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CHANGES IN ENZYME BINDING AND ACTIVITY DURING AESTIVATION IN THE FROG *NEOBATRACHUS PELOBATOIDES*

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Abstract—1. The proportion of aldolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) associated with the particulate fraction of a cell was measured in aestivating and non-aestivating *Neobatrachus pelobatoides*.

2. Reduced binding of these enzymes was found in the brain, indicating lower glycolytic flux. This was not correlated to metabolic rate suggesting that glycolytic rate was reduced in this tissue in the early stages of aestivation, possibly due to a change in fuel use.

3. Measurement of total enzyme levels showed that the liver of aestivating frogs had less GAPDH and less aldolase than non-aestivating frogs.

INTRODUCTION

Surviving extended periods without available food, water or oxygen is a problem faced by many organisms. Fuel supplies are often extended by (a) storing more fuel, (b) utilising more efficient biochemical pathways, or (c) depressing ATP turnover rates. The data from studies of phylogenetically diverse organisms suggest that all of these processes contribute to the ability of animals to survive long periods of starvation. However, metabolic arrest mechanisms, i.e. a decrease in ATP turnover rates, give the greatest protection against transient environmental deficiencies (Hochachka and Guppy, 1987).

Metabolic arrest requires the co-ordinated reduction of the rates of all or most cell functions to produce a new homeostasis in the hypometabolic state. Much work has been done with anoxia-tolerant organisms. During anoxia a dependence on anaerobic metabolism would lead to rapid fuel depletion and accumulation of end products. To survive extended anoxia these organisms depress their metabolic rate, and therefore require an absence of the Pasteur effect (the activation of glycolysis during anoxia, mainly by allosteric effectors). This absence is termed the "reverse Pasteur effect". Glycolysis during anoxia is characterized by the solubilization of glycolytic enzymes. Normal allosteric activation of glycolysis is thereby overcome and glycolytic rate is reduced (Storey, 1985).

The concept of metabolic regulation by association-dissociation of enzymes with the particulate fraction of the cell recognizes the importance of enzyme–enzyme interaction in the functioning of metabolic pathways *in vivo*. Previous studies have shown that enzymes *in vivo* form complexes with each other and with subcellular structural components (e.g. F-actin) which bring together consecutive enzymes of a pathway and thereby facilitate substrate flux through the pathway. Enzyme structure and kinetic properties may also depend upon binding/unbinding (Knull, 1978; Masters, 1981; Westrin and Backman, 1983; Srere, 1987). The physiological relevance of this phenomenon for glycolytic flux has been shown in a number of different species and situations. In the tissues of an anoxia-tolerant mollusc (*Busycotypus canaliculatum*) an aerobic to anaerobic transition leads to a decrease in the association of glycolytic enzymes with the particulate fraction. The likely result is a decrease in enzyme and pathway organization promoting a decrease in glycolytic flux (Plaxton and Storey, 1986). An increase in the per cent of enzymes bound to particulate matter has been found in both working and ischaemic mammalian muscle and heart (Clarke *et al.*, 1980, 1984; Walsh *et al.*, 1981; Choate *et al.*, 1985) and in trout white muscle (but not trout heart) during exercise (Brooks and Storey, 1988). A decrease in glycolytic enzyme binding was observed in the barred sand bass during starvation (Lowery *et al.*, 1987).

Species of the Australian frog genus *Neobatrachus* are well adapted to survive extended dry periods, showing fossorial behaviour, aestivation during dry periods and the accumulation of shed skin to form a cocoon while in aestivation (Lee and Mercer, 1967). The dry periods can last from 6 months to several years and during this time the aestivating frog must survive on its own water and energy stores. We have found that the Western Australian frog *N. pelobatoides* readily forms cocoons and has a metabolic depression to 15–20% of normal resting VO_2.

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Dehydration during aestivation is greatly reduced by cocoon formation and burrowing. Temperature remains constant in the burrow and soil oxygen concentration probably remains at atmospheric levels adjacent to the buried frog as it does in the spadefoot toad (*Scaphiopus*) (Seymour, 1973a).

Regulation of metabolism by enzyme interactions has not been studied previously in an animal that depresses its metabolic rate, but which is not under anoxic stress, or any other immediate environmental stress. We have used aldolase (E.C. 4.1.2.13) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (E.C. 1.2.1.12) to look for indications of this type of glycolytic control in tissues of the aestivating and non-aestivating frog.

MATERIALS AND METHODS

Chemicals

Biochemicals and coupling enzymes were obtained from Boehringer Mannheim and from Sigma Chemical Company. All reagents were of analytical grade.

Animals

N. pelobatoides were captured in the Darling Range, near Perth, Western Australia from May to August 1987, and were kept in sand filled aquaria in the dark with access to water. They were not fed after capture and aestivating frogs were induced to aestivate within 7 days of capture.

Frogs were induced to aestivate by removal from water. They were placed in plastic containers with a small hole in the lid which allowed some evaporative water loss, simulating the slow dehydration that occurs in an underground chamber. Slow dehydration is necessary for successful cocoon formation, enabling the frog to survive a long estival period without fatal loss of body water through the skin.

Three groups of frogs, all captured at different times, were used for the experiment: (1) the frogs designated for biochemical studies consisted of short- and long-term estivators. The short-term estivators were in aestivation for a period of 6 days–2 weeks while the long-term estivators were in aestivation for 9–12 weeks. Only 4 long-term estivators survived for biochemical studies, the cause of death was not dehydration but a liver degeneration. (2) Six control frogs, fully hydrated, and six cocooned aestivating frogs (at 7 weeks aestivation) were used for the tissue water content data. (3) The total body weight of eight frogs was followed over 10 weeks of aestivation.

Oxygen uptake

Metabolic rates of resting and aestivating frogs were measured by closed system respirometry over a 1–2 day period before biochemical studies. Animals were weighed, then sealed in a 500 ml plastic container for a prescribed time period (approximately 24 hr). A 50 ml gas sample was then removed for determination of per cent O_2 content. The air sample was slowly injected into a Beckman model E2 paramagnetic O_2 analyser, with water and CO_2 removed by passage through a silica gel-ascarite column. VO_2 values (ml $O_2\ g^{-1}\ hr^{-1}$; STP dry) were calculated after Seymour (1973b). To ensure that resting metabolic rate was not unduly influenced by any movement by the frog three consecutive measurements were taken and the lowest used. Maximum (active) rate of O_2 consumption was measured by manually rotating the frog in a metabolic chamber (Walsberg, 1986).

Tissue water content

The water content of tissues was measured in a separate group of frogs. Tissues were removed and the wet weight measured. They were then dried at 65°C to constant weight to give the dry weight.

Calculation of enzyme activity per gram adjusted for water loss, in tissues of aestivating frogs

The rate of whole frog dehydration was constant during aestivation and it was assumed that the rate of tissue dehydration was also constant. The water content of the tissues was then calculated based on the time spent in aestivation. From this the enzyme activity per gram for each tissue was adjusted to give activity per gram as if no dehydration had occurred.

Measurement of enzyme binding to the particulate fraction

The method of Clarke *et al.* (1984) was adapted to separate bound and soluble enzyme. This procedure involved homogenization in an essentially ion free medium, which preserves enzyme binding to particulate structures, enabling separation of bound and soluble enzymes by centrifugation. Frogs were decapitated, and the ventricle of the heart, liver, brain and gastrocnemius (gastroc) dissected out, weighed and homogenized with a Kinematica GmbH polytron on power control 3, in ice-cold 0.25 M sucrose and 0.1 mM dithiotreitol for 15 sec (heart, liver and brain) or 25 sec (skeletal muscle). Tissues were always removed in the same order. The approximate time taken between killing the frog and homogenization of the tissues was: (decapitation at 0 sec), heart 70 sec, liver 120 sec, brain 150 sec, and muscle 200 sec.

A 170 μl sample of each homogenate was centrifuged for 2 min (plus 2 min deceleration) at 4°C in a Beckman airfuge at 50,000 g. The supernatant was removed and stored on ice. The pellet was resuspended in the sucrose medium and spun under the same conditions. The supernatants were combined, and subsequently diluted in 3 vols of an ionic buffer, containing 100 mM potassium phosphate (pH 7.5), 1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM fructose-6-phosphate and 0.1 mM ATP, and stored on ice for later measurement of enzyme activity in the soluble fraction of the cell. The pellet was twice extracted with the ionic buffer by resuspension and centrifugation, and the combined extracts were assayed for the amount of enzyme activity that was bound to particulate structures in the cell. A separate sample of the homogenate was diluted in 4 vols of ionic buffer, and assayed to determine both per cent recovery of activity and activity per gram.

Enzyme assays

The assays were performed at 25°C by monitoring NADH oxidation at 340 nm on a Varian DMS 200 spectrophotometer. Assay conditions used were:

Aldolase. Tris–HCl buffer (70 mM) (pH 8.0), 2 mM fructose-1,6-bis phosphate, 0.15 mM NADH and excess triose phosphate isomerase and glycerol-3-phosphate dehydrogenase.

Glyceraldehyde-3-phosphate dehydrogenase. Tris–HCl buffer (60 mM) (containing 60 mM KCl and 6 mM $MgCl_2$, pH 7.5), 5 mM glycerate-3-phosphate, 2 mM Mg-ATP, 0.15 mM NADH and excess phosphoglycerokinase.

A blank assay, in which the substrate was excluded, was used to measure non-specific oxidation of NADH and this was subtracted from the test activity.

Technique validation

Two experiments were performed to test the efficacy of the technique in separating bound and soluble enzyme: (a) an initial homogenization was performed in the ionic buffer instead of 0.25 M sucrose buffer. This resulted in a loss of any detectable amounts of "bound" enzyme. (b) Following two extractions of the soluble enzymes with sucrose buffer, a third extraction with the sucrose buffer was performed. This did not extract any more enzyme activity whereas a subsequent extraction with the ionic buffer did extract enzyme activity. Recovered activity (the sum of soluble and particulate activity) was determined to be not statistically different from activity assayed directly from the homogenate

(Student's t-test) with the exception of heart aldolase, where recovered activity was significantly lower. This may have been due to difficulties in homogenization of the small heart, and subsequent separation and assay of the low activity aldolase. Recovery of heart GAPDH activity, which has more activity per gram than aldolase, was complete.

RESULTS

Oxygen uptake

The oxygen uptake for *N. pelobatoides* measured for this study is presented in Table 1. The frogs in aestivation for 6 days–2 weeks had a metabolic rate approximately one-third that of the resting frogs. Those in aestivation for 9–12 weeks had a metabolic rate approximately one-seventh of resting (ranging from one-fifth to one-eighth). Table 1 also shows the active metabolic rate. In deep aestivation the metabolic rate is 2.2% of the active rate.

Animal weights

Weight loss during aestivation was considerable. The average initial weight was 7.44 g and declined to 90% (±SE 2.5) initial mass by 2 weeks, 85% (±SE 3.1) by 4 weeks, 72.7% (±SE 3.0) by 6 weeks and 59.7% (±SE 3.1) by 10 weeks ($n = 8$) Using Student's paired t-test weight loss was significant at each 2-week interval. The weight of frogs in aestivation drops at a fairly constant rate with time. The probable cause is that the hole in the container in which the frogs were kept is the limiting factor in evaporative water loss (EWL). At a later stage in aestivation this rate may decrease as the cocoon thickens and EWL is reduced.

Tissue weights and water loss

Table 2 shows tissue mass as a proportion of total body mass for control and for 7-week aestivating frogs. Note that the average weight of the aestivating frogs declines to 67.3% of control mass. Therefore a direct comparison of tissue weights between control and aestivating frogs requires that the per cent mass value for the aestivating frogs must be multiplied by 0.673. Table 2 was used to calculate the dry per cent mass of the tissues in aestivation [(100 − % water) × proportion of body weight]. A comparison between the control and aestivating frogs showed that there was not a decrease in dry weight of liver, gastroc. or brain and a small, but not significant, decrease in the ventricle. The gastroc. lost the most water, 9.3% in the 7 weeks. The liver lost 6.3% and the brain 3.4% of water content. The ventricle had the least water loss of the four tissues, 2.8% in 7 weeks.

Table 1. The rate of oxygen consumption in active, resting and aestivating *N. pelobatoides*

	VO_2 (ml g^{-1} hr^{-1}) ± SE	n
Maximal	0.748 ± 0.072	18
Resting	0.113 ± 0.010	22
Aestivating		
6 days–2 weeks	0.0384 ± 0.005	10
9–12 weeks	0.0167 ± 0.002	4

All groups significantly different from each other. (Student's t-test, $P \leq 0.05$.)

Table 2. Percentage composition of tissues by weight and water content

	Controls (hydrated)		Aestivating (7 weeks)	
	% mass	% water	% mass	% water
Liver	2.15	71.4	2.909	65.1
Ventricle	0.272	81.7	0.319	78.9
Gastroc.*	0.764	79.4	0.878	69.9
Brain	0.341	85.0	0.487	81.6
Whole body	100		100†	

*Single gastrocnemius used.
†Average aestivating mass is 67.3% of control mass.
$n = 6$.

Enzyme activity per gram

Table 3 shows enzyme activity per gram wet weight in the first and second column. There was a significant reduction in liver GAPDH activity and an increase in muscle and ventricle aldolase activity per gram of tissue in aestivating frogs. No significant changes in enzyme levels occurred in the brain. If aestivating activities are adjusted for water loss (column 3) the changes in muscle and ventricle aldolase activity per gram are not significantly different.

Tissue enzyme capacity

The only change in total enzyme activity occurred in the liver, in which the activity of both enzymes decreased with aestivation (Table 4).

Enzyme partitioning

The degree of enzyme binding to the subcellular particulate fraction in the tissues for resting and aestivating animals is presented in Table 5. In the brain of aestivating frogs the proportion of aldolase and GAPDH bound activity decreased significantly to about one-third of resting levels. Within the aestivating frogs, the binding of aldolase and GAPDH in the brain is not correlated with oxygen uptake, and for this reason percent binding data is combined into one aestivating group. In the liver and heart no significant changes in enzyme binding occurred. There was a small, but significant increase in the bound activity of GAPDH in skeletal muscle of aestivating animals.

Table 3. Enzyme activity per gram in non-aestivating and aestivating *N. pelobatides*

	Non-aestivating	Aestivating (actual values)	Aestivating (adjusted for water loss)
Aldolase			
Liver	1.5 ± 0.18 (14)	1.2 ± 0.16 (14)	1.1 ± 0.16 (14)
Ventricle	5.2 ± 0.57 (11)	6.8 ± 0.43 (14)*	6.3 ± 0.41 (14)
Gastroc.	36 ± 3.2 (14)	54 ± 6.5 (14)†	44 ± 5.2 (13)
Brain	5.8 ± 0.39 (13)	6.5 ± 0.30 (13)	5.7 ± 0.25 (13)
GAPDH			
Liver	18 ± 3.5 (14)	11 ± 1.2 (14)‡	9.8 ± 3.4 (14)§
Ventricle	27 ± 4.4 (10)	24 ± 3.4 (13)	22 ± 3.2 (13)
Gastroc.	130 ± 17 (14)	143 ± 18 (13)	126 ± 18 (13)
Brain	15 ± 1.9 (13)	16 ± 1.3 (13)	14 ± 1.1 (13)

Data are means ± SE, n values in brackets. Enzyme activity is μmol min^{-1} g^{-1} wet tissue.
*Resting and aestivating significantly different by Student's t-test, $P = 0.033$; †$P = 0.02$; ‡$P = 0.05$; §$P = 0.023$.

Table 4. Enzyme capacity (total amount of enzyme) in the tissue of aestivating and non-aestivating *N. pelobatoides*

	Non-aestivating	Aestivating
Aldolase		
Liver	0.265 ± 0.04 (14)	0.163 ± 0.03 (14)*
Ventricle	0.125 ± 0.02 (11)	0.133 ± 0.02 (14)
Gastroc.	4.080 ± 0.48 (14)	4.325 ± 0.67 (14)
Brain	0.128 ± 0.01 (13)	0.135 ± 0.01 (12)
GAPDH		
Liver	3.135 ± 0.62 (14)	1.421 ± 0.23 (14)†
Ventricle	0.644 ± 0.12 (10)	0.414 ± 0.05 (13)
Gastroc.	16.33 ± 2.8 (13)	11.62 ± 1.6 (14)
Brain	0.369 ± 0.06 (13)	0.334 ± 0.03 (13)

Data are means ± SE, *n* values in brackets. Enzyme activity is μmol/min in the whole tissue.
*Resting and aestivating differ significantly by Student's *t*-test, $P = 0.042$; †$P = 0.016$.

DISCUSSION

Neobatrachus pelobatoides is a vertebrate estivator, with the capacity to depress metabolism to 15% of the normal resting level and to less than 3% of the active level. We report here two biochemical mechanisms which could participate in this metabolic depression: reduced enzyme levels and decreased enzyme binding.

Tissue enzyme levels are a good indication of the metabolic scope of a tissue (Blomstrand *et al.*, 1986). However the values presented in Table 3 should take into account the loss of water from the tissues. The loss of water in a tissue without the loss of enzyme would have the result of increasing enzyme activity per gram without an actual increase in activity *per se*. Therefore we have included enzyme activity values adjusted for water loss, assuming that the majority of weight loss during aestivation is due to dehydration. This assumption is sound, as a 7.44 g frog with a metabolic rate of 0.0384 ml $O_2\,g^{-1}\,hr^{-1}$, using only lipid as fuel, would use 0.05 g of lipid to fuel metabolism for 2 weeks. This is less than 10% of the weight loss over 2 weeks of aestivation. Frogs without food but with available water show no significant weight loss for several weeks (Withers, unpublished). Table 3 shows that liver GAPDH activity actually decreases, even though the liver lost a considerable amount of water. The real muscle and ventricle enzyme content remains unchanged during aestivation. The frog may not reduce the level of muscle glycolytic enzymes during aestivation because rainfall could occur at any time and the frog then be required to perform the very strenuous activity of digging out of its burrow. A similar argument can be applied to the ventricle which may have an increased circulatory workload during aestivation (see below). The brain activity per gram is unchanged. Total enzyme levels (Table 4) do not change in heart, muscle or brain, but both aldolase and GAPDH decrease in liver with aestivation. This supports the above evidence that aestivating liver has decreased levels of GAPDH.

Table 5. Percentage of enzyme activity associated with the particulate fraction of the cell in tissues of *N. pelobatoides*

	Resting	Aestivating
Aldolase		
Liver	10 ± 2.5 (9)	7 ± 1.0 (13)
Ventricle	24 ± 5.2 (6)	24 ± 2.8 (13)
Gastroc.	9 ± 1.4 (9)	12 ± 1.8 (14)
Brain	13 ± 2.6 (8)	5 ± 0.77 (14)†
GAPDH		
Liver	8 ± 2.1 (9)	6 ± 1.3 (14)
Ventricle	20 ± 5.6 (6)	16 ± 3.1 (12)
Gastroc.	7 ± 0.93 (9)	10 ± 1.1 (14)*
Brain	22 ± 4.4 (7)	7 ± 0.99 (14)†

Data are means ± SE, *n* value in brackets.
*Values are significantly different from corresponding resting values by the Mann–Whitney test, $P < 0.05$; †$P < 0.01$.

If glycolytic potential is reduced in the liver, would gluconeogenic rate be affected? Possibly not. The gluconeogenic capacity of frog liver is not known, but assuming rates similar to fish liver slices and using the data of Mommsen *et al.* (1985) an estimated rate would at most be 0.1 μmol glucose formed from lactate per g/min. The activity of GAPDH in non-estivating liver is at least 100-fold higher (Table 3). The 40% reduction in activity would therefore easily allow resting levels of gluconeogenesis. Similarly aldolase is more than 10-fold higher than the estimated gluconeogenic rate.

The degree of binding to cellular particulate structures of two glycolytic enzymes susceptible to this means of enzyme regulation was measured in the four tissues to ascertain how this mechanism might be utilized in aestivation. Enzyme binding is only decreased in the brain. Lipid is a primary metabolic fuel for aestivating spadefoot toads (Seymour, 1973c; van Beurden, 1980), presumably fueling aerobic catabolism in most organs. Brain, however, typically depends upon carbohydrate oxidation for its energy requirements. The cause of decreased aldolase and GAPDH binding could be a switch from glycolysis to fatty acid metabolism and not necessarily a direct result of overall tissue metabolic rate reduction. There was no indication that enzyme binding decreased in correlation to metabolic rate. This lack of correlation could indicate that most of the reduction in glycolytic rate in the brain occurs early in aestivation. The absence of significant changes in the level of enzyme binding in the heart is in accordance with indications that aestivating frogs which form cocoons do not have a lowered heart rate during aestivation, in fact, heart rate may even increase. This is the case in *Pyxicephalus adspersus*, the rationale being that the loss of the skin as a site of respiratory exchange due to the thickening cocoon increases the demand for pulmonary blood flow (Loveridge and Withers, 1981). In contrast, the heart rate of non-cocoon forming aestivating frogs declines during aestivation reflecting decreased metabolic rate (Whiteford, 1969; Seymour, 1973a). The reason for the small increase in binding of GAPDH in skeletal muscle is unknown; it could be due to increased concentration of soluble enzyme in this tissue caused by dehydration.

Brain glycolytic depression would be of considerable importance to carbohydrate regulation during aestivation although the metabolism of the brain accounts for only 2–8% of basal metabolic rate of the non-primate vertebrates (Mink *et al.*, 1981; Armstrong, 1983). If the brain used only glucose, and accounted for 5% of total oxygen uptake, then it would require 1 mg of glucose per day. A 0.17 g liver (the average resting liver weight) of a freshly captured *N. pelobatoides* contains about 6 mg of glycogen (unpublished data). A 5-fold increase in liver

glycogen levels prior to aestivation would still only allow a 1 month aestivation period with a non-metabolically depressed brain. If the non-aestivating frog brain is glucose dependent, the depression of glycolysis in the brain, whether the result is a depression of metabolism in general, or a switch from carbohydrate to another fuel (such as lipid or ketone bodies), is a vital aspect of a successful aestivation strategy.

There are two important considerations in the investigation of metabolic depression in aestivating frogs. The first is that metabolic rate declines slowly over many days and metabolic depression can last for several years. Long term adaptations to aestivation are therefore available to the frog. Secondly, the aestivating frog is not anoxic and therefore anaerobic pathways are not the only pathways involved in energy production. Metabolic depression mechanisms can therefore be applied to a greater range of metabolic loci, and probably require a greater range of different mechanisms.

REFERENCES

Armstrong E. (1983) Relative brain size and metabolism in mammals. *Science* **220,** 1302–1304.

Beurden E. K. van (1980) Energy metabolism of dormant Australian water-holding frog (*Cyclorana platycephalus*). *Copeia* **1980,** 787–799.

Blomstrand E., Ekblom B. and Newsholme E. A. (1986) Maximum activities of key glycolytic and oxidative enzymes in human muscle from differently trained individuals. *J. Physiol.* **381,** 111–118.

Brooks S. P. J. and Storey K. B. (1988) Subcellular enzyme binding in glycolytic control: *in vivo* studies with fish muscle. *Am. J. Physiol.* **255,** R289–R294.

Choate G. L., Lan L. and Mansour T. E. (1985) Heart 6-phosphofructo-1-kinase, subcellular distribution and binding to myofibrils. *J. biol. Chem.* **260,** 4815–4822.

Clarke F. M., Shaw F. D. and Morton D. J. (1980) Effect of electrical stimulation post mortem of bovine muscle on the binding of glycolytic enzymes. *Biochem. J.* **186,** 105–109.

Clarke F. M., Stephan P., Huxham G., Hamilton D. and Morton D. J. (1984) Metabolic dependence of glycolytic enzyme binding in rat and sheep heart. *Eur. J. Biochem.* **138,** 643–649.

Hochachka P. W. and Guppy M. (1987) *Metabolic Arrest and the Control of Biological Time*, 227 pp. Harvard University Press, Cambridge, MA.

Knull H. R. (1978) Association of glycolytic enzymes with particulate fractions from nerve endings. *Biochim. biophys. Acta* **522,** 1–9.

Lee A. K. and Mercer E. H. (1967) Cocoon surrounding desert-dwelling frogs. *Science* **157,** 87–88.

Loveridge J. P. and Withers P. C. (1981) Metabolism and water balance of active and cocooned African bullfrogs *Pyxicephalus adspersus*. *Physiol. Zool.* **54,** 203–214.

Lowery M. S., Roberts S. J. and Somero G. N. (1987) Effects of starvation on the activities and localization of glycolytic enzymes in the white muscle of the barred sand bass *Paralabrax nebulifer*. *Physiol. Zool.* **60,** 538–549.

Masters C. J. (1981) Interactions between soluble enzymes and subcellular structure. *Biochemistry* **11,** 105–143.

Mink J. W., Bluemenschime R. J. and Adams D. B. (1981) Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am. J. Physiol.* **241R,** 203–212.

Mommsen T. P., Walsh P. J. and Moon T. W. (1985) Gluconeogenesis in hepatocytes and kidney of Atlantic salmon. *Molec. Physiol.* **8,** 89–100.

Plaxton W. C. and K. B. Storey (1986) Glycolytic enzyme binding and metabolic control in anaerobiosis. *J. Comp Physiol.* **156B,** 635–640.

Seymour R. S. (1973a) Gas exchange in Spadefoot toads beneath the ground. *Copeia* **1973,** 452–460.

Seymour R. S. (1973b) Physiological correlates of forced activity and burrowing in the Spadefoot toad, *Scaphiopus hammondii*. *Copeia* **1973,** 103–115.

Seymour R. S. (1973c) Energy metabolism of dormant Spadefoot toads (*Scaphiopus*). *Copeia* **1973,** 435–445.

Srere P. A. (1987) Complexes of sequential metabolic enzymes. *A. Rev. Biochem.* **56,** 89–124.

Storey K. B. (1985) A re-evaluation of the Pasteur effect: new mechanisms in anaerobic metabolism. *Molec. Physiol.* **8,** 439–461.

Walsberg G. E. (1986) Comparison of two techniques for estimating the maximum aerobic capacity of amphibians. *Herpetologica* **42,** 389–394.

Walsh T. P., Masters C. J., Morton D. J. and Clarke F. M. (1981) The reversible binding of glycolytic enzymes in ovine skeletal muscle in response to tetanic stimulation. *Biochim. biophys. Acta* **675,** 29–39.

Westrin H. and Backman L. (1983) Association of rabbit muscle glycolytic enzymes with filamentous actin. *Eur. J. Biochem.* **136,** 407–411.

Whitford W. G. (1969) Heart rate and changes in body fluids in aestivating toads from xeric habitats. In *Physiological Systems in Semiarid Environments* (Edited by Hoff C. C. and Riedesel M. L.), 1st edn, pp. 125–133. University of New Mexico Press, Albuquerque, New Mexico.