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METABOLIC REGULATION OF SKELETAL MUSCLE ENERGY METABOLISM DURING EXERCISE

by

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ABSTRACT

The metabolic and biochemical factors involved in the regulation of fuel and pathway selection, at appropriate rates and times, during elevated metabolic demands remains to be resolved. Therefore, the purpose of this investigation was to examine the metabolic interrelationships involved in energy provision and its regulation during exercise at different intensities. Specifically, fuel selection and its control by adenine nucleotides and cytosolic redox and were investigated in red and white muscle of rainbow trout during various progressive intensities of exercise. The control of glycolysis was examined with respect to glycolytic enzyme disequilibrium. The effects of substrate limitations and end product accumulations were examined with regard to fatigue. Finally, the buffering capacity and various potential buffering constituents were investigated. It would appear that the energy turnovers required to perform the varied exercise intensities in this study were achieved by selecting the fiber type, fuel and pathway for optimizing ATP production rate versus substrate and proton accumulation. The purine nucleotide cycle was found to be operational within both fiber types. Fuel selection appeared to be intimately related to myofibrillar ATPase activation with free ADP acting as the metabolic signal to coordinate the phasing in of appropriate fuels/pathways at appropriate rates. HK, phos, PFK and PK were identified as regulatory enzymes in both fiber types. As well, the GPDH.PGK complex also appeared to exhibit regulation when glycogen was limiting and this regulation appeared to have been induced by a decreased ATP/ADP$_t$ ratio. The redox state of the NAD couple became more oxidized in both tissues when muscle glycogen was low. This finding was attributed to an induced shift in the equilibrium of LDH in the direction of NAD and lactate.

The simultaneous ATP/ADP$_f$ induced disequilibrium of the PGK reaction would inhibit flux through the GPDH.PGK complex. Skeletal muscle buffering was found to be dominated by protein, inorganic phosphate and histidine related compounds. Thus, these metabolic and biochemical adjustments, allowed a coordinated integration of fiber type, fuel and pathway selection, to achieve the appropriate coupling of myofibrillar ATPase activity to ATP turnover, while minimizing substrate depletion and proton accumulations.

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List of Abbreviations

Acid-Base

B	buffer capacity
CH	crude homogenate
HMW	high molecular weight
HRC	histidine related compounds
IDe	ion difference extracellular
IDr	ion difference red muscle intracellular
IDw	ion difference white muscle intracellular
LMW	low molecular weight
NMR	nuclear magnetic resonance
SN	supernatant

Chemical Compounds

AMP	adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
CoASH	acetyl-coenzyme A
Cr	creatine
Cr_c	compensated creatine
DNFB	dinitrofluorobenzene
EGTA	ethyleneglycol-bis-(B-amino-ethyl ether) N,N'-tetraacetic acid
DHAP	dihydroxyacetone phosphate
DPG	1,3-diphosphoglycerate

FDP	fructose-1,6-phosphate
F2,6BP	fructose-2,6-bisphosphate
F6P	fructose-6-phosphate
GAP	glyceraldehyde-3-phosphate
GAP_f	free glyceraldehyde-3-phosphate
GP	glycerol 3-phosphate
GTP	guanosine triphosphate
G1P	glucose-1-phosphate
G6P	glucose-6-phosphate
IAA	iodoacetic acid
IMP	inosine monophosphate
La^-	lactate
NAD, NADH	nicotinamide adenine dinucleotide (oxidized, reduced)
NADP, NADPH	nicotinamide adenine dinucleotide phosphate (oxidized, reduced)
NH_3	ammonia
NH_4	ammonium ion
PCA	perchloric acid
PCr	phosphocreatine
PCr_c	compensated phosphocreatine
PEP	phosphoenolpyruvate
Pi	inorganic phosphate
Pi_c	compensated inorganic phosphate
PMSF	phenolmethylsulfonyl flouride
PYR	pyruvate
3PG	3-phosphoglycerate
2PG	2-phosphoglycerate

Enzymes

Ald	aldolase
CS	citrate synthase
enol	enolase
GPDH	glyceraldehyde 3-phosphate dehydrogenase
HK	hexokinase
ICDH	isocitrate dehydrogenase
LDH	lactate dehydrogenase
OGDH	oxyglutarate dehydrogenase (ketoglutarate dehydrogenase)
PFK	phosphofructokinase
PGI	phosphoglucoisomerase
PGK	phosphoglycerate kinase
PGM	phosphoglyceromutase
PGM*	phosphoglucomutase
phos	phosphorylase
PK	pyruvate kinase
TPI	triose phosphate isomerase

Locomotory

BS	burst swimming
EMG	electromyographic
ES	exhaustive swim
PS	prolonged swimming
PSS	prolonged steady swimming
PSS-30	30 minute prolonged steady swim
PSS-7	7 minute prolonged steady swim
PUS	prolonged unsteady swimming
SS	sustained swimming
TBF	tail beat frequency
Ucrit	critical swimming velocity

Physical and Thermodynamic

Em	membrane potential
G	Gibbs Free Energy
H	enthalpy
Keq	equilibrium constant
Kd	dissociation constant
pK	dissociation constant
R	gas constant

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INTRODUCTION

Fuel Selection and Fatigue

A variety of constraints are involved in the structural and biochemical design for regulation of fuel selection at various exercise intensities. Different fiber types exist with their variations in ultrastructure, metabolite levels are altered, enzyme activities are adjusted or occur as specific isozymes and mechanisms exist to negate metabolically generated deleterious end products. These constraints serve to integrate metabolic functioning during work to prevent muscular dysfunction due to fatigue. Although fatigue has many etiologies, it can be defined in functional terms as an inability to generate sufficient power to continue to perform work at a given rate. High intensity exercise requires high rates of ATP turnover and eventually results in fatigue, with the time course of fatigue being related to work intensity. These high ATP turnovers are achieved by phosphagen hydrolysis and anaerobic glycolysis which result in substrate depletion and metabolic end product accumulations of creatine, inorganic phosphate, lactate and hydrogen (H^+) ions. Of these, substrate depletion and proton accumulations have been the most thoroughly investigated with regard to fatigue, but osmotic, ionic and charge disturbances which accompany this energy provision must be considered (Hochachka 1985).

Substrate depletion is recognized as a major contributor to fatigue during long term endurance work (Hermansen et al. 1967; Hultman 1967; Hultman 1978) and during very high intensity exercise which is primarily fueled by phosphagen hydrolysis (McGilvery 1975). Proton accumulation has been implicated in the fatigue process during short term high intensity exercise

(Toews et al. 1970; Dawson et al. 1978; Sutton et al. 1981). Although the source of the protons during anaerobic glycolysis has yet to be resolved (Krebs et al. 1975; Gevers 1977, 1979; Hochachka and Mommsen 1983; Portner et al. 1984), there appears to be no question as to the 1:1 stoichiometric relationship between lactate and H^+ production. Increased H^+ concentrations have been associated with alterations to both the contractile and glycolytic machinery resulting in decreased times to fatigue (Fitts and Holloszy 1976; Stevens 1980). However, Hochachka (1985) has recently suggested that protons per se, may play important role(s) during oxygen (O2) limiting periods. It was suggested that protons may; (1) create more favourable conditions for the unloading of oxygen; (2) facilitate phosphagen hydrolysis and glycolysis ; and (3) enhance lactate efflux. Thus the importance of investigating the effects of end product accumulations in exercise studies which require high rates of ATP turnover is emphasized.

The myotomal muscle mass of fish provides an ideal model to investigate the metabolic interrelationships involved in energy provision and regulation during exercise at different intensities. This is because it comprises essentially two spatially and functionally different fiber types, red and white (Johnston 1981). White muscle, which comprises the bulk of the myotomal muscle mass, is predominantly a fast-twitch gylcolytic tissue that is thought to be recruited during activity which requires high rates of metabolic energy turnover (Johnston 1981). Conversely, red muscle appears as a longitudinal strip running beneath the lateral line and is an aerobic slow-twitch oxidative tissue that is thought to be recruited during longer term steady state swimming (Johnston 1981). Within many species, including salmonids, the white muscle is in reality a mosaic muscle as red fibers constitute a small percentage (ie. 1 percent in trout) of this muscle mass

(Proctor et al. 1980) and must be considered when interpreting physiological and metabolic data. The evidence for this division in function comes from a variety of sources. Myofibrillar and sarcoplasmic reticulum ATPase activities have shown that white and red muscle have the biochemical properties of fast-twitch and slow-twitch muscle respectively (Johnston 1982b). As well, other differences between these two fiber groups with respect to fiber diameter, ultrastructure, enzyme activities, capillarization, myoglobin and number of mitochondria support this division of function (Bone 1966; Bilinski 1974; Johnston 1982a).

Intramuscular Acid-Base Regulation

A dominant theme in the evolution of vertebrate acid-base regulation is the physicochemical basis for maintenance of the configuration and charge of proteins necessary for the regulation of cellular pH over a narrow range (about 1 pH unit from neutrality). Three major changes have evolved to minimze the impact of changes in intracellular pH. These include (1) mechanisims to effux protons from the cell into the blood and/or regulate the influx of neutralizing ions into the cell (Koch et al. 1981), (2) utilization of the protons by metabolic processes so that the net proton production is matched by the net proton utilization (Krebs et al. 1975) and (3) absorbing protons by intracellular buffers (Parkhouse and McKenzie 1984).

These functionally different muscle fiber types are associated with a differential capacity for generating and consuming protons. In general, white muscle metabolism can be considered a net proton generator, whereas red muscle is not because the protons generated are stoichiometrically matched to proton consumption by mitochondrial oxidative phosphorylation (Krebs et al.

1975). This is one reason why white muscle is thought to have a higher buffering capacity than red muscle as was implied from earlier studies on a wide range of vertebrates (Castellini and Somero 1981). Although the major buffering components have rarely been adequately identified, a great deal of attention has focused on the imidazole moiety of histidine, particularly in dipeptides such as anserine and carnosine (Davey 1960; Somero 1981; Morris and Baldwin 1984; Parkhouse et al. 1985). However, the precise roles of histidine related compounds in vertebrate skeletal muscle buffering remains perplexing, in part because all species (Christman 1976; Castellini and Somero 1981; Morris and Baldwin 1984), including fishes (Abe 1981, 1983a, 1983b), show a wide variation in both their concentrations and their choice of dominant histidine related compounds.

The major buffering constituents have been classified into three components: physico-chemical buffering, consumption or production of non-volatile acids and transmembrane fluxes of protons or bicarbonate (Siesjo and Messeter 1971). The buffering capacity of in vitro preparations consists of the physico-chemical buffering component, which includes the buffering within the cell as a consequence of proton association with bases (Roos and Boron 1981). As such, most of the criticism surrounding the use of the crude homogenate titration method (in vitro) for assessing buffer capacity have inferred that it does not represent in vivo buffering. It neglects the transmembrane fluxes of protons and bicarbonate as well as metabolic reactions associated with enzymatic activity. Within human vastus lateralis muscle, Sahlin (1978) has suggested that bicarbonate could contribute as much as 15 to 18 percent (12 umol/g/pH) of total tissue buffer capacity in vivo during exercise. Whereas these criticisms may be true for most animals, fish white muscle may provide the exception due to its ability to retain lactate

and protons (Holeton et al. 1983; Turner et al. 1983; Milligan and Wood 1985) as well as possessing low bicarbonate levels (Heisler 1978). Conversely, fish red muscle, because of its higher bicarbonate content (Heisler 1978) and greater capillarization, would be subject to these same criticisms.

Within skeletal muscle, the major buffering constituents have been identified as protein (Bate-Smith 1938; Woodbury 1965), histidine related compounds (Davey 1960; Somero 1981) and inorganic phosphate (Burton 1978). Assuming either little or no proteolysis during short-term exercise, as is thought to be the case during high intensity, short duration swimming in fish (Driedzic and Hochachka 1978), the buffering constituents protein and histidine related compounds would contribute equivalent buffering power when assessed either in vivo or in vitro. However, the inorganic phosphate contribution to buffering would depend on its metabolic production during exercise. Using the fact that total buffer capacity is the sum of the individual buffer actions, a total in vivo buffer capacity can be estimated based on the sum of the physico-chemical buffering components plus the production or consumption of acids or bases. Similarily, the 1:1 stoichiometric relationship between lactate and protons allows an alternative in vivo buffer capacity value to be calculated based on the intramuscular pH change and lactate accumulation during exercise provided all the lactate is accounted for (Sahlin 1978).

Regulation of Metabolism

The regulation of metabolism in fish muscle appears to be similar to mammalian muscle. Fish muscles possess a full complement of enzymes for fermentative and oxidative metabolism, with the relative capacities of these

pathways varying between fiber types as expected (Crabtree and Newsholme 1972; Johnston 1977; Walton and Cowey 1982). Fish possess little adipose tissue and lipids are stored within the liver and muscle with red muscle stores being twice those of white muscle (Bone et al. 1966; Lin et al. 1974). ATP is stored in similar quantities to mammalian muscle (Driedzic and Hochachka 1978) but PCr levels were higher than most other animals. However, unlike mammalian muscle, fish red muscle contained an equivalent or even greater amount of endogenous glycogen than white muscle (Johnston 1981). Glycogen depletion and lactate accumulations during high intensity exercise in fish white muscle suggested an active glycolytic pathway (Driedzic and Hochachka 1976; Wokoma and Johnston 1977; Guppy et al. 1978; Driedzic et al. 1981). This pathway appeared to be under standard regulatory control similar to mammalian muscle (Driedzic and Hochachka 1976). The conversion of phos b to phos a was mediated by Ca^{++} (Yamamoto 1968) while PFK activity has been shown to be modulated by a variety of factors including adenylates (Freed 1971; Snudgen and Newsholme 1975; Newsholme et al. 1977) and fructose 2,6 bisphosphate (F2,6BP) (Dobson et al. 1986) but unlike mammalian muscle, lactate and proton efflux is slow from this tissue (Holeton et al. 1983; Turner et al. 1983; Milligan and Wood 1985). However, no information exists on the extent and control of glycolysis in red muscle, while most of the information gathered on white muscle was from studies conducted at very high intensity workloads. The purine nucleotide cycle appeared to be active within fish white muscle (Driedzic and Hochachka 1976) but the extent of its action in red muscle is unknown.

A great deal of evidence has suggested that adenine nucleotide metabolism plays a prominent role in metabolic regulation. It has been shown to be involved in; (1) mitochondrial respiratory control (Chance and Williams

1955, 1956; Slater et al. 1973; Jacobus et al. 1982); (2) the modulation of many glycolytic enzymes (Bloxham and Lardy 1973; Sols 1979,1981; Racker 1981); (3) phosphorylation and dephosphorylation reactions (Randle 1981); and (4) the coordination of myofibrillar ATPase activity with appropriate metabolic pathway functioning (Hochachka 1985). It has also been suggested that oxidative phosphorylation is the master process controlling the cytosolic redox state of the NAD couple as a consequence of the cytosolic phosphorylation potential (ATP/ADP.Pi) (Stubbs et al. 1972). In this regard, decreased oxygen tension at the mitochondrial level, as occurs during exercise in human muscle, is reflected in a more reduced cytosolic NAD couple (Sahlin 1985). Within fish muscle, large alterations in adenine nucleotides were found to accompany exercise (Driedzic and Hochacka 1976) and as such could potentially be involved in the regulation of energy metabolism through either direct effects on the above processes or indirectly via alterations in the cytosolic redox state of the NAD couple. As such, fish muscle presents an excellent model to study the design and regulation of fuel selection for exercise at various power outputs.

Locomotion and Muscle Function

Swimming in fish has been classified as sustained, prolonged or burst and refers to both the duration prior to fatigue and the type of swimming performance with velocity and tail beat frequency (TBF) being related to the length of the fish (Hoar and Randall 1978; Stevens 1979). In general, sustained swimming (SS) is characterized by long duration (greater than 200 minutes), low intensity, steady swimming at a constant TBF. Prolonged swimming (PS) refers to higher intensity swimming which is of greater than 20 seconds and less than 200 minutes duration. This type of swimming is

characterized by both steady (PSS) and unsteady (PUS) swimming with the degree of steady swimming being directly related to prior activity and duration while being inversely related to intensity. Burst swimming (BS) refers to very high intensity swimming which is of less than 20 seconds duration and is characterized by burst/glide patterns of propulsion (Hoar and Randall 1978; Stevens 1979).

The swimming velocity at which a fish can no longer maintain PS behavior has been identified as the critical swimming velocity (Ucrit) and it appears that red muscle can support locomotion up to 80 percent of this velocity. Above 80 percent, the mosaic muscle has been shown to be activated (Johnston et al. 1977b). As swimming velocity approached and exceeded this critical velocity, there appeared to be an increased reliance on white muscle (Johnston et al. 1977b; Bone 1978) which may at first be aerobic but which in the latter phases required increased glycolytic energy production. Therefore, this Ucrit appears to be analogous to maximal oxygen consumption whereby aerobic metabolism can support the energy needs up to approximately 80 percent of maximal oxygen uptake with the demands of higher work intensities being met by increased glycolytic functioning. Few investigations have examined metabolic energy provision during PS as the velocity surpasses the Ucrit in fish red and white muscle. However, electromyographic (EMG) evidence (Hudson 1973; Greer-Walker and Pull 1973; Kiceniuk 1975; Johnston et al. 1977b; Bone 1978) and training studies (Johnson and Moon 1980a,b) suggested that red muscle was recruited during SS with an increased reliance on white muscle as swimming intensity was increased (Bone 1978; Rome et al. 1984). These physiological data have been supported with biochemical investigations at sustained (Johnson and Goldspink 1973; Bilinski 1974) and at burst swimming velocities (Stevens and Black

1966; Dreidzic and Hochachka 1976; Wokoma and Johnston 1981). Thus, this velocity provides the opportunity to investigate the designs and constraints of energy provision during high intensity exercise within red and white muscle as the system is progressively stressed. However, when fish in a respirometer were subjected to a large increase in work intensity, there was an immediate recruitment of high energy phosphagen sources (Dobson et al. 1986) and propulsion is characterized by burst/glide patterns, until either fatigue or until the TBF is matched to work demands (Hoar and Randall 1978). To achieve steady swimming performance, the work increments must be small and of long enough duration to prevent any lapses into unsteady swimming behavior. This steady swimming therefore, provides a model which can be progressively stressed, instigating alterations in fuel selection which must conform to the structural and biochemical make-up of the animal.

Therefore the purpose of this investigation was to examine the metabolic interrelationships involved in energy provision and its regulation during exercise at different intensities. Specifically, fuel selection and its control by adenine nucleotides and cytosolic redox were investigated in red and white muscle of rainbow trout during progressive intensities of exercise. The control of glycolysis was examined with respect to glycolytic enzyme disequilibrium. The effects of substrate limitations and end product accumulations were examined with regard to fatigue under the different exercise intensities. Finally, the proton sequestering capacity and roles of various potential buffering constituents were investigated.

MATERIALS AND METHODS

ANIMALS

Metabolite, Tissue and Plasma Ion Studies. Rainbow trout (150 fish) weighing 200-250 grams, 26-28 cm. in length, were obtained from a single stock of a local supplier. They were kept in circulating (10 cm/s) dechlorinated tap water at 10-12°C for one month prior to the experiments and fed every other day with high protein trout pellets.

Histidine Related Compound Study. Rainbow trout and Pacific blue marlin were used for this investigation. The rainbow trout weighed 250-300 grams and were obtained from a local supplier. They were kept in dechlorinated tap water at 8-12°C for at least two months prior to the start of the experiment. During this time, they were fed every other day with high protein trout pellets. The Pacific blue marlin, weighing 50-100 kg were caught off Hawaii during the Annual Billfish Tournament in August 1983.

SWIMMING ASSESSMENT

All fish used in the metabolite, tissue and plasma ion studies were assessed for their swimming capabilities to obtain a homogeneous experimental population. During the month prior to the experiments, all fish were familarized with the respirometer and those demonstrating incorrect swimming behavior discarded. On a second occasion, all remaining fish were subjected to an exercise test to fatigue to assess their swimming capabilities. This test consisted of the following: a 5 minute period at 0.8 bodylengths/second

(BL/s) followed by 10 minute periods of 2.4, 2.6, 2.8 and 3.0 BL/s. This was immediately followed by a final period at 3.2 BL/s until fatigue. Fatigue was taken as an inability to maintain the given velocity and thus avoid the shock grid. The fish were grouped according to their swimming capacities and only those fish fatiguing after 3 minutes at 3.0 BL/s and before 4 minutes at 3.2 BL/s were retained. A total of 29 fish demonstrated swimming capacities within this range. Three animals from this group were assessed for their critical swimming velocity (Ucrit) (Brett 1964). Essentially this test consists of swimming the animals for 30 minutes at various incremental speeds (initial 2.0 BL/s; increments of 0.2 BL/s every 30 minutes) until the animal was unable to complete the 30 minute work interval at a given speed. Then the Ucrit was determined according to the formula:

$$Ucrit = Ui + (ti/tii \times Uii)$$

where Ui is the highest velocity maintained for the prescribed time, Uii is the velocity increment, ti is the duration of the fatigue velocity and tii is the prescribed period of swimming. The Ucrit was found to be 2.88±0.03 BL/s (mean±SE). All these exercise tests were completed at least one week prior to the initiation of the experiments.

EXPERIMENTAL PROTOCOL

Metabolite Studies. Prior to testing, fish were placed in a darkened plexiglass box maintained with dechlorinated tap water at 10±1°C overnight. Animals were transferred from this box to the respirometer in the dark. Struggling was minimal and frequently non-existent. All exercise tests were completed in 3 days and performed in low light between 1100 and 1600 hours. The experiment consisted of a 5 minute (27 cm/s; 1 BL/s) re-familarization period followed by a PSS of 30 minutes duration (PSS-30). This PSS-30

consisted of three 10 minute work intervals at 65, 70 and 75 cm/s (2.4, 2.6, 2.8 BL/s) respectively and was designed to maximally stress the red musculature without inducing unsteady swimming behavior. These velocities correspond to approximately 83, 90 and 97 percent of their Ucrit. The PSS-30 was immediately followed by a second prolonged steady swim at 85 cm/s (3.2 Bl/s; 108 percent of Ucrit) until this speed could not be maintained (mean±SE; 7.0±1.3 min) and will be referred to as PSS-7. This protocol allows direct comparison to the previous metabolic state (PSS-30), providing insights into the metabolic regulation of fuel selection associated with an increased power output, substrate depletion and fatigue. Immediately following this swim bout, fish were subjected to a prolonged unsteady swim bout which was designed to elicit exhaustion. To achieve this, they were made to swim at the maximal speed they could perform until they could no longer maintain a velocity (55 cm/s; 2 BL/s) equivalent to low aerobic swimming (mean±SE; 42±3 min). The fish demonstrated a swimming behavior of repeated bursts interspersed steady swimming and this bout will be referred to as the exhaustive swim (ES). This protocol was designed to allow direct comparison between different types of fatigue associated with the metabolic consequences of fuel and pathway selection to achieve appropriate energy turnovers. Fish were sacrificed prior to the exercise (pre-ex;PE; n=5) after the 30 minute PSS-30 (n=6), when the fish were unable to maintain 85 cm/s (PSS-7; n=5) or 55 cm/s (ES; n=5). Fish were quickly decapitated upon completion of the experimental protocol (usually less than 8 seconds) with liver, red and white muscle being rapidly excised. These tissues were immediately freeze clamped with stainless steel tongs cooled to the temperature of liquid nitrogen. White and red muscle were always obtained posterior to the caudal fin and frozen within 20 and 45 seconds, respectively. Livers were frozen within 40 seconds in all cases. Samples

were stored at -80°C until analysis.

Plasma Ion Study. Rainbow trout being used for this investigation were cannulated 24-48 hours prior to the experiment (n=5). A PE-50 polyethylene catheter was inserted into the dorsal aorta under MS-222 (tricaine methane sulfonate, 1:15,000 w/v solution) anaesthesia. Following cannulation, all fish were placed in a darkened plexiglass box maintained with dechlorinated tap water at 10+1 C until initiation of the experiment. Fish were transferred to the respirometer in the dark. The experiment consisted of a 5 minute, 27 cm/s re-familiarization period followed by a burst swim at 85 cm/s until the fish could not maintain this velocity and thus avoid the shock grid. Blood samples were obtained pre- and post exercise.

Histidine Related Compound Study. Five non-exercised rainbow trout were killed by decapitation with the red and white muscle being quickly excised. These tissues were then frozen in isopentane (2-methylbutane) cooled to the temperature of liquid nitrogen. This was to maintain the integrity of the proteins upon freezing. The tissues were stored at -80°C until analysis. Upon capture, the red and white muscle was quickly excised from five Pacific blue marlin, placed on ice and frozen at -80°C as soon as possible.

BIOCHEMICAL ANALYSES

Metabolite Extraction. Metabolite extraction was by a modification of the method of Bergmeyer (1974). Briefly, tissue sections were powdered under liquid nitrogen and added to ice cold 0.6 N perchloric acid (PCA) to a final dilution of 6 volumes (v/w). The PCA extracts were homogenized for 2 x 15 seconds at maximum speed with a tissue homogenizer (Polytron PT-10). Two 100

ul aliquots were immediately frozen in liquid nitrogen for later determination of glycogen. The remaining PCA extract was centrifuged for 3 minutes (white) and 5 minutes (red) at 11,000 g in a microcentrifuge (Eppendorf) at 4°C. The supernatant was neutralized with saturated trizma base (Tris) and immediately frozen in liquid nitrogen. The neutralized extracts were stored at -80°C until analysis (less than 48 hours).

Chromatography. High performance liquid chromatography (HPLC) was used to measure PCr, Cr, the nucleotides and histidine related compounds. For these determinations, three separate chromatographic procedures were employed on a HPLC system (Spectra Physics 8000B). Ultra-violet light absorbing compounds were monitored with a Spectraflow 773 (Kratos Analytical Instruments) detector unit. PCr, Cr and nucleotide separations were performed on a Brownlee (4.6 mm x 22 cm) Aquapore AX-300 column, 5 um particles with a Brownlee (4.6 mm x 3 cm) MPLC, AX-300, 10 um particle guard column. PCr, Cr, and nucleotide monophosphates were determined on a single run. A 2 ml/min (800 psi) isocratic flow rate was used with a 50 mM KH_2PO_4 (pH 3.0) mobile phase at 55 C with detection at 210 nm. The column was cleaned of bound di- and triphosphates once every hour with 600 mM KH_2PO_4 (pH 2.5), at a flow rate of 2 ml/min (800 psi) for 10 minutes. Regeneration was complete in 20 minutes. Nucleotide di- and triphosphates were determined with a gradient mobile phase and detection at 254 nm. Elution was isocratic at 55°C for 4 minutes using 50 mM KH_2PO_4 (pH 2.3) followed by a linear gradient (50-600 mM KH_2PO_4, pH 2.5) for 10 minutes. This was followed by a final isocratic mobile phase (600 mM KH_2PO_4, pH 2.5) for 10 minutes at a flow rate of 2 ml/min (1000 psi). Histidine related compounds were determined as described previously (Abe 1981) using a Zipax SCX column (0.21 x 50 cm), 10 ul sample loop and UV detection at 210 nm.

Elution was isocratic at 35°C for 10 minutes followed by a linear gradient (12-30 mM phosphate) at a flow rate of 1 ml/min. The coeficient of variation in analyses was always less than 5 percent.

Spectrophotometry. All determinations were performed on a Pye Unicam SP8-400 spectrophotometer in duplicate with standards being analyzed for each metabolite measured. The coefficient of variation in analyses was always less than 5 percent. The metabolites were measured enzymatically by linking them to reactions using NADH/NAD^+ or NADPH/$NADP^+$ and following the reaction at 340 nm. Labile phosphates were analyzed first. Unless otherwise stated, the assays used are essentially those of Bergmeyer (1974) with slight modifications. Inorganic phosphate (Pi) was determined colorimetrically by the method of Black and Jones (1983). Glycogen was digested according to the procedure of Keppler and Decker (1974) with standards being run to test the effectiveness of this procedure. Protein levels were determined using a modification of the method of Lowry et al. (1951). The modification incorporated solubilization of proteins with 1 percent sodium dodecyl sulfate. Taurine levels were determined on 1 ml. aliquots of the supernatants (HRC study). These supernatant fractions were deproteinized by the addition of 130 ul of 8 percent PCA. After centrifugation, aliquots were applied to a clean up column to remove other amino acids (Stabler and Siegal 1981) and the elutate and three 1 ml washings (water) collected. The combined elutates and washings were neutralized with 2N KOH and aliquots of this solution analyzed for taurine by the phtalaldehyde-urea method.

Calculated Metabolite Concentrations. The NMR studies have found PCr content to be 85 (Meyer et al. 1982; Meyer et al. 1985; Shoubridge et al. 1984) and 68 (Meyer et al. 1982; Meyer et al. 1985) percent of total PCr plus Cr at

rest in white and red muscle respectively. White and red muscle PCr, Cr and Pi contents were compensated for on this basis, with the change in PCr content being considered to be similar under the different exercise loads in a specific tissue type until ATP content was found to demonstrate a large decline. These compensated values were then used to calculate a minimum free ADP content in each fiber type.

Metabolite concentrations which occur at too low levels and/or as free/bound compounds can be calculated from their equilibrium constants (Keq) or by a combination of equilibrium constants using easily measured metabolites provided the reaction(s) are in equilibrium. Equilibrium constants are calculated under standard conditions at pH 7.0 and are known to be affected by many factors including ionic strength, temperature, free Mg^{++} and pH. In these investigations, with the exception of temperature effects on the LDH reaction, only the effects of pH on the equilibrium constants were considered as any attempt to correct for all these variables would lead to substantial error. The use of the Gibbs Free Energy equation:

$$\Delta G^{\circ\prime} = -RT \ln Keq^{\circ\prime}$$

allows estimation of a new Keq " at a different pH. To calculate a new Keq " the Gibbs Free Energy equation can be rewritten:

$$\frac{\Delta G^{\circ\prime\prime} - \Delta G^{\circ\prime}}{RT} = \ln \frac{(1+Ks/1x10^{-7})}{(1+Ks/\Delta H^{+})} - \ln \frac{(1+Kp/1x10^{-7})}{(1+Kp/\Delta H^{+})}$$

where: $Ks = H^{+}$ dissociation constant of the substrates

$Kp = H^{+}$ dissociation constant of the products

upon rearranging:

$$\Delta G^{\circ\prime\prime} = RT \ln \frac{(1+Ks/1x10^{-7})}{(1+Ks/\Delta H^{+})} - \ln \frac{(1+Kp/1x10^{-7})}{(1+Kp/\Delta H^{+})} + \Delta G^{\circ\prime}$$

therefore: $\Delta G^{\circ\prime\prime} = -RT \ln Keq^{\circ\prime\prime}$ at the new pH

upon rearranging: $\ln Keq°" = -\Delta G°"/RT$

$$Keq°" = 1/\ln Keq°"$$

The LDH reaction was also corrected for temperature since a Keq was available at 16 C ($Keq = 1.11 \times 10^{-12}$) (Hakala 1956) and the measured enthalpy of the reaction (Curtin and Woledge 1978) gave a similar value at 10 C suggesting that the enthalpy was a linear relationship for this reaction. Temperature was corrected for by the use of the Gibbs-Helmholtz equation:

$$\log Keq = - H/2.3RT + C$$

where: H=enthalpy, R=gas constant, T=temperature (°K) and C is a constant.

The apparent equilibrium constants of the PGK, CK and PK reactions are Mg^{++} independent above approximately 1 mM concentrations.

The equilibrium constants under standard conditions and at pH 6.5 are given in Table 1. These values were calculated from the H^{+} dissociation constants found in Table 2.

Table 1. Definitions and values of equilibrium constants.

Apparent Constant	Keq pH 7.0	pH 6.5
$K_{CK} = \frac{(ATP)(Cr)}{(ADP)(PCr)(H^+)}$	$1.66 \times 10^9\ M^{-1}$	$5.25 \times 10^9\ M^{-1}$
$K_{HK} = \frac{(G6P)(ADP_f)}{(Glu)(ATP)}$	5.5×10^3	
$K_{PGM} = \frac{(G6P)}{(G1P)}$	0.055	0.057
$K_{PGI} = \frac{(F6P)}{(G6P)}$	0.430	0.430
$K_{PFK} = \frac{(FDP)(ADP_f)}{(F6P)(ATP)}$	800	822
$K_{Ald} = \frac{(DHAP)(GAP_f)}{(FDP)}$	1×10^{-4}	1.92×10^{-4}
$K_{TPI} = \frac{(DHAP)}{(GAP_f)}$	12	9.3
$K_{GPDH} = \frac{(DPG)(H^+)}{(GAP_f)(Pi)}$ NADH/NAD	0.51×10^{-7}	1.13×10^{-7}
$K_{PGK} = \frac{(ATP)(3PG)}{(ADP_f)(DPG)}$	3.6×10^3	1.79×10^3
$K_{GPDH.PGK} = \frac{(ATP)(3PG)(H^+)}{(ADPf)(GAPf)(Pi)}$ NADH/NAD	1.83×10^{-4}	2.02×10^{-4}
$\frac{K_{GPDH.PGK}}{K_{LDH}} = \frac{(3PG)(ATP)(Lac)}{(GAP_f)(ADP_f)(Pi)(Pyr)}$	$1.68 \times 10^6\ M^{-1}$	$0.59 \times 10^6\ M^{-1}$
$K_{PGM} = \frac{(2PG)}{(3PG)}$	0.170	0.219
$K_{enol} = \frac{(PEP)}{(2PG)}$	3.0	2.59
$K_{PK} = \frac{(ATP)(Pyr)}{(ADP_f)(PEP)}$	2.98×10^4	2.04×10^4
$K_{LDH} = \frac{(Pyr)(NADH)(H^+)}{(Lac)(NAD)}$	$1.09 \times 10^{-12}\ M$	$3.44 \times 10^{-12}\ M$

Values are from Burton (1957), Noltman (1972), Bohme (1975), Cornell et al. (1979), Veech et al. (1979), Connett (1985).

Table 2. H^+ dissociation constants of histidine related compounds, glycolytic intermediates, inorganic and organic phosphates.

Compound	pK	kd
Pi^{2-}	6.81	1.55×10^{-7}
$MgATP^-$	5.21	6.17×10^{-6}
ATP^{3-}	6.95	1.12×10^{-7}
$MgADP^-$	5.30	5.01×10^{-6}
ADP^{2-}	6.78	1.66×10^{-7}
AMP^-	6.45	3.55×10^{-7}
PCr^{2-}	4.50	3.16×10^{-5}
Cr	4.10	8.00×10^{-5}
IMP^-	6.25	5.62×10^{-7}
NH_3	9.50	3.16×10^{-10}
Histidine	6.00	1.00×10^{-6}
Anserine	7.03	9.33×10^{-8}
Carnosine	6.83	1.48×10^{-7}
G1P	6.13	7.41×10^{-7}
G6P	6.11	7.76×10^{-7}
F6P	6.11	7.76×10^{-7}
FDP	5.95,6.15	1.12×10^{-6},7.08×10^{-7}
DHAP	6.52	3.02×10^{-7}
GAP	6.82	1.51×10^{-7}
DPG	7.40,7.99	3.98×10^{-8},1.02×10^{-8}
3PG	6.20	6.31×10^{-7}
2PG	6.65	2.24×10^{-7}
PEP	6.40	3.98×10^{-7}
PYR	2.55	2.82×10^{-3}

La	3.66	2.19×10^{-4}

Values are from Bate-Smith 1938; Phillips et al. 1965; Alberty 1969; Curtin and Woledge 1978; Gadian et al. 1981.

The concentration of GAP in tissues is very low and similar in magnitude to the concentration of enzymes for which it is a substrate (Veech et al. 1979; Ottaway and Mowbray 1977). Thus the amount of bound and free cytosolic GAP must be calculated. Estimates of free GAP have been made using the measured DHAP, which has been shown to represent the free cytosolic concentration (Veech et al. 1979; Connett 1985) and the Keq of TPI which has been determined in vivo (Connett 1985) and corrected for pH changes (Table 1).

$$KTPI=(DHAP)/(GAP)$$

Though total cytosolic ADP concentration is easily measured the amount considered to be free is much lower (Veech et al. 1979; Jacobus et al. 1982; Shoubridge et al. 1984; Meyer et al. 1985). Estimates of free ADP have been made by using the Keq of the CK reaction (Table 1).

$$K_{CK}=(ATP)(Cr)/(ADP)(PCr)(H^+)$$

Free ADP was calculated using the measured (ADP_{fm}) and compensated (ADP_{fc}) contents of the reactants. All metabolite values measured are reported as umol/g w/w of tissue. In order to make the metabolite ratio's directly comparable to the Keq's measured in vitro, all metabolite values were converted to umol/g cell water. The percentage of tissue weight taken to be cell water was 78 percent under all conditions. Percent water was assessed on pre and post freeze dryed, freeze clamped white muscle (Table 3). This value was very similar to previous values determined for liver and muscle (Krebs and Veech 1969).

Table 3. Cell water content of rainbow trout white muscle.

	Pre-ex	PSS-30	PSS-7	ES
n	(5)	(6)	(5)	(5)
Cell Water	77.8 +0.5	77.6 +0.3	77.7 +0.6	78.3 +0.5

Values are means+SE expressed as percent.

Energy Turnover. Estimated energy turnovers (umol ATP/g/min) from fats, glycolytic and high energy phosphagen sources were calculated for rainbow trout red and white muscle during the PSS-30 and PSS-7. Glucose fermentation contribution was based on lactate production not attributable to muscle glycogen fermentation with 2 ATP being generated per glucose unit fermented. Glucose oxidation contribution was based on liver glycogen and blood glucose utilization (PSS-30=3.7 umol/ml; PSS-7=3.7 umol/ml) not accounted for by glucose fermentation with 36 ATP being generated per glucose unit oxidized. Blood glucose values are estimated from a previous investigation (Dobson, Mommsen and Hochachka, unpublished observations) where blood glucose values decreased from 19.1 to 8.2 umol/ml with an exhaustive exercise regimen similar to the present one. The glycogen fermentation contribution was based on the change in muscle glycogen accounted for by lactate production with 3 ATP being generated per glucosyl unit fermented. The glycogen oxidation contribution was based on the change in muscle glycogen not accounted for by lactate production with 37 ATP being generated per glucosyl unit oxidized. Fat oxidation contribution was based on the oxygen uptake data of Randall and Daxdoek (1979) assuming a linear relationship between workload and oxygen uptake with Ucrit being equivalent to maximal oxygen uptake. The muscle oxygen uptake was converted to moles of O2 by:

$$PV=nRT$$

where P=140 mmHg at 10°C, V=0.00371 l, R=0.08205 l atm deg^{-1} mol^{-1} and T=283°K. Since 1 mole of O2 produces 6 mol ATP, an energy turnover due to oxidation of substrate can be calculated for muscle. The energy due to combustion of fats was assumed to be equal to the difference between the total muscle oxidation derived ATP turnover and the red plus white muscle glucose plus glycogen oxidation derived ATP turnover. Values are based on a 200 g fish which would contain 132 g white muscle, 2 g red muscle and 2 g liver (Randall and Daxboeck 1982). It is assumed that only half the red or white muscle is active at any time. The stored elastic component was not taken into account. Blood volume was assumed to be 5 ml/100g (Stevens 1968) with 83 and 9.4 percent of this volume during exercise being distributed within white and red muscle respectively (Neumann et al. 1983). The red muscle was assumed to have a 20 fold higher capacity to oxidize fats/proteins than white muscle based on maximal enzyme activities (Crabtree and Newsholme 1972) and free fatty acid oxidation rates (Bilinski 1963; Jonas and Bilinski 1964).

Intramuscular pH. Tissue sections were powdered under liquid nitrogen and added to an ice cold salt solution (pH 7.2) containing in mmol/l: 145 KCl, 10 NaCl, 1 iodoacetic acid (IAA), 20 NaF^-, 5 EGTA, 5 DNFB and 1 PMSF in a 1:10 dilution (v/w). This solution had a very low buffering capacity. These extracts were homogenized for 2 x 15 seconds at maximum speed with a polytron PT-10 tissue homogenizer. The suspensions were then centrifuged in an eppendorf microcentrifuge at 11,000 g for 30 seconds. Aliquots of this supernatant were used for pH determination at 10°C on a digital acid-base analyzer (Radiometer PHM 72) equipped with a microelectrode unit (Radiometer Type E 5021). Validity of this technique was ascertained by comparison with

pH values determined on the suspensions. All determinations were performed in duplicate.

Cytosolic Redox Potential. The free cytosolic redox potential was calculated from the temperature and pH adjusted Keq of the LDH reaction as outlined by Williamson et al. (1967).

$$K_{LDH}=(Pyr)(H+)/(lac) \times (NADH)/(NAD)$$

Upon rearranging:

$$\frac{NAD}{NADH} = \frac{(Pyr)(H+)}{(Lac)K_{LDH}}$$

Equilibrium/Nonequilibrium. Mass action ratios were calculated and divided by their respective equilibrium constants for the glycolytic reactions under all exercise states in white and red muscle. A plot of the log of these results (log (mass action ratio/Keq)) was used to demonstrate the amount of deviation from equilibrium for a given reaction.

Regulatory Enzymes. The crossover theorem provides a method for localizing interactions or regulatory sites in complex enzyme systems (Williamson 1969). For an enzyme to be regulatory, it must be nonequilibrium and demonstrate a change in substrate in the opposite direction to flux. However, caution must be exercised when interpreting crossover plots as errors in the identification of regulatory enzymes can occur due to modulators (Williamson 1969; Rolleston 1972). Crossover plots are presented for the glycolytic intermediates of red and white muscle under all exercise conditions.

Ion Analyses, Distribution and Differences. Blood samples were centrifuged in a microcentrifuge (Eppendorf) at 11,000 g to separate the plasma. An aliquot of this plasma was deproteinized with 200 ul of 0.6 N PCA and centrifuged at 11,000 g for lactate determinations. The remaining plasma was

frozen in liquid nitrogen and stored at -80°C for determination of Na^+, K^+, Ca^{++}, Mg^{++} and Cl^-. Aliquots of muscle extracts were also analyzed for these ions with the Tris/PCA contamination being taken into account. Na^+ and K^+ were analyzed by with a flame photometer. Ca^{++} and Mg^{++} levels were determined with an Atomic Absorption Spectrophotometer. Measurement of Cl^- was essentially by the method of Ramsay (1955) employing titration with silver nitrate. Cellular and extracellular ion concentrations were calculated according to the procedure of Macchia and Polimini (1982). This procedure requires four variables: tissue ion content, plasma ion concentration, wet and dry tissue weights. The extracellular compartment was assumed to be 94.1 ml/kg (Milligan and Wood 1985). The ion difference was the difference between the concentrations of cations and anions measured in mEq/l (Stewart 1981). In this investigation the ion difference was defined as:

$$IDe = (Na^+)+(K^+)+(Ca^{++})/2+(Mg^{++})/2-(Cl^-)-(La^-)$$

$$IDw=(Na^+)+(K^+)+(Ca^{++})/2+(Mg^{++})/38-(Cl^-)-(La^-)-(PCr^{2-})$$

$$IDr=(Na^+)+(K^+)+(Ca^{++})/2+(Mg^{++})/20-(Cl^-)-(La^-)-(PCr^{2-})$$

The concentrations of plasma divalent cations Ca^{++} and Mg^{++} and intracellular Ca^{++} were divided by 2 assuming that 50 percent were bound to molecules such as protein (Jackson and Heisler 1982). The content of cellular Mg^{++} was divided by 38 (white) or 20 (red) respectively to compensate for the amount bound by intracellular proteins (assuming a free Mg^{++} of approximately 1 mM; Velsco et al. 1973; Connett 1985). It was assumed that during exercise, the content of protein bound divalent cations remained constant. Any change in Ca^{++} or Mg^{++} was therefore in the form of free ions and/or Ca- or Mg- lactate complexes (Cannon and Kibrick 1938). This assumption was based on the counteracting effects of acidosis and hypercalcemia (Herbert and Jackson 1985). Ca- and Mg-lactate complexes

do not affect the ion difference calculation.

BUFFERING ANALYSES

Tissue Fractionation. For the histidine related compound study, red and white muscle were separated into a series of fractions with each fraction containing one less buffering constituent than the preceeding fraction. To achieve this, a crude homogenate (CH) was prepared by homogenizing (Polytron PT-10) 2-3 grams of muscle tissue in 10 volumes of ice cold salt solution containing (in mM) 145 KCl, 10 NaCl and 5 iodoacetic acid. The homogenate was centrifuged at 0°C for 20 minutes at 48,000 g. The pellet was homogenized in the same volume and processed as above. This procedure was repeated twice and the washings and supernatant (SN) were combined. The pellet was retained and resuspended in 10 ml. of salt solution. Aliquots of 5-10 ml. of the combined supernatants (SN plus washings) were applied to a Sephadex G-25 (coarse) column (1.9 x 25 cm.) and eluted at room temperature with salt solution. Elution of the high molecular weight fraction (HMW) was followed at 280 nm; for the low molecular weight fraction 260 nm was used as the indicator. The collected fractions were stored at 4°C and analyzed as soon as possible (less than 24 hrs).

Histidine Related Compounds. Method A. Tissue extracts were prepared with slight modifications according to Stein and Moore (1958). Briefly, 1 g of tissue was homogenized (Polytron PT-7) in 5 ml of picric acid. After centrifugation, the tissue was resuspended in 5 ml of picric acid and the procedure repeated. The supernatants were combined and applied to a Dowex 2X8 (200-400 mesh, Cl^- form) column (0.5 x 1.5 cm.). The column was washed with 0.01 N HCl and the elutate stored at 4°C. The elutates were

evaporated to dryness and brought to 2 ml (trout) and 10 ml (marlin) with high performance liquid chromatography (HPLC) grade water.

Histine Related Compounds. Method B. One gram of marlin white muscle was homogenized with 5 ml. of 8 percent PCA and centrifuged. The precipitate was homogenized in the same volume of PCA and centrifuged. The combined supernatants were neutralized with 2 N KOH and stored at 4°C as protein-free extracts. The supernatant was applied to a 2.5 x 10 cm. copper-sephadex G-25 (fine) column (prepared by equilibrating the Sephadex overnight in 0.08 M copper sulphate) to specifically bind histidine related compounds. The column was washed with water and the eluate, assumed to contain other amino acids and phosphates, was collected. The histidine related compounds were eluted with 0.01 N HCl. Both fractions were stored at 4°C. The concentrations of histidine related compounds were determined as described previously (above). The content of histidine related compounds of the marlin white muscle PCA extracts were similar to that of the picric acid extracts suggesting comparability of the two methods.

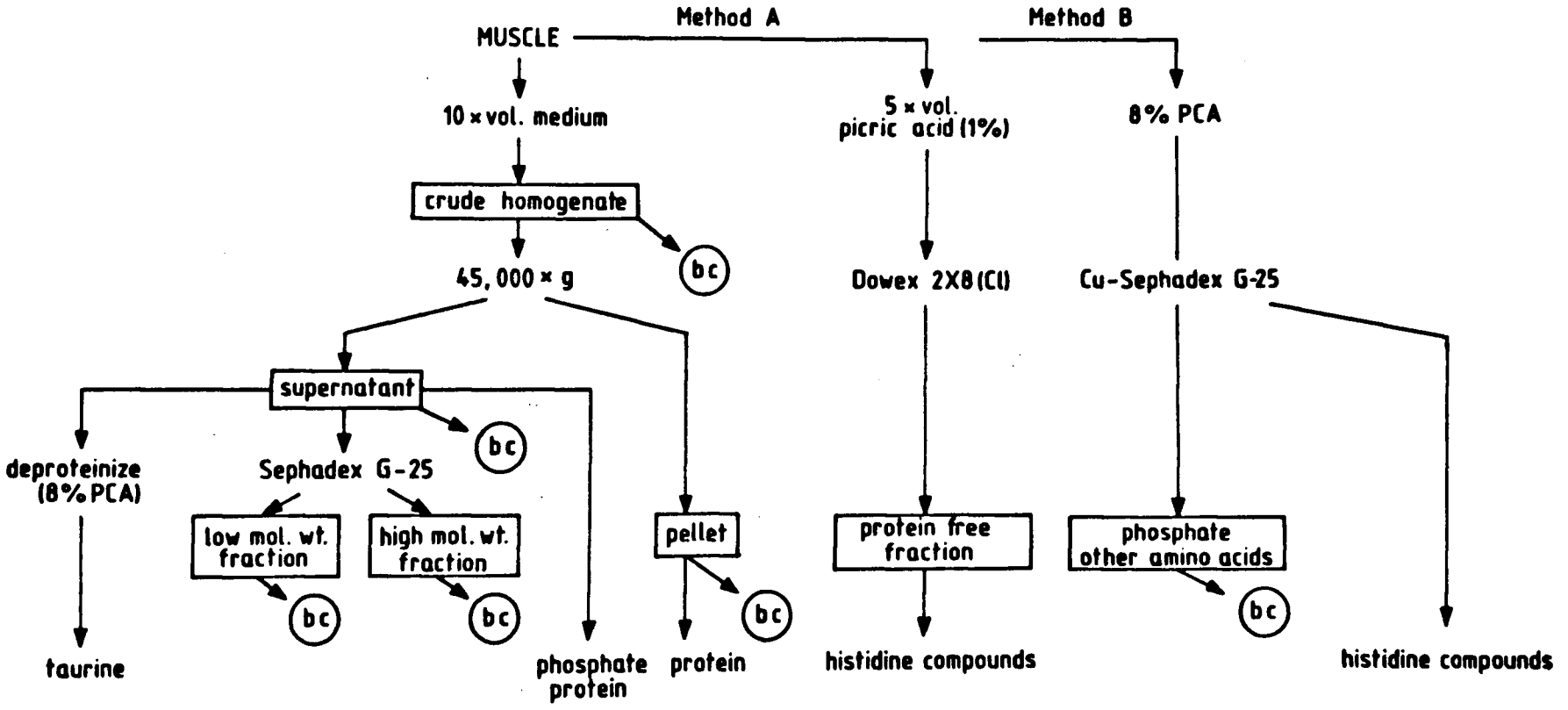
Buffer Capacity. Buffer capacities were determined on all isolated fractions. To further assess the contribution of histidine related compounds to total cellular buffering, salt solutions containing the experimentally determined marlin white muscle tissue levels of histidine related compounds were prepared. In the same way, representative taurine concentrations (see below) were also prepared. These salt solutions containing their respective histidine related compounds or taurine levels were empirically titrated for the calculation of their buffer capacities. Buffer capacity was assessed by a modification of the method of Davey et al. (1960). All preparations were adjusted to pH 6.00±0.05 and titrated to pH 8.00±0.05 with NaOH. Reliability

was ascertained by retitration of the extracts following pH readjustment to 6.00±0.05. Buffer capacity was determined as the number of OH ions required per gram of tissue to change the pH 1 unit. Buffer capacities were determined over the pH ranges 6.0-7.0, 6.5-7.5 and 7.0-8.0. A schematic representation of the histidine related compound study is contained in Figure 1. Alternatively, an in vivo estimate of trout white muscle buffer capacity was calculated during the PSS-7. This was achieved by assuming a 1:1 stoichiometric relationship between lactate and H+. By knowing the change in intramuscular pH, the buffer capacity was calculated as:

$$B = La^{-}/\Delta pH$$

As well, a further estimate of trout white muscle buffering was made by calculating the buffering due to association of H^{+} with bases and the buffering due to enzymatic activity.

Figure 1. Fractionation scheme used to separate the different tissue components. bc, measurement of buffer capacity.


MUSCLE
Method A
Method B
10 × vol. medium
5 × vol. picric acid (1%)
8% PCA
crude homogenate
bc
45,000 × g
Dowex 2X8(Cl)
Cu-Sephadex G-25
supernatant
bc
deproteinize (8% PCA)
Sephadex G-25
low mol. wt. fraction
high mol. wt. fraction
pellet
protein free fraction
phosphate other amino acids
bc
bc
bc
bc
taurine
phosphate protein
protein
histidine compounds
histidine compounds

RESULTS

FUEL SELECTION

The metabolic alterations associated with the various work intensities in liver, white and red muscle are illustrated in Figure 3 and outlined in detail in Tables 4, 5 and 6 respectively.

30 Minute Prolonged Steady Swim (PSS-30)

The results are presented relative to pre-exercise values.

Phosphagen and Nucleotide Metabolism. During the 30 minute prolonged steady swim, red muscle demonstrated a high rate of phosphagen and nucleotide metabolism relative to white muscle (Figure 3). Large decrements in PCr (96 percent) and ATP (40 percent) were observed for red muscle while changes in these metabolites were minor in white muscle. Associated with these alterations were increases of 1.8 and 1.3 fold in red muscle ADP and AMP levels respectively. These changes resulted in a decrement of 40 percent in the total adenylate pool (Figure 2) with the IMP increases (1.5 fold) accounting for the decrease in adenine nucleotides (Figure 2). White muscle total adenylate pool remained relatively constant (Figure 2) despite increases of 80 percent in IMP content. GTP levels fell approximately 40 percent in both tissues while Pi increased 4 and 8 fold in white and red muscle, respectively.

Glycogenolysis. The 30 minute prolonged steady swim resulted in an active recruitment of red fibers motor units as evidenced by the large decline in

stored glycogen (97 percent) to very low levels (0.6 umol/g). As well, white muscle glycolytic energy provision and to a far lesser degree, liver derived glucose, contributed to the energy requirements of this workload (Figure 3). White muscle glycogen content was found to have decreased 31 percent while liver glycogen had declined 7.3 umol/g. Associated with these declines in liver glycogen, were increases of 80 and 230 percent in white and red muscle glucose contents. The anaerobic glycolytic end product lactate was found to have increased 340 and 70 percent in white and red muscle respectively. These increases in lactate, were less than would be expected, had all the glycogen been fermented anaerobically.

Krebs Cycle Intermediates. Malate and fumarate levels increased dramatically in both tissue types exhibiting, increases of 1.3 to 4.5 fold. However, citrate levels were found to remain relatively stable during this workload.

7 Minute Prolonged Steady Swim (PSS-7)

Under the conditions of this experiment, all animals had performed the PSS-30 immediately prior to this exercise intensity. The results are therefore presented relative to the PSS-30 metabolite concentrations unless otherwise specified.

Phosphagen and Nucleotide Metabolism. During the higher intensity (108 percent Ucrit) 7 minute prolonged steady swim which followed the PSS-30, white muscle high energy sources were actively recruited while red muscle PCr and ATP appeared to initiate replenishment. Within white muscle, PCr content was found to have decreased 62 percent (13 umol/g) to 20.8 umol/g, 55 percent lower than the pre-exercise value. This corresponded to an average rate of

PCr depletion (1.86 umol/g/min) which was 14 fold greater than the PSS-30 rate. ATP levels had decreased a further 11 percent resulting in the ATP content being reduced 20 percent from pre-exercise levels. ADP levels were increased 1.6 fold to 1.15 umol/g. AMP content increased 1.9 fold such that its concentration was now 3.5 times its pre-exercise levels. A 3.3 fold elevation in IMP content to 1.8 umol/g was observed with this level of IMP being 6 times the pre-exercise value. GTP content remained at a constant value 35 percent lower than its pre-exercise concentrations. Inorganic phosphate and NH4 contents were elevated 3.3 (Table 4) and 2.2 fold respectively such that these values were 12.9 and 3.1 times their pre-exercise levels. In contrast, red muscle PCr values, although remaining 94 percent below pre-exercise values were found to have increased 1.6 fold while ATP levels were elevated 12 percent. Associated with these alterations were decreases in ADP of 20 percent, increases in AMP of 50 percent and increases of 18 percent (3 umol/g) for inorganic phosphate. IMP values increased 20 percent bringing the total increase to 7.2 times its pre-exercise value. GTP content increased 50 percent (0.016 umol/g) to a value only 17 percent less than pre-exercise levels.

Glycogenolysis. The higher intensity 7 minute prolonged steady swim resulted in a white muscle glycogen content decrement of 65 percent to 5.6 umol/g, a value 75 percent lower than its pre-exercise concentration. The average rate of glycogen depletion (1.49 umol/g/min) exceeded the PSS-30 rate by 6.2 fold. Lactate concentration increased 3.3 fold to 33 umol/g, an 11 fold elevation from pre-exercise values with its rate of accumulation being 25 times the PSS-30 rate. In red muscle, glycogen content remained low (0.5 umol glucosyl units/g) while lactate and glucose decreased 17 (1.5 umol/g) and 38 (1.27 umol/g) percent respectively. Glucose levels still remained 45 percent

higher than pre-exercise concentrations. During the PSS-7, liver glycogen values decreased 11 percent (18.6 umol/g), a depletion amount 3 times greater than was achieved during the PSS-30. The final glycogen values were 15 percent (25.9 umol/g) less than pre-exercise content. This depletion of liver glycogen occurred at a mean rate (2.66 umol/g/min) 11 times greater than during the PSS-30. Associated with this decline in liver glycogen, were increases of 2.4 fold to 11 umol/g in liver glucose, a value 3.2 times its pre-exercise concentration. Despite this increase in availability of blood glucose, white muscle glucose levels remained relatively constant.

Krebs Cycle Intermediates. During the PSS-7, both white and red muscle demonstrated increased contents of malate, fumarate and citrate such that these intermediates were now elevated 1.3 to 6 fold above pre-exercise levels.

Exhaustive Swim (ES)

The ES protocol immediately followed the PSS-30 and PSS-7 exercise regimens. The results are therefore presented relative to the PSS-7 metabolite contents unless otherwise specified.

Phosphagen and Nucleotide Metabolism. The exhaustive swim resulted in a further recruitment of white and red muscle high energy sources. In white muscle, PCr was found to have decreased 87 percent with this depletion resulting in PCr levels at exhaustion being only 5 percent (1.8 umol/g) of the pre-exercise concentration. ATP levels decreased 54 percent such that ATP content was reduced 65 percent (4.61 umol/g) from pre-exercise levels. No significant change was found in ADP content but AMP values increased 2

fold; ADP and AMP being 1.5 and 7.2 times their pre-exercise levels. IMP increased 2.4 fold bringing the total elevation to 14.5 fold (4.3 umol/g). The total adenylate pool decreased 50 percent but the total adenylate plus IMP pool was unchanged (Figure 2). NH_4^+ concentration was increased 2 fold such that ES levels (6.37 umol/g) were elevated 6.4 times pre-exercise values. Phosphate levels increased 88 percent (26 umol/g) while GTP levels were decreased 19 percent (0.007 umol/g). GTP content was depressed 40 percent (0.024 umol/g) from pre-exercise levels. In red muscle, further decrements in PCr (67 percent) and ATP (31 percent) occurred during this work intensity (ES) with final concentrations being 2 (0.4 umol/g) and 46 (1.6 umol/g) percent of pre-exercise values respectively. Associated with these changes were increases in ADP (32 percent), AMP (55 percent) and inorganic phosphate (6 percent) (Table 5) resulting in levels 1.9, 3.0 and 9.9 times their pre-exercise values. IMP content increased 77 percent to a final concentration (2.33 umol/g) 3.2 fold greater than its pre-exercise level. The total adenylate pool was decreased 45 percent but the total adenylate plus IMP pool was unchanged from pre-exercise values (Figure 2). GTP levels were decreased 31 percent (0.015 umol/g) with the final content (0.034 umol/g) being 42 percent lower than its pre-exercise value.

Glycogenolysis. White and red muscle glycogen contents declined to values less than 1 percent of their pre-exercise concentrations (Tables 4 and 5). Associated with these declines in glycogen were increases in white and red muscle lactate levels. White muscle lactate concentration increased 1.3 fold to 43 umol/g, a 14.3 fold increase from pre-exercise content. In red muscle, lactate content increased 50 percent to 10.8 umol/g, a value 2.1 times the pre-exercise level. Liver glycogen decreased 23 percent (36.6 umol/g) while glucose levels remained constant. Final glycogen concentrations were

decreased 34 percent (62.5 umol/g) and glucose levels increased 320 percent of their respective pre-exercise values. White and red muscle glucose contents remained relatively constant despite the large increase in glucose availability. However, the white muscle lactate levels observed, were greater than would have been expected from the amount of glycogen fermented suggesting that some lactate must have been derived from glucose.

Krebs Cycle Intermediates. In both white and red muscle, malate and fumarate contents were found to be elevated substantially from pre-exercise levels (3 to 10 fold). However, citrate contents were found to be equivilent to pre-exercise values for both tissue types.

Table 4. Content of metabolites and pH in rainbow trout white muscle.

Metabolite n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
PCr	19.9±1.6	15.9±0.9	2.9±0.7	1.8±0.6
PCr_c	37.8±1.6	33.8±0.9	20.8±0.7	1.8±0.6
Cr	24.6±1.0	29.7±1.4	42.7±0.2	43.4±1.8
Cr_c	6.7±0.3	11.8±1.4	24.8±0.2	43.4±1.8
ATP	7.26±0.11	6.57±0.22	5.82±0.5	2.65±0.25
ADP	0.70±0.01	0.68±0.04	1.15±0.07	1.05±0.05
AMP	0.021±0.001	0.039±0.007	0.073±0.012	0.152±0.035
Pi	21.1±2.6	26.8±1.9	47.6±3.2	55.7±1.8
Pi_c	2.3±0.4	8.9±1.4	29.7±2.8	55.7±1.8
IMP	0.30±0.05	0.53±0.09	1.78±0.27	4.34±0.26
NH_4^+	1.04±0.05	1.43±0.15	3.20±0.42	6.37±0.19
GTP	0.054±0.012	0.039±0.003	0.037±0.004	0.030±0.007
Glucose	1.02±0.14	1.86±0.55	2.19±0.51	2.16±0.44
Glycogen	23.3±1.0	16.0±1.6	5.6±1.1	0.2±0.04
Lactate	3.0±0.4	10.1±1.1	33.0±0.6	42.9±3.0
Malate	0.13±0.07	0.25±0.07	0.25±0.03	0.39±0.06
Fumarate	0.01±0.002	0.04±0.01	0.06±0.01	0.08±0.01
Citrate	0.30±0.01	0.28±0.02	0.35±0.05	0.28±0.03
pH	6.97±0.04	6.93±0.03	6.65±0.03	6.56±0.04

Values are means ± SE expressed in umol/g (w/w). Glycogen was calculated in glucose units. c, compensated metabolite.

Table 5. Content of metabolites and pH in rainbow trout red muscle.

Metabolite n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
PCr	5.2±0.7	0.8±0.4	1.2±0.2	0.4±0.2
PCr_c	18.6±0.8	0.8±0.4	1.2±0.2	0.4±0.2
Cr	22.1±1.0	26.5±0.8	26.0±1.9	26.5±0.3
Cr_c	8.8±0.4	26.5±0.8	26.0±1.9	26.5±0.3
ATP	3.43±0.18 d	2.02±0.27	2.26±0.32	1.57±0.32
ADP	0.65±0.07	1.18±0.08	0.84±0.04	1.11±0.17
AMP	0.106±0.012	0.137±0.014	0.207±0.023	0.321±0.055
Pi	14.1±1.2	21.1±2.9	21.5±2.4	22.7±2.1
Pi_c	2.3±1.3	21.1±2.9	21.5±2.4	22.7±2.1
IMP	0.73±0.11	1.09±0.18	1.31±0.15	2.33±0.28
NH_4^+	1.60±0.14	1.57±0.16	1.71±0.08	2.88±0.30
GTP	0.059±0.003	0.033±0.007	0.049±0.002	0.034±0.003
Glucose	1.46±0.19	3.38±0.55	2.11±0.14	2.95±0.05
Glycogen	18.1±2.5	0.6±0.3	0.5±0.2	<0.1
Lactate	5.2±0.8	8.8±1.1	7.3±0.9	10.8±1.4
Malate	0.20±0.10	0.32±0.03	0.40±0.05	0.65±0.1
Fumarate	0.03±0.01	0.04±0.01	0.08±0.02	0.08±0.02
Citrate	0.40±0.05	0.34±0.05	0.44±0.05	0.44±0.09
pH	6.89±0.02	6.92±0.04	6.88±0.02	6.81 (1)

Values are means ± SE expressed as umol/g (w/w). Glycogen was calculated in glucose units. c, compensated metabolites.

Table 6. Content of metabolites in rainbow trout liver.

Metabolite n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
Glycogen	183.6±35.6	176.3±34.1	157.7±36.8	121.1±8.5
Glucose	3.53±0.85	4.69±0.51	11.14±1.22	11.44±1.12
G6P	0.12±0.03	0.20±0.04	0.77±0.06	0.82±0.07
F6P	0.02±0.01	0.04±0.01	0.12±0.01	0.14±0.02
2PG	0.09±0.01	0.08±0.01	0.08±0.01	0.08±0.01
PEP	0.11±0.01	0.11±0.02	0.08±0.004	0.06±0.004
Pyruvate	0.17±0.01	0.15±0.01	0.16±0.01	0.14±0.01
Lactate	1.5±0.2	2.2±0.1	2.1±0.3	4.0±0.6
Alanine	3.54±0.41	3.03±0.94	1.55±0.28	2.48±0.39

Values are mean ± SE expressed as umol/g (w/w). Glycogen was calculated in glucosyl units.

Figure 2. The adenylate pool (sum of ATP+ADP+AMP) and adenylate plus IMP pool in rainbow trout muscle. A=ADP_m; B=ADP_{+m}; C=ADP_{+c}.

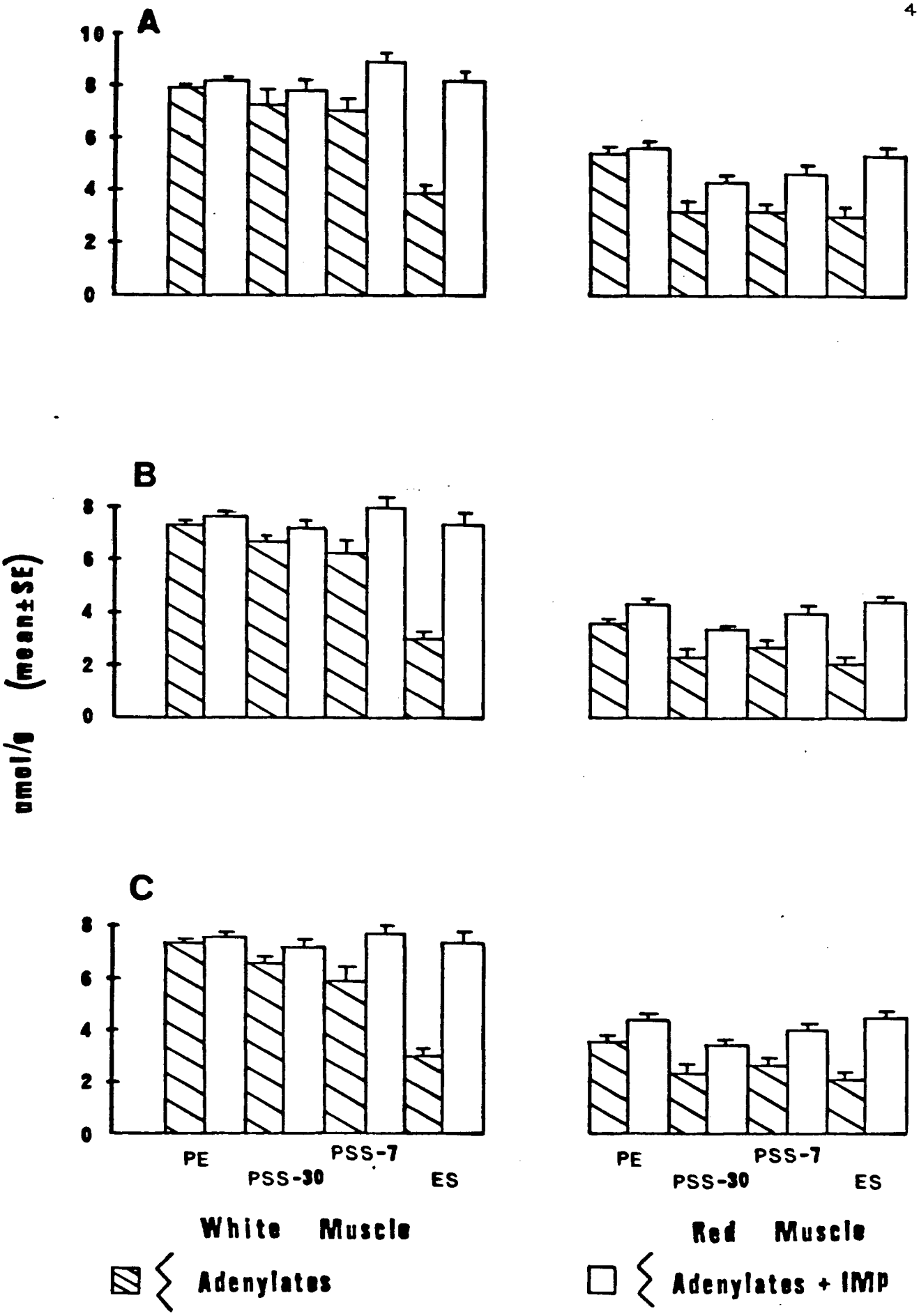
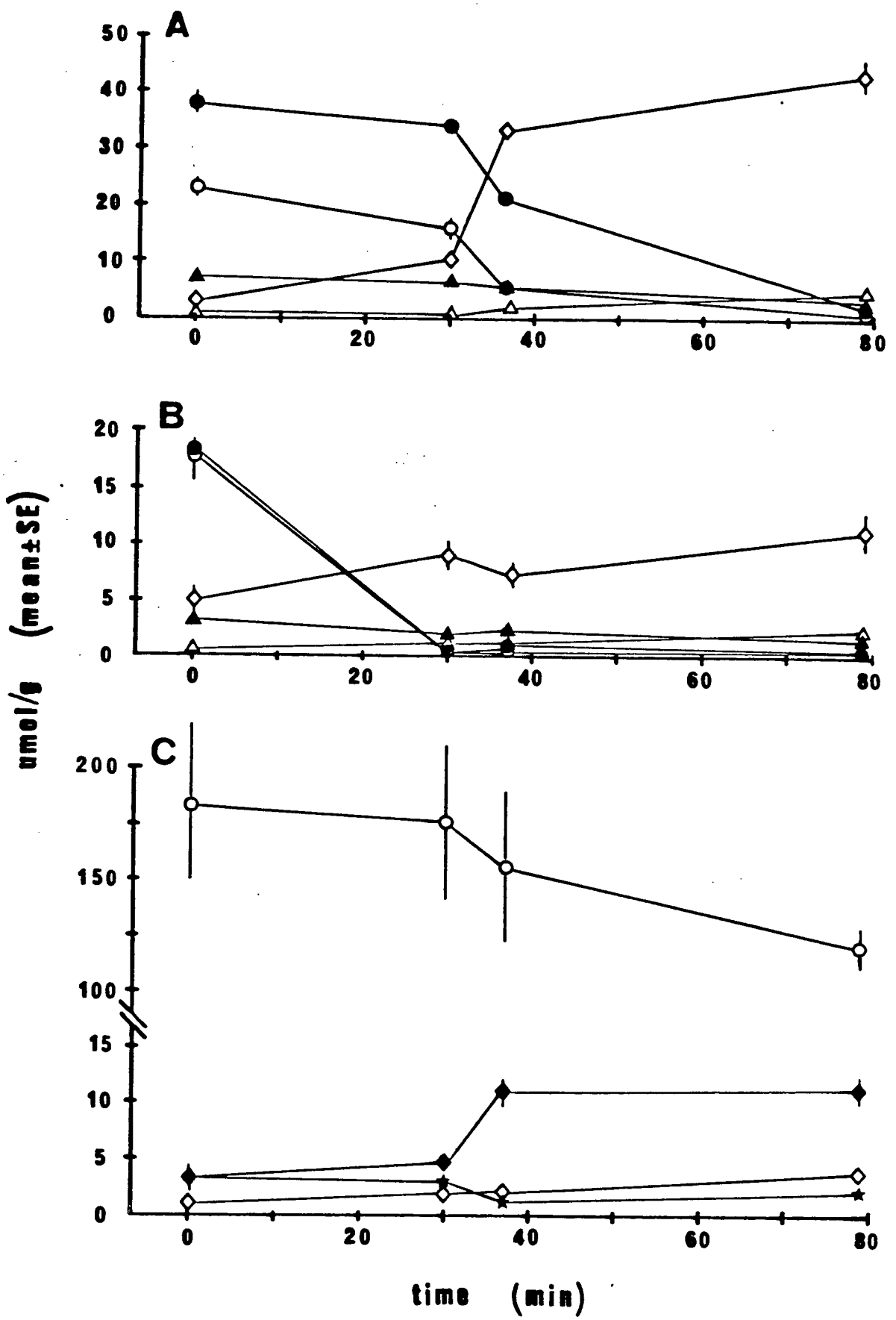

A
B
C
10
8
6
4
2
0
umol/g (mean±SE)
PE
PSS-30
PSS-7
ES
White Muscle
Red Muscle
Adenylates
Adenylates + IMP

Figure 3. Alterations in metabolite contents of liver, white and red muscle of trout. A. Alterations in rainbow trout white muscle glycogen, PCr, ATP, lactate and IMP contents. B. Alterations in rainbow trout red muscle glycogen, PCr, ATP, lactate and IMP contents. C. Alterations in rainbow trout liver glycogen, glucose, lactate and alanine contents. (○ glycogen; ◆ glucose; ● PCr; ▲ ATP; ◇ lactate; △ IMP; ★ alanine)


A
B
C
50
40
30
20
10
0
20
15
10
5
200
150
100
0
20
40
60
80
umol/g (mean±SE)
time (min)

GLYCOLYTIC INTERMEDIATES

White and red muscle glycoltic intermediate contents are contained in Tables 7 and 8 respectively. During the PSS-30, white muscle G1P, G6P, F6P, FDP and pyr levels were found to increase from 48 to 233 percent. The remaining white muscle glycolytic intermediates remained at pre-exercise levels. When white muscle glycolytic energy provision was furher activated (PSS-7), G1P, G6P, F6P and PYR concentrations demonstrated higher accumulations. As well, GP and 3PG demonstrated increases in content while FDP content declined although remaining 32 percent above pre-exercise values. DHAP, GAP, GAP+, DPG, 2PG and PEP concentrations were found to remain relatively constant. During the ES which resulted in very low levels of glycogen (Table 4), all white muscle glycolytic intermediates with the exception of GP, DPG and PYR exhibited lower contents than those of the PSS-30 although G1P, G6P and F6P levels were still elevated when compared to pre-exercise values. FDP content was found to have fallen to values 75 percent lower than pre-exercise levels.

During the PSS-30, red muscle glycolytic intermediate contents demonstrated relatively minor variations (Table 8) despite a large decline in glycogen concentration (Table 5). G1P was found to decline while G6P content increased and FDP levels decreased to 47 percent of pre-exercise levels. GP and pyr contents increased while 2PG content decreased. During the PSS-7, red muscle demonstrated declines in the contents of G6P and F6P while FDP remained depressed as GP and pyr levels were increasing. The ES resulted in decreases in all red muscle glycolytic intermediates with the exceptions of GAP, GAP+ and DPG which all remained at pre-exercise levels and GP and PYR contents which were elevated.

Table 7. Content of glycolytic intermediates in rainbow trout white muscle.

Intermediate	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
	n			
G1P	0.25±0.06	0.40±0.08	0.64±0.04	0.31±0.07
G6P	0.59±0.97	0.97±0.16	1.71±0.21	0.73±0.24
F6P	0.07±0.01	0.15±0.03	0.27±0.04	0.15±0.05
FDP	1.28±0.13	1.90±0.12	1.69±0.31	0.32±0.08
DHAP	0.21±0.03	0.19±0.02	0.23±0.03	0.17±0.02
GP	0.50±0.03	0.52±0.08	0.80±0.02	0.95±0.11
GAP	0.05±0.01	0.06±0.02	0.003±0.005	0.03±0.01
GAP$_f$	0.018±0.002	0.017±0.002	0.022±0.003	0.017±0.002
DPG	0.06±0.01	0.08±0.02	0.06±0.01	0.16±0.03
3PG	0.55±0.05	0.59±0.03	0.96±0.11	0.32±0.01
2PG	0.08±0.02	0.08±0.02	0.06±0.01	0.01±0.004
PEP	0.06±0.004	0.04±0.01	0.04±0.003	0.01±0.003
PYR	0.03±0.005	0.10±0.02	0.14±0.02	0.33±0.05

Values are mean±SE expressed as umol/g w/w.

Table 8. Content of glycolytic intermediates in rainbow trout red muscle.

Intermediate	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
G1P	0.41±0.02	0.20±0.06	0.18±0.06	0.14±0.03
G6P	0.45±0.08	0.54±0.10	0.31±0.04	0.33±0.06
F6P	0.10±0.01	0.08±0.02	0.04±0.004	0.02±0.004
FDP	0.55±0.09	0.26±0.02	0.25±0.03	0.12±0.03
DHAP	0.19±0.05	0.19±0.05	0.15±0.06	0.14±0.02
GP	0.62±0.10	0.71±0.16	0.98±0.55	1.21±0.32
GAP	0.03±0.02	0.04±0.01	0.04±0.02	0.06±0.01
GAP_f	0.017±0.002	0.016±0.004	0.013±0.005	0.015±0.002
DPG	0.06±0.01	0.05±0.01	0.05±0.01	0.06±0.01
3PG	0.16±0.03	0.20±0.03	0.20±0.05	0.11±0.05
2PG	0.11±0.02	0.05±0.01	0.05±0.01	0.03±0.01
PEP	0.04±0.01	0.03±0.01	0.03±0.01	0.02±0.01
PYR	0.05±0.01	0.07±0.01	0.10±0.02 b	0.11±0.002

Values are mean±SE expressed as umol/g w/w.

METABOLIC REGULATION

Free ADP contents were calculated for both the measured and compensated metabolite data of the CK reaction. Free ADP pre-exercise contents were very similar between fiber types when calculated using either the measured or compensated metabolite data, although the ADP_{fm} contents were 8-10 fold higher than the ADP_{fc} values (Table 9). Free ADP contents were found to increase in both tissues with increasing exercise intensity. Following the ES, both red and white muscle free ADP contents were similar and represent approximately 20 percent of the total ADP. An inconsistency occurred as free ADP in white muscle was found to demonstrate its largest increase during the PSS-7 (ADP_{fm}) and ES (ADP_{fc}) respectively. However, red muscle free ADP increased extensively during the PSS-30 when using either the measured or compensated metabolite data (Table 9).

ATP/ADP ratios were calculated for the total (measured), free measured and free compensated ADP contents (Table 9). Pre-exercise white muscle ATP/ADP ratios were approximately 2 fold greater than red muscle. Similar patterns of decline in the ATP/ADP ratio were exhibited for both tissues between the various procedures of calculating this ratio (ADP_m; ADP_{fm}; ADP_{fc}). The ATP/ADP ratio demonstrated by white muscle after the ES was similar to the ATP/ADP ratio in red muscle following the PSS-30, PSS-7 and ES.

Phosphorylation potentials (ATP/ADPxPi) were calculated for the total, free measured and free compensated ADP contents (Table 9). The pre-exercise phosphorylation potentials were 1.4 to 2.9 fold larger in white than red muscle. Similar decreases in phosphorylation potentials between the methods

of assessment were noted for white and red muscle. The ES phosphorylation potentials were very similar between tissues.

The free cytosolic redox state of the NAD couple was calculated in rainbow trout muscle from the measured contents of pyr and lactate employing the equilibrium constant of the LDH reaction (Table 1) and the values are presented in Table 10. White and red muscle NAD/NADH ratios remained at pre-exercise levels until after the ES and PSS-30, respectively. During these exercise intensities, the NAD/NADH ratio increased 2.8 to 3.6 fold in white and red muscle respectively.

PCr/Pi ratios were calculated for the measured and compensated metabolite data (Table 10). Measured PCr/Pi ratios were very low for all experimental states in both tissues. The compensated PCr/Pi ratio's were 16.8 and 8.1 for white and red muscle pre-exercise values respectively. These ratio's (PCr_c/Pi_c) declined to very low values following the ES in white and the PSS-30 in red muscle.

Table 9. Measured and calculated free cytoplasmic ADP content, ATP/ADP ratio and cytosolic phosphorylation potentials in rainbow trout muscle.

	Muscle	Pre-ex n (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
ADP_m (umol/g)	W	0.70±0.01	0.68±0.04	1.15±0.07	1.05±0.09
	R	0.65±0.01	1.18±0.08	0.839±0.04	1.11±0.17
ADP_{fm} (umol/g)	W	0.057±0.004	0.065±0.004	0.290±0.063	0.203±0.056
	R	0.073±0.012	0.189±0.010	0.229±0.022	0.229±0.021
ADP_{fc} (umol/g)	W	0.007±0.001	0.012±0.002	0.019±0.002	0.203±0.056
	R	0.008±0.001	0.189±0.010	0.229±0.022	0.229±0.021
ATP/ADP_m	W	10.4±0.2	9.8±0.2	5.1±0.4	2.6±0.2
	R	5.4±0.5	1.7±0.2	2.7±0.4	1.5±0.4
ATP/ADP_{fm}	W	129.6±9.5	103.0±4.5	24.5±5.9	18.1±5.1
	R	52.4±8.9	10.6±1.3	10.4±1.6	7.5±2.1
ATP/ADP_{fc}	W	1025±98	582±66	312±21	18.1±5.1
	R	457±24	10.6±1.3	10.4±1.6	7.5±2.1
$\frac{ATP}{ADP_m.Pi}$ (M^{-1})	W	553±73	371±24	110±12	46±3
	R	383±33	149±21	140±20	74±23
$\frac{ATP}{ADP_{fm}.Pi}$ (M^{-1})	W	6950±1100	3938±367	500±164	316±81
	R	3682±513	813±96	542±103	370±127
$\frac{ATP}{ADP_{fc}.Pi_c}$ (M^{-1})	W	451200±44800	60900±8900	11000±1400	316±81
	R	155000±30700	813±96	542±103	370±127

Values are mean±SE. W, white muscle; R, red muscle.

Table 10. Calculated free cytosolic redox state and PCr/Pi ratio's in rainbow trout muscle.

	Muscle n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
NAD/NADH	W	749±153	1068±82	828±106	2076±458
	R	961±145	888±132	2027±173	1525±142
PCr/Pi	W	1.04±0.12	0.6±0.06	0.06±0.02	0.03±0.01
	R	0.37±0.04	0.04±0.02	0.06±0.01	0.02±0.01
$\frac{PCr_c}{Pi_c}$	W	16.8±1.5	3.4±0.3	0.7±0.1	0.03±0.01
	R	8.1±0.4	0.04±0.02	0.06±0.01	0.02±0.01

Values are mean±SE. W, white muscle; R, red muscle.

Equilibrium versus Nonequilibrium

The degree of deviation from equilibrium was assessed for the glycolytic enzymes using both the measured and compensated metabolite data in white (Figures 5 and 6) and red (Figures 7 and 8) muscle. Similar results were noted within each fiber type for the measured and compensated metabolite data. Within both tissue types, the enzymes HK, PGM, PFK, Ald and PK appear to be out of equilibrium under all experimental conditions. Of these, HK, PFK and PK demonstrate the largest deviation from equilibrium in both tissues. With increasing workload, these nonequilibrium enzymes demonstrate relatively similar patterns of deviation. However, the equilibrium enzymes GPDH and PGK which act together in vivo (Lehninger 1975), demonstrate a deviation from equilibrium as exercise intensity increases. This change to nonequilibrium occurred during the ES for white muscle and for all exercise intensities in red muscle. The combined GPDH.PGK/LDH reaction demonstrated similar deviations from equilibrium as the GPDH.PGK reaction.

Figure 4. Deviations of trout white muscle glycolytic enzymes from equilibrium based on compensated metabolite data. A=Pre-ex; B=PSS-30; C=PSS-7; D=ES.

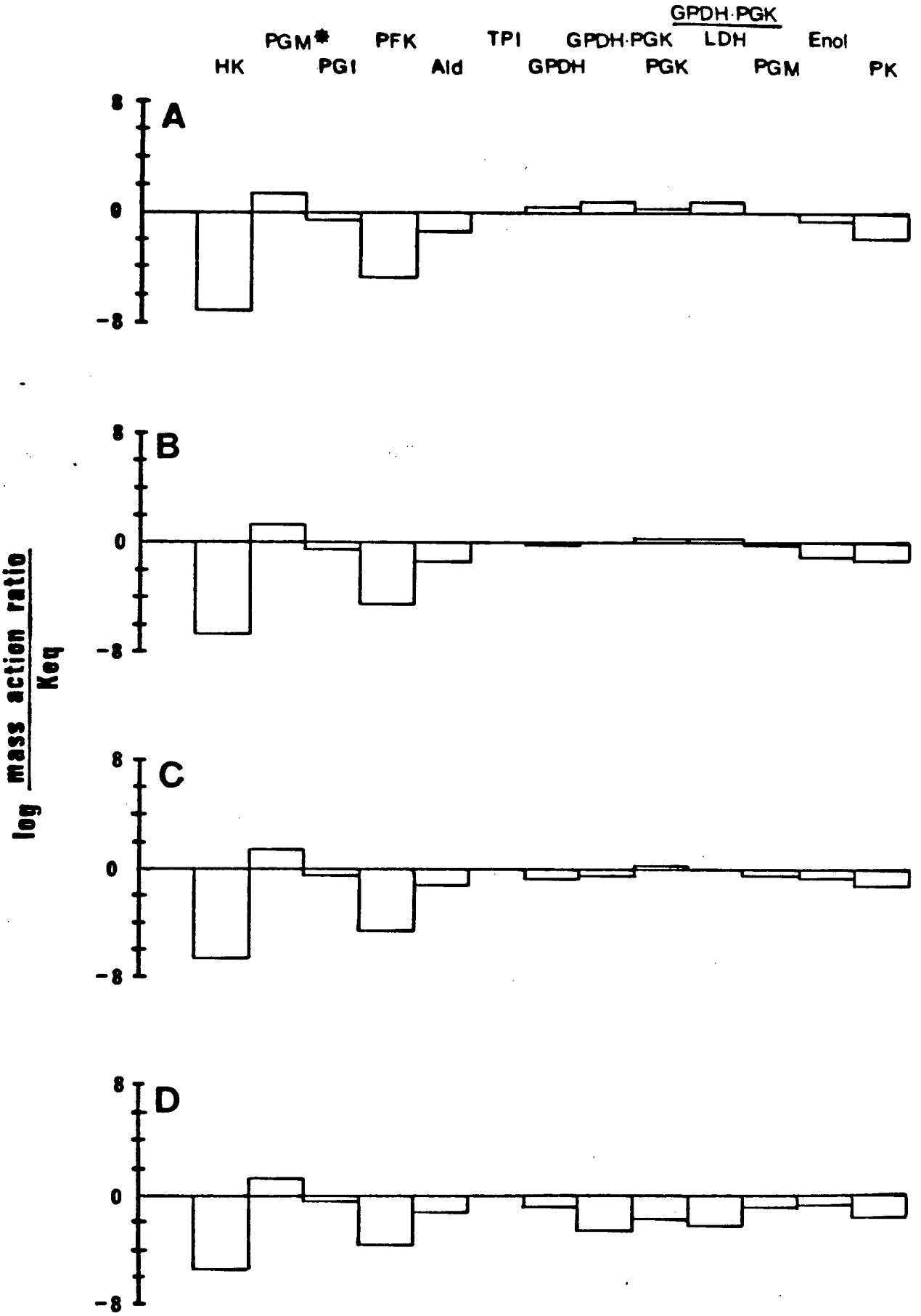

GPDH·PGK
PGM*
PFK
TPI
GPDH·PGK
LDH
Enol
HK
PGI
Ald
GPDH
PGK
PGM
PK
A
B
C
D
8
0
-8
log mass action ratio / Keq

Figure 5. Deviations of trout white muscle glycolytic enzymes from equilibrium based on measured metabolite data. A=Pre-ex; B=PSS-30; C=PSS-7; D=ES.

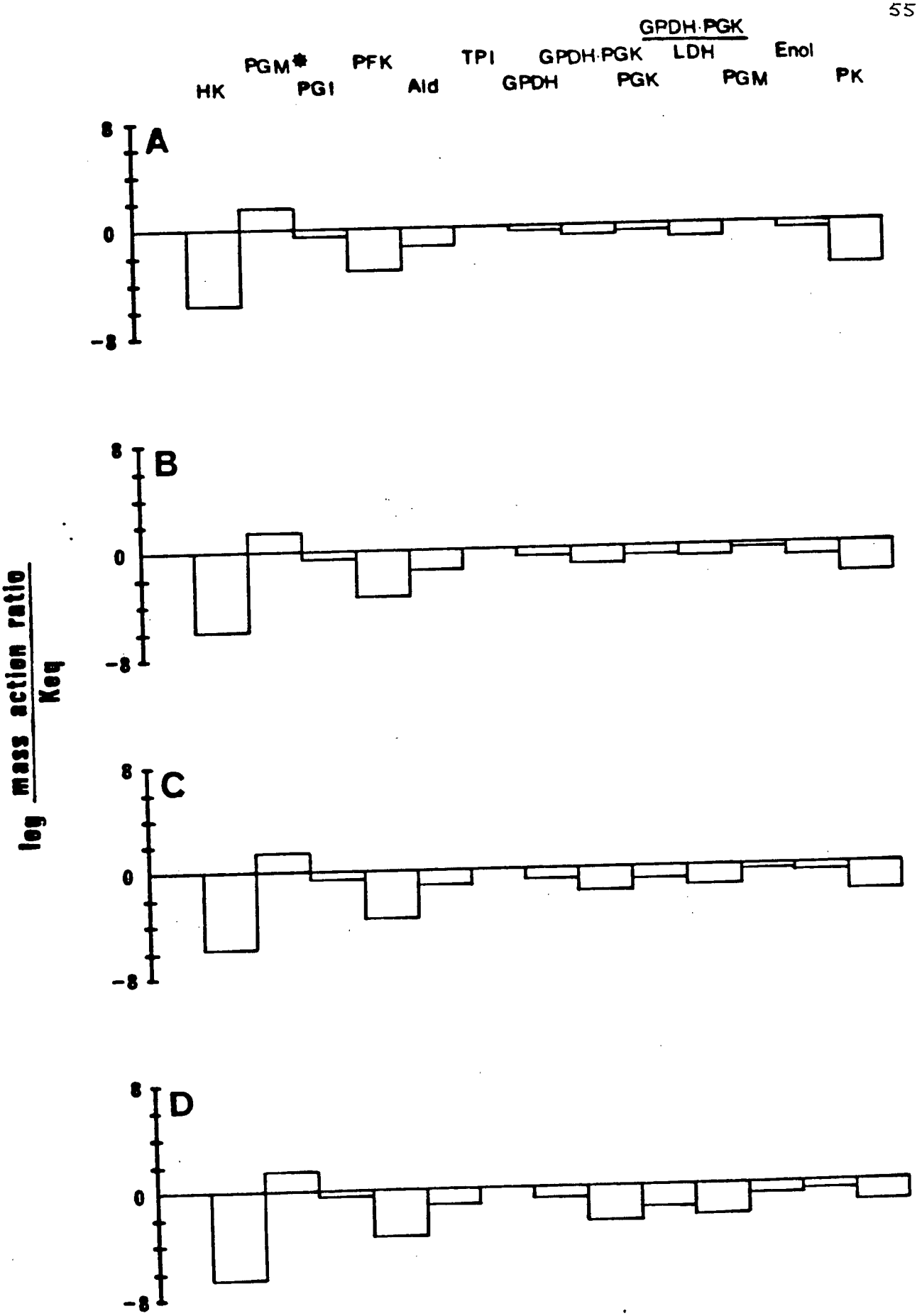

GPDH·PGK
HK
PGM*
PGI
PFK
Ald
TPI
GPDH
GPDH·PGK
PGK
LDH
PGM
Enol
PK
A
B
C
D
8
0
-8
log mass action ratio / Keq

Figure 6. Deviations of trout red muscle glycolytic enzymes from equilibrium based on compensated metabolite data. A=Pre-ex; B=PSS-30; C=PSS-7; D=ES.

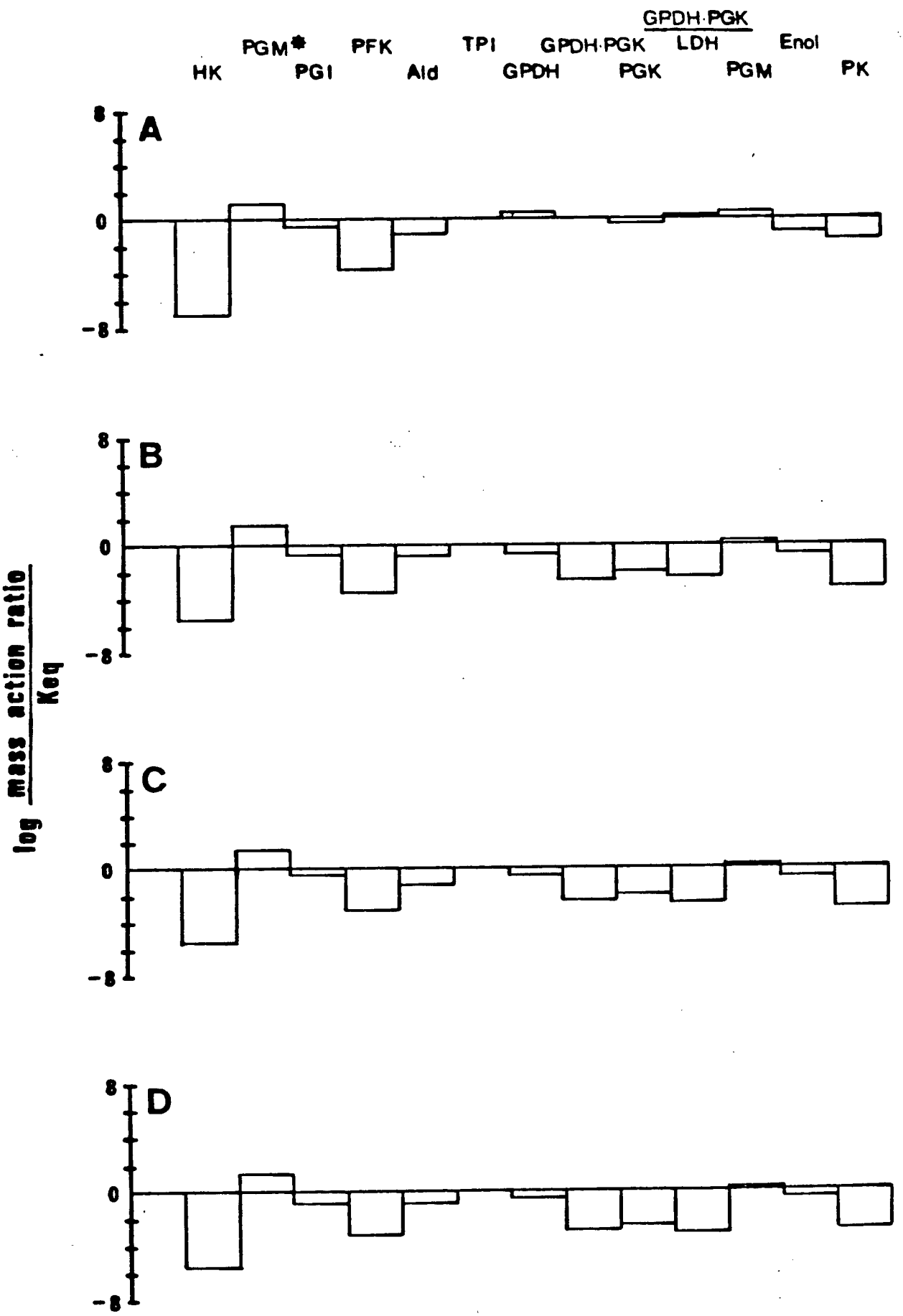
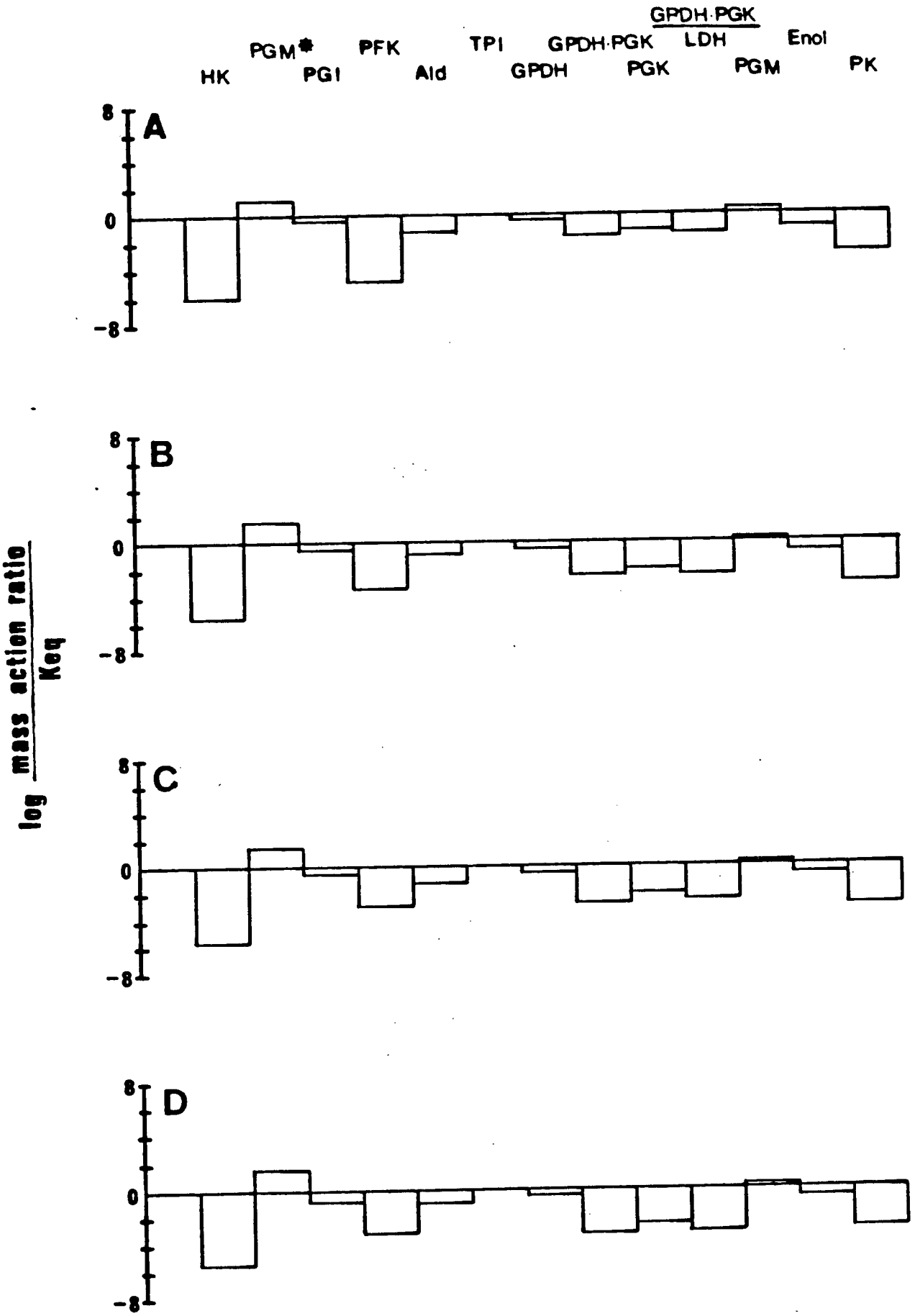

GPDH·PGK
PGM*
PFK
TPI
GPDH·PGK
LDH
Enol
HK
PGI
Ald
GPDH
PGK
PGM
PK
A
B
C
D
8
0
-8
log Mass action ratio / Keq

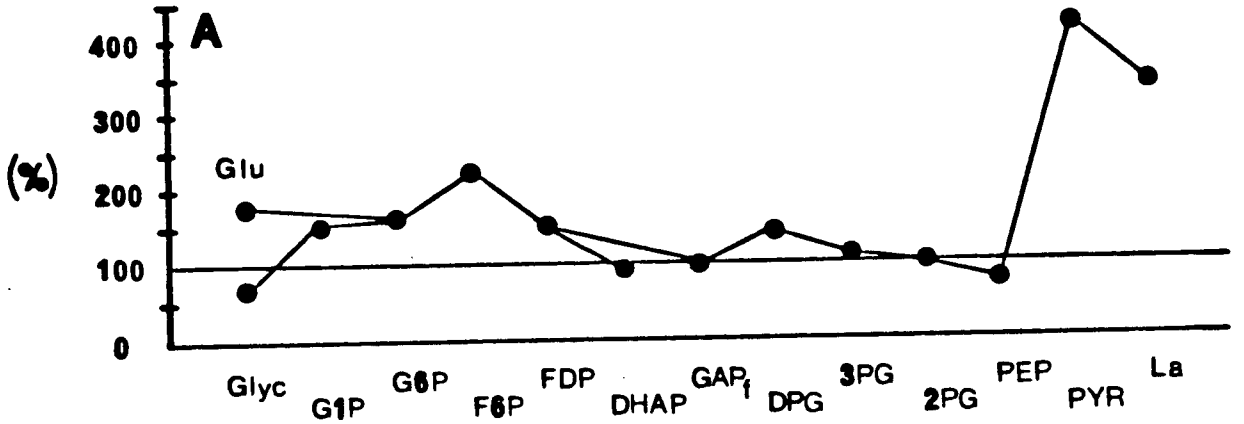
Figure 7. Deviations of trout red muscle glycolytic enzymes from equilibrium based on measured metabolite data. A=Pre-ex; B=PSS-30; C=PSS-7; D=ES.


GPDH·PGK
HK
PGM*
PGI
PFK
Ald
TPI
GPDH
GPDH·PGK
PGK
LDH
PGM
Enol
PK
A
B
C
D
8
0
-8
log mass action ratio / Keq

Regulatory Enzymes

Potential regulatory enzymes were assessed using the crossover theorem as outlined by Williamson (1969) and discussed in detail by Rolleston (1972). Pathway intermediates are plotted as a percent of control values on the ordinate with the sequence of intermediates on the abscissa and potential regulatory enzymes are identified whenever the axis is crossed. However, not all regulatory enzymes demonstrate a crossover and not all crossovers denote a regulatory enzyme (Rolleston 1972). All glycolytic intermediate contents were plotted versus their preceeding exercise values and versus the pre-exercise contents (Figures 9 and 10). Within white muscle, phos and PK were identified as potential regulatory enzymes under all conditions with the exception of phos in the ES versus PSS-7 condition (Figure 8). No apparent regulation was found to occur for HK and PFK within white muscle while the GPDH.PGK complex was found to exhibit an apparent regulatory interaction following the ES (Figure 8). Red muscle in contrast, demonstrated no regulation at phos and apparent regulation at the GPDH.PGK complex under all exercise states (Figure 9). HK was found to be potentially regulatory following glycogen depletion to less than 1 umol/g (Figure 9) while PK demonstrated similar regulatory potential as in white muscle (Figures 9 and 10).

Figure 8. Crossover plots showing the interactions caused by increasing workloads on trout white muscle glycolysis. A=PSS-30 versus Pre-ex; B=PSS-7 versus Pre-ex; C=ES versus Pre-ex; D=PSS-7 versus PSS-30; E=ES versus PSS-7.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(%)
200
100
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Glu
Glyc
G1P
G6P
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FDP
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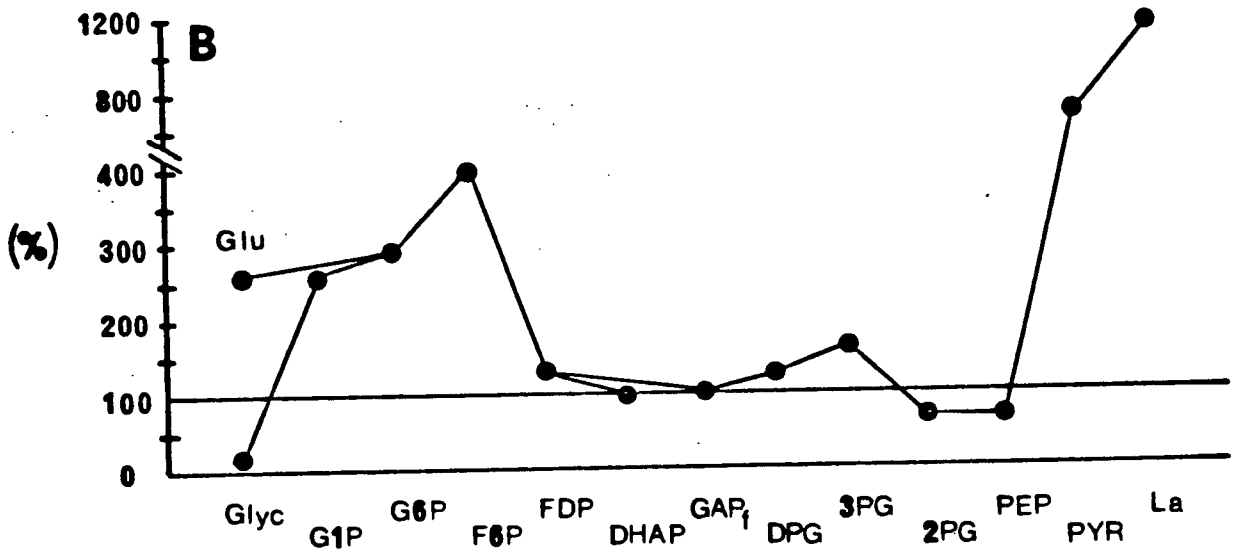
DPG
3PG
2PG
PEP
PYR
La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800
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(%)
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200
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Glyc
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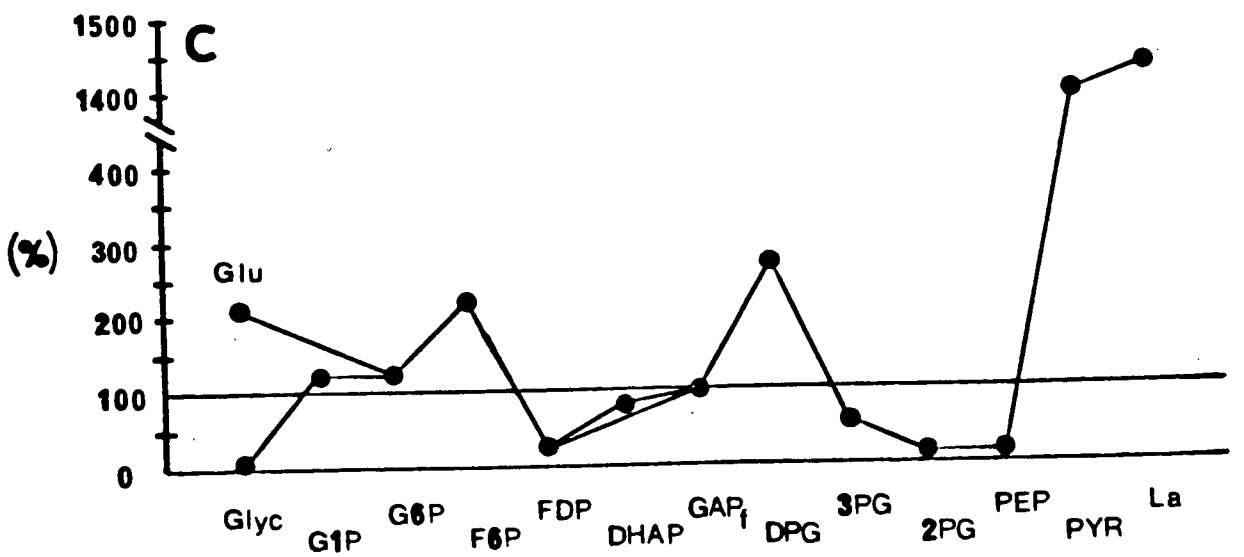
PYR
La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(%)
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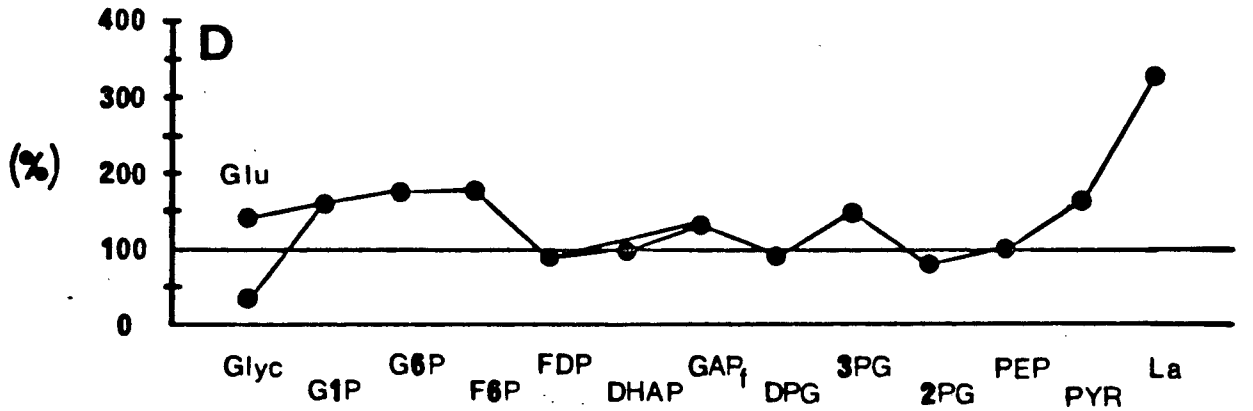
PEP
PYR
La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(%)
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Glyc
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G6P
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DPG
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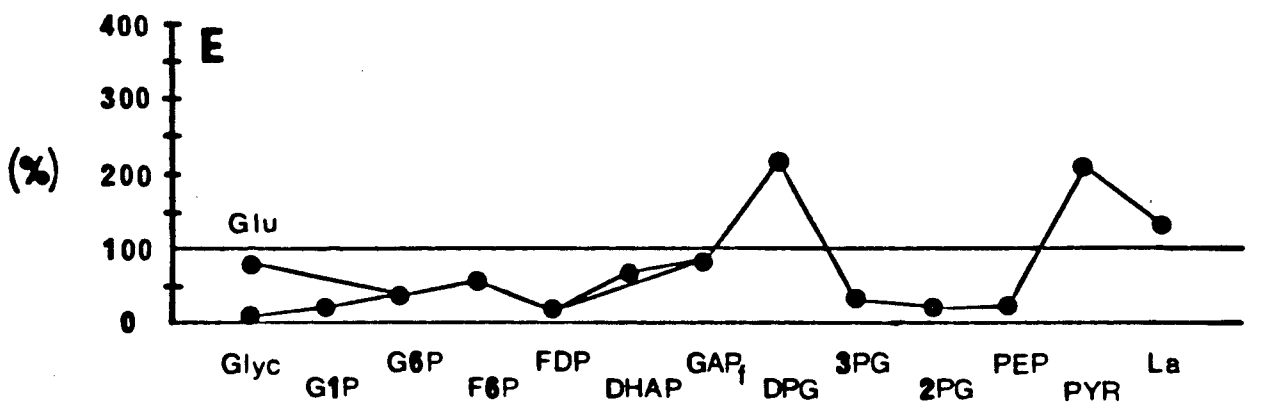
2PG
PEP
PYR
La


E
400
300
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100
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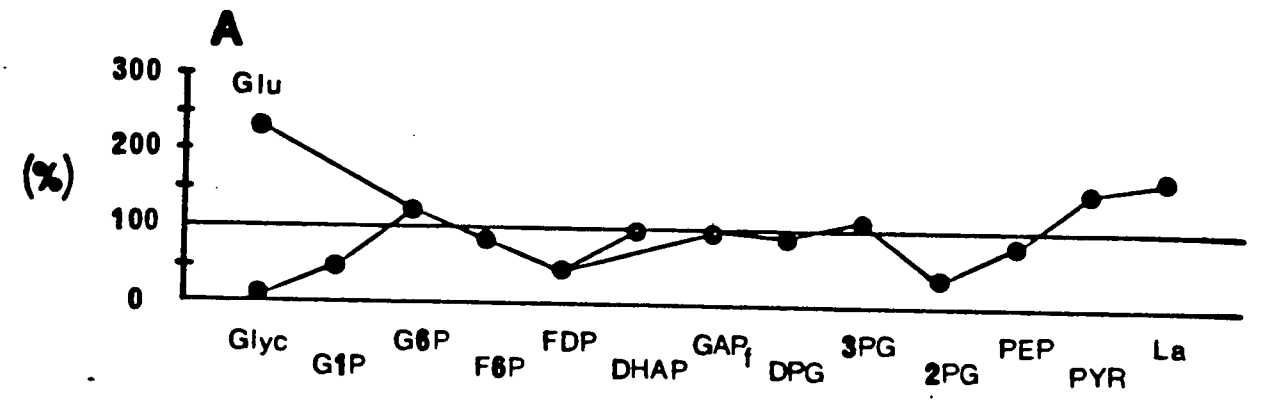
(%)
Glu
Glyc
G1P
G6P
F6P
FDP
DHAP
GAP$_f$
DPG
3PG
2PG
PEP
PYR
La

Figure 9. Crossover plots showing the interactions caused by increasing workloads on trout red muscle glycolysis. A=PSS-30 versus Pre-ex; B=PSS-7 versus Pre-ex; C=ES versus Pre-ex; D=PSS-7 versus PSS-30; E=ES versus PSS-7.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100
0
(%)
Glu
Glyc
G1P
G6P
F6P
FDP
DHAP
GAPf
DPG
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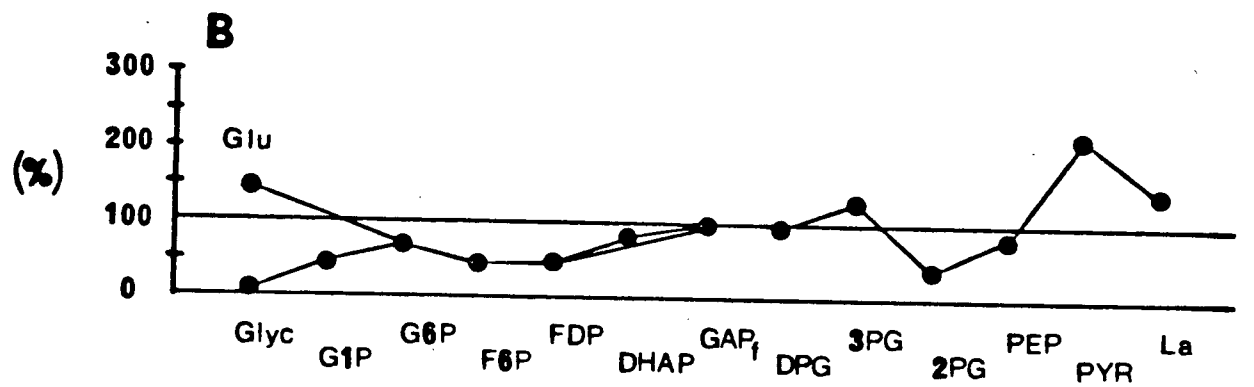
2PG
PEP
PYR
La


B
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PYR
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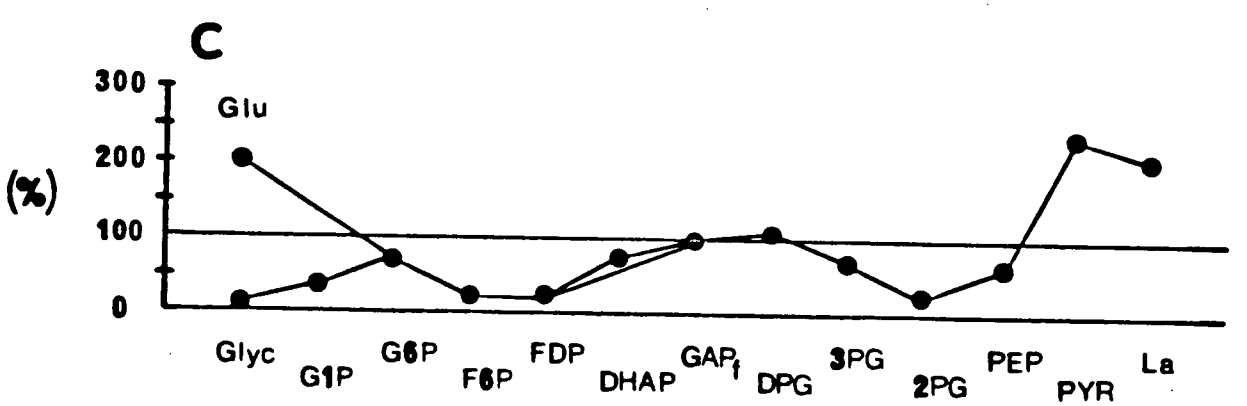
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0
(%)
Glu
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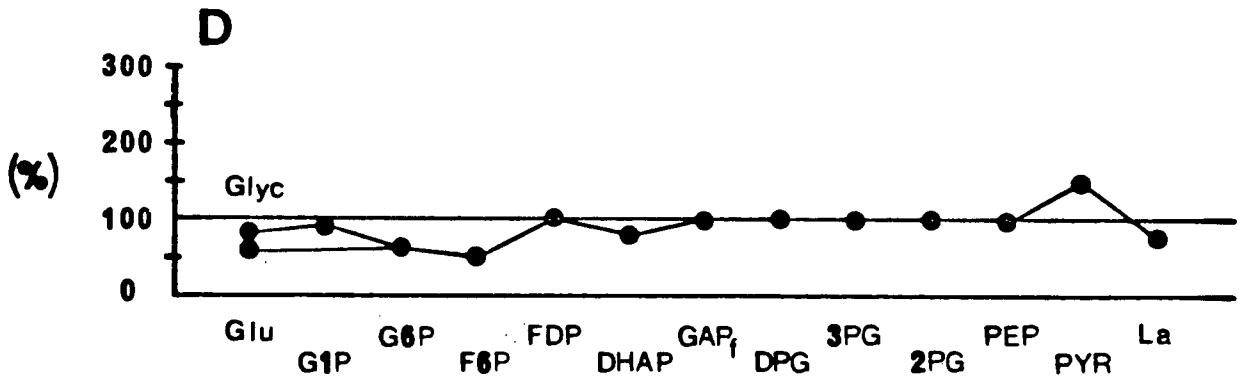
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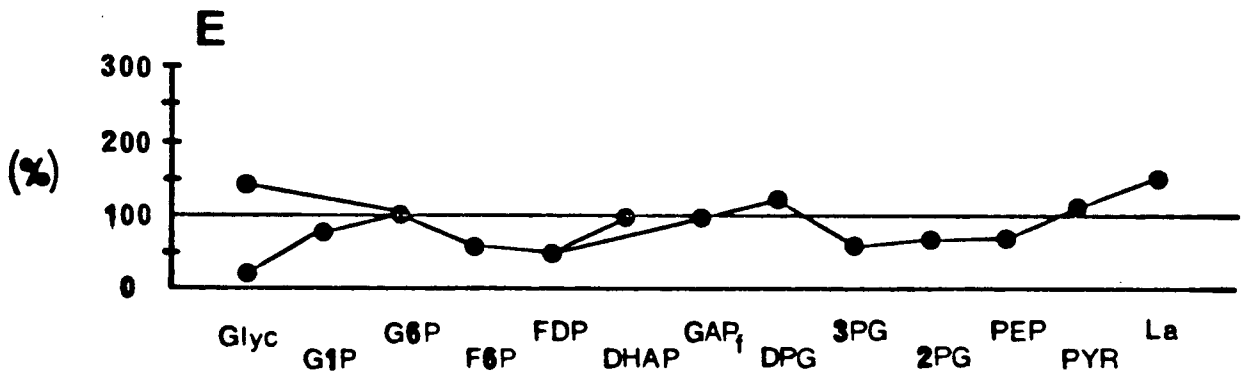
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DPG
3PG
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PEP
PYR
La

Energy Turnover

The maximal contribution of aerobic metabolism to energy turnover in this animal is estimated in Table 11. This aerobic contribution is 2.2 fold greater in red as opposed white muscle with mean values of 138.1 and 153.4 umol ATP/g muscle/min during the PSS-30 and PSS-7 respectively. Estimated metabolic energy turnover from all sources and the relative contributions of these sources to energy turnover in red and white muscle are presented in Table 12. During the PSS-30, aerobic energy turnover predominated both red and white muscle energy metabolism. Glycogen and glucose oxidation accounted for 14 percent of the energy turnover achieved by red muscle with this oxidation being essntially attributable to glycogen oxidation. Fat oxidation accounted for 86 percent of the energy turnover. In white muscle, 7 percent of the energy turnover was derived from glycogen and glucose oxidation with the glucose oxidation contribution being insignificant when expressed relative to per gram of muscle. Fat oxidation accounted for 92 percent of the energy turnover. The total ATP production for the PSS-30 equalled 18,624 umol ATP/min for the exercising muscle.

During the PSS-7, red and white muscle energy turnover increased 10 and 20 percent respectively (Table 12). However this increased white muscle energy turnover was less than was achieved by red muscle at either exercise intensity (Table 12). Fat oxidation accounted for 99+ and 92 percent of the energy turnover generated by red and white muscle respectively. Within white and red muscle glucose oxidation increased 6.5 and 5.2 fold respectively. White muscle glycogen fermentation increased 8.0 umol ATP/g/min, a 12.3 fold increase. The total ATP production during the PSS-7 was equal to 22,330 umol ATP/min for the exercising musculature.

Table 11. Estimated oxygen uptake and aerobic energy turnover (umol ATP/g/min) of rainbow trout.

		Pre-ex	80% Ucrit	90% Ucrit	100% Ucrit
Total O2 uptake (ml/min/Kg)		0.41	2.97	3.34	3.71
Muscle O2 uptake (ml/min/Kg)		0.22	2.59	2.88	3.24
Muscle O2 uptake (ml/min/Kg muscle)	a	0.33	3.87	4.36	4.84
Muscle O2 uptake increase (ml/min/Kg)			2.37	2.56	2.84
Muscle O2 uptake increase (ml/min/Kg muscle)	a		3.54	3.82	4.24
Energy Turnover by muscle (umol ATP/g muscle/min)	b		122.7	138.1	153.4
Energy Turnover by RM	c		267.5	301.1	334.5
Energy Turnover by WM	c		120.5	135.6	150.7

Data are from Randall and Daxdoeck 1979. RM, red muscle; WM, white muscle.
a Assumes white muscle constitutes 66 percent and red muscle 1 percent of the fishes total body mass (200g).
b Assumes that PV=nRT where P=140 mmHg, V=volume (l), R=0.08205 l atm/deg/mol and T=283°K.
c Assumes red muscle oxidizes substrates at a rate 20 fold greater than white muscle but blood flow to white muscle is 9 fold greater than red muscle.

Table 12. Estimated energy turnover (umol ATP/g/min) from fats, glycolytic and high energy phosphagen sources in rainbow trout red and white muscle during the PSS-30 and PSS-7.

Fuel/Pathway		PSS-30		PSS-7	
		R	W	R	W
glucose fermentation	a	-	-	-	0.62
glucose oxidation	b	0.17	0.02	0.89	0.13
glycogen fermentation	c	0.78	0.72	-	8.86
glycogen oxidation	d	41.32	9.32	-	-
PCr hydrolysis		0.41	0.13	-	3.72
ATP hydrolysis		0.20	0.04	-	0.22
Fat oxidation	e	259.61	126.26	333.61	150.57
Total Energy Turnover (umol ATP/g/min)		302.8	136.5	334.5	164.1
Total ATP Production (umol ATP/g/fish muscle)		18,624		22,330	

a Based on lactate production not attributable to muscle glycogen fermentation with 2 ATP being generated per glucose fermented.
b Based on liver glycogen and blood glucose utilization (PSS-30=3.7 umol/ml; PSS-7=3.7 umol/ml) not accounted for by glucose fermentation with 36 ATP being generated per glucose oxidized.
c Based on the change in muscle glycogen accounted for by lactate production with 3 ATP being generated per glucosyl unit fermented.
d Based on the change in muscle glycogen not accounted for by lactate production with 37 ATP being generated per glucosyl unit oxidized.
e Based on the increase in oxygen uptake of the working muscle and assuming that the red muscle oxidizes substrates at a rate 20 fold greater than red muscle.

Note: Values are based on a 200g fish which would contain 132g white muscle, 2g red muscle and 2g liver (Randall and Daxboeck 1982). It is assumed that only half of the red and white muscle is active at any time. The stored elastic component was not taken into account. Blood volume was assumed to be 5 mls/100g (Stevens 1968) with 83.2 and 9.4 percent of this volume during exercise being distributed within white and red muscle respectively (Neumann et al. 1983). R, red muscle; W, white muscle.

FATIGUE

Within white muscle, PCr depletion occurred as lactate and inorganic phosphate accumulated (Figure 10). Similarily, red muscle PCr contents decreased as inorganic phosphate and lactate concentrations increased, although the elevation in lactate was low compared to white muscle increments (Figure 10). White muscle ATP levels decreased slightly during the PSS-30 and PSS-7 while declining to low values (2.65 umol/g) after the ES (Table 1). Red muscle ATP content decreased to low levels during the PSS-30 and remained low for both the PSS-30 and PSS-7 (Table 2). Simultaneously, ADP content increased in both fiber types as ATP concentrations decreased (Tables 1 and 2). Glycogen levels were found to be low and to remain depressed in red muscle after the PSS-30 (Table 2). In contrast, white muscle glycogen levels remained relatively high until after the ES (Table 1). The corresponding muscle pH values refected these perturbations in metabolism (Figure 10). White muscle pH was maintained during the PSS-30 and dropped to values of 6.65±0.04 for the PSS-7 and 6.56±0.04 for the ES regimes (Figure 10). Alternatively, red muscle pH was maintained during both the PSS-30 and PSS-7 protocols demonstrating a slight decrease during ES (Figure 10).

The metabolic end products accumulations of Cr, Pi and lactate in white and red muscle are presented in Figure 11. Creatine accumulation was roughly equivilent to the amount of PCr hydrolyzed in both the red and white muscle (Tables 4 and 5). Inorganic phosphate levels increased relatively proportionally to Cr content in red and white muscle during all exercise states in the former and after the PSS-30 in the latter (Figure 11). However, inorganic phosphate levels increased to a greater extent than could be accounted for by ATP and PCr hydrolysis in white muscle after the PSS-7

and ES bouts (Tables 1 and 3). Lactate accumulations were far greater in white than red muscle under all exercise conditions (Figure 11).

No differences existed between the pre-exercise and any exercise state for tissue water content which averaged 78 percent (Table 3). Plasma, tissue, extracellular and intracellular ion contents are reported in Table 13. Only K^+ and lactate were observed to change within the plasma and extracellular compartments. Na^+, K^+ and lactate were found to increase while Cl^- and PCr^{2-} decreased in the white muscle intracellular compartment. Na^+, K^+, Ca^{++}, Cl^- and lactate content increased while PCr^{2-} content decreased in the red muscle intracellular compartment. Measured and hypothetical white and red muscle intracellular and extracellular ion differences are reported in Table 14. Hypothetical ion difference, were calculated assuming only changes in lactate or lactate and PCr^{2-} with all other ions remaining at their pre-exercsie levels. Pre-exercise values were found to be 51.1, 85.5 and 11.2 for white muscle, red muscle and the extracellular compartments, respectively. The lactate accumulations and PCr^{2-} hydrolysis resulted in large perturbations in charge within all three compartments. Accumulations and/or shifts of Na^+, K^+, Ca^{++}, Mg^{++} and Cl^- failed to compensate for these metabolically induced perturbations in charge. In fact, alterations in these ions resulted in the intracellular compartment becoming more positive in both tissues and less positive or even negative in the extracellular compartment. Despite these large changes in charge and ionic composition, the calculated membrane potentials, though more negative, remained relatively constant (Table 15).

Table 13. Rainbow trout ion concentrations.

Ion	Compartment Muscle		Exercise State Pre-ex	PSS-30	PSS-7	ES
Na^+		P	135.1±3.4	134.1±2.7	132.7±2.7	134.1±2.7
		E	141.1±3.7	140.1±2.7	138.4±2.7	140.1±2.7
	W	T	29.2±2.2	36.5±1.8	30.0±2.4	35.6±0.5
		I	12.3±0.9	19.7±1.0	13.3±1.1	18.9±0.3
	R	T	34.1±2.1	40.1±2.8	49.1±1.0	51.4±2.2
		I	17.2±1.1	23.3±1.6	32.4±0.7	34.6±1.5
K^+		P	1.78±0.17	2.49±0.27	3.19±0.27	3.19±0.27
		E	1.85±0.17	2.59±0.27	3.33±0.27	3.33±0.27
	W	T	149.4±3.9	171.8±7.4	159.0±6.8	187.2±6.4
		I	149.1±3.9	171.5±7.4	158.7±6.8	186.9±6.4
	R	T	138.9±9.0	167.1±7.4	147.2±5.8	169.2±11.5
		I	138.6±9.0	166.8±7.1	146.9±5.8	159.9±11.5
Ca^{++}		P	4.57±0.03	4.74±0.10	4.91±0.13	4.91±0.13
		E	4.77±0.03	4.94±0.10	5.11±0.13	5.11±0.13
	W	T	5.62±0.74	5.15±0.67	5.18±0.41	4.62±0.33
		I	5.04±0.66	4.56±0.59	4.56±0.36	4.00±0.29
	R	T	4.21±0.45	3.33±0.15	4.38±0.47	6.56±0.94
		I	3.68±0.39	2.73±0.12	3.77±0.40	5.95±0.85
Mg^{++}		P	1.34±0.03	1.38±0.03	1.38±0.03	1.38±0.03
		E	1.41±0.03	1.44±0.03	1.44±0.03	1.44±0.03
	W	T	38.7±1.7	35.3±1.5	38.3±0.9	37.1±0.4
		I	38.6±1.7	35.2±1.5	38.2±0.9	37.0±0.4
	R	T	20.4±0.4	17.9±0.6	20.5±0.5	20.9±0.4
		I	20.3±0.4	17.8±0.6	20.4±0.5	20.8±0.4

Cl^-	P	127.7±4.0	131.0±3.4	135.1±2.7	135.1±2.7
	E	133.3±4.0	137.1±3.4	141.0±2.7	141.0±2.7
W	T	25.9±4.1	23.4±4.1	19.4±1.5	21.3±1.5
	I	13.1±2.1	10.2±1.8	7.5±0.6	8.2±0.6
R	T	37.2±3.6	32.1±0.8	33.8±5.6	44.6±6.7
	I	18.8±1.8	14.0±0.4	13.1±2.2	17.2±2.6
La^-	P	1.4±0.4	2.8±0.5	4.1±0.6	7.8±0.7
	E	1.5±0.4	2.9±0.5	4.3±0.6	8.1±0.7
W	T	3.9±0.5	12.9±1.4	42.3±0.8	55.0±3.8
	I	3.7±0.5	12.6±1.4	41.8±0.8	54.0±3.7
R	T	6.7±1.0	11.3±1.4	9.4±1.2	13.8±1.8
	I	6.5±1.0	10.9±1.4	8.8±1.1	12.8±1.7
PCr^{2-}	P	-	-	-	-
	E	-	-	-	-
W	T	97.0±4.2	86.6±2.4	53.4±1.8	4.6±1.6
	I	97.0±4.2	86.6±2.4	53.4±1.8	4.6±1.6
R	T	47.8±2.0	2.0±1.0	3.0±0.6	1.0±0.6
	I	47.8±2.0	2.0±1.0	3.0±0.6	1.0±0.6

Values are mean±SE expressed in mEq/l. PSS-30 plasma values were assumed to be equal to the mean of the pre-ex and PSS-7 values. The ES plasma values were assumed to be equivilent to PSS-7 values with the exception of lactate which is taken from a separate investigation (Dobson, Mommsen and Hochachka unpublished observations). Plasma water, tissue water and extracellular volume were assumed to be 95.8 percent (Milligan and Wood 1985), 78 percent (Table 3) and 9 percent (Milligan and Wood 1985) under all conditions. P, plasma; E, extracellular; T, tissue; I, intracellular; W, white muscle; R, red muscle.

Table 14. Measured and hypothetical rainbow trout white and red muscle intracellular and extracellular ion differences.

Condition	Control Pre-ex	Hypothetical (La^-)	Hypothetical (La^- + PCr^{2-})	Measured
White Muscle Intracellular				
PSS-30	51.1	42.2	52.6	85.1
PSS-7	51.1	13.0	56.6	72.6
ES	51.1	0.8	93.2	142.0
Red Muscle Intracellular				
PSS-30	85.5	81.1	126.9	165.6
PSS-7	85.5	83.2	128.0	157.3
ES	85.5	79.2	126.0	167.5
Extracellular				
PSS-30	11.2	9.8	-	5.9
PSS-7	11.2	8.4	-	-0.3
ES	11.2	4.6	-	-2.4

$IDe=(Na^+)+(K^+)+(Ca^{++}/2)+(Mg^{++}/2)-(Cl^-)-(La^-)$

$IDw=(Na^+)+(K^+)+(Ca^{++}/2)+(Mg^{++}/38)-(Cl^-)-(La^-)-(PCr^{2-})$

$IDr=(Na^+)+(K^+)+(Ca^{++}/2)+(Mg^{++}/20)-(Cl^-)-(La^-)-(PCr^{2-})$

Hypothetical ion differences are calculated assuming only changes in La^- or La^- plus PCr^{2-} from pre-exercise values. IDe, ion difference extracellular; IDw, ion difference white muscle; IDr, ion difference red muscle.

Table 15. Calculated rainbow trout red and white muscle membrane potentials.

Muscle	Pre-ex	PSS-30	PSS-7	ES
White	-65	-71	-76	-76
Red	-56	-64	-64	-59

Values are means expressed as millivolts. Values are calculated from the Goldman Hodgekin Katz equation:

$$Em=RT/Fx\ln \frac{PK\ (K^+)o+PNa\ (Na^+)o+PCl\ (Cl^-)i}{PK\ (K^+)i+PNa\ (Na^+)i+PCl\ (Cl^-)o}$$

where PK^+, PNa^+ and PCl^- represent the relative permeability coeficients. These values were taken from frog sartorius muscle and are 0.5, 0.005 and 1 respectively (Hodgkin and Horowicz 1959). Mean K^+, Cl^- and Na^+ contents were used for all calculations. ()o, concentration outside; ()i, concentration inside.

Figure 10. Metabolic alterations in PCr^{2-}, La^{-}, Pi^{2-} and pH in rainbow trout muscle.

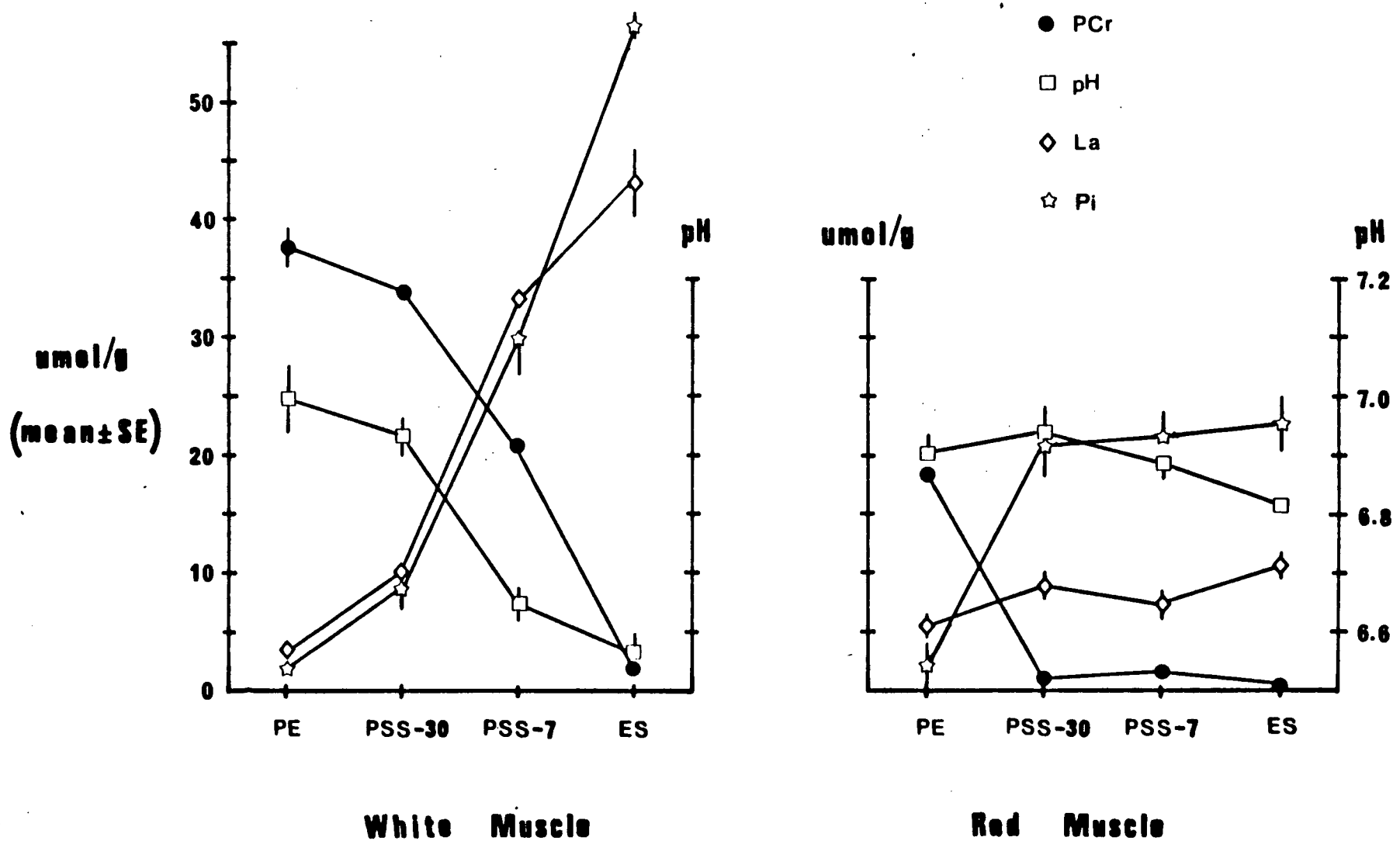
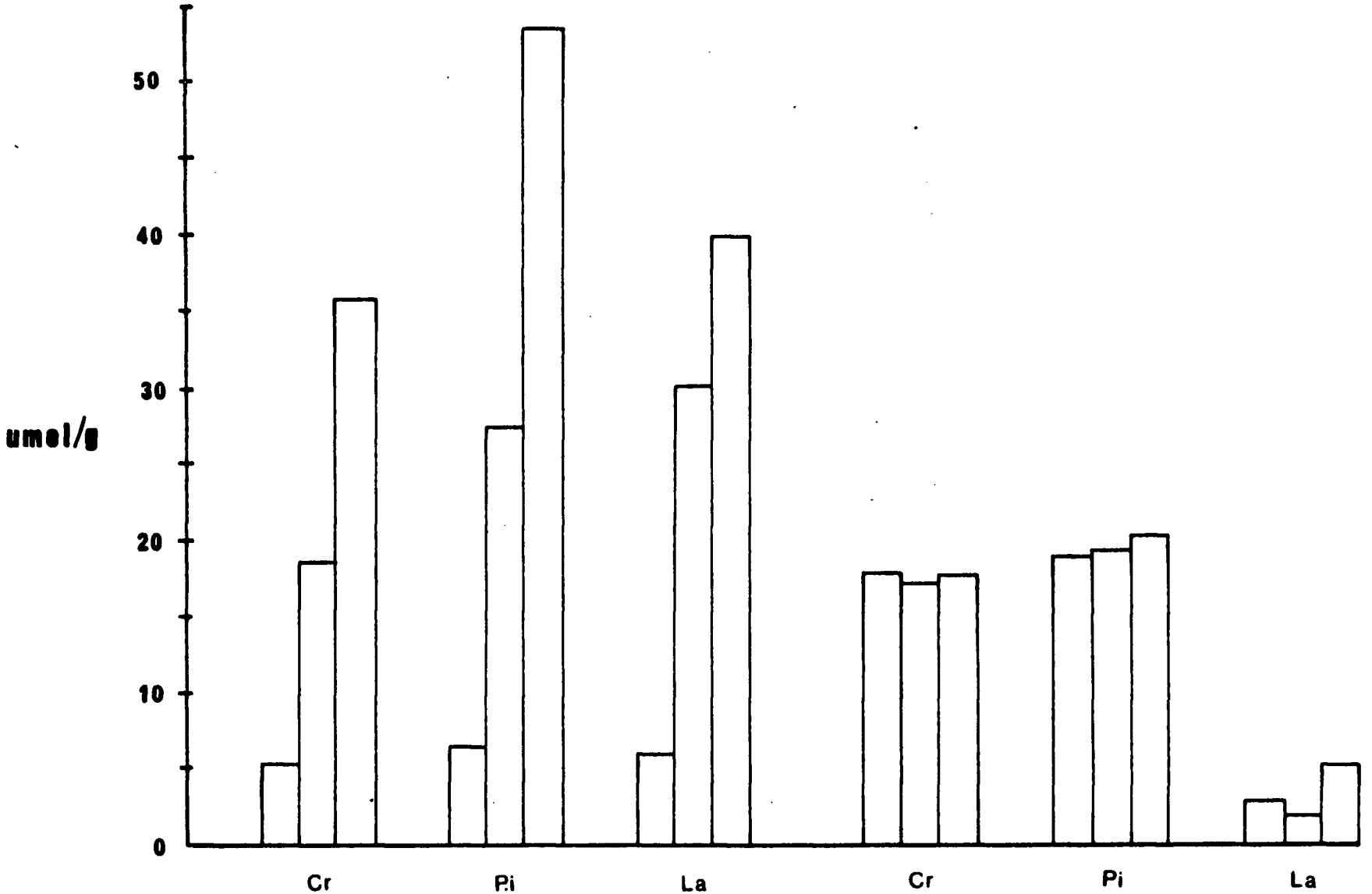

PCr
pH
La
Pi
umol/g
(mean±SE)
umol/g
pH
50
40
30
20
10
0
7.2
7.0
6.8
6.6
PE
PSS-30
PSS-7
ES
PE
PSS-30
PSS-7
ES
White Muscle
Red Muscle

Figure 11. Metabolic end product accumulations of creatine, inorganic phosphate and lactate in rainbow trout muscle.


umol/g
50
40
30
20
10
0
Cr
Pi
La
Cr
Pi
La
PSS-30
PSS-7
ES
White Muscle
Red Muscle

PROTON SEQUESTERING MECHANISMS

Crude Homogenate Buffer Capacity. Marlin red and white muscle buffer capacity was about 2 fold higher than the respective muscles of trout and within each species the buffering capacity of white muscle was twice that of red muscle (Table 16). In both species the buffering capacity of white muscle was found to be relatively constant over the pH range 6.0-7.5. Trout red muscle appeared to maintain its ability to absorb protons up to pH 8.0, whereas marlin red muscle did not (Table 16).

Histidine Related Compounds. Comparable results were obtained for the predicted, titrated and calculated histidine related compound buffer capacity of marlin white muscle (Table 17). The calculated histidine related compound buffer capacity was equal to the difference between the low molecular weight and phosphate plus other amino acid fraction buffer capacities. The predicted histidine related compound buffer capacity was based on the tissue content and pK characteristics. Since no differences were found between these methods, a predicted histidine related compound buffer capacity was assessed for marlin red and trout red and white muscle (Table 18). Based on these findings, the histidine related compounds contribute from as high as 65 percent for marlin white muscle over the pH range 7.0-8.0 (Table 19) to as low as 5 percent for trout red muscle over the pH range 7.0-8.0 (Table 20). The histidine related compound buffer capacity of white and red muscle was about sixfold higher in marlin than trout, regardless of the pH range. This difference is reflected in the relative tissue levels of the histidine related compounds (Table 17). A general trend of increased histidine related compound buffering in the pH range 6.5-7.5 was observed for all tissues (Table 18).

Protein Buffering. The buffering capcity of the pellet fraction, assumed to consist of myofibrillar protein, was comparable in all tissues (Table 16) and remarkably similar to the calculated myofibrillar protein buffering (Table 18). This calculated myofibrillar protein buffering was based on the difference between the crude homogenate and supernatant fraction buffer capacities (Table 16). The similarity in values is taken as evidence that the fractionation procedure did not alter the charge or pK characteristics of the myofibrillar protein. The comparable values found in all tissues can be attributed to the similar protein concentrations of their pellet fractions (Table 22). When the buffering due to myofibrillar protein was expressed relative to the crude homogenate buffer capacity, values ranged from a low of 8 percent over the pH range 7.0-8.0 for marlin white muscle (Table 19) to a high of 39 percent over the pH range 6.0-7.0 for trout red muscle (Table 20). This contribution was found to vary inversely with maximal buffer capacity of the muscle tissue. In all tissues, the buffering contribution attriutable to myofibrillar protein decreased as pH increased from 6.0-8.0 (Table 18).

The buffer capacity of the high molecular weight fraction, assumed to consist of mainly soluble proteins, was 1.5 fold greater in both white and red muscle of marlin than in trout (Table 16). These values were very similar to the calculated soluble protein buffering (Table 18) assessed as the difference between the supernatant and low molecular weight fraction buffer capacities. Due to the similar values, it would appear that the fractionization procedure did not alter the charge and pK characteristics of the soluble protein. When expressed relative to total tissue (crude homogenate) buffer capacity, the soluble protein contributes only 6 to 16 percent for all tissues (Tables 19 and 20). The buffer capacity of the

soluble protein appears to reflect the differences found in the supernatant protein content of the various fractions (Table 22).

Phosphate Buffering. Marlin white muscle phosphate buffering capcity was assessed on the fraction isolated from the quantification method B for histidine related compounds. The contribution of other amino acids to this buffering was considered to be negligible. An estimate of the other tissue's phosphate buffering capacity was calculated as the difference between the buffer capacity of the low molecular weight fraction and the histidine related compound plus taurine buffering (Table 18). The buffering that could be assigned to phosphate showed higher values in the white than in red muscle for both species (Table 18). These differences seem to reflect those found in tissue phosphate content (Table 22). The ability of the phosphate to buffer the alkali load decreased with increasing pH in all tissues (Table 18). The relative contribution of phosphate to total tissue buffer capacity ranged from 14 percent in marlin white muscle over the pH range 7.0-8.0 to 50 percent for trout white muscle in the pH range 6.0-7.0 (Tables 19 and 20).

Taurine Buffering. The red muscles of trout and marlin demonstrated high levels of taurine (Table 22). With increasing pH, the buffering ability of taurine increased (Table 18) to levels where it could contribute 27 percent of trout red muscle total tissue buffer capacity in the pH range 7.0-8.0 (Table 20).

The relative contribution of the various buffering constituents to total tissue buffering is illustrated in Figure 12.

Table 16. Buffer capacity of various fractions separated from white and red muscle of marlin and trout.

Fraction	Muscle	Marlin			Trout		
	pH	6-7	6.5-7.5	7-8	6-7	6.5-7.5	7-8
Crude Homogenate							
	W	96.4 ±8.5	97.3 ±5.8	76.1 ±4.4	63.3 ±3.7	56.7 ±2.2	43.4 ±1.7
	R	56.8 ±5.3	54.6 ±2.9	43.4 ±1.7	30.1 ±2.1	29.0 ±2.2	27.3 ±3.0
Pellet							
	W	12.0 ±1.9	8.9 ±1.3	6.5 ±1.0	14.9 ±2.7	13.3 ±2.2	10.7 ±1.5
	R	16.8 ±2.2	14.3 ±1.7	12.1 ±1.9	11.8 ±0.5	10.4 ±0.5	8.5 ±0.4
Supernatant							
	W	84.4 ±8.9	88.5 ±6.3	69.6 ±4.5	48.4 ±1.5	43.7 ±3.2	32.7 ±0.9
	R	40.0 ±7.2	39.5 ±3.6	30.8 ±2.7	18.3 ±1.9	18.6 ±2.2	18.4 ±2.7
High Molecular Weight							
	W	11.1 ±1.8	9.9 ±1.6	7.4 ±1.2	7.0 ±1.0	5.5 ±1.0	4.2 ±0.9
	R	8.0 ±0.7	6.2 ±0.7	4.4 ±0.7	4.2 ±0.2	3.3 ±0.2	2.3 ±0.2
Low Molecular Weight							
	W	74.1 ±6.8	77.9 ±5.3	59.9 ±4.2	40.2 ±1.2	36.2 ±1.4	25.9 ±1.6
	R	32.0 ±2.7	31.2 ±2.9	27.9 ±4.1	16.5 ±2.0	15.9 ±1.9	15.3 ±1.6

Values are means±SD expressed in umol NaOH/g/pH. W, white muscle; R, red muscle.

Table 17. Comparison of predicted, titrated and calculated histidine related compound and phosphate buffer capacities of marlin white muscle.

HRC	pH 6-7	pH 6.5-7.5	pH 7-8
Predicted a	50.2±5.7	59.3±6.8	46.4±5.4
Titrated b	52.3±8.2	66.0±8.5	57.6±7.5
Calculated c	39.6±8.9	60.6±5.7	49.2±6.7
Phosphate plus Other Amino Acids	33.2±9.5	21.4±8.9	11.0±4.5

Values are means±SD expressed in umol NaOH/g/pH. HRC, histidine related compounds.
a Based on pK characteristics of anserine (pK=7.03), carnosine (pK=6.83) and histidine (pK=6.0) and the tissue HRC contents.
b Representative marlin white muscle HRC concentrations were titrated.
c Low molecular weight fraction - phosphate plus other amino acid fraction

Table 18. Buffer capacity of histidine related compounds, phosphate, myofibrillar protein, soluble protein and taurine in white and red muscle of marlin and trout.

Compound	Muscle	Marlin			Trout		
	pH	6-7	6.5-7.5	7-8	6-7	6.5-7.5	7-8
HRC a							
	W	39.6 ±8.9	60.6 ±5.7	49.2 ±6.7	7.9 ±0.3	10.5 ±0.4	7.5 ±0.3
	R	11.3 ±3.3	12.3 ±3.8	9.4 ±3.0	2.3 ±0.6	2.0 ±0.7	1.4 ±0.6
Phosphate b							
	W	34.5 ±8.8	17.3 ±6.2	10.7 ±4.5	32.3 ±1.4	25.7 ±1.9	18.4 ±1.8
	R	19.6 ±4.4	16.9 ±7.8	12.3 ±8.8	12.9 ±1.9	11.6 ±1.9	6.5 ±1.8
Myofibrillar Protein c							
	W	12.0 ±1.2	8.8 ±0.9	6.5 ±0.6	17.9 ±2.1	13.0 ±1.5	10.7 ±1.2
	R	16.8 ±1.9	15.1 ±1.7	12.6 ±1.4	11.8 ±1.8	10.4 ±1.6	8.9 ±1.4
Soluble Protein d							
	W	10.4 ±1.2	10.6 ±1.2	9.7 ±1.1	8.2 ±0.5	7.5 ±0.4	6.8 ±0.4
	R	7.9 ±1.6	8.3 ±1.7	2.9 ±0.6	1.8 ±0.4	2.7 ±0.6	3.1 ±0.7
Taurine e							
	W	-	-	-	-	-	-
	R	1.1 ±0.2	2.0 ±0.4	6.2 ±1.3	1.3 ±0.2	2.3 ±0.3	7.4 ±0.9

Values are means±SD expressed in umol NaOH/g/pH. HRC, histidine related compounds. a) Marlin white muscle values are based on the difference between low molecular weight (LMW) and phosphate plus taurine buffering. Marlin red and trout red and white values are based on the pK's and tissue levels of these compounds (Table 17). b) Phosphate buffering=LMW-HRC plus taurine buffering. c) Myofibrillar protein buffering=crude homogenate-supernatant buffering (Table 20). d) Soluble protein buffering=supernatant-LMW buffering. e) Values are based on the relative tissue contents to titrated taurine buffer capacity.

Table 19. Percent relative contributions of various buffering constituents to total tissue buffering in marlin and trout white muscle.

Buffering Constituent	Marlin 6-7	Marlin 6.5-7.5	Marlin 7-8	Trout 6-7	Trout 6.5-7.5	Trout 7-8
Tissue	100	100	100	100	100	100
Myofibrillar Protein	12	9	8	27	23	25
Soluble Protein	11	11	13	12	13	16
Phosphate	36	18	14	50	45	42
HRC	41	62	65	11	19	17
Taurine	-	-	-	-	-	-

HRC, histidine related compounds. The contribution of other amino acids was considered to be negligible.

Table 20. Percent relative contributions of the various buffering constituents to total tissue buffering in marlin and trout red muscle.

Buffering Constituent	Marlin 6-7	Marlin 6.5-7.5	Marlin 7-8	Trout 6-7	Trout 6.5-7.5	Trout 7-8
Tissue	100	100	100	100	100	100
Myofibrillar Protein	30	28	29	39	36	33
Soluble Protein	14	15	7	6	9	11
Phosphate	34	31	28	43	40	24
HRC	20	22	22	8	7	5
Taurine	2	4	14	4	8	27

HRC, histidine related compounds. The contribution of other amino acids was considered to be negligible.

Table 21. Concentrations of histidine related compounds found in white and red muscle of trout and marlin.

Species	Muscle	His	L-MeHis	Car	Ans	Total
Trout	W	2.57 ±0.50	+	+	17.16 ±0.83	19.73 ±0.68
	R	0.59 ±0.18	+	+	3.02 ±0.57	3.61 ±0.72
Marlin	W	15.86 ±16.70	+	2.65 ±2.41	104.80 ±11.87	124.50 ±13.94
	R	4.79 ±0.48	+	+	21.13 ±7.35	26.12 ±7.25

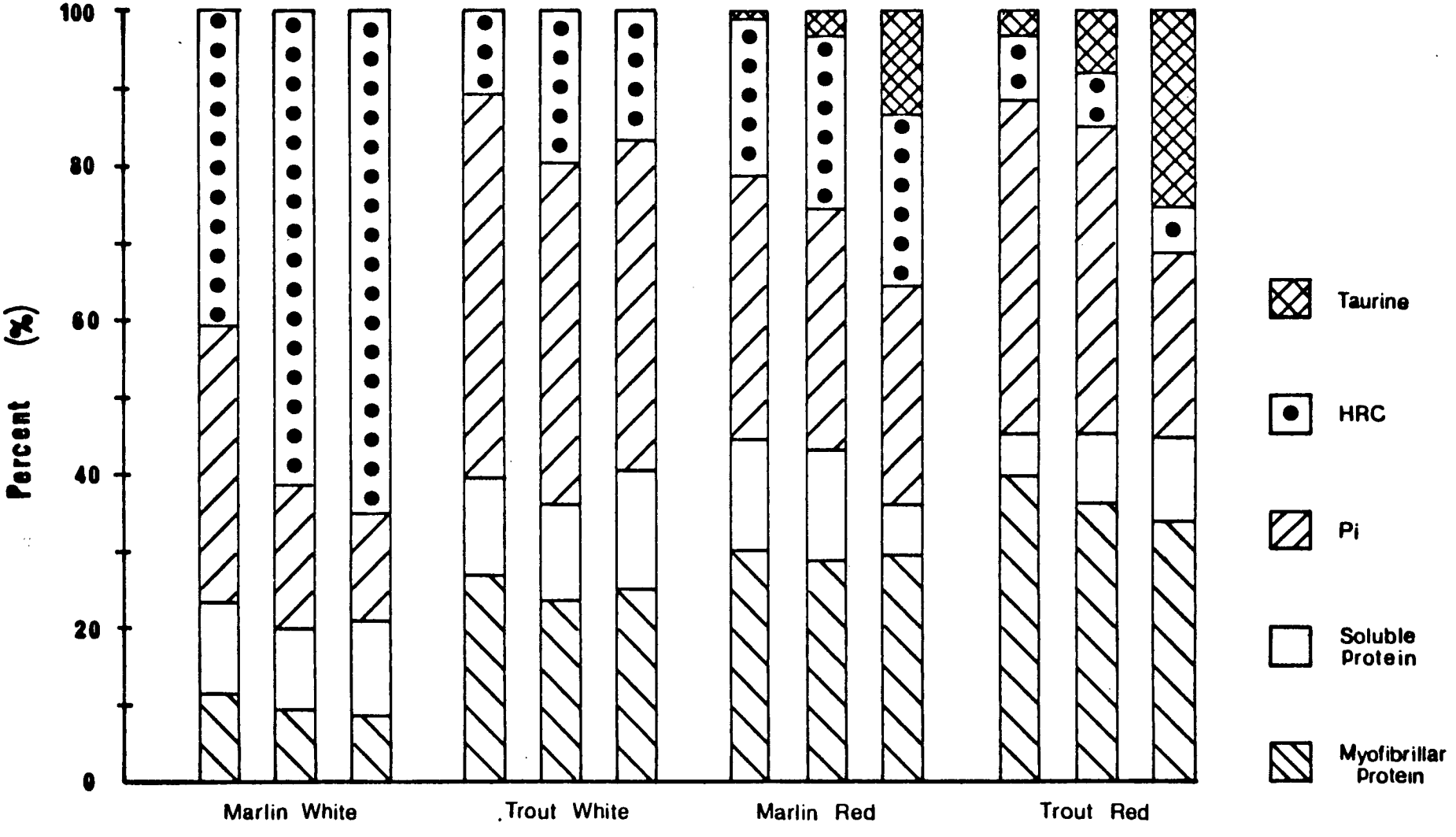
Values are means±SD expressed in umol/g (w/w). + refers to trace amount

Table 22. Concentrations of protein, inorganic phosphate and taurine found in the white and red muscle of marlin and trout.

Compound		Fraction	Marlin		Trout	
			W	R	W	R
Protein	(mg/g)	Crude Homogenate	125.4 ±10.6	108.3 ±8.6	115.5 ±12.2	99.1 ±8.3
		Pellet	61.1 ±5.9	54.0 ±6.1	54.0 ±6.2	49.9 ±7.6
		Supernatant	69.6 ±7.9	61.5 ±12.5	58.4 ±4.3	43.7 ±9.4
Inorganic Phosphate	(umol/g)	Supernatant	62.4 ±6.8	45.1 ±2.4	64.4 ±4.3	34.5 ±7.9
Taurine	(umol/g)	Supernatant	<3.0	44.7 ±8.5	<6.0	53.4 ±6.0

Values are mean±SD.

Figure 12. Percent relative contributions of the various buffering constituents to total tissue buffering in marlin and trout muscle.



Buffer Capacity

IN VIVO INTRAMUSCULAR BUFFERING

Tissue levels of the white muscle buffering constituents ATP, ADP, AMP, IMP and Pi are contained in Table 4, while the content of the glycolytic intermediates are found in Table 7. Tissue contents of the histidine related compounds are reported in Table 21. An estimate of white muscle buffering was made by calculating the buffering due to association of protons with bases (Table 23) and the buffering due to enzymatic activity (Table 24) following the burst exercise regime. Estimates of bicarbonate buffering are based on the low white muscle tissue contents (Heisler 1978) while the protein contribution to buffering (26.1 umol/g/pH) was assumed to be equivilent to that of the histidine related compound study (Table 18).

The total buffering of all the constituents analyzed due to proton association with bases was 40.9 umol H^+/g/pH (Table 23). The major contributor to this buffering appears to be protein which accounted for 64 percent of the total physico-chemical buffering. Histidine related compounds contributed 6.7 umol H^+/g/pH (16 percent) of this buffering while the inorganic phosphate was capable of absorbing 4 umol H^+/g/pH (10 percent). These three buffering constituents account for 90 percent of the buffering due to association of protons with bases. The other buffering constituents: bicarbonate, ATP, IMP and the glycolytic intermediates account for the remaining 10 percent of the physico-chemical buffering.

The total buffering of all the constituents analyzed due to enzymatic activity was 25.3 umol H^+/g/pH (Table 24). The major contributor to this buffering was the inorganic phosphate released during phosphate hydrolysis reactions. Release of Pi from the breakdown of PCr and ATP could

account for 12.1 umol H^+/g/pH, while the phosphate released from the other reactions could account for 10.4 umol H^+/g/pH. Phosphate therefore accounted for 89 percent of the buffering due to production or consumption of acids or bases (enzymatic activity). Ammonia production would absorb 1.8 umol H^+/g/pH such that phosphate and ammonia would account for 96 percent of the buffering due to enzymatic activity. The remaining buffering can be principally attributed to the accumulation of glycolytic intermediates, in particular G6P.

The total in vivo buffer capacity was estimated to be 66.2 umol H^+/g/pH (Table 25). Physico-chemical buffering accounted for 62 percent of this buffering potential. The principal buffers were assessed to be protein (39 percent), histidine related compounds (10 percent) and inorganic phosphate (40 percent). The remaining buffering was attributed to other physico-chemical and enzymatic buffering (6 and 4 percent, respectively) (Figure 13).

The titrated in vitro buffer capacity was found to be 63.3±3.7 umol H^+/g/pH (Table 16). The relative contributions of protein (39 percent), histidine related compounds (11 percent) and inorganic phosphate (50 percent) to total titrated buffer capacity are presented in Figure 13. The in vivo calculated (lactate) buffer capacity was found to be 88.1 umol H+/g/pH (Table 25). A comparison of percent relative contributions of the various buffering constituents to titrated, estimated and calculated total buffer capacities is contained in Figure 13.

Table 23. Estimates of proton absorbing potential of rainbow trout white muscle due to association of H+ ions with bases during the PSS-7.

Buffering Constituent	H+ (umol/g)	Buffer Capacity (umol H+/g/pH)
HCO_3 a	-0.26	1.0
ATP	-0.09	0.86
IMP	-0.06	0.12
Pi b	-1.32	3.96
NH_3	—	—
Ans c	-2.35	6.19
His c	-0.19	0.53
Protein d	-6.78	26.10
G1P	-0.04	0.19
G6P	-0.09	0.47
F6P	-0.01	0.07
FDP	-0.16	0.82
DHAP	-0.03	0.10
3PG	-0.06	0.30
GP	-0.08	0.25
Total	-11.52	40.9

Only those buffering constituents contributing greater than 0.01 buffering units (umol H+/g/pH) have been included.
a Calculated from estimated change in intracellular bicarbonate during exercise (Heisler 1978). All other values are calculated base on the lowest content measured and the change in concentrations of H+ buffering constituent associated with their respective pK's (Table 2).
b Calculated from the Pi content after the PSS-30 and a pK of 6.81.
c Calculated from the respective white muscle tissue contents (Table 21) and their respective pK's (Table 2).
d Assumed to be equivilent to the calculated soluble plus myofibrillar protein buffering (Table 18).

Table 24. Estimates of proton absorbing potential of rainbow trout white muscle due to enzymatic activity during the PSS-7.

Buffering Constituent	Metabolite Content (umol/g)	H+ (umol/g)	Buffer Capacity (umol H+/g/pH)
PCr a	13.0	-7.68	11.55
ATP	0.75	-0.03	0.50
IMP	1.25	-0.36	0.22
Pi b	7.05	-4.16	6.26
NH_4	1.8	-1.8	1.80
G1P	0.25	-0.06	0.15
G6P	0.74	-0.17	0.45
F6P	0.12	-0.03	0.07
FDP	-0.21	0.05	-0.13
DHAP	0.04	-0.02	0.03
3PG	0.37	-0.10	-0.24
GP	0.29	-0.14	0.24
Total	25.51	-12.69	21.1

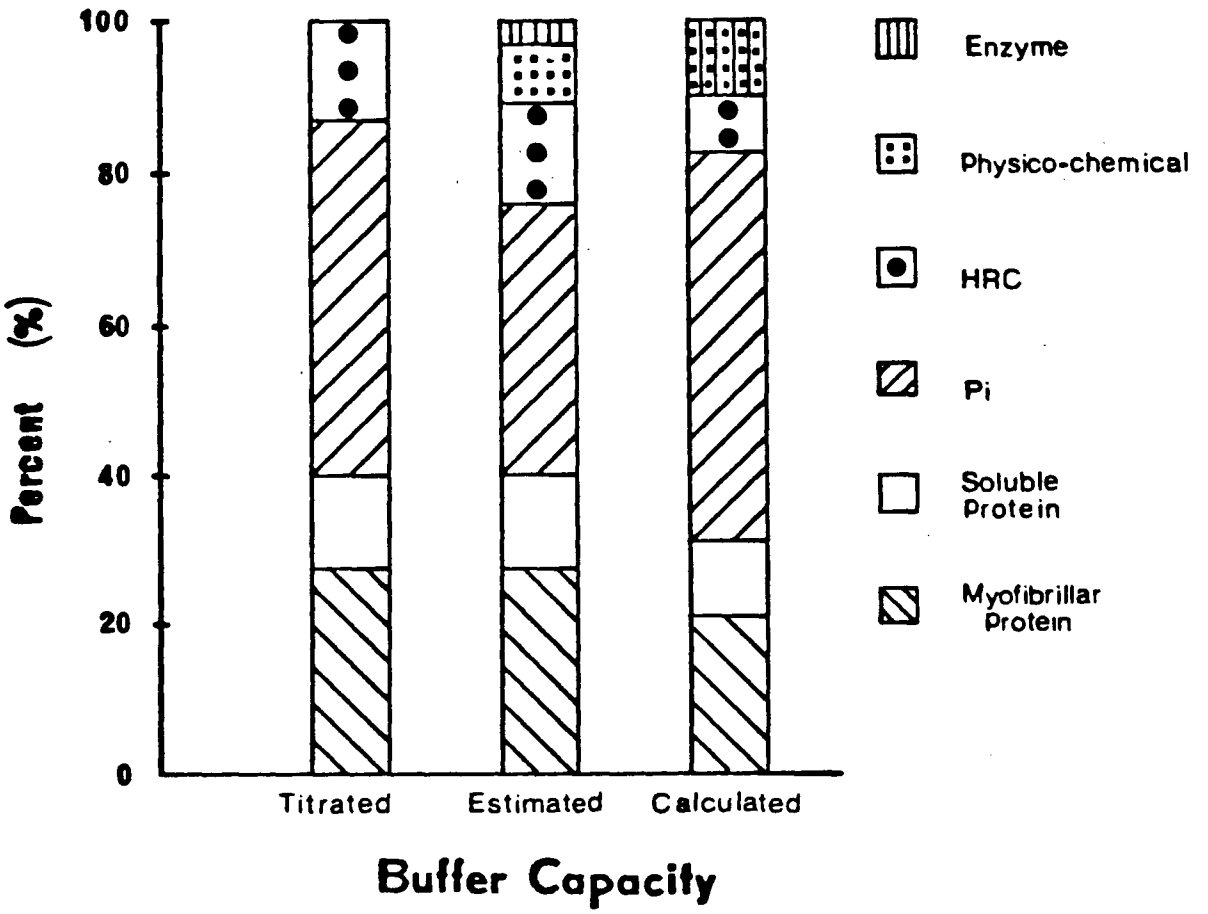
Only those buffering constituents contributing greater than 0.01 buffering units (umol H^+/g/pH) have been included.
a Calculated from the change in PCr content and a pK of 6.81 for Pi.
b Calculated from the change in Pi not attributable to PCr and ATP hydrolysis.
All other values are based on their change in concentration and their respective pK characteristic (Table 2).

Table 25. Comparison of titrated, calculated (lactate) and estimated (pK characteristics) buffer capacities of trout white muscle over the pH range 7.0 to 6.0.

Buffer Capacity (umol H^+/g/pH)		
Titrated	Calculated	Estimated
63.3±3.7 a	88.1 b	62.0 c

a Value is taken from the trout white muscle crude homogenate buffer capacity (Table 16).
b Value is calculated assuming a 1:1 coupling of H^+ ion production to lactate accumulation. $B = H^+/\Delta pH$
c Value is estimated as the buffering due to proton association with bases plus the buffering due to enzymatic activity.

Figure 13. Comparison of the relative contributions of the various buffering constituents to the titrated, calculated and estimated buffer capacities of trout white muscle over the pH range 7.0 to 6.0.


100
80
60
40
20
0
Percent (%)
Titrated
Estimated
Calculated
Buffer Capacity
Enzyme
Physico-chemical
HRC
Pi
Soluble Protein
Myofibrillar Protein

DISCUSSION

These findings demonstrated the integrative nature of fuel selection in skeletal muscles of rainbow trout during exercise. The regulation of this integrative fuel selection, appeared to revolve around adenine nucleotide metabolism with classical glycolytic control. Fatigue was associated with substrate and end product limitations in energy turnovers, to sustain the given work intensity. However, a substantial proton sequestering capacity within fish muscle, due predominantly to inorganic phosphate, protein and histidine related compounds, allowed high accumulations of lactate with comparatively minor perturbations to intramuscular pH.

FUEL SELECTION

Phosphagen and Adenylate Metabolism

PCr has an important role in energy metabolism as this metabolite is involved in the generation of ATP via the reaction catalyzed by creatine kinase (CK) and ATP thus formed maintains ATP levels during periods of high energy turnover (Hochachka 1985). To achieve this function, its P group must be transferable to ADP at high rates and at appropriate times as dictated by myosin ATPase activation due to their functional coupling. This is accomplished by having very high activities of the cytosolic (MM) isozyme of CK with its appropriate kinetic properties (Dawson et al. 1978; Gadian et al. 1981; Hochachka et al. 1983). In this regard, the Kd values for PCr are 72 and 32 mM for the binary and ternary complexes respectively, while for ADP they are 0.2 and 0.06 mM respectively (Jacobs and Kuby 1980). CK has been identified as the most abundant sarcoplasmic protein found in fish muscle

(Gosselin-Rey et al. 1968) with the MM isozyme being found in both red and white muscle of carp (Watts 1973). This enzyme is largely controlled by the concentration of adenylates and pH (Watts 1973). However, increasing concentrations of the glycolytic intermediates G6P, F6P, PEP, pyruvate and lactate have all been shown to inhibit fish muscle CK activity (Taame et al. 1979).

Based on the kinetics, PCr content under most conditions would not be saturating and the enzyme is therefore maximally responsive to changes in PCr content (Jacobs and Kuby 1980). Alternatively, recent evidence suggested that the ADP (free ADP; ADP_f) available to participate in this and other reactions occurs in much lower concentrations than is measured enzymatically. Estimates of resting free ADP generally fall in the range from 1 (less than 0.01 umol/g; Jacobus et al. 1982; Shoubridge et al. 1984) to 10 (approximately 0.07 umol/g) percent of measured ADP concentrations with the majority of investigators calculating free ADP to be at the latter end of this range (Dawson et al. 1977; Wilkie 1981; Meyer et al. 1985). In the present investigation, free ADP was calculated for both the measured and compensated metabolite data resulting in values ranging from 7 to 70 uM for pre-exercise red and white muscles (Table 9). Remarkably similar values of free ADP were demonstrated between fiber types when calculated with either the measured or compensated metabolite data. Free ADP contents were found to increase in both fiber types with exercise, these increases being minimal until PCr contents were low as would be expected due to the high CK and AK activities found in fish muscle (Noda et al. 1975; Johnston 1982a). Therefore the evidence from this study suggests that free ADP would not be saturating to the CK reaction allowing responsiveness to the free ADP increases which accompany exercise. As well, the high affinity of CK for ADP

would make it very competitive for ADP such that during the early stages of work the CK reaction would be driven in the direction of ATP formation (Gadian et al. 1981). Thus both enzyme content and enzyme kinetic properties favour high rates of ~P transfer to ADP. However, since PCr levels are not saturating and since they diminish during high energy turnover, these rates must rapidly decline (Hochachka 1985). The differences between red and white muscle in their ability to maintain ATP levels (Tables 4 and 5) may be due to the differences in PCr levels (Shoubridge et al. 1984; Meyer et al. 1985), the differences in enzyme activities (Hochachka et al. 1983) and to the large equilibrium constant for ATP formation of the CK reaction (Dawson et al. 1978; Gadian et al. 1981). This allows PCr to be almost completely converted to ATP prior to any decline in ATP concentrations. In red muscle, very low levels of PCr remained after the PSS-30 (<1 umol/g) and the ATP content was decreased. In contrast, white muscle at the end of the PSS-7 protocol, still contained modest PCr contents and therefore a relatively high ATP content. The ES resulted in both red and white muscle PCr depletion to very low values with the resultant ATP declines.

Enzymatic determination of PCr content in freeze clamped tissues has resulted in much lower concentrations than are determined by nuclear magnetic resonance (NMR) (Busby et al. 1978; Kushmerick and Meyer 1985; Meyer et al. 1985). It has been found that freezing per se results in PCr hydrolysis but not ATP hydrolysis (Meyer et al. 1985). The NMR investigations have found PCr content to be 85 (Meyer et al. 1982; Shoubridge et al. 1984; Meyer et al. 1985) and 68 (Meyer et al. 1982; Meyer et al. 1985) percent of total PCr and Cr at rest in white and red muscle respectively. These values are in agreement with the human values of 75-78 percent for this heterogeneous muscle (Dawson et al. 1978; Chance et al. 1981). These NMR investigations

use these facts to assess the true resting PCr content by measuring the ATP and PCr plus Cr contents in freeze clamped tissues and relating this information to their NMR spectras. In the present investigation, white and red muscle PCr contents were assessed based on these percentages (Tables 4 and 5) and found to be greater than previously recorded mammalian (Chance et al. 1981; Shoubridge et al. 1984; Kushmerick and Meyer 1985; Meyer et al. 1985) and fish (Dreidzic et al. 1981) values due to their greater total Cr plus PCr pool. PCr contributed only a small percentage of the metabolic energy turnovers required for these exercise intensities in both red and white muscle (Table 12). It is therefore apparent that the role of PCr during this type of exercise is one of ATP buffering.

The compensated PCr/Pi ratios observed in this study (Table 10) are similar to previously reported resting values (Chance et al. 1981; Dawson et al. 1977; Meyer et al. 1985) and demonstrate similar relationships with increasing workload to the electrically stimulated gastrocnemius muscle of rats (Shoubridge et al. 1984). Chance et al. (1981) observed a linear relationship between the PCr/Pi ratio and workload down to a ratio of approximately 1. They suggested that mitochondrial respiration was responsive to either these changes or the increasing Pi content. However, depletion of PCr resulted in lower ratios than have been reported previously due to the high initial PCr levels and the exhaustive exercise regime performed (Table 10). The linear relationship observed by Chance et al. (1981) no longer exists at these lower ratios demonstrating an uncoupling of myofibrillar ATPase activity to mitochondrial respiration.

The decreases in white muscle ATP content noted in this study (Table 4) are of similar magnitude to values found in previous studies on exercising

fish (Dreidzic and Hochachka 1976). No previous studies of fish exercising have reported ATP decrements in red muscle (Table 5) but, hypoxia has been found to elicit this response following one hour of decreased oxygen tension (20 Torr) (Dunn 1985). Accompaning these decreases in ATP are increases in ADP and AMP, although their elevations are insufficient to maintain the adenylate pool (Figure 2). Increasing ADP concentrations activate the adenylate kinase (AK) reaction by mass action such that the ADP increases are low (Dreidzic and Hochachka 1978). Therefore, AK functions to minimize alterations in the ratio ADP x Pi/ATP, thus maintaining a high free energy of ATP hydrolysis (Lowenstein 1972; Dreidzic and Hochachka 1978). However, although free ADP changes are minimized, concurrent alterations in the contents of ATP, PCr and Pi resulted in large changes in phosphorylation potential ($ATP/ADP_f.Pi$) (Table 9) and a subsequent decline in the free energy available from ATP hydrolysis.

Purine Nucleotide Cycle

At these workloads, which exceeded the tissues aerobic capabilities, GTP levels were found to decline. The GTP inhibition of 5'-AMP deaminase is therefore removed while increasing ADP activation is occurring. The concerted action of AK and 5'-AMP deaminase in response to the removal of GTP inhibition and ADP activation account for the decrease in the adenylate pool and the elevations of IMP and ammonia (Tables 4 and 5). The 5'-AMP deaminase reaction is one step in the reaction span termed the purine nucleotide cycle (Lowenstein 1972). According to this cycle, IMP further reacts with GTP and aspartate to form adenylsuccinate which in turn is converted to AMP and fumarate by the actions of adenylsuccinate synthetase and adenylsuccinase (Lowenstein 1972). It has been shown that this cycle functions in concert

with glycolysis in mammalian white but not red muscle (Meyer and Terjung 1979; Meyer et al. 1980). Furthermore, acidosis has been found to activate AMP deaminase in white muscle (Dudley and Terjung 1985). However, Driedzic and Hochachka (1978) suggested that in teleost white muscle the cycle acts as two separate arms with; (1) IMP accumulation during activity and (2) replenishment of the adenylate pool during recovery. On the other hand, the present investigation demonstrated decrements in the adenylate pool (Figure 2) as IMP accumulated while simultaneously AMP and fumarate were found to increase (Tables 4 and 5). As well, IMP continued to accumulate in red muscle during the PSS-7 while PCr, ATP, AMP and fumarate levels all increased (fumarate demonstrating its largest increase) (Table 5). It thus appears that 5'-AMP deaminase activity predominates over adenylsuccinate synthetase activity during exercise resulting in an accumulation of IMP. During the PSS-7, red muscle GTP content returned to pre-exercise values and would inhibit 5'-AMP deaminase serving to activate adenylsuccinate synthetase. Thus it would appear that the purine nucleotide cycle is operative in fish red and white muscle but the production of IMP and its reconversion to AMP occur at different rates during exercise and recovery. This difference between mammalian and trout red muscle in the functioning of the purine nucleotide cycle, may be related to the higher contractile (Johnston 1982b) and glycolytic (Johnston 1977) capacities of fish muscle. Although many roles for the purine nucleotide cycle have been postulated (Aragon and Lowenstein 1980), it would appear that its primary function is conservation of the adenine nucleotide pool (Figure 2).

These findings in regard to the purine nucleotide cycle, AK and CK stress two important functions of adenylate metabolism during exercise. First, a mechanism exists which attempts to prevent a reduction in the free energy of

ATP hydrolysis by controling ADP increases when ATP is reduced and secondly, the importance of ADP in the control of energy metabolism becomes apparent.

Phospagen Replenishment

Although this finding is tenuous, it was interesting to find an initiation of phosphagen replenishment within red muscle during the PSS-7 (Table 5) as electromyograph (EMG) recordings suggest that this muscle is still actively involved in force production (Hudson 1973; Bone 1978). To achieve this, the energy turnover requirements would have to be met by an increased white muscle energy turnover contribution which would allow a small percentage of the red muscle energy provision to be used for substrate replenishment. This appears to have occurred in the present investigation, although the metabolic costs are closely matched to the metabolic requirements such that the phosphagen replenishment is minor (Table 12). It is interesting to note that during the PSS-7, red muscle content of ADP decreased and GTP increased (Table 5) which could effectively inhibit AK and 5'-AMP deaminase. As well, citrate levels were increased, potentially acting to inhibit PFK along with decreasing ADP. Carbon flux through anaerobic glycolysis appeared to be reduced as evidenced by the lower lactate levels. It is therefore postulated that a small percentage of the red muscle energy turnover is used to replenish PCr and ATP while the increased white muscle energy provision supplies the necessary energy for the elevated workload. These red muscle ATP costs appear to be met chiefly by liver derived glucose and/or fatty acids since all other precursors were substantially depleted (Table 12). Priority of replenishment appears to have been given to ATP and PCr as glycogen levels remained low (Figure 3).

Glycolytic Energy Provision

Glycolytic energy provision can be achieved either anaerobically or aerobically with the concominant enhancement in ATP yield. Most of the glycolytic enzymes have been found in red and white muscles of trout (Johnson 1977, Walton and Cowey 1982) and both tissues have demonstrated the capacity to utilize glycogen, although at varying rates (Johnston and Goldspink 1973; Johnston and Moon 1980a,b). Red muscle glycogen decreased to very low values after all three exercise protocols (<1 umol/g) and could not be accounted for by lactate production (Table 5). Previous investigators have shown increased blood concentrations of lactate, presumably from white muscle and to a lesser extent red muscle (Johnston and Moon 1980a,b), but these levels (even assuming all blood lactate came from red muscle) plus tissue contents, still could not account for the amount of glycogen broken down in this tissue. Glycogen was found to contribute 14 percent of the energy turnover generated by red muscle (Table 12). Glycogen breakdown in white muscle occurred at all exercise intensities to values below 1 umol/g after the ES. The white muscle lactate accumulation could account for all the glycogen breakdown during the PSS-7 and ES, but not during the PSS-30 (Table 5). Since the release of lactate from white muscle is slow (Holeton et al. 1983; Turner et al. 1983; Milligan and Wood 1985), this descrepancy in lactate accumulation to glycogen breakdown is taken as evidence that white muscle can oxidize glycogen. Two possibilities exist to explain this descrepancy; (1) lactate oxidation within white muscle (glycogen oxidation indirectly) and/or (2) glycogen oxidation directly. The question as to whether this glycogen oxidation occurs directly and/or indirectly via lactate oxidation is uncertain. Evidence for the white muscle capacity to oxidize fuels comes from isolated mitochondrial studies, perfused trout hind parts and isolated muscle slices. In these

investigations, white muscle mitochondria oxidized pyruvate at a rate roughly equivalent to heart mitochondria (Donaldson 1985) and the perfused hind parts oxidized glucose at a rate of 80 nmol/100g/min (Moen and Klungsoyr 1981). Lactate has also been shown to be oxidized in isolated white muscle slices at a rate of 1/10th to 1/5th that of red muscle (Bilinski and Jonas 1972). Whatever the mechanism, this aerobic combustion of glycogen appears to contribute approximately 7 percent of the white muscle and total energy turnovers during the PSS-30 (Table 12).

Regulation of Glycolysis

The functional coupling of myofibrillar ATPase to the energy producing pathways should be emphasized before initiating any discussion of the regulation of glycolysis. In this regard, ATP the substrate of the former is the product of the latter and during muscular activity the rate of ATP required by the myofibrillar ATPases, dictates which energy producing pathways and to what extent each is activated. Evidence for this comes from reconstituted glycolytic systems where glycolytic flux can be increased by simply adding ATPases (Wu and Davis 1981). The myofibrillar ATPase activity of trout white muscle is approximately 3 fold higher than red, with the myofibrillar protein constituting 2 times the cell volume in white as opposed to red muscle (Johnston and Moon 1980b). Therefore the potential rate of ATP turnover due to myofibrillar ATPase activity would be 6 fold higher in trout white muscle and is reflected in the higher glycolytic enzyme activities (Johnston 1977).

The regulation of glycolysis was examined in both tissues by traditional techniques as outlined by Rolleston (1972) using analysis of enzyme

disequilibrium and applications of the crossover theorem. The crossover theorem identifies regulatory sites in complex enzyme systems and was first used by Chance and Williams (1955) to identify crossover points for the interaction of ADP with the respiratory chain of coupled mitochondria. An enzyme will be identified as regulatory if; (1) it is nonequilibrium, (2) its slope changes in the same direction as flux, (3) it demonstrates a crossover and (4) its substrate concentration changes in the opposite direction to flux (Williamson 1969).. However, strict adherence to these rules in multimodulated enzyme systems can lead to errors in the identification of regulatory sites due to the high complexity of pathway regulation. The interaction of various modulators at the different regulatory sites can mask the apparent crossover logic. This has led to the development of the Fault Theorem which assesses the allosteric modulators influence on the reaction as well (Rolleston 1972).

Phos, HK, PFK and PK have been identified by in vitro techniques as regulatory enzymes when either glycogen or glucose acts as the substrate for glycolysis (for reviews see: Bloxham and Lardy 1973; Sols 1979, 1981; Claus et al. 1984). Similarily, numerous investigations have identified these enzymes as regulatory in glycolysis in red blood cells, tumor ascities cells, heart, red and white muscle. In the present investigation, HK, PFK and PK have been identified as potential regulatory enzymes due to their degree of disequilibrium (Tables 4-7). Similarily, phos may act as a regulatory enzyme due to its assumed nonequilibrium status (Krebs 1981). However, it was surprising to find the GPDH.PGK complex exhibiting nonequilibrium kinetics in both tissues with increasing exercise intensity (Tables 4-7). Since this finding contrasts that found in the rat, where the GPDH.PGK complex within the gastrocnemius, soleus and plantaris maintains equilibrium with increasing

exercise intensity (Dobson et al. 1986), it is apparent that they may constitute a further regulatory complex within fish muscle.

The identification of control by the two enzymes (phos and HK) initiating entry of substrate into the glycolytic pathway is complicated by the merging of their carbons at G6P. Increasing G6P concentrations inhibit both HK and phos activities while serving to activate PFK (Sols 1981). Since G6P controls its own rate of formation from HK, G6P contents are thought to always change in the opposite direction to the rate of its formation from HK and therefore demonstrate no crossover point for this reaction (Rolleston 1972). When fish muscle myofibrillar ATPases are activated at rates which require glycolytic energy provision, muscle glycogen is the preferred fuel due to the much higher enzyme activities of phos than HK (Johnston 1977). Providing muscle glycogen is not limiting, the activation of phos by Ca^{++}, cAMP and epinephrine, which have been shown to accompany exercise in fish (Nakano and Tomlinson 1967), ensures that phos is activated at an appropriate rate and G6P accumulates and inhibits HK. Therefore, phos demonstrates regulatory properties provided muscle glycogen levels are not limiting as was demonstrated for trout white muscle (Figure 8). When muscle glycogen levels are limiting, HK may demonstrate regulatory properties as was observed for the trout red muscle (Figure 9). The observed slope which opposes flux, would tend to indicate inhibition at the HK reaction. However, the large difference in maximal enzyme activity between HK and PFK (Johnston 1977) would allow a new lower steady state of G6P to exist by matching the carbon flux through the two reactions.

PFK has repeatedly been shown to be regulatory for glycolysis (for reviews see: Bloxham and Lardy 1973; Ramaiah 1976; Goldhammer 1979; Sols

1979, 1981; Claus et al. 1984) demonstrating in vitro multimodulation by substrates, products and cofactors. However, no apparent regulation was demonstrated at PFK for either tissue in this study (Figures 8 and 9). This is misleading since FDP contents were found to increase when glycogen levels were not limiting suggesting activation of PFK. Furthermore, the allosteric modulators ATP, ammonia, Pi, ADP and AMP (Bloxham and Lardy 1973) were found to be altered in the appropriate direction to cause activation. However, F6P content has frequently been observed to change in the same direction as flux through the PFK reaction owing to the influx of hexose phosphates from glycogen (Williamson 1965; Driedzic and Hochachka 1976; Guppy et al. 1979). Phos demonstrates a 2.5 fold greater activity than PFK in both tissues and therefore could potentially account for these elevated F6P contents. As well, within red muscle, F6P contents were below pre-exercise values as would be expected when glycogen levels were limiting the influx of hexose phosphates (Figure 9). Furthermore, the F6P contents observed in this investigation (Tables 4 and 5) serve to stabilize the PFK aggregates and thereby amplify activation (Bloxham and Lardy 1973). The F6P saturation curve is sigmoidal demonstrating a Km in the 0.1 mM range and an increase in its concentration would lead to an increase in its rate of utilization by PFK while reversing ATP substrate inhibition (Danforth 1965). The falling ATP levels would lead to reduced substrate inhibition and to an increased affinity for F6P while the observed increased FDP and ADP contents, would serve to stimulate their own rate of formation resulting in a further activation of PFK (Sols 1981).

Insulin or epinephrine induced activation of glycolysis in the perfused rat hind limb found F2,6BP to increase 2 to 4 fold (Blackmore et al. 1982). F2,6BP has been shown to be the most potent activator of PFK thus far known

(Furuya and Uyeda 1982) demonstrating several critical effects on PFK; (1) It increases the affinity for F6P while decreasing the strength of F6P site-site cooperative interactions thereby making the F6P saturation curves less sigmoidal by moving them to the left; (2) It increases the affinity for the co-substrate ATP while reversing the inhibition caused by high levels of ATP; (3) It reverses inhibition by citrate and is synergistic in its effects with other positive modulators (ie, AMP and Pi) (Uyeda et al. 1981). Nevertheless, the possibility that F2,6BP is involved in PFK activation within fish muscle remains to be investigated.

PFK activity is integrated with PK and PGK activities since the ADP thus formed can act as a substrate for these reactions. Furthermore, FDP is a feed forward activator of PK in lower vertebrates and serves to couple PFK and PK functioning, a situation whish is potentiated as pH decreases (Storey and Hochachka 1974). PK demonstrates marked positive cooperativity in the binding of is substrates, with the Kd for ADP binding being lowered from the non-physiological range to 0.2 to 0.3 mM by the addition of PEP. Similarily, the Kd for PEP is also lowered from the non-physiological range to values between 0.01 and 1 mM on the addition of ADP (Dann and Britton 1978). Therefore the binding of one substrate leads to an order of magnitude increase in the affinity for its co-substrate with PK and PFK being integrated due to FDP and ADP activation of PK. The evidence for PK activation comes from the crossover analysis whereby PEP levels are lowered and PYR accumulates in both red and white muscle (Figures 8 and 9).

PGK demonstrates high activities in muscle and displays a high affinity for DPG (uM range) which suggests that this enzyme is saturated with substrate under most conditions (Krietsch and Bucher 1970). However, its

affinity for its co-substrate (ADP) has an apparent Km in the physiological range and would therefore be maximally responsive to changes in ADP content (Krietsch and Bucher 1970). In vivo, this enzyme functions as a complex with GPDH and is thought to be in equilibrium under most conditions (Lehninger 1975). However, within the erythrocyte, ouabain has been shown to induce inhibition of flux through this complex (Minakami and Yoshikawa 1966). In that investigation, an increased ATP/ADP ratio suggested the possibility of an ADP limitation at the PGK site. Increased Pi concentrations have also been shown to decrease flux through the GPDH.PGK complex relative to increased flux through PFK in the erythrocyte (Rose et al. 1964). Here it was suggested that the high Pi concentration shifted the mass action ratio of GPDH in favour of DPG and NADH, effectively inhibiting GPDH. In the present investigation, the GPDH.PGK complex was found to be out of equilibrium and to demonstrate crossover points (Figures 8 and 9) when PCr and glycogen contents were depleted to very low levels in both tissues (Figure 3). At these times, free ADP (Table 9), Pi (Tables 4 and 5) and NAD/NADH ratios (Table 10) were high while ATP levels were low. However, the NAD/NADH ratio does not appear to be involved in the regulation at this site as the combined GPDH.PGK/LDH reaction which factors out redox from the calculation (Table 1), was also found to be displaced from equilibrium (Figures 4-7). Therefore, it appears that the low ATP/ADP_f ratio (Table 9) results in the disequilibrium of the PGK reaction by shifting its mass action ratio in the direction of DPG and ADP, effectively inhibiting flux through the PGK.GPDH complex. An uncoupling of myofibrillar ATPase to glycolysis would have occurred due to an inability to maintain the correct ATP/ADP ratio as a consequence of an insufficient rate of carbon substrates.

Within the red muscle, although control of glycolysis would appear to

have been shared by HK, PFK, GPDH.PGK and PK as in white muscle, the low glycogen content following the PSS-30 resulted in HK acting as the flux generator. Because of its low activity relative to the other glycolytic enzymes and due to the increased flux as indicated by the elevated pyruvate levels, all intermediates appear to have established a lower steady state (Figure 9). This situation seems to have been reflected in the fish white muscle following the exhaustive swim (Figure 8).

Redox Balance

It was interesting to note that both the red and white muscle cytosolic compartments became more oxidized (increased NAD/NADH ratio), attaining maximal values around 2000 when muscle glycogen was depleted (Table 10). These findings are similar to those of Jobsis and Stainsby (1968) on the gastrocnemius-plantaris group and gracilis muscles of the dog following electrically stimulated 5 twitch per second contractions. Jobsis and Stainsby (1968) attributed the oxidation of NADH + H^+ to a temporary imbalance between the rates of pyruvate production by aerobic glycolysis and pyruvate utilization by the Krebs cycle. In contrast, Sahlin (1985) has found human quadriceps femoris muscle to become more reduced following exhaustive exercise performed at maximal oxygen uptake which would suggest either local hypoxia, an inability of the respiratory chain to oxidize NADH + H^+ at a sufficient rate and/or a limitation to mitochondrial membrane shuttling.

During anaerobic glycolysis, there is a need to continuously reoxidize NADH formed at the GPDH reaction. This is usually thought to be achieved by a 1:1 functional coupling between GPDH and LDH, although conditions during

the initial stages of glycolysis have been found to be unsuitable for NAD regeneration by LDH (Guppy and Hochachka 1978). Initially the pH is high, making the Km for pyruvate high, while pyruvate content is low. As pH decreases and pyruvate increases, LDH serves to maintain redox due to the acidic pH optimum of LDH in the forward direction and a lowering of the Km for pyruvate (Guppy and Hochachka 1978). However, when glycogen levels are low, these kinetic properties of LDH could continue to convert the elevated pyruvate to lactate due to: (1) the much higher LDH than PDH activity and (2) the induced shift of the LDH equilibrium in the direction of lactate and NAD formation. Since pyruvate levels are still elevated and GAP content depressed under this condition (Tables 7 and 8), an imbalance could have occurred between the functional coupling of GPDH and LDH reactions resulting in the free cytosolic NAD couple becoming more oxidized.

Lipid Metabolism

Lipids as a fuel source were not accounted for in this study but it has been demonstrated that free fatty acids (FFA) derived from triglycerides serve as a major aerobic fuel for energy metabolism in mammalian (Shaw et al. 1975; Hickson et al. 1977) and fish muscle (Krueger et al. 1968). The disposition of lipid in fish is different than mammals as fish possess negligible adipose tissue and the lipids are stored within the liver and muscle (Farkus 1967; Bilinski 1969). As well, the fat content of red muscle is usually twice that of white muscle (Bone 1966; Lin et al. 1974) with the fat being stored both intra- and extracellularly in red muscle. Furthermore, these red muscle intracellular lipids may be totally surrounded by mitochondria (Lin et al. 1974). While fat is a poor fuel for white muscle, red muscle demonstrates a high ability to utilize fat, demonstrating at least

10 fold higher rates of FFA oxidations in salmonids (Bilinski 1963; Jonas and Bilinski 1964). As well, high activities of carnitine palmitoyl transferase have been found in fish red muscle with the activity of this enzyme being 20 fold greater in red as opposed white muscle (Crabtree and Newsholme 1972).

To assess the relative contribution of the various substrates to energy provision of locomotion at different velocities, an estimate of the contribution of fats to energy turnover was calculated based on the maximal oxygen consumption data of fish muscle (Randall and Daxboeck 1979). Extrapolating their oxygen uptake data (for fish of comparable size to this study) to 100 percent Ucrit gave a maximal oxygen uptake of 3.71 ml O2/min/kg with 3.24 ml O2/min/kg being attributable to exercising muscle, an increase of 2.84 ml O2/min/kg (17.2 mmol O2/min/kg) above resting values. These values are very similar to the reported oxygen uptake value of Neumann et al. (1983) following strenuous exercise (3.1 ml O2/min/kg). Assuming a formation of 6 umol ATP/mmol O2/g of muscle and subtracting the glucose plus glycogen derived aerobic energy turnover, gave the energy turnover due to the oxidation of fats. The oxidation of fats predominated the energy provision in both red and white muscle at these workloads (Table 12). The citrate synthase activities found in fish muscle are more than sufficient to account for these observed oxidation rates in both red and white muscle (Walton and Cowey 1982).

Regulation of Krebs Cycle Flux

Within this study, further evidence for an activation of aerobic combustion of fuels within white and red muscle were the increases in the Krebs cycle intermediates malate and fumarate. Aspartate and alanine aminotransferase enzymes have been identified in fish muscle (Bell 1968) and could serve to augment Krebs cycle intermediates by forming oxaloacetate (Hochachka and Storey 1975) from which the carbon can be redistributed throughout the intermediates. Augmentation of Krebs cycle spinning, as would occur during exercise, could be accomplished in three ways; (1) Having mitochondrial isozymes with appropriate catalytic and regulatory properties; (2) Having enzymes which occur in low and high activity states; and (3) Having enzymes which are not saturated with substrate (Hochachka et al. 1983). The Krebs cycle enzymes do not appear to occur as isozymes but citrate synthase (CS), isocitrate dehydrogenase (ICDH) and 2-oxoglutarate dehydrogenase (OGDH) do appear to undergo a transition from a low to high activity state. These enzymes are collectively under allosteric regulation by the adenylates and CoASH (Hochachka et al. 1983). CS is inhibited by ATP and the decreasing ATP levels (Tables 4 and 5) along with increasing CoASH would serve to activate the enzyme. The observed increases in ADP (Table 9) would activate ICDH since ADP is a specific activator of this enzyme. As well, CS is limited by low concentrations of oxaloacetate while ICDH and OGDH are probably limited by isocitrate and 2-oxoglutarate availability, respectively (Hochacka and Somero 1984). Since CS, ICDH and OGDH appear to be limited by low levels of substrates, the increased concentrations of Krebs cycle intermediates serve to increase Krebs cycle spinning. Therefore, the regulation of Krebs cycle flux during exercise would appear to be under the control of the adenylates,

CoASH and levels of Krebs cycle intermediates.

Respiratory Control

Respiratory control refers to the coordination of mitochondrial oxidative phosphorylation with the ATP demands of the cytoplasm. Initially, the graded rates of respiration between State 3 and State 4 were preposed to be a function of ADP availability (Chance and Williams 1955; 1956). Subsequently, Klingenberg (1961) suggested that respiratory control was regulated by the cytoplasmic phosphorylation potential (ATP/ADP.Pi) and this theory has substantial support (Wilson et al. 1974; Nishiki et al. 1978; Stubbs et al. 1978; van der Meer et al. 1978). Finally, respiratory control was postulated to be simply a function of the ATP/ADP ratio (Slater et al. 1973; Davis and Lumeng 1975; Lemasters and Sowers 1979; Bohnensack 1981).

The lower estimates of ADP (free ADP) available to participate in reactions has cast considerable doubt on the latter two theories. The estimates of free ADP (Veech et al. 1979; Jacobus et al. 1982; Shoubridge et al. 1984; Meyer et al. 1985) are similar to this investigation and give rise to phosphorylation potentials and ATP/ADP ratios (Table 9) which are from 1 to several orders of magnitude higher than previous estimates (Veech et al. 1979). Similar ATP/ADP$_e$ ratios were found in this study but estimates of phosphorylation potentials were even higher due to the lower estimates of Pi (compensated). These new values fall in the ranges where only low rates of mitochondrial respiration are measured and the question arises as to how mitochondria can actively respire under presumably inhibitory conditions of high phosphorylation potentials or high ATP/ADP ratios? To resolve this question, Jacobus et al. (1982) experimentally separated the availability of

ADP to the F1-ATP synthetase and the exogenous ATP/ADP ratio. The rate of State 3 respiration was directly controlled by the ADP concentration, with little or no correlation to either cytosolic phosphorylation potentials or cytosolic ATP/ADP ratios. This suggested that respiratory control is regulated by availability of ADP to the F1-ATP synthetase mediated by the kinetic properties of the adenine nucleotide translocase (Lemasters and Sowers 1979). It was interesting to note that the Km values for heart mitochondria were in the 15 uM range (Jacobus et al. 1982). The values observed for free ADP (ADP_{fc}) in this investigation fell in this range and would suggest that mitochondrial respiration would be maximally responsive to increases in free ADP concentration. These results lend further support to the limitation of oxidative phosphorylation by the adenine nucleotide translocator rate. However, other factors including oxygen, Pi, substrate availability and matrix ATP/ADP ratios must be considered when examining the cellular control mechanisms involved in the regulation of oxidative phosphorylation.

Protein Metabolism

Proteins are known to contribute up to 10 percent of the total energy requirements during long-term exercise in mammals. The protein contribution to the energy requirements of exercise were not considered in this investigation, but the use of amino acids as fuels has been shown during starvation (Johnston and Goldspink 1973) and migration (Mommsen et al. 1980) in fish. The decreased alanine content and increased glycolytic intermediates within the liver suggested that the alanine may be active within the trout providing further hepatic glucose as fuel. However, since no studies have shown amino acid catabolism within fish during exercise and

because of the high rates of work (greater than 85 percent Ucrit) performed in this study, it would appear safe to assume that the protein contribution to energy provision was minimal. However, this does not preclude the possibility that protein catabolism could contribute substantially to sustained swimming energy requirements.

Hepatic Glucose

It was interesting to note that liver glycogen values decreased very little during the PSS-30 when red muscle glycogen values were falling rather markedly (Figure 3). This is in accord with the evidence that fish muscle does not utilize blood glucose very well as fuel (Walton and Cowey 1982). However, blood glucose levels have been found to decrease during exercise (Dobson, Mommsen and Hochachka, unpublished observations) and experiments using radioactive labels have demonstrated glucose uptake capabilities in both red and white muscle (Moen and Klungsoyr 1981). The increased red muscle glucose content following the PSS-30 (Table 5) suggested that hepatic glucose was involved in the energy provision since only low levels of glucose 6-phosphatase have been found in fish muscle (Walton and Cowey 1982). As well, the elevated glucose levels in both red and white muscles under all exercise states suggested a further involvement of blood glucose in energy production within the exercising muscles (Tables 4 and 5). Further evidence for this came from the high rate of liver glycogen breakdown during the PSS-7 (Figure 3). A previous investigation found blood glucose to decrease from 19.5 to 8.2 umol/ml during an exhaustive swim similar to the present study (Dobson, Mommsen and Hochachka, unpublished observations). The liver glycogen decrement observed in that study would have increased blood glucose levels 8.7 umol/ml assuming a liver weight of 2 grams and a blood volume of 5

mls/100g. Based on these values, approximately 210 umol of glucose would have been consumed over the whole swim bout (PSS-30+PSS-7+ES) resulting in a glucose removal rate of 2.7 umol/min. Though the hexokinase levels are low in fish muscle (white muscle 0.03-1.6; red muscle 0.14-2.6 umol glucose/min/g) (Johnston and Moon 1980a, Walton and Cowey 1982), they are sufficient to account for this hypothetical rate of removal when blood distribution is taken into account (Neumman et al. 1983).

Energy Turnover

It was of interest to find that; (1) The energy turnover required to perform the PSS-7 was approximately 1.2 times that of the PSS-30; and (2) The rate of energy turnover generated in white muscle was less than red muscle. In the former, the increase in the rate of work was 1.2 fold (mean velocity PSS-30=90 percent Ucrit; PSS-7=108 percent Ucrit) demonstrating a remarkable matching of energy turnover to power output. In the latter case, high speed burst exercise has been found to elicit a energy turnover of 375-1200 umol ATP/g/min in white muscle, far greater than red muscle (Dobson et al. 1986). It would appear that the metabolic energy turnovers required to perform the varied exercise intensities in this study were achieved by selecting the fiber type, fuel and pathway for optimizing ATP production rate versus substrate depletion and proton accumulation.

Role of ADP in Integrating Fuel Selection

The phasing in of the appropriate metabolic pathways at appropriate rates as exercise intensity increases, appears to be intimately related to the rate of myofibrillar ATPase activities. Although many regulatory signals may be

involved (Hochachka and Somero 1984), alterations in free ADP with increasing myofibrillar ATPase activities appears to play a prominant role through its actions on: (1) mitochondrial respiration; (2) the glycolytic enzymes PGK and PK; and (3) CK. It should be emphasized that a competition exists for ADP between all enzymes for which it is a substrate as these enzymes generally occur in the 10^{-4} M range while free ADP occurs in the 10^{-6} M range (Ottaway and Mowbray 1977). Initially, when mitochondria of non-working muscle are in State 4, flux through the electron transfer system is low because of limiting ADP or Pi content. As muscle work begins, mitochondria enter State 3 demonstrating a linear relationship in mitochondrial respiration and phosphorylation as ADP and/or Pi contents increase (Jacobus et al. 1982). This linear relationship probably accounts for the linear relationship observed by Chance et al. (1981) between work rates and PCr/Pi ratios. When State 3 capacity is surpassed, the further increases in ADP and Pi create conditions favouring CK and glycolytic competition for substrate. Initially the high activity of CK and its affinity for ADP ensures that this pathway predominates (Hochachka 1985). However, as work continues PGK and PK demonstrate a higher affinity for ADP than CK and a functional coupling occurs between glycolysis and myosin ATPase. This is achieved in the case of PK by lowering its Kd for PEP from a non-physiological range to values between 0.01 and 1 umol/g on the addition of ADP. As well, PEP has the same effect on the binding of ADP (Dann and Britton 1978). These constraints are recognizable in this study where free ADP levels were originally around those necessary for low rates of mitochodrial respiration. Subsequently, free ADP demonstrated a different rate of increase between fiber types which would lead to a coordinated activation of pathways and fiber recruitment. Therefore, the rate of myofibrillar ATPase induced alterations in ADP availability which accompany exercise ensures the phasing in of appropriate

pathways at appropriate rates.

Regulation of Exercise Metabolism by Compartmentation

The evidence for compartmentation of metabolism comes from a variety of sources and supports the concept that cell metabolism is a highly organized matrix of pathways where physical position of enzymes and changes in enzyme activity affect metabolite activities. With regard to glycolysis, evidence exists demonstrating specific localization and order of enzymes in the I-bands of the muscle fiber (Arnold et al. 1969; Segal and Pette 1969) with the equilibrium between bound and soluble forms being highly regulated. This in itself, suggests localization of glycolytic enzymes into 2 compartments (soluble and bound), which have been demonstrated within a single smooth muscle cell (Lynch and Paul 1983; Paul 1983). In this system, two pathways of glycolysis are functionally and discrete enough so that the glycogen derived glucosyl units do not enter the same pool of glycolytic intermediates that are used in the glucose derived lactate and vice versa. Thus, two lactate forming pathways exist, which are semi independent and use different precursors, while forming non-mixing pools of identical intermediates. This calls for a far more complicated metabolic control system than the traditional regulatory models of glycolysis.

The cellular concentrations of glycolytic enzymes far exceeds (approximately 100 fold) the cellular concentrations of glycolytic intermediates, which is opposite to conditions for most in vitro studies of enzyme kinetics. Based on this premise, Srivastava and Berhnard in a series of investigations (Weber and Berhnard 1982; 1985a,b) have demonstrated the direct transfer of the glycolytic intermediate DPG to GPDH and the GPDH

transfer of NADH to LDH with these reactions proceeding at a substantially faster rates than when NADH and DPG were supplied from solution. These rates were further enhanced 20 fold by saturating 3PG concentration which acted as a specific effector of the transfer process itself. Although this discussion is far from encompassing, it serves to illustrate the potential advantage of direct transfer of metabolites. It is postulated that during activated glycolysis, GPDH may be involved in a direct coupling for the transfer of DPG and NADH to PGK and LDH, effectively accelerating carbon flux through glycolysis by shifting the equilibrium from soluble to bound enzyme system. In this regard, it was interesting to note that hypoxia and ischemia in mammalian heart, resulted in an increased binding of glycolytic enzymes leading to increased activity and flux (Clarke et al. 1984). This system would not preclude the operation of a soluble enzyme glycolytic pathway under the traditional regulatory control but provide a second, bound glycolytic enzyme pathway (or parts of the glycolytic pathway), where the integration of the two pathways would achieve a coordinated transition from low to high flux rates.

The findings in this study are interpreted as being based a soluble enzyme system under classical metabolic regulation which requires that the flux changes and concentration changes be qualitatively and quantitatively consistent with a soluble model. The currently available NMR data suggest that those phosphate compounds in high concentration behave as if they are free in solution. The association of enzymes may not preclude a free solution equilibrium approach to the analysis since the effects are primarily to increase rates at low substrate concentrations and promote combined equilibrium conditions. Thus this approach to the data analysis is, in light of the current state of knowledge, the most reasonable. However, simple

calculation of free/bound glycolytic intermediate concentrations from equilibrium constants reveals how dramatic changes in the concentration of free intermediates has very little effect on enzyme disequilibrium. As well, the evidence that mitochondrial respiratory control is mediated by free ADP concentrations lends support to the concept that the equilibrium between soluble and bound enzyme/metabolite complexes serves not only to affect metabolite concentrations but as a potential regulatory mechanism itself. Similarily, the amount of bound ADP may be important in driving the mitochondrial, myofibrillar and sarcoplasmic reticulum CK isozymes which have been found to lie in close proximity to the mitochondrial translocator, M-line and Ca^{++} ATPase, respectively (Jacobus and Lehninger 1973; Bessman and Geiger 1981; Many and Kay 1978; Levitskii et al. 1977). Thus, the importance of compartmentation is apparent and subsequent investigations into metabolic regulation of exercise must address these findings. However, these findings in no way invalidate either previous or the present investigation into the regulation of metabolism during exercise, as these studies reflect whatever is occurring in the tissue, single or multiple pools.

FATIGUE

As the energy turnover requirements increased, there was a greater reliance on white fibers and an increased use of phosphagen and anaerobic glycolytic energy production to meet these needs (Tables 4 and 5). This was evidenced by the large drops in red muscle phosphagens and glycogen during the PSS-30 exercise while white muscle remained relatively inactive (Figure 3). At the higher workloads, white muscle phosphagen and glycogen levels were depleted while lactate accumulated. The intramuscular pH values reflected these perturbations in metabolism (Figure 10). The same energy

sources (ATP and PCr hydrolysis, anaerobic fermentations and oxidative metabolism) were used by both red and white muscle during exercise to achieve the metabolic energy turnovers necessary to maintain muscular work at given rates (Table 12). Subsequent depletion of intramuscular glycogen has been found to coincide with exhaustion during prolonged exercise (Hermansen et al. 1967; Hultman 1967; Hultman 1978). However, during either intermittent or short term high intensity exercise, fatigue was always found to occur prior to glycogen depletion (Saltin and Karlsson 1971; Hermansen and Vaage 1977). Similar findings were demonstrated in the present investigation in both red and white tissues (Tables 4 and 5).

PCr content was found to decrease in both red and white tissues during high intensity exercise (Figure 10). No relationship was found between PCr levels and tension development during intense exercise (Dawson et al. 1978). As well, during recovery experiments where PCr content was not restored, further contractions could be elicited although subsequent fatigue occurred at a greatly accelerated rate (Harris et al. 1976; Sahlin et al. 1979). It would appear that PCr principally functions to defend changes in ATP content during high intensity exercise and is directly involved in the fatigue process when very high rates of energy turnovers are required. Many investigators have found ATP content to decline during intense exercise (Hultman 1967; Hermansen 1971; Vaage et al. 1978) but few studies have assessed the time course of this decline. Wilkie (1981) reported ATP levels to remain relatively constant until fatigue was very advanced at which time ATP content fell to 25 percent of control values in frog gastrocnemius muscle. This suggests that ATP decrements per se are not the major factor in muscle fatigue, since ATP alterations did not parallel the changes in force generation. As well, ATP levels at fatigue are not diminished to a level

which should impair muscular contractions (Tables 4 and 5). Further evidence that ATP levels are not a causal factor in fatigue came about in recovery experiments where recovery in the contractile parameters occurred without any change in ATP levels (Harris et al. 1976; Sahlin et al. 1979).

Associated with these decrements in ATP content are increases in ADP and inorganic phosphate concentrations (Tables 4 and 5). Dawson et al. (1978) found changes in force generation and cross-bridge cycling to parallel alterations in ADP and inorganic phosphate contents. Hultman et al. (1981) has suggested that the increased concentration of ADP could both slow down the cross-bridge detachment and reduce the activity of the sarcoplasmic reticulum Ca^{++} ATPase. Inorganic phosphate has also been found to reduce the amplitude of stretch induced activation of insect fibrillar flight muscle (White and Thorson 1972) and increase the relaxation rate of skinned smooth muscle (Guth and Junge 1982). It would appear that ADP and inorganic phosphate accumulations may play a role within the fatigue process.

Most discussions of end-product accumulations during high intensity exercise have focussed on protons and lactate. An often overlooked fact is that PCr hydrolysis is coupled to myosin ATPase and therefore results in the accumulation of the guanidino compound Cr (or in the case of molluscan muscle, arginine) and inorganic phosphate (Figure 11). As with protons and lactate, it is imperative that these metabolic end-products not be deleterious. One reason why Cr is not deleterious is that its only metabolic fate is reconversion to its original phosphagen form (Walker 1979). However, inorganic phosphate is involved in many enzyme reactions in intermediary metabolism and a need to control its accumulation may place limits on the amount of PCr which can be stored (Hochachka et al. 1983). Fish white muscle

contains the highest PCr levels reported and because of this, may reflect the high lactate and Pi, contents of exhausted muscle.

It would appear that depletion of PCr could lead to ionic and charge perturbations since the breakdown of PCr which is present in the form PCr^{2-} would require production of a divalent counterion. Cr itself is uncharged and would therefore leave the cell in an anion gap. Hochachka (1985) has suggested that the coupling of the creatine kinase reaction to myosin ATPase negates this problem as inorganic phosphate (Pi^{2-}) is equivilent to PCr^{2-} in terms of charge. As well, he emphasizes that a second function of Pi^{2-} accumulation is buffering during proton generating metabolism. This is where the problem occurs, as PCr^{2-} and Pi^{2-} though demonstrating similar charge, have widely different pK values of 4.5 and 6.81 respectively (Table 2). In the physiological pH range, PCr^{2-} would be fully dissociated while Pi^{2-} would bind protons thereby altering its charge. If PCr^{2-} hydrolysis occurs without any proton accumulations, then Pi^{2-} would indeed negate the anion gap. However, should proton levels increase while PCr^{2-} hydrolysis is occurring (as would occur if anaerobic glycolysis were activated), Pi^{2-} would buffer the protons potentially leaving the cell in an anion gap. Fortunately, associated with the proton production is the accumulation of the anerobic end product lactate (La^{-}). La^{-} demonstrates a low pK (Table 2) and would as with PCr^{2-} be fully dissociated in the physiological pH range. In the present investigation, lactate accumulations were insufficient to compensate for the changes in PCr^{2-} content within red and white muscle (Figure 11). However, the PCr^{2-} hydrolysis would result in an equivilent release of Pi^{2-}. This Pi^{2-} in excess of the amount necessary to negate proton accumulations would be available to

maintain charge as was suggested by Hochachka (1985). Therefore it would appear that a combination of PCr^{2-} hydrolysis resulting in Pi^{2-} accumulations and lactate production serve to maintain constancy of charge.

Pre-exercise intracellular and extracellular concentrations of Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Cl^{-} were similar to previously reported values for fish plasma (Holeton et al. 1983), frog (Hodgkin and Horowitz 1959) or mammalian muscle (Sembrowich et al. 1983). Herbert and Jackson (1985) recently found alterations in these ions associated with anoxia to compensate the lactate accumulations in turtle plasma. However, in this investigation, alterations in Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Cl^{-} associated with exercise (Table 13), resulted in large changes in charge within the red and white muscle intracellular and extracellular compartments (Table 14). Fluid shifts could not account for these ionic perturbations. However, the techniques used in this investigation to assess intracellular ion content may have masked any local intracellular distribution. As well, the ion binding characteristics may have been altered such that the content of free ions may be quite different. Alternatively, a change in protein configuration and charge may occur negating the accumulation of positive charge within the intracellular compartment. Accepting these uncertainies, it would appear from the present investigation that fatigue was not associated with any alteration in charge. The evidence for this conclusion comes from the finding that the ion difference was greater in white muscle after the PSS-30 exercise than PSS-7 exercise protocols.

Despite these perturbations in charge and ion content, membrane potential was remarkably similar between fiber types and and after the various exercise intensities (Table 15). This was assuming a constant permeability coefficent

for each ion which may have been in error since various factors are known to alter membrane permeability. Lactate permeabilities are affected by external lactate concentrations (Koch et al. 1981) while Na^+ and K^+ permeability are affected by external calcium concentrations (McWilliams and Potts 1978). However, extracellular calcium concentration remained constant and would therefore not affect the ionic permeabilities. Fatigue was not associated with the white muscle after the PSS-30 exercise despite a drop in membrane potential. Thus it would appear that fatigue was not due to any change in membrane potential. However, the uncertinies in this calculation render this conclusion somewhat questionable.

Red muscle pH was relatively constant during all exercise intensities while white muscle pH was only maintained during the PSS-30 exercise regime. Subsequent decreases in white muscle pH occurred during the PSS-7 and exhaustive exercise bouts (Figure 10). The values reported in this investigation are similar to both the resting and working muscle pH values reported for human (Sahlin 1978), mammalian (Roos 1971; Hoult et al. 1974; Heisler 1975) and frog (Malan et al. 1976; Dawson et al. 1977). However, higher values have been found by Milligan and Wood (1985) using the DMO method on isolated trout trunk preparations (pH approximately 7.3). Three mechanisms exist for minimizing the decrements in intramuscular pH; (1) Efflux of protons from the cell into the blood and/or influx of neutralizing ions into the cell (Koch et al. 1981); (2) Stoichiometrically matching the protons generated to proton consumption by the aerobic combustion of fuels (Krebs et al. 1975); and (3) Absorption of protons by intracellular buffers (Parkhouse et al. 1985; Abe et al. 1985). At higher workloads, metabolic energy turnover cannot be matched by the aerobic combustion of fuels and proton production exceeds proton consumption with the resultant drop in pH.

These drops in pH, if of sufficient magnitude have been associated with reduced rates of glycolysis (Toews et al. 1970; Sutton et al. 1981), decreased times to fatigue (Fitts and Holloszy 1976; Stevens 1980) and decreased force generation by isolated muscle preparations (Dawson et al. 1978). However, Trivedi and Danforth (1966) have shown that increasing AMP and F6P lowers the pH optimum for glycolysis. As well, Dobson et al. (1986) has found F2,6BP increases to negate the inhibitory effects of decreasing pH on PFK activity. Re-examination of the time courses of pH changes and aberations in muscle function reveal that: (1) proton accumulations can occur before significant glycolytic activation (Fitts and Holloszy 1976); (2) the highest rates of glycolysis occur while protons are accumulating at their highest rates (Sahlin et al. 1981); and (3) glycolytic rate remains high and unchanged despite continued proton production and lactate formation (Dawson et al. 1978). Recently, a carrier-mediated lactate transfer system has been found to operate in skeletal muscle (Koch et al. 1981). This carrier-mediated transfer of lactate is pH dependent and appears to be an antiport system, lactate anions exchanging for OH^- ions (Dubinsky and Racker 1980; Koch et al. 1981). Hochachka and Mommsen (1983) therefore argue that proton production may; (a) create more favourable conditions for the unloading of oxygen; (b) facilitate phosphagen hydrolysis; (c) establish a pH optimum for glycolysis since inhibition of glycolysis only occurs at low pH values; and/or (d) facilitate lactate efflux due to the pH dependency of the carrier-mediated lactate transfer. There can be no question as to the benefits of proton accumulation on the Bohr shift or the rate of phosphagen hydrolysis, but the idea of establishing a pH optimum for glycolysis is misleading. This implies that decreasing pH increases the glycolytic rate. It is not the pH that optimizes the glycolytic rate but the action of

positive modulators which counteract the inhibitory effects of proton accumulations. Similarily, within fish white muscle lactate is retained negating the potential role of facilitated lactate efflux in this species.

In the present investigation, the fish were unable to continue to function at the PSS-7 swimming speed while substrate (glycogen) levels remained high. This inability to maintain a sufficiently high metabolic energy turnover could have resided in either the contractile or glycolytic machinery and appears to be related to the proton, ADP, inorganic phosphate and/or lactate accumulations. The matching of La^- production to PCr^{2-} hydrolysis and subsequent Pi^{2-} accumulations appears to negate potential metabolically induced charge perturbations. However, ionic alterations accompaning exercise were found to affect the intracellular and extracellular compartments charge characteristics, although these changes did not appear to alter membrane potential. Since proton inhibition of glycolytic rate appears to be counteracted by increased positive modulators, the proton, ADP and Pi induced alterations in the contractile machinery appear to be the most probable cause of fatigue during the PSS-7 swim while substrate depletion appears to be the primary cause of fatigue during the ES.

SKELETAL MUSCLE BUFFERING

The PSS-7 workload resulted in an inability of the fish to maintain the required velocity and appears to be intimately related to the effects of proton accumulations on the contractile machinery. Thus the ability of fish muscle to absorb protons generated during high intensity exercise was examined. High concentrations of imidazole based compounds exist in fish

muscle and the results support the role of these histidine related compounds as intracellular buffers. There are several lines of evidence from this investigation and others that support this conclusion. (1) The striking differences between red and white muscle buffering capacities (Table 16) and the concentrations of histidine related compounds (Table 21) with both species demonstrating similar five fold differences between their respective tissues in these parameters. (2) Marlin also showed six fold higher levels of histidine related compounds (Table 21) and buffer capacities in both its red and white muscle compared with trout (Table 16). (3) Histidine related compounds demonstrate pK characteristics which are consistent with their physiological role as buffers. The pK values are 6.0, 6.83 and 7.04 (histidine, carnosine, anserine, respectively) and in the case of anserine, its pK characteristics can account for the elevated buffer capacities found in the 6.5 to 7.5 pH range. (4) These two species operate in widely different water temperatures (marlin 25°C; trout 4-15°C) and therefore it is paramount that if similar, their choice of buffers not be temperature sensitive, since temperature alters the protonation state of many compounds. The imidazole based histidine related compounds are perfectly suited for this function as they conserve their protonation state under all conditions of temperature variation, thereby retaining their buffering potential over the physiological pH range (Somero 1981).

The higher buffer capacity observed for marlin skeletal muscle tissue was expected based on the swimming behavior of this species. These values are as high as any reported for fish species (Castellini and Somero 1981) and reflect their histidine related compound levels. The other principal components of total tissue buffer capacity appear to be protein and phosphate, while taurine may contribute within red muscle in the alkaline pH

range (Table 18). Proteins have long been recognized as a major buffer within skeletal muscle (Woodbury 1965). The comparable myofibrillar protein content of the tissues accounts for their similar non-soluble protein buffer capacities (Table 22). Previous investigators have shown this myofibillar protein buffering to be histidine based (Woodbury 1965; Morris and Baldwin 1984); this finding appears to be confirmed in this study, because the buffering capacity of this fraction decreased with increasing pH (Table 16). However, compared to soluble histidine related compounds, the myofibrillar protein histidine contribution to buffering is small (Table 18). The soluble protein buffering contribution relative to total tissue buffering capacity was remarkably similar across the species. An inverse relationship was demonstrated for the relative contribution of protein buffering to total tissue buffering. This suggests that buffering constituents other than protein are responsible for the elevated buffering capacities of the marlin red and white tissues. Of these, the aforementioned histidine related compounds are the most important but phosphate may have a potential role. Free phosphate can contribute to buffering since it demonstrates a pK (6.81) in the physiological pH range. During the tissue homogenization, hydrolysis of ATP and PCr would lead to an increase in free phosphate, thereby elevating the tissue buffer capacity and the phosphate buffering contribution. Thus, differences in buffering may be partially attributable to the higher phosphagen levels found in white as opposed to red tissues.

Note that the elevated buffer capacity of marlin white and red muscle (Table 16) could be totally attributed to their higher tissue levels of histidine related compounds (Tables 21). The high concentration of anserine in marlin white muscle parallels the situation found in tuna in which similarily high concentrations of histidine were found (80-90 umol/g) (Abe

1983b). It is interesting that both species belong to the same relative sub-order group (Scombriodidei), having similar swimming behavior and yet appear to have different histidine related compounds acting as their principal buffers. This may be misleading since these compounds are metabolically interconvertable (Aonouma et al. 1969). In tuna, a shift in the histidine related compounds has been observed during starvation. Free histidine decreased to 4 and 34 (5 and 12 days starvation, respectively) percent of controls but, the concomitant increase in the levels of anserine and carnosine led to a net decrease of only 30 to 40 percent of the control total histidine related compound pool (Abe and Hochachka 1986). Similarly in sockeye salmon, in which white muscle is the primary source of amino acids utilized during spawning migration (Mommsen et al. 1980), high anserine levels (around 40 umol/g white muscle) are maintained throughout the spawning run. Free histidine, on the other hand, shows a more than 10 fold drop (from 3.6 to 0.3 umol/g) during migration (Mommsen, French and Hochachka 1980). These shifts could be explained by a metabolic disposition of histidine to anserine and carnosine to prevent the degradation of free histidine during starvation. Anserine synthesis requires the methylation of histidine, which is in effect a way of protecting this reserve, since at least in rats, it cannot be converted back to methylhistidine (Aonouma et al. 1969). Thus the marlin may simply convert its free histidine into the more metabolically stable anserine dipeptide to conserve its buffering potential.

There is little information on the physiological functions of histidine related compounds other than buffering. Like taurine, histidine is only slowly metabolized and therefore does not seem to play a role in energy metabolism. In rat muscle, the half-life of carnosine was 29 days and 200 fold that of histidine while the half-life of anserine could not be

determined (Tamaki et al. 1977). Recent studies indicate a slow turnover of histidine in trout muscle although its uptake from blood into the tissue proceeds relatively rapidly (Abe and Hochachka 1986).

The histidine related compounds were found to contribute substantially to total tissue buffer capacity (Tables 19 and 20). It is known that histidine related compounds can complex copper (Brown 1981) and that this chelation would negate their potential proton absorbing capacity. As well, other roles for histidine related compounds have been proposed: they have been identified as myosin ATPase activators (Avena and Bowen 1969), as activators of sarcolemma Na+,K+ ATPase (Boldyrev and Petukhov 1978), as stimulators of fructose 1,6 bisphosphatase (Ikeda et al. 1980) and as a mechanism for transporting copper for the activation of cytochrome oxidase (Brown 1981). Thus the entire pool of histidine related compounds may not be free to participate as buffers. However, in fish species which possess high concentrations of these compounds, it is apparent that their dominant role is that of physiological buffers with the differences in levels of these compounds principally accounting for the buffering capacity differences found between the species and fiber types.

In Vivo Buffering Capacity

Similar values were found for the trout white muscle titrated (in vitro) and estimated (in vivo) buffer capacities (Table 25). At first glance, this finding would appear to suggest that fish white muscle crude homogenate (titrated) buffer capacities represent in vivo buffering. However, the in vitro crude homogenate titration technique assesses the maximal potential buffer capacity of the tissues, whereas the in vivo estimation of buffer

capacity only assess the buffering which occurred during the exercise. This implies that some potential buffers are unavailable to negate proton accumulations during the exercise. Intuitively this makes sense, as intermediary metabolism generates weak acids (glycolytic intermediates and inorganic phosphate) as glycogen, PCr and ATP are depleted. PCr and ATP generated inorganic phosphate can potentially be the most important buffering constituent during exercise (Burton 1978). As well, during periods of high intensity exercise, PCr hydrolysis serves to defend ATP declines (Hochachka 1985). Therefore this potential buffer (Pi) is made available during periods of high proton generation.

The evidence for this hypothesis comes from the examination of the contribution of inorganic phosphate to buffering. As was suggested by Burton (1978), the contribution of inorganic phosphate to buffering increases greatly once exercise has been initiated. In the present investigation, inorganic phosphate demonstrated minor variation in its contribution to buffering, 22.4 in vivo (Tables 23 and 24) versus 32.3 in vitro(Table 18) (umol H+/g/pH). However, PCr and ATP contents at the end of the PSS-7 exercise were still 20.8 and 5.8 (umol/g) respectively (Table 4). During the ES, these values were found to decline to 1.8 and 2.7 umol/g for PCr and ATP respectively (Table 4). These alterations would increase the in vivo inorganic phosphate contribution to buffering approximately 21 umol H+/g/pH and the total tissue buffering to about 83 umol H+/g/pH. Therefore it would appear that the in vivo buffer capacity is greatly infuenced by the metabolic status of the muscle.

The contribution of protein buffering was considered to be equivalent in both the titrated and estimated buffer capacities. This was based on the

assumption of no proteolysis during the exercise protocol. This assumption may be in error as fish muscle has the potential to use proteins as fuels for work (Johnston and Goldspink 1973). However, these findings have usually been found in combination with starvation (Johnston and Goldspink 1973; Mommsen et al. 1980) and are therefore thought to be unlikely in this investigation. Protein buffering has been demonstrated to be histidine based (Woodbury 1965; Morris and Baldwin 1984) and the content of histidine necessary to produce the 26.1 umol H+/g/pH of buffering is approximately 125 umol/g or 13 percent. As well, histidine related compounds were found to contribute remarkably similar amounts to the titrated (7.9±0.3 umol H+/g/pH; Table 18) and estimated (6.7 umol H±/g/pH; Table 23) buffer capacities. Thus it is apparent that proteins and histidine related compounds constitute consistent and important buffering components.

The calculated (lactate) buffer capacity exceeded both the titrated and estimated buffer capacity values demonstrating a value similar to the estimated maximal white muscle buffering capacity (Table 25). However, the ES demonstrated afurther decrease in pH to approximately 6.55 and an increase in lactate content to 43 umol/g. These values would result in a calculated buffer capacity of approximately 110 umol H^+, far in excess of the theoretical maximal buffer capacity. This finding was similar to that of Sahlin et al. (1978) for human vastus lateralis muscle and was not unexpected based on the pH range over which the buffer capacity was determined. This pH range (6.91-6.65) revolves around the two principal buffers, anserine (pK 7.04) and inorganic phosphate (pK 6.81). Thus the change in pH during the PSS-7 exercise centered around the point of greatest buffering potential within the cell and would produce a falsely high buffer value.

It would therefore appear that restraint must be exercised when attempting to imply that the crude homgenate titrated buffer capacity reflects in vivo buffering. However, the dominance of buffering by protein, histidine related compounds and inorganic phosphate does allow a reasonable estimate of maximal potential in vivo buffering. These buffering constituents represent approximately 90 and 80 percent of the maximal potential buffering capacity in white and red muscle respectively. The lower percentage for red is due to the greater contribution of ammonia to its buffering. Based on these findings, buffer capacity values of 83 and 55 (umol H^+/g/pH) are estimated for rainbow trout white and red muscle respectively. These values are relatively high and reflect the capacity of this animal to accumulate lactate and protons.

SUMMARY AND CONCLUSIONS

ATP hydrolysis, phosphagen hydrolysis, oxidative and anaerobic fermentative based energy provision occurred at all exercise intensities with swimming velocity dictating the extent to which each fiber type, fuel and pathway was used. Thus, the metabolic energy turnovers required to perform these workloads were achieved by using the same fuels (ATP, PCr, glycogen, glucose and fats) and varying the metabolic pathway contribution in both red and white muscle. The inability to generate sufficient energy turnover to continue to work resulted in fatigue under the PSS-7 and ES regimes. However, the etiologies of the fatigue were different. During the ES and PSS-7 regimes, substrate and end product limitations respectively, would appear to have been related to the inability to continue functioning. During both the PSS-30 and PSS-7, white muscle was found to be activated to a lesser extent than red muscle. However, the PSS-7 resulted in a further activation of white muscle glycolytic energy provision while red muscle energy turnover requirements were achieved by predominantly aerobic combustion of fuels. The purine nucleotide cycle was found to be operational in both fiber types although the formation of IMP and replenishment of adenine nucleotides appear to operate at differential rates. Lipid metabolism dominated energy provision at these power outputs although complete depletion of phosphagen and glycogen accompanied the ES in both red and white muscle. It would appear that the metabolic energy turnovers required to perform the varied exercise intensities in this study were achieved by selecting the fiber type, fuel and pathway for optimizing ATP production rate versus substrate and proton accumulation.

The regulation of fuel selection appears to be intimately related to

myofibrillar ATPase activation with free ADP acting as a metabolic signal to coordinate the phasing in of appropriate fuels/pathways at appropriate rates. This metabolic control exerted by free ADP is accomplished in the case of high energy turnovers by the competition of CK and the glycolytic enzymes PGK and PK for this substrate. During lower intensity workloads, mitochondrial respiration exerts a high affinity for ADP and because of the low concentrations of free ADP, is maximally responsive to changes in the concentrations of this metabolite. As myofibrillar ATPase activity increases, free ADP content availability increases creating conditions favouring CK and glycolytic competition for the substrate. However, concurrent alterations in ATP, PCr and Pi result in large changes in phosphorylation potential (ATP/ADP.Pi) and a subsequent decline in the free energy available from ATP hydrolysis. HK, phos, PFK and PK are all subject to allosteric regulation by adenine nucleotides and were identified as regulatory enzymes in both fiber types. However, the GPDH.PGK complex also appears to exhibit regulation when glycogen is limiting and this regulation appears to be induced by a decreased ATP/ADP_f ratio. An uncoupling of myofibrillar ATPase to glycolysis would have occurred due to an inability of glycolysis to maintain an appropriate ATP/ADP_f ratio as a consequence of insufficient carbon substrate. The free cytosolic redox state of the NAD couple became more oxidized in both tissues when muscle glycogen was limiting and this finding was attributed to an induced shift in the Keq of LDH in the direction of NAD and lactate by the elevated pyruvate and low pH. The simultaneous ATP/ADP_f induced disequilibrium of the PGK reaction would inhibit flux through the GPDH.PGK complex and allow an imbalance to occur in the free cytosolic redox state of the NAD couple. It would therefore appear that adenine nucleotides play a central role in the coordination of metabolism during exercise.

Proton induced alterations in the contractile machinery appeared to be the most probable cause of fatigue during the 7 minute higher intensity prolonged steady swim. Histidine related compounds were found to play a dominant role in buffering within trout and marlin muscle with differences in the levels of these compounds principally accounting for the buffering capacity differences found between the species and fiber types. However, in vivo buffer capacity was dominated by protein and the inorganic phosphate released during phosphate hydrolysis and transfer reactions. Histidine related compounds contributed a fixed small percentage of the trout white muscle buffering during the exercise.

Thus, these metabolic and biochemical adjustments, allowed a coordinated integration of fiber type, fuel and pathway selection, to achieve the appropriate energy turnovers for the coupling of myofibrillar ATPase activity to ATP turnover, while optimizing ATP production rate versus substrate depletion and proton accumulations.

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Appendix I

Determination of creatine and phosphocreatine levels in skeletal muscle tissues at rest and during exercise by isocratic anion exchange high performance liquid chromatography.

Introduction

Phosphocreatine (PCr) and creatine (Cr) are important components of skeletal muscle during bouts of high intensity exercise where PCr serves as an energy substrate. PCr is very labile and special care must be taken to prevent hydrolysis during storage and preparation of biological samples. Lowry et al. (1964) has described enzymatic procedures for the determination of PCr and Cr but, these methods involve multiple enzymes and cofactors, besides being tedious. Previously, Harmen et al. (1982) has described a method for the separation of creatine phosphate and the adenine nucleotides by anion exchange high performance liquid chromatography (HPLC), but each run took considerable time (28 minutes) and was followed by a long column regeneration time. As well, no creatine values were reported for this method. This was because the bases adenosine, inosine and hypoxanthine overlap the Cr peak giving falsely elevated values.

More recently, McMahon and Lutz (1984) have been able to moniter PCr levels in brain tissues by isocratic reverse phase, ion pair HPLC. This procedure can be performed in a short time (7 minutes) and appears well suited for tisues exhibiting low phosphate levels. However, the high values of phosphate and phosphorylated compounds present in skeletal muscle render this system inappropriate. As well, no values are again reported for creatine.

For the purpose of this investigation, we have chosen to examine exercising fish muscle since the body musculature comprises two functionally and spatially different fiber types, red and white. As well, these tissues possess a highly active purine nucleotide cycle which would result in elevated levels of IMP and AMP during high intensity exercise. The following procedure describes a rapid and accurate method for the analysis of PCr, Cr, NAD and the nucleotide monophosphates in skeletal muscle tissue by isocratic anion exchange HPLC.

Methods and Materials

(see Dissertation methodology; Animals-Metabolite Studies; Metabolite Studies; Metabolite Extraction; Chromatography)

Results and Discussion

Separation of Cr, PCr, inosine, adenosine, NAD and nucleotide monophosphate standards are presented in Figure 1. Figures 2 and 3 demonstrate the hydrolysis of PCr, a shift to creatine and an elevation of IMP and AMP in white and red muscle respectively, as a result of vigorous physical activity. As previously mentioned, fish muscle possesses a very active purine nucleotide cycle which is responsible for the elevated IMP values which accompany exercise. Inosine levels can be seen to have increased within red muscle during the exercise as a result of adenosine deaminase activity, the enzyme responsible for converting adenosine into inosine. Adenosine has become available during the exercise via the action of AMP deaminase and the purine nucleotide cycle. However, the use of this procedure to assess inosine levels can only be qualitative due to the overlapping of the hypoxanthine peak which would be expected to contribute

approximately a 2-5 percent error at physiological levels.

NAD levels were found to be comparable to reported mammalian values determined enzymatically (Jobsis and Stainsby 1968; Sahlin 1985) and did not change in either tissue during the exercise. Nevertheless, the PCA extraction procedure employed in the tissue preparation would have converted all the reduced NAD into its oxidized form and therefore appropriate precautions must be employed if this metabolite is to be assessed. NADH cannot be detected by this procedure due to its low extinction coefficient at this wavelength.

The measured PCr and Cr levels were compared to enzymatically determined values in Table 1. The levels obtained by these two methods were comparable with no differences being observed on samples stored at -80 C and those analyzed immediately after extraction.

Table 1. Resting Cr and PCr values in white muscle of rainbow trout.

Method	White Muscle	
	Cr	PCr
n	(5)	(5)
HPLC	24.6 ±2.2	19.9 ±3.5
Enzymatic Assay	27.5 ±3.9	17.5 ±0.8

Values are mean±SD expressed as umol/g wet weight.

By using a lower strength anion exchange column than was employed by Harmsen et al. (1982), Cr is separated from the interfering bases. As well, the use of an isocratic potassium phosphate elutant provides for a more accurate deterination of nucleotide monophosphates, due to lower background noise in comparison to the elevated potassium phosphate levels required for the gradient mode chosen by Harmsen et al. (1982). The nucleotide di- and triphosphates are strongly bound to the column and require higher concentrations of potassium phosphate to be eluted. However with time, these compounds begin to elute even under the 50 mM potassium phosphate elutant. No interference from these compounds was observed if the column was washed with 600 mM potassium phosphate (pH 2.5) for 10 minutes after every hour of use. This metod therefore allows a rapid and sensitive method for the determination of Cr, PCr and nucleotide monophosphates while simultaneously monitering the functioning of the purine nucleotide cycle.

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Figure 1. Separation of Cr, PCr, adenosine, inosine, NAD and nucleotide monophosphates (10 ul). 1=0.1 mM adenosine; 2=1 mM Cr; 3=0.1 mM inosine; 4=1.5 mM CMP; 5=0.1 mM NAD; 6=1.5 mM AMP; 7=1.5 mM UMP; 8=1.5 mM IMP; 9=1 mM PCr; 10=1.5 mM GMP.

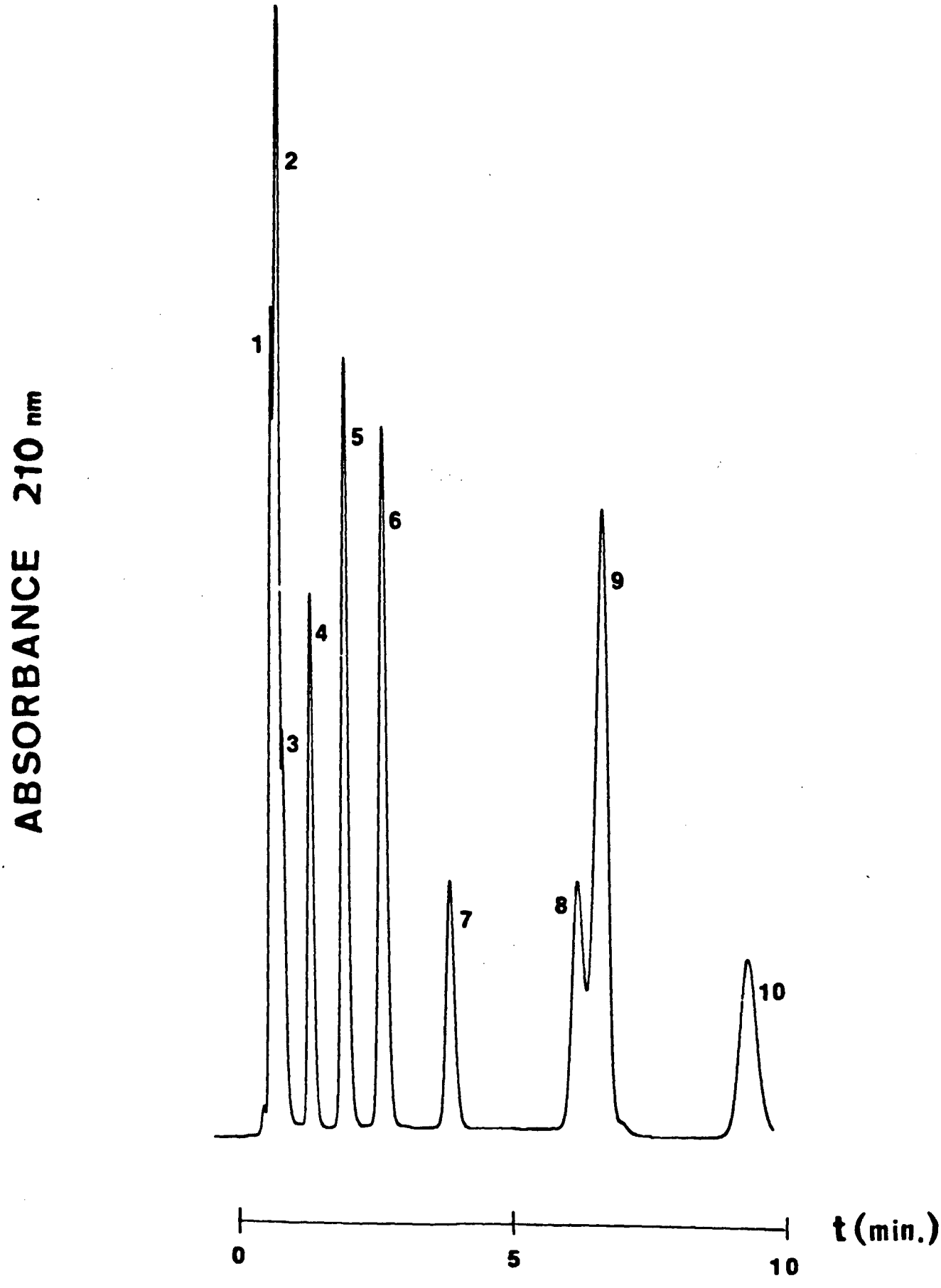

ABSORBANCE 210 nm
1
2
3
4
5
6
7
8
9
10
0
5
10
t (min.)

Figure 2. Separation of Cr, PCr, adenosine, inosine, NAD and nucleotide monophosphates in trout white muscle.

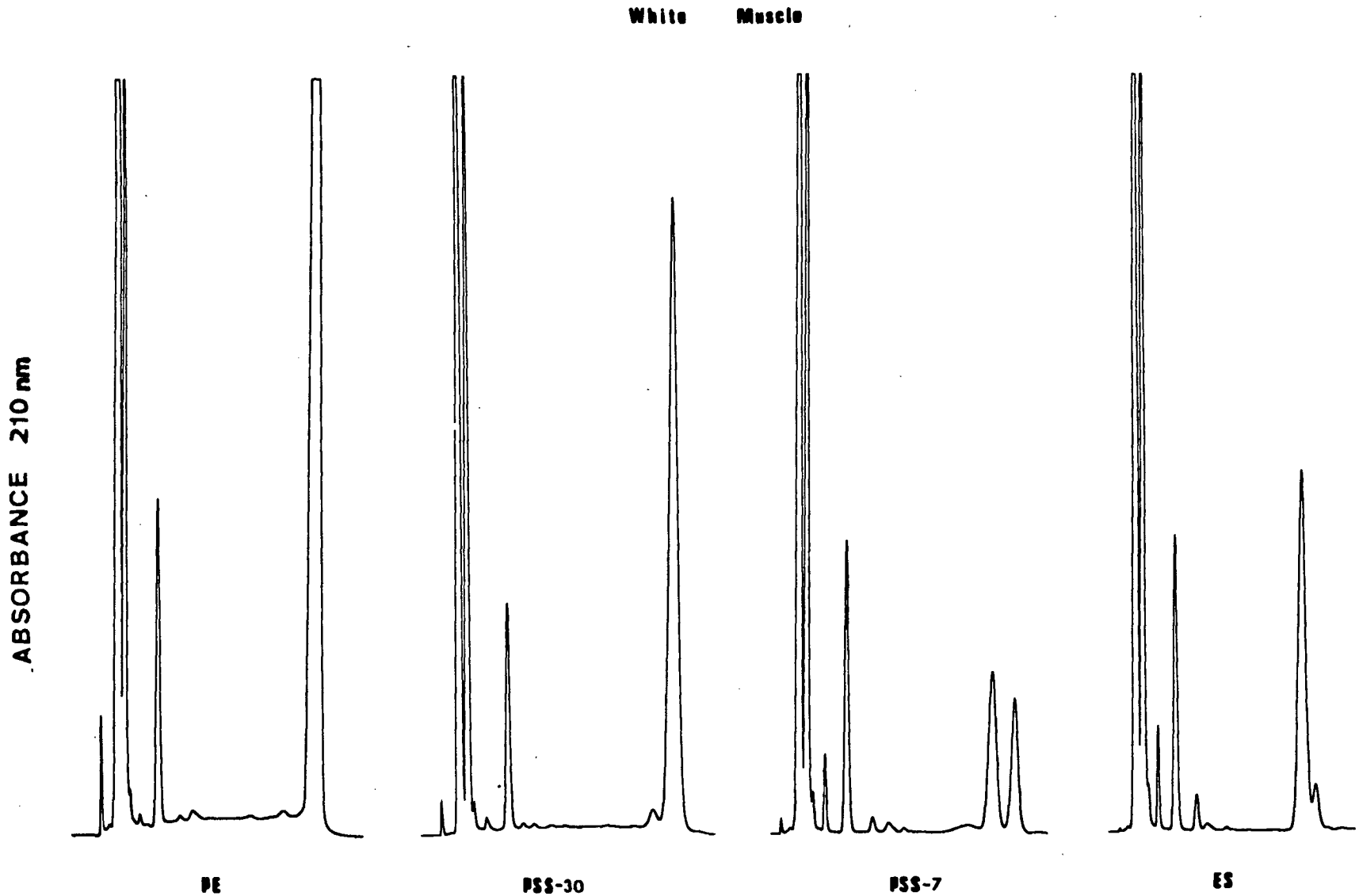
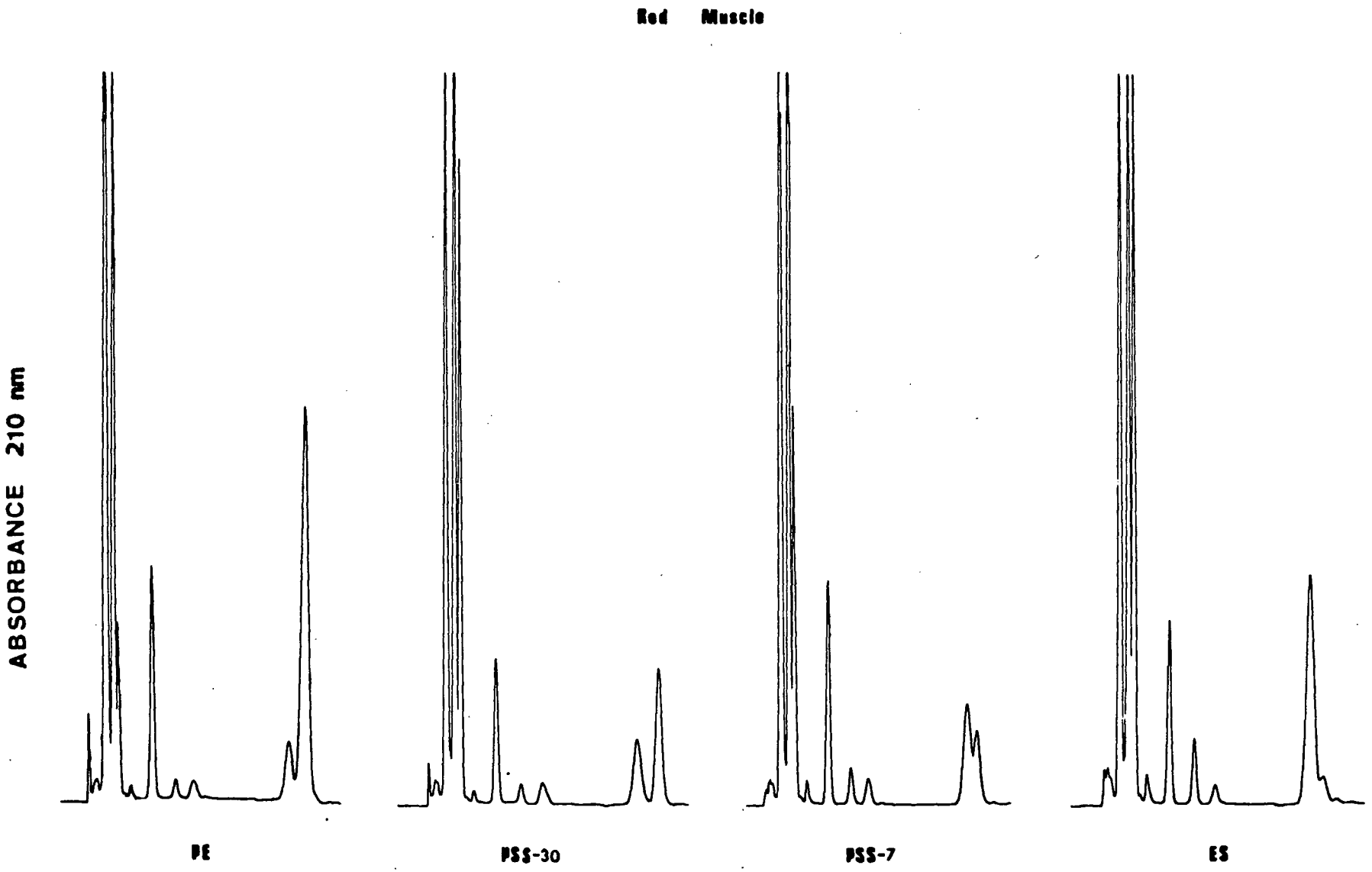

White Muscle
ABSORBANCE 210 nm
PE
PSS-30
PSS-7
ES

Figure 3. Separation of Cr, PCr, adenosine, inosine, NAD and nucleotide monophosphates in trout red muscle.


Red Muscle
ABSORBANCE 210 nm
PE
PSS-30
PSS-7
ES

Appendix II

Assessment of inorganic phosphate levels in freeze clamped frozen and perchloric acid extracts of trout white muscle.

The high levels of inorganic phosphate observed in this study, were considerably higher than expected based on ^{31}P NMR in vivo investigations, while being relatively similar to enzymatically determined inorganic phosphate (Pi) contents. Recently, Meyer et al. (1985) demonstrated the elevated Pi contents determined enzymatically to be attributable to PCr hydrolysis upon rapid freezing. To assess this possibility in these results, ^{31}P NMR spectra of freeze clamped frozen and freeze clamped PCA extracts of trout white muscle were determined. Although these results are merely qualitative, it appears certain that the colormetric assay of Black and Jones (1983) reflected accurately the Pi content in the tissues after freeze clamping. Therefore the elevated Pi contents can be attributed to the PCr hydrolysis upon rapid freezing and/or prior handling (see attached spectra).

References

Black, M.J. and M.E. Jones. 1983. Inorganic phosphate determination in the presence of labile phosphate: Assay for carbamyl phosphate phosphatase activity. Anal.Bioch. 135:233-238.

Meyer, R.A., T.R. Brown and M.J. Kushmerick. 1985. Phosphorus nuclear magnetic resonance of fast- and slow-twitch muscle. Am.J.Physiol. 248:C279-C287.

Figure 1. ^{31}P NMR spectra of rainbow trout freeze clamped white muscle at -5°C.

JOB NO: 85-320 W. PARKHOUSE

SAMPLE: FROZEN MUSCLE

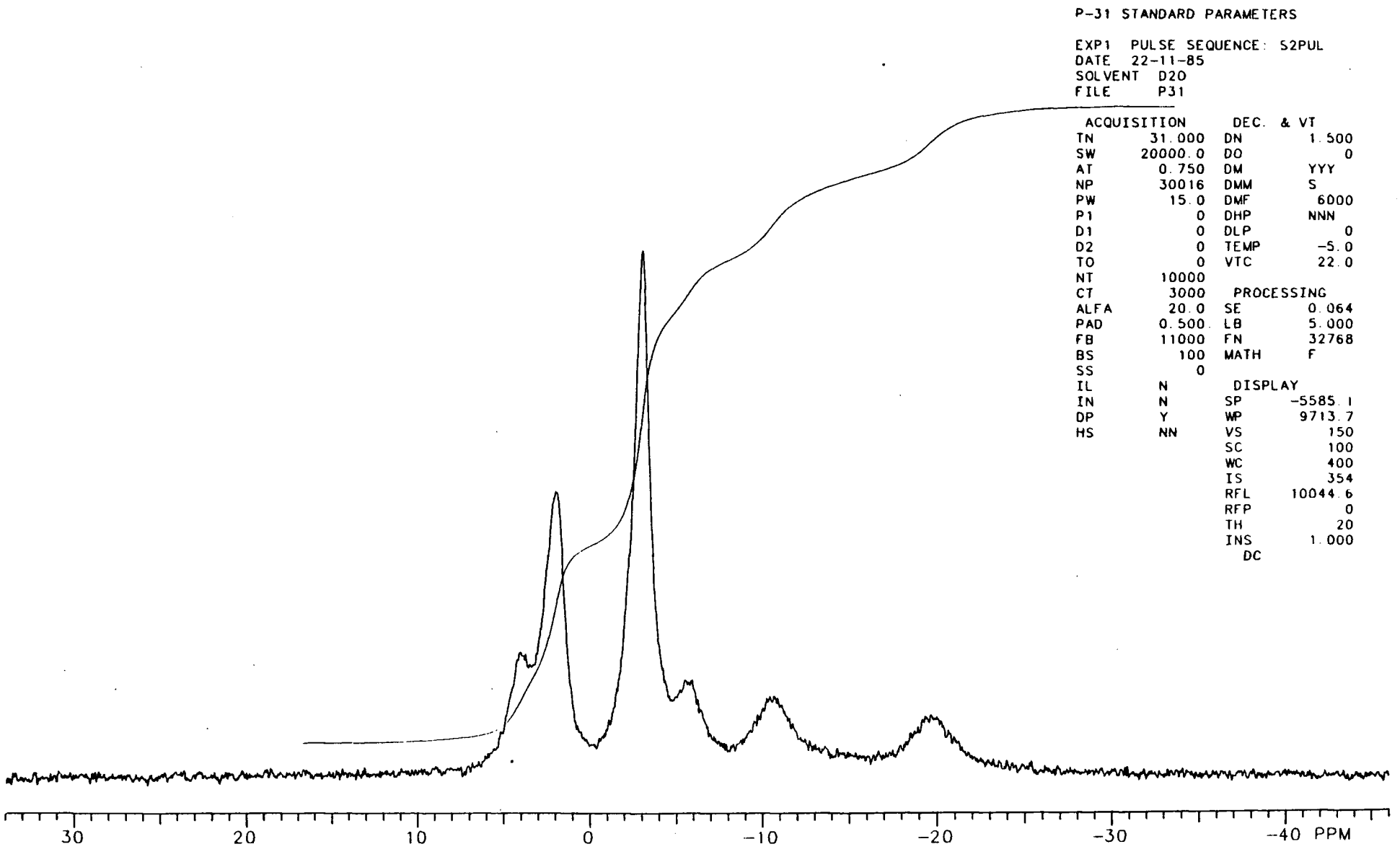
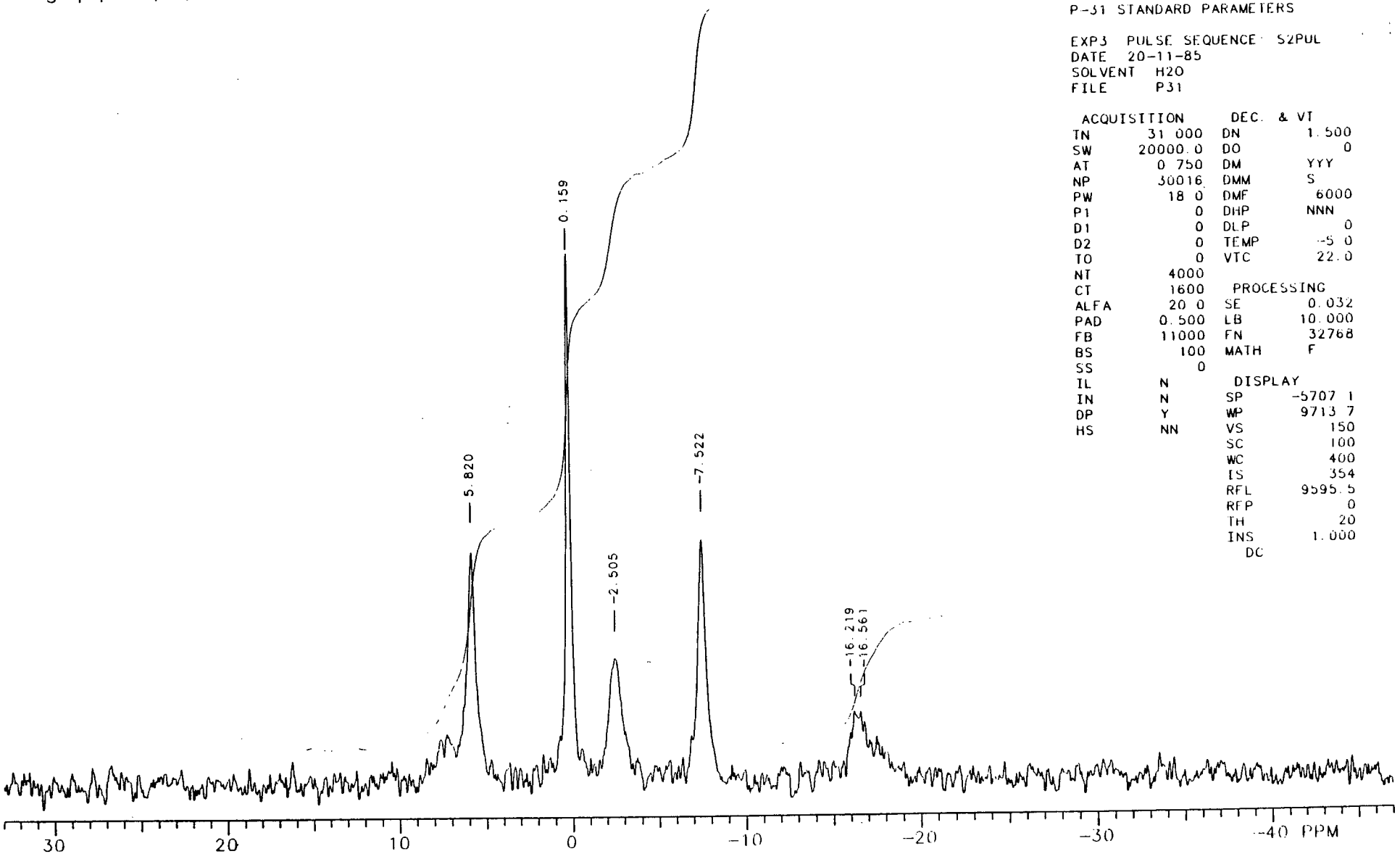


Figure 2. ^{31}P NMR spectra of rainbow trout freeze clamped white muscle PCA extract at -5°C.

JOB NO. 85-319 W. PARKHOUSE

31P FT SPECTRUM AT -5 C.



P-31 STANDARD PARAMETERS

EXP3 PULSE SEQUENCE S2PUL
DATE 20-11-85
SOLVENT H2O
FILE P31

ACQUISITION		DEC. & VT	
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AT	0 750	DM	YYY
NP	30016	DMM	S
PW	18 0	DMF	6000
P1	0	DHP	NNN
D1	0	DLP	0
D2	0	TEMP	-5 0
TO	0	VTC	22.0
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CT	1600	PROCESSING	
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BS	100	MATH	F
SS	0		
IL	N	DISPLAY	
IN	N	SP	-5707 1
DP	Y	WP	9713 7
HS	NN	VS	150
		SC	100
		WC	400
		IS	354
		RFL	9595.5
		RFP	0
		TH	20
		INS	1.000
		DC	

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Appendix III

Activities (umol/g wet weight/min) of glycolytic enzymes in rainbow trout red and white muscle.

Enzyme	Red Muscle	White Muscle
Phosphorylase	14-22	48-70
Hexokinase	0.14-2.6	0.03-1.6
Phosphofructokinase	9	24
Pyruvate Kinase	110-1225	90-310
Lactate Dehydrogenase	340-800	23-200

Values are from Crabtree and Newsholme (1972); Snugden and Newsholme (1973); Johnston (1977).

Appendix V

Statistical Analyses

The results of this investigation can only discussed in descriptive terms because of the large number of variables and relatively small sample size per group would render the validity of any statistical anaylsis questionable. However, as a check of the trends discussed, an analysis of variance was used to evaluate possible intergroup differences. The Tukey (HSD) test was used on variables exhibiting significant omnibus F ratios to identify where group differences existed.

Content of metabolites and pH in rainbow trout white muscle.

Metabolite n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
PCr	19.9±1.6	15.9±0.9 c	2.9±0.7 b	1.8±0.6 b
PCr_c	37.8±1.6 c	33.8±0.9 c	20.8±0.7 c	1.8±0.6 c
Cr	24.6±1.0	29.7±1.4 a	42.7±0.2 b	43.4±1.8 b
Cr_c	6.7±0.3 c	11.8±1.4 c	24.8±0.2 c	43.4±1.8 c
ATP	7.26±0.11	6.57±0.22	5.82±0.5 a	2.65±0.25 c
ADP	0.70±0.01	0.68±0.04	1.15±0.07 b	1.05±0.05 b
AMP	0.021±0.001	0.039±0.007	0.073±0.012	0.152±0.035 c
Pi	21.1±2.6 c	26.8±1.9 c	47.6±3.2 c	55.7±1.8 c
Pi_c	2.3±0.4 c	8.9±1.4 c	29.7±2.8 c	55.7±1.8 c
IMP	0.30±0.05	0.53±0.09	1.78±0.27 c	4.34±0.26 c
NH_4^+	1.04±0.05	1.43±0.15	3.20±0.42 c	6.37±0.19 c
GTP	0.054±0.012	0.039±0.003 b	0.037±0.004 b	0.030±0.007 b
Glucose	1.02±0.14	1.86±0.55	2.19±0.51	2.16±0.44
Glycogen	23.3±1.0 c	16.0±1.6 c	5.6±1.1 c	0.2±0.04 c
Lactate	3.0±0.4 c	10.1±1.1 c	33.0±0.6 c	42.9±3.0 c
Malate	0.13±0.07	0.25±0.07	0.25±0.03	0.39±0.06 b
Fumarate	0.01±0.002	0.04±0.01 a	0.06±0.01 b	0.08±0.01 b
Citrate	0.30±0.01	0.28±0.02	0.35±0.05	0.28±0.03
pH	6.97±0.04	6.93±0.03	6.65±0.03 b	6.56±0.04 b

Values are means ± SE expressed in umol/g (w/w). Glycogen was calculated in glucose units. c, compensated metabolite.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from other 3 groups

Content of metabolites and pH in rainbow trout red muscle.

Metabolite n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
PCr	5.2±0.7 d	0.8±0.4 a	1.2±0.2 a	0.4±0.2 a
PCr_c	18.6±0.8 d	0.8±0.4 a	1.2±0.2 a	0.4±0.2 a
Cr	22.1±1.0 d	26.5±0.8 a	26.0±1.9 a	26.5±0.3 a
Cr_c	8.8±0.4 d	26.5±0.8 a	26.0±1.9 a	26.5±0.3 a
ATP	3.43±0.18 d	2.02±0.27	2.26±0.32	1.57±0.32
ADP	0.65±0.07	1.18±0.08 c	0.84±0.04	1.11±0.17 c
AMP	0.106±0.012	0.137±0.014	0.207±0.023 a	0.321±0.055 d
Pi	14.1±1.2 d	21.1±2.9 a	21.5±2.4 a	22.7±2.1 a
Pi_c	2.3±1.3 d	21.1±2.9 a	21.5±2.4 a	22.7±2.1 a
IMP	0.729±0.110	1.090±0.180	1.313±0.145 a	2.330±0.278 d
NH_4^+	1.60±0.14	1.57±0.16	1.71±0.08	2.88±0.30 d
GTP	0.059±0.003	0.033±0.007 c	0.049±0.002	0.034±0.003 c
Glucose	1.46±0.19	3.38±0.55 a	2.11±0.14	2.95±0.05 a
Glycogen	18.1±2.5 d	0.6±0.3 a	0.5±0.2 a	$<$0.1 a
Lactate	5.2±0.8	8.8±1.1 c	7.3±0.9	10.8±1.4 c
Malate	0.20±0.10	0.32±0.03	0.40±0.05 a	0.65±0.1 d
Fumarate	0.03±0.01	0.04±0.01	0.08±0.02 b	0.08±0.02 b
Citrate	0.40±0.05	0.34±0.05	0.44±0.05	0.44±0.09
pH	6.89±0.02	6.92±0.04	6.88±0.02	6.81 (1)

Values are means ± SE expressed as umol/g (w/w). Glycogen was calculated in glucose units. c, compensated metabolites.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from Pre-ex and PSS-7
d Significantly different from other 3 groups

Content of metabolites in rainbow trout liver.

Metabolite n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
Glycogen	183.6±35.6	176.3±34.1	157.7±36.8	121.1±8.5 b
Glucose	3.53±0.85	4.69±0.51	11.14±1.22 b	11.44±1.12 b
G6P	0.12±0.03	0.20±0.04	0.77±0.06 b	0.82±0.07 b
F6P	0.02±0.01	0.04±0.01	0.12±0.01 b	0.14±0.02 b
2PG	0.09±0.01	0.08±0.01	0.08±0.01	0.08±0.01
PEP	0.11±0.01	0.11±0.02	0.08±0.004	0.06±0.004 c
Pyruvate	0.17±0.01	0.15±0.01	0.16±0.01	0.14±0.01
Lactate	1.5±0.2	2.2±0.1	2.1±0.3	4.0±0.6 c
Alanine	3.54±0.41	3.03±0.94	1.55±0.28 b	2.48±0.39

Values are mean ± SE expressed as umol/g (w/w). Glycogen was calculated in glucosyl units.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from other 3 groups

Content of glycolytic intermediates in rainbow trout white muscle.

Intermediate	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
G1P	0.25±0.06	0.40±0.08	0.64±0.04 c	0.31±0.07
G6P	0.59±0.97	0.97±0.16	1.71±0.21 c	0.73±0.24
F6P	0.07±0.01	0.15±0.03	0.27±0.04 c	0.15±0.05
FDP	1.28±0.13	1.90±0.12 a	1.69±0.31	0.32±0.08
DHAP	0.21±0.03	0.19±0.02	0.23±0.03	0.17±0.02
GP	0.50±0.03	0.52±0.08	0.80±0.02 b	0.95±0.11 b
GAP	0.05±0.01	0.06±0.02	0.003±0.005	0.03±0.01
GAP_f	0.018±0.002	0.017±0.002	0.022±0.003	0.017±0.002
DPG	0.06±0.01	0.08±0.02	0.06±0.01	0.16±0.03 c
3PG	0.55±0.05	0.59±0.03	0.96±0.11	0.32±0.01 c
2PG	0.08±0.02	0.08±0.02	0.06±0.01	0.01±0.004 c
PEP	0.06±0.004	0.04±0.01	0.04±0.003	0.01±0.003 c
PYR	0.03±0.005	0.10±0.02 a	0.14±0.02 a	0.33±0.05 c

Values are mean±SE expressed as umol/g w/w.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from other 3 groups

Content of glycolytic intermediates in rainbow trout red muscle.

Intermediate	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
G1P	0.41±0.02 c	0.20±0.06 a	0.18±0.06 a	0.14±0.03 a
G6P	0.45±0.08	0.54±0.10	0.31±0.04	0.33±0.06
F6P	0.10±0.01	0.08±0.02	0.04±0.004 b	0.02±0.004 b
FDP	0.55±0.09 c	0.26±0.02 a	0.25±0.03 a	0.12±0.03 a
DHAP	0.19±0.05	0.19±0.05	0.15±0.06	0.14±0.02
GP	0.62±0.10	0.71±0.16	0.98±0.55	1.21±0.32
GAP	0.03±0.02	0.04±0.01	0.04±0.02	0.06±0.01
GAP_f	0.017±0.002	0.016±0.004	0.013±0.005	0.015±0.002
DPG	0.06±0.01	0.05±0.01	0.05±0.01	0.06±0.01
3PG	0.16±0.03	0.20±0.03	0.20±0.05	0.11±0.05
2PG	0.11±0.02 c	0.05±0.01 a	0.05±0.01 a	0.03±0.01 a
PEP	0.04±0.01	0.03±0.01	0.03±0.01	0.02±0.01
PYR	0.05±0.01	0.07±0.01	0.10±0.02 b	0.11±0.002 b

Values are mean±SE expressed as umol/g w/w.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from other 3 groups

Measured and calculated free cytoplasmic ADP content, ATP/ADP ratio and cytosolic phosphorylation potentials in rainbow trout muscle.

	Muscle	Pre-ex n (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
ADP_m (umol/g)	W	0.70±0.01	0.68±0.04	1.15±0.07 b	1.05±0.09 b
	R	0.65±0.01	1.18±0.08 c	0.839±0.04	1.11±0.17 c
ADP_{fm} (umol/g)	W	0.057±0.004	0.065±0.004	0.290±0.063 b	0.203±0.056 b
	R	0.073±0.012 c	0.189±0.010 a	0.229±0.022 a	0.229±0.021 a
ADP_{fc} (umol/g)	W	0.007±0.001	0.012±0.002	0.019±0.002 a	0.203±0.056 c
	R	0.008±0.001 c	0.189±0.010 a	0.229±0.022 a	0.229±0.021 a
ATP/ADP_m	W	10.4±0.2	9.8±0.2	5.1±0.4 c	2.6±0.2 c
	R	5.4±05 c	1.7±0.2 a	2.7±0.4 a	1.5±0.4 a
ATP/ADP_{fm}	W	129.6±9.5	103.0±4.5	24.5±5.9 b	18.1±5.1 b
	R	52.4±8.9 c	10.6±1.3 a	10.4±1.6 a	7.5±2.1 a
ATP/ADP_{fc}	W	1025±98 c	582±66 c	312±21 c	18.1±5.1 c
	R	457±24 c	10.6±1.3 a	10.4±1.6 a	7.5±2.1 a
$\frac{ATP}{ADP_m.Pi}$ (M^{-1})	W	553±73 c	371±24 c	110±12 c	46±3 c
	R	383±33 c	149±21 a	140±20 a	74±23 a
$\frac{ATP}{ADP_{fm}.Pi}$ (M^{-1})	W	6950±1100 c	3938±367 c	500±164 b	316±81 b
	R	3682±513 c	813±96 a	542±103 a	370±127 c
$\frac{ATP}{ADP_{fc}.Pi_c}$ (M^{-1})	W	451200±44800 c	60900±8900 c	11000±1400 c	316±81 c
	R	155000±30700 c	813±96 a	542±103 a	370±127 b

Values are mean±SE. W, white muscle; R, red muscle.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from other 3 groups

Calculated free cytosolic redox state and PCr/Pi ratio's in rainbow trout muscle.

	Muscle n	Pre-ex (5)	PSS-30 (6)	PSS-7 (5)	ES (5)
NAD/NADH	W	749±153	1068±82	828±106	2076±458 c
	R	961±145	888±132	2027±173 c	1525±142 c
PCr/Pi	W	1.04±0.12 c	0.6±0.06 c	0.06±0.02 b	0.03±0.01 b
	R	0.37±0.04 c	0.04±0.02 a	0.06±0.01 a	0.02±0.01 a
PCr_c Pi_c	W	16.8±1.5 c	3.4±0.3 c	0.7±0.1 c	0.03±0.01
	R	8.1±0.4 c	0.04±0.02 a	0.06±0.01 a	0.02±0.01 a

Values are mean±SE. W, white muscle; R, red muscle.
a Significantly ($p<0.05$) different from Pre-ex
b Significantly ($p<0.05$) different from Pre-ex and PSS-30
c Significantly ($p<0.05$) different from other 3 groups

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