# Tissue Enzyme changes in air-exposed American eels

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AlaAt | Alanine aminotransferase |
| AspAt | Aspartate aminotransferase |
| ATP, ADP, AMP | Adenosine tri-, di- and mono-phosphate |
| BCA | Bicinchoninic acid |
| cAMP | Cyclic adenosine monophosphate |
| EDTA | Ethylenediamine tetraacetate |
| EGTA | Ethyleneglycol bis(β-aminoethyl ether) tetraacetate |
| $F1,6P_2$ | Fructose-1,6-bisphosphate |
| F2,6P | Fructose-2,6-bisphosphate |
| F6P | Fructose-6-phosphate |
| FBPase | Fructose-1,6-bisphosphatase |
| G6P | Gluose-6-phosphate |
| G6PDH | Gluose-6-phosphate dehydrogenase |
| GP | Glycogen phosphorylase |
| GP a | Glycogen phosphorylase a |
| GP b | Glycogen phosphorylase b |
| GS | Glycogen synthase |
| GS I | Glycogen synthase I |
| GS D | Glycogen synthase D |
| HK | Hexokinase |

| | |
|---|---|
| $I_{50}$ | Concentration of inhibitor that inhibits the maximal activity of an enzyme by 50 % |
| $K_a$ | Concentration of activator that produces half maximal enzyme activity |
| $K_m$ | Concentration of substrate at which the enzyme reaction proceeds at 50% of its maximal velocity |
| LDH | Lactate dehydrogenase |
| $NAD^+$, NADH | Oxidized and reduced forms of nicotinamide adenine dinucleotide |
| $NADP^+$, NADPH | Oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate |
| PEP | Phosphoenolpyruvate |
| PEPCK | Phosphoenolpyruvate carboxykinase |
| PFK | Phosphofructokinase |
| PK | Pyruvate kinase |
| PMSF | Phenylmethylsulfonyl fluoride |
| $pO_2$, $pCO_2$ | Partial pressure of oxygen, carbon dioxide |
| $S_{0.5}$ | Concentration of substrate at which the enzyme reaction proceeds at 50 % of its maximal velocity |
| UDPG | Uridine diphosphate glucose |

## ABSTRACT

American eels (*Anguilla rostrata*) are known to leave the water for excursions onto land despite lacking specialized air breathing organs. Other studies have established that the eel suffers from acidosis and hypoxemia as a result of this air exposure, but the survival of the eels despite these conditions demonstrates a large degree of tolerance to these effects. This thesis will investigate the changes in tissue enzymes of the American eel as a result of air exposure. It is the hypothesis of this experiment that under conditions of air exposure eels will undergo a metabolic reorganization including an initial Pasteur effect and metabolic depression indicated by changes in enzyme activities and metabolite concentrations. Eels were air exposed for 3, 5 or 10 days in boxes ensuring constant temperature and humidity. Maximal activities were determined in white muscle, gill and posterior kidney tissues harvested immediately following the particular air exposure period; as a result of technical difficulties, liver was unavailable for study. Enzymes assayed included those key in glycolysis (PFK, PK, LDH), glycogen metabolism (GPase and GSase) and gluconeogenesis (GDH, AspAT, AlaAT, and PEPCK). In addition, kinetic analysis was undertaken for PFK and PK in harvested posterior kidney. Blood collected at the time of harvest was analyzed for glucose, lactate and cortisol to determine circulating levels. Muscle samples were also analyzed for glycogen content. Although changes in enzyme rates, enzyme kinetics and metabolites were observed to result from the air exposure, it could not be concluded that the changes were part of a concerted metabolic depression.

## INTRODUCTION

### A. Background

The American eel (*Anguilla rostrata*) is peculiar as a teleost in its habit of making excursions onto land. They have been observed to move through damp vegetation near the rivers and ponds where they are typically found. It is thought that the eel may leave the water to migrate from pond to pond, or in search of food along shorelines. Fisherman often keep eels alive in a box for days at a time, covered only with a wet sack to prevent desiccation. The eel demonstrates an ability to survive outside of its normal environment where few other fish can. In the absence of its normal aquatic medium, how does the eel provide its tissues with oxygen? Or is this not necessary?

Various forms of adaptive morphologies are observed in species of air breathing fish. Adaptations vary from modified gills preventing collapse during air exposure and increased vascularization of the buccal and pharyngeal cavities (*Symbranchus marmoratus* and *Hypopomas,* Carter and Beadle 1931), to folding and papillations of buccal cavity to increase vascular surface (*Electrophorus electricus,* Johansen et al 1968), to the development of labyrinth-like organs from outgrowths in branchial arches as typified in members of the Anabantidae (Johansen 1970). Structural adaptations for air breathing are also found in the gastrointestinal tract, where the stomach or part of the small intestine are modified to allow gas exchange between swallowed air and the blood (*Plecostomus plecostomus,* Carter 1935; *Callichthys* and *Hoplosternum,* Carter and Beadle 1931). The swim bladder of the lungfish is found to be highly modified, resembling the lung of lower vertebrates (Johansen 1970).

Air breathing fish also exhibit a variety of physiologically adaptive features. Increased oxygen capacity, increased tolerance to $CO_2$ , $CO_2$ elimination at sites other than the gills, and increased $O_2$ absorbance are found in fish utilizing air as an oxygen source (Johansen 1970). These vary according to the relative importance of air breathing to water breathing (facultative to obligate air breathers) and specific environmental factors.

Fish capable of withstanding hypoxic conditions will alter their respiration behavior to suit the conditions. When conditions become hypoxic, some species of Amazonian fish (i.e. *Colossoma macropomum*) will skim the top layer of water, and flush the gills with this oxygen rich water, in addition to the air passing over the gills (Val 1995). During hypoxia, goldfish have been observed to "inhale" surface water and air bubbles at the water surface, effectively increasing blood $pO_2$ and blood $O_2$ saturation (Burggren 1982).

The American eel, however, does not appear to have any specialized morphology or physiology to allow for air breathing. Eels leave the water of their own accord, not necessarily forced out by anoxic conditions to use air as a secondary oxygen source as do facultative air breathing fish. When respiring aerially, the eel gulps air into the opercular cavity, trapping it, and allowing the gills to perform gas transfer with the air (Berg and Steen 1965, 1966). The gills are designed for effective gas transfer with flowing water and not stagnant air. Oxygen consumption cannot be maintained during aerial respiration as in other species (*Channa maculata*, Yu and Woo 1987) and rapidly decreases (Berg and Steen 1965), as does heart rate (Hyde *et al.* 1987). During breath holding (up to 5 min.; Berg and Steen 1965) $pO_2$ and $pCO_2$ between the blood and trapped air quickly equilibrate

(Hyde *et al.* 1987) and diffusive conductance decreases with the collapse of the gill lamellae. Although the eel skin does consume oxygen in air (Berg and Steen 1965), it has been shown to be poorly vascularized and is not likely to contribute to vascular gas exchange (Kirsch and Nonette 1977, Hyde *et al.* 1987). The increase in arterial $CO_2$ tension and decrease in arterial $O_2$ tension demonstrate the low capacity the eel has for aerial gas transfer (Hyde *et al.* 1987). In an attempt to prevent water loss the eel minimizes urine flow (Hyde and Perry 1987). Acid-base regulation is lost as the gills are not available for ion or gas exchange, and the kidney does not act to significantly reduce the acid load. Hyde and Perry (1987) suggest the eel has a limited capacity for acid-base regulation in air or in water, and because the kidney plays a minor role, the loss of the gills as a regulator has profound effects. In air, the gills cannot take advantage of the high solubility of $CO_2$ in water for the excretion of $CO_2$ (Bridges 1988). Eels do not regulate blood or red blood cell pH as can most fish species, including amphibious fish (Johansen 1970). Generally speaking, in the presence of decreasing blood pH, fish regulate red blood cell pH via adrenergic stimulation (Nikinmaa *et al.* 1987). The red blood cells of the American eel do not have the ability to regulate pH adrenergically (Hyde and Perry 1990). Blood cell pH conforms to that of the blood and decreases as metabolic acids accumulate and respiratory $CO_2$ is retained resulting in blood acidosis (Hyde *et al.* 1987). Increasing $pCO_2$ and decreasing pH impact upon the Bohr and Root effects of blood, worsening the anoxic conditions by decreasing oxygen binding affinity of hemoglobin. The arterial $O_2$ content declines to levels below that which is required to support metabolic requirements aerobically (Hyde *et al.* 1987).

Among other factors, the balance of adenosine triphosphate (ATP) utilization and production whether via aerobic or anaerobic metabolism is important to the condition of the animal at a cellular level. When ATP consumption exceeds production, there is membrane depolarization resulting from the inactivity of energy driven ion pumps, and $Ca^{++}$ channels are activated, the increased $Ca^{++}$ levels having a number of deleterious effects (Storey 1987). Different strategies are employed by animals under conditions of declining oxygen levels to maintain a balance between ATP production and ATP utilization. The $O_2$ regulating animal consumes oxygen at a constant rate during decreasing oxygen availability until a point is reached where aerobic metabolism rapidly switches to increased rates of anaerobic glycolysis in an effort to match ATP production to ATP demand (Storey and Storey 1990). This strategy, termed the Pasteur effect, is only a short term solution to meeting energy demands (Storey 1985*a*). The Pasteur effect is observed widely across species, from as first observed in yeast to mammalian tissues (Ramaiah 1974). Anaerobic metabolism, however, is a fundamentally inefficient metabolic strategy for animals surviving sustained anoxic conditions (Hochachka *et al.* 1973). The number of ATP molecules generated from each glucose molecule drops from 36 during oxidative metabolism to 2 during fermentation with lactate as the major end product (Hochachka and Somero 1984). Facultative anaerobes, a modification of the $O_2$ conformer, rely on aerobic metabolism at high $O_2$ tensions, but as the availability of oxygen decreases, so does oxygen consumption. Total ATP output is maintained by increasing fermentative glycolysis (Storey and Storey 1990). The principle strategy allowing for the survival of the facultative anaerobe in the absence of a Pasteur effect is a reduction in energy requirements of the animal. This eliminates a need for increased glycolysis, and may occur with a decrease in glycolysis (Storey 1985*a*). Fish utilizing this method can survive periods of anoxia at 0° C lasting as long as several months, while producing anaerobic endproducts other than lactate. (van den Thillart 1982).

For maintained metabolic depression, a lower balance of ATP generation and utilization must be attained. Animals typically reduce physical activities such as movement, feeding and digestion, heart rate, and respiration to limit energy demands. At the cellular level, a general depression of cellular processes limits ATP consumption. This is accomplished in part by limiting the production of ATP. The decreased activity of the few reactions generating ATP, either by limiting substrate (other than ATP) or decreasing enzyme activity, decreases the activity of the many ATP consuming reactions in the cell. Also important is the depression of ATP independent reactions, as although they may not directly effect the energy balance (i.e. facilitated diffusion of ions), they may be linked to ATP dependent reactions (i.e. ATP dependent ions pumps) and imbalance may have deleterious consequences. Mechanisms must therefore be well developed in control and coordination for transition to metabolic depression.

Under hypoxic conditions, aerobic metabolism continues to dominate over aerobic metabolism (van den Thillart 1982). As the severity of hypoxia increases, anaerobic metabolism plays an increasingly important role. During short term hypoxia (as well as anoxia) anaerobic metabolism is increased and the general response is an accumulation of lactate and decreased glycogen and creatine phosphate stores (van den Thillart and van Waarde 1985). Continued exposure to non-lethal levels of hypoxia may result in adaptation. Adaptation can be attributed to improved oxygen extraction, higher anaerobic capacity, or changes in enzyme pathways (van den Thillart and van Waarde 1985). Both increasing anaerobic capacity and altering enzyme pathways may involve modification of enzyme activities.

In anoxic conditions, the lack of oxygen deactivates the electron transport chain, by not being available as the terminal electron acceptor, preventing oxidative phosphorylation. ATP can still be generated from reactions linked to pathway substrate catabolism. The most utilizable substrate under anoxia is glucose from glycogen. Lipid oxidation involves a $O_2$ dependent mechanism (de Zwaan 1977) and is not operational under low oxygen conditions. Fermentation of carbohydrates is not limited by this problem. The fermentative reactions of glycolysis produce NADH linked to the transfer of phosphate from phosphorylated substrates to adenosine diphosphate (ADP), not to ATP generation by oxidative phosphorylation. The endproducts of carbohydrate fermentation in vertebrates is generally lactate, the fate of which is generally oxidation after oxygen returns. Invertebrate facultative anaerobes often produce altered endproducts to eliminate the dead-end production of lactic acid of the vertebrates (Hochachka and Somero 1984). Alternate energy producing pathways resulting in propionate, succinate and imino acids from glucose are also known to occur (Hochachka and Somero 1984). The goldfish has been found to convert lactate to ethanol and $CO_2$ in white muscle, preventing problems with lactate accumulation (Shoubridge and Hochachka 1981).

The production of ATP occurs along pathways consisting of enzymatic reactions catalyzing steps which are described as being either near-equilibrium or non-equilibrium.

Near-equilibrium reactions occur at a rate where the substrates and products are close to equilibrium and possess a relatively high maximal activity. Enzymes catalyzing non-equilibrium reactions operate at a maximal rate not great enough to allow the substrate and product to equilibrate (Newsholme and Start 1973). Rates of reaction preceding and following a non-equilibrium reaction create substrate and remove product faster than enzyme can act to bring the two to equilibrium. These reactions become the rate limiting, or flux generating steps of a pathway, and are typified by a low maximal activity, low mass action ratio, and regulation by allosteric (and sometimes covalent) modification. Because the flux of a pathway is determined at this point, the reactions following in the pathway adjust according to the flux generating reaction (Newsholme and Crabtree 1986). The following near-equilibrium reactions can allow large changes in flux to be transmitted through these reactions without complex regulation (Newsholme and Crabtree 1986). The measurement of the maximal activities of enzymes allows for the classification of enzymes in a particular pathway according to their activity, and identification of those steps acting as non-equilibrium steps in the tissue (Newsholme and Start 1973). Changes in the maximal activities of non-equilibrium enzyme activities then reflects changes in the flux through the pathway.

Because of the control the non-equilibrium steps have over the flux of a pathway, these enzymes are the principal points of regulation, including during periods of metabolic depression in facultative anaerobes. Mechanisms for metabolic rate depression include covalent modification of enzymes by phosphorylation or dephosphorylation (Storey and Storey 1990). Covalent modification is a good strategy for widespread control of enzymes within a cell for the following reasons; 1) major changes in the activities of affected enzymes occur, 2) process acts within a short time frame, 3) is widely applicable to control diverse enzymes within the cell, and 4) regulation of kinases and phosphatases by intracellular second messengers and extracellular messengers is in place in all animal cells (Storey 1988). The phosphorylation of enzymes causing metabolic depression resulting from anoxic conditions is likely due to activities of protein kinases (Storey and Storey 1990). *In vitro* experiments with goldfish liver have demonstrated that mammalian cyclic adenosine monophosphate (cAMP)-dependent protein kinase will elicit the same changes in phosphofructokinase (PFK) and pyruvate kinase (PK) kinetics as anoxic conditions (Rahman and Storey 1988). This mechanism in not necessarily conserved among species. In the marine whelk, anoxia is mediated by cyclic guanosine monophosphate (cGMP)-dependent protein kinases and not by the cAMP-dependent protein kinase (Storey and Storey 1990). These types of mediations are often responsive to extracellular control such as hormones (Storey 1985*a*).

The enzyme glycogen phosphorylase (GPase) was the first enzyme found to be activated or deactivated by reversible phosphorylation by Cori and Cori in the 1940's, covalently modifying the enzyme by phosphorylation from the inactive (AMP dependent) b to the active a form (Cohen 1980). Control over GPase activity leads to control over the release of substrate (glucose-1-phosphate) for the reactions of glycolysis from endogenous glycogen stores. Changes in GPase activity reflect changes in glycogen utilization (Storey 1987) as glycogenolysis is the generating step for glycolysis. Rapid alterations in the active form of GPase activities leads to immediate changes in glycogen utilization. Under anoxic conditions, covalent modification is utilized by both facultative anaerobes to decrease GPase activity (Storey 1987) and by $O_2$ regulators to increase GPase activity (Harris *et al.* 1986).

Phosphorylation/dephosphorylation also acts to modify (as opposed to activation or deactivation) the kinetics of other enzymes. Under anoxic conditions, PFK and PK are covalently modified by phosphorylation to produce less active forms (Storey 1985*a*). The kinetics of the enzymes are altered in addition to the decrease in maximal rate. The alteration of PFK activity is vital to altering glycolytic rate, and has been recognized as being the most important site of control (Hochachka 1980), largely responsible for the anti-Pasteur effect observed to occur in molluscs during anoxia (Storey and Storey 1990). Phosphorylated PFK shows greater ATP inhibition, and decreases activation by fructose-2,6-bisphosphate (F2, $6P_2$), a potent activator of PFK (Storey 1984). Phosphorylated PK has reduced affinity for phosphoenolpyruvate (PEP), its substrate, reduced activation by fructose-1, 6-bisphosphate (F1, $6P_2$), and increased inhibition by alanine (Plaxton and Storey 1984). The enzymes have increased sensitivity to endproducts, and decreased activation, both acting to minimize the flux of the pathway. Suppression of glycolytic metabolism by covalent modification of PFK and PK not only alter the rate of glycolysis, but may allow for an increase in flux along the reverse pathway called gluconeogenesis to increase glucose production. Depressing the glycolytic pathway allows the non-equilibrium enzymes catalyzing the reverse reactions (i.e. phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase)) to dominate, and the pathway becomes anabolic instead of catabolic. In this way PK is not only important in reducing glycolytic flux, but is also important in regulating glycolytic vs. gluconeogenic flux (Engstrom 1978).

Despite the fact that metabolic rates are much lower during anoxia, energy must be generated for fundamental metabolic requirements, even if at a basal level. Rainbow trout under hypoxic conditions maintain glucose flux indicating the fuel source for glycolysis is endogenous, while both lactate concentrations and flux increase indicating activation of anaerobic metabolism (Dunn and Hochachka 1986, Haman 1996).

Increases in lactate concentrations become significant after 24 h of air exposure in eels (Hyde *et al.* 1987). Anoxic goldfish release glucose from hepatic glycogen stores, reserving white muscle glycogen for fuel for swimming (Shoubridge and Hochachka 1981). Eels appear to utilize both liver and muscle glycogen during air exposure. Hepatic glycogen content was found to be consumed to a greater degree, dropping from approximately 120 to 10 µmol /g tissue in 72 h of air exposure, where muscle glycogen content decreased from approximately 18 to 11 µmol/g tissue (Moon *et al.* in prep.). Other fish protect liver glycogen stores in reduced oxygen environments (1 h hypoxic mudskippers (*C. maculata*), Yu and Woo 1987; anoxic Crucian carp (*Carassius carassius*), Nilsson 1990; hypoxic cod (*Gadus morhua*), Claireaux and Dutil 1992), and are related to lower activities of GPase. Eventual death resulting from anoxia is related to decreased hepatic glycogen and increased GPase activity (*C. carassius* L., Hyvärinen *et al.* 1985, Nilsson 1990; *A. rostrata*, Moon *et al.* in prep.) and increased lactate levels (Moon *et al.* in prep.). In contrast, long term hypoxia in the mudskipper resulted in decreased liver glycogen without effect in GPase activity (Yu and Woo 1987). It has been suggested by Moon and Foster (1995) that teleosts in general maintain liver glycogen as a last-resort fuel or as an energy source for response to additional stress, and once depleted, death occurs. This has been reported in American eels which after remaining unfed for 6 months demonstrate no alteration in the carbohydrate status of the tissues (Moon 1983). In an apparent attempt to prevent the usage of liver glycogen stores, insoluble myofibrillar protein is depleted and the whole body turnover of amino acids in the eel increases (Moon 1983). The protection of liver glycogen stores becomes evident in air exposed eels when returned to normoxic environments, and liver glycogen is rapidly restored (Moon *et al.* in prep.).

Although gluconeogenesis is not thought to contribute significantly to circulating glucose initially, when glucose levels fall and glycogen stores are depleted (or are being protected), pathways switch to gluconeogenesis as substrates lactate, glycerol, fructose and amino acids begin to accumulate. Rainbow trout (*Oncorhynchus mykiss*) will typically rely primarily on glycogenolysis for a source of glucose until hepatic stores near depletion at which time gluconeogenic flux will increase (Mommsen *et al.* 1988). The source of gluconeogenic substrate may come from the diet of the fish, but in starved and in exercised starved animals, amino acids from the proteolysis of muscle become primary substrates (Suarez and Mommsen 1987), and cortisol may be important in providing these amino acids. Teleosts typically synthesize glucose from lactate more readily than from other sources, in part due to the presence of cytosolic PEPCK (Moon *et al.* 1985, Mommsen and Suarez 1984, Moon 1988), suggesting an importance of the Cori cycle (muscle lactate → liver glucose → muscle glycogen). Hepatocytes from Japanese eels (*Anguilla japonica*) demonstrate a greater versatility, able to synthesize glucose from lactate, pyruvate and alanine at similar rates (Hayashi and Ooshiro 1979). The lactate typically found in increased concentrations in the plasma of anoxic fish (Jørgensen and Mustafa 1980, van den Thillart 1986, Storey 1987) may activate the gluconeogenic pathway. Activation by lactate has been found to be hyperbolic, peaking at 3 mM in the European eel (*Anguilla anguilla*) (Jankosky *et al.* 1984). High performance fish such as tuna (*Katsuwonus pelamis*) have lactate turnover rates similar to that of mammals, and very quick lactate clearance rates, but less than 5% is recovered as glucose (Weber *et al.* 1986). As much as 35% of the lactate carbon can be metabolized in the eel, but makes up only 1% of the total glucose production (Cornish and Moon 1985). This small percentage of glucose from lactate suggests a relative insignificance in the Cori cycle. Lactate turnover rates *in vivo* do not account for rates of clearance (Cornish and Moon 1985, Weber *et al.* 1986). However, muscle lactate clearance during recovery has been demonstrated to coincide with glycogen replenishment (see Milligan 1996). Glycolytic activity must be limited to allow gluconeo- or glycogenesis to occur. Reversal of PK has been suggested, as rates are high enough that a reversal of less than 2% would be sufficient to explain muscle glycogenesis (Moyes *et al.* 1992). An alternative to PK reversal is a depression of PK activity to allow a bypass via PEPCK (Figure 1). In fish, the white muscle isozyme of PK resembles that of mammalian liver PK and may be regulated by at least by small metabolites (*Mugil cephalus*, Bannister and Anastasi 1976; *A. rostrata*, Moon and Hulbert 1980*a*, 1980*b*). The regulatory abilities of the white muscle isozyme suggests that reversal of the glycolytic pathway is possible. In air exposed eels, concentrations of the potent PK activator F1,6$P_2$ was found to decrease after 24 h of air exposure, which could decrease PK activities (Moon *et al.* in prep).

When faced with a stress, rapid increases in glucose availability are required to meet the increased metabolic demand of tissues. Catecholamines and cortisol are both found to increase following a stress (Mazeaud *et al.* 1977), and through their hormonal effects act to regulate glucose availability.

Catecholamine levels increase markedly in arterial blood in response to various stresses, of particular interest, during exposure of fish to low environmental oxygen (Butler *et al.* 1979, Boutilier *et al.* 1988). In fish epinephrine and norepinephrine are released primarily from the chromaffin cells found in the posterior cardinal veins (Epple *et al.* 1989). The mechanism of action involves β-adrenoreceptor binding (Mommsen *et al.* 1988, Wright *et al.* 1989), activation of adenylate cyclase causing an increase in intracellular cAMP (Morales *et al.* 1982, Morata *et al.* 1982) and subsequent activation of protein kinases.

The action of the catecholamines has various effects on the metabolism of the cell and tissue, including hyperglycemic effects in fish. (deRoos and deRoos 1978, Morata *et al.* 1982, Janssens and Lowery 1987, Mommsen *et al.* 1988). Primary activation varies between species, epinephrine in some species (*Ictalurus melas*, Brighenti *et al.* 1987), norepinephrine in others (*Sebastes caurinus*, Danulat and Mommsen 1990). Hepatic PK activity is inhibited and both total and active form of glycogen phosphorylase (GPase) activities are increased in response to both catecholamine administration and hypoxia (Wright *et al.* 1989). Alteration of enzyme activities occurs by phosphorylation or dephosphorylation, and suggests an activation of glycogenolysis, inhibition of glycolysis and/or activation of gluconeogenesis (Wright *et al.* 1989). The result is increased glucose production by stimulated glycogenolysis (Danulat and Mommsen 1990) and gluconeogenesis (Morata *et al.* 1982, Janssens and Lowery 1987, Janssens and Waterman 1988, Mommsen *et al.* 1988, Wright *et al.* 1989, Danulat and Mommsen 1990,). Gluconeogenic glucose production may occur independent of glycogenolysis (Morata *et al.* 1982, Porthe-Nibelle and Lahlou 1987), so that catecholamine stimulated hyperglycemia may occur without effecting glycogen stores (Wright *et al.* 1989).

Cortisol is known to exert effects on teleost intermediary metabolism, including glycogenolysis and gluconeogenesis. Cortisol uptake by cells occurs by two methods acting simultaneously. Diffusion by the liposoluble glucocorticoid occurs, and is accelerated by a low affinity temperature sensitive transporter (Porthe-Nibelle and Lahlou 1981). Following binding with its nuclear receptor, the complex binds to DNA and regulates gene transcription via a specific interaction with steroid binding domains (van der Boon *et al.* 1991),

Metabolic effects of cortisol administration are either short or long term. Hyperglycemia can be quickly elicited, and gluconeogenesis from protein can be increased over a longer period of time. Effects of cortisol administration is apparent after two hours (Chan and Woo 1978) inducing hyperglycemia in many species of fish by altering enzyme activities (*A. japonica*, Chan and Woo 1978; *A. anguilla*, Lidman *et al.* 1979; *Fundulus heteroclitus*, Leach and Taylor 1980; *I. melas*, Brighenti *et al.* 1987; *Salvelinus fontinalis*, Vijayan *et al.* 1991; *Hemitripterus americanus*, Vijayan *et al.* 1993). Other reports have found that cortisol administration does not elicit an increase in blood glucose (Foster and Moon 1986*a*, Andersen *et al.* 1991), and is associated with a general metabolic depression (Foster and Moon 1986*a*). The European eel was found to increase liver glycogen and decrease liver protein as the amino acids from protein catabolism is incorporated for glycogen deposition following cortisol administration (Lidman *et al.* 1979). This is suggested to be due to increased gluconeogenic activity and decreased glucose utilization. Elevated cortisol levels have been associated with catabolism of muscle protein (Andersen *et al.* 1991), and increased concentrations of α amino acids in the blood (Chan and Woo 1978). Mobilization of amino acids can occur with concentrations as low as 1 μM (*Cyprinus carpio*, Janssens and Waterman 1988). The increase in circulating amino acids serves as an increase in substrate for gluconeogenesis in various tissues. In the teleost, cortisol also causes an increase in the activity of transaminases (Lidman *et al.* 1979, Davis *et al.* 1985) and the gluconeogenic pathway (Renaud and Moon 1980), utilizing the increased levels of substrate. FBPase activity increases and PFK activity decreases inhibition of glycolysis and stimulation of the gluconeogenic pathway (Freeman and Idler 1973, Vijayan *et al.* 1991). Increased transaminase activity varies among species of teleost. In rainbow trout and brook char (*S. fontinalis*) alanine aminotransferase (AlaAT) and aspartate aminotransferase (AspAT) activities increase with elevated cortisol levels (Freeman and Idler 1973), but do not change in *F. heteroclitus* (Leach and Taylor 1982). Even when activated, activities of the various transaminases are not necessarily activated to the same degree, as seen in the Japanese eel (*A. japonica*) (Chan and Woo 1978). Cortisol may alter, via changes in transaminase activities, the preferred substrate for *de* *novo* synthesis of glucose. Foster and Moon (1986) reported that although cortisol did not increase amino acid availability, the preferred substrate of gluconeogenesis shifted from lactate to amino acids.

In some cases, hyperglycemia may be due to the increase in gluconeogenesis independent of changes in glycogenolysis resulting from elevated cortisol. In the sea raven, cortisol induces gluconeogenesis without affecting glycogen breakdown (Vijayan *et al.* 1993). Elevated cortisol in the eel causes a decrease in liver protein, the released amino acids utilized by gluconeogenesis to increase glycogen stores (Lidman *et al.* 1979).

It should be noted that the variance in reported effects of both catecholamines and cortisol are due to not only species variation, but also due to experimental protocol. The effects noted in isolated hepatocytes often vary from whole animal experiments, and is likely due to the interaction of the suite of control hormones and mechanisms found *in vivo*. Cortisol, for example, is known to have permissive effects on intermediary metabolism, increasing the number of hepatic β receptors, increasing epinephrine responsiveness (Reid *et al.* 1992).

The eel is not necessarily a good air breather, but it does survive. Air exposed eels have the same problems of waste accumulation as other aquatic animals do during air exposure, but does not have the oxygen extraction ability truly amphibious fish possess. The eel is apparently tolerant to the problems of air exposure. One possible explanation to this tolerance may be a general metabolic depression as in the facultative anaerobe.

## B. Goals of the Investigation

The general aim of this thesis is to examine the metabolic changes in the American eel associated with extended periods of air exposure.

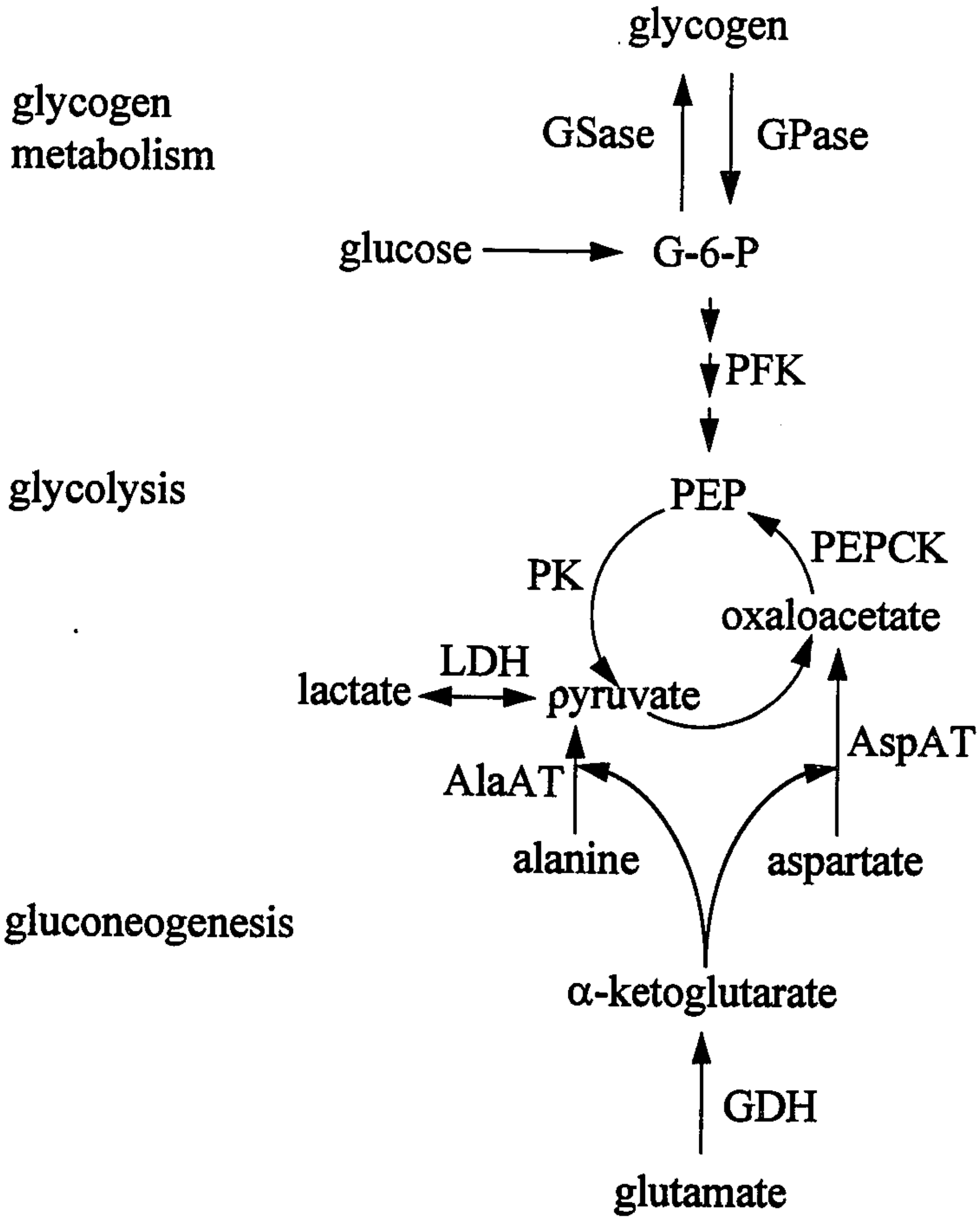
Maximal activities of representative enzymes of different metabolic pathways from different tissues will be estimated, and differences arising as a result of air exposure will give insight into the reorganization of these metabolic pathways. Changes in enzyme activities are not necessarily due to alterations in the amount of enzyme present. In addition, key enzymes may have their activities modified by phosphorylation or dephosphorylation. Enzymes such as GPase and glycogen synthase (GSase) phosphorylate and dephosphorylate between active and inactive forms. Changes in % active form of these enzymes reflect a change in phosphorylation state, allowing for alterations in activity without changing the quantity of total enzyme present. The total activity, activity of the active form, and the percent active form will be determined for these enzymes. Activity of key enzymes of glycolysis may also be modified by phosphorylation or dephosphorylation, including PFK and PK. This will be determined by examining the kinetics associated with these enzymes, in addition to maximal activities.

Tissues to be examined are white muscle, gill and posterior kidney tissue. Liver tissue, despite its significant contribution to whole animal metabolism was not analyzed due to

Metabolite levels in the blood and tissue which reflect changes in metabolism will also be examined. Changes in metabolite concentrations (glucose and lactate) will support enzymic evidence of pathway reorganization, as well as indicate the degree of stress the animal is experiencing.

It is the hypothesis of this thesis that tissues of the American eel will undergo metabolic reorganization as a result of extended air exposure. Specifically, I predict the eel tissues will initially switch from aerobic to anaerobic metabolism, followed by a depression in the activities in the key enzymes of glycogenolysis and glycolysis (Figure 1) as air exposure continues. As glycogen stores are depleted, an increase in gluconeogenic activity (Figure 1) will follow. The initial switch to anaerobic metabolism will be marked by increased glycogenolysis seen by changes in the active form of GPase and by increased activity of glycolytic enzymes, including a change in their kinetic properties and a decrease in the active form of GSase. In addition, plasma glucose concentrations will decrease and lactate concentrations will increase. The subsequent metabolic depression will be marked by decreased glycogenolysis and glycolysis indicated by changes in maximal activities and enzyme kinetics. Glycogen stores are expected to decrease, and gluconeogenic activity will increase. The stress experienced by the eel is expected to increase during the experiment, as indicated by increasing levels of cortisol and lactate. The changes observed in enzymes and metabolites during air exposure in the eel may differ between tissues.

**Figure 1.** Simplified pathway displaying enzymes measured in reactions involved in glycogen metabolism, glycolysis and gluconeogenesis.


glycogen metabolism
glycolysis
gluconeogenesis
glycogen
GSase
GPase
glucose
G-6-P
PFK
PEP
PK
PEPCK
oxaloacetate
LDH
lactate
pyruvate
AlaAT
AspAT
alanine
aspartate
α-ketoglutarate
GDH
glutamate

# METHODS AND MATERIALS

## A. Experimental Animals and Chemicals

American eels (*Anguilla rostrata*) were obtained from the eel ladder associated with the Saunders Hydroelectric Dam in Cornwall, Ontario; the ladder is maintained by the Ontario Ministry of Natural Resources and Ontario Hydro. Eels were transported on ice to the University of Ottawa where they were held in large fibreglass aquaria. The aquaria were supplied with flowing, aerated and dechlorinated City of Ottawa tap water kept at 10°C. The eels were exposed to a photoperiod of 12 h light: 12 h dark. Eels were maintained in this way for no less than two weeks, no more than 6 months, and were kept unfed at all times.

Eels used varied in weight from 84 to 179 g (mean = 114.0 ± 19.0 (SEM) g; n=36), and temperatures were maintained between 10 and 12°C throughout the experiment, whether under control or air exposed conditions.

All biochemicals and coupling enzymes were obtained from Sigma Chemical Co. (St. Louis, MO), ICN Pharmaceuticals Inc. (Montreal, PQ), or Boehringer-Mannheim Corp. (Montreal, PQ). Distilled, deionized water was used throughout for the preparation of solutions.

## B. Exposure Protocol

Air exposure boxes consisted of an opaque acrylic chamber(volume = 6 L, Perspex), the floor perforated with drilled holes and placed inside a second chamber (see Figure 2). The second chamber was supplied with flowing water and aerated vigorously with compressed air. The aerated water flowing beneath the false floor served to maintain constant temperature, humidity and to supply the chamber with a constant source of fresh air.

Control eels were kept in identical boxes with the water level elevated so that the animal was completely submerged at all times.

Animals were kept in these boxes in either control or experimental conditions for 3, 5 or 10 days.

## C. Tissue Extraction

At the appropriate time periods, animals were removed from the boxes, quickly stunned by striking the caudal region, and a blood sample quickly collected by caudal puncture into a heparinized syringe before killing the animal by decapitation. Plasma was prepared by centrifugation (12 000 g, Fischer, Micro Centrifuge Model 235B) for 5 min, decanting the plasma and freezing it at -80°C. Tissues were quickly excised and frozen in liquid nitrogen, and held at -80°C until analysis.

Enzyme extracts were prepared by homogenizing the frozen tissue samples using a Kinematica CH-6010 (Kriens, Luzern) in a homogenizing buffer (50 mM imidazole-HCl, pH 7.8, 15 mM β-mercaptoethanol, 100 mM KF, 5 mM EDTA, 5 mM EGTA, 10 mM $K_2HPO_4$, 20 % glycerol, 0.1 mM PMSF) at a ratio of 1:4 or 1:5 depending on the tissue. Homogenates were centrifuged at 12 000 g for 5 min using a SS-34 rotor in a Sorvall RC28S or RC5C centrifuge at 5°C, and the supernatant decanted into a clean tube.

Homogenates were either used immediately for analysis or filtered through a bed of fine Sephadex G-25 (Sigma) in a syringe with homogenate to gel ratio of 1:10 (v:v). These gel columns were spun in a table top centrifuge for 1.5 min to collect the tissue filtrate. For the analysis of phosphofructokinase (PFK), homogenates were first acidified to a pH of 6 and held on ice for 20 min before gel filtration to remove any fructose-2, 6-phosphate (F2, 6P), a potent PFK-activator, and then the pH was raised back to 7.8 during the gel filtration (van Shaftingen 1984; Storey 1987).

## D. Enzyme Assays

Maximal activities of PK, PFK, LDH, AlaAT, AspAT, GDH, PEPCK, GPase and GSase in white muscle, PK, PFK, LDH, AlaAT, AspAT, GDH, PEPCK, GPase and GSase in posterior kidney and PK, PFK, GPase and GSase in gill tissue were estimated. Preliminary experiments established optimal substrate concentrations for each enzyme in each tissue. Controls were run for all assays (absence of critical substrate) and any blank value was subtracted to yield final activities. In addition, the percentage of the enzyme in the active form was determined for GPase and GSase. The kinetic properties of PK and PFK in kidney were investigated. Optimal activity conditions were determined to be as follows:

**Pyruvate kinase** (PK; EC 2.7.1.40): 50 mM imidazole (pH 7.8), 100 mM KCl, 10 mM $MgCl_2$, 2.5 mM ADP, 0.15 mM NADH, and excess LDH. PEP concentrations are noted in Table 1. For the determination of kinetic properties, the same conditions were used, with varying concentrations of PEP, alanine, and F1, 6P as described in the Results. Samples were gel filtered through Sephadex G-25.

**Phosphofructokinase** (PFK; EC 2.7.1.11): 50 mM imidazole (pH 7.8), 175 mM KCl, 17.5 mM $MgCl_2$, 0.15 mM NADH, and excess aldolase, $\alpha$-G3PDH and TPI. ATP and F6P concentrations are noted in Table 1. For the determination of kinetic properties the same conditions were used with varying concentrations of F6P, ATP, F2,$6P_2$, and AMP as described in the Results. Samples were acidified and gel filtered. Commercial coupling enzymes are supplied in the presence on ammonium sulfate, which acts as an activator of PFK. Ammonium sulfate was removed from all coupling enzymes by desalting through a spin column of fine Sepahadex G-25 equilibrated in homogenizing buffer.

**Lactate dehydrogenase** (LDH; EC 1.1.1.27): 50 mM imidazole (pH 7.8), 0.15 mM NADH. Pyruvate concentrations are noted in Table 1.

**Alanine aminotransferase** (AlaAT; EC 2.6.1.2): 50 mM imidazole (pH 7.8), 0.15 mM NADH, 10 mM $\alpha$-KG, and excess LDH. Alanine concentrations are noted in Table 1.

**Aspartate aminotransferase** (AspAT; EC 2.6.1.1): 50 mM imidazole (pH 7.8), 0.15 mM NADH, 7 mM $\alpha$-KG, 40 mM L-aspartate and excess MDH.

**Glutamate dehydrogenase** (GDH; EC): 50 mM imidazole (pH 7.8), 0.15 mM NADH, 14 mM $\alpha$-KG, 1 mM EDTA, 0.1 mM ADP and 250 mM $NH_4Cl$.

**Phosphoenolpyruvate carboxykinase** (PEPCK; EC 4.1.1.32): 50 mM imidazole (pH 7.8), 0.15 mM NADH, 1 mM $MnCl_2$, 0.2 mM dGDP and excess MDH. $NaHCO_3$ and PEP concentrations are noted in Table 1. Only muscle samples were gel filtered.

**Glycogen phosphorylase** (GPase; EC 2.4.1.1): Assay conditions for total GPase (a + b) activity were 50 mM phosphate buffer (pH 7.0), 10 mM EDTA, 15 mM $MgSO_4$, 0.5 mM $NAD^+$, 0.01 mM G1, $6P_2$, 2.0 mg/ml dialyzed oyster glycogen and excess PGM and

G6PDH. AMP concentrations are noted in Table 1. For the measurement of the active (GPase a) form, 5 mM caffeine was added to the reaction mixture. Samples were gel filtered. % GPase a represents the percent GPase a activity of total GPase activity.

**Glycogen synthase** (GSase; 2.4.1.11): Assay conditions for the active I form maximal rates were 50 mM imidazole (pH 7.8), 0.15 mM NADH, 100 mM KCl, 10 mM $MgCl_2$, 2 mM dialyzed oyster glycogen, 5 mM PEP, and excess PK and LDH. UDPG concentrations are noted in Table 1. For total GSase activity (I + D), 10 mM G6P was included in the assay mixture. Samples were gel filtered. % GSase I represents the percent GSase I activity of total GSase activity.

GSase activity in white muscle (active and total enzyme activities) was determined by measuring the amount of [U-$^{14}$C] UDPG (Amersham Life Science Inc., Oakville, ON) incorporated into glycogen using the method of Thomas *et al.* (1968). Tissue samples were spotted on Whatman Filter Paper (No. 31 ET) and radioactive glycogen precipitated in ethanol; the dried papers added to 5 ml of ACS II and counted (Rackbeta 1215, LDK; Uppsala, Sweden). Total enzyme is measured by adding exogenous G6P, a stimulator of inactive GSase to the substrate buffer. Synthase I was measured in the same buffer in the absence of G6P. Final concentrations of the reaction mixture is as follows: I form- 0.55 mM UDPG, 24.5 mM glycylglycine, 1.1 mM EDTA, 11 mM $Na_2SO_4$, 1.1 % dialyzed oyster glycogen, 138 069 dpm/ml [U-$^{14}$C] UDPG; I + D forms- 50 mM tris-HCl, 20 mM UDPG, 5 mM G6P, 0.75% (w/v) dialyzed oyster glycogen, 3.6 mM EDTA, and 127 778 dpm/ml [U-$^{14}$C] UDPG.

All assays, except white muscle GSase, were conducted by monitoring changes in the concentration of NADH or NADPH at 340 nm using a Beckman DU65 spectrophotometer or a Ceres 900 (Biotek Instruments Inc., Winooski VT) microplate reader coupled to a 386 computer running Biotek KinetiCalc software900 (Biotek Instruments Inc., Winooski VT). Assays were initiated by adding either the tissue homogenate or a substrate to the final reaction mixture. White muscle enzyme activities were assayed at 14 ± 1 °C in the spectrophotometer, gill and posterior kidney enzyme activities at 21 ± 1 °C in the plate reader. Final volumes were 1 ml and 250 μl for the cuvette and microplate wells respectively.

## E. Plasma and Tissue Metabolite Measurements

Cortisol, lactate and glucose concentrations were determined in plasma. Blood samples were centrifuged (2 000 g, 5 min) immediately following collection to separate the red blood cells from the plasma, and the plasma decanted, and frozen at -80°C until assayed.

Lactate was measured using the following reaction:

$$\text{Lactate} + \text{NAD}^+ \xleftrightarrow{LDH} \text{Pyruvate} + \text{NADH}$$

The reaction is driven to completion by trapping pyruvate produced from lactate by LDH with hydrazine, measuring the increase in absorbance of NADH at 340 nm, and comparing the result to a standard curve. Reaction media concentrations were:

15 U/ml LDH, 2.5 mM NAD$^+$ in diluted glycine/hydrazine buffer.

Glucose was measured according to the reactions:

$$\text{Glucose} + \text{ATP} \xrightarrow{HK} \text{G6P} + \text{ADP}$$

$$G6P + NADP^+ \xrightarrow{G6PDH} 6\text{-}PG + NADPH + H^+$$

During this oxidation, an equimolar amount of $NADP^+$ is reduced to NADPH. The increase in absorbance at 340 nm is directly proportional to the glucose concentration. Reaction media concentrations were: 10 mM ATP, 0.82 mM $NADP^+$, excess G6PDH and hexokinase in triethanolamine-HCl (TRA) buffer (0.3 M TRA, 4.05 mM $MgSO_4$, pH 7.05).

Cortisol was measured using a commercially available radioimmunoassay kit (Immuno Corp.) according to Andersen *et al.* (1991). Antibody raised in rabbits is covalently bound to the inner surface of polypropylene tubes. Radioactive (added) and non-radioactive cortisol (from the sample or standard) compete for binding sites on the antibody. Because the antibody is bound to the tube, any antigen bound to antibody is also bound to the tube. With increased levels of non-radioactive cortisol, there is less binding of the radioactive cortisol. Following the aspiration of any free antigen and radioactivity, the amount of antibody-bound $^{125}I$ is determined using a gamma counter (Packard Cobra Auto Gamma Counter, Model 5003; Packard Instrument Co., Meriden CT), and the concentration of cortisol from the sample is determined graphically from a cortisol standard curve.

Muscle glycogen content was determined by amyloglucosidase hydrolysis of the sample, and measuring the glucose produced. Ice cold 8% perchloric acid (PCA) was added to cold, freshly homogenized (see Tissue Extraction) tissue (1:1) and quickly frozen in liquid $N_2$. Additional homogenate was centrifuged (see Tissue Extraction) PCA was added to supernatant and frozen. Homogenized samples were neutralized with 1 M

$KHCO_3$ (2:1) and incubated with amyloglucosidase (1 mg/ml 0.2 M acetate buffer, pH 4.8) (1:20, sample: amyloglucosidase) at 40°C for 2 h with shaking. Incubation was stopped by adding 8% PCA 2:1 to the reaction mixture. The resulting supernatant of the incubation was collected following centrifugation and analyzed for free glucose and glycogen-glucosyl units using the glucose assay previously described. Free glucose in the sample is measured in the supernatant of the homogenate, and subtracted from the total measured in the homogenate to yield glycogen values in glucosyl units per g tissue.

## F. Protein Determination

Protein concentration was determined using the bicinchoninic acid assay (BCA) based the ability of protein to reduce $Cu^{+2}$ to $Cu^{+1}$ in an alkaline medium, and the ability of BCA to preferentially complex with $Cu^{+1}$ ions (Smith *et al.* 1985). Bovine serum albumin was used as the standard.

## G. Kinetics and Statistics

For the determination of enzyme activity as nmol of substrate converted per min per mg of protein ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$), kinetic data from the Ceres 900 plate reader was entered into the Micro Plate Analysis program (MPA) (Brooks 1994) which performed a linear regression on the OD/time readings. Kinetic data from the Kinetics SoftPac program of the DU-65 spectrophotometer performed a linear regression analysis on the data, presenting the data as OD/time readings. One unit of enzyme activity is defined as the amount of activity that converts 1 μmol of substrate to product per minute.

$S_{0.5}$ values were determined by fitting data from the MPA program to the Hill equation using a non-linear least square regression program (Brooks 1992). A modified Hill equation that introduced a $V_0$ term (rate of activity at an activator concentration of 0) was used for determining $K_a$ values (Brooks 1992). The inhibitor constant values ($I_{50}$) were obtained from plots of rate vs. [inhibitor] (Brooks 1992).

All data are presented as means ± SEM. Statistics were analyzed using the SigmaStat statistical package. The data for each time of air exposure was subjected to analysis by a Students t-test (2-tailed, $p \leq 0.05$) compared to the control for that time. Data that did not pass equal variance testing was analyzed by the Mann Whitney Rank Sum test. Each control and test condition were also subjected to a One Way Analysis of Variance (ANOVA) with time as the factor. Where F-values indicated significance ($p \leq 0.05$), means were compared using the Student-Newmann-Keuls test. If data for the ANOVA did not pass normality tests, testing proceeded using Kruskal-Wallis, and where significance was observed ($p \leq 0.05$), Dunn's Test (pairwise) was used to compare means.

**Figure 2.** Diagram of air exposure box showing relative location of eel, water and air flow beneath the false bottom. Water leaves via outlet, air is bubbled through water into the holding chamber. Control boxes have lower water outlet blocked to increase water level, allowing the eel to remain submerged.

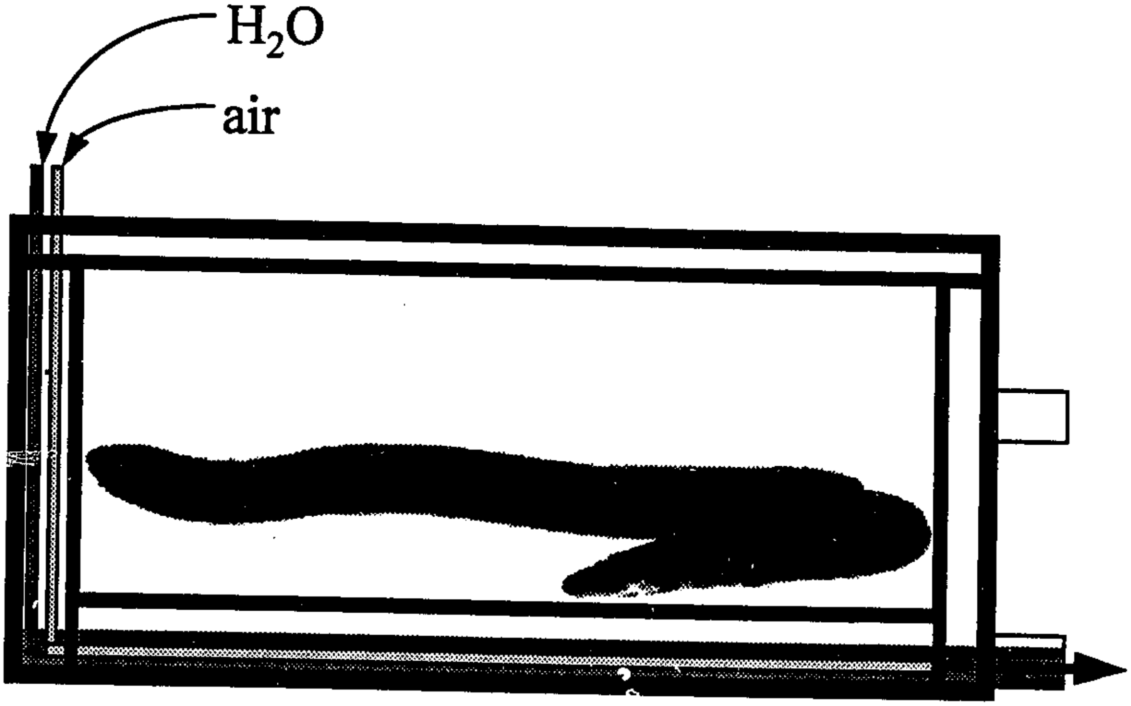

$H_2O$
air

**Table 1.** Concentrations of key substrates in reaction buffer. See Methods and Materials for details.

| Enzyme | Solution (mM) | Tissue | | |
|---|---|---|---|---|
| | | Kidney | Gill | White Muscle |
| PK | PEP | 5 | 5 | 5 |
| PFK | ATP | 7 | 5 | 2 |
| | F6P | 5 | 10 | 10 |
| LDH | pyruvate | 20 | -- | 30 |
| AlaAT | alanine | 100 | -- | 300 |
| PEPCK | $NaHCO_3$ | 40 | -- | 20 |
| | PEP | 10 | -- | 5 |
| GPase | AMP | 10 | 0.2 | 10 |
| GSase (a) | UDPG | 6 | 80 | -- |
| GSase (a + b) | UDPG | 20 | 80 | -- |

## RESULTS

### A. Effect of Air Exposure on Activities of Enzymes in the White Muscle

Maximal activities of 7 different enzymes and the activities of total and active forms of GSase and GPase, were measured in tissue homogenates of *A. rostrata* white muscle from animals in water (control) or air exposed (test) for periods of 3, 5 or 10 days. The enzymes assayed are found in pathways including glycolysis, gluconeogenesis, and glycogen metabolism (glycogenesis/glycogenolysis).

Activities of the measured glycolytic enzymes are presented in Figure 3. There were no significant differences in the maximal activities of PFK, PK or LDH either as a result of air exposure (relative to control conditions) or of the duration of the experiment (control or air exposed.

The enzymes of glycogen metabolism are presented in Figures 4 and 5. GPase a activities were significantly higher in air exposed compared with control eels. There were no changes in GPase (a + b) activities due to air exposure or to the duration of the experiment. There was a significant decrease in GPase a (active form) activity from 5 to 10 days in air exposed animals. At 5 and 10 days, the percent GPase a was significantly higher in air exposed than in the control animals. There was also a significant decrease in the % GPase a from 5 to 10 days in air exposed animals. There was a significant increase in total GSase (I + D) activities at 10 days of air exposure compared with controls. Total GSase activities of air exposed animals significantly decreased from day 3 to 5 of the experiment, and increased significantly from day 5 to 10. In the control condition, total GSase activity decreased significantly from 3 days to 5 and 10 days, but not from 5 to 10 days. At 5 and 10 days, activities of GSase I (active form) were significantly higher in air exposed animals. There were no significant changes in the % active form of GSase.

Enzymes of gluconeogenesis are presented in Figure 6. The only significant change observed in these enzymes is an increase in PEPCK activity in air exposed animals from day 5 to 10 of the experiment.

### B. Effect of Air Exposure on Activities of Enzymes in the Gill Tissue

The maximal activities of PK and PFK, and activities of total and active forms of GPase and total activity of GSase were measured in tissue homogenates of gill tissue from control and air exposed at 3, 5 and 10 days. These enzymes in part represent glycolysis and glycogen metabolism.

The enzymes represented in Figure 7 are associated with glycolysis. The only significant change observed was an increase in the maximal activity of PK in eels air exposed for 5 days relative to control animals at the same day. This is a direct affect of a relative decrease in activities of the control activities at day 5, not an increase in air exposed enzyme.

Activities of GPase and GSase are presented in Figures 8 and 9. There were no significant changes in total GPase (a + b), GPase a, or % GPase a. No significant changes occurred in total GSase (I + D) activities. The active form of GSase (I) was determined to be 100%, and therefore not included in the results.

### C. Effect of Air Exposure on Activities of Enzymes in the Posterior Kidney

The maximal activities of 7 different enzymes, and total and active forms of GSase and GPase were estimated in tissue homogenates of *A. rostrata* posterior kidney. The enzymes represent pathways including glycolysis, gluconeogenesis, and glycogen metabolism.

Glycolytic enzyme activities are presented in Figure 10. There were no significant changes in the maximal activities of PFK or PK of either as a result of air exposure (relative to control conditions) or of duration of the experiment. Maximal activities of LDH were significantly lower in air exposed compared with control eels 5 days. A significant increase in maximal activities occurred at day 5 relative to the 3 and 5 day values. These significant changes are a result of the large increase in activity in the control condition at day 5 as there were no differences between air exposed groups over the 3 periods.

Enzyme activities of GPase and GSase are presented in Figures 11 and 12. There were no significant changes in total GPase (a + b), GPase a, or % GPase a. At day 10, the total GSase (I + D) was significantly higher in air exposed compared with control eels. No other significant changes were noted in GSase I activities or % GSase I.

Gluconeogenic enzyme activities are presented in Figure 13. A significant decrease in GDH activities from day 3 to 10 of the air exposed condition was observed in addition to a significantly lower activity relative to control at day 5. AspAT activities were also significantly lower then control at day 5, but this is due to a significant increase in control activities from day 3 to 5, followed by a return to the 3 day value at day 10. There was no significant difference between day 3 and day 10. No significant changes were observed for either AlaAT or PEPCK activities.

## D. Effect of Air Exposure on the Kinetic Properties of Phosphofructokinase and Pyruvate Kinase in the Posterior Kidney

### 1. Phosphofructokinase

Table 2 summarizes the kinetic properties of PFK from the posterior kidney of *A. rostrata*. Enzyme affinity for the substrate F6P did not change as a result of air exposure, relative to control or to time. The sensitivity to inhibition by ATP did significantly decrease in the control animals from day 3 to 10, but not in the air exposed group. There was no significant change in sensitivity to the allosteric activator AMP, but there was a significant increase in the sensitivity of the enzyme to the allosteric activator F2, $6P_2$ compared to the control group at day 3.

### 2. Pyruvate Kinase

Table 3 summarizes the kinetic properties of PK from the posterior kidney of *A. rostrata* collected from animals in air exposed or control conditions for 3, 5 and 10 days. Enzyme affinity for the substrate PEP was significantly greater in the air exposed animals than the control animals at day 3, but differences are very small. There was no significant change in the sensitivity to inhibition by alanine, or activation by the allosteric activator F1, $6P_2$. Complete activation was observed, however, in both the air exposed and control conditions with concentrations of F1, $6P_2$ as low as 0.01 mM.

## E. Effect of Air Exposure on Plasma and Tissue Metabolites

Plasma was separated from the red blood cells, and glucose, lactate and cortisol concentrations determined as in Methods and Materials.

Plasma glucose concentrations (Figure 14A) of animals air exposed for 3 days were significantly higher than the control group at the same time. Plasma lactate concentrations (Figure 14B) were also significantly higher in the air exposed eels at 3 days, as well as 10 days as well. No other changes in plasma glucose or lactate were observed.

Cortisol concentrations (Figure 15) in air exposed animals were significantly increased at day 5, followed by a decrease back to levels observed at day 3. No significant changes were noted in cortisol concentrations in the control animals, or from day 3 to 10 in air exposed eels.

Glycogen content was determined in the white muscle (Figure 16). Air exposed eels had a significantly lower glycogen content than that of control eels. Eels air exposed for 5 or 10 days had significantly less glycogen than eels air exposed for 3 days. Control eels had significantly lower glycogen content after 5 days of the experiment than control eels following 3 or 10 days.

**Figure 3.** Maximal activities of enzymes associated with glycolysis in *A. rostrata* white muscle from animals in control and air exposure conditions for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values.

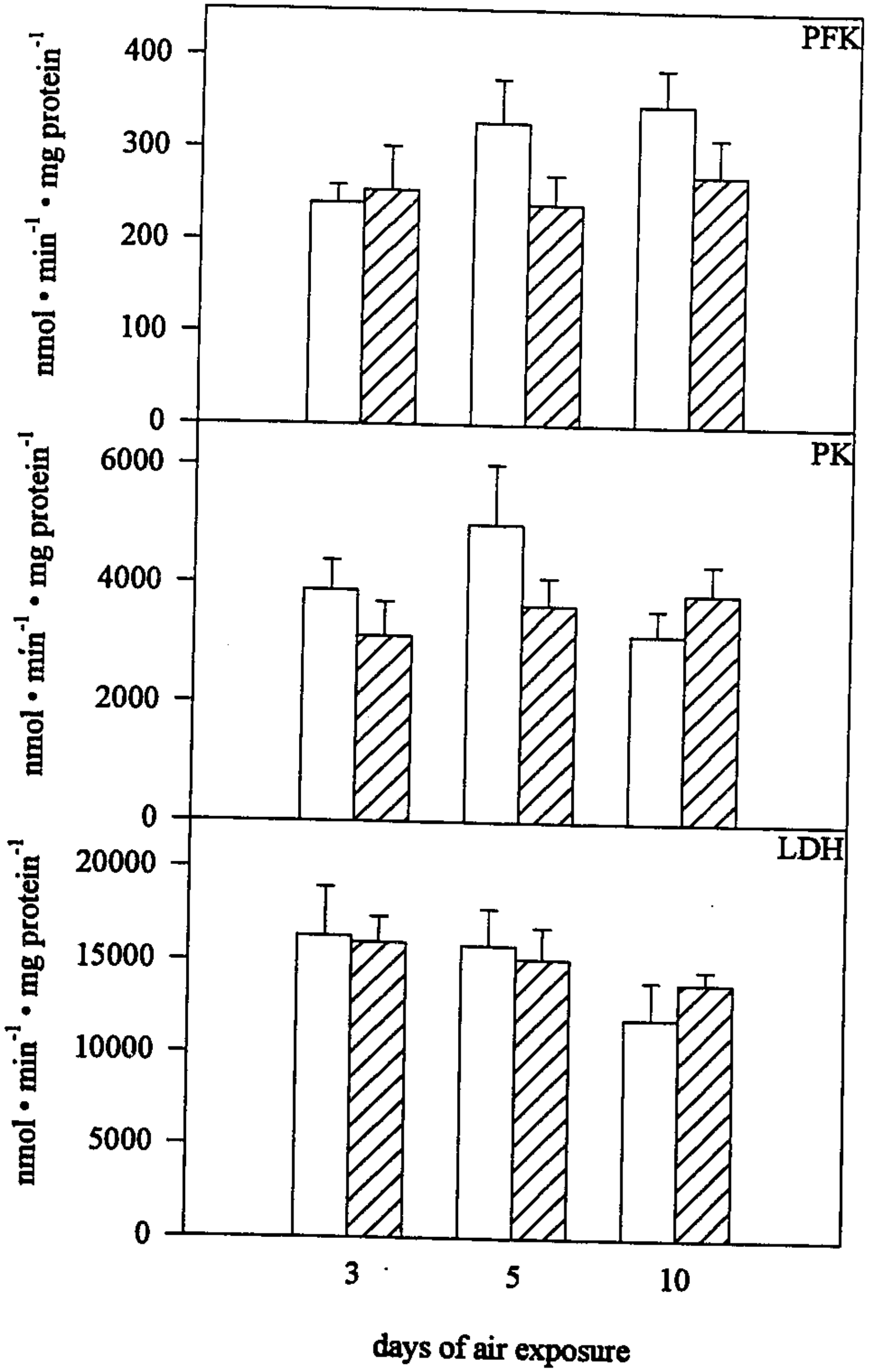

PFK
PK
LDH
nmol • min$^{-1}$ • mg protein$^{-1}$
400
300
200
100
0
6000
4000
2000
0
20000
15000
10000
5000
0
3
5
10
days of air exposure

**Figure 4.** Activity of total (a + b) and active (a) form, and percent a glycogen phosphorylase in *A. rostrata* white muscle from animals in control and air exposed conditions for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$. On graphs demonstrating significant change within a treatment, days sharing a common letter are significantly different by One Way ANOVA, $p < 0.05$.

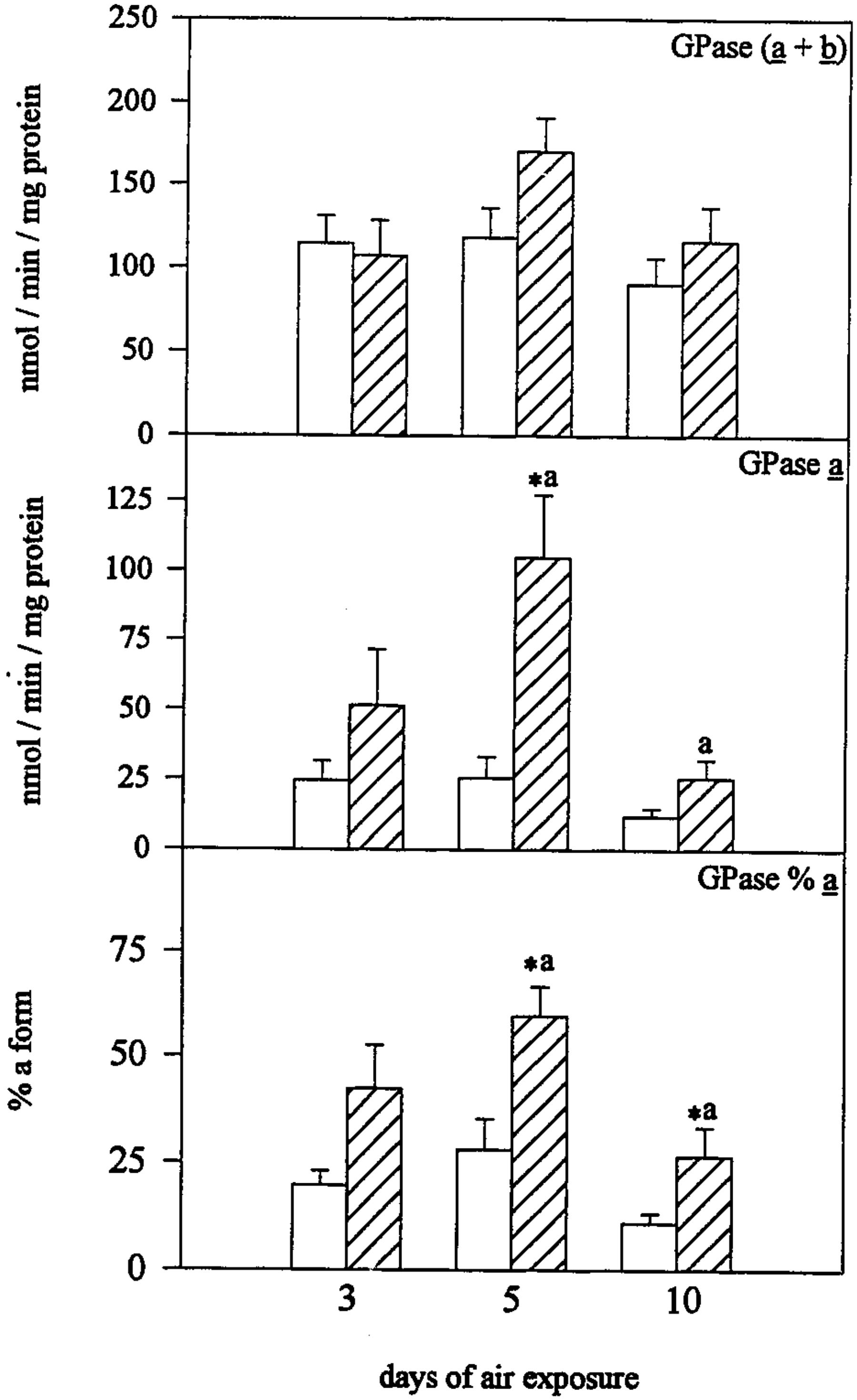

250
200
150
100
50
0
nmol / min / mg protein
GPase (a + b)
125
100
75
50
25
0
nmol / min / mg protein
GPase a
*a
a
75
50
25
0
% a form
GPase % a
*a
*a
3
5
10
days of air exposure

**Figure 5.** Activity of total (I + D) and active (I) form, and percent I of glycogen synthase in *A. rostrata* white muscle from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$. On graphs demonstrating significant change within a treatment, days sharing a common letter are significantly different by One Way ANOVA, $p < 0.05$.

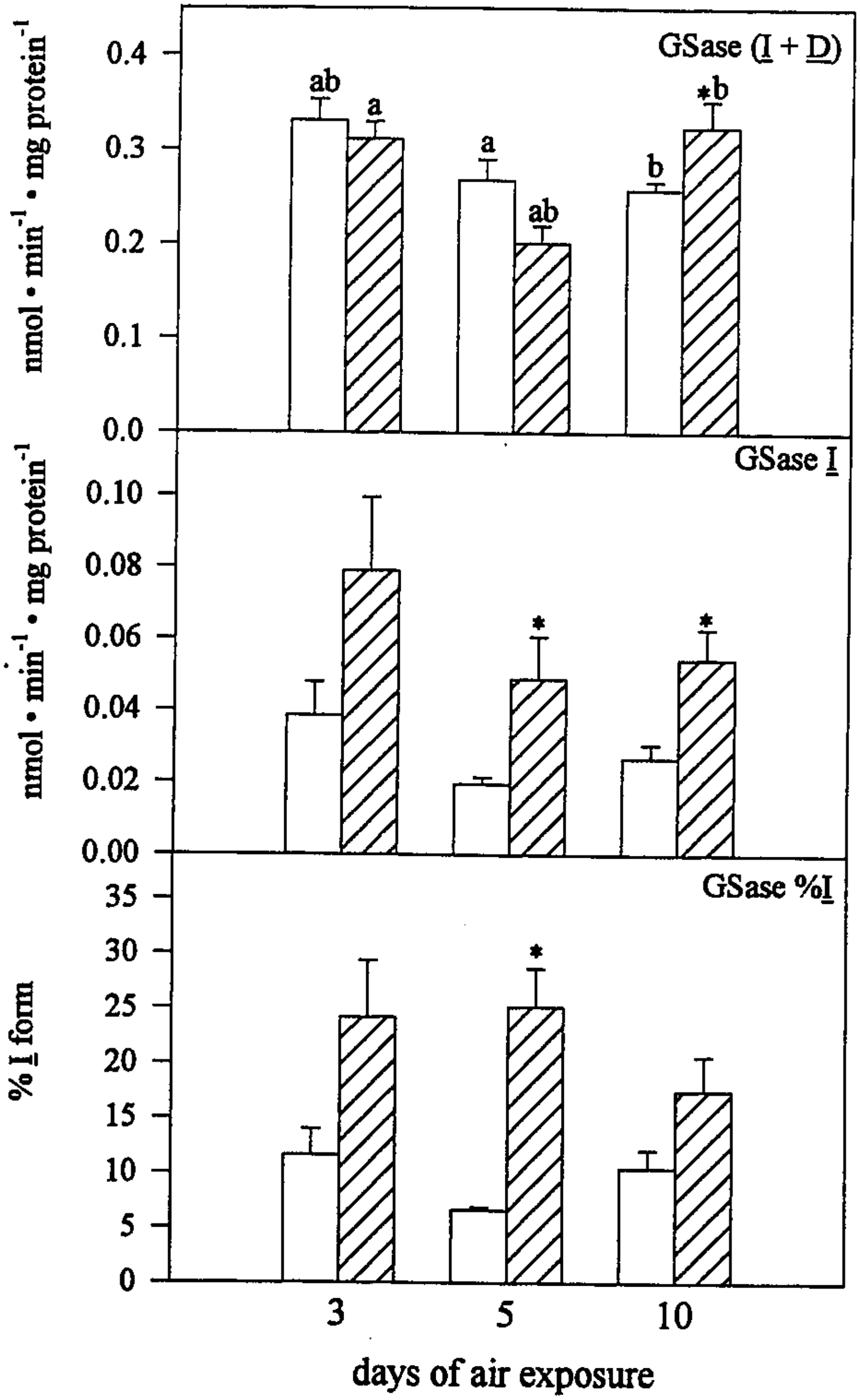

GSase (I + D)
nmol • min⁻¹ • mg protein⁻¹
0.4
0.3
0.2
0.1
0.0
ab
a
a
ab
b
*b
GSase I
nmol • min⁻¹ • mg protein⁻¹
0.10
0.08
0.06
0.04
0.02
0.00
*
*
GSase %I
% I form
35
30
25
20
15
10
5
0
*
3
5
10
days of air exposure

**Figure 6.** Maximal activities of enzymes associated with gluconeogenesis in *A. rostrata* white muscle from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$.

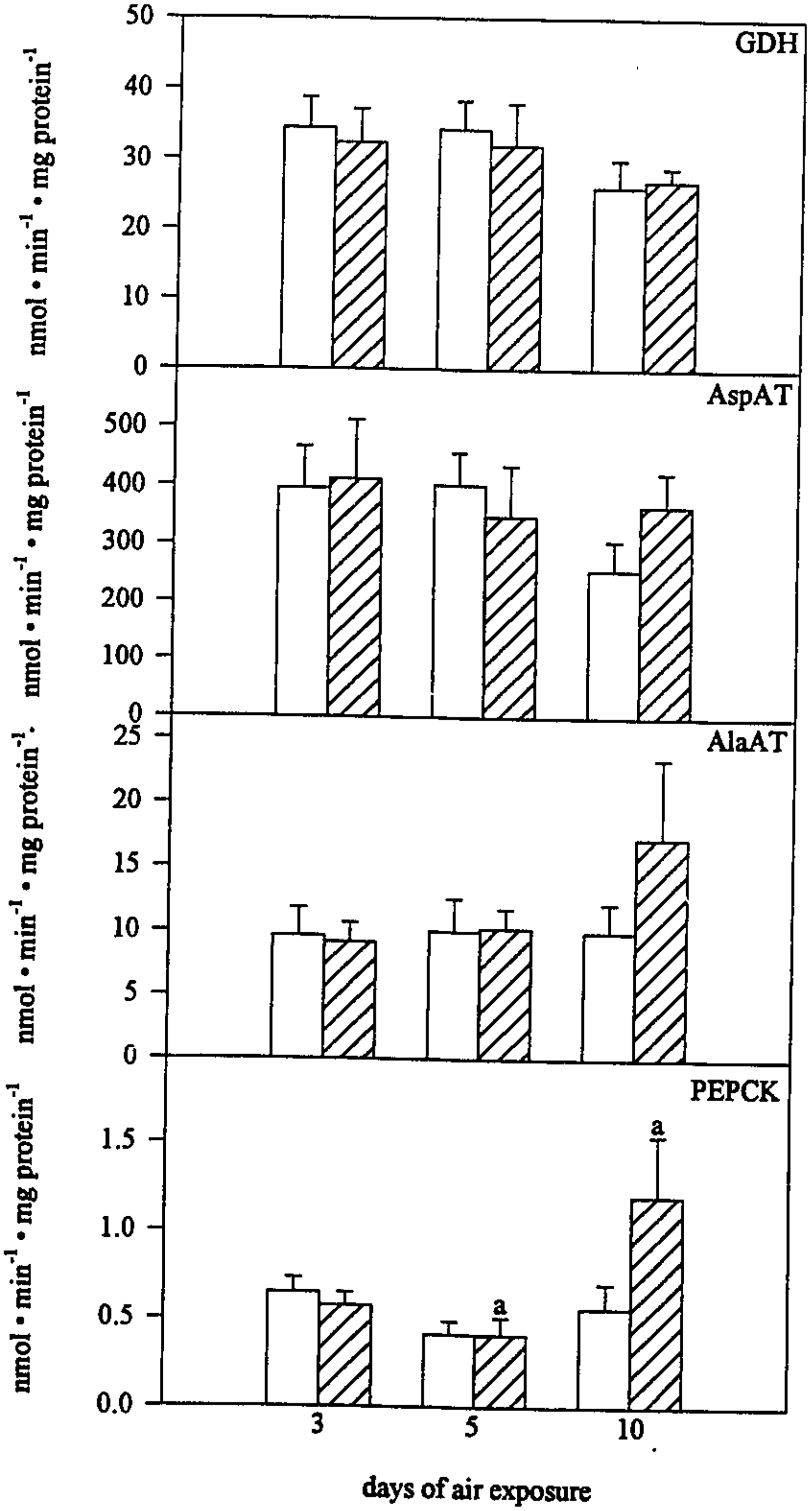

GDH
nmol • min⁻¹ • mg protein⁻¹
0
10
20
30
40
50
AspAT
nmol • min⁻¹ • mg protein⁻¹
0
100
200
300
400
500
AlaAT
nmol • min⁻¹ • mg protein⁻¹ •
0
5
10
15
20
25
PEPCK
nmol • min⁻¹ • mg protein⁻¹
0.0
0.5
1.0
1.5
a
a
3
5
10
days of air exposure

**Figure 7.** Maximal activities of enzymes associated with glycolysis in *A. rostrata* gill tissue from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$.

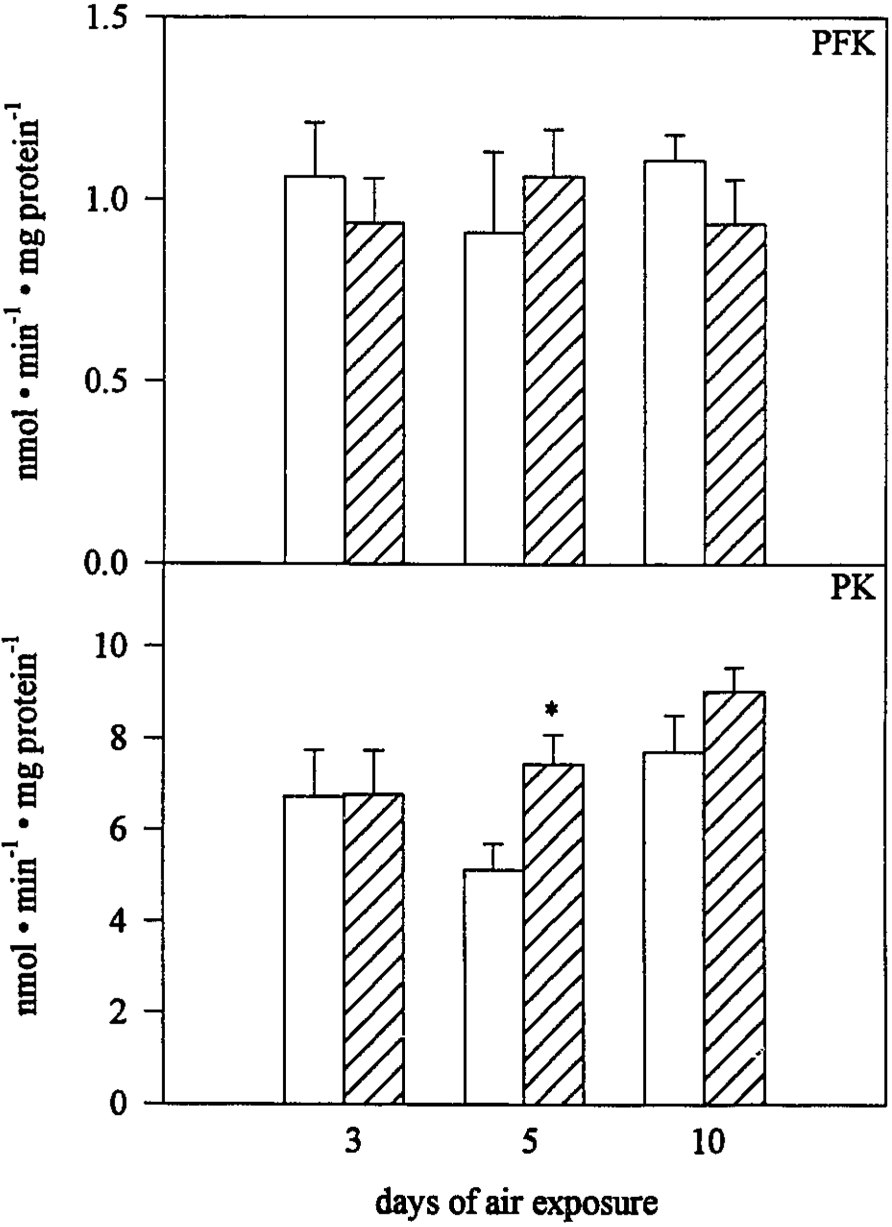

1.5
1.0
0.5
0.0
nmol • min$^{-1}$ • mg protein$^{-1}$
PFK
10
8
6
4
2
0
nmol • min$^{-1}$ • mg protein$^{-1}$
PK
*
3
5
10
days of air exposure

**Figure 8.** Activity of total (a + b) and active (a) form, and percent a of glycogen phosphorylase in *A. rostrata* gill tissue from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values.

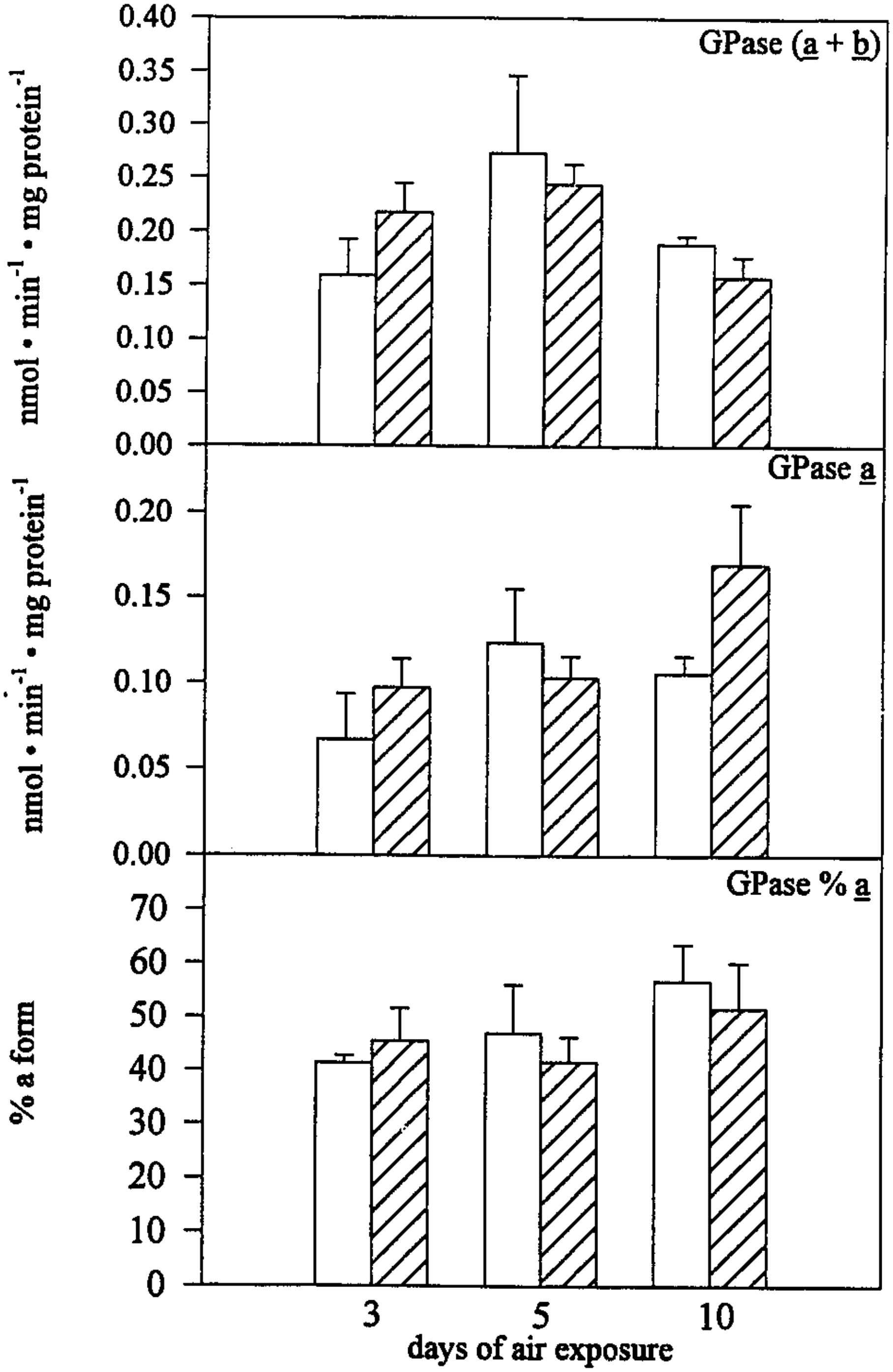

GPase (a + b)
nmol • min⁻¹ • mg protein⁻¹
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
GPase a
nmol • min⁻¹ • mg protein⁻¹
0.20
0.15
0.10
0.05
0.00
GPase % a
% a form
70
60
50
40
30
20
10
0
3
5
10
days of air exposure

**Figure 9.** Activity of total (I + D) of glycogen synthase in *A. rostrata* gill tissue from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values.

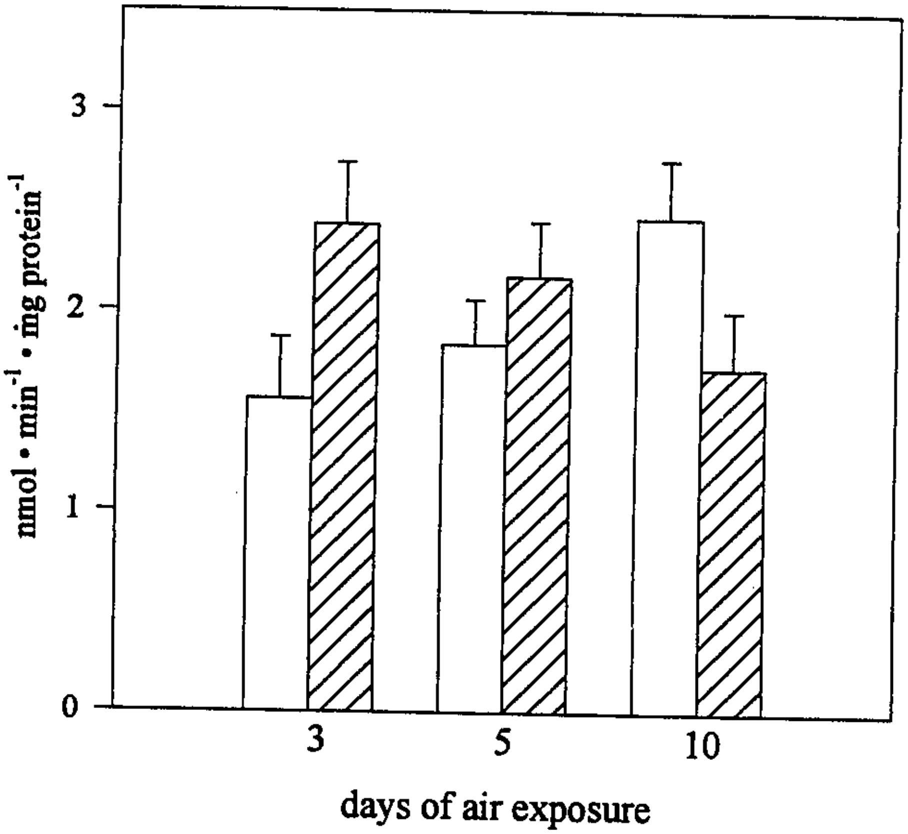

nmol • min$^{-1}$ • mg protein$^{-1}$
0
1
2
3
3
5
10
days of air exposure

**Figure 10.** Maximal activities of enzymes associated with glycolysis in *A. rostrata* posterior kidney from animals in control and air exposed conditions for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$. On graphs demonstrating significant change within a treatment, days sharing a common letter are significantly different by One Way ANOVA, $p < 0.05$.

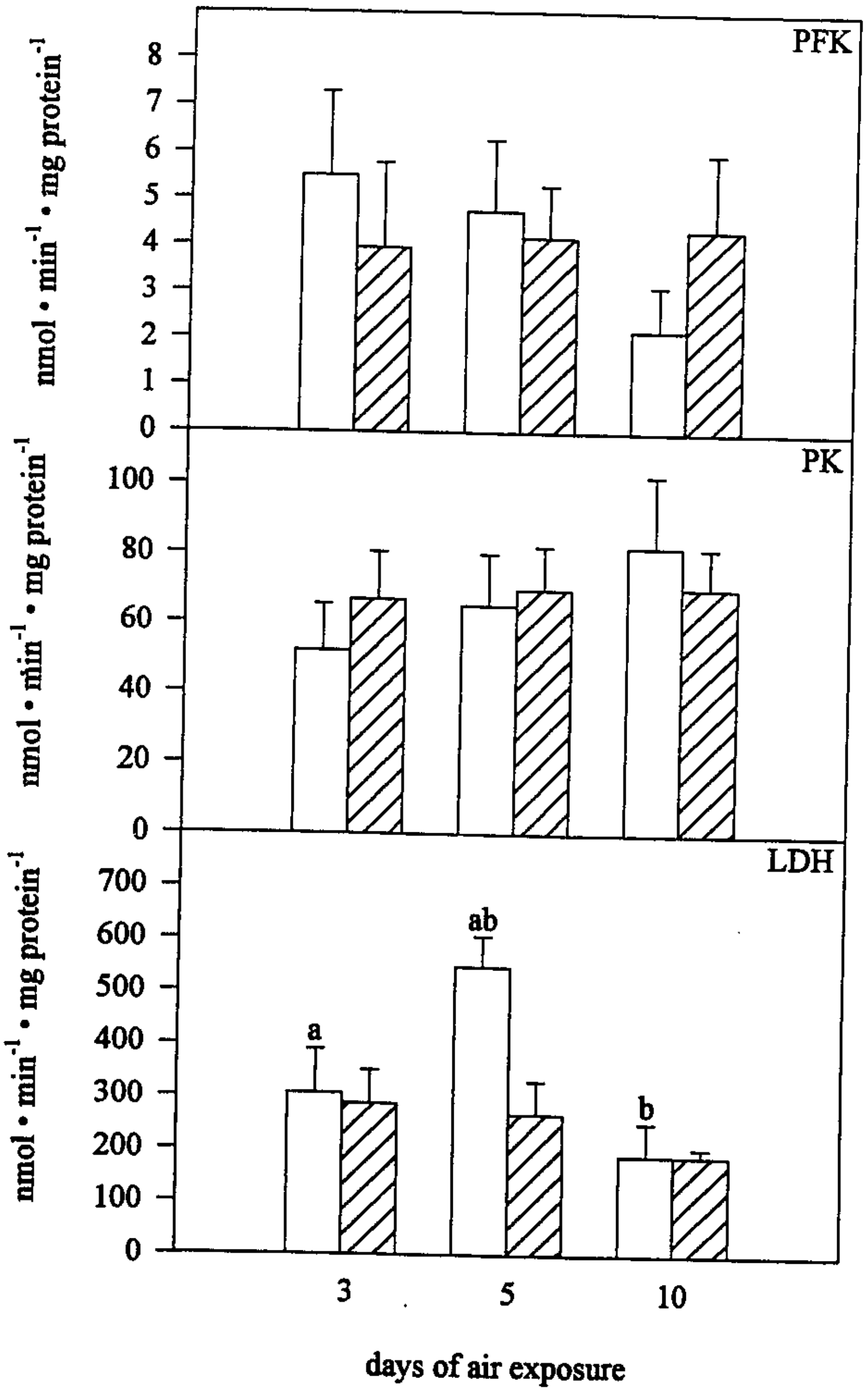

PFK
nmol • min$^{-1}$ • mg protein$^{-1}$
0
1
2
3
4
5
6
7
8
PK
nmol • min$^{-1}$ • mg protein$^{-1}$
0
20
40
60
80
100
LDH
nmol • min$^{-1}$ • mg protein$^{-1}$
0
100
200
300
400
500
600
700
a
ab
b
3
5
10
days of air exposure

**Figure 11.** Activity of total (a + b) and active (a) form, and percent a of glycogen phosphorylase in *A. rostrata* posterior kidney from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values.

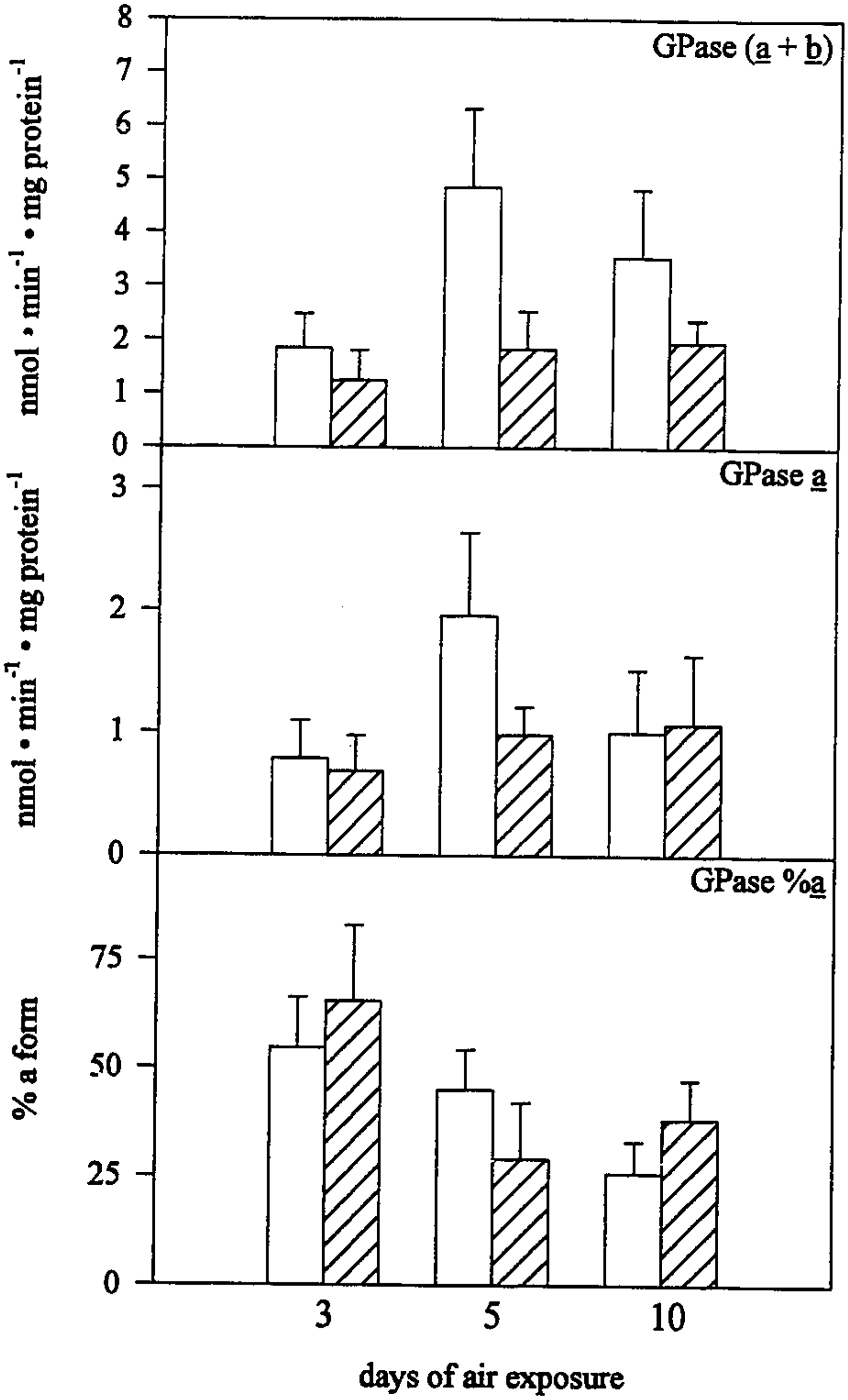

GPase (a + b)
nmol • min-1 • mg protein-1
0
1
2
3
4
5
6
7
8
GPase a
nmol • min-1 • mg protein-1
0
1
2
3
GPase %a
% a form
0
25
50
75
3
5
10
days of air exposure

**Figure 12.** Activity of total (I + D) and active (I) form, and percent I of glycogen synthase in *A. rostrata* posterior kidney from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$.

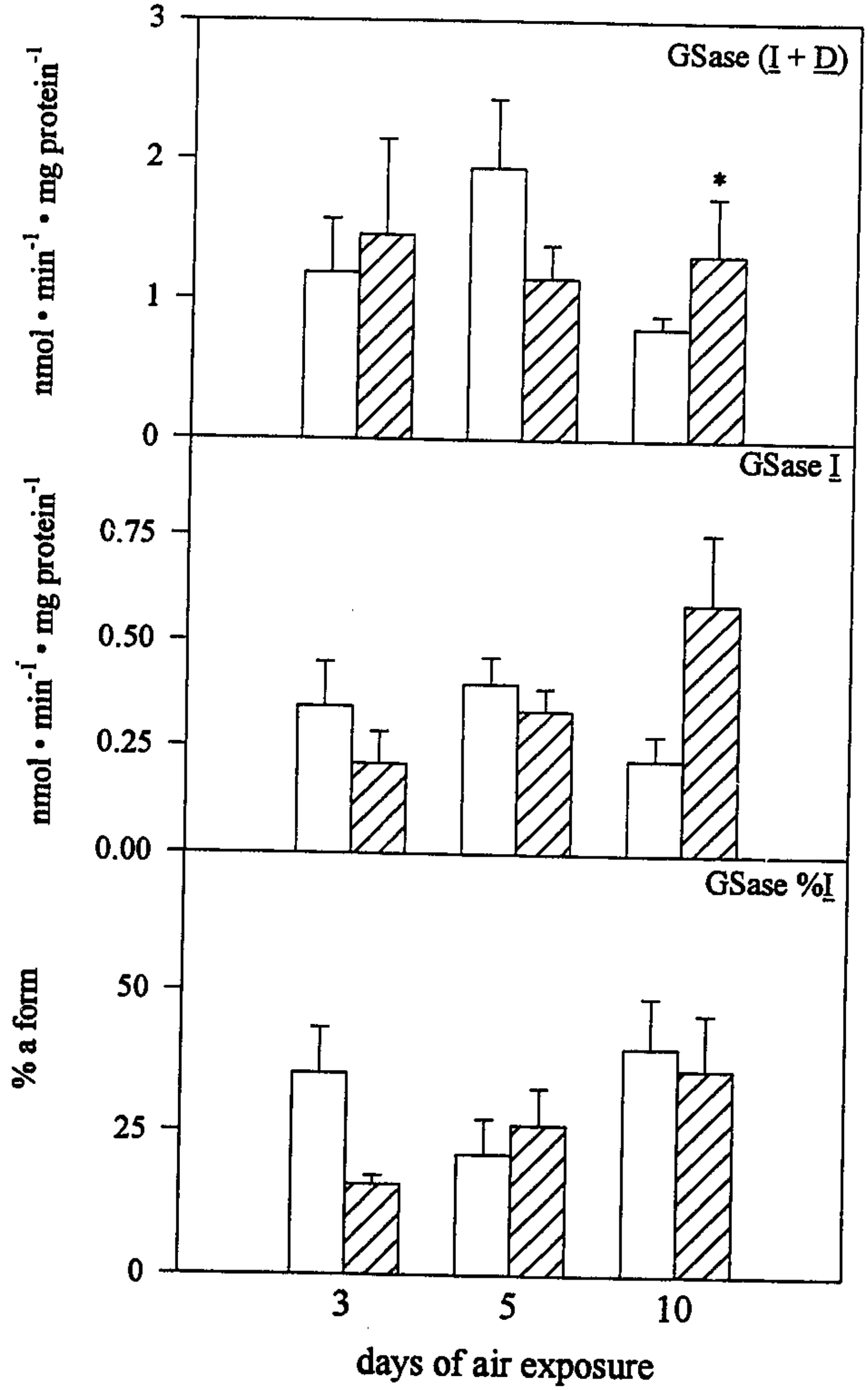

GSase (I + D)
nmol • min⁻¹ • mg protein⁻¹
3
2
1
0
*
GSase I
nmol • min⁻¹ • mg protein⁻¹
0.75
0.50
0.25
0.00
GSase %I
% a form
50
25
0
3
5
10
days of air exposure

**Figure 13.** Maximal activities of enzymes associated with gluconeogenesis in *A. rostrata* posterior kidney from animals in control and air exposed conditions, for 3, 5 and 10 days. Activities are nmol/min/mg protein, means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$. Significant changes within a treatment are demonstrated by days sharing a common letter. (One Way ANOVA, $p < 0.05$.)

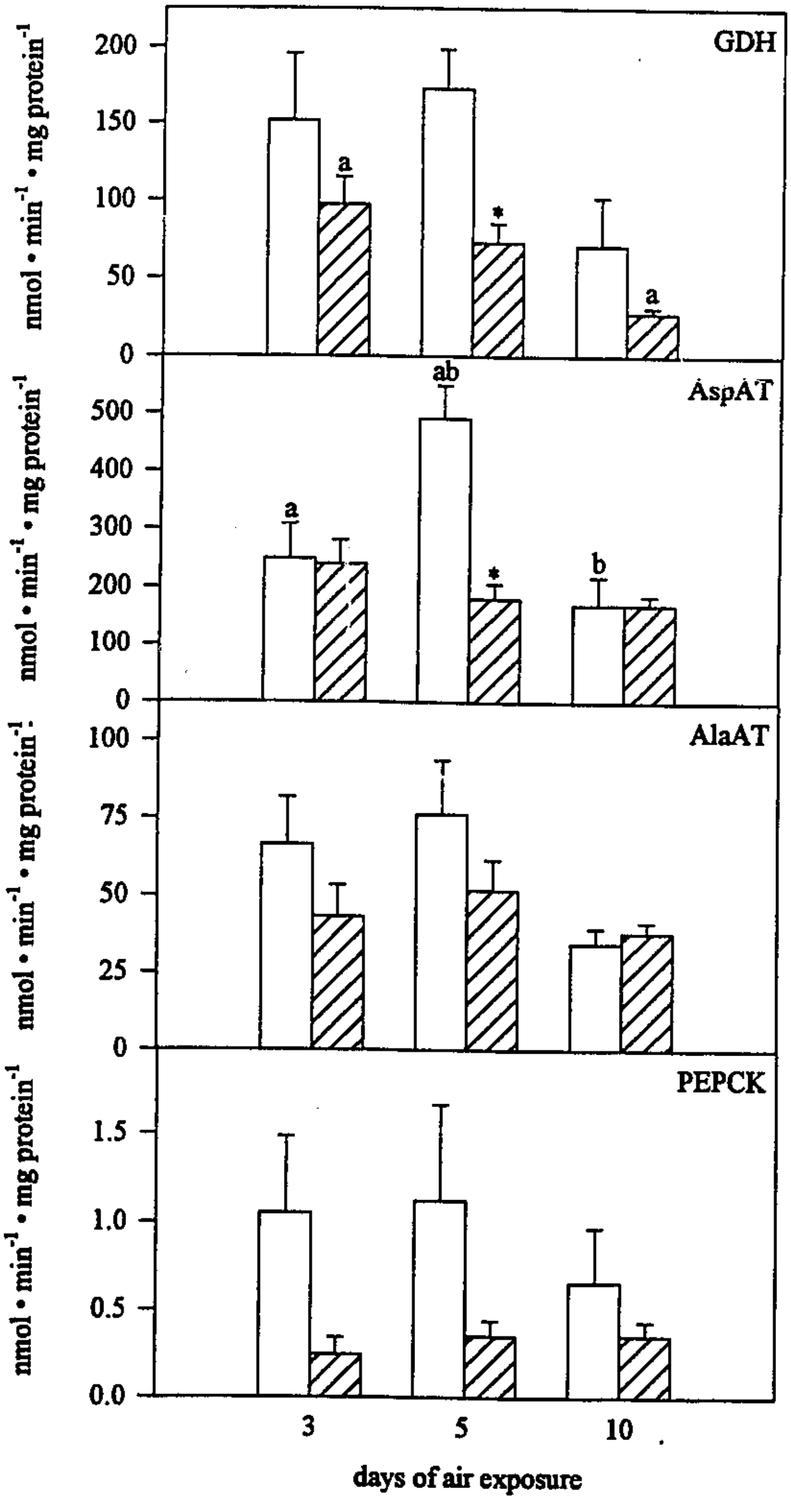

GDH
AspAT
AlaAT
PEPCK
nmol • min⁻¹ • mg protein⁻¹
200
150
100
50
0
500
400
300
200
100
0
100
75
50
25
0
1.5
1.0
0.5
0.0
a
*
a
ab
a
*
b
3
5
10
days of air exposure

**Figure 14.** Concentration of glucose (A) and lactate (B) measured in the plasma of *A. rostrata* from animals in control and air exposed conditions for 3, 5 and 10 days. Values are means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$.

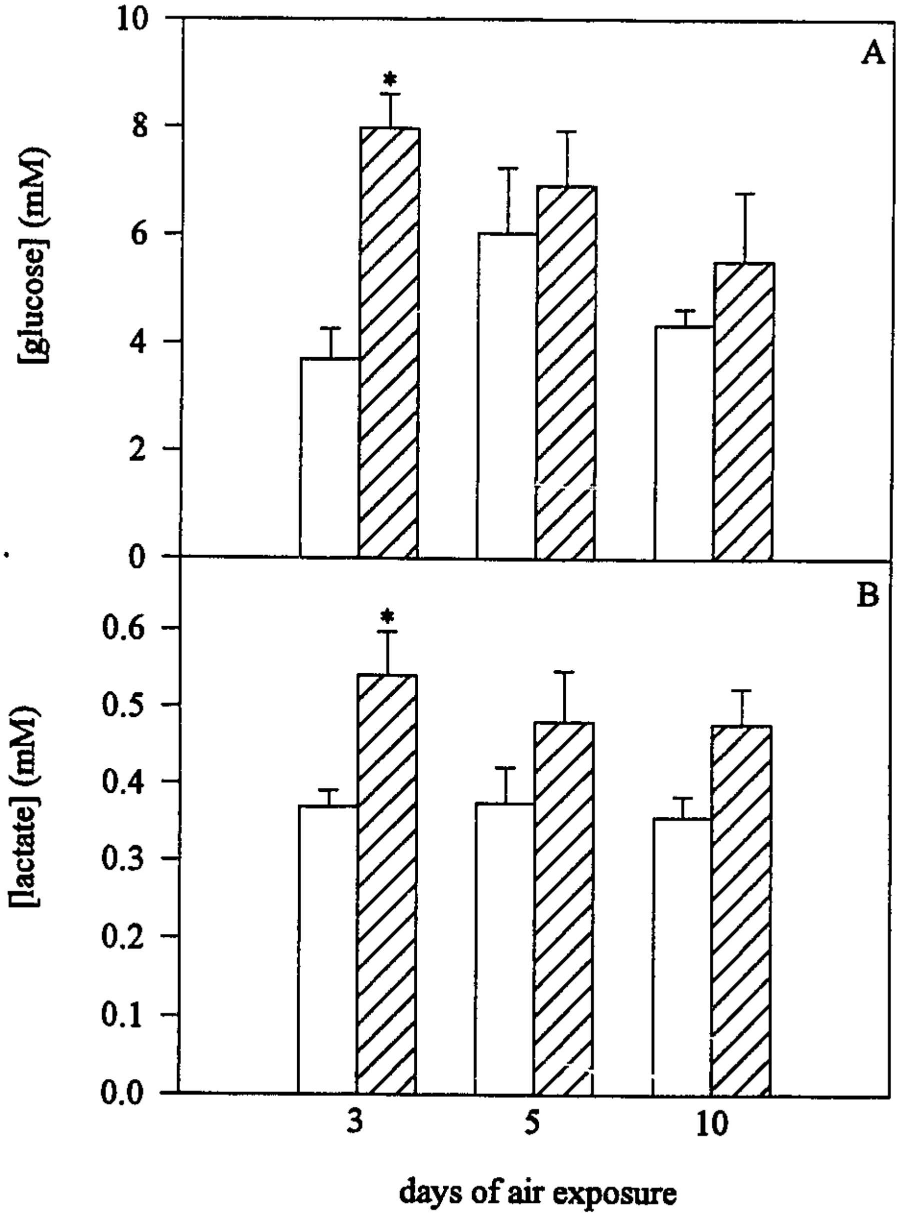

A
B
[glucose] (mM)
10
8
6
4
2
0
[lactate] (mM)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
3
5
10
days of air exposure

**Figure 15.** Concentration of cortisol measured in the plasma of *A. rostrata* from animals in control and air exposed conditions for 3, 5 and 10 days. Values are means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. On graphs demonstrating significant change within a treatment, days sharing a common letter are significantly different by One Way ANOVA, $p < 0.05$.

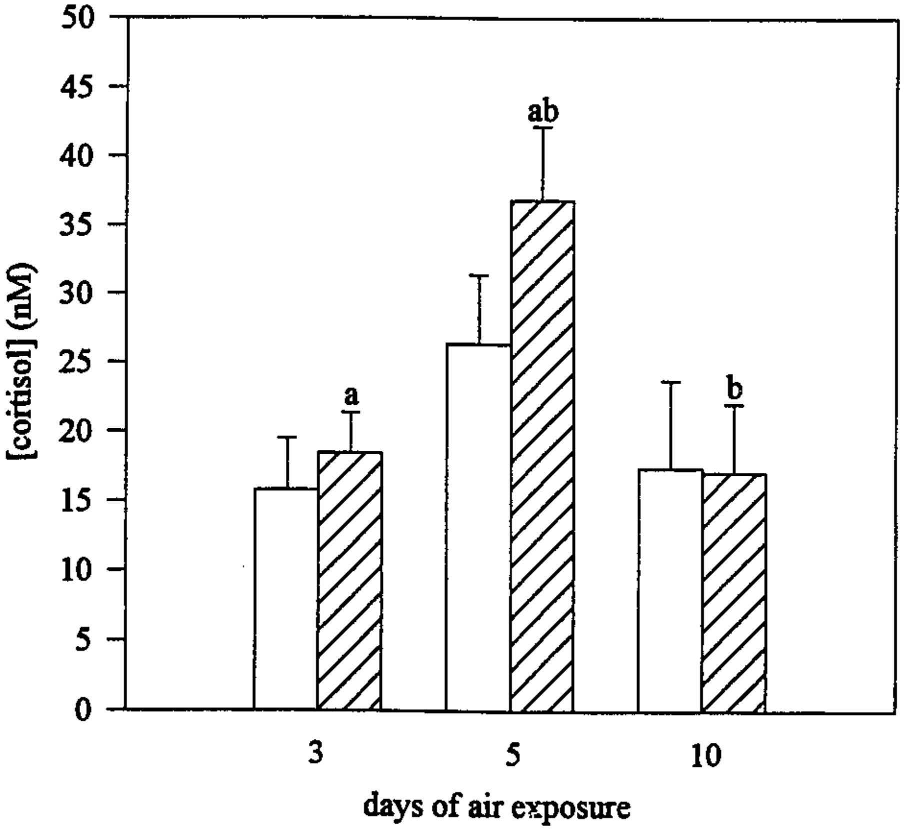

50
45
40
35
30
25
20
15
10
5
0
[cortisol] (nM)
ab
a
b
3
5
10
days of air exposure

**Figure 16.** Concentration of glycogen measured in the white muscle of *A. rostrata* from animals in control and air exposed conditions for 3, 5 and 10 days. Values are means ± SEM, n=5-6. Open bars indicate control values, hashed bars indicate air exposed values. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$. On graphs demonstrating significant change within a treatment, days sharing a common letter are significantly different by One Way ANOVA, $p < 0.05$.

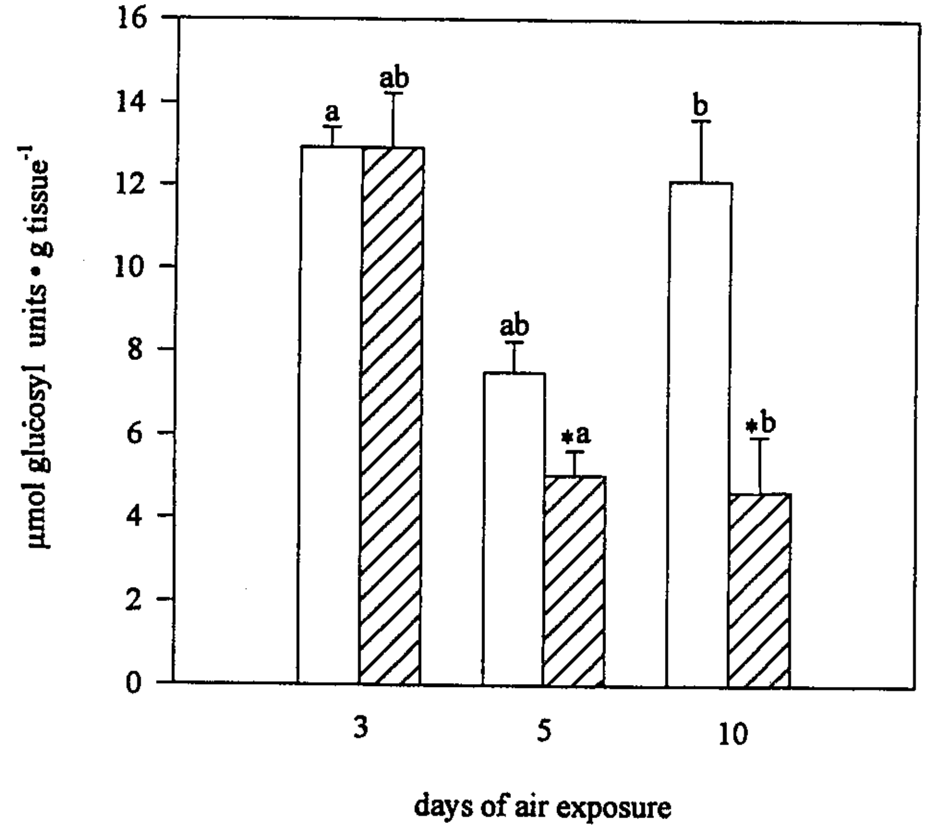

16
14
12
10
8
6
4
2
0
μmol glucosyl units • g tissue$^{-1}$
a
ab
ab
*a
b
*b
3
5
10
days of air exposure

**Table 2.** Kinetic properties of phosphofructokinase measured in *A. rostrata* posterior kidney from animals in control and air exposed conditions for 3, 5 and 10 days. Values represent means ± SEM, n=5-6. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$. On graphs demonstrating significant change within a treatment, days sharing a common letter are significantly different by One Way ANOVA, $p < 0.05$.

| | | time of air exposure | | |
|---|---|---|---|---|
| | | 3 days | 5 days | 10 days |
| $S_{0.5}$ F6P | control | 4.80 ± 0.70 | 12.50 ± 4.82 | 11.54 ± 4.70 |
| | air exposed | 7.42 ± 2.78 | 4.46 ± 0.98 | 3.84 ± 0.62 |
| nH F6P | control | 1.65 ± 0.59 | 1.08 ± 0.11 | 1.66 ± 0.47 |
| | air exposed | 1.27 ± 0.23 | 1.55 ± 0.49 | 3.25 ± 0.88 |
| $I_{50}$ Mg-ATP | control | 2.59 ± 0.71$^{a}$ | 1.48 ± 0.43 | 1.38 ± 0.45$^{a}$ |
| | air exposed | 0.64 ± 0.24 | 0.86 ± 0.24 | 0.60 ± 0.19 |
| Ka AMP | control | 2.04 ± 0.73 | 1.67 ± 0.15 | 2.60 ± 0.52 |
| | air exposed | 2.49 ± 0.43 | 2.07 ± 0.44 | 3.15 ± 0.28 |
| Ka F2,6P | control | 1.60 ± 0.46 | 0.73 ± 0.20 | 1.14 ± 0.63 |
| | air exposed | 0.88 ± 0.21* | 0.80 ± 0.15 | 0.95 ± 0.23 |

**Table 3.** Kinetic properties of pyruvate kinase measured in *A. rostrata* posterior kidney from animals in control and air exposed conditions for 3, 5 and 10 days. Values are means ± SEM, n=5-6. * Significantly different from the corresponding control value by Student's t-test (2-tailed), $p < 0.05$.

| | | time of air exposure | | |
|---|---|---|---|---|
| | | 3 days | 5 days | 10 days |
| $S_{0.5}$ PEP (mM) | control | 0.36 ± 0.02 | 0.34 ± 0.02 | 0.36 ± 0.01 |
| | air exposure | 0.38 ± 0.02* | 0.36 ± 0.02 | 0.38 ± 0.02 |
| nH PEP | control | 1.47 ± 0.10 | 1.40 ± 0.13 | 1.61 ± 0.07 |
| | air exposure | 1.49 ± 0.09 | 1.45 ± 0.08 | 1.41 ± 0.12 |
| $I_{50}$ Ala (mM) | control | 46.26 ± 10.40 | 48.30 ± 25.86 | 37.47 ± 5.61 |
| | air exposure | 61.22 ± 22.04 | 69.27 ± 22.99 | 34.50 ± 3.98 |

# DISCUSSION

## A. White Muscle

Skeletal muscle represents the largest glycolytic potential in tissues of the teleost (Knox *et al.* 1980). Small alterations in muscle metabolism may reflect a significant shift in the metabolism of the whole animal. Certain molluscs have demonstrated the same metabolic response in the glycolytic pathway to anoxia that we expected to see in eels. *Otala lactea* has been shown to exhibit a short term activation of glycolysis followed by a long term depression in glycolytic rate in foot muscle (Churchill and Storey, 1989). The marine whelk (*Busycotypsus canaliculatum*) responded similarly to anoxia (Storey *et al.* 1990). The glycolytic pathway is regulated at the enzymatic level by PFK and PK (Williamson, 1965). In the cockle (*Cardium tuberculatum*) foot muscle anoxia decreased the activity of both PFK and PK, depressing glycolytic flux (Meinardus-Hager *et al.* 1989). In goldfish (*Carassius auratus*) muscle, anoxia has been associated with a decrease in the maximal activity of PFK (Rahman and Storey, 1987) decreasing pathway flux. No significant changes, however, were observed in the glycolytic enzymes of eel white muscle (Figure 3). The ratio of PFK to PK was roughly 1:10, which appears to be correlated with maximal glycolytic flux in muscle tissue (Zammit *et al.* 1978), suggesting the maximal rate of glycolysis was not affected during air exposure. In studies involving closely related air breathing and water breathing species (*Hoplerythrimus unitaeniatus* and *Hoplias malabaricus, Arapaima gigas* and *Osteoglossum bicchosum*) from the Amazon, similarities in the kinetics of PK and LDH were thought to relate to phylogeny rather than similar respiratory habits (Fields *et al.* 1978; French and Hochachka, 1978). The air breathing eels in this study did not significantly alter their glycolytic pathway enzymatically. Changes in metabolic rate may have been due to changes in effector concentration, and may have occurred without changes in the enzymes themselves or their activities in the absence of these effectors. Anoxia caused an increase in inhibitors of PFK activities in flounder muscle (*Platichthys flesus* L., Jørgensen and Mustafa, 1980), effectively decreasing glycolytic flux. F1,6$P_2$, a potent activator of PK, was found to decrease in eel muscle following 24 h or air exposure (Moon *et al.* in prep.).

Glycogen phosphorylase activity provides a good indication of the relative activity of glycogenolysis as a contributor to anaerobic carbohydrate metabolism (Storey, 1987). Glycogen is a major source of endogenous substrate for energy producing pathways. The rate at which glycogen is degraded and/or synthesized is an important component to the metabolic changes an animal undergoes during stress. It was expected that the initial stress would increase glycogenolysis, with increased anaerobic metabolism. The ensuing metabolic depression would have be reflected in decreased glycogenolysis. Species of bivalve molluscs have demonstrated a variety of metabolic changes in muscle resulting from anoxic conditions. In *Littorina littorea* GPase a and total GPase activities decreased after 12 h of anoxia, but returned to normal levels after 24 h (Russell and Storey 1995). The same study showed a decrease in total GSase, and an increase of % GSase I. *Mytilus edulis* appeared to balance metabolic depression with anoxia induced fermentation in favour of depression maintaining a low percentage of GPase a (6%-8%) during anaerobiosis (Ebberink and Salimans 1982). Goldfish have been observed to maintain percentage GPase a and total GPase activity in white muscle, in which glycolysis was determined to be functioning at maximal capacity during anoxia (Storey 1987). Activity estimation in the muscle of eels indicated changes in GPase activities (increased GPase a activities and % GPase a at 5 and 10 days; decreased in GPase a activities and % GPase a from 5 to 10 days; refer to Figure 4) occurred concurrently with changes in GSase activities (increased total GSase from 5 to 10 days; higher total activities at 10 days; GSase I activities at 5 and 10 days and % GSase I at 5 days; refer to Figure 5) as a result of air exposure. The decreasing percentage GPase a and GPase a activity over the last 5 days of the experiment may reflect a trend towards glycogen conservation. The changes in GSase, however, may reflect changes in GPase. Shulman *et al.* (1995) suggested that GSase adapts to glycogenolytic flux, and is controlled by the activity of the hexokinase reactions and glucose transporters. The activity of GSase may be adapted towards the maintenance of G6P concentrations during changes in flux of the different pathways (including glycolysis) that G6P feeds into (Shulman and Rothman 1996). If this is true, the response observed in eel muscle would indicate increased glycogenolysis in muscle as a result of air exposure followed by a depression in activity (although still greater than control values). It should be noted that the changes observed in glycogenolysis occurred concurrently with changes in the glycogen content in the muscle (Figure 16).

The gluconeogenic potential of muscle is thought to be limited, the highest potential being found in the liver and gills (Knox *et al.* 1980). The presence, however, of gluconeogenic enzymes in the muscle does suggest some potential for *de novo* glucose production (Suarez and Mommsen 1987). The low rates observed in this experiment (Figure 6) suggest that the gluconeogenic potential is low. The increase of PEPCK activity from 5 to 10 days may suggest an increase in gluconeogenic potential despite the lack of change in the other gluconeogenic enzymes. These values from air exposed eels did not change from control values, and the lack of a decrease in the rate controlling enzymes of glycolysis (PFK and PK; Figure 6) suggest that any change is minimal at best. Despite this, the obligate air breather *Arapaima* was found to have higher levels of GDH and AspAT in muscle than in the closely related obligate water breather aruana (Hochachka *et al.* 1978a).

White muscle is, by its nature, anoxia tolerant attributed to the very low metabolic rate commonly associated with resting white muscle, in combination with ATP and creatine phosphate pools. The eel has a metabolic rate and $O_2$ consumption rate at the low end of teleosts, comparable to other anoxia tolerant fish such as carp and goldfish (Peyraud-Waitzenegger and Soulier 1989). It may be that the $O_2$ requirements of resting white muscle during air exposure in eels was met by the $O_2$ extracted from air, or that continued anaerobic metabolism met the energy requirements of the resting muscle. This may not mirror the muscle metabolism of an eel respiring aerially and actively moving through damp grass. The inactivity of the large muscle mass is apt to greatly reduce the animal's overall energy and oxygen demands relative to that of a moving animal. Edwards and Cech (1990) found that respiration of the intertidal fish, the monkeyface prickleback (*Cebidichthys violaceus*), is lower in air than water. They suggested that this may to some extent be due to the sluggish nature of the fish during air exposure, whereas intertidal fish which remain active during air exposure were able to maintain aquatic oxygen consumption. This may prove to be a factor in active air exposed eels.

## B. Gill Tissue

Glycolysis has been found to be an active pathway in gill tissue although not to the same extent as in the muscle, heart, or brain (Knox *et al.* 1980). In experiments involving goldfish, PFK activities did not change, but anoxia did decrease activation of PFK by its potent activator F2,6$P_2$ (Rahman and Storey 1988). The same experiments demonstrated anoxia induced decreases in $S_{0.5}$ (PEP), decreases in inhibition by alanine and decreases in maximal activities of PK. These modifications in the key enzymes of glycolysis result in less active forms and in a depression of flux through the pathway. In the air exposed American eel, however, no change in the maximal activities of the glycolytic enzymes examined were observed (Figure 7). It is possible that PK and PFK have been altered by phosphorylation and kinetics other than maximal velocity are affected, as mentioned in goldfish previously. Alterations can also be affected, as in muscle, by changes in small effector molecule concentrations. Decreases in F2,6$P_2$ were found to occur in goldfish gill tissue exposed to anoxic conditions (Storey 1987). The gills of the water breathing aruana were found to have higher activities of PFK and PK than the closely related water breather *Arapiama* (Hulbert *et al.* 1978*a*). Yet the water breather *Hoplias* and related facultative air breather *Hoplerythrimus* were found to have similar PFK and PK activities (Hulbert *et al.* 1978*b*).

Glycogenolysis in the gill tissue also was not affected by air exposure, as measured by changes in GPase and GSase. Figures 7 and 8 illustrate how the activities associated with GPase and total GSase remained constant from 3 to 10 days of air exposure. GSase I and % GSase I were not determined in gill tissue, as no method of blocking the conversion of GSase D to GSase I was found for the assay, and values of % GPase I were found to be essentially 100% of GSase activity. In the same way, gills from goldfish exposed to anoxic conditions did not differ from control values of % GPase a or GPase a activity, although there was a decrease in total GPase (a + b) (Storey 1987).

From these results, the enzymes measured involved with glycogen metabolism and glycolysis of the American eel were not affected by extended periods of air exposure. It is possible that the key enzymes of glycolysis and glycogenolysis in the gills of the American eels were either modified (via phosphorylation or dephosphorylation) without effecting maximal activity, or were not affected by air exposure. The latter may be the case as it is conceivable that metabolic depression would not have been observed in gill tissue of an air exposed eel. Gas transfer occurs across the gills during air exposure, and energy levels must be maintained to keep the cellular processes of the epithelial cells operating so as to maximize $O_2$ extraction in an attempt to maintain $O_2$ uptake. $O_2$ consumption at a cellular level may not be limited due to the proximity to the source.

## C. Posterior Kidney

The kidney of the teleost does not demonstrate a large glycolytic potential relative to the muscle, heart or brain (Knox *et al.* 1980). The only changes observed in the glycolytic enzymes of the eel are based on a large increase in the activity in LDH of control animals at 5 days (Figure 10), which is difficult to explain. Lactate to pyruvate flux may have been increased to utilize blood lactate, but it is not clear why this would be occurring control animals. The water breather aruana was found to have much higher activities of both PFK and PK than the related air breather *Arapaima* (Hochachka *et al.* 1978*b*). LDH levels were considerably higher in *Arapaima,* but this was found to be related to increased lactate metabolism. Although in the case of the Amazonian fish, air breathers had a higher anaerobic potential, air exposure of American eels was not found to increase pathway potential.

The results from kinetic analysis of PFK (Table 2) and PK (Table 3) illustrate the consistency of the pathway during air exposure. Anoxia has been shown to induce covalent modification of PFK to produce a less active enzyme characterized by decreased $K_m$, increased inhibition by ATP, and decreased activation by AMP and F2,6P (Storey 1984). The activation of PFK by F2,6P did decrease with air exposure at 3 days, although this did not remain significantly lower for the duration of the air exposure. Because the altered Ka by F2,6P appears transient, and is the only indication of phosphorylation, the data suggests PFK of the posterior kidney of eels was not modified during air exposure. PK phosphorylated under anoxic conditions is characterized by decreased $K_m$ and increased inhibition by alanine (Plaxton and Storey 1984). As with PFK, the no alteration of PK activity was determined kinetically. Following 3 days of air exposure PK had increased $S_{0.5}$ values, indicating reduced phosphorylation and increased activity, but was not maintained throughout the air exposure. The kinetic analysis of kidney PFK and PK failed to demonstrate any changes to the key enzymes of glycolysis, indicating that no change in pathway potential occurred as a result of air exposure.

The kidney does contain glycogen, but much less then that of the liver (Moon and Foster 1995). Glycogenolytic flux does not appear to be altered in the American eel (refer to Figures 11 and 12). Although total GSase (I + D) was higher than control at 10 days, there was no change in the active form of the enzyme, and there were no changes in GPase activities. This may reflect the relative insignificance of the kidney's contribution to whole animal glycolytic metabolism.

Although gluconeogenic enzymes are present in the kidney, the contribution to whole animal metabolism is questionable (Moon and Foster 1995). This is associated with the low potential found in the tissue due to the greater glycolytic enzyme activity and low gluconeogenic activity relative to other tissues (Moon and Foster 1995). During extended periods of hypoxia, however, Jørgensen and Mustafa (1980) reported that flounder kidney contained high activities of gluconeogenic enzymes. In addition, a stable energy charge (ATP/ADP/AMP ratio) and stable total adenylates in kidney tissue during extended periods of hypoxia suggested that kidney may replace the liver as the principle gluconeogenic organ. The changes observed (Figure 13) do not suggest a high or increased gluconeogenic capacity with air exposure. The decrease in GDH activities from 3 to 10 days in air exposed eels, in addition to the lower levels at 5 days compared to control values, contrast with findings from studies conducted on Amazonian fish. The obligate air breather *Arapaima* had GDH levels sevenfold that of the related water breather aruana (Hochachka *et al.* 1978*b*). The changes in activities of AspAT are based on a large increase in the activity of control eels at 5 days of air exposure, similar to that of LDH in this same tissue. The increase in AspAT activities may have provided substrate for gluconeogenesis, but as with LDH, why this would have occurred in control animals cannot explained at this time.

It has been suggested that the kidney may contribute significantly to the gluconeogenesis of the whole animal in the case of some species of teleost (Jørgensen and Mustafa 1980). Although the kidney has not been shown to operate as a gluconeogenic tissue, this study has not shown gluconeogenesis to be activated elsewhere in the animal, and therefore the gluconeogenic capabilities of the posterior kidney of an air exposed eel can be neither refuted nor concluded. The kidney is not functional as an excretory organ as the animal prevents water loss during air exposure (Hyde *et al.* 1987). The elimination of its kidney function may also result in a loss in metabolic contribution, and be reflected by the absence of alteration in enzyme activities.

### D. Metabolites

Reduced environmental oxygen elicits variable responses in terms of circulating glucose, but more constant responses in lactate levels among species of teleosts studied. Increases (*C. maculata*, Yu and Woo 1987), decreases (*Pleuronectes platessa*, White and Fletcher 1989; *O. mykiss*, Wright *et al.* 1989) and maintenance (*A. anguilla*, van Waarde *et al.* 1983) of glucose concentrations have been reported. Even among species, variation has been observed. Dalla Via *et al.* (1994) reported that glucose concentrations in 50% of anoxic animals (*Solea solea*) did not change, while 50% had values 10 fold that of normoxic animals. Lactate, however, consistently increased with hypoxia and/or anoxia (Berg and Steen 1965, van Waarde *et al.* 1983, Hyde *et al.* 1987, Yu and Woo 1987, White and Fletcher 1989, Dalla Via *et al.* 1994, Moon *et al.* in prep.).

Glucose and lactate concentrations (Figure 14) in eel plasma did not change as expected with air exposure. Other researchers have reported that plasma glucose concentrations do not change, while plasma lactate increases in eels exposed to air or hypoxic/anoxic water, although the time course of these experiments was much shorter (4-10 h, van Waarde *et al.* 1983; 36 h, Hyde *et al.* 1987; 72 h, Moon *et al.* in prep.). The stability of glucose levels was likely due to glycogenolysis from hepatic (van Waarde *et al.* 1983) or muscle stores (Moon *et al.* in prep.).

Increases in blood lactate resulting from air exposure of eels was first noted by Berg and Steen (1965). Plasma lactate tends to increase with the duration of the oxygen reduction until either the animal is returned to normoxia, at which time the lactate levels rapidly return to normal, or the animal expires (Hyde *et al.* 1987, Yu and Woo 1987, Moon *et al.* in prep.). The lack of lactate accumulation in my experiment is a curious observation. An increase in anaerobic metabolism consumes the available glucose producing primarily lactate (Hochachka and Somero 1984, Dalla Via *et al.* 1994). Lactate flux increases in the air exposed eel, although this is not likely to account for the near-normal levels observed, as the eels determined to have increased lactate flux also had increased blood lactate concentrations (Moon *et al.* in prep.). Changes in tissue enzyme activities do not suggest an increased potential in gluconeogenesis from lactate. Liver enzyme data is not available for this experiment, and therefore an assumption of rapid lactate clearance by hepatic gluconeogenesis and/or glyconeogenesis can not be supported. Yet, if this was the case, it is not likely that lactate levels would have remained at concentrations similar to control values of this and other experiments (Hyde *et al.* 1987, Moon *et al.* in prep.). Anoxic goldfish have the ability to convert lactate to ethanol and

$CO_2$, both of which are easily lost to the water (Shoubridge and Hochachka 1981), but no other teleost has conclusively been shown to produce ethanol. Release of other endproducts into the circulation has been reported in other teleosts during hypoxia (Shoubridge and Hochachka 1981, van Waarde *et al.* 1982) as well as in eels where blood alanine was found to rise 2-3 fold, but occurred together with a 5 fold increase in lactate (van Waarde *et al.* 1983). Although other endproducts may have been produced and released into the circulation, it is odd that this would have occurred to the exclusion of increased lactate production. Yu and Woo (1987) reported that after prolonged hypoxia, liver lactate of *C. maculata* returned to normoxic values in the absence of significant lactate excretion, suggesting *in situ* gluconeogenesis or oxidation, and was reflected in a slight decrease in plasma lactate. The fate of lactate in the air exposed eel would be more clearly elucidated with data from hepatic enzyme activities, and data from amino acid concentrations in the blood and tissues.

Effects of cortisol administration include increasing metabolic rates to elevate serum glucose (Leach and Taylor 1980) and in *A. japonica* acted to increase oxygen consumption and depress the respiratory quotient (Chan and Woo 1978). It was expected that cortisol levels would remain elevated relative to that of control animals throughout the duration of the air exposure stress. Cortisol levels, however, did not respond to air exposure (Figure 15). Levels did increase at 5 days, then decrease again at 10 days, but as there were no differences measured between control and air exposure at any time during the experiment, these changes could not be attributed to air exposure. Archaic fish such as Siberian sturgeon (*Acipenser baeri*) have been reported to respond to hypoxic stress as other fish, increasing both catecholamines and cortisol levels (Maxime *et al.* 1995). In rainbow trout, catecholamines increased in response to environmental hypoxia, and acted to counteract the effects of hypoxia to maintain blood glucose (Wright *et al.* 1989). Teleosts more tolerant to stress respond similarly, but to a reduced degree. During severe acidosis from hypercapnia in eels, catecholamines were observed to increase, yet remain at low levels (< 1.0 nM, Hyde and Perry 1990). If data concerning catecholamines can be generally extrapolated to changes in cortisol levels, the maintenance of cortisol levels may be explained. It does not appear that the animal was stressed to a degree, either by $O_2$ deficiency or metabolic acidosis associated with air exposure (Hyde *et al.* 1987), to significantly increase cortisol levels. The levels determined in this experiment are very high relative to other studies (Hyde *et al.* 1990). Levels may be falsely high due to blood sampling following removal from the eel box, a procedure which may cause the animal great stress. Handling stress causes increases in cortisol levels in rainbow trout (Vijayan and Moon 1992) and may have masked actual cortisol concentrations and any differences due to air exposure.

The site from which glycogen is mobilized during hypoxia varies between species. Anaerobic metabolism in the flounder (Jørgensen and Mustafa 1980), and eel (van Waarde *et al.* 1983, Moon *et al.* in prep.) occurs with glycogenolysis of both muscle and liver glycogen stores. Hagfish appear to use liver glycogen primarily (Foster and Moon 1986*b*), whereas carp have been reported to initially rely on muscle glycogen stores (Johnston 1975). In rainbow trout, muscle glycogen decreases by as much as 50 % during severe hypoxia (Boutilier *et al.* 1988). Muscle glycogen was observed to decrease significantly after 72 h of air exposure in eels (Moon *et al.* in prep.), and decreased by as much as 70 % in eels exposed to hypoxic/anoxic water (van Waarde *et al.* 1983). Eels appear to protect hepatic glycogen stores, by either mobilizing muscle stores first (Moon 1983) or by reestablishing glycogen stores once the animal is returned to normal environmental conditions (Moon *et al.* in prep.). In this experiment, the glycogen content of eels air exposed for 5 or 10 days was less than that of controls and that of air exposed eels at 3 days, which were identical to control animals. Immature eels have a low turnover of glucose suggesting that carbohydrates play a minor role in the fasting of the immature eel (Cornish and Moon 1980). The initial condition of the glycogen stores is known to play a role in the survivability of the animal during periods of stress (Moon and Foster 1995). Differences in glycogen store condition is likely in part responsible for variation between reports of anoxia endurance as well as variation between animals within an experiment.

Liver metabolism and metabolite status are vital components of metabolism studies. Because of the small size of the livers, and problems not anticipated with enzyme assay development, analysis of the liver was not reported in this thesis. The magnitude of this problem was realized and a repetition of the study was attempted, but not completed. The animals were surviving a maximum of 5 days of air exposure. The eels used in the second experiment were purchased from a fisherman on the St. Lawrence River in September, and were much larger in size (≈ 500 g) and believed to be resident eels, not migrating eels captured on the eel ladder of a hydro dam as used in the first experiment. The health of the eels was also questionable. The experiments performed by Hyde *et al.* (1987) were repeated in attempt to determine the cause of the eel death during air exposure. Eels were cannulated via the pneumogastric artery under anesthetic and following a minimum of 24 h recovery, eels were air exposed. Blood samples were collected prior to air exposure, and at 12, 24, 48, 36, 72 h (or as long as animals survived). Blood parameters (pH, $pO_2$, total $O_2$, $pCO_2$, total $CO_2$, $HCO_3$, hematocrit) changed as expected. Blood lactate increased dramatically, moreso than other reports (Hyde *et al.* 1987, Moon *et al.* in prep.), and blood glucose dropped sharply. Literature does not report any other findings were blood glucose drops as rapidly, and indicated a serious problem with the physiology of the eel. This may be due to depleted glycogen stores before the application of the stress due to poor health. Berg and Steen (1965) reported that reactions causing accumulation of $H^+$ and lactic acid at 15 °C were almost absent at 7 °C. The temperature of the water used in the experiment increased from near 10 °C to near 15 °C due to changes at the facilities, and may in part be responsible for the reduced air exposure tolerance. It became apparent that data collected from an experiment employing these eels would not be representative of the physiology of a healthy eel. Because of the timing, no more eels could be collected until the summer of the following year, and analysis of the tissues harvested in the original experiment continued and are presented in this thesis.

## E. CONCLUSIONS

The American eel has been reported to possess a lower $O_2$ consumption and metabolism than that of most teleosts, comparable to that of other hypoxia/anoxia tolerant fish such as goldfish and carp (Peyraud-Waitzenegger and Soulier 1989). The white muscle of the eel represents a large portion of the body mass (Hulbert and Moon 1978). Small changes in glycogen content and enzyme activities may reflect a large impact upon the rest of the animal with regard to energy status. The utilization of endogenous energy stores is indicated by decreased glycogen (Figure 16) and increased GPase a activities (Figure 4) following 5 days of air exposure. After 10 days, GPase activities returned to control values, and glycogen levels stabilized at a lower level. At these lower levels, % GSase I and activity were significantly higher than control, possibly indicating an attempt to maintain minimum glycogen levels. This may indicate an activation of other processes for incorporation as energy sources. At this point, liver glycogen may have been utilized, or hepatic gluconeogenesis may have increased. These actions would be indicated by analysis of hepatic metabolism and glycogen stores, muscle protein and circulating amino acid determination. The increase in muscle PEPCK activity (Figure 6) may indicate increasing gluconeogenic potential. Although white muscle is not thought to contribute significantly to gluconeogenesis, this observation should not be ignored.

There is little evidence of pathway reorganization in the air exposed eel. Although there is evidence of increased glycogenolysis in white muscle, this was likely the provision of energy for the tissue and/or whole animal. PEPCK activity does increase, but as glycolysis was not depressed, gluconeogenic activation can not be concluded. Results from gill tissue and posterior kidney provided no evidence of pathway reorganization. The absence of lactate accumulation in the plasma suggests that a switch to anaerobic metabolism did not take place. Analysis of liver metabolism may find an active gluconeogenic pathway utilizing the lactate generated by anaerobic metabolism, although it is unlikely that gluconeogenesis would account for the total absence of lactate accumulation.

The changes in enzyme activities of the white muscle, gill tissue, and posterior kidney do not indicate a Pasteur effect in the air exposed eel. This may have be initiated with the initial air exposure of the eel or be evident from liver analysis, but was not evident after 24 h of air exposure in my experiment. Moon *et al.* (in prep.) did not find metabolite indications of Pasteur effect following 12 h of air exposure. Decreases in liver and muscle glycogen stores could not be positively attributed to a significant Pasteur effect in their experiment, as in my experiment.

The changes in enzyme activities of the white muscle, gill tissue, and posterior kidney do not indicate a metabolic depression in the air exposed eel. The lack of change in enzyme kinetics of PFK and PK support this conclusion. Although the enzymes may be depressed by increased pH, or by effector molecules, this was not determined in this experiment. The metabolic requirements were apparently being met by either the $O_2$ extracted from the air, or by anaerobic metabolism acting at a rate accomplished by enzyme activities prior to air exposure. However, indications of metabolic depression may be found with the analysis of liver metabolism. As a large glycogen store and primary site for gluconeogenesis, the liver is a key to carbohydrate metabolism. Tissue differences resulting from air exposure would become more evident with liver metabolism analysis.

Changes in enzyme rates, enzyme kinetics and metabolites were observed to result from the air exposure, yet it could not be concluded that the changes were part of a concerted metabolic depression. The eel did utilize muscle glycogen stores as an energy source but the lack of lactate accumulation in the plasma suggests that there is no switch to anaerobic metabolism. Future research including liver metabolism would likely fully describe the metabolism of the air exposed eel.

# REFERENCES

Andersen, D. E., Reid, S. D., Moon, T. W. & Perry S. F. (1991). Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Orcorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **48**, 1811-1817.

Bannister, J. V. & Anastasi, A. (1976) The effect of fructose 1,6-diphosphate on pyruvate kinase from white muscle of three species of Mediterranean fish. *Comp. Biochem. Physiol.* **55B**, 449-451.

Berg. T. & Steen, J. B. (1965). Physiological mechanisms for aerial respiration in the eel. *Comp. Biochem. Physiol.* **15**, 469-484.

Berg. T. & Steen, J. B. (1966). Regulation of ventilation in eels exposed to air. *Comp. Biochem. Physiol.* **18**, 511-516.

Boutilier, R. G., Dobson, G., Hoeger, U. & Randall, D. J. (1988). Acute exposure to graded levels of hypoxia in rainbow trout *Salmo gairdneri*: metabolic and respiratory adaptations. *Respir. Physiol.* **71**, 69-82.

Bridges, C. R. (1988) Respiratory adaptations in intertidal fish. *Amer. Zool.* **28**,79-96.

Brighenti, L., Puviani, A. C., Gavioli, M. E. & Ottolenghi, C. (1987). Mechanisms involved in catecholamine effect on glycogenolysis in catfish isolated hepatocytes. *Gen. Comp. Endocrinol.* **66**,306-313.

Brooks, S. P. J. (1992). A simple computer program with statistical tests for the analysis of enzyme kinetics. *Biotechniques.* **13(6)**, 906-911.

Brooks, S. P. J. (1994). A simple computer program for the analysis of enzyme kinetics obtained from a microplate reader. *Biotechniques.* **17(6)**, 1154-1151.

Burggren, W.W. (1982). "Air gulping" improves blood oxygen transport during aquatic hypoxia in the goldfish *Carassius auratus*. *Physiol. Zool.* **55(4)**, 327-334.

Butler, P. J., Taylor, E. W. & Davidson, W. (1979) The effect of long term, moderate hypoxia on acid-base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula*. *J. Comp. Physiol.* **132**, 297-303.

Carter, G. S. (1935). Reports of the Cambridge expedition to British Guiana 1933. Respiratory adaptations of the fishes of the forest waters, with descriptions of the accessory respiratory organs of *Electrophorus electricus* L. and *Plecostomus plecostomus* L. *J. Linnean Soc. London.* **39**, 219-233.

Carter, G. S. & Beadle, L. C. (1931). The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in the fishes. *J. Linnean Soc. London.* **37**, 327-366.

Chan, D. K. O. & Woo, N. Y. S. (1979). Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. *Gen. and Comp. Endocrin.* **35**, 205-215.

Churchill, T. A. & Storey, K. B. (1989). Intermediary energy metabolism during dormancy and anoxia in the land snail *Otala lactea*. *Physiol. Zool.* **62(5)**, 1015-1030.

Claireaux, G. & Dutil, J. D. (1992). Physiological response of the Atlantic cod (*Gadus morhua*) to hypoxia at various environmental salinities. *J. Exp. Biol.* **163**, 97-118.

Cohen, P. (1980). *Recently discovered systems of enzyme regulation by reversible phosphorylation*. Elvsevier/North Holland Biochemical Press, Amsterdam.

Cornish, I. & Moon, T. W (1985). Glucose and lactate kinetics in the American eel *Anguilla rostrata*. *Am. J. Physiol.* **249**, R67-R72.

Dalla Via, J., van den Thillart, G., Cattani, O. & de Zwann, A. (1994). Influence of long-term hypoxia exposure on the energy metabolism of *Solea solea*. II. Intermediary metabolism in blood, liver and muscle. *Mar. Ecol. Prog. Ser.* **111**, 17-27.

Danulat, E. & Mommsen, T. P. (1990). Norepinephrine: a potent activator of glycogenolysis in rockfish hepatocytes. *Gen. Comp. Endocrinol.* **78**, 12-22.

Davis, K. B., Torrance, P., Parker, N. C. & Suttle, M. A. (1985) Growth, body composition and hepatic tyrosine animotransferase activity in cortisol-fed channel catfish, *Icatalurus punctatus* Rafinesque. *J. Fish. Biol.* **27**, 177-184.

deRoos, R. & deRoos, C. C. (1978). Elevation of plasma glucose levels by catecholamines in elasmobranch fish. *Gen. Comp. Endocr.* **34**, 447-452.

de Zwann, A. (1977). Anaerobic energy metabolism in bivalve molluscs. *Oceangr. Mar. Biol. Ann. Rev.* **15**, 103-187.

Dunn, J. F. & Hochachka, P. W. (1986) Metabolic responses of trout (*Salmo gairdneri*) to acute environmental hypoxia. *J. Exp. Biol.* **123**, 229-242.

Dunn, J. F. & Hochachka, P. W. (1987) Turnover rates of glucose and lactate in rainbow trout during acute hypoxia. *Can. J. Zool.* **65**, 1144-1148.

Ebberink, R. H. M. & Salimans, M. (1982). Control of glycogen phosphorylase activity in the posterior adductor of the sea mussel *Mytilus edulis*. *J. Comp. Physiol.* **148**, 27-33.

Edwards, D. G. & Cech Jr., J. J. (1990). Aquatic and aerial metabolism of juvenile monkeyface prickleback, *Cebidichthys violaceus*, an intertidal fish of California. *Comp. Biochem. Physiol.* **96A(1)**, 61-65.

Engstrom, L. (1978). The regulation of liver pyruvate kinase by phosphorylation-dephosphorylation. *Curr. Top. Cell. Regul.* **13**, 29-51.

Epple, A., Hathaway, C. B., & Nibbio, B. (1989). Circulatory catecholamines in the eel: origins and functions. *Fish. Physiol. Biochem.* 7, 273-278.

Fields, J. H. A., Driedzic, W. R., French, C. J. & Hochachka P. W. (1978). Some kinetic properties of skeletal muscle pyruvate kinase from air-breathing and water-breathing fish of the Amazon. *Can. J. Zool.* **56**, 751-758.

Foster, G. D. & Moon, T. W. (1986*a*).Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). *Fish Physiol. Biochem.* **1**, 113-124.

Foster, G. D. & Moon, T. W. (1986b). Enzyme activities in the Atlantic hagfish, *Myxine glutinosa*: changes with captivity and food deprivation. *Can. J. Zool.* **64**, 1080-1085.

Freeman, H. C. & D. R. Idler. (1973). Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdnerii*) and the brook trout (*Salvelinus fontinalis*). *Gen. Comp. Endocrinol.* **20**,69-75.

French, C. J. & Hochachka, P. W. (1978). Lactate dehydrogenase isozymes from heart and white muscle of water-breathing and air-breathing fish from the Amazon. *Can. J. Zool.* **56**, 769-773.

Haman, F. (1996). Effects of acute hypoxia and cold exposure on glucose and fatty acid fluxes in rainbow trout (*Oncorhynchus mykiss*). M.Sc. thesis. University of Ottawa, Ottawa, Canada.

Harris, K., Walker, P.M., Mickle, D.A.G., Harding, R., Gately, R., Wilson, G.J., Kuzon, B., McKee, N., & Romaschin, A. D. (1986). Metabolic response of skeletal muscle to ischemia. *Am. J. Physiol.* **250**, H213-H220.

Hayashi, H. & Ooshiro, Z. (1979). Gluconeogenesis in isolated liver cells of the eel, *Anguilla japonica. J. Comp. Physiol.* **132**, 343-350.

Hochachka, P. W., Fields, J. & Mustafa, T. (1973). Animal life without oxygen: basic biochemical mechanisms. *Amer. Zool.* **13**, 543-555.

Hochachka, P.W. (1980). *Living without oxygen*. Harvard University Press, Cambridge, Mass.

Hochachka, P. W., Guppy, M., Guderley, H. E., Storey, K. B. & Hulbert, W. C. (1978a). Metabolic biochemistry of water- vs. air-breathing fishes: muscle enzymes and ultrastructure. *Can. J. Zool.* **56**, 736-750.

Hochachka, P. W., Moon, T. W., Bailey, J. & Hulbert, W. C. (1978b). The osteoglossid kidney: correlations of structure, function, and metabolism with transition to air breathing. *Can. J. Zool.* **56**, 820-832.

Hochachka, P. W. & Somero, G. N. (1984). *Biochemical Adaptation.* Princeton University Press, Princeton, NJ.

Hulbert, W. C. & Moon, T. W. (1978). General characteristics and morphology of eel (*Anguilla rostrata* L.) red and white muscle. *Comp Biochem. Physiol.* **61A**, 377-382.

Hulbert, W. C., Moon, T. W. & Hochachka, P. W. (1978a). The osteoglossid gill: correlations of structure, function and metabolism with transition to air breathing. *Can. J. Zool.* **56**, 801-808.

Hulbert, W. C., Moon, T. W. & Hochachka, P. W. (1978b). The erythrinid gill: correlations of structure, function, and metabolism. *Can. J. Zool.* **56,** 814-819.

Hyde, D. A., Moon, T. W. & Perry, S. F. (1987). Physiological consequences of prolonged aerial exposure in the American eel, *Anguilla rostrata*: blood respiratory and acid-base status. *J. Comp. Physiol.* **157B**, 635-642.

Hyde, D. A. & Perry, S. F. (1987). Acid-base and ionic regulation in the American eel (*Anguilla rostrata*) during and after prolonged aerial exposure: Branchial and renal adjustments. *J. Exp. Biol.* **133**, 429-447.

Hyde, D. A. & Perry, S. F. (1990). Absence of adrenergic red cell pH and oxygen content regulation in American eel (*Anguilla rostrata*) during hypercapnic acidosis in vivo and in vitro. *J. Comp. Physiol.* **159B**, 687-693.

Hyvärinen, H., Holopainen, I. J., & Piironen J. (1985). Anaerobic wintering of crucian carp (*Carassius carassius* L.) - I. Annual dynamics of glycogen reserves in nature. *Comp. Biochem. Physiol.* **82A**, 797-803.

Jankosky, D., Hotopp, W. & Seibert, H. (1984). Influence of thermal acclimation on glucose production and ketogenesis in isolated eel hepatocytes. *Am. J. Physiol.* **246**, R471-R478.

Janssens, P. A. & Lowery, P. (1987). Hormonal regulation of hepatic glycogenolysis in the carp, *Cyprinus carpio. Am. J. Physiol.* **252**, R653-R660.

Janssens, P. A. & Waterman, J. (1988). Hormonal regulation of gluconeogenesis and glycogenolysis in carp (*Cyprinus carpio*) liver pieces cultured in vitro. *Comp. Biochem. Physiol.* **91A**, 451-455.

Johansen, K. (1968). Air breathing fishes. *Sci. Am.* **219**, 102-111.

Johansen, K. (1970). Air breathing in fishes. *In* "Fish Physiology" (W. S. Hoar & D. J. Randall, Eds.), Vol. IV, pp.361-411. Academic Press, New York.

Jørgensen, J. B. & Mustafa, T. (1980). The effect of hypoxia on carbohydrate metabolism in flounder (*Platichthys flesus* L.) --II. High energy phosphate compounds and the role of glycolytic and gluconeogenic enzymes. *Comp. Biochem. Physiol.* **67B**, 249-256.

Kirsch, R. & Nonnotte, G. (1977). Cutaneous Respiration in three freshwater teleosts. *Resp. Physiol.* **29**. 339-354.

Knox, D., Walton, M. J. & Cowey, C. B. (1980). Distribution of enzymes of glycolysis and gluconeogenesis in fish tissues. *Mar. Biol.* **56**, 7-10.

Leach, G. J. & Taylor, M. H. (1980). The role of cortisol in stress-induced metabolic changes in *Fundulus heteroclitus. Gen. Comp. Physiol.* **42**, 219-227.

Leach, G. J. & Taylor M. H. (1982). The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. *Gen. Comp. Endocrinol.* **48**,76-83.

Lidman, U., Dave, G., Johansson-Sjöbeck. M. L., Larsson, Å. & Lewander, K. (1979). Metabolic effects of cortisol in the European eel, *Anguilla anguilla* (LeSueur). *Comp. Biochem. Physiol.* **63A**,339-344.

Maxine, V., Nonnette, G., Peyraud, C., Willot, P. & Truchot, J. P. (1995). Circulatory and respiratory effects of an hypoxic stress in the Siberian sturgeon. *Resp. Physiol.* **100**, 203-212.

Mazeaud, M. M., Mazeaud, F. & Donaldson, E. M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* **160(3)**, 201-212.

Meinardus-Hager, G., Gabbott, P. A. and Gäde, G. (1989). Regulatory steps of glycolysis during environmental anoxia and muscular work in the cockle, *Cardium tuberculatum*: control of low and high glycolytic flux. *J. Comp. Physiol.* **159B**, 195-203.

Milligan, C. L. (1996) Metabolic recovery from exhaustive exercise in rainbow trout. *Comp. Biochem. Physiol.* **113A**, 51-60.

Mommsen, T. P., & Suarez, R. K. (1984). Control of gluconeogenesis in rainbow trout hepatocytes: role of pyruvate branchpoint and phosphoenolpyruvate - pyruvate cycle. *Mol. Physiol.* **6**, 9-18.

Mommsen, T. P., Walsh, P. J., Perry, S. F. & Moon, T. W. (1988) Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. *Gen. Comp. Endocrinol.* 70, 63-73.

Moon, T. W. (1983). Metabolic reserves and enzyme activities with food deprivation in immature American eels *Anguilla rostrata* (LeSueur). *Can. J. Zool.* **61**, 802-811

Moon, T. W. (1988). Adaptation, constraint, and the function of the gluconeogenic pathway. *Can. J. Zool.* **66**, 1059-1068.

Moon, T. W. & Foster, G. D. (1995). Tissue carbohydrate metabolism, gluconeogenesis and hormonal and environmental influences. *In* "Biochemistry and Molecular Biology of Fishes" (P. W. Hochachka & T. P. Mommsen, Eds.), Vol. 4, pp. 65-100. Elsevier, New York.

Moon, T. W. & Hulbert, W. C. (1980*a*). Characteristics of pyruvate kinases isolated from tissues of the American eel, *Anguilla rostrata* LeSueur. *Comp. Biochem. Physiol.* **65B**, 283-289.

Moon, T. W. & Hulbert, W. C. (1980*b*). Regulatory properties of pyruvate kinases isolated from tissues of the American eel, *Anguilla rostrata* LeSueur. *Comp. Biochem. Physiol.* **65B**, 291-296.

Moon, T. W., Walsh, P. J., & Mommsen, T. P. (1985). Fish hepatocytes: a model metabolic system. *Can. J. Fish. Aq. Sci.* **42**, 1772-1782.

Morales, A. E., Garcia-Rejon, L. & de la Higuera, M. (1990). Influence of handling and /or anesthesia on stress response in rainbow trout. Effects on liver primary metabolism. *Comp. Biochem. Physiol.* **95A**,87-93.

Morata, P., Vargos, A. M., Pita, M. L. & Sanchez-Medina, F. (1982). Involvement of gluconeogenesis in the hyperglycemia induced by glucagon, adrenaline, and cAMP in rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **73A**,379-381.

Moyes, C. D., Shulte, P. W. & Hochachka, P. W. (1992). Recovery metabolism of trout white muscle: the role of mitochondria. *Am. J. Physiol.* **262**, R295-R304.

Newsholme, E. A. & Crabtree, B. (1986). Maximum catalytic activity of some key enzymes in provision of physiologically useful information about metabolic fluxes. *J. Exp. Zool.* **239**, 159-167.

Newsholme, E. A. and Start C. (1973). *Regulation in metabolism*. London, England: John Wiley and Sons.

Nikinmaa, M., Steffensen, J. F., Tufts, B. L. & Randall, D. J. (1987). Control of red cell volume and pH in trout: effects of isoproterenol, transport inhibitors and extracellular pH in bicarbonate/carbon dioxide-buffered media. *J. Exp. Zool.* **242**, 273-281.

Nilsson, G. E. (1990). Long-term anoxia in crucian carp: changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. *J. Exp. Biol.* **150**, 295-320.

Peyraud-Waitzenegger, M. & Soulier, P. (1989) Ventilatory and circulatory adjustments in the European eel (*Anguilla anguilla* L.) exposed to short term hypoxia. *Exp. Biol.* **48**, 107-122.

Plaxton, W. C. & Storey, K. B. (1984). Purification and properties of aerobic and anoxic forms of pyruvate kinase from red muscle tissue of the channeled whelk, *Busycotypus canaliculatum. Eur. J. Biochem.* **143**, 257-265.

Porthe-Nibelle, J. & Lahlou, B. (1981). Mechanisms of glucocorticoid uptake by isolated hepatocytes of the trout. *Comp. Biochem. Physiol.* **69B**,425-433.

Rahman, M. S. & Storey, K. B. (1988). Role of covalent modification in the control of glycolytic enzymes in response to environmental anoxia in goldfish. *J. Comp. Physiol.* **157B**, 813-820.

Ramaiah, A. (1974). Pasteur effect and phosphofructokinase. *Curr. Top. Cell. Regul.* **8**,297-345.

Reid, S. D., Moon, T. W. & Perry, S. F. (1992). Rainbow trout hepatocyte ß-adrenoreceptors, catecholamine response, and effects of cortisol. *Am. J. Physiol.* **262**,R794-R799.

Renaud, J.M. & Moon, T. W. (1980). Characterization of gluconeogenesis in hepatocytes isolated from the American eel, *Anguilla rostrata* LeSueur. *J. Comp. Physiol.* **135B**,115-125.

Russell, E. L. & Storey, K. B. (1995). Anoxia and freezing exposure stimulate covalent modification of enzymes of carbohydrate metabolism in *Littorina littorea. J. Comp. Physiol.* **165B**, 132-142.

Shoubridge, E. A. & Hochachka, P. W. (1981). The origin and significance of metabolic carbon dioxide production in the anoxic goldfish. *Mol. Physiol.* **1**, 315-338.

Shulman, R. G., Bloch. G., & Rothman, D. L. (1995). In vivo regulation of muscle glycogen synthase and the control of glycogen synthesis. *Proc. Natl. Acad. Sci. USA*. **92**, 8535-8542.

Shulman, R. G. & Rothman, D. L. (1996). Enzymatic phosphorylation of muscle glycogen synthase: A mechanism for maintenance of metabolic homeostasis. *Proc. Natl. Acad. Sci. USA*. **93**, 7491-7495.

Smith, D. G., Duiker, W. & Cooke, I. R. C. (1983). Sustained branchial apnea in the Australian short-finned eel, *Anguilla australis*. *J. Exp. Zool.* **226**, 37-43.

Smith, D. G., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. & Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85.

Storey, K. B. (1984). Phosphofructokinase from foot muscle of the whelk, *Busycotypus canaliculatum*: Evidence for covalent modification of the enzyme during anaerobiosis. *Arch. Biochem. Biophys.* **235**, 665-672.

Storey, K. B. (1985*a*). Fructose-2,6-bisphosphate and anaerobic metabolism in marine molluscs. *FEBS Lett.* **181**, 245-249.

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

Storey, K. B. (1987). Tissue-specific controls on carbohydrate catabolism during anoxia in goldfish. *Physiol. Zool.* **60(5)**, 601-607.

Storey, K. B. (1988). Suspended animation: The molecular basis of metabolic depression. *Can. J. Zool.* **66**, 124-132.

Storey, K. B., Kelly, D. A., Duncan, J. A. & Storey, J. M. (1990). Anaerobiosis and organ-specific regulation of glycolysis in a marine whelk. *Can. J. Zool.* **68**, 974-980.

Storey, K. B. & Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Quarterly Review of Biology.* **65(2)**, 145-174.

Suarez, R. K. & Mommsen, T. P. (1987). Gluconeogenesis in teleost fishes. *Can. J. Zool.* **65**, 1869-1882.

Thomas, S. A., Schlender, K. K. & Larner, J. (1968). A rapid filter paper assay for UDPG glucose-glycogen glucosyltransferase including an improved biosynthesis of UDP-$^{14}$C-glucose. *Anal. Biochem.* **25**, 486-499.

Val, A. L. (1995) Oxygen transfer in fish: morphological and molecular adjustments. *Brazilian Journal of Medical and Biological Research.* **28**, 1119-1127.

van den Thillart, G. (1982). Adaptations of fish energy metabolism to hypoxia and anoxia. *Mol. Physiol.* **2**, 49-61.

van den Thillart, G. (1986). Energy metabolism of swimming trout (*Salmo gairdneri*). *J. Comp. Physiol.* **156**, 511-520.

van den Thillart, G. & van Waarde, A. (1985). Teleosts in hypoxia: aspects of anaerobic metabolism. *Mol. Physiol.* **8**, 393-409.

van der Boon, J., van den Thillart, G. E. E. J. M. & Addink, A. D. F. (1991). The effects of cortisol administration on intermediary metabolism in teleost fish. *Comp. Biochem. Physiol.* **100A**, 47-53.

van Schaftingen, E. (1984). D-fructose-2,6-bisphosphate. *In* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, Ed), pp. 335-341. Chemie, Weinheim.

van Waarde, A., van den Thillart, G. & Kesbecke, F. (1983). Anaerobic energy metabolism of the European eel, *Anguilla anguilla* L. *J. Comp. Physiol.* **149**, 469-475

Vijayan, M. M., Ballantyne, J. S. & Leatherland, J. F. (1991). Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. *Gen. Comp. Endocrinol.* **82**,476-486.

Vijayan, M. M., Foster, G. D. & Moon, T. W. (1993). Effects of cortisol on hepatic carbohydrate metabolism and responsiveness to hormones in the sea raven *Hemitripterus americanus*. *Fish Physiol. Biochem.*

Vijayan, M. M. & Moon, T. W. (1992). Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquatic. Sci.* **49**, 2260-2266.

Weber, J-M, Brill, R. W., Hochachka, P. W. (1986). Mammalian metabolite flux rates in a teleost: lactate and glucose turnover in tuna. *Am. J. Physiol.* **250**, R458-R458.

White, A. & Fletcher, T.C. (1989). The effect of physical disturbance, hypoxia and stress hormones on serum components of the plaice, *Pleuronectes platessa* L. *Comp. Biochem. Physiol.* **93A**, 455-461.

Williamson, J. R. (1965). Glycolytic control mechanisms: inhibition of glycolysis by acetate and pyruvate in isolated perfused rat heart. *J. Biol. Chem.* **240**, 2308-2321.

Wright, P.A., Perry, S. F. & Moon. T. W. (1989). Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. *J. Exp. Biol.* **147**,169-188.

Yu, K. L. & Woo, N. Y. S. (1987). Metabolic adjustments of an air-breathing teleost, (*Channa maculata*), to acute and prolonged exposure to hypoxic water. *J. Fish. Biol.* **31**, 165-175.

Zammit, V. A., Bei, L. & Newsholme, E. A. (1978). Maximum activities and effects of fructose biphosphate on pyruvate kinase from muscles or vertebrates and invertebrates in relation to the control of glycolysis. *Biochem. J.* **174**, 989-998.