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Intermediary Energy Metabolism during Dormancy and Anoxia in the Land Snail *Otala lactea*

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Abstract

Metabolic responses to dormancy and anoxia were assessed in foot muscle and hepatopancreas of the land snail Otala lactea *(Pulmonata, Helicidae). In both states cellular energetics (arginine phosphate content, adenylate energy charge) were maintained at a high level, fueled during estivation by aerobic metabolism and during anoxia by carbohydrate fermentation. D-lactate was the major product of anaerobiosis in hepatopancreas, but in foot muscle D-lactate, L-alanine, and succinate accumulated (in net amounts of 13.5, 3.5, and 1.8 μmol g^{-1} wet weight, respectively). Changes in the concentrations of glycolytic intermediates were compared for both short- and long-term stress: 2 and 14 h of exposure to N_2 gas atmosphere, 3 and 22 d of dormancy at 22° C. Both stresses appeared to include glycolytic activation in the short term in foot muscle, with crossover analyses indicating regulatory control at the phosphofructokinase, aldolase, and pyruvate kinase loci. Over the long term, however, this was reversed and a glycolytic rate depression was observed as part of the overall metabolic rate depression of these states. In foot muscle, inhibition at phosphofructokinase and pyruvate kinase was apparent during anoxia, whereas aldolase and pyruvate kinase were the key sites of inhibitory control during estivation. In hepatopancreas, phosphofructokinase was the primary locus for inhibitory control during both prolonged anoxia and estivation. These data suggest that common molecular mechanisms underlie glycolytic rate depression during dormancy and anaerobiosis.*

Introduction

With limited abilities to physically elude environmental extremes, gastropod molluscs have developed a range of physiological and biochemical ad-

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aptations that permit them to endure stress. Chief among these is metabolic rate depression, a retreat into dormancy whenever conditions are unfavorable for active life. This can occur in response to aridity, low temperature, or lack of oxygen, and the characteristics of estivation, hibernation, and anaerobiosis have been studied in many laboratories (for review, see Machin 1975; Livingstone and De Zwaan 1983; Riddle 1983). By lowering metabolic rate to values ranging from 5% to 30% of the standard resting rate, molluscs can survive for days without oxygen or for months in dormancy when conditions are too dry or too cold (Herreid 1977; Horne 1979; Famme, Knudsen, and Hansen 1981; Shick, De Zwaan, and de Bont 1983; Vorhaben, Klotz, and Campbell 1984; Barnhart and McMahon 1987).

The molecular mechanisms controlling metabolic rate depression have only recently come under study (Storey 1985*a,* 1988*a;* Hochachka and Guppy 1987). Biochemical controls applied at key regulatory sites reorganize metabolism and permit rapid entry into and arousal from dormancy. To date, these have been most thoroughly investigated for facultative anaerobiosis in marine molluscs, particularly for the gastropod whelk, *Busycotypus canaliculatum* (reviewed by Storey 1985*a,* 1988*a*). Here, despite a dependence on carbohydrate fermentation for anaerobic energy production, glycolytic rate is depressed and no Pasteur effect is seen (De Zwaan and Wijsman 1976; Storey 1985*a*). The mechanisms involved include (*a*) modification of the activity state of regulatory enzymes by protein phosphorylation or dephosphorylation, (*b*) enzyme and pathway control by reversible associations of enzymes with the particulate fraction of the cell, and (*c*) control of carbohydrate utilization for anabolic purposes via fructose-2,6-P_2 regulation of phosphofructokinase (Plaxton and Storey 1984, 1985, 1986; Storey 1984, 1985*b,* 1988*b;* Storey et al. 1990). Recent studies have shown that these mechanisms also apply to metabolic rate depression as it occurs in anaerobiosis in goldfish and diving turtles and in hibernation in small mammals (Storey 1988*a*).

The pulmonate land snail *Otala lactea* inhabits seasonally arid environments and spends many months of the year in a dormant state. Respiration, acid-base balance, and evaporative water loss during dormancy have been extensively analyzed (Barnhart 1983, 1986*a,* 1986*b;* Barnhart and McMahon 1987, 1988). Dormancy is characterized by hypoxia, hypercapnia, extracellular acidosis, and metabolic depression (aerobic metabolic rate reduced by as much as 85%) (Barnhart 1986*b;* Barnhart and McMahon 1988). Long periods of apnea are interrupted by short periods of rapid gas exchange (Barnhart and McMahon 1987). In the laboratory, this species can be readily induced to enter dormancy by the removal of food and water; conversely,

arousal occurs within minutes when these are reintroduced. *Otala lactea* also has a substantial tolerance for anoxia. We reasoned, therefore, that this was an excellent model animal in which to assess the metabolic controls of estivation versus anaerobiosis and to determine whether common regulatory mechanisms underlie metabolic depression in both of these situations. The present study initiates an analysis of metabolic depression in *O. lactea* by investigating the effects of dormancy and of anoxia on cellular energetics and the glycolytic pathway.

Material and Methods

Chemicals

All biochemicals and coupling enzymes were purchased from Sigma Chemical Co., St. Louis, Missouri, or Boehringer Mannheim Corp., Montreal, Quebec.

Animals

Snails, *Otala lactea,* were provided by Dr. C. Barnhart from an introduced population in Los Angeles County, California. Animals were held in the laboratory at 22°C in covered plastic containers lined with moist paper towels. Snails were fed cabbage and carrots every 10–12 d. Active snails were identified 24 h after the introduction of food and were divided into three experimental groups: (1) controls: active snails, sampled immediately, (2) estivation: active snails were removed to a glass jar without food or water but with ample aeration and were allowed to reenter a dormant state at 22°C with subsequent sampling after 3 or 22 d of dormancy, and (3) anoxia: active snails were placed in an airtight container that had been previously flushed with a 95% N_2/5% CO_2 gas mixture; after the container was closed, gassing was continued for a further 2 min. Animals were sampled after 2 and 14 h of anoxia at 22°C. Foot muscle and hepatopancreas were rapidly excised from control or experimental animals and frozen in liquid nitrogen (foot was freeze-clamped with precooled tongs). Tissues were then transferred to −80°C for storage until use.

Tissue Extraction and Metabolite Assay

Samples of frozen tissue (about 300 mg) were ground to a powder under liquid nitrogen using a mortar and pestle. Samples were rapidly weighed

and then homogenized 1:5 w/v in perchloric acid (6% containing 1 mM EDTA) at −8°C using a Polytron PT10 homogenizer. Homogenates were centrifuged at 8,000 × *g* for 15 min at 4°C. Supernatants were removed and neutralized by addition of a solution containing 3 M KOH, 0.3 M Tris base, and 0.4 M KCl. After a second centrifugation to remove precipitated $KClO_4$, an aliquot of the neutralized extract was removed for immediate assay of pyruvate and P-enolpyruvate; the remainder of the extract was frozen in liquid nitrogen and transferred to −80°C for storage.

Metabolite Assays

All metabolites were quantified by coupled enzyme assays using an Aminco-Bowman spectrofluorometer (for foot) or a Pye Unicam SP 8-100 spectrophotometer (for hepatopancreas). Most assays were those of Lowry and Passonneau (1972); arginine phosphate was measured using assay conditions described for creatine phosphate but with the substitution of arginine kinase. Succinate was determined as described by Williamson and Corkey (1969) but using ATP and ATP-linked succinyl-CoA synthetase.

Lactate Dehydrogenase Assay

Fresh tissues were homogenized 1:5 w/v in 20 mM imidazole-HCl buffer, pH 7.0 containing 15 mM 2-mercaptoethanol and 0.1 mM phenylmethylsulfonyl fluoride. After centrifugation at 27,000 × *g* for 15 min at 4°C, lactate dehydrogenase activity was measured in the resulting supernatant. Activity was measured in the forward direction using reaction conditions: 20 mM imidazole buffer, pH 7.0, 0.15 mM NADH and 2 mM pyruvate. To determine whether the enzyme was D- or L-lactate-specific, activity in the reverse direction was tested using 20 mM Tris buffer, pH 8.5, 0.5 mM NAD, and 5 mM D-lactate or L-lactate.

Statistics

Results are presented as means ± SEM. Tests for significant differences between groups used the Student's *t*-test.

Results

Table 1 shows the effects of 14 h of anoxia and 3 d of dormancy on phosphagen and adenylate contents in *Otala lactea* foot muscle and hepatopancreas.

TABLE 1
Effect of anoxia and dormancy on phosphagen and adenylate levels in Otala lactea *tissues*

	Control	14 h Anoxia	3 d Dormancy
Foot muscle:			
Arginine phosphate ...	1.72 ± .15 (10)	2.00 ± .17 (7)	. . .
ATP	.83 ± .08 (7)	.87 ± .04 (9)	1.16 ± .15 (6)
ADP	.62 ± .03 (4)	.33 ± .04 (10)	.84 ± .10 (9)
AMP	.34 ± .03 (4)	.13 ± .02 (9)	.23 ± .05 (6)
Energy charge	.64	.78	.71
Hepatopancreas:			
Arginine phosphate ...	1.00 ± .08 (6)	1.88 ± .24 (5)	2.52 ± .33 (7)
ATP	.66 ± .10 (7)	.68 ± .20 (4)	.82 ± .20 (5)
ADP	.24 ± .08 (5)	.25 ± .07 (8)	.59 ± .12 (7)
AMP	.23 ± .07 (6)	.13 ± .03 (7)	.18 ± .04 (6)
Energy charge	.69	.76	.70

Note. Values are μmol/g wet weight, mean ± SEM, with *n* values for the number of individual snails sampled shown in parentheses. Energy charge is defined as [ATP + ½ ADP]/[ATP + ADP + AMP].

Neither condition affected the energy status of the tissues. Both phosphagen content and energy charge remained high under anoxia or dormancy.

Table 2 shows the levels of fermentative substrates (glucose, aspartate) and anaerobic products in tissues of *O. lactea*. Under anoxic conditions both tissues showed elevated glucose content. Anoxic foot muscle rapidly catabolized aspartate and accumulated L-alanine and succinate; the net increases in these end products were 3.5 and 1.75 μmol/g wet weight, respectively, compared with a 1.3 μmol/g decrease in aspartate. However, the major product of anaerobic metabolism in foot muscle was D-lactate, a net accumulation of 13.5 μmol/g occurring over 14 h of anoxia. Not surprisingly, *O. lactea* tissues contained a D-lactate-specific lactate dehydrogenase; enzyme activity was 110 μmol pyruvate utilized min^{-1} g^{-1} in foot and 28 units g^{-1} in hepatopancreas. In hepatopancreas, D-lactate was also the primary end product of anaerobic metabolism. Low amounts of alanine also accumulated in anoxic hepatopancreas, but succinate was not produced and only a minor amount of aspartate was utilized.

Estivation in *O. lactea* is not an oxygen-limited state (Barnhart and McMa-

TABLE 2

Effect of anoxia and dormancy on levels of substrates and end products in Otala lactea *tissues*

	Control	2 h Anoxia	14 h Anoxia	3 d Dormancy	22 d Dormancy
Foot muscle:					
Glucose	.36 ± .03 (19)	1.68 ± .12 (9)[a]	1.66 ± .22 (8)[a]	.15 ± .02 (10)[a]	.23 ± .03 (6)[b]
D-lactate	.28 ± .03 (8)	4.07 ± .37 (10)[a]	13.8 ± .91 (6)[a]	.31 ± .02 (9)	.95 ± .03 (6)[a]
Aspartate	1.72 ± .12 (6)	.44 ± .17 (4)[a]	.44 ± .05 (5)[a]	. . .	2.03 ± .20 (6)[b]
Alanine	.63 ± .22 (5)	2.81 ± .20 (4)[a]	4.10 ± .68 (5)[a]	. . .	.95 ± .16 (5)
Succinate	1.90 ± .43 (4)	2.86 ± .11 (4)[b]	3.65 ± .29 (4)[b]	. . .	1.99 ± .18 (4)
Hepatopancreas:					
Glucose	.27 ± .04 (8)	1.19 ± .04 (10)[a]	1.25 ± .08 (14)[a]	.13 ± .01 (8)[a]	.37 ± .04 (6)[b]
D-lactate	.64 ± .02 (8)	2.22 ± .17 (10)[a]	6.57 ± .44 (5)[a]	.60 ± .09 (10)	2.04 ± .27 (5)[a]
Aspartate	.85 ± .10 (6)	.97 ± .11 (5)	.49 ± .07 (6)[b]	. . .	.84 ± .16 (6)
Alanine	1.43 ± .21 (3)	1.71 ± .06 (6)	2.73 ± .36 (5)[b]	. . .	.43 ± .08 (5)[a]
Succinate	2.60 ± .20 (6)	2.62 ± .13 (5)	2.51 ± .20 (3)	. . .	2.43 ± .20 (5)

Note. Values are μmol/g wet weight, means ± SEM, with *n* values for the number of individual snails sampled in parentheses. Significantly different from corresponding control values, [a]$P < 0.005$; [b]$P < 0.05$.

hon 1988), and, not unexpectedly, few changes in the levels of substrates or products of fermentative metabolism were seen during dormancy (table 2). The aspartate pool was not depleted, and neither alanine nor succinate accumulated over 22 d of dormancy. Glucose content was reduced in foot muscle of estivating snails and in hepatopancreas after 3 d of dormancy. Both tissues accumulated minor amounts of D-lactate during dormancy, but levels after 22 d were less than the amounts accumulated after 2 h of anoxia.

Table 3 shows levels of glycolytic intermediates in *O. lactea* tissues after both short and long periods of anoxia and estivation. Figure 1 converts this information to crossover diagrams (Williamson 1970) that show the relative changes in levels of each intermediate in two situations: the effect of short-term stress compared to control values (set at 100%) and the effect of long-term stress compared to values for the short term (set at 100%).

Changes in the contents of glycolytic intermediates in foot muscle after 2 h of anoxia quite clearly indicate an activation of glycolysis with regulatory control at both the PFK and PK loci (table 3, fig. 1*a*). Glucose-6-phosphate (G6P) content was elevated (suggesting enhanced glycogenolysis or glucose utilization), but despite this the content of fructose-6-phosphate (F6P), the substrate of PFK, decreased. Levels of the PFK product, fructose-1,6-bisphosphate (FBP), remained the same, but the contents of triose phosphates increased, indicating increased flux through the PFK and aldolase loci. At the PK locus, pyruvate content was elevated, indicating increased PK activity. The situation reversed, however, when anoxia was extended to 14 h. The F6P content increased, whereas that of FBP and the triose phosphates decreased. Phosphoenolpyruvate (PEP) content rose, and pyruvate content dropped. Both the PFK and PK loci showed negative crossovers (a rise in substrate content above 100% and a drop in product content below 100% of the values at 2 h anoxia) when data for 14 h anoxia were compared with values at 2 h. This indicates reduced enzyme activity at both loci relative to the situation at 2 h of anoxia.

In hepatopancreas, less pronounced changes in glycolytic intermediates were seen during anoxia (table 3, fig. 1*b*). G6P levels had increased after 2 h of anoxia, in line with an elevated glucose content (table 2). Other hexose intermediates of glycolysis were not altered, nor were glyceraldehyde-3-phosphate (GAP) or dihydroxyacetonephosphate (DHAP) contents. PEP and pyruvate levels were increased after 2 h, however, suggesting an activation of PK early in anoxia. After 14 h of anoxia, regulatory control appeared to shift to the PFK locus, a drop in FBP content suggesting inhibition at this locus as anoxia became extended.

Dormancy, like anoxia, produced changes in the levels of glycolytic inter-

TABLE 3

Effect of anoxia and dormancy on levels of glycolytic intermediates in Otala lactea *tissues*

	Control	2 h Anoxia	14 h Anoxia	3 d Dormancy	22 d Dormancy
Foot muscle:					
Glucose-6-P	88 ± 4 (12)	172 ± 6 (10)[a]	54 ± 8 (10)[a,c]	102 ± 6 (8)[b]	62 ± 9 (6)[a,c]
Fructose-6-P	16 ± 1 (7)	<7	10 ± 1 (8)[a]	<7	7 ± 3 (4)[a]
Fructose-1,6-P_2	10 ± 1 (15)	10 ± 1 (7)	8 ± 1 (10)	10 ± 1 (8)	27 ± 2 (4)[a,c]
Glyceraldehyde-3-P	17 ± 7 (13)	35 ± 2 (10)[b]	5 ± 1 (7)[c]	42 ± 2 (10)[a]	17 ± 2 (6)[c]
Dihydroxyacetone-P	11 ± 1 (16)	34 ± 3 (10)[a]	12 ± 1 (10)[c]	37 ± 5 (9)[a]	19 ± 3 (6)[a,d]
P-enolpyruvate	193 ± 28 (8)	277 ± 21 (6)[b]	333 ± 22 (6)[a,d]	216 ± 43 (9)	186 ± 9 (6)
Pyruvate	72 ± 8 (6)	235 ± 51 (6)[b]	90 ± 4 (6)[b,d]	562 ± 66 (5)[a]	53 ± 5 (6)[b,c]
Hepatopancreas:					
Glucose-6-P	29 ± 5 (12)	54 ± 9 (4)[b]	79 ± 6 (4)[a,d]	<10	28 ± 7 (4)[c]
Fructose-6-P	<10	<10	<10	<10	35 ± 7 (6)[c]
Fructose-1,6-P_2	202 ± 14 (14)	200 ± 9 (8)	76 ± 6 (8)[a,c]	123 ± 12 (8)[a]	43 ± 6 (6)[a,c]
Glyceraldehyde-3-P	99 ± 15 (8)	94 ± 9 (10)	132 ± 22 (4)[d]	99 ± 5 (7)	101 ± 12 (6)
Dihydroxyacetone-P	123 ± 14 (8)	121 ± 12 (10)	133 ± 13 (9)	160 ± 18 (10)	123 ± 16 (6)
P-enolpyruvate	89 ± 10 (6)	152 ± 12 (6)[a]	133 ± 21 (10)	159 ± 20 (8)[b]	86 ± 9 (6)[d]
Pyruvate	106 ± 14 (6)	206 ± 16 (6)[a]	173 ± 10 (8)[a,d]	229 ± 34 (5)[a]	110 ± 16 (6)[c]

Note. Values are nmol/g wet weight, mean ± SEM, with n values for the number of individual snails sampled in parentheses. Significantly different from corresponding control value, [a]$P < 0.005$; [b]$P < 0.05$. Significantly different from the corresponding value for short-term stress, [c]$P < 0.005$; [d]$P < 0.05$.

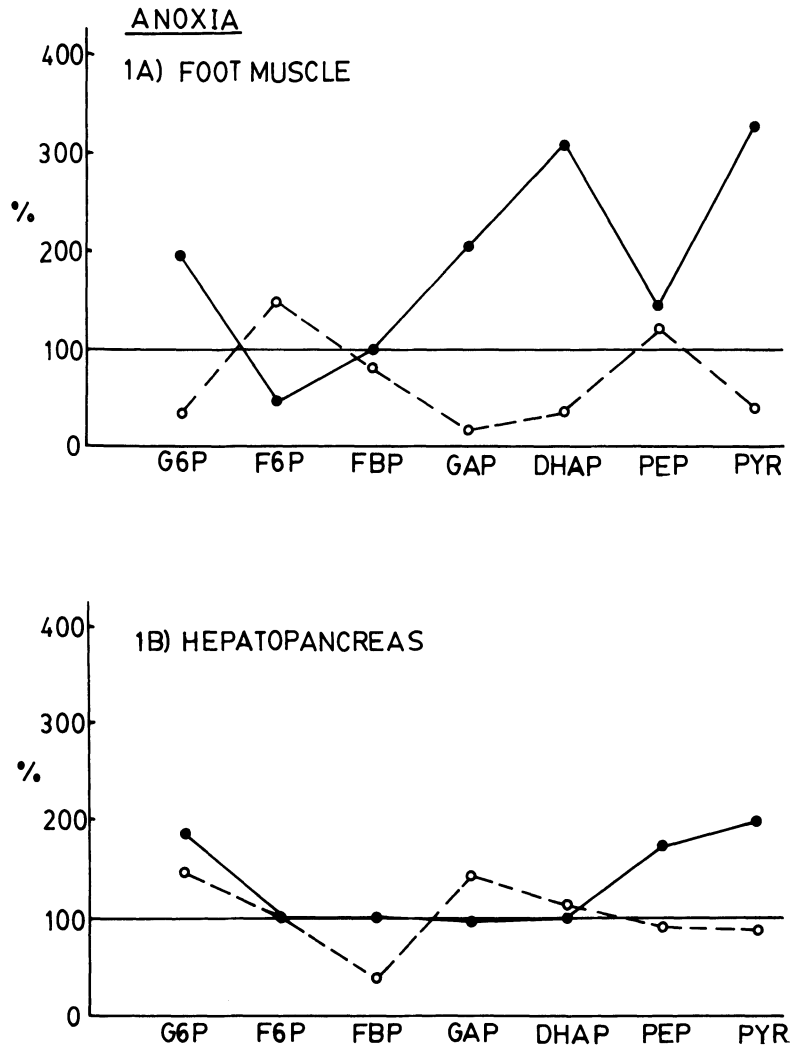

Fig. 1. Crossover diagrams (Williamson 1970) showing the effects of anoxia on the contents of intermediates of glycolysis in Otala lactea *foot muscle* (A) *and hepatopancreas* (B). *Data from table 3 have been converted to show the percentage change in the content of each intermediate at 2 h of anoxia versus aerobic control values set to 100% (— ● —), and at 14 h of anoxia versus 2 h data set to 100% (– – ○ – –). Abbreviations are:* G6P, *glucose-6-phosphate;* F6P, *fructose-6-phosphate;* FBP, *fructose-1,6-bisphosphate;* GAP, *glyceraldehyde-3-phosphate;* DHAP, *dihydroxyacetonephosphate;* PEP, *phosphoenolpyruvate;* PYR, *pyruvate.*

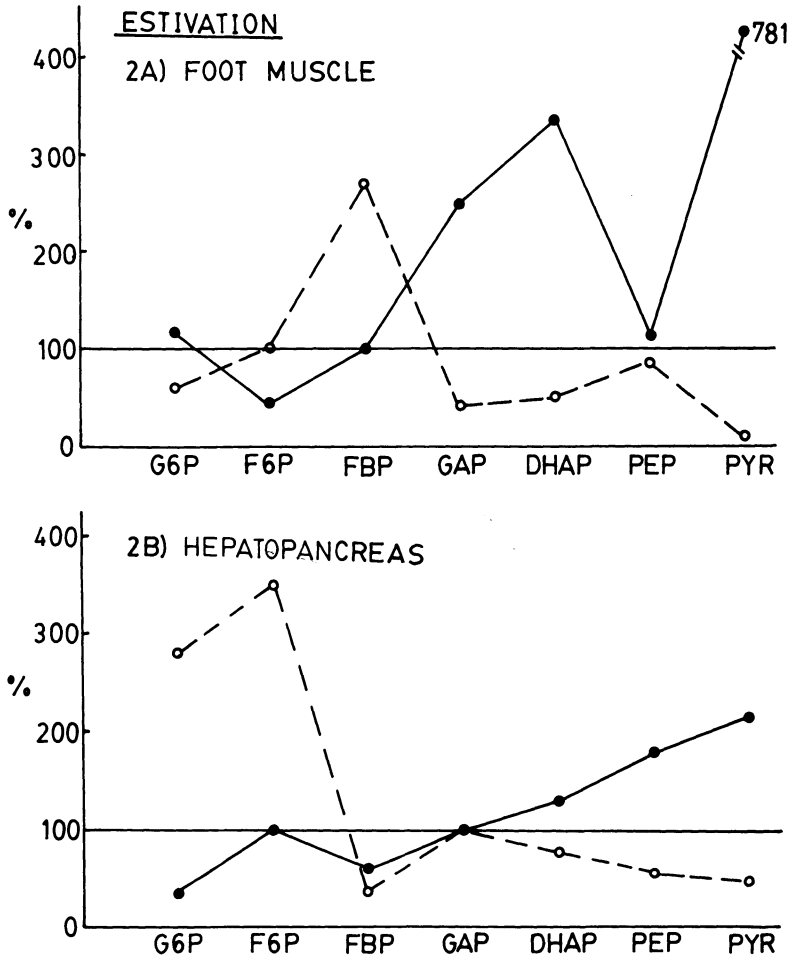

Fig. 2. Crossover diagrams showing the effects of dormancy on the contents of intermediates of glycolysis in Otala lactea *foot muscle (*A*) and hepatopancreas (*B*). Data from table 3 have been converted to show the percentage change in the contents of intermediates after 3 d of dormancy versus control values set to 100% (— ● —), and after 22 d of dormancy versus 3 d values set to 100% (- - ○ - -). Abbreviations are as in fig. 1.*

mediates in foot muscle that indicated an activation of PFK and PK under the short-term stress and a reversal of this when the stress was prolonged. Thus, after 3 d of dormancy, F6P content was reduced, FBP content remained constant, and triose phosphate contents were elevated (table 3). PEP content remained constant but pyruvate concentration rose eightfold. Crossover diagrams in figure 2*a* illustrate the activation at both PFK (and/or aldolase) and PK loci. After 22 d of dormancy a different situation was

seen. FBP levels had risen 2.7-fold, but contents of GAP and DHAP were strongly reduced, suggesting inhibitory action at aldolase. Inhibition at PK was also indicated by a tenfold drop in pyruvate content.

In hepatopancreas of dormant snails, control again appeared to be applied primarily at the PK locus when stress was short term; an activation of PK was indicated at the 3-d point (table 3; fig. 2*b*). After 22 d of dormancy, inhibitory control was apparent at the PFK locus; F6P content was increased and FBP content was reduced to produce a negative crossover at the PFK locus. Inhibition at the PK reaction was also suggested.

Discussion

Metabolism during anoxia in *Otala lactea* is similar in most respects to that reported for another land snail, *Helix pomatia* (Wieser 1981; Wieser and Platzer 1983), but *O. lactea* appears to have a better tolerance of oxygen lack. Thus, *O. lactea* foot muscle showed no signs of energy stress after 14 h of anoxia (table 1), whereas in *H. pomatia* arginine phosphate content of foot muscle had dropped by 90% and adenylate energy change had fallen to 0.45 (from 0.62) after 15–16 h of exposure to an N_2 gas atmosphere (Wieser and Platzer 1983). Both species accumulated D-lactate as the primary end product of anaerobic glycolysis, but both also showed the capacity, as is most highly developed in marine molluscs (Livingstone and De Zwaan 1983), for the production of alternative fermentative end products (alanine, succinate) and the utilization of aspartate as an anaerobic substrate. For *O. lactea,* the inverse relationship between aspartate depletion and alanine and succinate accumulation was clear in foot muscle; the same relationship occurs in foot muscle of the marine whelk during anoxia (Eberlee and Storey 1988). In hepatopancreas, however, alanine accumulation exceeded aspartate utilization and succinate was not built up. Total anaerobic end-product accumulation by hepatopancreas was less than half that in foot muscle; the very low accumulation of end products by hepatopancreas, compared to other organs, also occurs in other species (Eberlee and Storey 1988).

The metabolic responses by glycolysis in anoxia are biphasic. The short-term response was clearly an activation of glycolysis: glucose levels were elevated, and changes in the levels of substrates and products of the regulatory enzymes indicated an activation of PK in both foot and hepatopancreas along with an activation of PFK in foot. A regulatory site at aldolase in foot muscle might also be proposed since the products of this reaction build up substantially (see further discussion below). Subsequently, however, the

data indicate that glycolytic rate is depressed. This occurs despite the fact that anaerobic fermentations must still supply the full energy needs of the anoxic state. Data on product accumulation show that the rates of D-lactate, L-alanine, and succinate accumulation in foot drop from 1.90, 1.09, and 0.48 $\mu mol\ g^{-1}\ h^{-1}$ over the first 2 h of anoxia to an average of 0.81, 0.11, and 0.07 $\mu mol\ g^{-1}\ h^{-1}$, respectively, over the subsequent 12 h. Furthermore, significant changes in levels of glycolytic intermediates (table 3) result in negative crossovers at the PFK and PK reactions at 14 h as compared to the state at 2 h anoxia (fig. 1). This indicates enzyme inhibition at these loci and reduced pathway flux. The same short- versus long-term responses to anoxia are seen in organs of the marine whelk (Storey et al. 1990) and can be interpreted for both *O. lactea* and *Busycotypus canaliculatum* in the same way.

For animals with a wide tolerance of varying environmental oxygen concentrations the initial response to declining oxygen availability is a compensatory one; the rates of nonoxidative (fermentative glycolysis + phosphagen breakdown) ATP production increase as those of oxidative metabolism decline (Lutz, Rosenthal, and Sick 1985). The response has similarities to the Pasteur effect and is probably mediated by adenylate activation of regulatory enzymes of glycolysis, but is actually a response to hypoxia and not to anoxia (Lutz et al. 1985; Storey 1985*a*). The response probably persists in *O. lactea* until O_2 reserves in body fluids and the lung fall below a critical level. Subsequently, a conservation strategy is brought into play to extend survival time. A profound metabolic depression is initiated; typically, anoxic metabolic rate in marine molluscs is only 5%–20% of the aerobic resting rate (Famme et al. 1981; Shick et al. 1983; Storey 1985*a*). Part of the metabolic depression is glycolytic rate depression. The rate of fermentative ATP production is restricted and coordinated with restrictions placed on ATP use to reestablish homeostasis in the hypometabolic state. The present study clearly shows that anoxia-induced glycolytic rate depression occurs in *O. lactea,* and, as in other animals that can tolerate oxygen lack, this is undoubtedly part of a larger overall metabolic rate depression that underlies long-term anoxia survival.

By contrast, dormancy during estivation in *O. lactea* is an aerobic state. Episodes of oxygen uptake and carbon dioxide release from the animal are intermittent (such that hypoxia and hypercapnia occur), but oxidative metabolism clearly continues throughout dormancy although at a depressed rate compared to the active state (Herreid 1977; Barnhart and McMahon 1987). Stored carbohydrate appears to be the major fuel supporting basal metabolism in dormancy (Livingstone and De Zwaan 1983; Umezurike and Iheanacho 1983; Cedeno-Leon 1984), but D-lactate does not accumulate

(Barnhart 1986*a*; this study) and the aspartate/succinate pathway of anaerobic metabolism is not utilized.

The metabolic adjustments by glycolysis during dormancy have both similarities and differences to those seen during anoxia. Entry into dormancy was without the large increases in glucose or glucose-6-P contents that characterized anoxia. However, the changes in concentrations of glycolytic intermediates in foot muscle early in dormancy (3 d) indicated an activation of PFK, aldolase, and PK and suggested that glycolytic flux was increased relative to the control state. The reasons for this are not apparent in the present study but may be the consequences of specific metabolic reorganizations occurring over time during the fed/starved or active/dormant transitions. During long-term dormancy, however, glycolytic rate depression was indicated when data are compared either to the control state or to the state after 3 d of dormancy. For hepatopancreas, primary regulation of glycolytic flux was at the PFK locus, as it also was in prolonged anoxia. For foot muscle inhibitory controls on glycolysis were localized instead at the aldolase and PK reactions. The differing sites of control between the two tissues probably reflect the different metabolic functions of foot versus hepatopancreas. For example, in liver and liver-like organs of many animals, inhibitory control of glycolysis at PFK during starvation or domancy contributes to (*a*) channeling glycogenolysis into glucose export from the organ and (*b*) reducing the use of carbohydrate reserves for biosynthesis. Controls on PFK in muscle, however, are primarily used to regulate carbohydrate catabolism with respect to the ATP demands of muscle work. Situations such as entry into dormancy that require a change in glycolytic flux but occur without a disruption of adenylate levels may not, therefore, be mediated via regulation of PFK in muscle. Indeed, the present results indicate that muscle aldolase is functioning in a nonequilibrium manner in foot muscle during both anoxia and dormancy. The ratio of substrate:products [FBP:(GAP + DHAP)] is distinctly different with the changing metabolic states (table 3). Although aldolase is not considered to be a regulatory enzyme in the mammalian literature, regulation at aldolase is certainly key to the control of substrate utilization (carbohydrate versus lipid) by insect flight muscle (Storey 1980).

The regulation of metabolic depression is a major topic of recent research. Both signaling mechanisms (e.g., hormones, pH change) and the specific molecular processes that control individual enzymes or proteins are being studied. In marine molluscs, for example, anoxia-induced protein phosphorylation of PFK, PK, and 6-phosphofructo-2-kinase is key to glycolytic rate depression (Plaxton and Storey 1984, 1985; Storey 1984; Bosca and Storey 1989). Phosphorylation of all three is coordinated via the actions of a

single protein kinase (Brooks and Storey 1989). Changes in pH occurring during anoxia do not stimulate or prevent enzyme covalent modification (Brooks and Storey 1989), but they create cellular conditions under which the phosphorylated enzymes function less efficiently. A similar interplay of regulatory factors undoubtedly underlies glycolytic rate depression, and metabolic rate depression in general, in *O. lactea*. PK in *O. lactea* foot undergoes covalent modification during dormancy (R. Whitwam and K. Storey, unpublished results). Barnhart and McMahon (1988) have shown that oxygen consumption by active *O. lactea* is strongly depressed by hypercapnia, indicating that high CO_2 or its associated acidosis may be critical in triggering the events of metabolic depression or in creating a metabolic context that stabilizes the depressed state. Indeed, the presence of carbon dioxide in the nitrogen gas used in anoxia experiments may have helped to promote the transition to anaerobic metabolism and facilitate the observed glycolytic rate depression.

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