Pacific Science (1982), vol. 36, no. 3

The Properties and Functions of Alanopine Dehydrogenase and Octopine Dehydrogenase from the Pedal Retractor Muscle of Strombidae (Class Gastropoda)[1]

J. BALDWIN[2] and W. R. ENGLAND[2]

ABSTRACT: The pedal retractor muscles of Strombidae contain high activities of both alanopine dehydrogenase and octopine dehydrogenase, raising questions as to the functions of these two enzymes during muscle anoxia associated with locomotion. Alanopine dehydrogenase and octopine dehydrogenase were isolated from the pedal retractor muscle of *Strombus luhuanus*, and their structural and kinetic properties investigated. Alanopine dehydrogenase occurs as a single electrophoretic form with a molecular weight of approx. 42,000. Octopine dehydrogenase was electrophoretically polymorphic, existing as three alleles in the population of animals studied. The major form of the enzyme had a molecular weight of approx. 39,000. Both enzymes displayed similar pH optima for the forward (pyruvate reduction) reaction and similar K_m values for the common substrates pyruvate and NADH.

During bursts of leaping, both octopine and strombine/alanopine accumulated in the pedal retractor muscles of Strombidae. However, during recovery from exercise, only strombine/alanopine accumulated. Octopine was a potent inhibitor of the forward reaction catalyzed by octopine dehydrogenase, and may act to prevent further octopine production during the recovery phase. The results of this study show that both alanopine dehydrogenase and octopine dehydrogenase are functioning to catalyze the terminal step of anaerobic glycolysis during muscle anoxia associated with locomotion.

DURING SHORT-TERM BURSTS of anaerobic muscle work associated with locomotion, adenosine 5′-triphosphate (ATP) may be obtained from substrate-level phosphorylations coupled to the glycolytic degradation of carbohydrates. The final step of this pathway serves to reoxidize NADH produced by the glyceraldehyde phosphate dehydrogenase reaction, with pyruvate functioning as the terminal electron acceptor. In mollusk muscle, this pyruvate reduction may be catalyzed by at least three enzymes, which potentially could compete for a common pool of pyruvate and NADH. The enzymes are (1) lactate dehydrogenase (pyruvate + NADH + $H^+ \rightleftharpoons$ lactate + NAD^+); (2) octopine dehydrogenase (arginine + pyruvate + NADH + $H^+ \rightleftharpoons$ octopine + NAD^+ + H_2O); (3) alanopine dehydrogenase (alanine/glycine + pyruvate + NADH + $H^+ \rightleftharpoons$ alanopine/strombine + NAD^+ + H_2O).

It is generally held that competition between lactate dehydrogenase and octopine dehydrogenase is avoided by the presence in the muscle of much greater activities of one of these enzymes, or by kinetic and regulatory properties that result in the two reactions proceeding in opposite directions during aerobic–anaerobic transitions (Baldwin and Opie 1978, Fields et al. 1976*b*, Regnouf and van Thoai 1970, Storey 1977, Zammit and Newsholme 1976). However, in at least some

[1] This research was conducted as part of the *Alpha Helix* Cephalopod Expedition to the Republic of the Philippines, supported by National Science Foundation grant PCM 77–16269 to J. Arnold. This study was also supported in part by the Australian Research Grants Committee.

[2] Monash University, Department of Zoology, Clayton, Victoria 3168, Australia.

gastropods and bivalves, both octopine and lactate are produced within the same muscle, although during different physiological states associated with muscle anoxia (Baldwin, Lee, and England 1981; Gäde 1980).

Less information is available on the distribution, properties, and biological functions of alanopine dehydrogenase and possible competition between this enzyme and octopine and lactate dehydrogenases in mollusk muscles used in powering locomotion (Fields 1976; Fields and Hochachka 1981; Fields et al. 1980; de Zwaan, Thompson, and Livingstone 1980). Isolation and identification of the product strombine from the gastropod *Strombus gigas* (Sangster, Thomas, and Tingling 1975) led us to examine the distribution of the three dehydrogenases in pedal retractor muscles of a range of gastropods.

MATERIALS AND METHODS

Experimental Animals

Gastropods were collected on the Great Barrier Reef, and the Victorian Coast, Australia, and in the Tanon Strait, Philippine Islands, during the R/V *Alpha Helix* expedition. Animals used for muscle metabolite studies were held for up to 5 days at 23°C in outdoor circulating seawater tanks.

Determination of the Maximum Activities of Enzymes in Pedal Retractor Muscles

Fresh pedal retractor muscles were cut into small pieces and homogenized with 10 vol of ice-cold buffer [50 mM sodium phosphate, 10^{-4} M ethylenediaminetetraacetic acid (EDTA), 10^{-4} M 1,4-dithioerythritol (DTE), pH 7.5]. Triton X-100 (1% vol/vol) was added to an aliquot of the homogenate, which was then incubated on ice for 30 min, followed by centrifugation at 600 × *g* to remove cell debris. This supernatant was used for assaying hexokinase, glutamate oxaloacetate transaminase, α-glycerophosphate dehydrogenase, and citrate synthetase. The remaining portion of the original homogenate was centrifuged as above, and the supernatant was used for assaying alanopine dehydrogenase, octopine dehydrogenase, lactate dehydrogenase, and phosphofructokinase. All assays were completed within 90 min of tissue preparation.

Enzyme activities were measured with a Unicam SP1800 or Zeiss DM4 recording spectrophotometer, and cell temperatures were controlled with a circulating water bath. Citrate synthetase was assayed at 412 nm, and the other enzyme reactions were followed by absorbance changes at 340 nm due to oxidation of NADH or reduction of NADP. Suitable controls were run to allow for nonspecific activity, and all determinations were made in triplicate at 25°C, which approximates the habitat temperature of the animals examined. Assays were carried out with 2–25 μl of muscle extract in a total reaction volume of 1 ml. The composition of the reaction mixtures, selected to give maximum activities, were as follows: (1) octopine dehydrogenase: 5 mM sodium pyruvate, 20 mM arginine, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.0; (2) lactate dehydrogenase: 2.5 mM sodium pyruvate, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.4; (3) alanopine dehydrogenase: 5 mM sodium pyruvate, 200 mM glycine, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.0; (4) α-glycerophosphate dehydrogenase: 0.5 mM dihydroxyacetone phosphate, 0.2 mM NADH, 50 mM sodium phosphate buffer, pH 7.4; (5) phosphofructokinase: 0.1 mM NADH, 0.3 mM sodium cyanide, 6 mM fructose-6-phosphate, 2 mM AMP, 1 mM ATP, 5 mM magnesium chloride, 100 mM potassium chloride, 2 IU triosephosphate isomerase, 2 IU aldolase, 2 IU α-glycerophosphate dehydrogenase, 50 mM Tris–HCl buffer, pH 8.2; (6) hexokinase: 0.4 mM NADP, 7.5 mM magnesium chloride, 1 mM EDTA, 5 mM 2-mercaptoethanol, 1.5 mM potassium chloride, 2.5 mM ATP, 1.5 mM glucose, 10 mM creatine phosphate, 2 IU glucose-6-phosphate dehydrogenase, 2 IU creatinekinase, 50 mM Tris–HCl buffer, pH 7.5; (7) glutamate oxaloacetate transaminase: 0.2 mM NADH, 10 mM α-ketoglutarate, 20 mM aspartate, 0.1 mM pyridoxal-5-phosphate, 2 IU NAD-malate dehydrogenase, 50 mM Tris–HCl buffer, pH 7.5; (8) citrate synthetase: 0.5 mM

oxaloacetate, 0.1 mM acetyl CoA, 0.2 mM 5,5′-dithiobis-(2-nitrobenzoic acid), 50 mM Tris–HCl buffer, pH 8.0.

The rate measured with the octopine dehydrogenase and alanopine dehydrogenase reaction mixtures includes lactate dehydrogenase activity; thus, values were corrected by subtracting the rate due to lactate dehydrogenase alone when arginine or glycine was omitted from the assay.

Isolation of Alanopine Dehydrogenase and Octopine Dehydrogenase from the Pedal Retractor Muscle of Strombus luhuanus

All steps in the isolation procedures were carried out at 0–4°C; buffers contained 10^{-4} M EDTA and 10^{-4} M DTE. Pedal retractor muscles were homogenized in 5 vol of 50 mM sodium phosphate buffer, pH 7.0, using a Sorvall omni-mixer. The homogenate was centrifuged at 10,000 × *g* for 30 min, and the pellet discarded.

The portion of the supernatant precipitating between 0 and 55% ammonium sulfate saturation was collected by centrifugation and used for the isolation of octopine dehydrogenase. The precipitate was dissolved in a small volume of 10 mM sodium phosphate buffer, pH 6.5, and dialyzed against a large volume of the same buffer. The dialyzed sample was applied to a O-(carboxymethyl) (CM) cellulose column (Whatman CM23) equilibrated with the dialysis buffer, and octopine dehydrogenase activity was eluted by washing with this buffer. Pooled fractions from the CM-cellulose column were concentrated by ammonium sulfate precipitation and dialyzed against 25 mM Tris–acetate buffer, pH 8.4. The dialyzed sample was loaded onto a O-(diethylaminoethyl) (DEAE) cellulose column (Whatman DE22) equilibrated with the dialysis buffer. Octopine dehydrogenase activity was eluted as up to three separate peaks by using a linear gradient from 0 to 250 mM sodium chloride in the column equilibration buffer. The peaks were concentrated separately by membrane filtration (Amicon, PM10) and further purified by gel filtration on a Sephadex G100 column equilibrated with 50 mM Tris–HCl buffer, pH 7.6. Fractions with the highest specific activity were stored at 4°C in 80% saturated ammonium sulfate.

The portion of the original supernatant precipitating between 55 and 90% ammonium sulfate saturation was used for the isolation of alanopine dehydrogenase. This precipitate was subjected to CM-cellulose chromatography as described for octopine dehydrogenase. Pooled fractions from the CM-cellulose column were concentrated by ammonium sulfate precipitation and dialyzed against 25 mM Tris–HCl buffer, pH 8.0. The dialyzed sample was loaded onto a DEAE-cellulose column equilibrated with the dialysis buffer, and the enzyme was eluted as a single peak of activity with a linear gradient from 0 to 300 mM sodium chloride in the column equilibration buffer. Pooled fractions were concentrated by membrane filtration and further purified by gel filtration as described for octopine dehydrogenase. Fractions with the highest specific activities were stored at 4°C in 80% saturated ammonium sulfate.

Determination of Molecular Weight

The molecular weights of *Strombus luhuanus* alanopine dehydrogenase and octopine dehydrogenase were estimated by gel filtration on Sephadex G100. The column (2.5 × 85 cm) was run at 4°C, with 50 mM Tris–HCl buffer, pH 7.6, containing 100 mM sodium chloride. It was calibrated with ribonuclease A (13,700 mol wt), chymotrypsinogen A (25,000 mol wt), ovalbumin (43,000 mol wt), and bovine serum albumin (67,000 mol wt). The void volume was determined with Blue Dextran 2000 (Pharmacia gel filtration kit, low molecular weight). Samples were applied in 5 ml of buffer, and 5-ml fractions were collected at a flow rate of 30 ml/hr. Molecular weights were calculated from the plot of V_e/V_0 versus log molecular weight, where V_e is the elution volume of the protein and V_0 is the void volume of the column.

Protein Determinations

The protein concentrations of samples assayed during enzyme purification were determined by ultraviolet (UV) absorption using

the following relationship: protein concentration (mg/ml) = $1.55A_{280} - 0.75A_{260}$ (Layne 1957).

Electrophoresis

Alanopine dehydrogenase and octopine dehydrogenase preparations were run on cellulose acetate gels (Cellogel, Chemtron, Milano) using 100 mM Tris–citrate buffer, pH 7.5. The gels were stained for activity as described by Fields et al. (1976*b*), using strombine, alanopine, or octopine as substrates. Proteins were stained with Coomassie blue.

Determination of Muscle Metabolites

Entire pedal retractor muscles were rapidly removed and frozen in liquid nitrogen. The frozen tissue was crushed in a percussion mortar, and then ground to a fine powder under liquid nitrogen with a mortar and pestle. The muscle powder was extracted with 10 vol of ice-cold 0.6 M perchloric acid in a Sorvall omni-mixer. The perchloric acid extracts were centrifuged at 10,000 × *g* for 20 min at 4°C, and the supernatants were neutralized with 5 M potassium carbonate. After standing on ice for several hours, precipitated potassium perchlorate was removed by centrifugation. The samples were stored at −20°C until assayed.

Octopine was determined using the purified *Strombus luhuanus* octopine dehydrogenase. The reaction mixture contained 100 mM Tris–HCl buffer, pH 9.0, 100 mM hydrazine, 10 mM NAD^+, and 10 IU octopine dehydrogenase. Strombine and alanopine were assayed with the same reaction mixture, but using 10 IU alanopine dehydrogenase in place of octopine dehydrogenase. This assay did not distinguish between strombine and alanopine, and the results are presented as the sum of the two compounds (strombine/alanopine). Arginine was measured by the method of Gäde and Grieshaber (1975), and arginine phosphate as described by Grieshaber and Gäde (1976). Pyruvate was measured enzymatically using lactate dehydrogenase. Adenylates were determined with test kits (Boehringer Mannheim). Alanine and glycine were determined with an amino acid analyzer after the perchloric acid extracts were dried under vacuum and dissolved in an appropriate buffer.

Exercising Animals

Strombus luhuanus, *S. gibberulus*, and *Lambis lambis* were exercised in small tanks of seawater. Leaping behavior was initiated by adding crushed specimens of predatory *Conus* spp. to the water. The animals responded within several minutes by violently leaping about the tank using the pedal retractor muscle for propulsion. This activity continued at up to 16 leaps/min for 5–10 min, after which the animals withdrew into the shell and failed to respond when further attempts were made to elicit activity. Individuals used for recovery experiments were removed from the exercise tank, washed free of mucus, and placed in fresh aerated seawater, or in air, for the required time.

RESULTS

Enzyme Activities in Gastropod Pedal Retractor Muscles

The maximum activities of alanopine dehydrogenase, octopine dehydrogenase, and lactate dehydrogenase assayed in supernatants of pedal retractor muscles from a range of gastropods are shown in Table 1. Alanopine dehydrogenase was present in twelve of the eighteen families surveyed, with highest activities occurring together with high activities of octopine dehydrogenase in the Strombidae. In the Conidae, Cypraeidae, Cassidae, Cymatiidae, and Thaididae, significant activities of alanopine dehydrogenase occur in the absence of high activities of octopine dehydrogenase and lactate dehydrogenase.

On the basis of these results, the pedal retractor muscle of *Strombus luhuanus* was chosen for the purification of both alanopine dehydrogenase and octopine dehydrogenase. *Strombus luhuanus*, *S. gibberulus*, and *Lambis lambis* were used for metabolite studies be-

TABLE 1

MAXIMUM ACTIVITIES OF ALANOPINE DEHYDROGENASE, OCTOPINE DEHYDROGENASE, AND LACTATE DEHYDROGENASE IN PEDAL RETRACTOR MUSCLES OF GASTROPODS

	ALANOPINE DEHYDROGENASE	OCTOPINE DEHYDROGENASE	LACTATE DEHYDROGENASE
Strombidae, *Strombus aurisdianae*	163	410	13.8
S. canarium	240	241	12.1
S. gibberulus	90	702	13.0
S. labiatus	227	369	3.5
S. lentiginosus	269	442	25
S. luhuanus	230	585	5.4
S. sinuatus	153	143	12.3
Lambis lambis	139	606	15.2
L. millepeda	150	94	3.4
L. scorpius	55	266	9.8
Tibia martinii	61	111	6.8
Cymatiidae, *Cymatium pileare*	158	<0.2	2.7
Cypraeidae, *Cypraea tigris*	65	<0.2	0.7
Thaididae, *Thais tuberosa*	25.3	<0.2	<0.2
Conidae, *Conus arenatus*	19.5	<0.2	<0.2
Bursidae, *Bursa* sp.	15.6	5.2	4.5
Cassidae, *Cypraecassius rufa*	14.2	<0.2	1.4
Nassariidae, *Nassarius coronatus*	7.8	148	45
N. glansparticeps	4.5	87	21
Buccinidae, *Cantharus undosus*	4.4	64	3.9
Neritidae, *Melanerita atramentosa*	3.7	<0.2	1.2
Xenophoridae, *Xenophora crispa*	3.7	2.9	<0.2
Mitridae, *Mitra eremitarum*	2.3	<0.2	0.7
Muricidae, *Dicathais textilosa*	<0.2	40	1.8
Murex tribulus	<0.2	14	<0.2
Patellidae, *Cellana tramoserica*	<0.2	<0.2	<0.2
Siphonariidae, *Siphonaria diemenensis*	<0.2	<0.2	2.4
Trochidae, *Austrocochlea concamerata*	<0.2	<0.2	<0.2
Turbinidae, *Turbo argyrostomus*	<0.2	<0.2	<0.2
Volutidae, *Cymbiola vespertilio*	<0.2	<0.2	24.8

NOTE: Data expressed as μmole NADH/min/g wet wt muscle at 25°C.

TABLE 2

MAXIMUM ACTIVITIES OF ENZYMES IN PEDAL RETRACTOR MUSCLES OF *Strombus luhuanus*, *Strombus gibberulus*, AND *Lambis lambis*

ENZYME	*S. luhuanus*	*S. gibberulus*	*L. lambis*
Hexokinase	<0.1	< 0.1	<0.1
Phosphofructokinase	20.0 ±1.7	14.5 ± 0.5	9.5
α-Glycerophosphate dehydrogenase	0.40±0.03	1.3 ± 0.07	<0.1
Glutamate oxaloacetate transaminase	7.6 ±0.1	8.9 ± 1.5	
Citrate synthetase	0.41±0.02	0.47± 0.04	0.20
Alanopine dehydrogenase	245 ±5	89 ±10	140
Octopine dehydrogenase	576 ±9	651 ±51	615
Lactate dehydrogenase	<0.1	< 0.1	<0.1

NOTE: Data expressed as μmole substrate/min/g wet wt muscle at 25°C. Values for *S. luhuanus* and *S. gibberulus* are means ± ranges for determinations on two individuals. Values for *L. lambis* are means for three determinations on one individual.

cause of their availability and the ease with which the animals could be exercised. The maximum activities of a number of enzymes involved in aerobic and anaerobic energy metabolism in the pedal retractor muscles of *S. luhuanus*, *S. gibberulus*, and *L. lambis* are shown in Table 2. The small amount of lactate dehydrogenase activity in these muscle homogenates (Table 1) disappeared following dialysis, and is assumed to be an artifact resulting from the presence of arginine, alanine, and particularly glycine in the tissue extracts (see Table 8) serving as substrates for octopine dehydrogenase and alanopine dehydrogenase.

Purification of Alanopine Dehydrogenase and Octopine Dehydrogenase

Alanopine dehydrogenase was present as a single anodal electrophoretic band in pedal retractor muscles of all *Strombus luhuanus* examined. This band migrated at about twice the rate of octopine dehydrogenase, and the two enzymes were readily separated on DEAE-cellulose chromatography. Coincident bands of alanopine dehydrogenase activity were obtained when electrophoretic gels were stained with *MS*-alanopine or D-strombine. No band was observed when L-strombine was used as substrate. The final alanopine dehydrogenase preparation was estimated to be about 70% homogenous on the basis of protein and activity staining following electrophoresis. It had a specific activity of 200 IU/mg protein, and was free of octopine dehydrogenase activity.

Octopine dehydrogenase occurs as three electrophoretically distinguishable anodally migrating bands of enzyme activity in the pedal retractor muscles of *Strombus luhuanus*. Individual animals possess either one or two of these three bands. When purifying the enzyme from pooled samples, the three bands were separated as nonoverlapping peaks of activity by DEAE-cellulose chromatography. The major peak, accounting for up to 80% of the total octopine dehydrogenase activity in pooled samples of pedal retractor muscle, was used for structural and kinetic studies and for metabolite determinations. The purified enzyme gave single coincident protein and activity bands on electrophoresis, had a specific activity of 780 IU/mg protein, and was free of alanopine dehydrogenase activity.

TABLE 3

Strombus luhuanus ALANOPINE DEHYDROGENASE: APPARENT K_m VALUES FOR SUBSTRATES

SUBSTRATE	COSUBSTRATE	APPARENT K_m (mM)
Pyruvate	200 mM glycine, 0.2 mM NADH	3.1
	10 mM glycine, 0.2 mM NADH	2.9
	200 mM alanine, 0.2 mM NADH	2.8
	10 mM alanine, 0.2 mM NADH	2.4
Glycine	5 mM pyruvate, 0.2 mM NADH	200
	0.5 mM pyruvate, 0.2 mM NADH	200
Alanine	5 mM pyruvate, 0.2 mM NADH	90
	0.5 mM pyruvate, 0.2 mM NADH	70
NADH	200 mM glycine, 5 mM pyruvate	0.04
	10 mM glycine, 0.5 mM pyruvate	0.04
	200 mM alanine, 5 mM pyruvate	0.06
	10 mM alanine, 0.5 mM pyruvate	0.03
MS-Alanopine	5 mM NAD^+	3.2
	1 mM NAD^+	3.2
D-Strombine	5 mM NAD^+	2.8
	1 mM NAD^+	2.8
NAD^+	10 mM *MS*-alanopine	0.25
	0.5 mM *MS*-alanopine	0.40
	2 mM D-strombine	0.74

ASSAY CONDITIONS: forward reaction, 50 mM sodium phosphate buffer, pH 7.0; reverse reaction, 50 mM Tris–HCl buffer, pH 8.0. Assay temperature, 25°C. Apparent K_m values were determined from Lineweaver–Burk and Cornish–Bowden plots.

Molecular Weight of Alanopine Dehydrogenase and Octopine Dehydrogenase

Gel filtration on Sephadex G100 gave a molecular weight of 42,000 for *Strombus luhuanus* alanopine dehydrogenase. A value of 47,000 has been reported for the enzyme from oyster adductor muscle (Fields and Hochachka 1981). The molecular weight of 39,000 obtained for octopine dehydrogenase corresponds to the single subunit enzyme of bivalves (Baldwin and Opie 1978, Olomucki et al. 1972) and the enzymes from cephalopod mantle (Fields, Baldwin, and Hochachka 1976*a*).

Kinetic Properties of Strombus luhuanus *Alanopine Dehydrogenase*

EFFECT OF pH ON ACTIVITY: The pH profiles at saturating substrate concentrations show optima at about pH 7.0 for the forward (pyruvate reduction) reaction and 8.5 for the reverse reaction. The ratio of the maximum activities at these pH optima is 6 : 1 in favor of the forward reaction.

SATURATION KINETICS: The enzyme displayed Michaelis–Menten saturation kinetics for all substrates of both the forward and reverse reactions. Substrate inhibition did not occur at high concentrations of pyruvate, alanine, glycine, NAD^+, NADH, D-strombine, or *MS*-alanopine. No activity was observed when L-strombine was used as a substrate for the reverse reaction. The K_m (apparent Michaelis constant) values for these substrates are listed in Table 3 and can be compared with the substrate concentrations present in the *Strombus luhuanus* pedal retractor muscle (Table 8).

The effectiveness of the products of the forward reactions catalyzed by both alanopine dehydrogenase and octopine dehydrogenase in inhibiting alanopine dehydrogenase are given as K_i (inhibition constant) values in Table 4.

TABLE 4

Strombus luhuanus ALANOPINE DEHYDROGENASE: INHIBITION CONSTANTS (K_i) FOR THE FORWARD REACTION

SUBSTRATE	INHIBITOR	K_i (mM)
Pyruvate	Octopine	17.5
	D-Strombine	2.8
	MS-Alanopine	8.5
Glycine	Octopine	18
	D-Strombine	2.8
Alanine	Octopine	18
	MS-Alanopine	8.5
NADH	NAD^+	0.23

ASSAY CONDITIONS: 50 mM sodium phosphate buffer, pH 7.0, 25°C; K_i values were determined from Dixon plots using three concentrations of substrate and saturating concentrations of cosubstrates.

Kinetic Properties of Strombus luhuanus *Octopine Dehydrogenase*

EFFECT OF pH ON ACTIVITY: The pH profiles at saturating substrate concentrations were similar to those obtained for octopine dehydrogenases of bivalves and cephalopods (Baldwin and Opie 1978, Fields et al. 1976*a*), with a pH optimum of about 7.0 for the forward reaction and a broad peak at 9.4 for the reverse reaction. The relative activities under optimal conditions were in the ratio 21 : 1 in favor of the forward reaction.

SATURATION KINETICS: Michaelis–Menten saturation kinetics were obtained for all substrates. Substrate inhibition occurred at octopine concentrations above 5 mM, but arginine, pyruvate, NAD^+, and NADH were not inhibitory at high concentrations. The K_m values for these substrates are listed in Table 5. As with other molluscan octopine dehydrogenases (Fields et al. 1976*a*; Hochachka, Hartline, and Fields 1977), the K_m values for pyruvate and arginine were influenced by the cosubstrate concentration (arginine or pyruvate). Increasing the concentration of one substrate decreased the K_m value for the other.

The K_i values listed in Table 6 show that octopine was a potent inhibitor of the forward reaction, with K_i octopine about one-twelfth the highest octopine concentration measured in the *Strombus luhuanus* pedal retractor muscle (Table 8). This product inhibition was competitive with respect to both arginine and pyruvate. The effects of physiological concentrations of octopine on the K_m values obtained for these substrates are shown in Table 7.

TABLE 5

Strombus luhuanus OCTOPINE DEHYDROGENASE: APPARENT K_m VALUES FOR SUBSTRATES

SUBSTRATE	COSUBSTRATE	APPARENT K_m (mM)
Pyruvate	1 mM arginine, 0.1 mM NADH	5.0
	10 mM arginine, 0.1 mM NADH	3.0
	10 mM arginine, 0.05 mM NADH	3.2
Arginine	0.25 mM pyruvate, 0.1 mM NADH	20.0
	0.5 mM pyruvate, 0.1 mM NADH	11.0
	1 mM pyruvate, 0.1 mM NADH	6.0
	1 mM pyruvate, 0.05 mM NADH	5.5
NADH	20 mM arginine, 5 mM pyruvate	0.06
	1 mM arginine, 0.5 mM pyruvate	0.04
Octopine	0.1 mM NAD^+	0.18
	0.5 mM NAD^+	0.18
	1 mM NAD^+	0.18
NAD^+	1 mM octopine	0.32
	5 mM octopine	0.33

ASSAY CONDITIONS: forward reaction, 50 mM sodium phosphate buffer, pH 7.0; reverse reaction, 50 mM Tris–HCl buffer, pH 8.0. Assay temperature, 25°C. Apparent K_m values were determined from Lineweaver–Burk and Cornish–Bowden plots.

TABLE 6

Strombus luhuanus OCTOPINE DEHYDROGENASE: INHIBITION CONSTANTS (K_i) FOR THE FORWARD REACTION

SUBSTRATE	INHIBITOR	K_i (mM)
Pyruvate	Octopine	0.20
	D-Strombine	6.5
	MS-Alanopine	11
Arginine	Octopine	0.18
	D-Strombine	6.0
	MS-Alanopine	11
NADH	NAD^+	0.06

ASSAY CONDITIONS: 50 mM sodium phosphate buffer, pH 7.0, 25°C; K_i values were determined from Dixon plots using three concentrations of substrate and saturating concentrations of cosubstrates.

TABLE 7

Strombus luhuanus OCTOPINE DEHYDROGENASE: APPARENT K_m VALUES FOR ARGININE AND PYRUVATE AT VARIOUS OCTOPINE CONCENTRATIONS

	K_m (mM)	
OCTOPINE (mM)	ARGININE	PYRUVATE
0	5.7	3.0
0.25	14	9
0.5	25	14
1.0	40	30
2.0	67	50

ASSAY CONDITIONS: 50 mM sodium phosphate buffer, pH 7.0, 25°C; K_m arginine: 1 mM pyruvate, 0.2 mM NADH; K_m pyruvate: 10 mM arginine, 0.2 mM NADH. Apparent K_m values were determined from Lineweaver–Burk plots.

TABLE 8

MAXIMUM CONCENTRATIONS OF METABOLITES MEASURED IN PEDAL RETRACTOR MUSCLES OF *Strombus luhuanus*

SUBSTRATE	STATE OF ANIMAL	CONCENTRATION (μmole/g wet wt muscle)
Pyruvate	Resting in water	< 0.2
Pyruvate	Exercised	< 0.2
Glycine	Resting in water	147
Alanine	Resting in water	5.2
Octopine	Exercised	2.3
Alanopine/strombine	Recovery in air	5.9
Arginine	Resting in water	9.6
Arginine	Exercised	10.8

TABLE 9

CONCENTRATIONS OF STROMBINE/ALANOPINE, OCTOPINE, AND ARGININE PHOSPHATE IN PEDAL RETRACTOR MUSCLES OF *Strombus luhuanus*, *Strombus gibberulus*, AND *Lambis lambis* AT REST AND IMMEDIATELY FOLLOWING EXERCISE

SPECIES	MUSCLE STATE	*n*	CONCENTRATION (μmole/g wet wt muscle) STROMBINE/ ALANOPINE	OCTOPINE	ARGININE PHOSPHATE
Strombus luhuanus	Rest	5	0.5 (0.2–0.9)	0.5 (< 0.2–1.0)	11.5 (10.1–12.4)
	29 leaps	1	1.6	1.2	10.0
	33 leaps	1	2.2	1.6	8.2
Strombus gibberulus	Rest	6	0.3 (< 0.2–0.8)	0.7 (< 0.2–1.3)	10.2 (8.5–12.8)
	108 leaps	1	1.3	1.9	7.5
	125 leaps	1	2.9	2.5	6.5
Lambis lambis	Rest	2	0.3 (0)	0.4 (0.3–0.5)	6.6 (6.2–6.9)
	43 leaps	1	0.9	0.7	4.4
	52 leaps	1	1.0	1.6	2.9

NOTE: *n* is the number of individual animals assayed. Values for resting animals are means, with ranges in parentheses.

Effects of Activity on Pedal Retractor Muscle Metabolites

The effects of exercise on the concentrations of strombine/alanopine, octopine, and arginine phosphate in the pedal retractor muscles of *Strombus luhuanus*, *S. gibberulus*, and *Lambis lambis* (freeze-clamped immediately following activity) are shown in Table 9.

Concentrations of strombine/alanopine, octopine, arginine phosphate, and the energy charge in pedal retractor muscles of *Strombus luhuanus* at rest, immediately following exercise, and after 10 min and 30 min recovery in water and in air are shown in Table 10.

DISCUSSION

Enzyme Profiles in Pedal Retractor Muscles of Strombidae

Molluscan muscles used in powering locomotion differ markedly in the capacity to produce ATP under both aerobic and anaerobic conditions. These differences are reflected in the maximum activities of associated enzymes. High activities of phosphofructokinase, citrate synthetase, and α-glycerophosphate dehydrogenase or glutamate oxaloacetate transaminase occur in the mantle and fin muscles of active pelagic squids that use aerobic carbohydrate catabolism during prolonged periods of rapid swimming (Baldwin, this issue; Hochachka et al. 1975). Muscles that rely heavily on anaerobic metabolism during short bursts of maximal activity and have little capacity in sustaining high levels of aerobic work, show high activities of phosphofructokinase and octopine dehydrogenase, but lower activities of α-glycerophosphate dehydrogenase, glutamate oxaloacetate transaminase, and Krebs cycle enzymes. Examples include the scallop fast adductor muscle and the muscles of less active cephalopods (Alp, Newsholme, and Zammit 1976; Baldwin, this issue; Baldwin and Opie 1978; Fields et al. 1976*a*; Zammit and Newsholme 1976). Muscles with low maximum rates of both aerobic and anaerobic energy production have low activities of octopine dehydrogenase, phosphofructokinase, α-glycerophosphate dehydrogenase, glutamate oxaloacetate transaminase, and Krebs cycle enzymes (Alp et al. 1976, Baldwin and Opie 1978, Zammit and Newsholme 1976).

The relatively high activities of octopine dehydrogenase and phosphofructokinase,

TABLE 10

CONCENTRATIONS OF STROMBINE/ALANOPINE, OCTOPINE, ARGININE PHOSPHATE, AND ENERGY CHARGE IN PEDAL RETRACTOR MUSCLES OF *Strombus luhuanus* AT REST, IMMEDIATELY AFTER EXERCISE, AND DURING RECOVERY IN AIR AND IN WATER FOLLOWING EXERCISE

MUSCLE STATE	*n*	CONCENTRATION (μmole/g wet wt muscle) STROMBINE/ ALANOPINE	OCTOPINE	ARGININE PHOSPHATE	ENERGY CHARGE*
Resting in water	6	0.6 (0.2–1.0)	0.6 (<0.2–1.0)	10.8 (7.4–12.4)	0.90 (0.88–0.91)
Exercised	6	1.2 (0.5–2.2)	1.5 (0.8–2.3)	9.9 (8.2–11.9)	0.90 (0.89–0.91)
10 min recovery in water	4	1.2 (0.8–1.4)	1.2 (0.8–2.1)	9.5 (8.5–10.6)	0.90 (0.89–0.91)
10 min recovery in air	4	1.4 (1.1–2.2)	1.1 (0.4–1.9)	8.0 (6.5–8.6)	0.86 (0.83–0.89)
30 min recovery in water	4	2.2 (1.2–4.6)	1.4 (1.1–1.8)	9.2 (8.0–10.6)	0.87 (0.82–0.90)
30 min recovery in air	4	3.7 (2.3–5.9)	1.9 (1.3–2.2)	7.1 (6.2–9.0)	0.84 (0.79–0.89)

NOTE: *n* is the number of individual animals assayed. Values are means, with ranges in parentheses.
*Energy charge was calculated according to Atkinson (1977) as ([ATP] + [ADP]/2)/([ATP] + [ADP] + [AMP]).

and low activities of hexokinase, α-glycerophosphate dehydrogenase, glutamate oxaloacetate transaminase, and citrate synthetase in the pedal retractor muscles of *Strombus luhuanus*, *S. gibberulus*, and *Lambis lambis* (Table 2) are indicative of muscles that rely on anaerobic ATP production during short bursts of maximum activity, with octopine accumulating as an end product of anaerobic glycolysis.

Alanopine dehydrogenase does not occur at significant activities in the fast adductor muscle of the scallop *Placopeten magellanicus* (de Zwaan et al. 1980), or in the mantle, fin, arm, funnel or retractor muscles of cephalopods (Baldwin, this issue). The high activities of this enzyme in *Strombus* and *Lambis* muscles used for powering short-term bursts of rapid leaping suggest that strombine or alanopine, in addition to octopine, may accumulate during muscle anoxia associated with locomotion in these animals.

Electrophoretic Forms of Strombus luhuanus *Octopine Dehydrogenase*

The distribution of the three electrophoretic forms of octopine dehydrogenase among individual *Strombus luhuanus* pedal retractor muscles suggests that the enzyme is polymorphic, existing as three alleles. If the enzyme is monomeric, as suggested by the molecular weight of 39,000, then individuals homozygous for any one of the three alleles should give a single band of octopine dehydrogenase activity. Individuals heterozygous for any two of the three alleles should show two octopine dehydrogenase bands. As expected from this interpretation, no individuals possessing all three bands were observed. Similar genetic variation has been reported for octopine dehydrogenase in bivalve adductor muscle (Beaumont, Day, and Gäde 1980).

How Many Enzymes Are Responsible for Octopine, Strombine, and Alanopine Dehydrogenase Activity in Pedal Retractor Muscles of Strombidae?

Octopine dehydrogenase and strombine/alanopine dehydrogenase activities in the *Strombus luhuanus* pedal retractor muscle are catalyzed by different enzymes. These enzymes can be distinguished on the basis of ammonium sulfate precipitation, electrophoretic migration, ion exchange chromatog-

raphy, and gel filtration. In addition, antisera produced against *S. luhuanus* octopine dehydrogenase (Baldwin, this issue) inhibit octopine dehydrogenase but have no effect on the strombine/alanopine dehydrogenase activity.

Strombine dehydrogenase and alanopine dehydrogenase activities copurify through all stages of the isolation procedure, maintaining a constant activity ratio of 1 : 0.8 when 200 mM glycine and 200 mM alanine are used as substrates. The identical electrophoretic migration rates obtained when D-strombine or *MS*-alanopine are used as substrates for the reverse reaction provide further evidence that both strombine and alanopine dehydrogenase activities are catalyzed by a single enzyme.

Kinetic Properties of Alanopine Dehydrogenase and Octopine Dehydrogenase

The kinetic properties of the two enzymes were investigated to provide information on the probable directions of the reactions in vivo and on possible competition for common substrates.

The pH profiles obtained for both enzymes suggest that the forward reactions are favored throughout the physiological pH range.

Neither enzyme displayed substrate inhibition for the forward reaction. With lactate dehydrogenases, pyruvate inhibition has been used as a criterion for determining the direction of the reaction in vivo (Dawson, Goodfellow, and Kaplan 1964; Long, Ellington, and Duda 1979). Storey and Storey (1979*a*) distinguished between the physiological roles of mantle muscle and brain octopine dehydrogenase in the cephalopod *Sepia officinalis* on the basis of inhibition of the forward reaction at high pyruvate concentrations. The pyruvate saturation curves for *Strombus luhuanus* octopine dehydrogenase and alanopine dehydrogenase resemble the *Sepia* mantle enzyme, which is known to function in the direction of octopine formation during swimming (Storey and Storey 1979*b*). However, octopine dehydrogenase from the fast adductor muscle of the scallop *Pecten alba*, which also produces octopine during rapid contractions of the fast adductor muscle, is strongly inhibited at pyruvate concentrations above 1 mM (Baldwin and Opie 1978). This suggests that pyruvate inhibition per se is of limited value for predicting the direction of the reactions catalyzed by the *Strombus luhuanus* enzymes in vivo.

Both enzymes show similar K_m values for pyruvate and NADH when determined at physiological concentrations of the cosubstrates (Tables 3, 5, 8). The pyruvate concentrations measured in pedal retractor muscles of resting animals are well below the K_m values, while the NADH concentration is unknown. The K_m values for glycine and arginine approximate the physiological levels of these substrates, but the concentration of alanine is one-tenth the K_m value. Thus, glycine, rather than alanine, may serve as the major substrate for the alanopine dehydrogenase reaction, with strombine as the major product. If this is the case, then the activities of both octopine dehydrogenase and alanopine dehydrogenase may be regulated by the rate at which pyruvate and NADH are produced, thereby linking both reactions directly to the glycolytic flux during muscle anoxia. In addition, simultaneous increases in pyruvate from glycolysis and arginine from the hydrolysis of arginine phosphate may accelerate octopine production by increasing the affinity of octopine dehydrogenase for both substrates.

For the reverse reaction, the low K_m value for octopine relative to octopine concentrations in the exercised muscle may indicate that octopine dehydrogenase is better suited for the removal of the product of the forward reaction following anaerobic muscle work than is alanopine dehydrogenase.

Production of Strombine/Alanopine and Octopine in the Pedal Retractor Muscles of Strombidae

During bursts of rapid leaping, both strombine/alanopine and octopine accumulate together in the pedal retractor muscles of *Strombus luhuanus*, *S. gibberulus*, and *Lambis lambis*, while arginine phosphate concentrations fall (Table 9). These results show that the rate at which ATP is used during exercise exceeds the aerobic capabilities of the muscle. Under these conditions, additional ATP is obtained from the breakdown of arginine

phosphate and from anaerobic glycolysis, with both alanopine dehydrogenase and octopine dehydrogenase apparently acting to catalyze the terminal step in the glycolytic pathway.

Some mollusk muscles with limited aerobic capabilities use anaerobic glycolysis in regenerating arginine phosphate and reestablishing energy charge during recovery from exercise (Baldwin et al. 1981; Gäde, Weeda, and Gabbott 1978; Grieshaber 1978). In the pedal retractor muscle of *Nassarius coronatus*, which has high activities of both octopine dehydrogenase and lactate dehydrogenase (Table 1), only octopine is produced during activity and only lactate during the recovery phase (Baldwin et al. 1981). To determine the relative contributions of alanopine dehydrogenase and octopine dehydrogenase during recovery from exercise, metabolite levels were measured in pedal retractor muscles of *Strombus luhuanus* at rest, immediately following exercise, and after various periods of recovery in water and in air (Table 10).

In animals left to recover in aerated seawater following exercise, octopine and arginine phosphate concentrations remained at postexercise levels, energy charge fell slightly, and the concentration of strombine/alanopine increased with time. These results indicate that anaerobic glycolysis is used during the recovery phase, but with only alanopine dehydrogenase catalyzing the terminal reaction. A similar conclusion can be drawn from the results obtained for animals left to recover in air. When exposed to air, the gills cannot be irrigated with water, and oxygen uptake may be impaired. In this situation, the rate of anaerobic glycolysis apparently was not sufficient to maintain energy charge and arginine phosphate concentration at postexercise levels. Both arginine phosphate and energy charge fell, octopine levels remained constant, while strombine/alanopine concentrations increased to the highest values observed in this study.

An important question arising from these results relates to the independent regulation of octopine dehydrogenase and alanopine dehydrogenase activity during the recovery period. Presumably, mechanisms exist for the selective inhibition of octopine dehydrogenase following exercise. One way of achieving this would be through inhibition of octopine dehydrogenase, but not alanopine dehydrogenase by-products of the two pyruvate reductase reactions, once these had accumulated to sufficiently high concentrations during muscle work. The K_i values listed for these products (Tables 4, 6) show that octopine is a potent inhibitor of the forward reaction catalyzed by octopine dehydrogenase, but has little effect on alanopine dehydrogenase. For octopine dehydrogenase, the inhibition constant for octopine is about one-twelfth the concentration of octopine measured following exercise. This inhibition is competitive with respect to both substrates, with physiological concentrations of octopine (2 mM) increasing K_m arginine about twelvefold and K_m pyruvate seventeenfold (Table 7).

The inhibitor constant for NAD^+ is also lower for octopine dehydrogenase than for alanopine dehydrogenase. If NAD^+ concentrations in the pedal retractor muscle are similar to other tissues (about 1 mM; Edington 1970, Tischler et al. 1977), the low K_i NAD^+ values indicate that the cytoplasmic NAD^+/NADH ratio may also play a major role in regulating the activities of these two dehydrogenases.

Finally, while product inhibition provides a possible explanation for the accumulation of both octopine and strombine/alanopine during exercise but only strombine/alanopine during recovery from exercise, it is not at all clear why two different enzymes are used to catalyze the terminal step of anaerobic glycolysis in the pedal retractor muscle of Strombidae. Similar problems exist concerning the octopine dehydrogenases and lactate dehydrogenases that function independently during different physiological states associated with anoxia in the pedal retractor muscle of the gastropod *Nassarius coronatus* (Baldwin et al. 1981) and the foot of the bivalve *Cardium tuberculatum* (Gäde 1980).

ACKNOWLEDGMENTS

We thank members of the *Alpha Helix* Philippines expedition for providing many

of the mollusks used in this study, and the Heron Island Research Station for making their facilities available to us. Strombine and alanopine were kindly provided by J. Tempé.

LITERATURE CITED

ALP, P. R., E. A. NEWSHOLME, and V. A. ZAMMIT. 1976. Activities of citrate synthase and NAD^+-linked and NADP-linked isocitrate dehydrogenase in muscles from vertebrates and invertebrates. Biochem. J. 154:689–700.

ATKINSON, D. E. 1977. Cellular energy metabolism and its regulation. Academic Press, New York.

BALDWIN, J., and A. M. OPIE. 1978. On the role of octopine dehydrogenase in the adductor muscles of bivalve molluscs. Comp. Biochem. Physiol. 61B:85–92.

BALDWIN, J., A. K. LEE, and W. R. ENGLAND. 1981. The functions of octopine dehydrogenase and D-lactate dehydrogenase in the pedal retractor muscle of the dog whelk *Nassarius coronatus* (Gastropoda: Nassariidae). Mar. Biol. 62:235–238.

BEAUMONT, A. R., T. R. DAY, and G. GÄDE. 1980. Genetic variation at the octopine dehydrogenase locus in the adductor muscle of *Cerastoderma edule* (L.) and six other bivalve species. Mar. Biol. Lett. 1:137–148.

DAWSON, D. M., T. L. GOODFELLOW, and N. O. KAPLAN. 1964. Lactic dehydrogenase: Functions of the two types. Science 143: 929–933.

EDINGTON, D. W. 1970. Pyridine nucleotide oxidized to reduced ratio as a regulator of muscular performance. Experientia 26: 601–602.

FIELDS, J. H. A. 1976. A dehydrogenase requiring alanine and pyruvate as substrates from oyster adductor muscle. Fed. Proc. 35:1687.

FIELDS, J. H. A., and P. W. HOCHACHKA. 1981. Purification and properties of alanopine dehydrogenase from the adductor muscle of the oyster, *Crassostrea gigas* (Mollusca, Bivalvia). Eur. J. Biochem. 114:615–621.

FIELDS, J. H. A., J. BALDWIN, and P. W. HOCHACHKA. 1976*a*. On the role of octopine dehydrogenase in cephalopod mantle muscle metabolism. Can. J. Zool. 54:871–878.

FIELDS, J. H. A., H. GUDERLEY, K. B. STOREY, and P. W. HOCHACHKA. 1976*b*. The pyruvate branch point in squid brain: Competition between octopine dehydrogenase and lactate dehydrogenase. Can. J. Zool. 54:879–885.

FIELDS, J. H. A., A. K. ENG, W. D. RAMSDEN, P. W. HOCHACHKA, and B. WEINSTEIN. 1980. Alanopine and strombine are novel iminoacids produced by a dehydrogenase found in the adductor muscle of the oyster, *Crassostrea gigas*. Arch. Biochem. Biophys. 201:110–114.

GÄDE, G. 1980. The energy metabolism of the foot muscle of the jumping cockle, *Cardium ruberculatum*: Sustained anoxia versus muscular activity. J. Comp. Physiol. 137B:177–182.

GÄDE, G., and M. GRIESHABER. 1975. A rapid and specific enzymatic method for the estimation of L-arginine. Anal. Biochem. 66: 393–399.

GÄDE, G., E. WEEDA, and P. A. GABBOTT. 1978. Changes in the level of octopine during the escape response of the scallop, *Pecten maximus* (L.). J. Comp. Physiol. 124B:121–127.

GRIESHABER, M. 1978. Breakdown and formation of high-energy phosphates and octopine in the adductor muscle of the scallop, *Chlamys opercularis* (L.), during escape swimming and recovery. J. Comp. Physiol. 126B:269–276.

GRIESHABER, M., and G. GÄDE. 1976. The biological role of octopine in the squid, *Loligo vulgaris* (Lamarck). J. Comp. Physiol. 108B:225–232.

HOCHACHKA, P. W., P. H. HARTLINE, and J. H. A. FIELDS. 1977. Octopine as an end product of anaerobic glycolysis in the chambered nautilus. Science 195:72–74.

HOCHACHKA, P. W., T. W. MOON, T. MUSTAFA, and K. B. STOREY. 1975. Metabolic sources of power for mantle muscle of a fast swimming squid. Comp. Biochem. Physiol. 52B:151–157.

LAYNE, E. 1957. Spectrophotometric and turbidometric methods for measuring proteins. Pages 447–454 *in* S. P. Colowick and

N. O. Kaplan, eds. Methods in enzymology. Vol. 3. Academic Press, New York.

LONG, G. L., W. R. ELLINGTON, and T. F. DUDA. 1979. Comparative enzymology and physiological role of D-lactate dehydrogenase from the foot muscle of two gastropod molluscs. J. Exp. Zool. 207: 237–248.

OLOMUCKI, A., C. HUC, F. LEFEBURE, and N. VAN THOAI. 1972. Octopine dehydrogenase. Evidence for a single-chain structure. Eur. J. Biochem. 28:261–268.

REGNOUF, F., and N. VAN THOAI. 1970. Octopine and lactate dehydrogenases in mollusc muscles. Comp. Biochem. Physiol. 32:411–416.

SANGSTER, A. W., S. E. THOMAS, and N. L. TINGLING. 1975. Fish attractants from marine invertebrates. Arcamine from *Arca zebra* and strombine from *Strombus gigas*. Tetrahedron 31:1135–1137.

STOREY, K. B. 1977. Tissue specific isozymes of octopine dehydrogenase in the cuttle fish, *Sepia officinalis*. The roles of octopine dehydrogenase and lactate dehydrogenase in *Sepia*. J. Comp. Physiol. 115B:159–169.

STOREY, K. B., and J. M. STOREY. 1979*a*. Kinetic characterization of tissue-specific isozymes of octopine dehydrogenase from mantle muscle and brain of *Sepia officinalis*. Functional similarities to the M_4 and H_4 isozymes of lactate dehydrogenase. Eur. J. Biochem. 93:545–552.

———. 1979*b*. Octopine metabolism in the cuttle fish, *Sepia officinalis*: Octopine production by muscle and its role as an aerobic substrate for non-muscular tissues. J. Comp. Physiol. 131B:311–319.

TISCHLER, M. E., D. FRIEDRICHS, K. COLL, and J. R. WILLIAMSON. 1977. Pyridine nucleotide distributions and enzyme mass action ratios in hepatocytes from fed and starved rats. Arch. Biochem. Biophys. 184:222–236.

ZAMMIT, V. A., and E. A. NEWSHOLME. 1976. The maximum activities of hexokinase, phosphorylase, phosphofructokinase, glycerol phosphate dehydrogenases, lactate dehydrogenase, octopine dehydrogenase, phosphoenolpyruvate carboxykinase, nucleoside diphosphatekinase, glutamate oxaloacetate transaminase and argine kinase in relation to carbohydrate utilization in muscles from marine invertebrates. Biochem. J. 160:447–462.

DE ZWAAN, A., R. J. THOMPSON, and D. R. LIVINGSTONE. 1980. Physiological and biochemical aspects of the valve snap and valve closure responses in the giant scallop *Placopecten magellanicus*. II. Biochemistry. J. Comp. Physiol. 137B:105–114.