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Energy Metabolism in the Mantle Muscle of the Squid, *Loligo pealeii*

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Summary. 1. The concentrations of glycolytic and Krebs cycle intermediates, α-glycerophosphate, the adenylates, and free amino acids were determined in the mantle muscle of the squid, *Loligo pealeii,* at rest and after 10 s of vigorous swimming.

2. Exercise resulted in significant increases in the levels of glucose-6-phosphate, fructose-1,6-diphosphate, and pyruvate. Phosphofructokinase and pyruvate kinase were identified as control points of glycolysis. In the Krebs cycle, changes in the levels of isocitrate and α-ketoglutarate suggested a facilitation of the isocitrate dehydrogenase reaction.

3. The products of anaerobic metabolism in mantle muscle, in particular α-glycerophosphate and octopine, did not accumulate during exercise indicating the aerobic nature of "burst" swimming in these animals.

4. Exercise resulted in a drop in muscle proline concentrations of approximately 2 μmol/g wet wt. which was accompanied by an almost stoichiometric increase in alanine levels.

5. The changes in adenylate concentrations with exercise were dramatic: ATP fell to 39% of the value in resting muscle while ADP and AMP rose 3- and 6-fold, respectively. Arginine phosphate concentrations fell from 10 μmol/g wet wt. in resting to 1 μmol/g wet wt. in exercised muscle.

6. Muscular work in this squid appears to be accompanied by an activation of aerobic carbohydrate metabolism, the α-glycerophosphate cycle functioning to maintain cytoplasmic redox balance. During metabolic activation, the carbon skeleton of proline may be used to augment the pool size of Krebs cycle intermediates. The observed changes in arginine phosphate and AMP levels could be key in the activation of the reactions of glycolysis.

Introduction

Squid are fast swimming predators that use a jet propulsion mode of locomotion in which water is shot out of the mantle cavity by strong contractions of the mantle muscle (Young, 1975). The mantle muscle supports two kinds of work: slow swimming and respiratory movements, and the rapid bursts of speed needed to catch prey or evade predators. Amongst vertebrates these two types of muscular work would characteristically be performed by different muscle fibre types; steady state physical performance is almost always sustained by aerobic fat catabolism in mitochondria-rich red fibres while "burst" muscular activity is supported by anaerobic glycogen-based metabolism in white fibres (Drummond, 1966). Electron micrographs reveal, however, that the mantle muscle of squid is homogeneous in fibre type (Moon and Hulbert, 1975). Muscle cells are packed with mitochondria (Moon and Hulbert, 1975) and glycogen-like granules are present (Hochachka et al., 1975). Fat droplets are not present nor is the fat content of the tissue (1.5% of the wet weight) sufficient to indicate that muscle work is fat based (Hochachka et al., 1975). In addition, fat metabolizing enzymes, such as thiolase, are not detectable in the muscle. This, coupled with the high respiratory rates recorded for cephalopods (Ghiretti, 1966), R.Q. values approximating 1.0 (Redfield and Goodkind, 1929), and the high titres of glycolytic and Krebs cycle enzymes in the mantle muscle (Hochachka et al., 1975), indicates that the work performed by the mantle muscle is supported by aerobic carbohydrate metabolism. Indeed, when held out of water and therefore deprived of oxygen, the animal is incapable of contracting the mantle muscle and the muscle rapidly accumulates large amounts of pyruvate and α-glycerophosphate (Hochachka et al., 1975).

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To gain further insights into the nature and control of mantle muscle metabolism, we chose to compile a profile of changes in the concentrations of some intermediary metabolites during the initiation of muscular work. The study revealed that "burst" swimming in *Loligo* is accompanied by an activation of both glycolysis and the Krebs cycle. Anaerobic end products fail to accumulate indicating that work is completely aerobic. As in insect flight muscle (Sacktor, 1970), proline reserves are depleted at the initiation of muscle work; the carbon skeleton of this amino acid may likely be utilized to augment the pool size of Krebs cycle intermediates. The make-up of the adenylate pool is altered dramatically, a sudden pulse of AMP perhaps serving to rapidly activate metabolism.

Materials and Methods

Materials

All enzymes, cofactors, substrates, and reagents were purchased from Sigma Chemical Co., St. Louis, Mo. unless otherwise stated.

Animals

Squid, *Loligo pealeii*, (approx. 8″ long) were obtained from the Marine Biological Laboratory, Woods Hole, Mass. and held, for less than a day, in running, aerated sea water. Individual squid were vigorously exercised by chasing them (at what was probably their maximum speed) around a large sea table (running, aerated sea water) for approximately 10 s. The animals were caught while still swimming and dropped into a container of liquid nitrogen. Control, rested squid were obtained by allowing isolated individual squid to rest quietly at the bottom of small enclosed tanks (still sea water) for approximately 15 min. At rest the mantle muscle supports only the slow rhythmic contractions needed to fan water past the gills. By approaching the animals from behind, they can be caught in a net without struggle and dropped into liquid nitrogen before the mantle can contract. Essentially identical results for muscle metabolite levels were obtained when muscle was collected by freeze-clamping with tongs cooled in liquid nitrogen.

Tissue Preparation

Pieces of frozen mantle muscle (approx. 2 g) were ground into a powder using a mortar and pestle which were kept cold by the frequent addition of liquid nitrogen. After weighing, the frozen powder was homogenized in 3 volumes of 8% $HClO_4$ in 40% ethanol using a Sorvall Omni-Mixer (Williamson and Corkey, 1969). The mixture was kept cold by immersion in a dry ice:ethanol bath. The homogenate was transferred to a centrifuge tube and the precipitated protein removed by centrifugation at 25,000 *g* (4 °C) for 10 min. The supernatant was removed and neutralized to pH 5.5 with 3 M K_2CO_3 in 50 mM 2-(N-morpholino) ethanesulphonic acid (MES) (Hansford, 1974). Precipitated $KClO_4$ was removed by centrifugation as above. Alphaketoglutarate and arginine phosphate assays were performed immediately while other metabolites were measured in extracts which were stored at −80 °C until use.

Measurement of Metabolites

Glucose was measured by the method of Madin and Crowe (1975). All other intermediates were measured by coupled enzyme assays and the change in absorbance of the pyridine nucleotides followed at 340 nm using a Unicam SP1800 recording spectrophotometer. Glycolytic intermediates, adenylates, α-glycero-P, citrate, malate, isocitrate, and α-ketoglutarate were measured by the assays outlined by Lowry and Passonneau (1972). By substituting arginine phosphokinase (CalBiochem Co.) for creatine phosphokinase, the assays for creatine (buffer pH was altered to 8.0) and creatine phosphate, described by Lowry and Passonneau (1972), were converted into assays for arginine and arginine phosphate. Octopine was determined by a substitution of purified octopine dehydrogenase (Fields et al., 1976) for lactate dehydrogenase in the lactate assay of Lowry and Passonneau (1972). Succinate was measured by the method of Williamson and Corkey (1969). Amino acids (except arginine) were quantitated using a Techtron Amino Acid Analyser.

Statistics

The statistical significance of the differences between the rested and exercised metabolite levels was determined using the Student's T-test for the difference between means (Snedecor and Cochran, 1967).

Results

Table 1 shows the concentrations of selected glycolytic and Krebs cycle intermediates in the mantle muscle at rest and after 10 s vigorous swimming. A slight but not significant increase in muscle glucose concentrations is seen in the exercised state. Of hexose monophosphates, only glucose-6-P concentrations changed significantly during the transition from resting to active states. As in a variety of other muscles (Beis and Newsholme, 1975), glucose-6-P: fructose-6-P ratios approached the expected equilibrium value of 3:1 in the muscle at rest (assuming the measured values represent free concentrations in a homogeneous system) but during exercise the ratio suggests that phosphoglucoisomerase is displaced from equilibrium.

While fructose-6-P concentrations in the muscle did not change, the levels of fructose-1,6-diP rose 3-fold at the initiation of muscular work. The concentrations of dihydroxyacetone-P and glyceraldehyde-3-P underwent changes paralleling that of fructose-1,6-diP although neither increased in concentration as much as the hexose diphosphate. These changes, as well as a slight increase in muscle P-enolpyruvate concentrations occur in a variety of other muscle during the activation of glycolysis (Sacktor and Wormser-Shavit, 1966; Williamson, 1966; Rowan, 1975). The concentration of pyruvate in the muscle rose significantly during the 10 s of muscular work.

Although the measurements of Krebs cycle inter-

Table 1. Effect of exercise on the concentrations of glycolytic and Krebs cycle intermediates in squid mantle muscle. Exercised squid were subjected to 10 s vigorous swimming as described in the Experimental section. The values are the means ±S.D. of determinations on six individual animals

	Concentration (μmol/g wet wt.)	
	At rest	After exercise
Glucose	0.37	0.50
Glucose-1-P	0.01 ± 0.001	0.01 ± 0.004
Glucose-6-P	0.03 ± 0.01	0.08 ± 0.03[a]
Fructose-6-P	0.008 ± 0.001	0.01 ± 0.02
Fructose-1,6-diP	0.07 ± 0.04	0.20 ± 0.05[a]
Dihydroxyacetone-P	0.10 ± 0.01	0.12 ± 0.01
α-glycero-P	0.15 ± 0.03	0.15 ± 0.03
Glyceraldehyde-3-P	0.03 ± 0.02	0.04 ± 0.01
P-enolpyruvate	0.05 ± 0.01	0.06 ± 0.02
Pyruvate	0.05 ± 0.02	0.19 ± 0.01[a]
Lactate	0.13 ± 0.05	0.12 ± 0.06
Citrate	0.16 ± 0.09	0.10 ± 0.02
Isocitrate	0.07 ± 0.001	0.04 ± 0.02[a]
α-ketoglutarate	0.06 ± 0.02	0.12 ± 0.02[a]
Succinate	1.48 ± 1.00	1.30 ± 0.72
Malate	0.41 ± 0.13	0.35 ± 0.10
Octopine	< 0.1	< 0.1

[a] The value is statistically different from that in the muscle at rest, $P < 0.01$

Table 2. Effect of exercise on the concentrations of free amino acids in the mantle muscle of squid. Exercised squid were subjected to 10 s of vigorous swimming as described in the Experimental section. The values are the means ±S.D. of determinations on six individual animals

	Concentration (μmoles/g wet wt.)	
	At rest	After exercise
Lysine	0.84 ± 0.57	1.10 ± 0.39
Histidine	0.16 ± 0.01	0.16 ± 0.07
Aspartate	0.76 ± 0.10	0.85 ± 0.20
Threonine	0.76 ± 0.18	0.75 ± 0.11
Serine	0.34 ± 0.01	0.52 ± 0.24
Glutamate	1.49 ± 0.43	1.36 ± 0.24
Glycine	4.97 ± 3.02	6.70 ± 2.45
Cystine	0.61 ± 0.20	0.74 ± 0.20
Valine	0.44 ± 0.12	0.49 ± 0.07
Methionine	0.79 ± 0.36	0.75 ± 0.18
Isoleucine	0.45 ± 0.05	0.39 ± 0.18
Leucine	0.38 ± 0.16	0.27 ± 0.18
Tyrosine	0.26 ± 0.01	0.26 ± 0.01
Proline	3.15 ± 1.00	1.59 ± 0.53[b]
Alanine	3.80 ± 1.06	6.10 ± 1.25[a]
Arginine	15.24 ± 3.00	27.11 ± 5.50[b]

[a] The value is statistically different from that in the muscle at rest, $P < 0.05$;
[b] $P < 0.01$

mediates made in this study are of total, rather than mitochondrial, concentrations, mitochondrial changes often parallel changes in the total tissue concentration (Johnson and Hansford, 1975) and measurements of whole tissue changes are widely used to infer changes in the reaction rates of Krebs cycle enzymes (Safer and Williamson, 1973). In the exercised muscle, the concentrations of isocitrate decreased significantly with respect to the muscle at rest while the level of α-ketoglutarate rose significantly. On a cross-over plot (Williamson, 1970) of exercised as a percentage of rested metabolite levels, these changes resulted in a cross-over point at the isocitrate dehydrogenase reaction. The concentration of oxaloacetate in mantle muscle could not be measured, the true level of oxaloacetate being below the limits of the spectrophotometric assay (i.e. < 0.01 mM). Citrate concentration fell (although not significantly) in the manner expected if the aconitase reaction were close to equilibrium (Johnson and Hansford, 1975).

The concentrations of free amino acids in the muscle of rested and exercised squid are shown in Table 2. High tissue levels of taurine (> 30 mM) were detected but not quantitated. The levels of amino acids are similar to those found in the mantle muscles of other cephalopod molluscs, glutamate, glycine, proline, alanine, and arginine being the amino acids present in highest concentrations (Florkin, 1966; Robertson, 1965). Except for arginine, proline and alanine were the only amino acids to change significantly in concentration during the 10 s of muscular work. Proline concentrations fell by 1.6 μmol/g wet wt. while the levels of alanine rose by 2.3 μmol/g wet wt.

In Table 3 the effects of anoxia and of exercise on the concentrations of some metabolites in mantle muscle are contrasted. During vigorous exercise, the concentration of α-glycero-P in the muscle did not change. In contrast, however, anoxia resulted in a rapid accumulation of large amounts of α-glycero-P, a result also observed in the anoxic insect flight muscle (Sacktor, 1970) and during glycolytic work in vertebrate skeletal muscle (Edington et al., 1973). Lactate does not accumulate in squid muscle under either condition; this observation being consistent with the extremely low activities of lactate dehydrogenase in the muscle (Hochachka et al., 1975). Similarily, octopine, an alternate end product of glycolysis, does not accumulate during brief periods of "burst" muscular work in the squid (Table 1) although the compound has been demonstrated to accumulate when squid are swum to exhaustion (Grieshaber and Gäde, 1976). A build-up of alanine and pyruvate accompanies the activation of aerobic as well as anaerobic metabolism in a variety of muscles (Sacktor and Wormser-Shavit,

Table 3. A comparison of metabolite concentrations in the mantle muscle of squid at rest, after exercise, and during anoxia. Metabolite values in the anoxic muscle are taken from Hochachka et al. (1975) and are expressed in mM. Rested and exercised values, given in μmoles/g wet wt., are taken from Tables 1 and 2

	At rest	After exercise	During anoxia	
			After 10 s	After 30 min
α-glycero-P	0.15	0.15	4.5	11.0
Alanine	3.80	6.10	9.0	10.5
Pyruvate	0.05	0.19	0.4	0.5
Lactate	0.13	0.12	0	0
Malate	0.41	0.35	0.7	1.5
Succinate	1.48	1.30	–	3.5

Table 4. Effect of exercise on the concentrations of the adenylates, arginine, and arginine phosphate in squid mantle muscle. Exercised squid were subjected to 10 s vigorous swimming as described in the Experimental section. Values are the means ±S.D. for $n=6$ animals (for arginine phosphate $n=2$). Exercised values are each significantly different from their corresponding values in the muscle at rest, $P<0.01$

	Concentration (μmol/g wet wt.)	
	At rest	After exercise
Arginine phosphate	10.5	1.0
Arginine	15.2±3.0	27.1±5.5
ATP	5.3±0.6	2.1±0.6
ADP	0.7±0.2	2.2±0.4
AMP	0.3±0.2	1.8±0.7

1966; Rowan, 1975; Safer and Williamson, 1973). However, the accumulation of these two metabolites is not as great during exercise as in the anoxic muscle. Succinate, an end product of anaerobic metabolism in molluscs (Hochachka et al., 1973), accumulated along with its precursor, malate, in anoxic mantle muscle but not in the exercised muscle. This failure of various anaerobic end products to accumulate during muscle work in squid is, therefore, good evidence for suggesting that the muscle work required for "burst" swimming is supported by an aerobic metabolism.

Table 4 shows the changes in the muscle adenylate and phosphagen pools during muscular exercise in squid. Arginine phosphate reserves are almost completely depleted by the 10 s of muscular work and this depletion is met by an equal rise in arginine concentrations. While the total adenylate pool of the muscle remains constant during exercise, the levels of the individual adenylates changes greatly. The concentration of ATP dropped to a value of 39% of the resting level while ADP and AMP levels rose 3-fold and 6-fold respectively. Whether these changes are transient or maintained for the duration of muscle exercise is not known but the magnitude of the changes in ATP and AMP are far greater than those reported for any other working muscle systems.

Discussion

While several studies have suggested that squid muscle is endowed with a high capacity for aerobic metabolism (Moon and Hulbert, 1975; Hochachka et al., 1975), the degree to which swimming is supported by aerobic muscle metabolism has not previously been assessed. The indication of this study is that the aerobic capacity of the muscle is fully able to support the energy demands of high speed swimming. Typical end products accumulated in the anoxic squid muscle do not build up during exercise (Table 3). Of particular interest in this context is α-glycerophosphate. Squid mantle muscle, like insect flight muscle, utilizes the α-glycerophosphate cycle for transferring reducing equivalents from the cytoplasm into the mitochondria (Hochachka et al., 1975; Sacktor, 1970). Indeed, the absence of the aspartate-malate shuttle is supported by data in this study; changes in metabolite levels which characterize activation of the shuttle during muscle work in mammals (Safer and Williamson, 1973) do not occur in squid muscle. In a muscle relying on a functional α-glycerophosphate cycle, any inequality in the rates of α-glycerophosphate production in the cytoplasm and its utilization in the mitochondrial would result in an immediate build-up of α-glycerophosphate. This occurs during anoxic stress in both squid (Hochachka et al., 1975) and insect (Sacktor, 1970) muscle. The failure of α-glycerophosphate to accumulate in the exercised muscle coupled with a lack of octopine production indicates that aerobic metabolism in squid muscle can keep pace with the energy demands of swimming.

Previous studies have indicated that muscle metabolism in squid is fueled by the catabolism of stored carbohydrate (Hochachka et al., 1975; Moon and Hulbert, 1975). This reliance of carbohydrate is evidenced by the build-up of carbohydrate end products (pyruvate, α-glycerophosphate) during anoxia and the elevated levels of pyruvate, fructose-1,6-diphosphate, and glucose-6-phosphate during mantle muscle work (Tables 1 and 3). However, the storage polysaccharide of squid muscle appears to be an altered form of glycogen (Hochachka et al., 1975; Goudsmit, 1972) and to date quantitation of polysaccharide depletion during muscle work has proven impossible. Blood glucose may also play a role as an energy source;

the small increase in muscle glucose concentrations with exercise could indicate increased uptake of blood glucose. In addition to the mobilization of carbohydrate, the data in this study indicate that the hydrolysis of arginine phosphate reserves and the catabolism of proline also contribute to energy production in the mantle muscle during the initial stages of muscle activation.

The pattern of changes in the metabolite profile of squid mantle muscle resembles that reported for other working muscles and is indicative of a coordinated activation of both glycolysis and the Krebs cycle. Metabolic activation is accompanied by a rise in glucose-6-phosphate concentrations in some muscles (Edington et al., 1973; Rowan, 1975), including squid muscle, and may be indicative of an increased flow of carbon into the glycolytic pathway. Facilitation of the two key regulatory enzymes of glycolysis, phosphofructokinase and pyruvate kinase, is suggested. An increased concentration of product (fructose-1,6-diP, pyruvate) but not of substrate (fructose-6-P, P-enolpyruvate) of these reactions, coupled with an increase in glycolytic flux, is indicative of an activation of the enzymes (Williamson, 1970). This pattern of changes in the concentrations of glycolytic intermediates occurs in a variety of other muscles during metabolic activation (Sacktor and Wormser-Shavit, 1966; Williamson, 1966; Edington et al., 1973) and can be taken as evidence for the activation of phosphofructokinase and pyruvate kinase during muscle work in squid. Although it is difficult to pinpoint rate limiting steps in a cycle from steady-state measurements of intermediates (Randle et al., 1970), the "cross-over point" at the isocitrate dehydrogenase reaction of the Krebs cycle suggests that this reaction is strongly facilitated at the initiation of mantle muscle work. This, taken together with the non-equilibrium nature of NAD^+-dependent isocitrate dehydrogenase, implies that isocitrate dehydrogenase could be the major control point of the Krebs cycle in mantle muscle.

Activation of the Krebs cycle can be achieved not only by kinetic activation of the enzymes of the cycle but also by increasing the capacity of the cycle for handling 2-carbon, acetyl-CoA units by increasing the total pool size of Krebs cycle intermediates. The intramitochondrial concentration of oxaloacetate is extremely low (La Noue et al., 1972) and is believed to be rate-limiting to cycle activity under many conditions. The activation of aerobic metabolism is often coupled, therefore, to some mechanism designed to increase the pool size of oxaloacetate. Vertebrate muscle, burning carbohydrate, directly depletes aspartate reserves, funneling the carbon skeleton of this amino acid into the Krebs cycle. The subsequent increase in concentrations of Krebs cycle intermediates can be fully accounted for by aspartate depletion (Safer and Williamson, 1973). Insect flight muscle utilizes the carbon skeleton of proline for the same purpose (Sacktor, 1970). The depletion of proline, but not of aspartate, reserves during the initiation of muscle work in squid indicates that these two invertebrate aerobic muscles utilize the same mechanism for priming the Krebs cycle. Proline catabolism appears to be energetically advantageous during the initial stages of muscle work when metabolism has not yet been fully activated. The conversion of 1 mole of proline to 1 mole of oxaloacetate (α-ketoglutarate being produced by the glutamate-pyruvate transaminase reaction) is coupled to one flavoprotein-linked and four NAD^+-linked dehydrogenations for a total output of 15 moles of ATP (Hochachka and Somero, 1973). The corresponding conversion of aspartate to oxaloacetate in vertebrate tissues results in no production of ATP by the system. The depletion of either aspartate or proline reserves is coupled to the accumulation of alanine, the amino groups from these amino acids being transferred into the synthesis of alanine. The pyruvate needed for alanine synthesis appears to be derived from a temporary accumulation of pyruvate which occurs when the reactions of glycolysis are activated faster than those of the Krebs cycle (Safer and Williamson, 1973; Sacktor and Wormser-Shavit, 1966; Ruderman and Berger, 1974). Thus the depletion of proline reserves is coupled to an almost stoichiometric increase in alanine concentrations (Table 2). In some insect tissues (Bursell, 1967), pyruvate is derived from the decarboxylation of oxaloacetate, proline thus providing both the carbon skeleton and the amino group of alanine. This appears to be unlikely in squid muscle, however, the activities of oxaloacetate-decarboxylating enzymes in mantle muscle being very low (Hochachka et al., 1975).

The arginine phosphate reserves of mantle muscle are almost completely hydrolyzed during the first few seconds of muscle work (Table 4). Yet despite this, ATP concentrations in the muscle drop dramatically. Indeed, the changes in the make-up of the adenylate pool during the initiation of muscular work in squid are much greater than those reported for other working muscles in vivo. Most muscles accomplish the transition from resting to active states with little or no change in ATP concentrations (Neely et al., 1973; Sacktor and Hurlbert, 1966; Piras and Staneloni, 1969), the mobilization of phosphagen reserves serving to "buffer" ATP levels until activation of metabolism is complete. It is generally thought that tissues cannot recover if ATP levels fall too low and for this reason adenylate energy charge is maintained as constantly high as possible. *Loligo,* however, do re-

cover completely from the "burst" swimming to which they were subjected and indeed, can maintain this swimming speed for a much longer time period. The dramatic fall in ATP levels (to 39% of resting levels) measured in squid muscle after 10 s of swimming may be only transient, the physiological function of this fluctuation in ATP levels being to rapidly raise the concentration of AMP, a potent activator and deinhibitor of key enzymes of aerobic energy metabolism in squid muscle. The action of adenylate kinase in squid muscle translates the 2.5-fold drop in ATP concentration into a 6-fold increase in AMP levels (Table 4) (Storey, 1976). A "pulse" of AMP is also seen during metabolic activation in blowfly flight muscle, AMP concentration rising rapidly on the initiation of flight to a peak concentration 2.5-fold higher than that in resting muscle before falling back to a new steady state level (Sacktor and Hurlbert, 1966). A burst of AMP is thought to be instrumental in rapidly activating phosphofructokinase (Sacktor and Hurlbert, 1966), AMP being an extremely potent activator of both insect flight muscle and squid mantle muscle phosphofructokinase (Storey and Hochachka, 1975a). Activation of squid mantle phosphofructokinase is further promoted by the release of arginine-P inhibition of the enzyme at the initiation of work (the K_i for mantle muscle phosphofructokinase being 5 mM; Storey, unpublished data).

The adenylates may also be important in coordinating the activation of the α-glycerophosphate cycle in mantle muscle. Squid muscle α-glycerophosphate dehydrogenase is potently inhibited by ATP in vitro (Storey and Hochachka, 1975b). Deinhibition of the enzyme by falling levels of ATP coupled with Ca^{2+}-activation of mitochondrial α-glycerophosphate oxidase (Hochachka et al., 1975) would effectively activate the cycle at the initiation of muscle work and would likely produce an immediate, but transient, fall in NADH levels. A transient fall in NADH concentrations has been recorded in several muscles during the initiation of work (Edington et al., 1973; Jobsis, 1963; Chance and Weber, 1963) and indeed, the NAD/NADH ratio in squid muscle (calculated from dihydroxyacetone-P and α-glycero-P concentrations in Table 1 and the K_{eq} for α-glycerophosphate dehydrogenase; Veech et al., 1972) increases approximately 20% in the exercised state. The regulatory importance of a decrease in NADH levels during metabolic activation has been shown in previous studies which indicate that NADH is an important inhibitor of the activity of several enzymes in squid mantle muscle including phosphofructokinase, pyruvate kinase, glyceraldehyde-3-P dehydrogenase, adenylate kinase, and arginine kinase (Storey and Hochachka, 1975a, c, d; Storey, 1976; Storey, 1977). Release of NADH inhibition at the start of muscle contraction would therefore not only act to increase glycolytic flux directly but would also influence the activity of the enzymes involved in controlling the levels of two major regulatory metabolites: AMP and arginine-P.

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