC). Uptake rates for ^{14}C-labelled alanine, arginine, aspartate, glutamate, glycine and serine, presented singly at an initial concentration of 1 μmol l^{-1}, were estimated by observing disappearance of radioactivity from the ambient solution. Net entry of alanine, arginine, aspartate and serine was estimated by HPLC in parallel samples of the medium. There was no significant difference in the rate of influx of labelled substrate and net entry estimated by HPLC for these amino acids. Ligature of the anterior and posterior ends of the worms did not perceptibly modify rates of uptake. Distribution of radioactivity to the internal and external body wall, coelomocytes, gut and coelomic fluid was observed after 1 h of incubation in 1 μmol l^{-1} serine. Rates of exchange of labelled carbon were estimated for all combinations of these internal tissues by taking advantage of morphological features of *Glycera*. Amino acid pools in each of the tissues of the worms are described, based on HPLC analyses. Internal distribution of radioactivity derived from [^{14}C]serine is compared with total pools based on estimates of tissue volume and the normal serine content of free amino acid pools for each tissue. The distribution of the non-metabolized analogue cycloleucine is also described at the end of 1 h of incubation.

Introduction

Epidermal uptake of free amino acids (FAA) from dilute solution in ambient sea water has been reported for many marine invertebrates. Early work in the field employed carbon-labelled or tritiated substrates. Later, the reagent fluorescamine was employed which permitted analysis of amino acids in sea water at concentrations in the micromolar range or lower. However, limitations of these procedures made it impossible to exclude the possibility of exchange diffusion or passive leakage of FAA. Also, it was not possible to work with naturally occurring

Key words: amino acid uptake, *Glycera*, epidermal uptake, internal distribution, HPLC.

sources of FAA in the environment. A technique for HPLC separation and identification of amino acid derivatives in sea water was reported by Lindroth & Mopper (1979). This technique made it possible to work with substrate concentrations in the nanomolar range and to study rates of net entry into marine invertebrates from the complex mixtures which are characteristic of natural waters. HPLC was first applied to the study of epidermal uptake in marine invertebrates by Manahan, Wright, Stephens & Rice (1982). Subsequently, Stephens and coworkers have published a series of papers describing net removal of FAA based on HPLC analysis. Forms studied include larval and adult echinoderms, annelids and molluscs. Uptake of amino acids occurring naturally in the habitat of the animal concerned has been reported as well as uptake from test mixtures. When HPLC analysis is employed in combination with radiochemical observations, it is possible to compare the rate of influx of labelled substrates with their net entry (e.g. Davis & Stephens, 1984*a*). In most cases, the two rates are closely comparable. This permits exclusion of exchange diffusion and passive leakage in these cases. The field has been reviewed recently by Stephens (1988).

Gomme (1981) has suggested that the epidermal transport systems for small organic substrates might serve to limit passive losses along the very high tissue to medium concentration gradient which characterizes such systems. For example, FAA are typically present in intracellular pools at concentrations which range from 0·1 to 0·5 mol l^{-1}. In haemolymph or coelomic fluid, concentrations are of the order of 1–2 mmol l^{-1}. In nearshore surface waters, total FAA ranges from 0·1 to 1 μmol l^{-1}. Thus gradients of total FAA are roughly 10^6 (epidermal cells: medium) or 10^4 (body fluids: medium). Given these very large gradients, the possibility of passive leakage of small organic substrates is evident. Gomme measured passive entry rates for mannitol and concluded that such passive losses might be substantial. Thus epidermal uptake of FAA and glucose may well serve to limit such losses by active reabsorption of these compounds.

Most investigators working with amino acid transport in marine invertebrates have assumed that substrates acquired by uptake from the environment are subsequently translocated to internal tissues where they may be deaminated and oxidized or incorporated into macromolecules or otherwise transformed metabolically. There is evidence supporting both catabolic and anabolic metabolism of FAA acquired by epidermal uptake (production of $^{14}CO_2$ and incorporation of radioactivity into molecules insoluble in trichloroacetic acid). However, published evidence for translocation to deeper tissues after acquisition by epidermal uptake is exiguous. In reviews of the field, Gomme (1982, 1984, 1985) has drawn attention to the lack of evidence for such translocation and has suggested that it may not occur in annelids. Wright (1985) has made a similar suggestion concerning translocation after amino acid uptake by *Mytilus*.

The suggestion that epidermal transport may function to limit passive loss of small organic molecules is quite attractive and seems likely. However, there is no necessary conflict between this hypothesis and the existence of mechanisms for translocation of FAA acquired by uptake to deeper tissues. The issue merits direct

examination. Most workers in the field have interpreted epidermal influx of FAA as a potential nutritional supplement, providing amino nitrogen and reduced carbon for catabolic and anabolic metabolism by tissues throughout the organism. For this reason, rates of entry of amino acids *via* the epidermis have frequently been compared with rates of oxygen consumption and/or nitrogen excretion to assess the potential significance of this input. If FAA are limited to the surface epithelium after epidermal uptake, this approach is not defensible.

The aim of the present paper is to describe translocation of labelled amino acids to deeper tissues of *Glycera dibranchiata* after epidermal uptake. The morphology and organization of this worm permit resolution of rates of transfer between the substrate supplied in the ambient medium and the body wall (interior and exterior surfaces), coelomic fluid, coelomocytes and gut, and all paired combinations of these tissues. In the present work, short-term rates are determined and presented on the basis of distribution of radioactivity in internal tissues after 1 h of incubation in $1\,\mu mol\,l^{-1}$ serine and $1\,\mu mol\,l^{-1}$ cycloleucine. Translocation is compared with the normal pools of FAA in these tissues and the estimated volume of each. Rates of uptake from naturally occurring amino acid resources in the interstitial water of inshore sediments are estimated and compared with the oxygen consumption of the worms.

Materials and methods

Specimens of *Glycera dibranchiata* were collected commercially in Maine and shipped by air freight to California. Animals were maintained in the laboratory at 14°C in moist seaweed (*Fucus*). They were fully hydrated and acclimated to 22°C prior to use in experiments. All experiments were conducted in artificial sea water (MBL) prepared according to Cavanaugh (1956). Animals ranged in mass from 2·60 to 8·29 g, with an average of $5{\cdot}90 \pm 0{\cdot}38$ g ($N = 60$).

Glycera offers several advantages as an experimental animal. It has an extensive coelom, undivided by septa, in which haemoglobin-containing coelomocytes are suspended. Suspensions of the coelomocytes accumulate radiolabelled amino acids rapidly (Preston & Stephens, 1969). If a tube of body wall is prepared by severing the head and tail, the coelom can be flushed with sea water and both ends ligated. Such a preparation retains muscular tone for 24 h or more and takes up amino acids from the medium at nearly normal rates. The tube can be filled with sea water or with a suspension of labelled or unlabelled coelomocytes. These features make it possible to isolate and estimate rates of influx into and exchange between the various tissues of the worm.

Uptake of amino acids was determined as follows. Animals were washed in sterile MBL (filtered through a 0·2 μm pore diameter membrane filter) and placed individually in 100 ml of sterile MBL. 2 μCi of ^{14}C-labelled amino acid was added together with sufficient ^{12}C-labelled amino acid to provide an initial concentration of $1\,\mu mol\,l^{-1}$. Samples were taken periodically after addition of the worm.

Radioactivity in the medium was measured using a Beckman CPM-100 scintillation counter. In some cases, parallel samples were taken for analysis by HPLC. HPLC analysis was also used to determine rates of net entry of amino acids from a mixture of 18 amino acids, each present at an initial concentration of $1\,\mu mol\,l^{-1}$. The procedure for HPLC determination of amino acids is essentially that described by Jones, Paabo & Stein (1981) with minor changes in pH and gradient programme to improve separation on the column used (Ultrasphere ODS, 150×4·6 mm, 5 μm packing). Amino acids were derivatized prior to injection with an *o*-phthaldialdehyde reagent. Amino acids were identified on the basis of elution times established with authentic standards; concentrations were estimated from peak area compared with standards. Stephens & Manahan (1984) and Davis & Stephens (1984*b*) provide a detailed account of methods and gradients. The same procedure was followed for HPLC analysis of pools of FAA in coelomocytes, body wall, gut and coelomic fluid after suitable dilution of 80 % ethanol extracts. The cell volume of the body wall was corrected for extracellular space determined by [^{14}C]inulin distribution. Isolated portions of the body wall were incubated with labelled inulin. The incubation medium was sampled hourly until no further changes in activity were observed. Other tissue volumes and the volume of coelomic fluid were estimated on the basis of mass.

Production of $^{14}CO_2$ was determined in some experiments where labelled substrates were presented to the worms, which were then incubated in 100 ml of sterile MBL in a flask closed with a rubber stopper. A ground-glass rod, dipped in concentrated KOH, was suspended in the flask to absorb CO_2 evolved. At the end of the incubation period, a sample of the medium was removed for scintillation counting and 3 ml of concentrated HCl was injected, killing the worm and driving CO_2 into the gas phase where it was absorbed by the KOH. The glass rod was rinsed into scintillation cocktail and radioactivity was determined.

The rate of uptake of labelled substrate into the external and internal surfaces of the body wall was determined as follows. A portion of the body wall, not including the retractable gills, was clamped in an Ussing-type chamber. An area of 7 mm^2 was exposed to sterile MBL placed on each side of the body wall. Labelled substrate ($1\,\mu mol\,l^{-1}$) was added to either side. Mixing and aeration was accomplished by bubbling air into the solution. A 0·5-ml sample was withdrawn from each side at the beginning and end of the incubation period for counting. The rate of uptake of labelled substrate by a suspension of coelomocytes in MBL was estimated by disappearance of radioactivity from the medium sampled periodically during incubation.

A fraction of the radioactivity in the tissues of the worm is converted into compounds insoluble in ethanol and trichloroacetic acid (TCA). Radioactivity in fractions soluble and insoluble in 5 % TCA is reported for tissues at the end of 1 h of incubation in $1\,\mu mol\,l^{-1}$ [^{14}C]serine. The latter fraction was determined by dissolving the precipitate after extraction with TCA in a tissue solubilizer (TS-2, Research Products International Corp., IL). Counts were corrected for background and quench in all cases where radioactivity was measured.

Some observations used a tube of the muscular body wall of *Glycera*. The head and tail of the worm were severed and the resulting tube, containing the gut, was rinsed thoroughly with sterile MBL. After ligature of one end, the relevant test solution (sterile MBL, a suspension of labelled coelomocytes in sterile MBL, fresh coelomic fluid and coelomocytes) was introduced with a syringe and the other end ligated.

The ratio of the surface area of the body wall and the gills was measured as follows. The length and diameter of exposed segments of the body wall from the anterior and posterior third of worms were measured under a dissecting microscope with an ocular micrometer. The length and diameter of the exposed gills from the same segments were also measured. Areas were calculated treating the segments and the gills as cylinders. The calculated ratios were then averaged. The average ratio was $5{\cdot}35 \pm 0{\cdot}18{:}1$ ($N = 6$).

Statistical analysis was performed using one-way analysis of variance or Student's *t*-test. Calculation of entry rates of substrate is based on the assumption of first-order exponential depletion of the medium. Substrate concentrations are well below half-saturation parameters (K_t) reported for *Glycera* which range upwards from $200\,\mu\text{mol}\,l^{-1}$ (Chien, Stephens & Healey, 1972).

Results

The total intracellular pools of FAA in coelomocytes, body wall and gut of *Glycera* do not differ significantly but their qualitative composition differs (Table 1). The FAA content of coelomic fluid is two orders of magnitude less than that of the cells (Table 1).

Fig. 1 presents data for uptake of serine from an initial concentration of $1\,\mu\text{mol}\,l^{-1}$ by *Glycera*. Similar observations were made using alanine, arginine and aspartate as substrates. In no case was the rate of disappearance of radioactivity significantly different from the net entry determined by HPLC ($P > 0{\cdot}05$, $N = 4$).

Entry rates, measured by HPLC, for labelled alanine, arginine, aspartate, glutamate, glycine and serine presented individually at $1\,\mu\text{mol}\,l^{-1}$ are not significantly different from their respective net entry rates in combination ($P > 0{\cdot}05$) (Table 2).

There is no difference ($P > 0{\cdot}05$, $N = 8$) in the rate of uptake of ^{14}C-labelled serine in intact and ligated worms, indicating that uptake of substrate is primarily epidermal (Fig. 2).

After 1 h of incubation in ^{14}C-labelled serine approximately 95·4 % of the radioactivity was recovered in the various fractions (Table 3). For labelled serine, $1{\cdot}08 \pm 0{\cdot}29$ % of the radioactivity was recovered as $^{14}CO_2$ ($N = 14$), giving a total recovery in excess of 96 %. Recovery for glycine was comparable but production of $^{14}CO_2$ was somewhat larger, $2{\cdot}79 \pm 0{\cdot}44$ % ($N = 7$).

Rates of uptake of labelled serine into the external and internal surfaces of the body wall were measured in the Ussing chamber. They were not significantly different ($P > 0{\cdot}05$) when substrate was presented at an initial concentration of

Table 1. *Amino acid pool in tissues of* Glycera

Amino acid	Coelomocytes mmol l^{-1}	%	Body wall mmol l^{-1}	%	Coelomic fluid mmol l^{-1}	%	Gut mmol l^{-1}	%
Asp	7·84 ± 0·21	2·35	12·63 ± 1·55	3·46	0·027 ± 0·005	1·33	13·22 ± 1·65	4·01
Glu	16·93 ± 1·85	5·07	6·54 ± 0·41	1·79	0·033 ± 0·006	1·63	10·67 ± 1·06	3·23
Asn	40·91 ± 1·05	12·25	95·30 ± 6·81	26·10	0·411 ± 0·065	20·32	33·45 ± 3·42	10·14
Ser	6·87 ± 0·60	2·06	40·26 ± 4·35	11·03	0·086 ± 0·008	4·25	5·72 ± 0·27	1·73
His-Gln	18·32 ± 1·29	5·47	1·16 ± 0·06	0·32	0·209 ± 0·006	10·33	2·70 ± 0·42	0·82
Gly	10·18 ± 1·86	3·05	65·70 ± 3·03	17·99	0·080 ± 0·007	3·95	24·45 ± 3·11	7·41
Thr	11·73 ± 1·76	3·51	4·43 ± 0·24	1·21	0·155 ± 0·008	7·66	7·37 ± 1·01	2·23
Arg	5·37 ± 1·67	1·61	17·39 ± 1·25	4·76	0·043 ± 0·011	2·13	20·73 ± 5·36	6·28
Tau	142·40 ± 10·97	42·62	110·89 ± 5·70	30·37	0·210 ± 0·018	10·40	111·56 ± 10·90	33·81
Ala	45·70 ± 3·07	13·70	0·28 ± 0·04	0·08	0·542 ± 0·036	26·74	50·11 ± 3·97	15·20
Tyr	2·13 ± 0·38	0·64	0·82 ± 0·05	0·22	0·011 ± 0·001	0·54	2·44 ± 0·37	0·74
Met	5·50 ± 0·21	1·65	3·34 ± 0·27	0·92	0·046 ± 0·005	2·27	3·00 ± 0·49	0·91
Val	6·61 ± 1·07	1·97	2·20 ± 0·19	0·60	0·044 ± 0·007	2·17	7·32 ± 0·96	2·22
Phe	2·86 ± 0·19	0·86	0·73 ± 0·02	0·20	0·025 ± 0·007	1·24	5·53 ± 0·98	1·67
Ile	3·78 ± 0·70	1·12	1·08 ± 0·07	0·29	0·027 ± 0·003	1·33	6·32 ± 1·44	1·92
Leu	4·86 ± 0·29	1·44	1·13 ± 0·09	0·31	0·041 ± 0·002	2·03	10·89 ± 2·67	3·30
Lys	2·09 ± 0·60	0·63	1·23 ± 0·19	0·34	0·033 ± 0·006	1·63	14·44 ± 1·94	4·38
Total	334·08 ± 27·77	100	365·11 ± 24·32	100	2·023 ± 0·201	100	329·96 ± 40·02	100

Concentrations are expressed in mmol l^{-1} ± S.E.M. ($N = 4$).
Values for body wall are corrected for inulin space (23·4 ± 3·2 %).

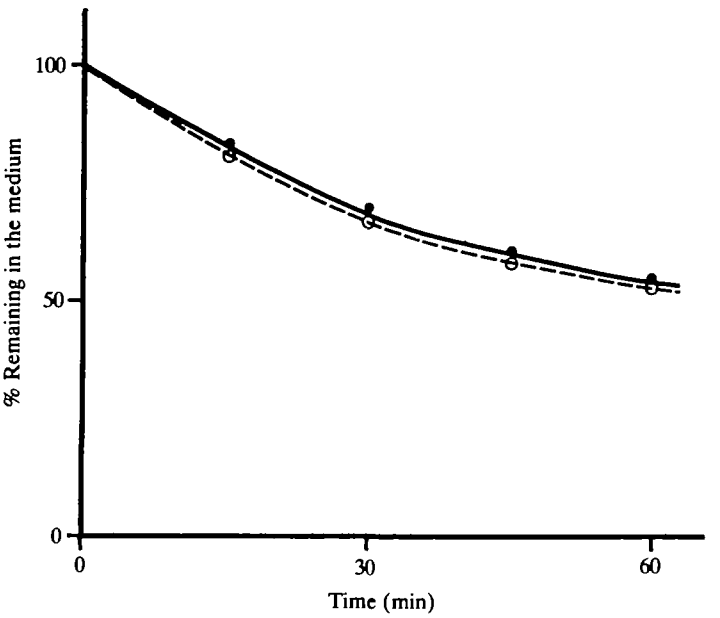

Fig. 1. Depletion curve of serine when presented to the worms at an initial concentration of 1 μmol l^{-1}, as measured by liquid scintillation counting (——) and HPLC (-----), $N = 4$. Each point has a deviation of less than 3 %.

1 μmol l^{-1}. The respective rates were 95·08 ± 7·83 ($N = 12$) and 80·67 ± 9·89 nmol $cm^{-2} h^{-1}$ ($N = 4$). Similar results were obtained using labelled glycine and asparagine.

Coelomocytes were washed and resuspended in MBL at the same density as that found in normal worms. The coelomocytes represent 30–45 % of the coelomic fluid by volume in the specimens used. They removed 94·2 ± 0·5 % of ^{14}C-labelled serine after 1 h of incubation at an initial concentration of 1 μmol l^{-1}. Table 4 compares the radioactivity transferred to the body wall and the gut when isolated tubes of body wall were filled with labelled coelomocytes resuspended in MBL with transfer of radioactivity in tubes filled with 1 μmol l^{-1} serine. Control observations showed that very little lysis of coelomocytes occurred. In both cases, the incubation period was 1 h. This establishes rates for exchange between coelomocytes and these tissues *via* the coelomic fluid, and the rates for transfer from the coelomic fluid to the gut and body wall. Transfer of labelled coelomocytes to the tube of body wall involves negligible lysis. In control observations, 0·4 % of the radioactivity in coelomocytes was liberated to the medium after passage of the suspension through the syringe used to inject the tubes of *Glycera* body wall.

To establish rates of transfer between the internal body wall and gut and the coelomic fluid and coelomocytes, tubes filled with 1 μmol l^{-1} of labelled serine and incubated for 1 h were drained, rinsed and filled with fresh blood. 11·35 ± 1·73 %

of the radioactivity was present in the coelomocytes and 0·71 ± 0·08 % was found in the coelomic fluid. Thus, total transfer *via* the coelomic fluid from the body wall into the coelomocytes and coelomic fluid was approximately 12·1 % during the observation period. The radioactivity in the gut increased by 1·21 ± 0·27 %.

Table 5 indicates complete recovery of labelled cycloleucine, which is not metabolized in the TCA-soluble fraction. Although the totals for the two substrates in coelomic fluid are different, the internal distribution of radioactivity after 1 h is broadly comparable in the two cases.

Table 6 compares radioactivity in internal tissues and coelomic fluid after 1 h in 1 μmol l^{-1} serine, with the distribution of radioactive cycloleucine after comparable exposure. The distribution of TCA-soluble radioactivity and total radioactivity are listed separately for serine. Although activity in the gut and in the coelomic fluid are significantly different, the overall distribution is similar.

The permeability of *Glycera* to 1 μmol l^{-1} [^{14}C]mannitol was 0·04 % of the rate of serine uptake. Stevens & Preston (1980) report less than 1 % for mannitol influx

Table 2. *Net rates of uptake of amino acids*

	Amino acids presented simultaneously	Amino acids presented singly	
Amino acid	Rate	Rate	*N*
Asp	17·01 ± 1·09	16·36 ± 0·96	6
Glu	15·63 ± 2·24	12·96 ± 2·14	11
Asn	13·27 ± 1·02		
Ser	21·05 ± 1·37	20·26 ± 1·28	16
His-Gln	14·07 ± 0·62		
Gly	12·77 ± 1·91	16·80 ± 1·08	9
Thr	14·03 ± 1·50		
Arg	11·74 ± 1·96	9·64 ± 0·94	15
Tau	17·36 ± 0·25		
Ala	19·38 ± 2·71	17·45 ± 1·28	4
Tyr	13·25 ± 1·03		
Met	8·01 ± 0·81		
Val	17·22 ± 1·48		
Phe	16·43 ± 0·12		
Ile	12·09 ± 1·08		
Leu	14·63 ± 0·57		
Lys	6·64 ± 0·55		
Total	224·58 ± 20·31		

The first pair of columns lists net entry rates of 18 amino acids estimated by HPLC analysis (means ± S.E.M., $N = 4$). Each amino acid was initially present at 1 μmol l^{-1} (total concentration, 18 μmol l^{-1}).

The second pair of columns lists rates of entry of ^{14}C-labelled amino acids presented individually at an initial concentration of 1 μmol l^{-1}.

Rates are expressed as nmol g body wall^{-1} h^{-1}.

The differences between the net entry from the mixture and the entry of individual labelled compounds are not significant ($P > 0·05$).

compared with their measured rate of uptake of labelled alanine. Since amino acids are charged substrates, it is not clear that the rate of mannitol entry is an appropriate index of passive flux of FAA.

Discussion

Glycera dibranchiata removes 16 amino acids from a mixture in which each amino acid is present at a concentration of 1 μmol l^{-1} (initial rates of removal given in Table 2). Histidine and glutamine were not separated in our HPLC analysis procedure but one and possibly both substrates were also removed from ambient

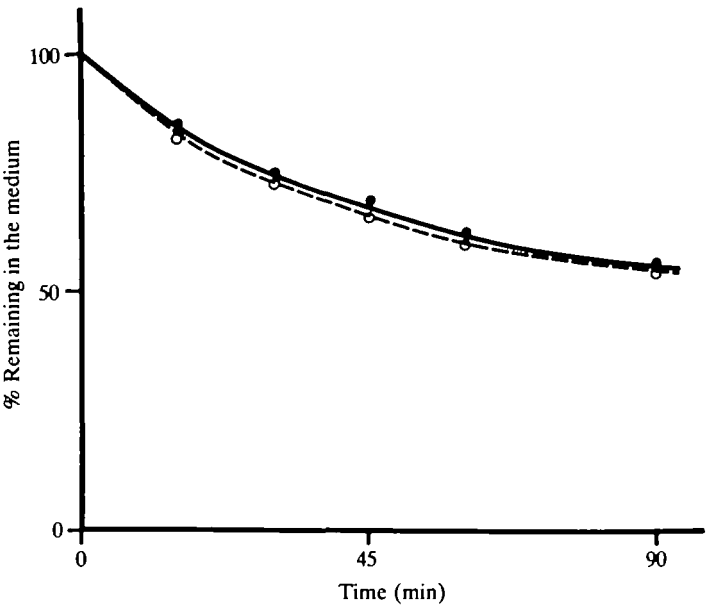

Fig. 2. Depletion curves of serine when presented to the worms at an initial concentration of 1 μmol l^{-1}. The ends of the worms were either freely open to the incubation medium (——) or restricted from the medium by ligation (-----), $N = 8$. Each point has a deviation of less than 3 %. Data are normalized to compensate for differences in mass of worms. Masses ranged from 5·4 to 6·7 g.

Table 3. *Distribution of radioactivity in tissues of* Glycera *after incubation in 1 μmol l^{-1} [^{14}C]serine for 1 h*

Body tissue	TCA-soluble fraction	*N*	TCA-insoluble fraction	*N*
Coelomic fluid	0·27 ± 0·05	14	0·09 ± 0·02	5
Coelomocytes	3·96 ± 0·60	14	5·60 ± 2·80	5
Body wall	83·74 ± 5·09	10	0·94 ± 0·11	5
Gut	0·71 ± 0·23	10	0·13 ± 0·04	5

Values are percentages of the total taken up by the worms ± S.E.M.

Table 4. *Radioactivity appearing in tissues of* Glycera *when tubes of body wall were filled with either 1 μmol l^{-1} [^{14}C]serine or coelomocytes previously exposed to 1 μmol l^{-1} [^{14}C]serine*

Body tissue	With preloaded coelomocytes	With serine solution
Body wall	23·11 ± 2·86	56·80 ± 4·86
Gut	4·39 ± 0·70	16·11 ± 2·64
Coelomic fluid	0·40 ± 0·05	

Exposure time was 1 h.
Values are percentages of total activity ± S.E.M. ($N = 4$).

Table 5. *Distribution of radioactivity in tissues of* Glycera *after incubation for 1 h in 1 μmol l^{-1} [^{14}C]serine or 1 μmol l^{-1} [^{14}C]cycloleucine*

Body tissue	Serine	Cycloleucine
Body wall	84·68 ± 5·20	84·86 ± 1·97
Coelomocytes	9·56 ± 3·40	12·51 ± 1·61
Coelomic fluid	0·36 ± 0·07	1·41 ± 0·25
Gut	0·84 ± 0·27	1·43 ± 0·17

The figures for serine are taken from Table 4 and include both TCA-soluble and TCA-insoluble radioactivity.
Values are percentages of the total activity disappearing from the incubation medium ± S.E.M. ($N = 4$).

Table 6. *Distribution of ^{14}C-labelled serine and cycloleucine in tissues and coelomic fluid*

	Body wall	Coelomocytes	Coelomic fluid	Gut
% mass	58·05 ± 1·37	6·12 ± 0·39	17·92 ± 1·51	17·91 ± 0·89
Serine/tissue	94·13 ± 1·99	1·70 ± 0·32	0·06 ± 0·01	4·11 ± 0·20
Serine/TCA-soluble	94·43 ± 5·74	4·47 ± 0·68	0·30 ± 0·07	0·80 ± 0·26
Serine/activity	88·72 ± 5·45	10·02 ± 3·56	0·38 ± 0·07	0·88 ± 0·28
Cycloleucine	84·86 ± 1·97	12·51 ± 1·61	1·41 ± 0·25	1·43 ± 0·17

The first row lists the mass of tissue of *Glycera* as a percentage of total mass.
The second row lists the percentage of total animal serine in each tissue calculated from values in Table 1 and the mass of each tissue (body wall corrected for intercellular space).
The third row gives TCA-soluble radioactivity after 1 h of exposure to [^{14}C]serine in the medium (from Table 3 expressed as a percentage of the total TCA-soluble radioactivity).
Row four lists the total radioactivity derived from [^{14}C]serine at the end of 1 h (from Table 3 expressed as a percentage of the total activity).
Row five lists concentrations of [^{14}C]cycloleucine in the tissues as a percentage of the total removed from solution.
Values are means ± S.E.M. ($N = 4$ for cycloleucine, N as listed in Tables 1 and 3 for other values).

solution. Rates of influx of four labelled substrates presented at a concentration of $1\,\mu mol\,l^{-1}$ were determined on the basis of disappearance of radioactivity from solution. Rates of net influx determined by net disappearance of substrate analysed by HPLC were not significantly different for alanine, arginine, aspartate or serine (Fig. 1).

The initial entry rates of labelled alanine, arginine, aspartate, glutamate, glycine and serine presented individually at $1\,\mu mol\,l^{-1}$ are not significantly different from net entry (HPLC) of these substrates presented as a mixture (Table 2). There does not appear to be perceptible interaction among rates of influx of these amino acids at these concentrations. The total concentration of FAA employed ($18\,\mu mol\,l^{-1}$) is low compared with the reported half-saturation parameters for amino acid uptake, so this is not unexpected. In no case was leakage of any amino acid from the animals into the medium observed with HPLC.

The uptake of serine is predominantly epidermal (Fig. 2). This is in accord with many observations conducted on a variety of soft-bodied marine invertebrates (G. C. Stephens, in press). Although a high percentage of radioactivity derived from labelled serine taken up from solution during a 1-h exposure is found in the body wall (approximately 85 %), smaller amounts are found distributed to internal tissues and the coelomic fluid. Since uptake *via* the mouth and anus is not significant, the remaining 15 % presumably enters across the body wall for internal distribution. The observations with the Ussing chamber indicated that there is no perceptible translocation across the thick, muscular body wall. This agrees with reports by Chien *et al.* (1972) and a more recent study by Chien & Rice (1985).

Chien & Rice (1985) used autoradiography of freeze-dried frozen sections of *Glycera dibranchiata* to study the distribution of radioactivity in the worms after epidermal uptake of ^{14}C-labelled amino acids. They found heavy labelling of the retractable gills and labelling of coelomocytes shortly after exposure to labelled substrates. Radioactivity detected in the general body surface was translocated across the thick, muscular body wall very slowly over a period of days. They interpret their results to indicate rapid translocation of FAA to the coelomocytes after epidermal uptake *via* the epithelium of the thin-walled gills. Presumably, the radioactivity found in the present study after 1 h of incubation in [^{14}C]serine and [^{14}C]cycloleucine enters *via* the gills. The result in the present report, based on observations employing an Ussing chamber, agrees with their conclusion that there is no short-term transfer of radioactivity across the general surface of the body wall.

Table 5 indicates that 84·7 % of radioactivity derived from labelled serine is found in the body wall. The remaining 15·3 % is in the coelomic fluid and internal tissues. The ratio is 5·54. The pathway of entry of the internal fraction is presumably the thin-walled gills. It is of interest to compare the ratio of radioactivity (external: internal) with the estimated ratio of surface areas (body surface: gill). The latter is $5{\cdot}35 \pm 0{\cdot}18$. The similarity of the two ratios suggests that there is no marked difference in distribution of carriers for FAA between the gills and the general surface of the body wall.

Radioactivity from coelomocytes exposed for 1 h to 1 μmol l^{-1} [^{14}C]serine is transferred to the interior of the body wall, gut and coelomic fluid (Table 4). These observations were made using tubes of the body wall (including the gut) after excising the head and tail of the worm and rinsing thoroughly. Experiments were also performed in which fresh blood was added to such a tube after it had previously been filled with 1 μmol l^{-1} serine in sterile MBL. In this case, 11·35 ± 1·73 % of the radioactivity derived from the previous exposure to labelled serine was found in the coelomocytes at the end of 1 h ($N = 5$). The comparable figures for coelomic fluid and gut were 0·71 ± 0·08 and 1·21 ± 0·27 %, respectively. These results indicate that exchanges continue to occur among the internal tissues of the worms. These data would modify the rates given in Fig. 3 to a minor extent, but they have not been included: the additional complexity which their inclusion would introduce does not seem justified. The existence of such ongoing exchanges

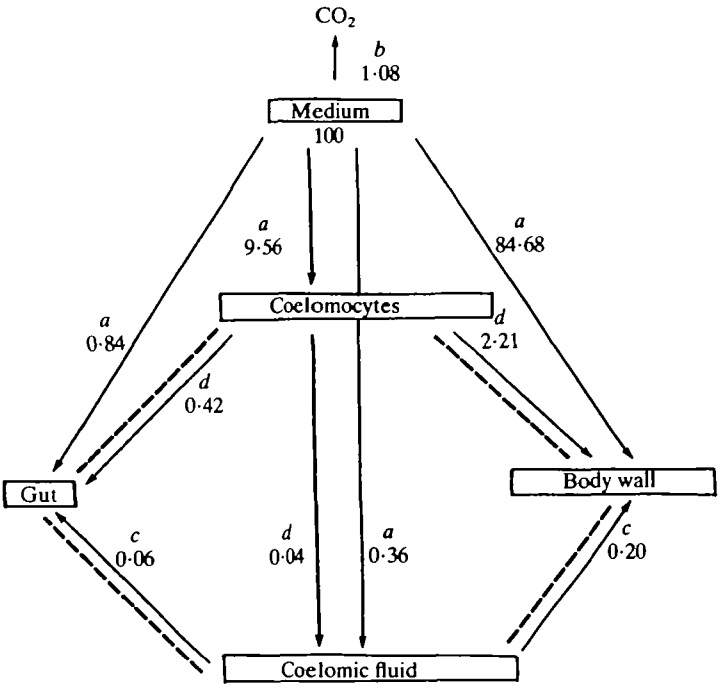

Fig. 3. Summary of rates for transfer of radioactivity derived from 1 μmol l^{-1} [^{14}C]serine. All rates are approximate and are based on levels of radioactivity at the end of 1 h. Letters (*a–d*) indicate the experimental basis for rate calculation as follows. (*a*) Uptake by intact worm and fractionation among tissues (total activity from Table 3). (*b*) Percentge of $^{14}CO_2$. (*c*) Uptake by body wall and gut when a tube of body wall was filled with 1 μmol l^{-1} [^{14}C]serine (Table 4). Values are obtained by multiplication of these two rates by 0·36, the percentage of radioactivity found in coelomic fluid (Table 3). (*d*) Transfer of radioactivity from coelomocytes preloaded with [^{14}C]serine for 1 h and injected into a tube of body wall (transfer rates from Table 4 multiplied by total radioactivity in coelomocytes from Table 3). The existence of continuing changes among internal tissues is indicated by dashed lines.

also implies that a more sophisticated mathematical analysis would be required to estimate true exchange rates among the tissues. Neither the quality of the data nor the basic point at issue justifies such analysis. The approximate rates in Table 4 are expressed as a fraction of the total radioactivity translocated to the interior of the worms and are incorporated into Fig. 3.

Fig. 3 is a flow sheet summarizing the approximate exchange rates between the external medium and the various tissues of the worm. An example will illustrate the procedure followed for obtaining such rates. 9·56 % of the radioactivity entering from the medium is found in the coelomocytes (Tables 3, 5). 23·2 % of the radioactivity from prelabelled coelomocytes introduced into a tube of body wall is transferred to the interior of the body wall (Table 4). Thus the rate of transfer, expressed in terms of the fraction entering the worm, is 2·2 %. The existence of continuing interchange of labelled substrates among internal tissues is indicated by dashed lines.

Table 6 compares the distribution of radioactivity in the tissues of *Glycera* at the end of 1 h of incubation in ^{14}C-labelled serine at an initial concentration of 1 μmol l^{-1} with the normal serine content in the FAA pools of the body wall, coelomocytes, coelomic fluid and gut. There is no reason to expect equilibrium distribution at the end of this short period. Also, labelled serine may well have been converted to other amino acids to an extent which has not been estimated. However, the data demonstrate that effective distribution of substrate to the internal tissues has taken place and the short-term distribution of radioactivity crudely approximates normal levels of the pools of free serine found in the various tissues. Similarly, the numerical values for exchange rates calculated in Fig. 3 are less important than the fact that such exchanges of substrates occur among all tissues of the worms and lead to the effective distribution of amino acids acquired by epidermal uptake.

The rates of entry of amino acids from the mixture of 18 amino acids (Table 2) can be compared with the rates of oxygen consumption of *Glycera*. Hoffmann & Mangum (1970) report a rate of oxygen consumption of 32·4 μl O_2 g^{-1} h^{-1}. Converting the total amino acid entry given in Table 2 to comparable units, net entry is 128 nmol g^{-1} h^{-1}. The generally accepted conversion factors for aerobic oxidation of amino acids are 1 ml O_2 is equivalent to 1 mg of a mixture of amino acids; the average relative molecular mass of such a mixture is taken to be 100. Thus the complete oxidation of 128 nmol of FAA would require 12·8 μl of O_2. If the metabolism of *Glycera* were completely aerobic and amino acids acquired by epidermal uptake were fully oxidized, amino acid uptake would account for almost 40 % of the substrate required to account for oxygen consumption. Neither of these assumptions is likely. Metabolism is partially anaerobic in some polychaete annelids even in well-aerated water and the environment of *Glycera* is certainly not well-aerated. Also, FAA acquired by epidermal uptake are clearly incorporated into TCA-insoluble compounds and therefore, presumably, contribute to anabolic as well as catabolic metabolism. However, there are no available methods which would allow a more cogent comparison of rates of amino acid

influx with metabolic requirements. Thus, we can only indicate that FAA acquired by uptake from the environment are in fact distributed to internal tissues of the worms and that they participate in the general metabolism in such a way as to reduce requirements for reduced carbon and amino nitrogen from ingestion.

The total concentration of the amino acid mixture on which the preceding calculation is based is $18\,\mu mol\,l^{-1}$. This is realistic in terms of the amino acids found in inshore interstitial waters. Recent values are given in Henrichs & Farrington (1979), Davis, Stephens & Rice (1985) and O'Dell & Stephens (1986). The only reported value from an area where *Glycera dibranchiata* is abundantly present is an old estimate of $74\,\mu mol\,l^{-1}$ (Stephens, 1963) based on a Moore–Stein analysis of interstitial water in shallow sediment in Massachusetts. Since *Glycera* is an errant predator, its body surface is freely exposed to amino acids in interstitial water.

We can conclude that *Glycera* has access to levels of FAA in its normal environment such as to provide a potentially valuable supplement to food ingested orally by uptake directly across the epidermis. These supplementary amino acids are rapidly and effectively distributed throughout the worms where they presumably contribute to the general metabolism of the animals.

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