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EPITHELIAL AMINO ACID TRANSPORT IN MARINE MUSSELS: ROLE IN NET EXCHANGE OF TAURINE BETWEEN GILLS AND SEA WATER

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SUMMARY

The exchange of taurine across epidermal epithelia of *Mytilus edulis* and *M. californianus* was studied using radiotracer and chromatographic (HPLC) methods. Gill levels of taurine in both species ranged from 60 to 70 μmol g^{-1} wet weight. Net uptake of taurine, determined using HPLC, occurred down to ambient concentrations as low as 10 nmol l^{-1}. The rate of taurine loss from mussels was about 0·02 μmol g^{-1} wet body weight h^{-1}, and when exposed to amino-acid-free sea water, external taurine concentration increased until a steady-state of about 15 nmol l^{-1} was achieved. Mussels accumulated inhibitors of taurine transport at rates which were directly related to their relative inhibitory capacities: β-alanine > β-aminobutyric acid $\simeq$ γ-aminobutyric acid (GABA). Addition of large concentrations (50–200 μmol l^{-1}) of GABA resulted in a rapid increase in taurine concentration in test solutions. This increase was consistent with a model in which GABA both competitively inhibits the reaccumulation of endogenous taurine lost from epidermal tissues, and accelerates the exchange diffusion of taurine from surface cells. We suggest that epidermal taurine transport in *Mytilus* assists in the maintenance of large intracellular taurine concentrations, and can serve to reaccumulate up to 30 % of the taurine lost from surface tissues by passive processes.

INTRODUCTION

Taurine is the principal constituent of the free amino acid pool in tissues of *Mytilus edulis* and a number of other marine bivalves. The concentration of taurine in the epidermal tissues of the gill and mantle is about 50–100 nmol g^{-1} fresh weight, with the total free pool of amino acid being about 75–150 nmol g^{-1} (Lange, 1963; Zurburg & de Zwaan, 1981). The total concentration of amino acids in near-shore waters is micromolar, or less (Braven, Evans & Butler, 1985), which leads to a large chemical gradient favouring loss of amino acid to surrounding sea water. Such a loss has been suggested to constitute a significant fraction of the total nitrogen excreted by mussels (e.g. Hammen, 1968; Bayne, 1973), and also to represent an important caloric loss under certain circumstances (Bayne, 1973; Bayne & Scullard, 1977).

Key words: amino acid transport, *Mytilus*, gills, epithelia.

The epidermal tissues of marine mussels can actively accumulate amino acids, including taurine, from dilute external solution (Manahan, Wright & Stephens, 1983; Jørgensen, 1983). Similar processes appear to be widespread among soft-bodied marine invertebrate phyla, and there has been considerable speculation on their role in whole animal nutrition (see Stephens, 1968, 1982), and, more recently, in reducing the loss of endogenous compounds from surface cells to surrounding sea water (Gomme, 1981*a*). We recently provided evidence in support of the latter phenomenon in the mussels, *M. edulis* and *M. californianus* (Wright & Secomb, 1984). Gill tissue from these animals has a transport process with a high degree of structural specificity for taurine and closely related structural analogues. A mathematical model of epidermal transport in the actively pumping gill was introduced and used to determine the kinetics of taurine uptake. The results of the model supported the idea that epidermal transport can reduce taurine loss from mussels.

The present study examines the exchange of taurine across the apical membrane of surface epithelial cells in *Mytilus*. We used chromatographic procedures to monitor net fluxes of taurine and related compounds into mussels, and to measure rates of taurine loss from these animals. The results indicate that taurine is lost from mussels, though at a lower rate than that reported in earlier studies. We also provide direct evidence that the epidermal transport pathway significantly reduces the rate of this loss from surface cells, and suggest that the transport process plays an important role in maintaining the high intracellular concentrations of taurine that are characteristic of the gill and mantle.

MATERIALS AND METHODS

Animals

Mussels (*M. edulis* and *M. californianus*) were purchased from commercial sources (Sea Life Supply, Monterey, California; Bodega Bay Marine Laboratory, Bodega Bay, California), and maintained in refrigerated aquaria (12°C) containing circulating, filtered artificial sea water (Instant Ocean). The mussels were not fed, and were used within 6 weeks of collection. The mussels used for intact animal studies were held in a room temperature (23°C) aquarium containing filtered artificial sea water for at least 24 h, but for less than 7 days, before being used. The valves of these animals were scraped free of encrusting organisms with a wire brush, and thoroughly washed with flowing distilled water.

Uptake experiments with intact mussels

The general protocol used for studies with intact animals was as follows. A mussel was placed into a 0·5-l plastic container filled with artificial sea water prepared from reagent grade salts (ASW; Cavanaugh, 1956). Usually, the animal quickly opened and began pumping activity in this 'pre-equilibration' solution. The medium was vigorously mixed by a stream of bubbles from an airstone. Uptake periods were begun by rapidly removing the pre-equilibration solution and adding a 'test solution' containing the desired concentration of amino acid substrate. Samples of the test solution were collected at appropriate intervals and assayed for their content of either

radiolabelled substrate, or chemically determined substrate, or both. In some experiments, rates of uptake were estimated by assuming that the disappearance of substrate was first-order in nature. This procedure was justified because the substrate concentrations used in these experiments ($< 260\,\text{nmol}\,l^{-1}$) were much less than the apparent Michaelis constants for transport of the compounds under study ($1{\cdot}5–50\,\mu\text{mol}\,l^{-1}$), and thus were within the nearly linear portion of the Michaelis–Menten substrate–velocity relationship.

Analysis of amino acids using high performance liquid chromatography (HPLC)

The procedure used was a modification of that described by Lindroth & Mopper (1979), and is described in detail elsewhere (Manahan *et al.* 1983). It involved derivatization of amino acids with *o*-phthaldialdehyde (OPA) to form fluorescent products that were separated on a reverse phase C-18 5-μm column (ODS; Beckman) and detected by a fluorescence monitor (Gilford). Individual amino acids were identified and quantified by comparison of retention times and peak areas (determined with a Spectraphysics recording integrator) to those of appropriate standards; the reproducibility of standard retention times and peak areas was better than 5 %. The detection limit for taurine, β-alanine (BALA), β-aminobutyric acid (BABA), and γ-aminobutyric acid (GABA) was approximately 0·7 pmol, corresponding to a concentration of $4\,\text{nmol}\,l^{-1}$ taurine in experimental samples; the detection limit for alanine, aspartate, glycine and serine (besides taurine, the major constituents of the free amino acid pool of *Mytilus* epidermal tissues) was approximately $1\,\text{nmol}\,l^{-1}$.

Preliminary studies revealed that taurine concentrations in both standards and experimental samples decreased rapidly with time regardless of the holding procedures used. Therefore, fresh standards were prepared every week, and experimental sea water samples were analysed as quickly as possible after collection (usually immediately, always within 2 h); 80 % ethanol extracts of gill tissue were stored for up to 2 weeks, and diluted with distilled water immediately prior to HPLC analysis. Because of the length of time for individual sample analysis, most of the results reported here were derived from analysis of single samples. However, when duplicate experimental samples were analysed, the results usually varied by less than 5 %.

Chemicals

[^{14}C]taurine (about $100\,\text{mCi}\,\text{mmol}^{-1}$) was purchased from Amersham. OPA was purchased from Cal-Biochem. All other chemicals were acquired from standard sources and were the highest grade available.

RESULTS

Amino acid content of Mytilus *gill tissue*

The total ethanol-extractable pool of amino acid in tissue from *M. edulis* and *M. californianus* was about $80\,\mu\text{mol}\,g^{-1}$ wet weight of tissue (Table 1). Approximately 99 % of this total consisted of five amino acids: taurine ≫ aspartate >

glycine ≃ alanine ≃ glutamate. In both species, taurine is the major constituent of the extractable pool (82 and 89 % for *M. edulis* and *M. californianus*, respectively).

Evidence for a loss of taurine from intact mussels

The taurine content of the gill tissue was consistent with an intracellular concentration in excess of 60 mmol l^{-1} cell water. Thus, there is a large concentration gradient in favour of a loss of taurine to the sea water that flows past the surfaces of the gill. When mussels were placed in amino-acid-free ASW, taurine often did appear, rising in concentration for 30–60 min until a 'steady-state' concentration, $[Tau]_{ss}$, was achieved. The value of $[Tau]_{ss}$ varied widely between individual mussels. For *M. edulis*, the range was from <4 nmol l^{-1} (i.e. undetectable) to 74 nmol l^{-1}, with an average of 14·3 ± 3·7 nmol l^{-1} (S.E., $N = 26$). For *M. californianus*, the range was from <4 nmol l^{-1} to 51 nmol l^{-1}, with an average of 21·1 ± 6·9 ($N = 6$). Because a subthreshold level of taurine was often noted even when it was quantitatively 'undetectable', we elected to assign a value of 4 nmol l^{-1} for $[Tau]_{ss}$ in those cases where the level was below the limit of taurine detection. The cause of the inter-animal variability for $[Tau]_{ss}$ is not clear, but it probably reflects differences in the rate of efflux of taurine from epidermal tissues rather than differences in the kinetics of taurine influx; inter-animal variability for amino acid uptake rates as measured using radioactively labelled substrates is small (S.D. 25 % or less; e.g. Wright & Secomb, 1984), compared to the variations in $[Tau]_{ss}$ noted above. It should be emphasized, however, that the value for $[Tau]_{ss}$ was very reproducible for any given animal. For example, four consecutive 1-h incubations of an intact *M. californianus* resulted in values for $[Tau]_{ss}$ of 36·1, 56·0, 61·2 and 51·2 nmol l^{-1}. After 14 h in a 500-ml test solution, the taurine concentration was 28·1 nmol l^{-1}.

It is also worth noting that, though taurine was often observed to be lost from mussels, the other major amino acids of the extractable pool showed no predictable pattern of loss from intact animals. Serine, for example, was often apparent as a contaminant in our nominally 'amino-acid-free' ASW, and was found in concentrations ranging from approximately 1 to 10 nmol l^{-1}. However, after exposure to an intact, actively pumping mussel, the concentration of serine (or other 'contaminants' present at time zero) often declined with time, in many cases becoming undetectable within 1 h. Thus, with the exception of taurine, the principal constituents of the free

Table 1. *Amino acid content of the gill of* Mytilus edulis *and* M. californianus

Amino acid	*M. edulis*	*M. californianus*
Alanine	2·9 ± 1·2*	1·0 ± 0·5
Aspartic acid	5·1 ± 1·7	5·0 ± 0·7
Glutamic acid	1·6 ± 0·2	1·5 ± 0·2
Glycine	3·9 ± 1·5	1·6 ± 0·6
Taurine	63·1 ± 10·1	73·9 ± 10·8

* μmol g^{-1} wet weight ±1 S.E. ($N = 4$).

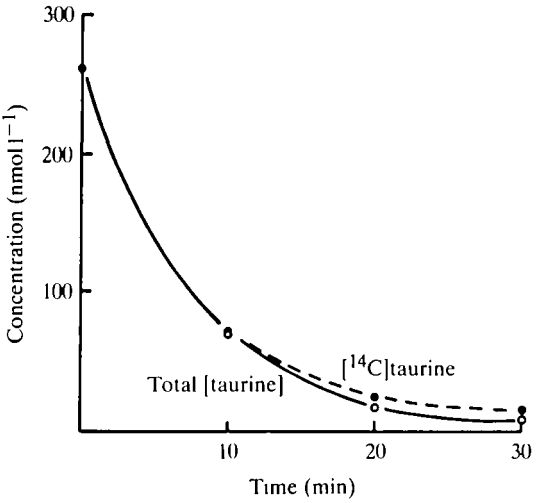

Fig. 1. Influx and net flux of taurine in an intact *Mytilus edulis*. The animal was placed into 500 ml of artificial sea water containing 260 nmol l^{-1} [^{14}C]taurine. Samples of the medium were collected and analysed for radioactivity (solid circles) or total taurine content (open circles).

amino acid pool of the gill do not appear to be lost to surrounding sea water under the conditions in our experiments.

Influx versus *net flux of taurine*

We have shown previously (Wright & Secomb, 1984) that the gills of *M. edulis* and *M. californianus* can accumulate radioactively labelled taurine. Because of the observation that taurine can be lost from epidermal tissues, albeit at low rates, it was of interest to compare the uptake of taurine, as determined from rates of removal of radioactively labelled taurine from test solutions, to the net accumulation of taurine, determined by chemically monitoring the total taurine concentration. The results of an experiment in which an intact *M. edulis* was exposed to 260 nmol l^{-1} [^{14}C]taurine are shown in Fig. 1. The rates of disappearance of the radiolabelled substrate, often used as a measure of unidirectional influx, and the chemically determined taurine, a measure of net flux, were virtually identical (at time zero, influx and net flux of 0·73 and 0·76 μmol g^{-1} gill h^{-1}, respectively). The implication of this observation is that efflux of taurine from the animal must have been much less than influx over a broad range of taurine concentration (i.e. 10–260 nmol l^{-1}). In experiments with three different animals, the average influx (expressed as a first order rate constant, k, ± 1 S.E.) was $0{\cdot}054 \pm 0{\cdot}023$ min^{-1}, while the average net rate of taurine clearance was $0{\cdot}058 \pm 0{\cdot}031$ min^{-1}. In these cases, the clearance of radiolabelled substrate was an adequate measure of both influx and net flux.

For those animals having $[Tau]_{ss}$ values in the >10 nmol l^{-1} range, there should be an increasing discrepancy between the rate of medium depletion of radiolabelled substrate and the disappearance of total taurine. Such a case is shown in Fig. 2. Preliminary tests with this animal revealed a $[Tau]_{ss}$ of 42 nmol l^{-1}. At time zero,

[^{14}C]taurine was added, resulting in an increase in the total taurine concentration to 96 nmol l^{-1}. Radioactivity in the medium quickly declined to an apparent concentration of less than 10 nmol l^{-1} (k at 30 min of 0·056 min^{-1}), similar to the situation shown in Fig. 1. However, total [taurine] declined at a slower rate ($k = 0{\cdot}015\ min^{-1}$), and reached a $[Tau]_{ss}$ of approximately 45 nmol l^{-1} within 90 min, a concentration similar to that observed in the preliminary test. In this case, because of the steady efflux of unlabelled taurine and the consequent decrease in the specific activity of [^{14}C]taurine in the medium, the accumulation of the labelled substrate did not provide an accurate measure of influx. Such observations emphasize the importance of assessing the relationship between uptake of labelled substrate and rates of net clearance.

It should be stressed that the disappearance of radiolabelled taurine and total taurine was a function of exposure of the test solution to the animal's tissues. When a mussel was bound with rubber bands to prevent pumping activity, the disappearance of taurine was less than 3 % of that noted after the animal was permitted to pump. Thus the observed uptake of taurine noted in these experiments was neither the result of uptake by surface bacteria or other organisms on the valves or test container, nor of binding to external surfaces of the animal.

Uptake of β-alanine, β-aminobutyric acid and γ-aminobutyric acid

Taurine is a β-amino acid (2-aminoethanesulphonic acid). In an earlier study (Wright & Secomb, 1984) we tested and verified that, though α-amino acids had no effect on taurine transport, several other β-amino acids, and several γ-amino acids,

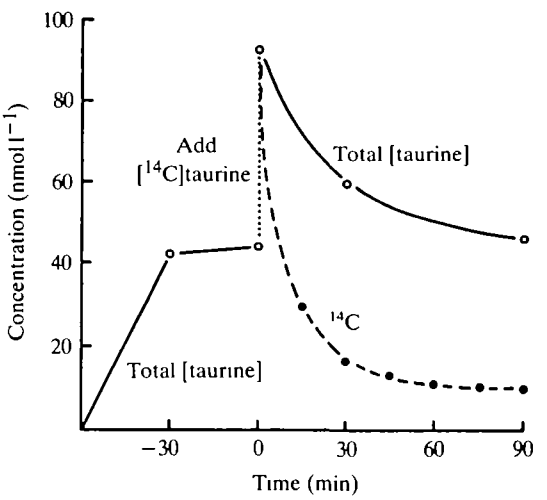

Fig. 2. Influx of [^{14}C]taurine in the face of efflux of endogenous taurine. An intact *Mytilus edulis* waas placed into 500 ml of amino-acid-free artificial sea water. Loss of taurine to the medium was monitored using HPLC (open circles). At 'time zero', [^{14}C]taurine was added, increasing the total taurine concentration to 96 nmol l^{-1}. Subsequent depletion of radioactivity (solid circles) and total taurine was monitored as in Fig. 1.

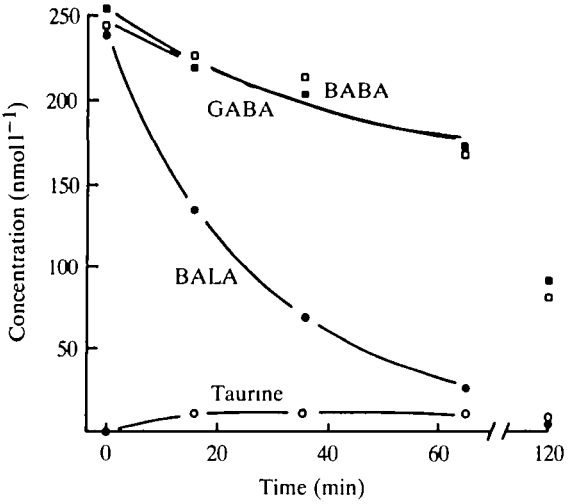

Fig. 3. Simultaneous uptake of β-alanine (BALA, solid circles), β-aminobutyric acid (BABA, solid squares) and γ-aminobutyric acid (GABA, open squares) into an intact *Mytilus edulis*. The mussel was placed into artificial sea water containing 250 nmol l^{-1} of each of the three test substrates. After the animal gaped and began pumping, a time zero sample was collected. Loss of endogenous taurine from the animal was indicated by the increase in taurine concentration (open circles) in the medium.

were effective inhibitors of [^{14}C]taurine uptake into isolated gill tissue. However, we did not provide direct evidence that these inhibitory compounds were themselves transported by gill tissue. To determine whether epidermal tissues can accumulate other 'taurine-like' substrates, we measured the simultaneous net clearance of 250 nmol l^{-1} β-alanine (BALA), β-aminobutyric acid (BABA) and γ-aminobutyric acid (GABA). The results of one such experiment are shown in Fig. 3. All three amino acids were removed from the test solution. BABA and GABA were cleared at similar rates (about 0·04 μmol g^{-1} h^{-1}), but clearance of BALA was approximately 10 times faster. Data of this type were used to estimate half-saturation constants (K_t^*) for the uptake of these substrates *via* the taurine transport pathway. This calculation assumes that (i) whole animal uptake is adequately described by the Michaelis–Menten equation, (ii) the concentrations of the three substrates were low enough for competitive interactions to be ignored, and (iii) the maximal rate of uptake (J_{max}) of each of these compounds is equal to the previously measured whole animal J_{max} for taurine uptake into *M. edulis* (expressed per gram of gill tissue, 12 μmol g^{-1} h^{-1}; Wright & Secomb, 1984). Rates of uptake (J) were calculated from the first order rate constants for clearance of a 250 nmol l^{-1} concentration of each compound. The half-saturation constants were then calculated by rearranging the Michaelis–Menten equation:

$$K_t^* = \frac{[S](J_{max} - J)}{J}, \quad (1)$$

where [S] is the substrate concentration. In experiments with four different animals, the mean K_t^* values (in μmol l^{-1}, ± 1 S.E.) for BALA, BABA and GABA were

$12{\cdot}9 \pm 0{\cdot}6$, $38{\cdot}5 \pm 11{\cdot}6$ and $44{\cdot}6 \pm 11{\cdot}8$, respectively. These numbers can be compared to the K_t^* for taurine uptake, $4{\cdot}7 \pm 0{\cdot}6\,\mu mol\,l^{-1}$, calculated from the rate of taurine depletion determined in experiments with three animals. This latter number is comparable to the value of $5{\cdot}3\,\mu mol\,l^{-1}$ determined by measuring rates of uptake into intact *M. edulis* from a variety of taurine concentrations (Wright & Secomb, 1984). Measurement of BALA, BABA and GABA clearance by four intact *M. californianus* resulted in calculated K_t^* values of $15{\cdot}4 \pm 2{\cdot}5$, $30{\cdot}6 \pm 3{\cdot}8$ and $52{\cdot}5 \pm 14{\cdot}3\,\mu mol\,l^{-1}$, respectively.

Effect of GABA on taurine efflux from Mytilus

The inhibitory effect of GABA on taurine uptake reported previously (Wright & Secomb, 1984), combined with the present observation that GABA was itself transported by mussels (Fig. 3), suggested that GABA could be used as a tool to examine loss of taurine from epidermal tissues. We have suggested that epidermal transport may play an important role in the reaccumulation of taurine lost from the gill to the passing water stream (Wright & Secomb, 1984). The addition of large concentrations of GABA (i.e. $>K_t^*$) to test solutions should reduce or eliminate this reaccumulation, through competition between GABA and taurine for a common transport pathway. In such a situation, taurine concentration should rise above normal $[Tau]_{ss}$ values. Fig. 4 shows the results of such an experiment. A mussel was placed into 500 ml of amino-acid-free ASW and a $[Tau]_{ss}$ of $11{\cdot}9\,nmol\,l^{-1}$ was

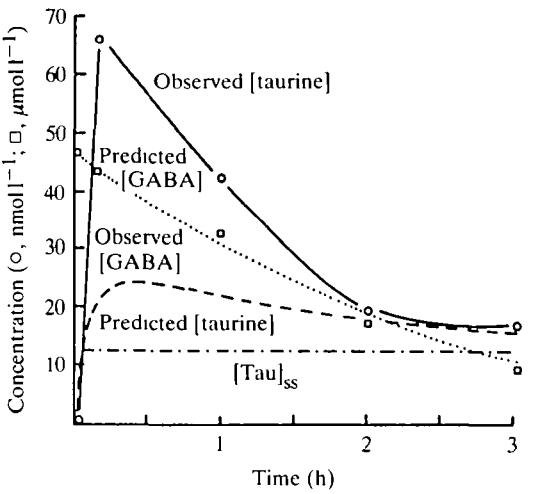

Fig. 4. Time course of the predicted relationship between addition of GABA and the release of taurine from an intact *Mytilus edulis*. Taurine concentrations are in $nmol\,l^{-1}$; GABA concentrations are in $\mu mol\,l^{-1}$. A 60-min 'pretest' provided the value for the taurine steady-state (horizontal dashed line, $[Tau]_{ss}$, $12\,nmol\,l^{-1}$). Dashed and dotted lines show the predicted time courses for change in concentration of taurine and GABA, respectively, according to equations 6 and 7 (see text). Open circles and squares indicate the actual concentrations of taurine and GABA, respectively, in the medium. Volume of the medium was 500 ml.

measured after 60 min. This solution was removed and replaced with ASW containing 46 μmol l^{-1} GABA. Ten minutes after the addition of the GABA, taurine was evident in the medium at a concentration of 65 nmol l^{-1}. Thereafter, the taurine concentration decreased (from 65 to about 20 nmol l^{-1} after 3 h), in parallel with the steady decline in GABA concentration in the medium (from 46 to 10 μmol l^{-1}). This 'secondary' reaccumulation of taurine also demonstrated that the presence of such a non-physiological concentration of GABA had no irreversible deleterious effects on either the taurine transport pathway or the epidermal tissue in general.

Control experiments verified that the 'GABA-induced' leak of taurine was a function of exposure of the epidermal tissues of the mantle cavity to GABA.When a mussel was bound with rubber bands and exposed to 50 μmol l^{-1} GABA, there was no appearance of taurine in the test solution. Thus GABA was not displacing taurine from binding sites on the shell of the animal. Likewise, when bound mussels were exposed to 50–100 nmol l^{-1} concentrations of [^{14}C]taurine in the presence of 50 μmol l^{-1} GABA, there was no consistent disappearance of either taurine or GABA.

Of particular significance was the observation that the only amino acid to appear in the medium when mussels were exposed to GABA was taurine; the other major constituents of the epidermal free amino acid pool (Asp, Gly, Ser, Ala) did not increase in concentration and remained at the very low ($<$10 nmol l^{-1}) concentrations observed in the 'steady-state' experiments. Thus the effect of GABA seems to involve a specific interaction with the $\beta(\gamma)$-amino acid pathway.

Analysis of the cause of the 'GABA-induced' taurine leak

While a competitive interaction between GABA and taurine could be expected to produce a pattern of taurine loss qualitatively similar to that shown in Fig. 4, there is an alternative possibility that should be considered, namely exchange diffusion. Examples of exchange processes have been reported in a wide variety of experimental systems (see Christensen, 1975). In the present case, the presence of a large concentration of GABA on the outside (or *cis* face) of the epidermal cells could result in an acceleration of a carrier-mediated backflux of taurine from the inside of these cells (i.e. from the *trans* face), with the result being a hetero-exchange of GABA for taurine.

The extent to which such an exchange process may be involved in the GABA-induced leak of taurine can be estimated by comparing the experimental observations to predictions of taurine loss and reaccumulation based on the assumption that there was no exchange process in effect. The following reasoning is used. At steady state, in the absence of any competing substrates, the influx of taurine is given by:

$$J_{in}^{T} = \frac{J_{max}^{T}\,[Tau]_{ss}}{K_T + [Tau]_{ss}}, \qquad (2)$$

where J_{in}^{T} is the influx from the steady-state taurine concentration, $[Tau]_{ss}$, J_{max}^{T} is the maximal rate of taurine uptake, and K_T is the Michaelis constant for taurine transport. Passive diffusion of taurine into epidermal tissues is very small over the

relevant concentration range (S. H. Wright, unpublished observations). However, given its very large intracellular concentration, the efflux of taurine (J^T_{out}) presumably includes both a passive leak component (J_{pl}), as well as the carrier-mediated backflux *via* the taurine transport pathway (J_{cl}). Thus:

$$J^T_{out} = J_{pl} + J_{cl}. \tag{3}$$

At steady state, $J^T_{in} = -J^T_{out}$, and therefore the rate of efflux is given by equation 2.

The addition of GABA to the medium will reduce the influx of taurine due to an interaction between GABA and taurine for a common transport pathway. Assuming this interaction is competitive in nature, the new influx of taurine will be given by (Segel, 1975):

$$J^T_{in} = \frac{J^T_{max}[Tau]}{[Tau] + K_T\left[1 + \frac{[GABA]}{K_G}\right]}, \tag{4}$$

where K_G is the Michaelis constant for GABA transport *via* the taurine transport pathway. With the addition of GABA and the resulting reduction in the influx of taurine, the system is no longer at steady state, i.e. $J^T_{in} \neq -J^T_{out}$, and the taurine concentration in the external medium can be expected to rise. However, while the taurine concentration rises, the GABA concentration should decrease due to uptake by the taurine transport pathway. The rate of GABA uptake, (J^G_{in}), is given by:

$$J^G_{in} = \frac{J^G_{max}[GABA]}{K_G + [GABA]}. \tag{5}$$

The inhibitory effects of taurine on GABA uptake can be ignored, considering the differences in concentration of these two compounds associated with the present set of experiments. Also, given the comparatively short time course of these experiments, the backflux of GABA should be small and was thus ignored.

The net rate of taurine release to the surrounding solution will be influenced by the rate of taurine influx, which will change as the concentrations of both taurine and GABA in the test medium change. For both taurine and GABA, the rate of change of the concentration in the medium is equal to the net rate of loss of these substrates from the animal. For taurine:

$$V\frac{d[Tau]}{dt} = A(J^T_{out} - J^T_{in}) = A\left[J_{pl} + J_{cl} - \frac{J^T_{max}[Tau]}{[Tau] + K_T\left[1 + \frac{[GABA]}{K_G}\right]}\right], \tag{6}$$

where V is the volume of the test solution and A is the amount of transporting tissue in the system (e.g. grams of gill tissue). For GABA:

$$V\frac{d[GABA]}{dt} = -AJ^G_{in} = -A\left[\frac{J^G_{max}[GABA]}{K_G + [GABA]}\right]. \tag{7}$$

In this analysis it is assumed that (i) the maximal rate of GABA uptake is equal to the J_{max} for taurine (i.e. whole animal uptake expressed per gram of gill,

$J^{T}_{max} = 12\,\mu mol\,g^{-1}\,h^{-1}$; Wright & Secomb, 1984), which was supported by observed rates of GABA clearance by intact *M. edulis* (from an average substrate concentration of $38{\cdot}8 \pm 1{\cdot}09\,\mu mol\,l^{-1}$, GABA uptake was $5{\cdot}9 \pm 0{\cdot}52\,\mu mol\,g^{-1}\,h^{-1}$; S.E., $N = 6$; from a concentration of $94{\cdot}4 \pm 1{\cdot}22\,\mu mol\,l^{-1}$, uptake was $9{\cdot}0 \pm 2{\cdot}54\,\mu mol\,g^{-1}\,h^{-1}$); (ii) the Michaelis constant (K_t) for taurine has the value obtained when gill morphology and pumping characteristics are taken into account ($1{\cdot}5\,\mu mol\,l^{-1}$; Wright & Secomb, 1984); and (iii) the K_t for GABA is equal to the half-saturation constant measured from the medium depletion studies described earlier ($45\,\mu mol\,l^{-1}$); clearance of GABA was low enough that this value should approach the 'true' value of the GABA Michaelis constant (Wright & Secomb, 1984).

The pair of coupled differential equations 6 and 7 may be solved numerically to predict taurine and GABA concentrations as a function of time. The data from the experiment shown in Fig. 4 were used to calculate a predicted profile of changes in taurine and GABA concentrations (dashed and dotted lines). The steady decline in the concentration of GABA in the medium over the 3-h course of the experiment was reasonably well described by the model. The transient peak in taurine concentration, however, exceeded by a factor of 3 that predicted by the model. After rising to a concentration of $65\,nmol\,l^{-1}$ (*vs* a predicted value of $20\,nmol\,l^{-1}$), the taurine concentration decreased to approach an asymptote at $[Tau]_{ss}$. The discrepancy between the predicted and actual loss of taurine cannot be accounted for by the influence of gill morphology and water flow on the kinetics of epidermal uptake (Wright & Secomb, 1984). When these factors are taken into account, the predicted transient rise in taurine concentration in the medium can be shown to be *less* than that depicted in Fig. 4, while the initial loss of taurine and the approach to the $[Tau]_{ss}$, as well as the uptake of GABA, are largely unaffected by the structure and pumping activity of the gill.

Fig. 5 presents the results from three similar determinations of net taurine and GABA fluxes that occurred in 1-h incubations. Though the decrease in GABA concentration is adequately described by the model, the taurine concentration was more than twice as large at every time point as that predicted by the model. As discussed in a later section, we feel that the consistent observation of this discrepancy between the model's prediction and the actual experimental observation is evidence for a taurine–GABA exchange occurring under these experimental conditions.

As the concentration of GABA in the medium was increased, the concentration of taurine appearing in the medium also increased. Fig. 6 shows the relationship between increasing GABA concentration ($50–200\,\mu mol\,l^{-1}$) and the amount of taurine lost from intact *M. californianus*, expressed as the increase in taurine concentration in test solutions. Superimposed on these data are the predicted taurine concentrations, as determined from the model. The observations are in qualitative agreement with the model's predictions; the taurine concentration should increase as GABA's competition with taurine for the transport process becomes increasingly more effective and is maintained at a higher level for longer periods of time. However, it is clear that the absolute increase in the amount of taurine lost from the animals due to the presence of GABA is greater than that predicted by the model.

DISCUSSION

These results demonstrate that the epidermal tissues of the marine mussels, *M. edulis* and *M. californianus*, have a transport process for taurine and structurally related compounds that is capable of a net accumulation of these molecules from

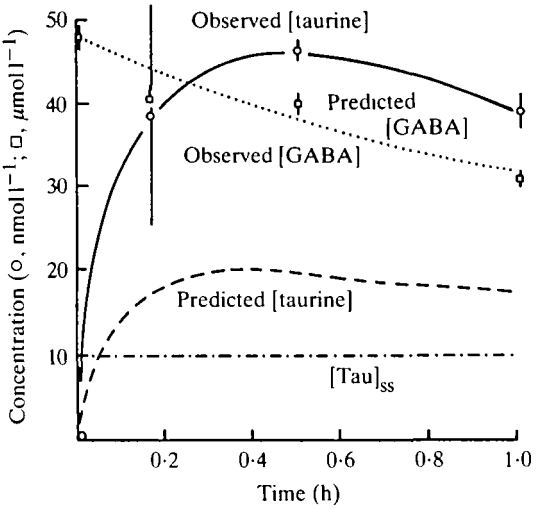

Fig. 5. Mean results of experiments with three intact *Mytilus edulis* showing the effect of 50 μmol l^{-1} GABA on release of taurine. Protocols were the same as in Fig. 4, except the experiments were conducted for 1 h. Vertical bars indicate ±1 S.E. Average wet weight of gill tissue was 1·4 g.

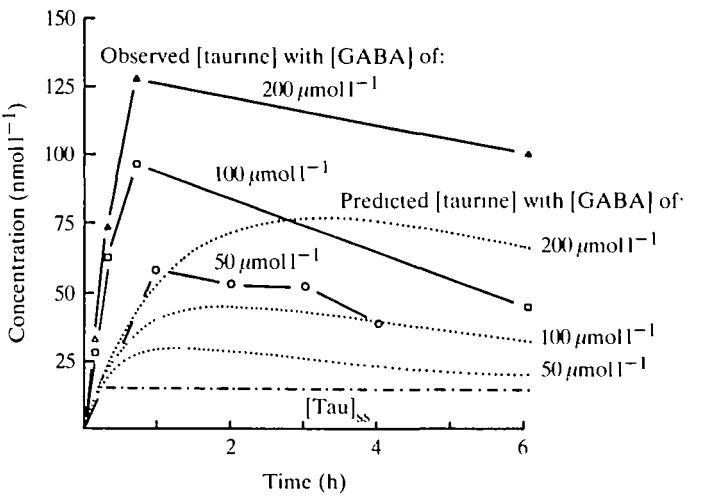

Fig. 6. Effect of increasing concentration of GABA on the release of taurine from intact *Mytilus californianus*. Experiments were performed on three different mussels, using either 50, 100 or 200 μmol l^{-1} GABA (preliminary studies indicated that *o*-phthaldialdehyde concentration in the HPLC procedure was not limiting through a total amino acid concentration in excess of 200 μmol l^{-1} in our samples). Protocols were similar to that described in Fig. 4. Average weight of gill tissue was 0·9 g.

ambient concentrations in the 5–10 nmol l^{-1} range. Nevertheless, mussels do appear to lose taurine to surrounding sea water, though the rate of loss is reduced through the action of the epidermal taurine transporter. When taurine uptake was blocked by addition of large concentrations of GABA, the rate of taurine loss increased. This increase was larger, however, than could be explained by a simple competitive interaction between GABA and taurine for a common transport pathway, suggesting that a mediated taurine efflux can be accelerated through *trans*-stimulation.

Taurine loss from mussels

There has been considerable speculation concerning the rate of amino acid loss from mussels and the impact such a loss has on these animals. The free amino acid content of epidermal tissues such as the gill and mantle is approximately 70–150 μmol g^{-1} wet weight (Table 1; Lange, 1963; Bayne, 1975; Zurburg & de Zwaan, 1981), leading Hammen (1968) to suggest that there should be a significant loss of amino acid from mussels (and other bivalves) across a presumed leaky cell membrane. A number of studies have shown that amino nitrogen is lost from mussels (e.g. Bayne, 1973; Bayne & Scullard, 1977), and Bayne (1975) demonstrated that the major constituent of this loss is taurine. Based upon the apparent rates of amino nitrogen excretion he measured, Bayne (1973) suggested that loss of amino acid represents an average caloric loss equivalent to 10 % of the animal's metabolic rate, which can increase to as much as 63 % under conditions of 'stress' (i.e. elevated temperature and starvation).

In the present study we confirmed that taurine can be lost from *Mytilus*. However, the apparent rate of taurine loss under our experimental conditions was much lower than that reported in these earlier studies. The only amino acid that we observed to be lost consistently was taurine, and then our analytical procedures could confirm a loss in only 14 of the 26 animals tested. The range of taurine steady-state concentrations developed by the animals in our study (4–75 nmol l^{-1}) corresponded to a rate of taurine loss from the animal of 0·02–0·40 μmol g^{-1} dry body weight h^{-1}. The average loss of taurine represented a caloric equivalent of approximately 4 % of a mussel's metabolic rate, lower than previous estimates. These calculations assumed that (i) the wet weight of *Mytilus* gill is 15 % of the total wet weight of the animal; (ii) dry weight is 20 % of total wet weight; (iii) the rate of oxygen consumption of a 1·33-g (dry weight) mussel is between 0·3 and 0·7 ml O_2 g^{-1} h^{-1} (Bayne, Thompson & Widdows, 1973); and (iv) 1 ml of O_2 consumed is equivalent to the oxidation of 1 mg of amino acid.

The cause of this discrepancy in the apparent rate of amino acid excretion from mussels is not clear. Our holding and experimental conditions placed the animals under the type of nutritional and temperature stress that should have maximized loss of amino nitrogen (Bayne, 1973; Bayne & Scullard, 1977). Nevertheless, in the present study, and in other studies employing similar methodologies (Manahan, Wright, Stephens & Rice, 1982; Manahan *et al.* 1983), we have not seen an amino acid leak of the size suggested by previous workers. One possible source of this discrepancy involves the analytical procedures used to measure amino acids. The

HPLC procedure used in the present study permits the identification and measurement of individual amino acids in water samples that have not undergone any handling other than the addition of the OPA reagent. In contrast, the techniques used in studies by other groups usually employed indirect estimates of amino nitrogen, such as the difference between total ninhydrin-positive material and total ammonia (Bayne, 1973), or the determination of total primary amines as a measure of amino acids (Bayne & Scullard, 1977). In the latter case, it is not clear to what extent the presence of ammonia interfered with the estimates of total amino acid concentration (see Jørgensen, 1980). Alternatively, the differences in apparent rates of amino acid loss could be real and reflect either seasonal or population differences, or both (Bayne & Scullard, 1977). Though our studies were carried out on animals collected from at least two different populations over the course of 1 year, such sources of variation must be considered a potential cause of the significant differences seen in the rate of amino loss from mussels.

Epidermal transport and the recycling of endogenous taurine

It has been suggested that epidermal transport processes in the bivalve gill could reduce diffusional losses of endogenous amino acids (Bamford & McCrea, 1975), as well as provide supplementary nutrients for these animals. The former suggestion was recently tested through the theoretical and experimental work of Gomme (1981*a*,*b*). He demonstrated that epidermal glucose transport can significantly reduce the loss of this substrate from the integument of the marine polychaete, *Nereis diversicolor*. His idea is equally pertinent to such transport processes in other systems, including the bivalve gill. In a previous study (Wright & Secomb, 1984), we introduced a mathematical model of amino acid transport in the bivalve gill that was formulated to take into account the geometry and fluid dynamics of this system, as well as the kinetics of the transport process, to predict the effect of epidermal transport on the recycling of endogenous amino acids. We estimated that the kinetics of taurine uptake were such that approximately 30–50 % of the taurine lost from tissues would be recovered by the action of the transporter. The results of the present study extend this model, and provide experimental evidence that epidermal transport can reduce the loss of substrate from mussels to the surrounding sea water.

The average efflux of taurine from *M. edulis* (J_o^T) was 0·12 μmol g^{-1} gill h^{-1}. This is shown in Fig. 7, in the form of a computer-generated contour plot representing the steady-state concentration of taurine in the gill of an actively pumping mussel, based on the model described by Wright & Secomb (1984). Fig. 7A shows the case in which the only source of taurine is efflux from the gill tissue and there is no reaccumulation of this material. The rate of taurine loss in this case would amount to approximately 5 % of the total taurine pool of the gill per day. Fig. 7B shows the effect of recovery of taurine by epidermal transport, i.e. a 35 % reduction in the loss from the animal.

Two underlying assumptions should be emphasized. First, we have assumed in this calculation, and throughout this study, that the kinetics of amino acid exchange

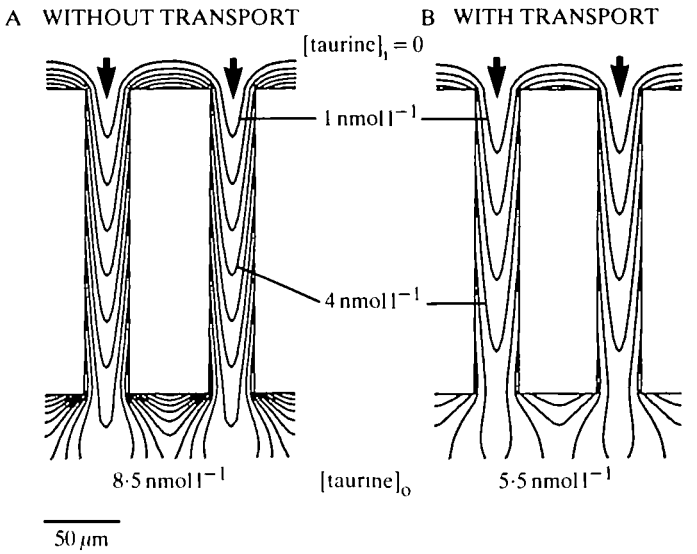

Fig. 7. Computer-generated contour plots showing the steady-state concentration of taurine in the water stream flowing between adjacent gill filaments as a result of the normal efflux of taurine from the gill epithelium in the (A) absence and (B) presence of normal epidermal taurine transport activity. Incoming (i.e. inhalant) sea water was assumed to have no taurine. Heavy arrows show the direction of water flow, from the frontal towards the abfrontal aspect of the gill filaments. See the text for a discussion of these plots, and Wright & Secomb (1984) for details of the mathematical model used. The following values were assumed in the model: peak water flow of 0·12 cm s^{-1}, taurine leak of 4×10^{-14} mol cm^{-2} s^{-1}, and for the case when taurine influx was considered, Michaelis constant of $1{\cdot}5\times10^{-9}$ mol cm^{-3} and J_{max} of 4×10^{-12} mol cm^{-2} s^{-1}.

are not influenced by the presence of microvilli or associated ultrastructural components of the apical membrane of gill cells. To the extent that these structures introduce a resistance to the exchange of amino acids from the cell surface to the bulk solution, our estimates of efflux will underestimate the true rate of loss of taurine from epidermal cells. Second, we have assumed for simplicity that the gill is the site for all taurine uptake and release. However, approximately 30% of whole animal uptake is due to other epidermal tissues, e.g. mantle (Jørgensen, 1983; Wright & Secomb, 1984). Moreover, the major site of amino acid release is not known. In the calculations depicted in Fig. 7 we assumed that the taurine efflux is uniformly distributed in the gill. However, renal excretion of taurine may be a significant component of whole animal efflux. Urine formation in lamellibranchs is of the order of 20–40 μl g^{-1} wet body weight h^{-1} (Martin, Harrison, Huston & Stewart, 1958; Hevert, 1984), and haemolymph concentration of taurine in *Mytilus* is approximately 0·4 mmol l^{-1} (Zurburg & de Zwaan, 1981). If none of the filtered taurine is reabsorbed (and there is no renal secretion of taurine), then the excretory loss of taurine from mussels could range from 5 to 10 nmol g^{-1} wet body weight h^{-1}). Given the placement of the excretory aperture in the suprabranchial chamber of the pallial cavity (see White, 1937), most of the solutes in the urine are probably lost from the animal to the exhalant water stream. Thus, to the extent that taurine and other amino

acids are lost in the urine rather than from epidermal cells, their recovery by epidermal transport will be reduced, and the model in Fig. 8, depicting our current understanding of transport and recycling of substrates, may require modification.

Implications of the influx–net flux relationship of epidermal transport

The observation that *Mytilus* can accumulate taurine from external concentrations as low as 10 nmol l^{-1} (e.g. Fig. 1) warrants discussion on three points: the energetics of the transport process; the apparent permeability of epidermal cells to taurine; and the significance of *trans*-stimulation in this system.

As indicated earlier, the taurine content of gill tissue in *Mytilus* is very high, consistent with an intracellular concentration of at least 70 mmol l^{-1}. It should be stressed, however, that this high concentration does not necessarily imply equally high *activity* of taurine in the cytoplasm of gill cells. Nevertheless, the well-documented role of taurine in isosmotic volume regulation in *M. edulis* (Lange, 1963; Gilles, 1979) argues against a significant fraction of this material being either bound or compartmentalized within the cell. Thus, until contrary evidence is found, it appears that the epidermal transporter can accumulate taurine against an electrochemical gradient in excess of 6×10^6:1 (i.e. 60 mmol l^{-1}(in):10 nmol l^{-1}(out). The driving force for transport against such a large gradient is presumed to come from the inwardly-directed electrochemical gradient for Na^+ (Preston & Stevens, 1982). We have discussed previously (Manahan *et al.* 1983) the view that, on thermodynamic

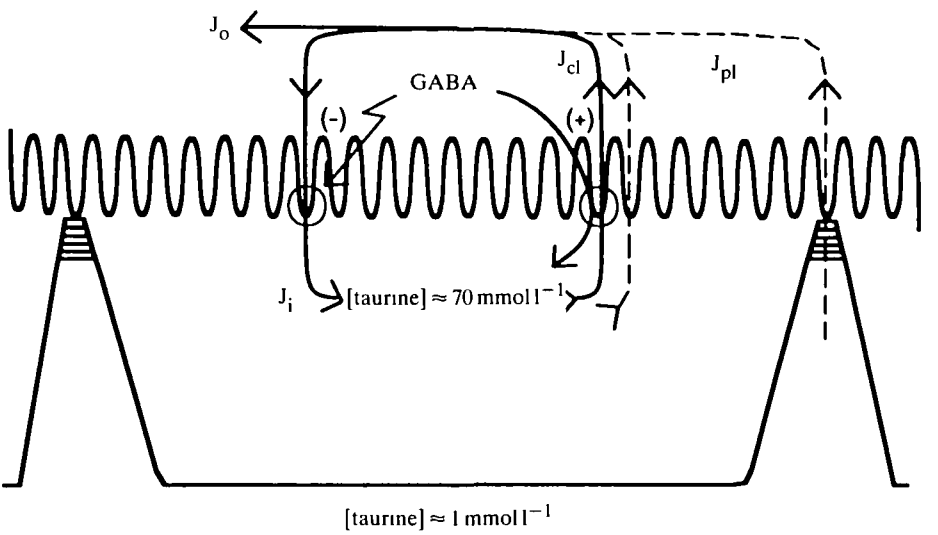

Fig. 8. Schematic representation of the processes associated with taurine fluxes across the apical membrane of the gill epithelium. J_i is the carrier-mediated influx of taurine and J_o is the total efflux of taurine from the gill, which includes carrier-mediated (J_{cl}) and passive (J_{pl}) components. The transporter is depicted twice, representing the two mechanisms by which GABA (or other taurine analogues) is believed to increase the loss of taurine from the gill, namely (a) a competitive interaction (indicated by the minus sign) and (b) an acceleration of efflux due to exchange *via* the transporter (indicated by the plus sign).

grounds, the maintenance of such concentration gradients of zwiterionic amino acids in mussel gills would necessitate a coupling of at least three Na^+ ions to the transport of each amino acid molecule. There is no direct experimental evidence for such a coupling in bivalve tissues; this is an important area for future research.

The observation of a measurable leak of taurine from mussels confirms that, despite the remarkable influx capabilities of epidermal tissues, there is a finite backflux of this molecule. There are at least two possible routes by which taurine may leave the tissue: a carrier-mediated route (presumably *via* reversal of the influx mechanism), and a passive, non-carrier-mediated flux (see Fig. 8). Since the concentration of taurine in haemolymph (about 1 mmol l^{-1}) is much greater than that in the surrounding sea water, this latter component could, in turn, include a paracellular flux from the branchial blood vessel of the gill filaments to sea water, in addition to diffusion of taurine from the cytoplasm across the apical membrane of the gill cells. At this point it is not possible to separate the total backflux into its individual components. The value for J_o^T for *M. edulis* was 0·12 μmol g^{-1} h^{-1}; or approximately 5×10^{-14} mol cm^{-2} s^{-1} (Wright & Secomb, 1984). If the entire backflux consisted of a passive leak across the apical membrane of gill cells, a conservative estimate of 50×10^{-6} mol cm^{-3} for intracellular taurine results in a predicted permeability coefficient (P_T) for taurine of 1×10^{-9} cm s^{-1}. If in fact we have overestimated the effective intracellular taurine activity, this value for P_T will be an underestimate of the true P_T. Conversely, to the extent that carrier-mediated or paracellular avenues of taurine movement dominate the backflux, or that excretory pathways result in a loss of taurine, this will be an overestimate of passive membrane permeability to taurine. Finally, this calculation did not take into account any increase of the actual surface area available for fluxes due to epidermal microvilli, and thus the 'true' P_T is likely to be a lower value than that calculated here. Nevertheless, a value of 1×10^{-9} cm s^{-1} is quite comparable to other values for amino acid permeability coefficients measured in red cells and liposomes (Young & Ellory, 1977).

The loss of taurine from mussels exposed to large concentrations of GABA ($>K_G$) was greater than that predicted using the assumption that GABA's only effect was competitive inhibition with taurine for a common transport pathway (Figs 4, 5, 6). A likely cause for the apparent 'acceleration' of taurine loss in the presence of GABA is exchange diffusion. In 'active' transport systems, such as that present in the gill for taurine and other amino acids, kinetic theory predicts that exchange processes can occur (Stein, 1967), but the 'coupling' of such exchange (i.e. the number of taurine molecules exchanged per molecule of GABA accumulated) need not be 1:1; that will be defined by the forward and back rate constants for all the intermediate steps associated with the transport process (Cuppoletti & Segel, 1975). It is clear from the present observations that influx and net flux can be tightly coupled in the gill. In the example shown in Fig. 5, the efflux of taurine due to exchange with GABA (i.e. observed taurine loss minus that predicted by the $[Tau]_{ss}$ of 10 nmol l^{-1}) was approximately 2% of the influx of GABA, i.e. approximately 50 GABA molecules were accumulated per taurine molecule lost. Subsequent studies can make use of the ability of HPLC procedures to examine the relationship between influx–efflux

coupling in a variety of transport pathways. Though it is unlikely that exchange processes such as that shown in Figs 4, 5, 6 play an important role in the routine transport activity of intact mussels, analysis of exchange diffusion could prove to be a valuable tool in studying the mechanism of epidermal amino acid transport in marine bivalves, and other marine invertebrates as well.

Role of taurine transport in Mytilus

The transport process for taurine in *Mytilus* gill is quite specific for taurine and immediately related structural analogues (Wright & Secomb, 1984; S. H. Wright, unpublished observations). The source of substrate for this specific pathway is not clear. The concentration of taurine in near-shore waters is low; it is not a major constituent of the total amino acid pool in natural waters, which appears to range from 20–200 $nmol\,l^{-1}$ (D. T. Manahan, personal communication) to 1–2 $\mu mol\,l^{-1}$ (Braven *et al.* 1985; Siebers & Winkler, 1984). It is probable that the taurine concentration in sea water to which mussels are exposed does not routinely exceed 10–15 $nmol\,l^{-1}$. Hence, it is unlikely that taurine transport serves any significant role in acquiring exogenous substrate to meet the nutritional needs of mussels (Wright, 1982). However, it is clear that the presence of the epidermal taurine transport pathway can reduce the rate of taurine loss from epidermal tissues. The transport capacity of the taurine pathway is great enough that the efficiency of recycling should remain fairly constant in the face of large increases in the rate of loss of taurine from epidermal cells; in the face of a 10-fold increase in taurine efflux, the gill would still recover approximately 35 % of this material. Such losses might occur transiently, the result of tissue damage occurring during normal pumping and filtering activity.

Regardless of the source of endogenous taurine, recycling this substrate will have an important nutritional impact on mussels. Though *M. edulis* does have the capacity to synthesize taurine (Bishop, Ellis & Burcham, 1983), the rates are very low and total taurine turnover in the gill of *M. edulis* has been estimated to be about 1 % per day (Jørgensen, 1983). It has been suggested that taurine is, effectively, a dietary requirement of mussels (Bishop *et al.* 1983). Thus, by reducing the loss of taurine, the transport process is reducing a significant caloric drain, as well as reducing the loss of an important osmolyte. It should also be stressed that there are at least three other pathways in the apical membrane of *Mytilus* gill specific for the transport of structurally distinct classes of amino acid (S. H. Wright, unpublished observations). These pathways may also play an important role in reducing diffusional losses of endogenous amino acid, and thereby aid in maintaining the large cellular content of amino acid characteristic of gill and other epidermal cells.

In conclusion, this study has shown that intact *M. edulis* and *M. californianus* can accumulate taurine from extremely dilute external solution. This uptake was observed to occur against an apparent chemical gradient in excess of 6 million to one. Nevertheless, the large concentration of taurine in gill tissue resulted in a measurable loss of taurine from mussels, though at rates lower than some previous estimates. The loss of taurine may be reduced by 30 % through the activity of the epidermal taurine transport process. We suggest that epidermal amino acid transport in marine

mussels plays a critical role in the maintenance of the high intracellular concentrations of taurine and other amino acids that are characteristic of bivalve tissues.

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