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TRANSPORT OF AMINO ACIDS BY ISOLATED GILLS OF THE MUSSEL *MYTILUS CALIFORNIANUS* CONRAD

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SUMMARY

The unidirectional influx of cycloleucine into *in vitro* preparations of gill tissue of the mussel, *Mytilus californianus*, was determined. Influx was found to be linear for at least an hour, and the kinetics of cycloleucine influx conformed to Michaelis–Menten type kinetics. The transport mechanism(s) for cycloleucine is relatively specific for the L-enantiomorph of neutral amino acids, and is capable of accumulating cycloleucine to intracellular concentrations much higher than those of the surrounding medium. Evidence is presented that the transport of amino acids by gill tissue plays a significant role in whole animal nutrition.

INTRODUCTION

The ability to accumulate amino acids from dilute solution across external epithelia is widespread among soft-bodied marine invertebrates. The last decade saw many studies involved with the potential ecological role of the transport process, the focus of these studies being the nutritive value of amino acid uptake (reviewed by Stephens, 1972) and the effects reduced salinity have on uptake (Stephens, 1964; Stephens & Virkar, 1966; Anderson & Bedford, 1973; Shick, 1973). However, there has been little work on the physiology of the transport process in marine invertebrates. If the importance of the accumulation of exogenous amino acids by an organism is to be understood, then an understanding of the physiology of the transport process itself is necessary. While the results of the studies to date can generally be interpreted to indicate significance of the uptake of amino acid in whole animal nutrition and osmoregulation, the frequent use of intact animals as experimental systems makes such interpretations suspect; the variables inherent in a whole animal study severely limit conclusions concerning cellular processes such as molecular transport.

A recent autoradiographic study by Péquignat (1973) of glycine accumulation by the mussel, *Mytilus edulis*, suggested that the gills of bivalves function not only in the mechanical transport of particulate organic matter, but also serve as a site for the initiation of digestion and absorption of particulate matter. With this in mind, the present paper is a report of an *in vitro* system utilizing gill tissue of the California coastal mussel, *Mytilus californianus* Conrad, with which a critical examination of the

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non-electrolyte transport mechanism of a marine bivalve can be made. The absorptive capacity of this tissue is examined through studies of its ultrastructure and the transport characteristics of the neutral amino acid analogue, 1-aminocyclopentane-1-carboxylic acid (cycloleucine).

MATERIALS AND METHODS

Mussels

Specimens of *Mytilus californianus* Conrad were collected from the open coast north of Bodega Head (California). Mussels were maintained in 15 gallon aquaria containing filtered, aerated sea water (salinity = 34‰) in a constant temperature room (12 °C) at the University of California, Davis. No attempt was made to feed the animals during the study, as preliminary studies revealed no variation in experimental results between animals maintained in the laboratory 8 weeks and freshly acquired mussels. Animals were usually used within 3 weeks of their collection.

Test solutions

All experiments were conducted in artificial sea water mixed from reagent grade salts and distilled water according to Cavanaugh (1954). Incubation media for the uptake experiments contained a known activity of ^{14}C-cycloleucine (New England Nuclear) and were brought to the desired substrate concentration by the addition of ^{12}C-cycloleucine (^{12}C-amino acids were purchased from either Sigma Corporation or the J. T. Baker Chemical Co.). For the experiments on the selectivity of the cycloleucine transport mechanism, a second ^{12}C-amino acid was added to this medium.

Cycloleucine influx experiments: typical procedure

Mussels were opened by cutting the posterior adductor muscle. The gills (four per animal) were excised and placed in a container of artificial seawater and allowed to equilibrate to room temperature (22 ± 1 °C). Two methods were employed to incubate the gill tissue in experimental media. For the experiments on the kinetics of cycloleucine influx, a length of thread, approximately 20 cm long, was tied around each gill and after 10 min of pre-incubation in artificial sea water, the gills were dipped into a beaker containing 200–1000 ml of sea water solution containing ^{14}C-cycloleucine and concentrations of ^{12}C-cycloleucine varying from 0·05 mM to 5 mM. After a prescribed interval of time, the gills were pulled from the solution, rapidly rinsed in two successive 0 °C sea water baths, and blotted on filter paper. A cork borer was used to cut discs of gill tissue, which were placed in scintillation vials containing 2 ml of 0·1 N-HNO_3. The radioactive compounds accumulated in the tissue were extracted directly in the scintillation vials for at least 2 h, after which time 15 ml of scintillation cocktail was added. The cocktail contained 2 parts toluene containing 6 g/l of the fluor, 2,5-diphenyl oxazole, to 1 part of the detergent, Triton X-100. Samples were counted on a Beckman 100-cpm liquid scintillation counter. To minimize the appearance of interanimal variability in influx rates, gill tissue from four different mussels was used at each time increment, and each scintillation vial contained 12 pieces of tissue randomly chosen from these four animals. In all influx experiments, initial and final radioactivity in the test solution was monitored.

The second method for incubating gill tissue was used in all experiments other than

those in the series measuring kinetics of cycloleucine influx. Discs of tissue were cut from the gills immediately after excision from the whole animal. Preliminary experiments revealed that the rate of cycloleucine influx was drastically reduced immediately after the cutting procedure. However, thereafter the rate increases with time, and when the isolated tissue discs were allowed to pre-incubate in artificial sea water for 20–30 min, the influx rates returned to those determined from whole gill experiments. Consequently, in all studies in which pre-cut tissues were used, the isolated discs were pre-incubated at room temperature for 30 min. They were then incubated in the appropriate experimental media, and the radioactivity accumulated by the isolated discs was determined as above.

Determination of intracellular volume and cycloleucine concentrations

Tissue discs from four different mussels were cut, blotted and quickly weighed to the nearest 0·1 mg. These samples were then dried in an oven at 60 °C for 24 h and reweighed to determine the dry weight of tissue discs. The difference between the wet and dry weights of each sample was considered a measure of total tissue water. In a separate test, extracellular space was estimated by using ^{14}C-inulin as an extracellular marker. Schultz, Foisz & Curran (1966) discuss the technique and assumptions of this method of determining extracellular volume. Whole gills were incubated in artificial sea water containing ^{14}C-inulin. The radioactivity in tissue discs cut from gills incubated for 1 and 1·5 h was determined as described above for ^{14}C-cycloleucine measurements, and did not vary significantly. Total intracellular water per tissue disc was assumed to be the difference between total tissue water per tissue disc and the ^{14}C-inulin space per disc. Intracellular concentrations of cycloleucine were determined based on the assumption that the amount of cycloleucine in the tissue as calculated from the accumulated radioactivity is in a free osmotic state within the entire intracellular volume.

RESULTS

Linearity of cycloleucine influx

In Fig. 1, the cumulative uptake of ^{14}C-cycloleucine by intact and isolated gill discs is plotted as a function of time of exposure to test solutions containing 0·05 mM cycloleucine. The data in this graph demonstrate that the two methods described for monitoring cycloleucine uptake provide closely comparable results. The uptake of isotope is linear, within experimental error, for at least 180 sec, and corresponds to an influx of cycloleucine of $0{\cdot}7 \times 10^{-7}$ moles/cm^2.hr. The observation that the lines shown in Fig. 1 extrapolate close to the origin suggests that the uptake of isotope is halted by the 0 °C sea-water baths and that negligible amounts of label are lost from the tissue during rinsing, cutting and/or blotting. Had a significant loss of isotope occurred during these procedures, it would have to have been a constant fraction of the total uptake for the lines to extrapolate to the origin; this possibility is considered unlikely. Also, these data suggest that surface binding of ^{14}C-cycloleucine to mucus does not contribute significantly to the calculated uptake of cycloleucine; such a process would probably occur rapidly and would be observed as a positive shift of the extrapolated lines away from the origin toward a positive intercept on the ordinate.

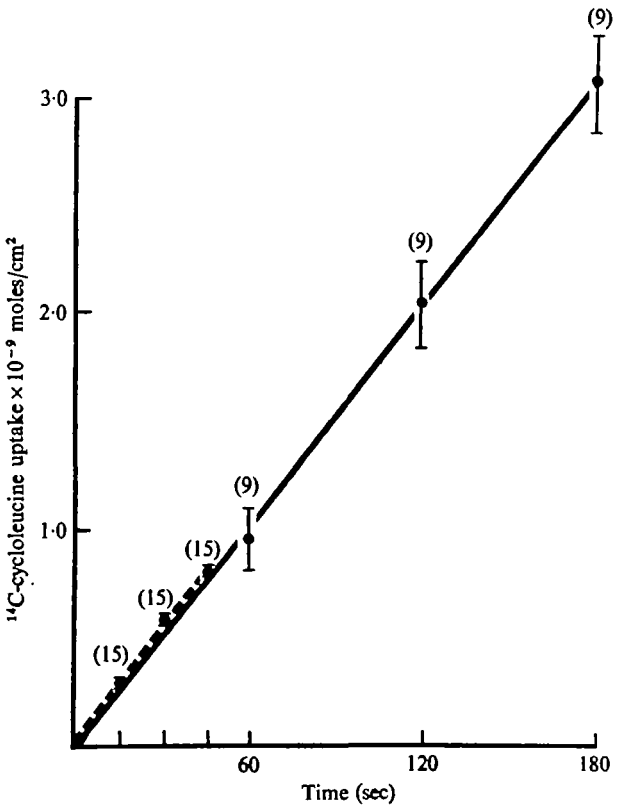

Fig. 1. Uptake of ^{14}C-cycloleucine by intact gills and isolated gill discs as a function of time. The solid line represents accumulation by isolated gill discs, the dashed line accumulation by whole gills. The concentration of cycloleucine in the test solutions was 0·05 mM. Numbers in parentheses are number of observations. Bars signify ± 2 S.E.M. Lines were fit by regression analysis.

Kinetics of cycloleucine influx

Fig. 2 shows the influx of cycloleucine over time at four different concentrations of cycloleucine. The rate of influx increases with increasing concentration of substrate in the test solution. However, the rate of influx does not increase linearly with increasing concentration, but instead displays a tendency towards saturation at high concentrations of cycloleucine. The apparent hyperbolic relation between the rate of influx and the concentration of cycloleucine in the surrounding medium can be adequately described by the Michaelis-Menten equation:

$$\mathcal{J}_c^i = \frac{\mathcal{J}_c^{i\max}[c]_m}{K_t + [c]},$$

in which $\mathcal{J}_c^i$ is cycloleucine influx, $\mathcal{J}_c^{i\max}$ is maximal influx, $[c]_m$ is the cycloleucine concentration in the test solution, and K_t is the apparent Michaelis constant (the value of $[c]_m$ at which $\mathcal{J}_c^i = \mathcal{J}_c^{i\max}/2$). The value for $\mathcal{J}_c^{i\max}$ ($3{\cdot}5 \times 10^{-7}$ moles/cm^2.h), and for K_t ($0{\cdot}25 \times 10^{-3}$ moles/l), were graphically determined by plotting $[c]_m/\mathcal{J}_c^i$ against $[c]_m$ (Woolf plot) (Fig. 3). This linear transformation of the Michaelis–Menten equation has advantages over the widely used Lineweaver–Burke reciprocal transformation,

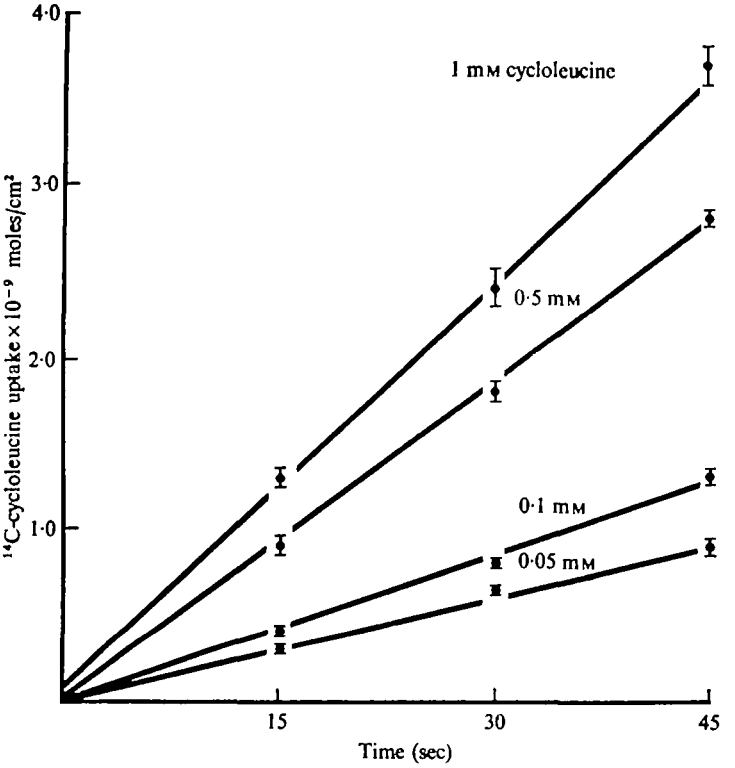

Fig. 2. Uptake of ^{14}C-cycloleucine by intact gills as a function of time and concentration of cycloleucine. Concentrations in test solutions were, 1, 0·5, 0·1, and 0·05 mM. Each point is the mean ± S.E.M. of five observations of uptake by tissue collected from four different animals.

since it does not place disproportionate emphasis on rate of influx at the lower substrate concentrations tested and thus yields more reliable kinetic parameters (Neame & Richards, 1972).

Specificity of the transport mechanism

The effect of nine different amino acids on the influx of cycloleucine by isolated tissue discs are compared in Fig. 4. In these experiments, tissue discs were exposed to test solutions containing ^{14}C-cycloleucine plus a second amino acid, at concentrations of 0·05 mM and 0·1 mM respectively. At these concentrations, the rates of cycloleucine influx in the presence of the L-isomers of the neutral amino acids alanine, leucine, isoleucine, valine, and phenylalanine, were inhibited by 72–80 %. D-isomers, on the other hand, seem considerably less effective at reducing the influx of cycloleucine; D-alanine inhibited influx by 44 %, while its L-enantiomorph inhibited influx by 75 %.

The basis amino acids L-arginine and L-lysine were much less effective at inhibiting the influx of cycloleucine and did not significantly reduce the rate of influx. Also, neither the dicarboxylic acid, L-aspartic acid, nor taurine significantly affected the influx of cycloleucine.

Examination of the data in Fig. 4 suggests that: (*a*) cycloleucine shares a common transport pathway with neutral amino acids; (*b*) the transport mechanism is relatively specific for L-isomers; and (*c*) the transport mechanism is relatively insensitive to basic and acidic amino acids.

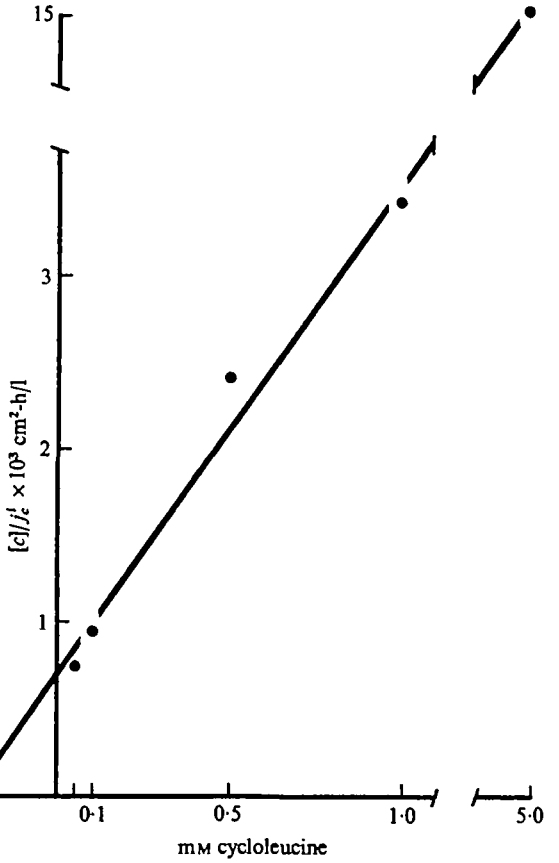

Fig. 3. Cycloleucine influx data presented in a linear transformation of the Michaelis–Menten equation. The abscissal intercept is $-K_t$. The slope of the line is $1/J_{mc}^{i\,max}$.

Concentrative transport of cycloleucine

To determine accurately the intracellular concentrations of accumulated cycloleucine, it was necessary to estimate the intracellular volume of the isolated gill discs used in these studies. In order to obtain some estimate of the variation in the size of the sample tissue, dry weights were measured for a series of 18 small (diameter 0·7 cm) and 21 large (diameter 1·1 cm) isolated tissue discs. The dry weights for the small discs averaged 2·8 ± 1·7 mg (S.D.) (3·7 ± 2·2 mg/cm²); the large discs averaged 6·0 ± 0·7 mg (S.D.) (3·2 ± 0·4 mg/cm²). Total tissue water of isolated tissue discs averaged 0·82 ± 0·11 ml/g wet weight (S.D.). The ^{14}C-inulin extracellular space was calculated to be 0·07 ± 0·01 ml/g wet weight (S.D.). While this figure seems low for an epithelial tissue, the fact that the calculated inulin space for a 1·5 h incubation was nearly identical to that obtained for a 1 h incubation (0·06 versus 0·08 ml/g wet weight, respectively) suggests equilibrium had been obtained between the ^{14}C-inulin concentration in the tissue and in the test solution. The difference between the mean total tissue water and the mean extracellular space resulted in an average value for the volume of the intracellular water in isolated gill tissue of 0·75 ml/g wet weight. If, however, our figures for the magnitude of the extracellular fluid are low, the resultant

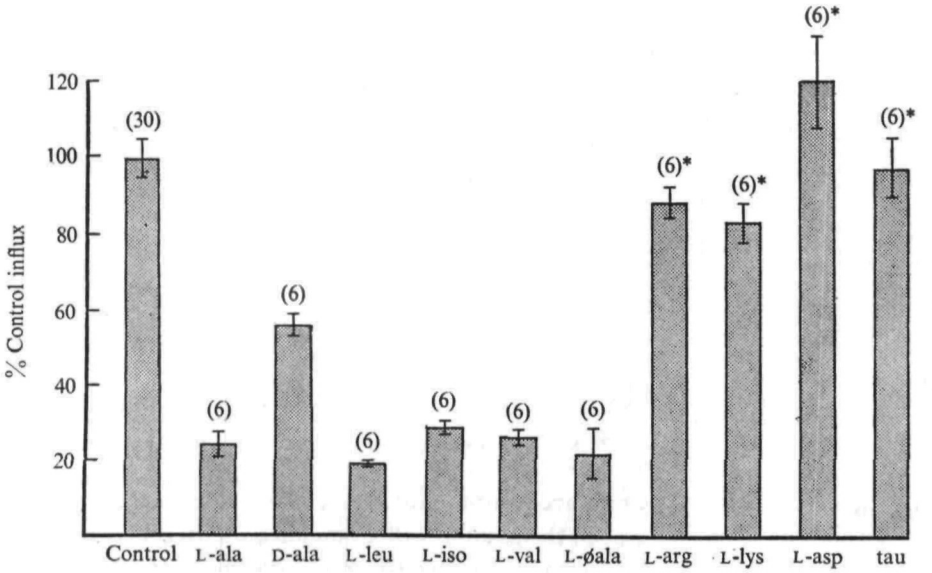

Fig. 4. The effect of the presence of a second amino acid on the influx of cycloleucine into isolated discs of gill tissue. Concentrations in the test media were cycloleucine, 0·05 mM, second amino acid, 0·10 mM. Bars signify ± S.E.M. Numbers in parentheses are number of observations. Asterisks (*) indicate non-significance ($P > 0·05$) of experimental rates of influx from control values.

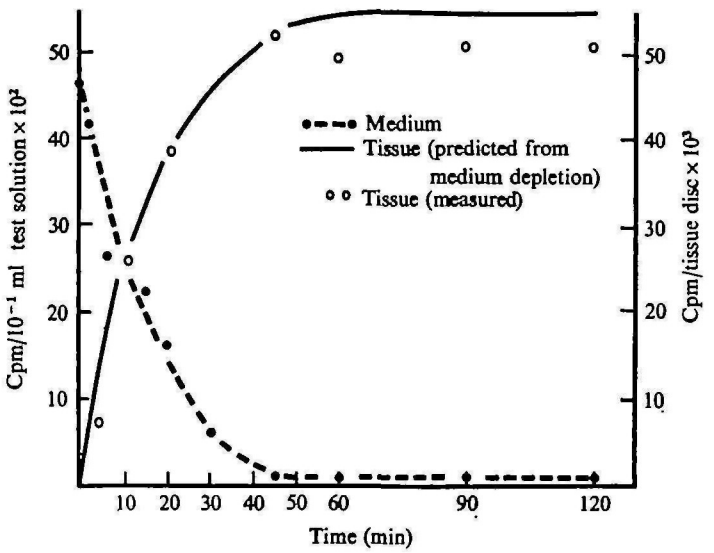

Fig. 5. The depletion of radioactivity in a test solution by isolated discs of gill tissue. The left ordinate is the amount of radioactivity remaining in a test solution of 0·05 mM cycloleucine containing 40 isolated tissue discs; the right ordinate is the amount of radioactivity accumulated by the tissue discs.

volume of cell water per isolated tissue disc would be an overestimate and consequently an underestimate of the intracellular concentration of cycloleucine.

When 40 isolated tissue discs (diameter 1·1 cm) were placed in 50 ml of a test solution containing 0·05 mM ^{14}C-cycloleucine, the radioactivity in the medium was

Table 1. *Effect of preloading isolated gill discs with cycloleucine on the influx of cycloleucine*

(The preincubation medium contained 1 mM cycloleucine. Control discs of tissue from the same organisms were preincubated in artificial sea water. The rate of influx was measured from a test solution containing 0·05 mM cycloleucine.)

Preincubation solution	Length of preincubation (min)	Cycloleucine influx ($\times 10^{-8}$ moles/ $cm^2 \pm$S.E.M.)	n*	p
Artificial sea water (control)	20, 40, 60	7·1 ± 0·5	15	—
1 mM cycloleucine	20	6·3 ± 0·4	4	> 0·1
1 mM cycloleucine	40	7·9 ± 0·3	4	> 0·1
1 mM cycloleucine	60	7·0 ± 0·5	4	> 0·1

* Number of observations.

rapidly depleted (Fig. 5). Concurrent tests for radioactivity in the isolated discs revealed a simultaneous increase in the amount of ^{14}C-cycloleucine accumulated in the tissue, which equilibrated at a steady-state cell-to-medium concentration ratio of approximately 2000:1.

While accumulation of radioactive cycloleucine is indicative of a net influx of cycloleucine, it does not necessarily indicate a net increase in intracellular amino acid content; countertransport, or 'exchange diffusion' could yield identical data. This possibility was tested indirectly in two ways. Isolated gill discs were pre-loaded with cycloleucine by preincubation in 1 mM ^{12}C-cycloleucine, after which the rate of influx of 0·05 mM cycloleucine was measured. Separate experiments demonstrated that the pre-loading procedure results in intracellular concentrations of cycloleucine in excess of 5 mM, providing at least a 100-fold chemical gradient against the influx of cycloleucine. In Table 1 the rates of influx into pre-loaded tissue are compared with rates obtained from parallel control experiments. If an exchange process were occurring, a trans-stimulation of cycloleucine influx would be expected as the intracellular concentration of cycloleucine increased (Heinz & Walsh, 1958). As the data in Table 1 demonstrate, the elevated concentrations of cycloleucine in the gill tissue did not significantly alter the rate of cycloleucine influx.

Fig. 6 presents the results of a second experiment in which isolated tissue discs were incubated for 60 min in a test solution containing 0·05 mM ^{14}C-cycloleucine. The volume of the test solution, 400 ml, was sufficiently large that the concentration of radioactivity did not significantly decline during the course of the experiment. As demonstrated in Fig. 6, after 60 minutes the isolated gill discs developed a $[\text{cycloleucine}]_{\text{cell}}/[\text{cycloleucine}]_{\text{medium}}$ ratio in excess of 100/1. If neutral amino acids were exchanged for this molar quantity of cycloleucine, the resulting increase in the external concentration of neutral amino acids should be evidenced by a decline in the rate of influx of cycloleucine. This was not the case, as the rate was unaffected for the entire time course of the experiment.

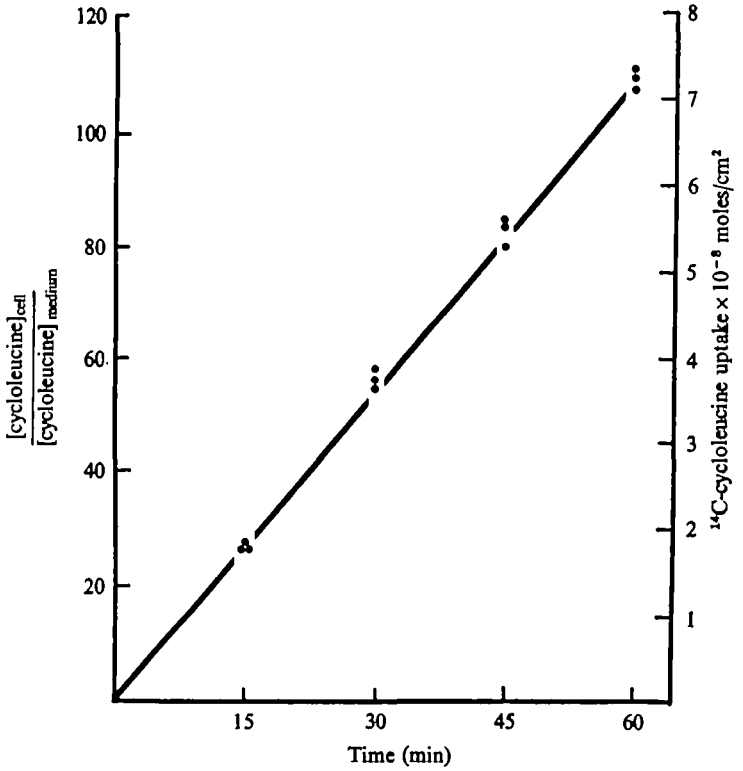

Fig. 6. The cell-to-medium cycloleucine concentration gradient (left ordinate) and the rate of cycloleucine influx into isolated discs of gill tissue (right ordinate) as a function of time. Concentration of cycloleucine in the test solution was 0·05 mM and was constant over the time course of the experiment. Each point is the accumulated radioactivity from three isolated tissue discs.

DISCUSSION

Uptake of cycloleucine

The observation that the rate of influx of cycloleucine reaches a maximum with increasing concentration of cycloleucine in the test solution agrees with the results of other studies on amino transport by soft-bodied marine invertebrates (Stephens, 1972). The $\mathcal{J}_c^{i\,max}$ of $3{\cdot}5 \times 10^{-7}$ moles/cm^2.h corresponds to approximately 3×10^{-5} moles/h.g wet weight of gill tissue. The latter figure is at least an order of magnitude greater than the V_{max} for glycine uptake by intact *Rangia* (Anderson & Bedford, 1973), falling within the higher range reported by Stephens (1972) for an assortment of marine invertebrates tested. However, as with the study on *Rangia*, the range reported by Stephens (10^{-7} to 10^{-5} moles/h.g wet weight) are from studies on whole animals. It is difficult to compare these rates to transport by gill tissue on a weight basis; the gills have a comparately high surface-to-volume ratio (therefore surface: weight ratio), resulting from their sheet-like structure.

The K_t for cycloleucine influx, $0{\cdot}25 \times 10^{-3}$ moles/l is within the midrange of K_t values reported by Stephens (1972) (5×10^{-5} to 2×10^{-3} moles/l). Approximate K_t's for the transport of other neutral amino acids by gill tissue can be obtained by the manipulation of data presented in Fig. 4. If it is assumed that the effect of the neutral

amino acids is a simple competition for a single transport site, it is possible to calculate approximate K_t's for the competing amino acids, using the following equation (Neame & Richards, 1972):

$$K_t = \frac{\mathcal{J}_c^{i'}}{\mathcal{J}_c^{i} - \mathcal{J}_c^{i'}} \times \frac{K_t[i]}{[c]_m \times K_t},$$

where K_t is the approximate Michaelis constant of the inhibitor, $\mathcal{J}_c^{i'}$ is the rate of influx of cycloleucine in the presence of the inhibitor, and $[i]$ is the concentration of the inhibitor in the test solution. The K_t's for the neutral amino acids used in this study ranged between 0·10 and 0·12 mM, in agreement with Stephens's (1972) data.

Mytilus gill tissue is capable of the concentrative transport of cycloleucine. It has been suggested by other workers that the fluxes of radioactive tracers are not accurate measures of net amino acid fluxes in marine invertebrates because it overlooks efflux and exchange diffusion of intracellular amino acids (Johannes, Coward & Webb, 1969). However, the results of our studies on the effect of pre-loading gill tissue with unlabelled cycloleucine and extended incubation periods in test solutions on the influx of ^{14}C-cycloleucine support the contention that concentrative transport of amino acids by *Mytilus* gills is not an artifact of exchange diffusion. These results are in agreement with several other studies which have demonstrated that uptake of amino acids across external epithelia results in a net influx of organic material (Wong, 1971; Stephens, 1972).

Specificity of transport

Neutral amino acids. It appears that *Mytilus* gill tissue possesses a transport pathway selective for the L-isomers of neutral amino acids. The data presented in Fig. 4 make it apparent that, at the concentrations used in these experiments, L-leucine, L-isoleucine, L-valine, L-alanine, and L-phenylalanine all exercise an approximately equal inhibitory effect on the influx of cycloleucine. While there are no comparable studies on marine bivalves, this is in agreement with the work of Wong (1971), who reported that cycloleucine influx into intact specimens of the annelid, *Stauronereis rudolphi*, was inhibited by the presence of either L-alanine, L-valine or L-phenylalanine. It is possible that influx of cycloleucine into *Mytilus* gills is mediated by more than one pathway. There is ample evidence for the existence of more than one transport pathway for cycloleucine in other species. For example, Harris & Read (1968) observed that the tapeworm, *Hymenolepis diminuta*, accumulates cycloleucine through a saturable process described by Michaelis–Menten type kinetics, and that neutral amino acids strongly inhibit its uptake. Two distinct systems for cycloleucine transport were identified, however, a L-phenylalanine-preferring system and a L-proline-preferring system.

The comparatively small inhibition of cycloleucine influx caused by the D-isomer of alanine is consistent with the results of Bamford & James (1972), who demonstrated that the carrier mechanism responsible for neutral amino acid transport by isolated gut of the echinoid, *Echinus esculentus*, is stereospecific for the L-enantiomorphs.

Basic amino acids. The ability of basic amino acids such as lysine and arginine to inhibit the transport of various neutral amino acids across mammalian intestine is well documented (Reiser & Christiansen, 1972; Munck & Schultz, 1969). Wong (1971) reported that although arginine had no inhibitory effect, lysine did slightly reduce

uptake of cycloleucine by intact *Stauronereis*. In our study, lysine and arginine seemed to slightly inhibit cycloleucine influx but statistical analysis showed that the inhibition was not significant.

Acidic amino acids. The influx mechanism seems to be insensitive to acidic amino acids, contrasting with Wong's (1971) findings with *Stauronereis*. But the transaminase activity of some epithelia (Neame & Wiseman, 1957) makes these data difficult to interpret. However, an interesting prediction was confirmed by the results of the taurine test. Schultz, Yu-tu & Strecker (1972), demonstrated that the interaction between neutral amino acids and the influx mechanism(s) of rabbit ileum involves the α-amino and α-carboxylate groups as well as the side chain. Taurine has no carboxylate group and thus might be expected to have a correspondingly small effect on the mechanism sensitive to neutral amino acids in *Mytilus* gills. The negligible effect of taurine on the influx of cycloleucine corroborated this prediction.

Conclusions: significance of transport by gills

The role that accumulated amino acids play in the energy budget of marine invertebrates has been discussed at length in several studies (Stephens, 1967, 1968, 1972; Johannes *et al.* 1969). Oxygen consumption is often used as a measure of energy requirements when estimates of the potential contribution of accumulated amino acids to nutrition are made (Stephens, 1968). Anderson & Bedford (1973) have made the only quantitative estimates of the nutritional role of accumulated amino acids in a bivalve. They found that glycine accumulation by intact *Rangia* could account for only 4% of the total oxygen requirement of that organism. However, *Rangia's* estuarine habitat makes it a poor candidate for comparisons of the importance of accumulated amino acids in marine bivalve nutrition. Stephens (1964) demonstrated that the rate of uptake of glycine by *Nereis limnicola*, an estuarine annelid, was much lower than that of the congeneric marine species, *N. succinea*. Also, the uptake of glycine by *N. succinea* was found to decrease dramatically at low salinities ($< 20‰$), a result which Stephens suggested was due to an incompatibility between the uptake mechanism and osmoregulation at low salinities.

The evidence suggests that accumulated amino acids play a considerably more important role in the nutrition of *Mytilus* than in *Rangia*. The oxygen consumption of isolated *Mytilus* gills is approximately 1·1 ml O_2/g.dry weight.h (unpublished results obtained through differential respirometry). From an external cycloleucine concentration of $5{\cdot}0 \times 10^{-5}$ moles/l the gills can accumulate 3 mg cycloleucine/g.dry weight.h. One ml of oxygen consumed is approximately equal to the oxidation of 1·0 mg of an average amino acid (Stephens, 1963). Thus the accumulation of a metabolizable amino acid at a rate similar to those recorded for cycloleucine could account for more than 250% of the oxygen consumption of the gill tissue. The gills, though, represent only 15% of the dry weight of the animal. If it is assumed that the gills are the only surface accumulating amino acids from the external solution, then this figure is certainly an overestimate of the potential contribution accumulated amino acids make to the mussels' nutrition. When, however, the figures for basal oxygen consumption of intact *Mytilus edulis* (Newell, 1970) are used in these calculations (0·4 ml O_2/g.dry weight.h), amino acid accumulation by the gills can still account for over 100% of the oxidative metabolism of *M. californianus*.

An external concentration of 5×10^{-5} M may seem high for a single amino acid in the marine environment. However, the choice of this as a representative concentration is based on three arguments. (1) Stephens (1972) has reported values between 5×10^{-5} and $2{\cdot}5 \times 10^{-4}$ moles/l for total free amino acids in interstitial water in mud flats and algal mats on wharf pilings, the latter being a similar environment to that in which *Mytilus* is found. (2) Stephens (personal communication) has discussed the relation between the K_t of a transport mechanism and the concentration of transported compounds in the environment. A transport mechanism with a K_t considerably larger or smaller than the environmental concentration has a correspondingly reduced efficiency and will tend to be eliminated by natural selection. This hypothesis is substantiated by the empirical correlation between the K_m's of enzymes and the levels of substrate they normally encounter (see Cleland, 1967; Hochachka & Somero, 1973, for examples). This argument suggests then that the K_t of transport mechanisms should be within an order of magnitude of concentrations in the environment. If a range of concentrations an order of magnitude below the K_t is examined (0·025–0·25 mM), then the transport of amino acids by gill tissue can account for 50–300 % of the oxygen consumption of *Mytilus*. (3) Finally, Péquignat (1973) and Pasteels (1969) presented evidence that *Mytilus* gills may normally encounter concentrations of amino acid considerably higher than those found in the surrounding sea water. They reported that gill epithelia of *Mytilus edulis* contain chymotrypsin (Péquignat, 1973), acidic phosphatase (Pasteels, 1969), and other digestive enzymes. Coupled with his demonstration of the direct assimilation of amino acids by gill epithelia, Péquignat suggested that the gills of marine bivalves may serve in the initiation of digestion and absorption of particulate matter.

It should be noted that we are not suggesting that all the amino acid accumulated by *Mytilus* is funnelled into oxidative pathways. In fact, Péquignat (1973) demonstrated that a considerable fraction of the glycine accumulated by the gills of *M. edulis* is assimilated into alcohol insoluble fractions. However, the observation that influx of amino acid is of the same order of magnitude as respiratory needs suggests that it may be a significant supplement to other well-established feeding mechanisms.

It may well be that the fluxes of amino acids across bivalve body surfaces contribute not only to their nutrition, but also to osmoregulatory phenomena. Recently, it has been suggested by Pierce & Greenberg (1972, 1973) that the efflux of amino acid across external body surfaces of bivalves is responsible for the observed decrease in intracellular amino acid content induced by external hypotonicities. However, they have not examined the accumulation of amino acids or the role that influx of amino acids may play in osmoregulation.

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