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# INFLUX AND TRANSEPITHELIAL FLUX OF AMINO ACIDS IN THE MUSSEL, *MYTILUS EDULIS*

BY MICHAEL A. RICE* AND GROVER C. STEPHENS

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

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## SUMMARY

The uptake of amino acids by the non-gill epithelia of the mantle cavity of *Mytilus edulis* L. was studied and compared with uptake by the gills. Amino acid entry rates and the subsequent distribution of amino acids to the other tissues of the animals were studied using high-performance liquid chromatography and radiochemical techniques. Uptake *via* the non-gill epithelia lining the mantle cavity was separated from uptake *via* the gill by employing a preparation in which the gills were surgically removed. Amino acid uptake by such animals was compared with that of suitably sham-operated controls.

In short-term experiments (up to 2 h), transfer of substrate from the gills to other tissues of the animal is extremely limited. Amino acids taken up by the non-gill epithelia of the mantle cavity are rapidly transferred to deeper tissues. Roughly 25 % of alpha-amino acids enter the animal *via* the non-gill epithelia. Estimates of total epithelial surface area for the gills and non-gill mantle epithelium are compared with entry rates of amino acid substrates *via* the two routes. The apparent densities of carriers for alanine and cycloleucine per unit area of surface are approximately equal for these two substrates. The density of taurine carriers per unit area of non-gill epithelium is apparently significantly higher than their density per unit area of gill epithelium. Finally, evidence is presented for differential sensitivity of taurine transporters in the non-gill epithelium to inhibition by alpha-amino acids.

## INTRODUCTION

There is a substantial literature concerning uptake of amino acids by marine and estuarine bivalves (see Stephens, 1983; Wright, 1982, 1985, for recent reviews). Net entry of free amino acids (FAA), both from dilute solution in sea water and from naturally occurring sources in the water column, has now been demonstrated by direct chemical methods (Manahan, Wright, Stephens & Rice, 1982; Siebers & Winkler, 1984; Stephens & Manahan, 1984). Such net influx has typically been interpreted in the context of its potential contribution as a nutritional supplement to

* Present address: Department of Fisheries and Aquaculture, University of Rhode Island, 35 Steamboat Avenue, Wickford, RI 02852, USA.

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traditional feeding pathways. It has been assumed that uptake was transepidermal, i.e. that solutes acquired from the environment by uptake into the epidermis were translocated to other tissues where they contributed generally to metabolism as sources of amino nitrogen and reduced carbon.

This interpretation has been called into question. Gomme (1982, 1985) and Wright (Wright & Secomb, 1984, 1986; Wright, 1985), working with the mussel *Mytilus edulis*, have suggested that uptake of FAA by the epidermis may play primarily or solely an osmotic role. Intracellular amino acid concentrations in marine and estuarine bivalves range from 0·2 to 0·5 mol $l^{-1}$, while extracellular fluids typically have total concentrations in the millimolar range. Normal environmental levels of FAA range from 0·1 to 1·0 $\mu$mol $l^{-1}$. The epidermis thus faces an impressive concentration gradient against which it must maintain its high concentration of organic osmolytes, primarily FAA. Wright and Gomme suggest that amino acid uptake functions primarily to retrieve passive losses of solute. They argue that, if unchecked, these would represent an energetically unacceptable continuous loss at rates which exceed the capacity of replacement by measured rates of food collection. In this interpretation, the net influx of FAAs from the environment is viewed as an unimportant side effect of this primary retrieval function and uptake plays a negligible supplementary nutritional role (Wright, 1985).

In this recent work, attention has been focused primarily on the role of the gills in uptake of FAA. The work of Famme (Famme, 1980*a*,*b*, 1981; Famme & Kofoed, 1980) on oxygen consumption by *Mytilus* has shown that the gills of this bivalve plays a minor role in oxygen consumption of the animal. The gills are poorly supplied with blood and the circulation is slow. Oxygen consumption is normal in animals with excised gills provided adequate perfusion of the mantle cavity is maintained artificially. Thus the function of the gills in oxygen consumption appears to be limited to the perfusion of the mantle cavity. The entry of oxygen presumably occurs *via* the epithelia lining the mantle rather than the gill. This body of work is reviewed and the interpretation is strengthened by Jørgensen, Möhlenberg & Sten-Knudsen (1986). This information suggests, by analogy, that translocation of FAA after uptake by the gill, if it occurs, may be a slow process for the same reasons as apply to oxygen consumption.

The suggestion that uptake of FAA by the gill serves to limit losses of organic osmolytes may very well be correct. However, there is no necessary incompatibility between this osmotic role and a role of uptake of dissolved organic solutes as a supplement to traditional feeding methods. The occurrence of significant translocation of organic solutes, acquired by epidermal uptake, to deeper tissues of the organism is a separate issue. It needs to be examined and accepted or rejected on the basis of direct evidence.

The translocation of organic solutes after epidermal uptake in molluscs has received little attention since Pequignat (1973) studied the internal distribution and assimilation of glucose and amino acids in *Mytilus edulis*. He reported evidence of internal distribution based on tissue digests as well as autoradiography. He also provided evidence for entry of FAA supplied in ambient sea water *via* the tissues

lining the mantle cavity as well as *via* the gill. However, his conclusions are based on very small samples and are qualitative in nature. Thus the subject merits further attention, particularly in view of the current interest in the issue of translocation.

The present work is an investigation of the transport role of tissues lining the mantle cavity and a quantitative comparison of the role of these epithelia in amino acid uptake with that of the gills of *Mytilus*. We also present evidence for rapid translocation of FAA to internal tissues of the animals.

## MATERIALS AND METHODS

Mussels were collected from floating docks in Newport Bay, Orange County, California. They were maintained without feeding in aquaria at 17°C for at least 1 week prior to use. Experiments were performed after acclimation to room temperature (22°C). Animals used in the various portions of this study ranged from 0·49 to 11·04 g shell-free wet mass. For experiments relating amino acid transport to transporting surface area, animals were selected to lie within the size range of 55–65 mm shell length, which corresponds to a wet mass of internal tissues of approximately 6 g. The external surface of the shell was cleaned of adhering organisms by vigorous brushing and thorough rinsing in clean sea water. Experiments were performed in artificial sea water (ASW) prepared according to the formulation of Cavanaugh (1956).

There is a possibility that the mussels found in southern California may be *Mytilus galloprovincialis* rather than *Mytilus edulis* (A. Southward, personal communication). Differences are subtle and we did not attempt specific identification of our animals. The population is presumably the same as that used in some of Wright's work but may be different from the animals employed by Gomme and Famme, which were collected in Denmark.

For some observations, the gills of the animals were surgically removed. A thin scalpel blade was carefully slipped between the mantle tissue and the internal surface of one valve and the adductor muscles were cut. The mussel was opened and the gills excised, taking care to avoid damage to the epithelia lining the mantle and underlying tissues. The valves were then clamped in a slightly gaping position and the mantle cavity irrigated with ASW using a peristaltic pump. Water was supplied *via* a Pasteur pipette introduced at the posterior margin of the mantle cavity. This is essentially the procedure of Famme & Kofoed (1980). The medium was changed repeatedly during the first few hours after excising the gills. Leakage of amino acids induced by tissue damage declined during this initial period to very low levels, as shown by high-performance liquid chromatography (HPLC) of the bathing medium. Taurine concentrations were the highest and reached an average of $830 \pm 105\ \text{nmol}\,\text{l}^{-1}$ ($N = 5$) (mean ± S.D.) during a 90-min observation period. Alpha-amino acids were present in trace amounts of less than $10\ \text{nmol}\,\text{l}^{-1}$. These low levels did not interfere significantly with observations carried out on the preparation.

HPLC analysis of FAA was based on the procedure of Jones, Paabo & Stein (1981) with slight modifications to improve separation with our columns (Stephens &

Manahan, 1984; Davis & Stephens, 1984). A Beckman CPM-100 scintillation counter was used for estimates of radioactivity and samples were corrected for background and quenching as necessary.

Influx of amino acids supplied in ASW was measured both by radiochemical techniques using carbon-labelled compounds and by HPLC in selected cases. Substrate was supplied in 250 ml of aerated ASW in a closed system. In mussels which had their gills excised, the mantle cavity was irrigated throughout the observations. Preliminary experiments were performed to investigate the relationship between irrigation rate and influx rate. Influx rate of amino acids would be expected to be a hyperbolic function of perfusion rate, analogous to oxygen uptake (Jørgensen *et al.* 1986). All subsequent observations on uptake were made at an irrigation rate of $2{\cdot}5\,l\,h^{-1}$, which results in an influx rate that falls along the asymptote of the putative hyperbolic curve (Fig. 1). The irrigation rate of $2{\cdot}5\,l\,h^{-1}$ also falls within the range of normal laboratory pumping rates for animals of the size specified (Wright & Stephens, 1978).

Single $^{14}C$-labelled amino acids were supplied at an initial concentration of $5\,\mu mol\,l^{-1}$ in most experiments. Radioactivity was adjusted using mixtures of $^{12}C$ and $^{14}C$ substrate to give an activity of $10\,nCi\,ml^{-1}$. Disappearance of labelled carbon from the medium and its appearance in the tissues was followed radiochemically. All rate calculations were based upon medium depletion of radiolabel.

In experiments to test interactions between pairs of substrates, a labelled amino acid was supplied at a concentration of $5\,\mu mol\,l^{-1}$. Its initial rate of influx was followed radiochemically and then $400\,\mu mol\,l^{-1}$ of a competing substrate was added to the medium. Rates of influx before and after the addition of the competing

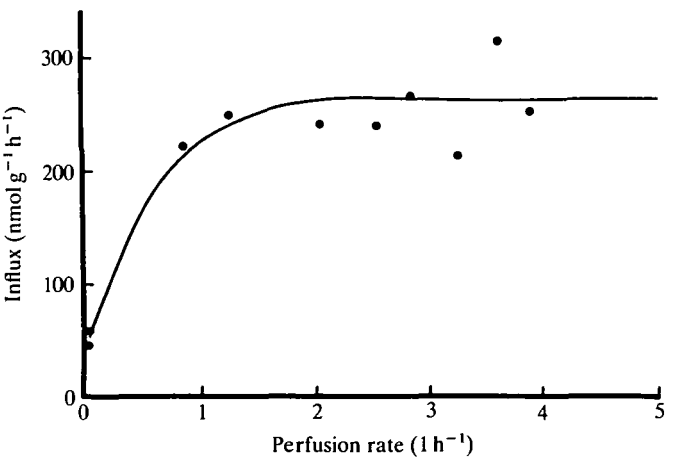


Fig. 1. Influx of $10\,\mu mol\,l^{-1}$ alanine as a function of perfusion rate of artificial sea water (ASW). *Mytilus edulis* (approximately 9 g each) with gills excised were placed in 250 ml of ASW containing radiolabelled alanine. Influx rates were determined by medium depletion of radiolabel over a 20-min period. Influx of alanine into non-perfused mussels was observed. This may be a result of ASW circulation in the experimental vessel by aeration. Line fitted by a nonlinear least-squares regression (Duggleby, 1981).

substrate were calculated based on assumed first-order depletion coefficients of radioactivity in the medium before and after the addition of the competing substrate. The medium was sampled at 5-min intervals. The assumed first-order depletion was independently verified in control observations of disappearance of label from the medium and net disappearance of substrate measured by HPLC.

For comparison of the relative contribution to amino acid uptake of the gills and other tissues lining the mantle cavity, animals of the specified size were selected and their adductor muscles were cut as described for animals whose gills were surgically removed. They were perfused at the same rate and the disappearance of added amino acid substrates was followed by scintillation counting.

Estimation of tissue levels of FAA was carried out as follows. Mussels were incubated in 5 $\mu$mol $l^{-1}$ labelled alanine for 2 h and the depletion of radioactivity in the medium was monitored. Activity in the tissues was determined by extraction and subsequent scintillation counting. Samples of the haemolymph were taken from the base of the foot and samples of other tissues were dissected and minced. Possible leakage of the external medium into the haemolymph during the experimental manipulations was estimated by incubating mussels for 30 min in ASW containing $^{14}$C-labelled inulin. Samples of haemolymph were then collected for scintillation counting. The radioactivity appearing in the haemolymph in comparison with known volumes of ASW was taken as an estimate of leakage of the external medium into the haemolymph. For tissues other than haemolymph, extracellular space was estimated in control observations by injection of $^{14}$C-labelled inulin into the base of the foot and measurement of radioactivity later. Extracellular volume was estimated by comparing levels of radioactivity in the tissues with those in known volumes of haemolymph as an approximation of tissue volume. Free amino acids were extracted from the tissues (haemolymph, adductor muscles, foot, mantle and gills) in 5% trichloroacetic acid made up in 50% ethanol. Estimates were corrected for intercellular haemolymph activity on the basis of the inulin estimates of extracellular space. The FAA in the tissue pools were measured by HPLC analysis of extracts of tissues dissected from control animals and corrected for intercellular space.

Estimates of the area of epithelia lining the mantle cavity were made as follows. An outline of one valve was traced on square-ruled paper, and the enclosed area was calculated and multiplied by two. This provisional estimate of valve area can be described by the linearized allometric equation $\log A = \log a + b \log W$, when A is area in $mm^2$ and W is wet mass of the soft tissues in g. The allometric parameters $a = 966{\cdot}0$ and $b = 0{\cdot}642$ were calculated using animals ranging in mass from 0·49 to 10·44 g. The coefficient of correlation for the least-square regression line was $r = 0{\cdot}97$ ($N = 40$). The curvatures of the major and minor axes of the shell were estimated by use of a flexible measuring tape. Corrections based on this curvature added $12{\cdot}8 \pm 2{\cdot}8$% (mean ± S.D., $N = 14$) to the area of the provisional estimate. The adductor muscles were cut, the visceral mass flattened and positioned dorsally by gentle stretching of the free mantle tissues, and the area occupied by the visceral mass was estimated from photographs. This reduced the epithelial area estimate by $12{\cdot}6 \pm 0{\cdot}9$% (mean ± S.D., $N = 5$). Finally, the visceral mass was allowed to hang

freely in the mantle cavity and photographed in this position. Its area in this position was estimated from photographs, multiplied by two, and added to the provisional estimate. This increased the total area by 34·9 ± 5·5 % (mean ± S.D., $N = 9$). Thus the area of the epithelial tissues lining the mantle cavity is 135·1 ± 6·2 % (S.D.; based on variance of sums rule) of the initial area calculated from the tracing. This area (approximately 2·7 times the projected area of one valve) was compared with estimates for the area of the surface of gill filaments available in the literature (Möhlenberg & Riisgard, 1979; Jørgensen, 1983).

## RESULTS

If mussels are carefully prepared, the unidirectional influx of alanine across the non-gill epithelia adequately reflects the net flux of alanine (Fig. 2). The HPLC data used to calculate the net flux of alanine indicated a modest loss of taurine from the animal. The final concentration of taurine in the medium was less than 1 $\mu$mol $l^{-1}$.

Table 1 presents data comparing the influx rates of alanine, cycloleucine and taurine into mussels with and without gills. The initial concentration of each of these substances was 5 $\mu$mol $l^{-1}$. The rate of influx was calculated at this concentration and expressed as $\mu$mol $h^{-1}$. Rates of entry into mussels with gills were not significantly different for the three substrates. Entry rates into mussels from which the gills had been removed were not significantly different for alanine and cycloleucine perfused

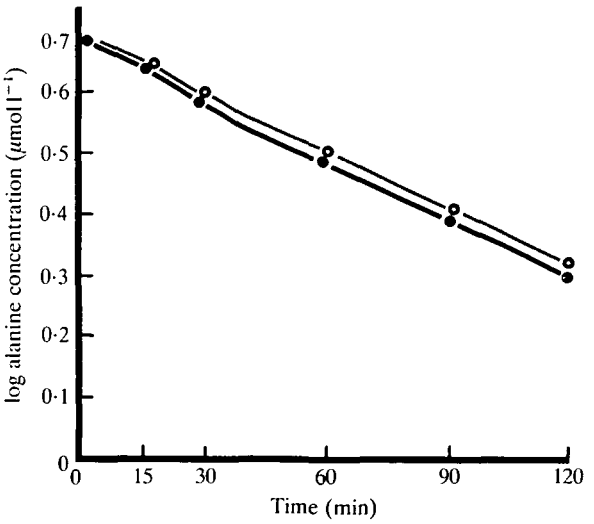


Fig. 2. Medium depletion of radiolabel and total alanine. *Mytilus edulis* (approx. 6 g) were placed in 250 ml of ASW containing 5 $\mu$mol $l^{-1}$ of radiolabelled alanine. Depletion of radiolabel and alanine were monitored by scintillation counting (closed dots and solid lines) and HPLC (stars and hatched lines), respectively. The rate of unidirectional influx of alanine, based on depletion of radiolabel, was 96·3 nmol $g^{-1}$ $h^{-1}$. The net influx of alanine, based on HPLC, was 91·7 nmol $g^{-1}$ $h^{-1}$. The correlation coefficients of the regressions used to calculate these values were each in excess of $r = 0·998$. Data are the means of duplicate determinations.

at the same flow rate ($N = 5$) (Fig. 3; Table 1). However, the entry of taurine into the group with gills excised is faster and significantly different from that observed with other substrates ($P < 0{\cdot}05$; $N = 16$).

Table 2 compares the distribution of alanine and cycloleucine in internal tissues of operated and sham-operated mussels after incubation in labelled substrate for 2 h at an initial concentration of 5 $\mu$mol $l^{-1}$. There is no significant difference in the distribution of radioactivity in tissues other than in the gills between the two groups. In the case of alanine, approximately 20% of the radioactivity appears in the six animals in internal tissue other than the gill. In the case of cycloleucine, the comparable figure is approximately 31%.

Radioactivity after incubation in labelled cycloleucine is significantly higher in the haemolymph ($P < 0{\cdot}05$) and foot tissues ($P < 0{\cdot}05$). This may reflect differences in

Table 1. *The rates of influx of various amino acids into mussels with gills intact and gills excised*

| Amino acid (5 $\mu$mol $l^{-1}$) | Influx rate ($\mu$mol $h^{-1}$ ± S.E.M.) Gills intact | Gills excised | %* |
|---|---|---|---|
| Alanine ($N = 5$) | 2·03 ± 0·27 | 0·64 ± 0·12 | 31·6 |
| Cycloleucine ($N = 5$) | 1·66 ± 0·31 | 0·43 ± 0·18 | 25·7 |
| Taurine ($N = 16$) | 1·72 ± 0·12 | 1·27 ± 0·10 | 73·8 |

Mussels were chosen so that external dimensions of the valves were approximately equal.
The wet mass for the soft tissues of each of the mussels was approximately 6 g.
*The rate in mussels with excised gills as a percentage of the rate in mussels with intact gills.

Table 2. *The distribution of alanine and cycloleucine in various tissues of mussels with gills intact and gills excised*

| Tissue | Gills intact | Gills excised |
|---|---|---|
| Distribution of alanine (nmol $g^{-1}$ ± S.E.M. $N = 6$) | | |
| Haemolymph | 12·93 ± 4·90 | 14·37 ± 4·35 |
| Mantle | 49·94 ± 3·05 | 53·00 ± 4·62 |
| Foot | 46·93 ± 14·53 | 54·63 ± 6·26 |
| PAM* | 11·86 ± 3·68 | 10·57 ± 3·19 |
| Gills | 550·62 ± 129·1 | |
| Distribution of cycloleucine (nmol $g^{-1}$ ± S.E.M. $N = 4$) | | |
| Haemolymph | 75·87 ± 4·50 | 62·32 ± 9·58 |
| Mantle | 55·64 ± 5·32 | 67·70 ± 13·62 |
| Foot | 124·48 ± 21·08 | 150·93 ± 30·78 |
| PAM* | 19·47 ± 6·14 | 13·61 ± 8·02 |
| Gills | 610·36 ± 125·50 | |

Units are nmol $g^{-1}$ tissue, except for haemolymph which are nmol $ml^{-1}$.
Results of Student's $t$-tests indicate no significant differences between the distribution of amino acids in non-gill tissues of mussels with gills intact and gills excised.
*PAM, posterior adductor muscle.

the affinity of transport carriers in the various tissues for the two substrates or possibly some tissue oxidation of alanine in the foot/byssus region. These possibilities are consistent with, but are not directly supported by, the data and were not pursued further.

The average area of the non-gill epithelia of 6-g mussels used in these experiments was approximately 40 $cm^2$. The total area of gill filaments was calculated to be 105 $cm^2$, based upon estimates available in the literature (Möhlenburg & Riisgard, 1979; Jørgensen, 1983). Thus, the total epithelial area is 145 $cm^2$ and the non-gill area as a percentage of the total area is 27·6 %.

In Fig. 4A, the initial entry of alanine estimated from the first three measurements of radioactivity in the medium (0, 5, 10 min) is compared with the rate of disappearance subsequent to the addition of 400 $\mu mol\,l^{-1}$ phenylalanine. In this case, the entry rate of labelled alanine was reduced to 3 % of the initial rate. Fig. 4B

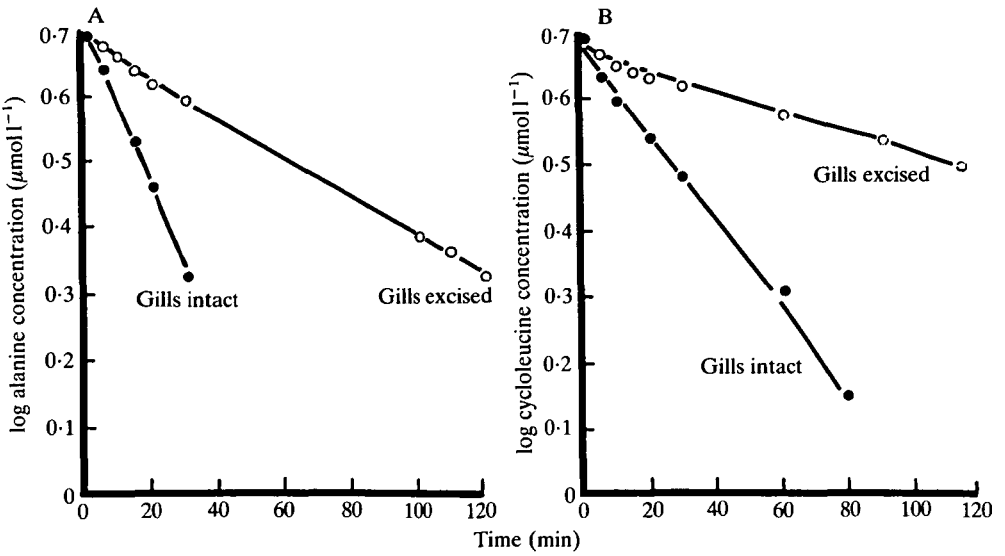


Fig. 3. *Mytilus edulis* with posterior adductor muscles cut were placed into 250 ml ASW and perfused at 2·5 l $h^{-1}$. (A) An example of the data collected showing influx of alanine from an initial concentration of 5 $\mu mol\,l^{-1}$. A mussel with gills intact was compared with one with gills excised. Medium depletion follows first-order kinetics. Depletion of 5 $\mu mol\,l^{-1}$ alanine by mussels with intact gills is described by a depletion constant of −0·027785 $min^{-1}$; the correlation coefficient of the linear regression is $r = 0{\cdot}9991$. The depletion constant for alanine in the case of mussels with gills excised is −0·007016 $min^{-1}$ with a correlation coefficient of $r = 0{\cdot}9993$. (B) An example of influx of cycloleucine from an initial concentration of 5 $\mu mol\,l^{-1}$. The depletion constant for cycloleucine in the mussel with intact gills is −0·01514 $min^{-1}$ with a correlation coefficient of $r = 0{\cdot}9968$; in the case of cycloleucine depletion by the mussel with gills excised, the depletion constant is −0·003636 $min^{-1}$ and the correlation coefficient is $r = 0{\cdot}9891$. All mussels used in these experiments had the same exterior valve dimensions and had an approximate wet mass of 6 g (valves excluded). In these examples, the influx into mussels with gills excised as a percentage of that into mussels with gills intact is 25·25 % for alanine, and 24·1 % for cycloleucine.

illustrates the result of adding 400 $\mu$mol $l^{-1}$ taurine. The resulting change in the rate of alanine uptake is negligible. The presence of 400 $\mu$mol $l^{-1}$ taurine influences the entry rates of aspartate, alanine, glycine, lysine and proline marginally, if at all (Table 3). The presence of 400 $\mu$mol $l^{-1}$ concentrations of these substrates reduces entry of taurine to about 50% of control rates. Entry of labelled taurine at a concentration of 400 $\mu$mol $l^{-1}$ is only a few percent of its entry rate at 5 $\mu$mol $l^{-1}$, indicating saturation of the carrier (or carriers) concerned with its entry at the higher substrate concentration.

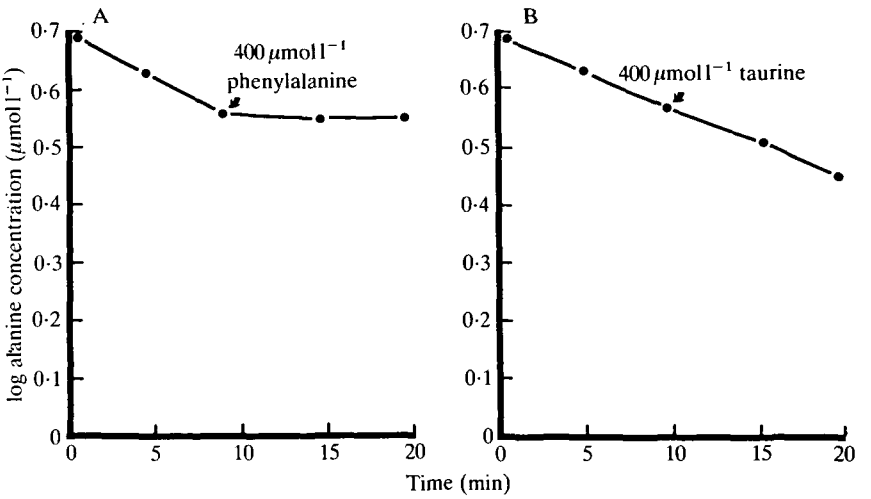


Fig. 4. *Mytilus edulis* with gills excised were placed into 250 ml of ASW and perfused at 2·5 l $h^{-1}$. (A) Influx of alanine from an initial concentration of 5 $\mu$mol $l^{-1}$. At 10 min, phenylalanine was added to the medium to give a final concentration of 400 $\mu$mol $l^{-1}$. An inhibition of 97% was observed. (B) Influx of alanine from an initial concentration of 5 $\mu$mol $l^{-1}$. However, upon addition of 400 $\mu$mol $l^{-1}$ taurine there was no observed inhibition. Mussels used in this example weighed approximately 9 g.

Table 3. *Partial inhibition of the influx of amino acids by taurine*

| Amino acid (5 $\mu$mol $l^{-1}$) | % inhibition by 400 $\mu$mol $l^{-1}$ taurine (mean ± S.E.M., $N = 3$) | Inhibitor (400 $\mu$mol $l^{-1}$) | % inhibition of influx of 5 $\mu$mol $l^{-1}$ taurine (mean ± S.E.M., $N = 3$) |
|---|---|---|---|
| Aspartate | 10·66 ± 5·33 | aspartate | 52·41 ± 5·54 |
| Alanine | 17·90 ± 10·83 | alanine | 62·03 ± 4·83 |
| Glycine | 14·43 ± 7·73 | glycine | 51·50 ± 3·69 |
| Lysine | 20·20 ± 7·94 | lysine | 62·09 ± 5·17 |
| Proline | 24·56 ± 9·31 | proline | 55·42 ± 3·63 |
| Taurine | 98·30 ± 1·48 | | |

Influx of 5 $\mu$mol $l^{-1}$ taurine was 'self inhibited' by the presence of 400 $\mu$mol $l^{-1}$ taurine.
Influx of 5 $\mu$mol $l^{-1}$ taurine was moderately inhibited by other amino acids.
Percent inhibition was calculated from the ratio of first-order depletion constants before and after the addition of the potential inhibitor to the medium.

Table 4. *Free alanine content of the various tissues determined by HPLC after 24 h extraction with 5 % trichloroacetic acid in 50 % ethanol*

| Tissue | Extractable alanine content (nmol $g^{-1}$ ± S.E.M., $N = 6$) Starved | 2-h incubation in 5 $\mu$mol $l^{-1}$ alanine |
|---|---|---|
| Gills | 1324·7 ± 694·9 | 2043·8 ± 760·7 |
| Mantle | 1319·2 ± 635·9 | 1397·3 ± 347·9 |
| Haemolymph | 127·7 ± 36·5 | 134·8 ± 30·7 |

None of the differences are statistically significant.

Table 4 presents data concerning the modification of FAA pools after a 2-h incubation of mussels in 5 $\mu$mol $l^{-1}$ alanine. There was no significant change in the alanine content of any of these tissues. This is not unexpected, since the predicted increase based on distribution of labelled alanine would not be significant in comparison with the baseline variability of alanine within the FAA pools. We observed considerable quantitative variability in the contribution of all of the constituent amino acids in our analyses of the FAA pools of *Mytilus* by HPLC.

## DISCUSSION

What is the general physiological state of the mussels with their adductor muscles cut and gills removed? Jørgensen (1975) reports that mussels recovered soon after the posterior adductor muscle had been severed, embyssed on the glass wall of an observation aquarium and moved around in a normal fashion.

The data suggest that the amino acid carriers responsible for the transport of alanine and cycloleucine are similarly distributed between the gill and non-gill epithelia (Table 1). They also suggest that there is a greater proportion of taurine carriers in the non-gill epithelia. If we use the estimate of the area of the non-gill epithelium as a percentage of the total surface area of the external epithelia, 27·6 %, and compare that figure with the rate of amino acid transport by mussels without gills as a percentage of transport by intact mussels (Table 1), we can conclude that the alpha-amino acid carriers responsible for transporting alanine and cycloleucine are distributed roughly equally within the gill and non-gill epithelia. Using the estimates for the surface area of the gill (105 $cm^2$) and non-gill (40 $cm^2$) epithelia, it is possible to calculate the area-specific transport rates for taurine. Using the influx rates for taurine from mussels with gills excised and gills intact (Table 1), the influx rate into gills is 4·3 nmol $cm^2$ $h^{-1}$, and into the non-gill epithelia the rate is 31·8 nmol $cm^2$ $h^{-1}$. Thus, the taurine carrier density on the non-gill epithelia is 7·4 times greater than on the gills. Jørgensen (1983) observed a qualitatively similar phenomenon with a different population of mussels of similar size, although he did not draw this conclusion from his data. In his material, the mantle surface represented 14·5 % of the total surface area (gill + mantle); approximately 32 % of the taurine entered *via* the mantle.

The physiological significance of a high taurine carrier density on the non-gill epithelial surface is unknown. Taurine is not normally found in significant concentrations in natural waters but is found in very high concentrations ($>100\,mmol\,l^{-1}$) in *Mytilus* tissues. In addition, the taurine carriers in the gill are different from the alpha-amino acid carriers in that they are highly specific for taurine. From these observations, Wright & Secomb (1984) have postulated that taurine carriers in the gills of *Mytilus* act to recapture substrate lost by diffusion. The taurine carriers in the non-gill epithelia may also act in this way. However, there is an apparent difference between the amino acid specificities of taurine carriers in the gill and non-gill epithelia. Wright & Secomb have shown very clearly that taurine carriers in the gill are highly specific for taurine. Our data suggest that high concentrations of alpha-amino acids result in significant inhibition of taurine influx (Table 3). The taurine carriers of mammalian heart (Huxtable, Laird & Lipincott, 1979) and mussel gills (Wright & Secomb, 1984) have different inhibition characteristics (particularly with respect to the taurine analogue guanidinoethyl sulphonate). However, there has been no description of a difference of carrier specificities in tissues of a single species. Additional work is necessary to elucidate the function of the apparent differentiation and differential distribution of taurine carriers between the gill and non-gill epithelia of *Mytilus*.

The data presented in Tables 1 and 2 can be used to relate total influx of amino acid to its distribution. The amount of alanine transported in 2 h is $1189{\cdot}1 \pm 22{\cdot}8$ nmol (S.E.M.) by mussels with gills intact and $768{\cdot}0 \pm 94{\cdot}9$ nmol (S.E.M.) by mussels with gills excised, which is $64{\cdot}5 \pm 9{\cdot}0\,\%$ of the amount transported by mussels with intact gills. The amount of cycloleucine transported in 2 h is $1129{\cdot}2 \pm 32{\cdot}5$ nmol (S.E.M.) by animals with intact gills and $514{\cdot}4 \pm 135{\cdot}9$ nmol (S.E.M.) by animals with gills excised, which is $45{\cdot}5 \pm 13{\cdot}8\,\%$ of the amount transported by mussels with intact gills. If we assume that the mass of gill tissue from a 6-g mussel is 800 mg, the ratio of amino acid distribution between the gill and non-gill tissue can be estimated. The alanine in the gills (Table 2), $550{\cdot}6\,nmol\,g^{-1}$, multiplied by 800 mg gives us a total of 440·5 nmol. The alanine in the non-gill tissue would be the total alanine influx in 2 h minus the content of the gills, or $1189{\cdot}1 - 440{\cdot}5 = 748{\cdot}6$ nmol. Thus the uptake of alanine by the non-gill tissue as a percentage of the total uptake would be $63{\cdot}0\,\%$. A similar calculation using the data for cycloleucine influx and the content of cycloleucine in the gill gives 488·3 nmol in the gill, 640·9 nmol in the non-gill tissue, with a non-gill/total uptake percentage of $56{\cdot}7\,\%$. The ratio of amino acid uptake by the non-gill tissue to the total uptake of amino acids is therefore quite comparable based upon either gill removal experiments or subtracting the amino acid content of the gill. These ratios based on influx over a 2-h period are considerably higher than the ratios presented in Table 1, which are based upon first-order depletion constants.

The removal of the gills does not affect the distribution of alanine or cycloleucine within the non-gill tissues of mussels (Table 2). This result suggests that there is very little, if any, transfer of amino acids from the gill to other tissues. These findings are analogous to the findings of Famme & Kofoed (1980) who reported that gill removal does little to diminish the oxygen consumption. Borradaile, Potts & Eastham (1935)

speculated that there would be limited exchange between the gills and other tissues of *Mytilus*. Their argument was based on the observation that the haemolymph vessel diameter in the gills is very much less than the vessel diameter in the mantle, so that circulation would be much less in the gills. Jørgensen (1983) found that very little tritiated taurine was exported from the gills between 1 and 24 h after exposure to the labelled substrate. Thus it appears that amino acids taken up by the gill are exported to other tissues extremely slowly.

Removal of the gills does not modify the rate of appearance of radiolabelled alanine or cycloleucine in the haemolymph (Table 2). These observations suggest that the amino acids transported by the non-gill epithelia are rapidly distributed to internal tissues. In a very rough fashion, the distribution of alanine among the various tissues follows the distribution of the non-metabolizable amino acid analogue cycloleucine. This suggests that over the 2-h observation period, the majority of the alanine remains in a free form. There is, however, very indirect evidence for some oxidation of the alanine substrate. There was no attempt to collect radiolabelled carbon dioxide. However, there was 24·1 % less alanine than cycloleucine present in the tissues of mussels with intact gills (Table 2), although the total entry of alanine over the 2-h period was slightly higher (Table 1). Likewise, there was 55·0 % less substrate present in mussels with gills excised. Thus the differences between the contents of alanine and cycloleucine may possibly reflect tissue oxidation. However, the large variation in the baseline FAA pool makes this a tentative suggestion.

In conclusion, the major findings of this study are as follows. There is limited short-term transfer of amino acids from the gill to non-gill tissues. Amino acids transported *via* the non-gill epithelia are rapidly transferred to subepithelial tissues. There is evidence that alpha-amino acid carriers are approximately equally distributed on the gill and non-gill epithelia. However, the taurine carriers per unit area on the non-gill epithelial surface exceed those on the gill. Finally, evidence is presented for differential sensitivity of taurine transporters in the non-gill epithelium to inhibition by alpha-amino acids.

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## REFERENCES

BORRADAILE, L. A., POTTS, F. A. & EASTHAM, L. E. S. (1935). *The Invertebrata: A Manual for Use by Students*. Cambridge: Cambridge University Press.

CAVANAUGH, G. M. (ed.) (1956). *Formulae and Methods IV, of the Marine Biological Laboratory Chemical Room*. Woods Hole, MA: Marine Biological Laboratory.

DAVIS, J. P. & STEPHENS, G. C. (1984). Uptake of free amino acids by bacteria-free larvae of the sand dollar, *Dendraster excentricus*. *Am. J. Physiol.* **247**, R733–R739.

DUGGLEBY, R. G. (1981). A non-linear regression program for small computers. *Analyt. Biochem.* **110**, 9–18.

FAMME, P. (1980*a*). Effect of shell valve closure by the mussel *Mytilus edulis* L. on the rate of oxygen consumption in declining oxygen tension. *Comp. Biochem. Physiol.* **67**A, 167–170.

FAMME, P. (1980*b*). Oxygen-dependence of the respiration by the mussel, *Mytilus edulis* L., as a function of size. *Comp. Biochem. Physiol.* **67**A, 171–174.

FAMME, P. (1981). Haemolymph circulation as a respiratory parameter in the mussel, *Mytilus edulis* L. *Comp. Biochem. Physiol.* **69**A, 243–247.

FAMME, P. & KOFOED, L. H. (1980). The ventilatory current and ctenidial function related to oxygen uptake in declining oxygen tension by the mussel, *Mytilus edulis* (L.). *Comp. Biochem. Physiol.* **66**A, 161–171.

GOMME, J. (1982). Laminar water flow, amino acid absorption, and amino acid recycling in the mussel gill. *Am. Zool.* **22**, 898 (Abstr.).

GOMME, J. (1985). The transport of exogenous organic substances by invertebrates. In *Comparative Physiology and Biochemistry: Current Topics and Trends:* vol. B, *Transport, Iono and Osmoregulation* (ed. R. Gilles & M. Gilles-Bailien). Berlin: Springer-Verlag.

HUXTABLE, R. J., LAIRD, H. E. & LIPINCOTT, S. E. (1979). The transport of taurine in the heart and the rapid depletion of tissue taurine content by guanidinoethyl sulfonate. *J. Pharmac. exp. Ther.* **211**, 465–471.

JONES, B. N., PAABO, S. & STEIN, S. (1981). Amino acid analysis and enzymatic sequence determination of peptides by an improved *o*-phthaldialdehyde precolumn labeling procedure. *J. Liq. Chromatog.* **4**, 565–586.

JØRGENSEN, C. B. (1975). On gill function in the mussel *Mytilus edulis*. *Ophelia* **13**, 187–232.

JØRGENSEN, C. B. (1983). Patterns of uptake of dissolved amino acids in mussels, *Mytilus edulis*. *Mar. Biol.* **73**, 177–182.

JØRGENSEN, C. B., MÖHLENBERG, F. & STEN-KNUDSEN, O. (1986). Nature of relation between ventilation and oxygen in filter feeders. *Mar. Ecol. Prog. Ser.* **29**, 73–88.

MANAHAN, D. T., WRIGHT, S. H., STEPHENS, G. C. & RICE, M. A. (1982). Transport of dissolved amino acids by the mussel, *Mytilus edulis*: demonstration of net uptake from seawater by HPLC analysis. *Science* **215**, 1253–1255.

MÖHLENBERG, F. & RIISGARD, H. U. (1979). Filtration rate, using a new indirect technique in thirteen species of suspension-feeding bivalves. *Mar. Biol.* **54**, 143–147.

PEQUIGNAT, E. (1973). A kinetic and autoradiographic study of direct assimilation of amino acids and glucose by organs of the mussel, *Mytilus edulis*. *Mar. Biol.* **19**, 227–244.

SIEBERS, D. & WINKLER, A. (1984). Amino acid uptake by mussels, *Mytilus edulis*, from natural seawater in a flow-through system. *Helgolander Meeresunters* **38**, 189–199.

STEPHENS, G. C. (1983). Dissolved organic material and the nutrition of marine bivalves. In *Proceedings of the 2nd International Conference on Aquaculture Nutrition* (ed. G. D. Pruder, C. J. Langdon & D. E. Conklin), pp. 338–357. Louisiana State University, Baton Rouge: World Aquaculture Society.

STEPHENS, G. C. & MANAHAN, D. T. (1984). Technical advances in the study of nutrition of marine mollusks. *Aquaculture* **39**, 155–164.

WRIGHT, S. H. (1982). A nutritional role for amino acid transport in filter-feeding marine invertebrates. *Am. Zool.* **22**, 621–634.

WRIGHT, S. H. (1985). Mechanism and physiological role of epidermal amino acid transport in marine bivalves. In *Comparative Physiology and Biochemistry: Current Topics and Trends:* vol. B: *Transport, Iono and Osmoregulation* (ed. R. Gilles & M. Gilles-Bailien). Berlin: Springer-Verlag.

WRIGHT, S. H. & SECOMB, T. W. (1984). Epidermal taurine transport in marine mussels. *Am. J. Physiol.* **247**, R346–R355.

WRIGHT, S. H. & SECOMB, T. W. (1986). Epithelial amino acid transport in marine mussels; role in net exchange of taurine between gills and seawater. *J. exp. Biol.* **121**, 251–270.

WRIGHT, S. H. & STEPHENS, G. C. (1978). Removal of amino acid during a single passage of water across the gill of marine mussels. *J. exp. Zool.* **205**, 337–351.