LACTATE AND PROTON DYNAMICS FOLLOWING STRENUOUS EXERCISE IN RAINBOW TROUT (SALMO GAIRDNERI) AND FLATHEAD SOLE (HIPPOGLOSSOIDES ELASSODON)

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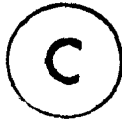

JEFFREY D. TURNER

A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

December 1981

LACTATE AND PROTON DYNAMICS FOLLOWING STRENUOUS EXERCISE IN RAINBOW TROUT (*SALMO GAIRDNERI*) AND FLATHEAD SOLE (*HIPPOGLOSSOIDES ELASSODON*)

MASTER OF SCIENCE (1981)
(Biology)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Lactate and Proton Dynamics Following Strenuous Exercise in Rainbow Trout (*Salmo gairdneri*) and Flathead Sole (*Hippoglossoides elassodon*)

AUTHOR: Jeffrey D. Turner, B.Sc. (Mount Allison University)

SUPERVISOR: Professor C.M. Wood

NUMBER OF PAGES: xi, 117

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ABSTRACT

Strenuous exercise in both rainbow trout (*Salmo gairdneri*) and flathead sole (*Hippoglossoides elassodon*) caused substantial blood acidosis of combined respiratory (protons due to CO_2 accumulation) and metabolic (protons due to metabolic acid accumulation) origin. The contribution of the respiratory component was maximal immediately following the cessation of exercise and was fully corrected within 1 h. The metabolic acid load in the blood reached a maximum at 0.5 - 1 h after exercise and required 8 - 12 h to recover fully.

Although lactic acid production by glycolysis generates stoichiometrically equivalent amounts of lactate and protons in muscle during exercise, their blood concentrations during recovery were quite different. Recovery in rainbow trout displayed a pattern in which lactate accumulated in the blood in excess of metabolic protons. The flathead sole exhibited the exactly opposite discrepancy where proton accumulation in the blood exceeded that of lactate. L(+)-lactic acid infusion experiments in both fish illustrated that preferential removal of either protons or lactate from the blood could not account for the observed lactate/proton discrepancies.

Experimentation using an isolated, perfused rainbow trout trunk showed that the myotome can differentially release lactate and protons into the extracellular space in response to the appropriate

extracellular signals. Through the manipulation of extracellular pH and P_{CO_2} it was possible to regulate the rate of proton efflux from the myotome. This and other evidence indicates that a differential release of protons and lactate from the muscle causes the observed discrepancies during *in vivo* recovery.

Simultaneous muscle and blood sampling *in vivo* suggests that most (≈ 90%) of the lactate and protons produced during exercise are retained within the myotome. This causes a water shift into the intracellular space which contracts the extracellular space, resulting in haemoconcentration and disturbance in plasma ion balance. The eventual fate of the lactate and proton load retained within the muscle would seem to be metabolic removal *in situ*, probably glyconeogenesis or oxidation.

A model is presented which can explain the complex events occurring during recovery from strenuous exercise. This model integrates the results from *in vivo* and *in vitro* experimentation and hypothesizes on the mechanism and control of lactate and proton utilization within the muscle cell and their movements between body compartments.

ACKNOWLEDGEMENTS

I would like to acknowledge and thank Dr. Chris Wood for his constant encouragement and assistance throughout this project. I wish to thank my committee members, Dr. E.L. McCandless, Dr. R.A, Morton and Dr. G.J.F. Heigenhauser for their many helpful suggestions and criticisms of the manuscript.

My grateful thanks also go to my colleagues and friends, Doug Spry for his good humor and wit which kept things in perspective; Mark Graham for his skillful assistance and support and to Louise Milligan and Marrance Rogano for making the lab work a cheerful experience.

Many thanks also go to Helve Hōbe of the University of Calgary, for her assistance and friendship during the sole project at Bamfield.

I am also grateful to Rob Gillies for his technical assistance which permitted the construction of countless inventions.

This project was supported by a National Science and Engineering Research Council Operating Grant to Dr. C.M. Wood and the Yates Scholarship to J. Turner.

Personal financial support was provided by McMaster University Teaching Assistantship.

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I. INTRODUCTION

Since the work of Stefano Lorenzeni in 1678, (Golarz de Bourne, 1966) it has been recognized that two distinct muscle fiber types exist, which are commonly referred to as red and white fibers. Mammalian muscles are composed of both fiber types interspersed throughout the musculature. Various muscles have different red/white ratios, but the concept of mixed fiber or mosaic muscle mass remains constant (Burke, Levine and Zajac, 1971). Muscles with a high red/white ratio are recruited during static or endurance work, while those muscles displaying a low red/white ratio are utilized for short term dynamic work of high intensity (Keul, Doll and Keppler, 1972).

In lower vertebrates, red and white fibers are often found in discrete muscle groups (Mosse and Hudson, 1977). In many fish, the red fibers are found in a thin superficial band located near the lateral line. These fibers are used during slow continuous swimming or cruising (Bone, 1966; Rayner and Keenan, 1967; Hudson, 1973). As the name suggests, red muscle is well vascularized (Stevens, 1968; Mosse, 1979) and contains myoglobin (Buttkus, 1963; Hamoir, Focant and Distèche, 1972). The high flow of oxygen rich blood and heme-containing myoglobin gives this tissue its red appearance and also provides it with oxygen. This oxygen is utilized by numerous mitochondria during the aerobic catabolism of fats and/or carbohydrates (George, 1962, Mosse, 1979).

In many fish, white muscle forms over 95% of the myotome (Nag and Nursall, 1972). It is used during vigorous bouts of strenuous activity associated with feeding or escape (Rayner and Keenan, 1967; Hudson, 1973). Oxygen delivery is limited by poor vascularization (Hamoir et al., 1972; Mosse, 1979). White muscle does however possess high titers of glycolytic enzymes (Hamoir et al., 1972; Johnston, 1977) and exceptionally high activities of lactate dehydrogenase (Bostrom and Johanssen, 1972). As one would expect, white muscle catabolism is predominately glycolytic, using carbohydrates as a metabolic fuel (Hudson, 1973; Mosse, 1979). The discrete nature of these red and white muscle groups in fish permit detailed examination of events occurring in a particular fiber species. Since this thesis is concerned with recovery from severe exercise in which white muscle activity predominates, the emphasis will be directed toward white muscle metabolism and physiology.

Recovery from exercise can be more fully understood by first examining the various physical and physiological principles involved in the exercise itself. Fast swimming in fish is a short lived event. In rainbow trout swimming faster than 2.5 body lengths per second (L/sec), the duration of activity is usually less than 60 seconds (Brett, 1964). The thrust for this swimming is generated by alternate contractions of the axial white musculature on either side of the fish's vertebral column. The maximum speed of swimming is limited by the velocity of these muscular contractions (Wardle, 1975). Of importance here is the inverse relationship found in muscle between force development and contractile velocity. As the contraction rate increases, the mechanical force development increases but the muscular efficiency of these contractions decreases

(Alexander, 1969). As fast swimming requires rapid contractions, these events are accomplished at a low efficiency. To move the same distance faster requires more energy.

Another consideration is that fish swim in water, a much more dense medium than air. Webb (1971a, 1975) has determined an expression for factors affecting drag in swimming fish. The greater the drag, the more energy the fish must expend to move.

$$D = \frac{1}{2} \rho S_W V^2 (4.94\ C_D)$$

D = load drag (dynes)

ρ = density of water (g/cm^3)

S_W = wetted surface area (cm^2)

V = swimming velocity (cm/sec)

C_D = drag coefficient

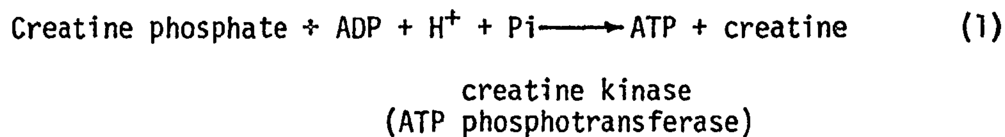
(4.94 is a constant for fish of subcarangiform swimming modes)

Evolution has altered the morphology of fast swimming fish to reduce drag. Many are streamlined, which reduces their drag coefficient, while others fold their pectoral or anal fins to reduce the wetted surface area. One important factor, however, remains unchanged: drag is proportional to the square of the velocity. If a fish wishes to swim twice as fast, he must generate 4x as much thrust to overcome the increased drag. This makes swimming faster energetically very costly.

As velocity increases linearly, drag increases exponentially, all at a time when muscular contractile efficiency is falling. To overcome these deficits, the fish requires enormous quantities of energy over a brief time period.

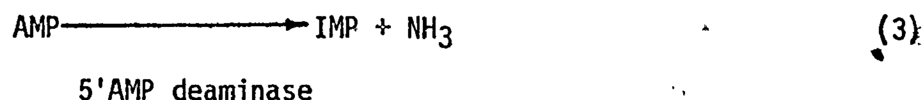
Energy for muscular contraction is derived from the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) plus inorganic phosphate (Pi). In fish white muscle, ATP concentrations are small, 4-5 μmoles g^{-1} fresh tissue (Jones and Murray, 1960; Driedzic and Hochachka, 1976). As this quantity of ATP would be totally consumed during only a few contractions (Davies, Cain and Delluva, 1959), there is obviously metabolic compensation to prevent ATP depletion. These mechanisms fall into two categories: (1) the reactions which occur simultaneously with the onset of exercise and maintain ATP levels during the first few seconds and (2) those metabolic pathways, such as glycolysis, which provide ATP during the remainder of the activity.

Initially, ATP levels are maintained by two reactions:



In resting trout muscle, creatine phosphate levels of 9.45 μmole g^{-1} fresh tissue have been reported (Tomlinson, Geiger and Kay, 1965). Exercise reduces this level to 2.10 μmole g^{-1} fresh tissue. This fact is not surprising as creatine kinase, the enzyme catalyzing reaction 1, is a very abundant protein in fish sacroplasm (Gosselyn-Rey, Hamoir and Scopes, 1968). As ADP accumulates, adenylate kinase generates further ATP via reaction 2 and adenosine monophosphate (AMP) accumulates. Since reaction 2 functions through mass action, any build up

of AMP would slow the rate of ATP formation. To prevent such an occurrance, AMP is deaminated to form inosine monophosphate (IMP) plus ammonia.



$$AMP \xrightarrow{\text{5'AMP deaminase}} IMP + NH_3 \quad (3)$$

The enzyme 5'AMP deaminase, is found in exceptionally high titers in fish white muscle (Driedzic and Hochacka, 1978). The overall effect of these reactions is to maintain ATP levels and allow muscular contraction to occur. They also provide AMP, ADP, IMP and NH_4^+ which are potent activators of certain metabolic pathways, especially glycolysis.

Glycolysis in fish white muscle utilizes glycogen as the primary metabolic fuel (Miller, Sinclair and Hochachka, 1959; Black et al., 1960). For example, Stevens and Black (1966) reported a rate of glycogen consumption of 40 µmole of glycogen glucose $gram^{-1}$ sec^{-1} or a 50% reduction in total muscle glycogen in 15 seconds, in trout subjected to severe exercise. Blood glucose is not utilized as a metabolic fuel for trout white muscle (Black, 1960). This is not surprising as white muscle lacks substantial hexokinase activity (Bostrom and Johansson, 1972; Van den Thillart, 1977). The net reactions of glycolysis produce 2 ATP and 2 lactic acid units per hexose unit. Glycolysis is energetically less efficient than oxidative phosphorylation, but it accomplishes two essential roles during the severe exercise condition. Firstly, glycolysis can produce large quantities of ATP quickly from muscle glycogen reserves and secondly,

glycolysis functions in the absence of oxygen. Blood glucose, free fatty acids and oxygen are therefore available for aerobically based tissues such as the brain, red muscle and liver.

The fact that lactic acid is produced during severe muscular activity has been known since the work of Ryffel (1909). Since then, much work has been devoted to the field of lactate production and accumulation. In mammals, lactic acid generation and movement between body compartments has been extensively studied. Resting man maintains a blood lactate load of 1.4-2.2 mM, which increases during severe exercise and exhaustion to a maximum of 10-19 mM after 5-10 minutes of recovery. As recovery progresses, lactate concentrations fall rapidly and return to resting levels in less than 60 minutes (Johnson and Edwards, 1937; Crescitelli and Taylor, 1944; Bergström *et al.*, 1971; and Karlsson, 1971). Similar *in vivo* studies with other mammalian species have yielded equivalent findings (Eggleton and Evans, 1930; Margaria and Edwards, 1933).

Lower vertebrates in general and fish in particular exhibit a much different blood lactate response to severe exercise. Black *et al.* (1962) severely exercised rainbow trout (*Salmo gairdneri*) and observed a resting blood lactate level of 0.5 mM which increased to 15 mM after exercise. The unusual feature was the time required to achieve maximum blood lactate levels. The peak was not obtained until some 2-4 hr after exercise and required 12-24 h to recover fully. This protracted time course for lactate accumulation and removal has been observed with many other fish and lower vertebrates (Black, Chiu, Forbes and

Hanslip, 1959; Black, Connor, Lam and Chiu, 1962; Black, Manning and Hayashi, 1966; Piiper, Meyer, and Drees, 1972; Wood, McMahon and McDonald, 1977; McDonald, McMahon and Wood, 1979; McDonald, Boutilier, and Toews, 1980; Wood, and Randall, 1981). These observations have sponsored many hypotheses which include circulating catecholamines, muscle perfusion, temperature and acid-base status.

Circulating catecholamines have been implicated in the control of lactic acid release from the muscles of exhausted plaice (Wardle, 1978). In this study, the release of lactate from muscle to blood could be experimentally manipulated through the use of β-adrenergic blockers and stimulators. Propanolol, a β blocker caused an enhanced and often lethal release of lactate whereas β stimulation with isoxsuprine suppressed lactate release.

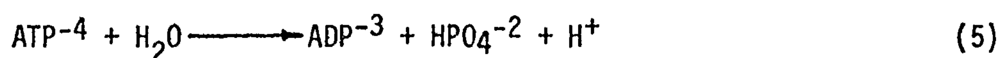
Impaired muscular perfusion has also been implicated in the protracted accumulation of lactate in fish blood (Stevens and Black, 1966; Stevens, 1968). Black et al. (1962) cited Hayashi's unpublished results which showed that circulation was impaired during and following exercise in trout. This sluggish blood flow could slow lactate release from the muscle.

The low body temperatures seen in most fishes has been a tempting explanation for slow lactate release (Black et al., 1959) as diffusion rates are lower at reduced temperature. However, Black et al., (1962), suggest that reduced diffusion rate is not a major component of slow release.

Acid-base status may affect lactate and proton movements. Mainwood and Worsley-Brown (1975) illustrated that lactate efflux from fatigued, isolated frog sartorius muscle could be experimentally manipulated by altering the acid-base status of the external medium. At high [HCO_3^-] of 25 mEq L^{-1} (pH 7.9) peak lactate efflux was 0.28 μmol $g^{-1}min^{-1}$, while a reduction in external [HCO_3^-] to 1 mEq L^{-1} (pH 6.8) resulted in a lactate efflux of only 0.12 μmol $g^{-1}min^{-1}$. There is no doubt all the above factors can affect lactate movements in specific circumstances; however, their relative importance to the overall control mechanism(s) remains to be established.

An interesting difference between mammals and lower vertebrates is the apparent manner in which the lactic acid leaves the myotome and accumulates in the blood. Lactate and H^+ are formed in stoichiometrically equivalent amounts during anaerobic exercise when lactate production via glycolysis is coupled with ATP hydrolysis (Krebs, Woods, and Alberti, 1975).

$$\text{glucose} + 2\ HPO_4^{-2} + 2\ ADP^{-3} \longrightarrow 2\ \text{lactate}^- + 2\ ATP^{-4} \quad (4)$$



$$ATP^{-4} + H_2O \longrightarrow ADP^{-3} + HPO_4^{-2} + H^+ \quad (5)$$

During recovery in man, the quantity of lactate in the blood always closely approximates the base deficit or metabolic acid load, which is representative of the quantity of metabolic protons buffered in the blood (Turrel and Robinson, 1942; Bouhury, Pool, Binkhorst and Leeuwen, 1966; Keul, Doll, and Keppler, 1972).

In lower vertebrates and invertebrates, the situation is often quite different. Observations during *in vivo* and *in vitro* experimentation have determined that two basic patterns exist, in which lactate accumulates in the blood either in excess of or in lesser amounts than the proton load. Piiper, Meyer and Drees (1972) exercised the elasmobranch, *Scyliorhinus stellaris*, to exhaustion with electric shocks and observed an increase in blood lactate levels to a high of 20 mEq L^{-1} at 6-8 h of recovery. The metabolic proton load ($\Delta H^{+}m$) remained below 5 mEq L^{-1} during the same period. As the authors suggest this discrepency can be explained either through excretion of protons or a differential compartmentalization of lactate and protons. Proton excretion into the environmental water was determined to be too small to account for the discrepancy between blood lactate and protons, so it was assumed that the protons were retained and buffered by the tissues. This retention of protons in relation to lactate was also observed in the isolated frog sartorius muscle (Mainwood and Worsley-Brown, 1975). In these studies, the muscles were exercised electrically and the efflux rate of protons and lactate determined with respect to the buffer capacity of the superfusing saline. With a high external buffering capacity (25 mM), lactate efflux rates ranged from 0.2-0.4 μmol $g^{-1}min^{-1}$ while proton efflux was .03-0.10 μmol $g^{-1}min^{-1}$. At a low external buffering capacity (1 mM) the proton efflux rate decreased to only 10% that of lactate. Further it was noted that conditions which increased lactate and proton efflux also assisted in maintaining the muscle's contractile integrity. Recovery from enforced activity in the Dungeness crab (*Cancer magister*)

reflected the same descrepancy between protons and lactate in the haemolymph (McDonald et al., 1979). These authors implicated the low buffering capacity of crab haemolymph when explaining a possible mechanism for reduced proton efflux. Wood and Randall (1981), exercised the land crab Cardisoma carnifex, and observed that during early recovery, lactate and proton loads were the same. Only after 1 h did a discrepancy form in which the protons were removed faster than the lactate. The possibility of $CaCO_3$ mobilization from the carapace, as was evidenced by elevations in haemolymph [Ca^{++}] could explain this discrepancy.

Another body of work presents a diametrically opposite viewpoint. Wood et al. (1977) found that during recovery from severe exercise in the starry flounder (Platichthys stellatus), protons were found in the blood in excess of lactate. The authors ascribed this discrepancy to an unmeasured organic acid. Beggs, Holeton and Crossman (1980) found very similar results during recovery from angling fatique in the muskellunge (Esox masquinongy). Benadé and Heisler (1978) electrically stimulated frog sartorius muscles and noted that during recovery, proton efflux exceeded that of lactate by a factor of 50. The authors cited differences in experimental approaches to explain the discrepancy between their work and that of Mainwood and Worsley-Brown (1975). A point of additional interest in this study was that an isolated mammalian tissue, the rat diaphragm also showed a more rapid proton efflux after exercise.

Studies of two lower vertebrates, the urodele *Cryptobranchus alleganiensis* (Boutilier *et al*., 1980) and the anuran *Bufo marinus* (McDonald *et al*., 1980) conclude that these animals accumulate protons in excess of lactate in their blood following exercise. These discrepancies are formed early during recovery and remain until 4-6 h, at which time the proton and lactate loads are similar. McDonald *et al*. (1980) attribute this phenomenon to either a more rapid efflux of protons into the blood in relation to lactate or to the fact that some unmeasured metabolic acid is moving into the blood.

Two major questions become apparent when one examines the field of lower vertebrate exercise physiology. Firstly, why is the time course of lactic acid accumulation in the blood so protracted when compared to mammalian systems? Secondly, what mechanism(s) regulate the movement of protons and lactate between different body compartments?

To explore these questions a series of studies were undertaken using two fish species; the rainbow trout (*Salmo gairdneri*) and the flathead sole (*Hippoglossoides elassodon*). These fish were chosen as they differ in their ecology and in their exercise performance capabilities. The rainbow trout is a carnivorous, pelagic, freshwater fish which spends much of its time cruising or sprinting during feeding or escaping predation. It is well adapted to both types of activity as it possesses both red and white muscle fiber types. Red muscle along the lateral line plus numerous axial red fibers compose some 24% of the caudal peduncle (Webb, 1975) and permit almost unlimited duration swimming at velocities below two body lengths per second. The caudal

peduncle was selected as a reference point as it is the region of maximal contribution of red fibers to the myotome. The axial musculature is predominantly composed of white fibers which provide the thrust for short term, very high velocity (15 L sec^{-1}) sprinting activity.

The flathead sole is a benthic marine form which spends much of its life buried motionless in the sand or slowly browsing on small mollusks (Hart, 1973). Flatfish possess very little red muscle, less than 10% of the caudal peduncle (Mannen, 1961). Further, the capillary density of both red and white muscle would be much lower than in the trout (Mosse, 1979). Even with total white muscle recruitment it is unlikely that this fish can attain swimming velocities higher than 3 L sec^{-1} (Priede and Holliday, 1980) and only for a brief time period.

By means of exhaustive exercise, it is possible to probe the strategies that these two fish employ to deal with the metabolic, acid-base and ionic alterations imposed by activity.

Two parallel studies, each consisting of three _in vivo_ experiments were undertaken using rainbow trout and flathead sole. The first experiment examined acid-base, metabolic and ionic alterations that occurred in the blood during the first 12 hrs of recovery from severe exercise. This experiment could determine the nature of any acidosis (metabolic/respiratory) and quantify the accumulation of metabolically produced protons. Blood lactate was examined in relationship to recovery time and proton accumulation. The major plasma ions were measured to determine if there existed any perturbations of the plasma electrolytes as a consequence of the disturbances in proton, lactate and HCO_3^- levels

seen in post-exercise acidosis. Haematological variables such as haemoglobin, % haematocrit and plasma protein were monitored to assess the effects of repetitive blood sampling and to follow water movements to and from the vascular space. Further, haemoglobin levels were used to calculate blood non-bicarbonate buffering capacity, a value used in the analysis of blood acid-base disturbances.

The second experiment utilized a terminal muscle biopsy technique to examine lactate and pyruvate concentrations in the muscle during recovery from severe exercise. Blood lactate and pyruvate measurements on the same animal simultaneous with the muscle biopsy could then establish the relationship between intracellular levels and blood levels of lactate and pyruvate. Plasma ammonia concentrations were also measured to assess the possible impact of its accumulation on blood acid-base status.

A third study examined the relative rates of removal of the lactate anion and protons from the blood space following a L(+)-lactic acid infusion. This procedure would identify any preferential removal of either ionic species from the blood and possibly offer a partial explanation for the discrepency between lactate and proton accumulation in the blood.

An additional in vitro experiment, using rainbow trout only, explored the magnitude and pattern of lactate and proton release from the muscle. By exercising an intact trout, then rapidly preparing it as an isolated perfused trunk (Wood and Shelton, 1975, Moen and Klungsoyr, 1981) it was possible to determine very accurately proton

and lactate movement from the muscle into the perfusate. Furthermore, this preparation permitted repetitive muscle biopsy which was not possible during *in vivo* work. It was also possible to probe the controlling mechanism(s) of lactate and proton release through the modification of perfusate composition, addition of pharmacological agents or alteration of perfusion flow.

II. MATERIALS AND METHODS

1.00 RAINBOW TROUT

1.10 Experimental Animals

Rainbow trout (*Salmo gairdneri*) of both sexes weighing 282±8g ($\bar{X}$±1 SEM, n=50) were purchased from Spring Valley trout farm, Petersburg, Ontario and acclimated to 15±1°C in dechlorinated, aerated fresh water for two weeks. During holding, fish were fed *ad libitum* on trout pellets (Martin Feed Mills, Elmira, Ontario) and were starved for at least seven days prior to experimentation.

Dorsal aortic catheters were implanted in all fish for chronic blood sampling. Cannulae consisted of Clay-Adams PE50 polyethylene tubing tipped with 22 gauge needles and filled with Cortland Saline (Wolf, 1963). Catheterization was performed while the animal was under 1:15,000 MS-222 (Sigma) anaesthesia on an operating table (Smith and Bell, 1964; Holeton and Randall, 1967). Following surgery, the fish were allowed to recover for a minimum of 24 hours in darkened acrylic boxes served with a water flow of 300-450 ml/min (Kobayashi and Wood, 1980).

1.20 Experimental Series

1.21 Blood Parameters During Recovery

The first experiment employed 13 fish to elucidate the acid-base, metabolite and ionic disturbances in the blood associated with

recovery from severe exercise. Exercise was achieved by quickly transferring the trout to a circular 500 l tank and chasing it vigourously with a stick for six minutes. By the end of the exercise period all fish had become sluggish and refractory to stimulation, indicating that this procedure produced severe exercise. The fish was then returned to its individual box and monitored over the following 12 h recovery period. Anaerobically drawn samples of 600 µl whole blood were taken at rest (control), immediately following exercise (0h) and at 0.5, 1, 2, 4, 8 and 12 h. Blood samples were replaced with an equal volume of Cortland saline (Wolf, 1963). The blood was analyzed for pHa, Ca_{CO_2} (whole blood and plasma), haematocrit, haemoglobin concentration, lactate concentration and plasma levels of sodium, potassium, chloride and total protein. It is noteworthy that five fish died from the effects of exercise during the first 4 h of recovery. The data from these fish were not utilized in this report, but will be presented elsewhere (Wood, Turner and Graham, in preparation).

1.22 Muscle Biopsy

The second study utilized the same exercise protocol to examine lactate and pyruvate gradients between muscle and blood at various recovery times. Furthermore, comparisons could be made between these gradients and other blood parameters. Twenty-nine fish were cannulated and exercised as in the previous study and placed in specially designed biopsy boxes. Each animal was used only once for a terminal muscle and blood samples. Sampling times included rest, 0, 1, 4,

8 and 12 h. Anaerobically drawn samples of 1000 µl whole blood were analyzed for pHa, Ca_{CO_2} (plasma and whole blood), haematocrit, haemoglobin, lactate and pyruvate concentrations and plasma levels of sodium, potassium, chloride, ammonia and total protein. Muscle biopsy occurred within three minutes of blood sampling.

The biopsy chambers, while of the same size as the standard acrylic boxes, were modified with a moveable plastic liner and large (5 cm diameter) port in the bottom. For biopsy sampling, the box was rapidly drained (< 2 sec) by opening the port while the plastic liner was pulled rapidly upwards. This trapped the fish on its side, pressed firmly against the acrylic lid and covered with only a thin film of water. Biopsy needles were then punched through a slit in the lid and through the underlying epaxial mass just posterior to the dorsal fin. This technique allowed lateral biopsy of the epaxial myotome without contamination from environmental water, major blood vessels or viscera. The biopsy needles were stainless steel trocars of ID=4.78 mm (Arnold-Nasco Ltd., Guelph, Ontario) mounted in duplicate, 1 cm apart in an acrylic holder. Each needle obtained a sample of 93.2±4.2 mg (n=41), which was immediately frozen in liquid nitrogen. The total elapsed time from initial positioning of the fish to freezing the sample was under five seconds.

1.23 Lactic Acid Infusion

The third study examined the removal rate of lactate and associated protons from the blood following a lactic acid infusion.

Eight animals were infused, by means of a dorsal aortic catheter, with 5 ml Kg^{-1} of 248±18(8) $\mu M ml^{-1}$ L(+)-lactic acid (Sigma) in 120 mM NaCl. The mean infused load was 1,242±93(8) $\mu M\ Kg^{-1}$. The infusion required 15 minutes and then a further five minutes were allowed for mixing before post-infusion sampling commenced (0h). (For further details see Kobayashi and Wood, 1980). The blood sampling technique and times were the same as in the first study except that the 12 h sample was omitted. Blood samples were analyzed for pHa, Ca_{CO_2} (whole blood and plasma), haematocrit, haemoglobin, and lactate concentrations and plasma levels of sodium, potassium, chloride and total protein. Lactate space was calculated by the method of Kobayashi and Wood (1980) which involved extrapolating Δlog lactate values to 0 h.

$$\frac{\text{lactate space}}{\text{ml Kg}^{-1}} = \frac{\text{infused lactate load } \mu\text{Eq Kg}^{-1}}{\text{lactate 0h}(\mu\text{Eq ml}^{-1}) - \text{lactate at rest}(\mu\text{Eq ml}^{-1})} \quad (6)$$

1.30 Isolated Perfused Trunk Experimentation

1.31 Perfusate. The perfusate utilized in this study was a modification of basic Cortland salmonid saline (Wolf, 1963) (Table 11).

To this basic saline was added 5.50 g dl^{-1} haemoglobin (Sigma type II, from washed lysed, dialyzed bovine erythrocytes) as a buffering system, plus 4% w/v polyvinylpyrrolidone (PVP) (Sigma, Technical grade) to simulate oncotic pressure and 50 iu ml^{-1} ammonium heparin (Sigma). The pH was altered by addition of 0.1 N NaOH.

Oxygen saturation was achieved by vigorously gassing the complete saline minus the haemoglobin with 0.29% CO_2 balance O_2 at 15°C±1°C for 45 minutes. The haemoglobin was quickly added and the complete per-

fusate placed under the same gas. Direct oxygenation of the complete saline was not performed because the haemoglobin foamed excessively. Nevertheless, P_{O_2} was maintained above 400 torr while P_{CO_2} averaged 2.30 torr and pH = 7.832 in the complete saline. The latter two values are very similar to that in arterial blood of resting trout (cf. Figure 2).

Preliminary experiments with this perfusate indicated that the oxygen transport capacity of the haemoglobin was severely impaired, and for this reason the equilibration gas used was CO_2 in oxygen, rather than in air. Gassing the perfusate with 100% O_2 for 60 min resulted in only 40% saturation of the haemoglobin, at a PO_2 of ≃ 400 torr, as measured by a Radiometer Hemoximeter (OSM 2) for a total perfusate O_2 content (ie., dissolved plus haemoglobin bound) of about 5.1 vol %. This compares with the normal trout values of about 7.8 vol % (Holeton and Randall, 1967). Normal mammalian haemoglobin is 90% saturated at a PO_2 of 80-100 torr (Guyton, 1981).

An alternate buffering system using imidazole (29.1 mM) proved non-productive as this solution, caused the isolated fish trunk to graphically illustrate the "tonic extensor seizure" which is characteristic of imidazole toxicity in mammals and birds (Nishie, Waiss and Keyl, 1969).

Variations from this basic perfusion protocol included addition of adrenaline or SITS or the preparation of a "chloride free" medium and preparations in which inflow rate was 3x normal or perfused with an "acidotic" saline. Since circulating catecholamines have been

implicated in lactate movement in vivo (Wardle, 1978), one trunk was perfused with saline plus $1x10^{-5}M$ L-adrenaline bitartrate (Sigma).

In two experiments, the anion transport inhibitor SITS (4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulphonic acid; BDH) was added to the perfusate at a concentration of $1x10^{-4}M$. This concentration of SITS has been shown specifically to inhibit anion transport in a number of tissues including fish RBC's and gill (Cabantchik and Rothstein, 1972; Haswell et al., 1978, Perry, Haswell, Randall and Farrell, 1981). If lactate movement is anion carrier mediated, then SITS should alter lactate efflux from the trunk.

Lactate movement into the bloodspace might be in concert with another anion moving inward. One possible counter ion could be chloride. To determine the effect on lactate movement of reduced chloride in the perfusate, a chloride-free medium was prepared (Table 1) and perfused into an isolated trunk.

Fish white muscle is generally considered to be under-perfused (Stevens, 1968). To explore the possible implications of this on lactate efflux, one trunk was perfused at 18.6 ml Kg^{-1} min^{-1}, a rate 3x higher than that used for all the other trunks.

The effects of acid-base status on lactate efflux was determined in four trunks during perfusion with a saline of pH 7.339 and P_{CO_2} set at 6.82 torr. Normal perfusate has a pH of 7.832 and P_{CO_2} of 2.30 torr.

TABLE 1. The composition of modified Cortland saline and of the chloride-free media used during isolated, perfused trunk experiments

Modified Cortland Saline (Wolf 1963)		Chloride-Free Media	
Salt	g L^{-1}	Salt	g L^{-1}
NaCl	7.25	Na_2SO_4	8.88
KCl	0.38	$K_2SO_4 \cdot 7 H_2O$	0.62
$CaCl_2 \cdot 2 H_2O$	0.23	$CaSO_4 \cdot 2 H_2O$	0.26
$MgSO_4 \cdot 7 H_2O$	0.23	$MgSO_4 \cdot 7 H_2O$	0.23
$NaHCO_3$	1.00	$NaHCO_3$	1.00
$NaH_2PO_4 \cdot 2 H_2O$	0.41	$NaH_2PO_4 \cdot 2 H_2O$	0.41
Ion	mEq L^{-1}	Ion	mEq L^{-1}
Na^+	139.5	Na^+	139.5
Cl^-	133.0	Cl^-	0.00*
K^+	5.1	K^+	5.9
Mg^{++}	1.8	Mg^{++}	1.8
Ca^{++}	3.0	Ca^{++}	3.0
$SO_4^=$	2.6	$SO_4^=$	134.8

* Actual measured chloride level 1.33±0.49 mEq L^{-1} (n = 6)

1.32 Tonometry

Before any accurate metabolic acid analysis ($\Delta H^{+}m$) could be calculated with an isolated trunk, it was necessary to determine the buffering capacity (β) for the perfusing media. Two millilitres of saline were placed in 50 ml Erlenmeyer flasks which were constantly agitated in a water bath of $15^{o}C \pm 1^{o}C$. The saline was equilibrated for 30 min with water vapour saturated CO_2 mixtures of known composition from pre-mixed analyzed cylinders. Equilibration gases included; 0.18, 0.29, 1.14 and 5.00% CO_2 with balance air or oxygen. Once equilibrated, 100 μl of saline was drawn anaerobically and analyzed for pH, P_{CO_2} and [HCO_3^-] (see Analytical Techniques II, 3.00). By plotting pH vs [HCO_3^-] the non-bicarbonate buffer line can be generated. The slope of this line, $\Delta HCO_3/\Delta pH$, equals the β value, Table 2, which is used in the calculation of perfusate metabolic acid load (equation 5, II, Sec. 3.00). The buffering capacity of the complete saline was 8.89 slykes (Table 2).

1.33 Perfusion Technique and Apparatus

Rainbow trout weighing 250-350 g were severely exercised as per II, 1.21, by manual chasing for six minutes. The fish was then quickly removed from the water and decapitated just posterior to the cleithrum. The colon and any mature gonads were tied off 2 cm from the vent, cut anteriorally to the ligature and along with the rest of the gastrointestinal tract, (including liver, spleen and stomach) were removed. The kidney was left intact as its removal caused extensive leakage of perfusate which would render the preparation useless. The

TABLE 2. The buffering capacities (β) of a variety of salines on which tonometry was performed.

Solution	Buffering Capacity β (slykes)	Correlation Coefficient	n
Cortland saline + 4% PVP + 5.50 g dl^{-1} haemoglobin	- 8.89	.994	12
Cortland saline + 4% PVP	- 1.58	.829	6
Cortland saline	- 3.19	.916	7
Cortland saline + 4% PVP + 0.198 g dl^{-1} imidazole*	-13.03	.983	14

* This solution was not used due to the toxic nature of the imidazole.

** This buffer capacity is a good estimate of β values for trout blood which range from -6 to -12 slykes (Wood et al., 1981).

trunk was weighed, and then a polyethylene catheter (Clay-Adams PE 160) was tied into the cut end of the dorsal aorta. Inflow pressure was measured by a Narco pressure transducer (Model RP-1500) linked to a Gilson polygraph (Model ICT-5H) and connected to T joint (Figure 1) 12 cm from the junction with the dorsal aorta. Inflow pressure was maintained at 20-40 cm H_2O with a perfusate flow rate of 6.0-8.0 ml Kg^{-1} min^{-1} from a Buchler polystaltic pump or Sage 220 constant flow syringe pump. These pressures were chosen as they represent dorsal aortic pressures in intact trout (Kiceniuk and Jones, 1977). Flow rates were a compromise between simulating *in vivo* dorsal aortic flow rates and those flows at which a measurable inflow-outflow difference for lactate could be detected. The flow rates used were approximately 60% the resting dorsal aortic flow in intact trout (Jones and Randall, 1978).

The preparation and perfusate were maintained at $15^{o}C \pm 1^{o}C$ in an environmentally controlled room or with appropriate water jacketting. This temperature was chosen as all the *in vivo* experimentation was done at $15^{o}C$.

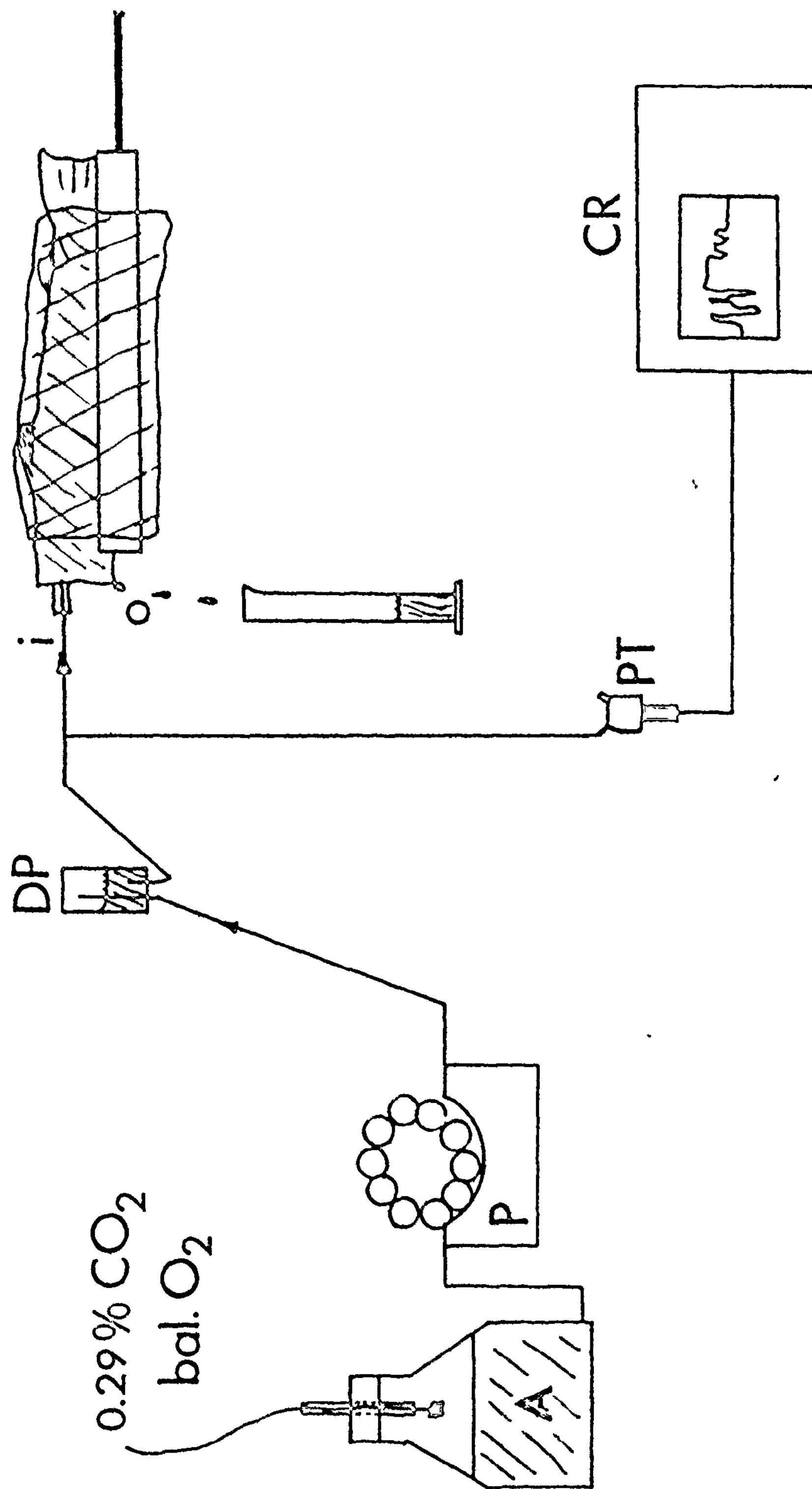
1.34 Experimental Protocol

The initial five minutes of perfusion were used to wash trapped blood out of the trunk. At the end of the wash period a muscle biopsy was taken (0 min) and the first of six successive flux periods started. Muscle biopsies were taken at the end of each flux period. The biopsy trocor (II, 1.22) was pushed manually though the epaxial musculature obtaining a muscle sample ($\sim$ 100 mg) which was frozen in

Figure 1

Perfusion Apparatus

Perfusate was stored in a 500 ml Aspirator bottle (A) where it was maintained under 0.29% CO_2 balance O_2. It was moved through flexible Tygon tubing (ID = 4 mm) by either a Buchler polystaltic pump (P) or Sage 220 constant flow syringe pump (P), then to a depulsator (DP) and T-joint. Connected to this T-joint was a polyethylene catheter (Clay-Adams PE 160) which was tied into the dorsal aorta of the trunk and served as an inflow to the preparation. In addition, the T-joint was connected to a Narco Pressure Transducer (PT) (Model RP-1500) linked to a Gilson polygraph (CR) (Model ICT-5H). The preparation was at a slight angle so that outflow saline from the venous circulation of the preparation dripped into a 100 ml graduated cylinder. The trunk was supported in air in a "V" shaped trough and covered with a moist towel.



0.29% CO2
bal. O2
A
P
DP
i
o
PT
CR

liquid nitrogen. Samples were taken starting at the most anterior region of the trunk, with additional biopsies taken at 1 cm intervals along the epaxial musculature. Occasionally during the biopsy procedure, the trocar would cut small segmental arteries within the myotome and despite attempts to plug the biopsy holes with cotton swabs slow leaks still occurred.

Inflowing perfusate was sampled as it left the Aspirator bottle (Figure 1) at a time midway through each flux period. The outflow was collected in a graduated cylinder (Figure 1) for each flux period (30 min), at the end of which time an aliquot of this collection was taken. Aliquots of each sample were analyzed for pH, total CO_2 and chloride concentration. L-lactate levels were measured on 500 µl of outflow perfusate deproteinated with 1000 µl of 6% $HClO_4$.

2.00 FLATHEAD SOLE

2.10 Experimental Animals

Flathead sole (*Hippoglossoides elassodon*) of both sexes weighing 223±10 g ($\bar{X}$ ± 1 SEM, n=60) were collected during a series of summer otter trawls of 30 minutes duration in Barkley Sound, off Bamfield BC. No mortalities were caused by capture stress or transportation. Fish were held in large sand covered tanks with flow through sea water at 11.5 ± 1°C for at least five days before experimentation. The animals were not fed.

Fish were anaesthetized on an operating table with 1:15,000 MS222 (Sigma) and the caudal artery chronically catherized with Clay-

Adams PE50, as described by Watters and Smith, 1973. The incision was dusted with "Furanace" (Nifurpironal, Dainippon Pharmaceutical) a fish antibiotic, and closed with silk sutures. Fish were recovered in pairs for a minimum of 48 h in covered, sand filled plastic mesh enclosures, (30 x 40 x 10) cm placed on a wet table with flow through water. Fish typically burrowed into the sand and remained motionless.

2.20 Experimental Series

2.21 Blood Parameters During Recovery

The first study employed 10 fish to examine the acid-base, metabolic and ionic disturbances associated with two exercise intensities. The two exercise protocols were moderate and exhausting. Moderate exercise was achieved by manually chasing the fish in a shallow oval tank for six minutes. Upon termination of exercise, the fish failed to respond to further tactile stimulation but was not exhausted as the use of electric shocks caused further swimming activity. Exhausting exercise was produced by chasing the fish with an electric wand, which consisted of 16 gauge copper wire unravelled into two fan shaped electrodes 12 cm apart and connected to a 60 VAC power supply. When the electric field approached the fish, a startle response lasting 5-20 seconds was elicited. Direct contact stimulation was strictly avoided. After six minutes of such exercise the fish was completely refractory to further stimulation and exhibited no righting response, indicating that exhaustion had occurred.

Upon completion of the exercise, the fish were returned to the enclosure, where they immediately burrowed into the sand. A blood sample of 600 µl was immediately drawn anaerobically and denoted 0 h. Subsequent samples were taken at 0.5, 1, 2, 4, 8 and 12 h. The blood removed was replaced with an equal volume of ammonia heparinized (51 iu/ml) Cortland saline (Wolf, 1963), adjusted to 160 mEq L^{-1} Na^+ with NaCl. Blood was analyzed for pHa, total CO_2 (whole blood and plasma), plasma concentrations of Na^+, Cl^-, K^+ and total osmolarity, haemoglobin content, % haematocrit and whole blood lactate. No fish perished during either exercise regime or recovery period.

2.22 Muscle Biopsy

The second study used both moderate and exhausting exercise to examine the muscle to blood lactate ratios as well as blood pyruvate and plasma ammonia levels. Thirty-three fish were cannulated, exercised and allowed to recover as in the first study. Eleven animals were fitted with venous catheters. Each animal was used only once for a single terminal muscle and blood sample. Sample times included rest, 0, 2 and 12 h after severe exercise and 0 and 12 h after moderate exercise. Anaerobically drawn samples of 1000 µl whole blood were analyzed for pHa, total CO_2 (whole blood and plasma), % haematocrit, haemoglobin, lactate and pyruvate concentrations and plasma levels of sodium, potassium, chloride and ammonia. Muscle biopsy occurred within 60 seconds of blood sampling. The fish were quickly removed from the water, placed on a sponge, wiped dry and two muscle samples were taken, by manually pushing two biopsy needles (Arnold-Nasco Ltd.,

Guelph, Ontario, ID = 4.79 mm) through the epaxial muscle, 5-7 cm from the head. The samples were frozen in a dry ice/ethanol mixture. All fish maintained their motionless defense posture while on the sponge. The total elapsed time from removal of the fish from the water to freezing the sample was less than five seconds.

2.23 Lactic Acid Infusion

The third study examined the removal rate of lactate and associated protons from the blood following a lactic acid infusion. Five fish were infused with 5 ml Kg^{-1} of 248±8 (5) mM of L(+)-lactic acid (Sigma) in 160 mM NaCl, by means of a caudal arterial catheter. The total lactate load delivered was 1,241±42 (5) μM Kg^{-1} After the infusion the catheters were rinsed with 2 ml Kg^{-1} 160 mM NaCl. The infusion required 10 minutes plus an additional five minutes to permit adequate mixing before the post-infusion sampling commenced (0 h) (for further details see Kobayashi and Wood, 1980). The blood sampling times and techniques were the same as in the first study except that the 12 h sample was omitted. Blood samples were analyzed for pHa, total CO_2 (whole blood and plasma), % haematocrit, haemoglobin and lactate concentrations, and plasma levels of sodium, potassium and chloride. The lactate space was calculated by the method of Kobayashi and Wood (1980) which involved extrapolating the Δlog lactate values at 0h.

3.00 ANALYTICAL TECHNIQUES

Blood pH_a was determined by injecting a 40 µl aliquot of whole blood into a Radiometer micro-electrode (type E5021) thermostatted to the experimental temperature and linked to a Radiometer pHM71 or pHM72 acid-base analyzer. Total CO_2 (Ca_{CO_2}) was measured on 50 µl samples of true plasma and whole blood using the Cameron method (1971) and a Radiometer CO_2 electrode (type E5036/0) at 37°C. From pH_a and Ca_{CO_2} measurements, plasma and blood bicarbonate and Pa_{CO_2} could be calculated by rearrangement of the Henderson-Hasselbalch equation for the bicarbonate/carbonic acid system:

$$pH_a = pk' + \log \frac{[HCO_3^-]}{\alpha CO_2 \cdot Pa_{CO_2}} \quad (7)$$

where: pH_a = arterial pH

Pa_{CO_2} = arterial PCO_2

αCO_2 and pK' from Severinghaus (1965)

$[HCO_3^-]$ = arterial bicarbonate concentration

$$[HCO_3^-] = CCO_2 - \alpha CO_2 \cdot Pa_{CO_2} \quad (8)$$

Then substituting for $[HCO_3^-]$ in equation 7 and solving for $PaCO_2$:

$$Pa_{CO_2} = \frac{CCO_2}{\alpha CO_2 \cdot [1 + \text{antilog}(pH - pk')]} \quad (9)$$

Metabolic acid load (ΔH^+m) can be calculated as described by Wood, McMahon and McDonald (1977) and McDonald, Hōbe and Wood (1980).

$$\Delta H^+m = [HCO_3^-]_I - [HCO_3^-]_F - \beta(pH_I - pH_F) \quad (10)$$

where the non-bicarbonate buffering capacity β for rainbow trout blood at 15°C is given and described by Wood, McDonald and McMahon (1981) and that for flathead sole at 12°C (Wood, personal communication, 1981).

$$\beta = -1.073[\text{haemoglobin}] - 2.48 \quad \text{rainbow trout} \quad (11)$$

$$\beta = -1.575[\text{haemoglobin}] - 1.88 \quad \text{flathead sole} \quad (12)$$

The subscripts I and F indicate initial and final values for whole blood $[HCO_3^-]$ and pH_a during a test period. Whole blood bicarbonate is used in these calculations as ΔH^+m is compared to whole blood lactate.

Percent haematocrit was measured by centrifuging 80 µl of blood in heparinized capillary tubes (Radiometer type D) at 5000 g for 2.5 minutes. This separated true plasma for Ca_{CO_2} determinations (see above); plasma was transferred anaerobically to the Cameron (1971) chamber. Haemoglobin content was determined colorimetrically on 20 µl of blood using the cyanomethaemoglobin method (Sigma Technical Bulletin No. 525). Total plasma protein was determined with a Goldberg Refractometer (American Optical).

Plasma concentrations of sodium and potassium were appropriately diluted and measured against known standards using flame photometry (EEL Mark II). Chloride was determined via coulometric titration (Radiometer CMT10). Total plasma osmolality was determined with a Wescor Vapour Pressure Osmometer (Model 5100C).

Blood lactate was measured on 100 µl of whole blood, deproteinized with 200 µl of chilled 6% $HClO_4$ and centrifuged at 9,000 g for 2.5 minutes. The supernatant was analyzed enzymatically for L-lactate (Sigma Technical Bulletin No. 826-UV). Similarly, blood pyruvate determinations used 400 µl of whole blood deproteinized with 800 µl of 6% $HClO_4$, centrifuged as above and the supernatant analyzed enzymatically for L-pyruvate (Sigma Technical Bulletin No. 726 UV). Plasma ammonia was measured on 67 µl of plasma *via* enzymatic analysis (Sigma Technical Bulletin No. 170-UV).

To determine muscle concentrations of lactate and pyruvate, individual muscle samples were trimmed of skin and blood-stained portions while frozen, weighed, then extracted in 1000 µl of chilled 6% $HClO_4$ with a glass homogenizer for five minutes. The homogenate was spun at 9,000 g for 2.5 minutes and the supernatant neutralized with KOH and Trizma buffer (Sigma) and analyzed for l-lactate and l-pyruvate as above. The values obtained were then corrected for lactate and pyruvate trapped in extracellular fluid using trout white muscle extracellular fluid volume estimates of 73.2 ml Kg^{-1} (Milligan and Wood, in press) and for the sole 91.6 ml Kg^{-1} (Batty and Wardle, 1979). Final concentrations were expressed as mmol Kg^{-1} fresh tissue, as it could not necessarily be assumed that all intracellular lactate and pyruvate were in solution in cell water. Cell water averaged 698 ml Kg^{-1} in trout white muscle (Milligan and Wood, unpublished).

Statistical significance was determined using the paired Student t-test with a two tailed significance of $P < .05$. Each animal

was used as its own control unless otherwise stated. Differences between groups were tested using the unpaired student "t"-test, again with significance limits set at $P < .05$.

III. RESULTS

1.00 RECOVERY IN RAINBOW TROUT

Severe exercise in rainbow trout caused major acid-base metabolite, and ionic disturbances, most of which were corrected during 12 h of recovery.

1.10 Blood Parameters Following Exercise

The first study examined alterations occurring in the blood during recovery. Blood acid-base disturbances were severe, (Figure 2). Blood pH_a fell from 7.880±0.014 (8) at rest to 7.37±0.031 (8) at 0 h, then rose slowly to control levels, overshot slightly at 8 h before returning to the control value at 12 h. Plasma bicarbonate concentration was 8.34±0.68 (8) mM at rest and was depressed post exercise, to a low of 3.58±0.29 mM at 1 h before returning to resting levels at 8 h. Pa_{CO_2} more than doubled from 3.03±0.22 (8) torr at rest to 7.79±0.64 (8) torr at 0 h but was rapidly corrected by some 76% in 30 min and completely recovered by 2 h. These data are indicative of a mixed respiratory and metabolic acidosis (Davenport, 1974) (Table 17).

By definition, blood metabolic acid load is equal to zero, at rest (Equation 10). Blood lactate levels averaged 0.23±0.09 (8) mEq L^{-1} at rest. In Figure 3, only the changes (Δ) in H^+m and lactate with respect to resting levels are illustrated. At 0 h, both ΔH^+m and lactate (ΔLa^-) were present in the blood in equivalent amounts, ΔLa^- at a concentration of 5.17±0.27 (8) mEq L^{-1} and ΔH^+m at 6.65±0.65 (8) mEq L^{-1}. As recovery progressed, lactate accumulated in excess of

Figure 2

Blood acid-base status (pH_a, $[HCO_3^-]_p$ and Pa_{CO_2})($\overline{X}$ ± 1 SEM; n = 8) in the rainbow trout prior to and following severe exercise.

R = rest; bar = indicates six minutes of severe activity; 0h = immediately post-exercise; 2,4h etc. = recovery time in hours.

(*) indicates a significant difference ($P < .05$) from rest (by paired "t" test).

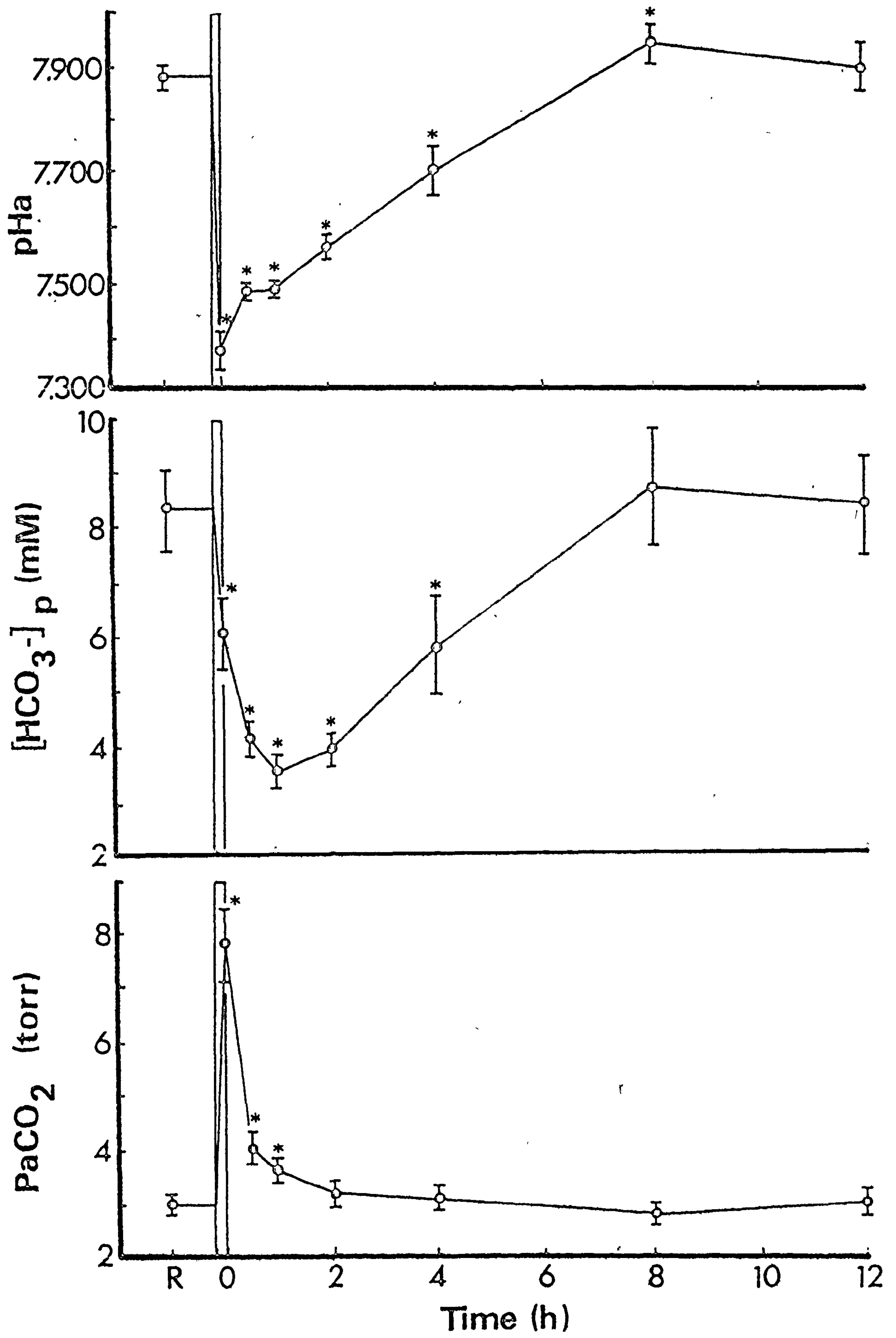
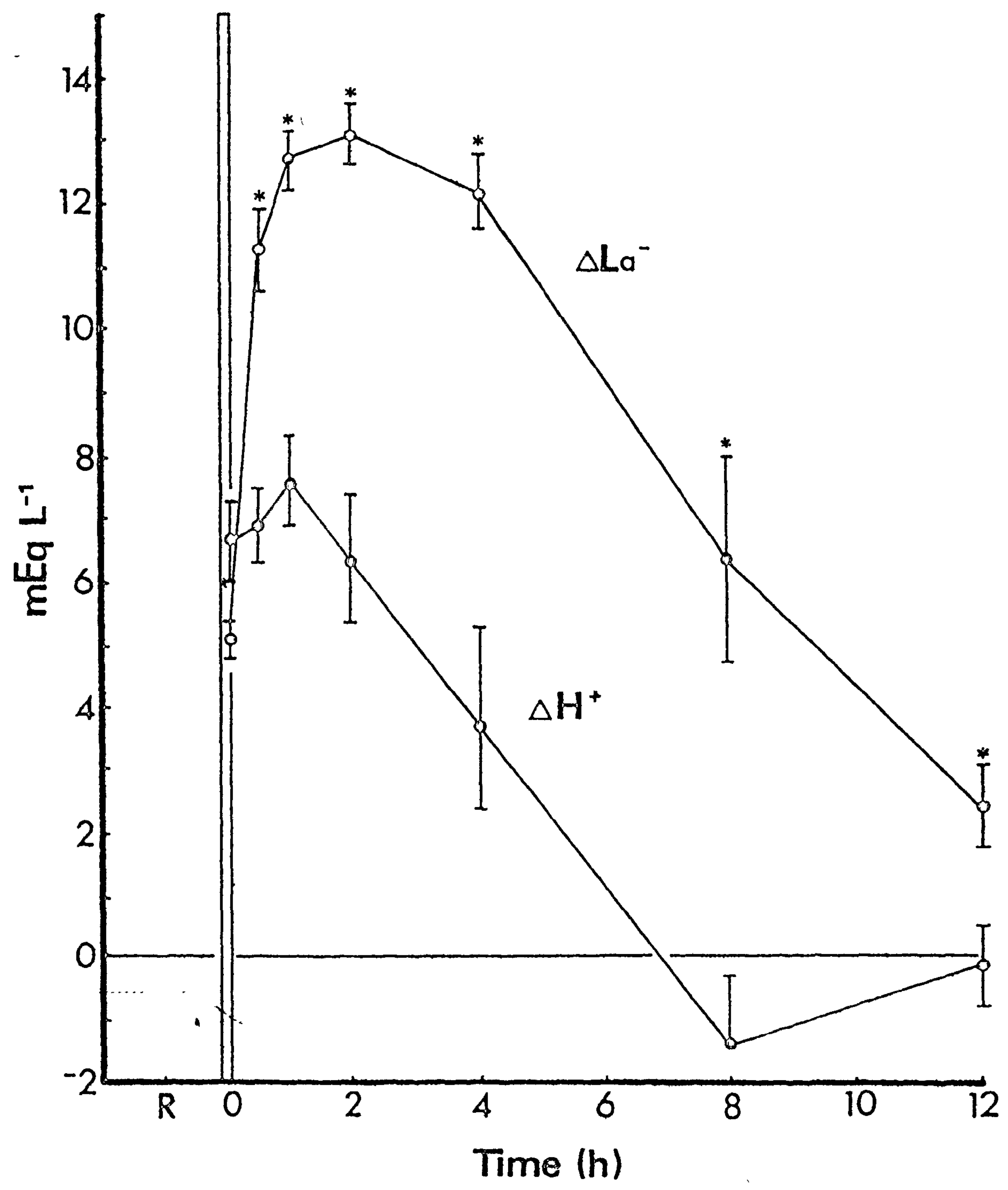

pHa
7.900
7.700
7.500
7.300
[HCO_3^-]p (mM)
10
8
6
4
2
$PaCO_2$ (torr)
8
6
4
2
R
0
2
4
6
8
10
12
Time (h)

Figure 3

Changes in blood lactate load (ΔLa^-) and metabolic acid load (ΔH^+) ($\overline{X} \pm 1$ SEM; $n = 8$), during recovery from severe exercise in rainbow trout.

R = rest; bar = indicates six minutes of severe activity, 0h = immediately post-exercise, 2,4h etc. = recovery time in hours.

(*) indicates a significant difference ($P < .05$) between ΔLa^- and ΔH^+ at each sample time (by paired "t" test).



14
12
10
8
6
4
2
0
-2
mEq L-1
ΔLa-
ΔH+
*
R
0
2
4
6
8
10
12
Time (h)

ΔH^+m. Lactate concentration was significantly greater than ΔH^+m at all times from 30 min to 12 h. By 2 h, $[\Delta La^-]$ had doubled to a maximum value of 13.15±0.39 (8) mEq L^{-1} while ΔH^+m had actually declined slightly to 6.30±0.83 (8) mEq L^{-1}. Between 2 h and 8 h, both components were removed from the blood stream at similar rates. By 8 h, ΔH^+m has been slightly overcorrected while ΔLa^- was still only reduced to one half its maximum. Lactate remained significantly elevated at the 12 h sample. This discrepancy between ΔLa^- and ΔH^+m could be caused by a preferential removal of protons from the blood space and/or by a slower proton release with respect to lactate release from the muscle mass.

Exercise caused significant increases in plasma concentrations of sodium, potassium and chloride (Figure 4). Sodium increased from 146.5±3.7 (8) mEq L^{-1} at rest to 166.3±4.6 (8) mEq L^{-1} at 0 h and returned to control levels by 4 h. Potassium increased from 2.50±0.13 mEq L^{-1} at rest to a maximum of 4.73±0.34 (8) mEq L^{-1} at 2 h and remained elevated until 12 h when it returned to resting levels. Chloride increased from 132±1.2 (8) mEq L^{-1} to 144±1.5 (8) mEq L^{-1} at 0 h, then decreased below control level to a low of 122±1.8 (8) mEq L^{-1} at 4 h before recovering by 12 h.

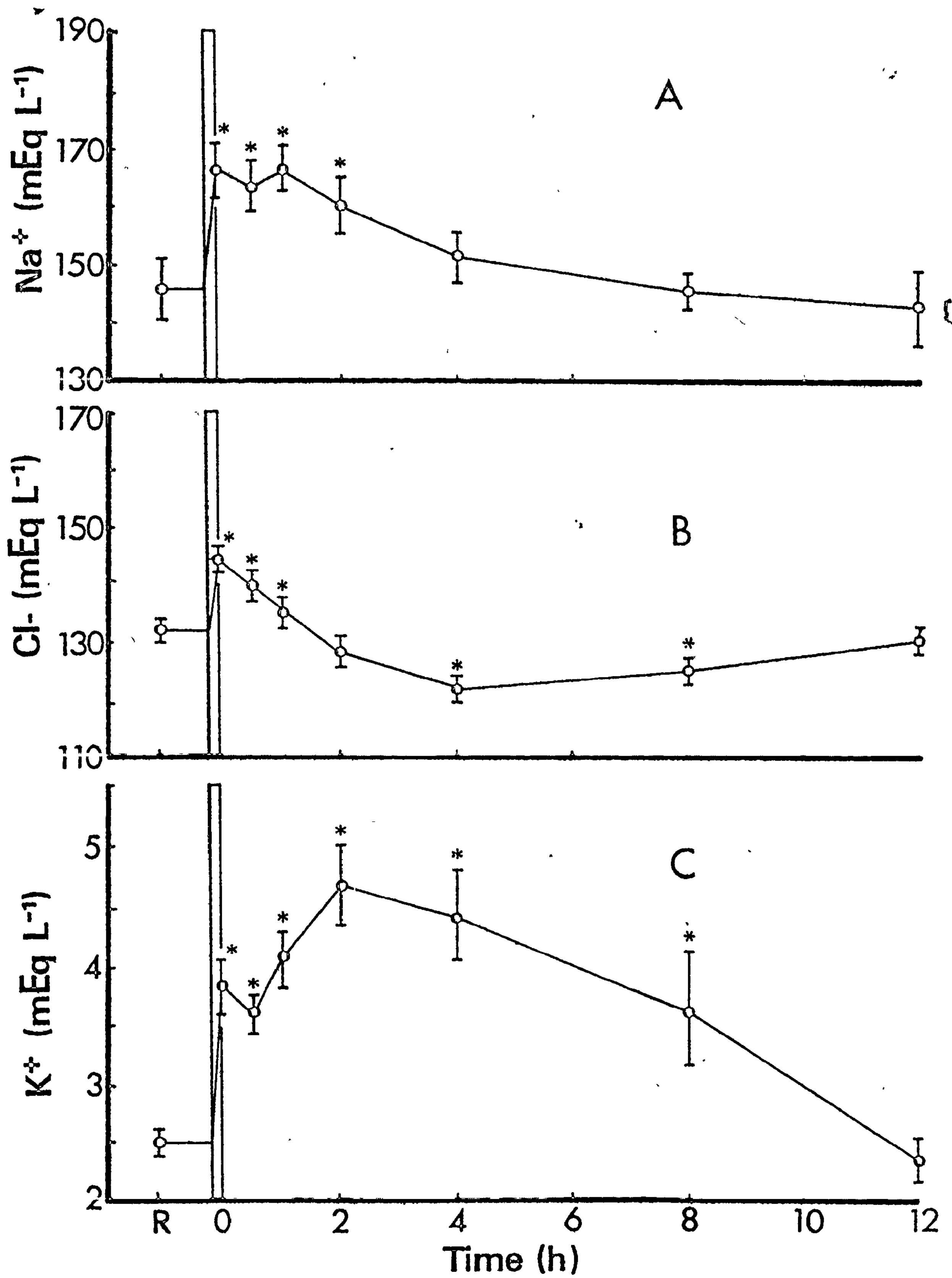
Percent haematocrit, haemoglobin and plasma protein concentrations increased during early recovery, in a pattern similar to that of plasma ions but slowly decreased thereafter due to sampling losses (Table 3). Mean corpuscular haemoglobin concentration (g haemoglobin ml^{-1} of packed erythrocytes) fluctuated insignificantly around 0.25± 0.02 g ml^{-1} throughout the experiment, indicating the absence of RBC

Figure 4

Plasma concentrations ($\overline{X} \pm 1$ SEM; $n = 8$) of sodium (A), chloride (B) and potassium (C), prior to and following severe exercise in the rainbow trout.

R = rest, bar = indicates six minutes of severe activity, 0h = immediately post-exercise, 2,4h etc. = recovery time in hours.

(*) indicates a significant difference ($P < .05$) from rest (by paired "t" test).



A
B
C
Na⁺ (mEq L⁻¹)
Cl- (mEq L⁻¹)
K⁺ (mEq L⁻¹)
190
170
150
130
170
150
130
110
5
4
3
2
R
0
2
4
6
8
10
12
Time (h)

TABLE 3. Haematocrit, mean corpuscular haemoglobin concentration, and total plasma protein ($\bar{X}$ ± 1 SEM; n = 8), prior to and following six minutes of severe exercise in rainbow trout.

0 h = immediately post-exercise; 0.5 h, 1 h etc. recovery times in hours.

TIME	REST	0 h	0.5 h	1 h	2 h	4 h	8 h	12 h
Haematocrit (%)	21.7 ±1.1	28.4* ±0.9	29.3* ±1.2	27.8* ±1.2	24.1 ±0.7	22.6 ±2.5	12.7* ±1.2	12.1* ±1.5
Mean Corpuscular Haemoglobin Concentration ($g\ ml^{-1}$)	0.25 ±.02	0.25 ±.02	0.25 ±.01	0.23 ±.01	0.24 ±.01	0.22 ±.02	0.28 ±.04	0.23 ±.01
Plasma Protein Concentration ($g\ 100\ ml^{-1}$)	2.6 ±0.2	2.8 ±0.2	2.9* ±0.2	2.8* ±0.3	2.6 ±0.2	2.4* ±0.3	2.2* ±0.2	2.0* ±0.2

* Indicates a significant difference ($P < 0.05$) from rest (paired "t" test).

swelling in contributing to the haematocrit changes.

1.20 Muscle Biopsy

In the second study, in which only a single terminal blood sample was drawn from each fish prior to muscle biopsy, all acid-base and ionic alterations were very similar to that of the first study and therefore have not been shown. The only exception was a smaller respiratory component immediately post exercise (Pa_{CO_2} = 4.80±1.09 (6) torr *vs* 7.79±0.64 (8) torr which initially reduced the severity of the acidosis [pHa 7.535±0.066 (6) *vs* 7.370±0.031 (8)]. The reason for these differences are unknown.

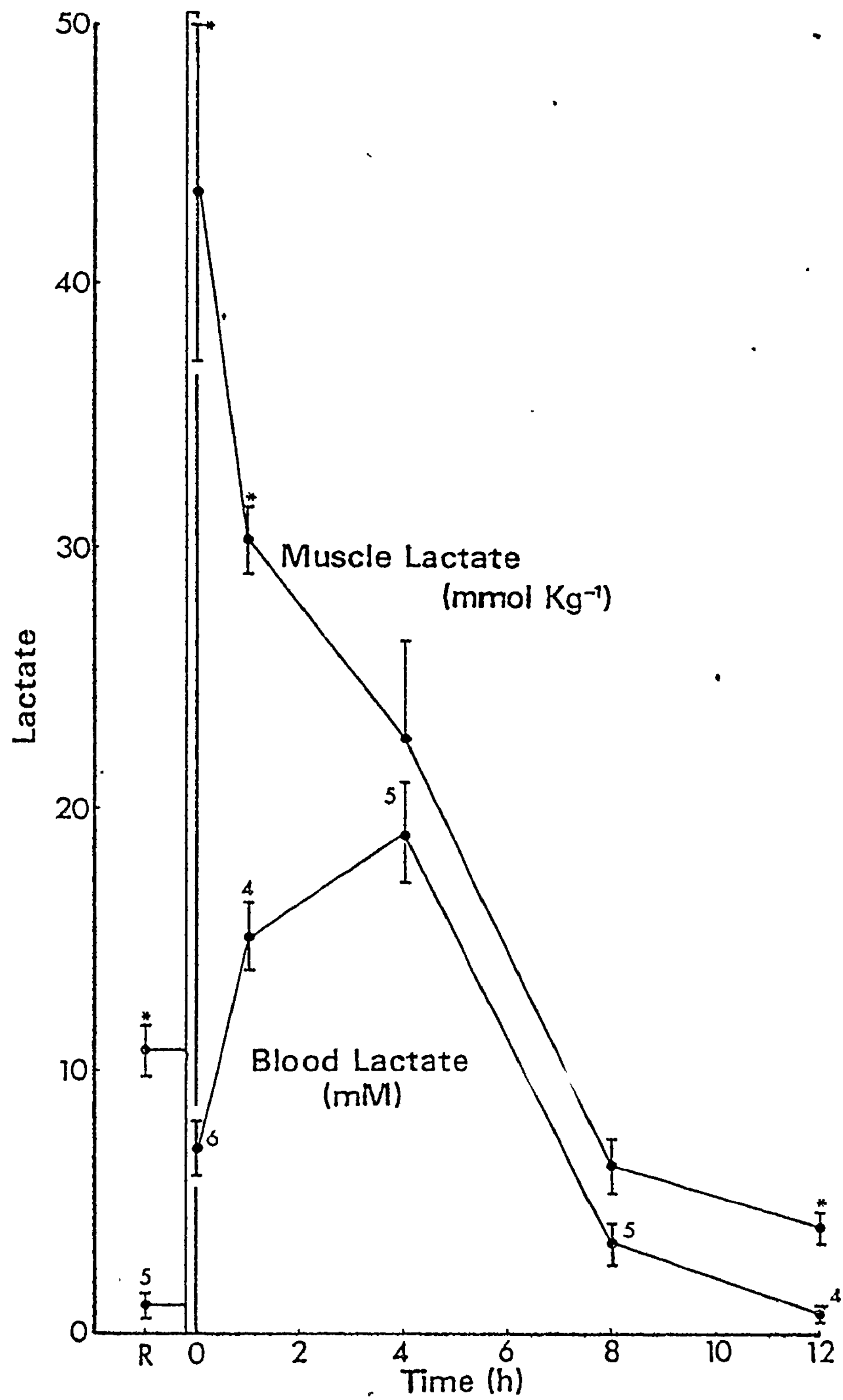
Resting muscle maintained a lactate level of 10.06±1.04 (5) mmol Kg^{-1} which increased to a maximum immediately after exercise of 43.60±6.44 (6) mmol Kg^{-1}. During recovery, muscle lactate decreased exponentially to a 12 h low of 4.04±0.33 (4) mmol kg^{-1}, significantly below resting levels (Figure 5). Blood lactate, initially 1.01±0.44 (5) mM increased much more slowly until an equilibrium was reached with the muscle lactate concentration at 4 h. At this time, blood lactate was 19.15±1.95 (5) mM and muscle lactate was not significantly different at 22.59±3.94 (5) mmol Kg^{-1}. During the subsequent recovery period, the decreases in muscle and blood lactate followed a similar pattern. These results show that muscle to blood lactate gradients are very high immediately after exercise but decline to very small values as blood lactate continues to rise. After 4 h, only a small gradient persists.

Figure 5

The relationship between muscle and blood lactate concentration ($\overline{X}$ ± 1 SEM; n) in terminally sampled rainbow trout, prior to and following severe exercise.

R = rest; bar = indicates six minutes of severe activity; 0h = immediately post-exercise, 2,4h etc. = recovery time in hours.

(*) indicate a significant difference ($P < .05$) between muscle and blood lactate levels (by paired "t" test).



50
40
30
20
10
0
Lactate
Muscle Lactate
(mmol Kg⁻¹)
Blood Lactate
(mM)
R
0
2
4
6
8
10
12
Time (h)
5
6
4
5
5
4

Muscle pyruvate was not significantly elevated by the exercise regime but it did fall significantly below resting levels as recovery progressed past 4 h (Table 4). Blood pyruvate levels reached a maximum at 1 h before returning to resting levels. An equilibrium was established between muscle and blood pyruvate at 1 h and remained for the duration of the experiment.

Plasma ammonia concentration peaked at 0 h then slowly returned to resting levels by 8 h. The effects of these changes on acid-base status would be minimal (Table 4).

1.30 Lactic Acid Infusion

In the third study lactic acid infusion placed equivalent quantities of protons (H^+) and lactate (La^-) in the bloodstream in order to test whether the results from the first study could be explained by differential removal rates of the two species from the blood. At 0 h, ΔLa^- was 10.10±0.83 (8) mEq L^{-1} while ΔH^+ was not significantly different at 8.67±1.10 (8) mEq L^{-1}. The removal rates of these species from the blood during the post-infusion period was shown to be not significantly different (Table 5). Therefore, the discrepancy found between ΔLa^- and ΔH^+m in the blood during recovery from exercise is not due to preferential removal.

Acid-base disturbances resulting from the infused lactic acid were severe but shortlived. Blood pH_a was depressed from a resting value of 7.912±0.020 (8) to 7.529±0.046 (8) at 0 h, but then increased sharply, returning to resting levels within 1 h. Similarly, $[HCO_3^-]_p$ dropped promptly from 9.20±0.41 (8) mM at rest to 4.85±0.58 (8) mM

TABLE 4. Muscle pyruvate, blood pyruvate and plasma ammonia concentrations ($\overline{X}$ ± 1 SEM; n) at rest and following six minute of severe exercise in rainbow trout. 0 h = immediately post-exercise; 1 h, 4 h etc. = recovery time in hours.

TIME	REST	0 h	1 h	4 h	8 h	12 h
Muscle Pyruvate (mmol Kg^{-1})	0.306* ±0.046 (4)	0.570* ±0.138 (5)	0.485 ±0.182 (4)	0.159Δ ±0.030 (4)	0.095Δ ±0.028 (5)	0.123Δ ±0.035 (5)
Blood Pyruvate (mM)	0.058* ±0.007 (5)	0.091* ±0.017 (6)	0.229Δ ±0.016 (4)	0.143Δ ±0.028 (5)	0.088 ±0.035 (5)	0.061 ±0.025 (4)
Plasma Ammonia (mM)	0.103 ±0.017 (3)	0.385Δ ±0.065 (6)	0.206Δ ±0.027 (4)	0.187 ±0.024 (5)	0.053Δ ±0.005 (5)	0.068 ±0.007 (7)

* Indicate significant difference ($P < .05$) between muscle and blood pyruvate (unpaired "t" test)

Δ Indicate significant difference ($P < .05$) from rest (paired "t" test)

TABLE 5. A comparison of the rates ($\overline{X} \pm 1$ SEM; n) of removal of lactate anions and metabolic protons from the blood of rainbow trout during the first 2 h after an L-lactic acid infusion

TIME	LACTATE $\mu mol\ L^{-1}\ min^{-1}$	PROTONS $\mu mol\ L^{-1}$ min	SIGNIFICANCE LEVEL
LACTIC ACID			
0 - 0.5 h	234 ± 32 (7)	224 ± 24 (8)	n.s.
0.5 - 1.0 h	33 ± 12 (7)	57 ± 9 (8)	n.s.
1.0 - 2.0 h	10 ± 3 (8)	4 ± 8 (8)	n.s.

at 0 h, and was completely recovered by 12 h. Pa_{CO_2} increased briefly from 3.10±0.17 (8) torr at rest to 4.13±0.19 (8) torr at 0 h but returned to control by 30 min (Figure 6). As would be expected, this acidosis was determined to be over 90% metabolic in origin (Davenport, 1974). Plasma concentrations of sodium and chloride remained constant throughout the recovery period (Table 6). Potassium was elevated significantly above resting levels from 0.5 h to 12 h . Since haemolysis was noted in all cases following infusions, elevations in plasma potassium are not surprising. Red blood corpuscle swelling was observed briefly at 0 h , after the infusion, as was indicated by a drop in mean corpuscular haemoglobin concentration (Table 6).

The lactate space in rainbow trout has been calculated to be 214±16 ml Kg^{-1}.

1.40 Isolated Perfused Trunk

All the trunks that were recovering from severe exercise became progressively more rigid. This observation of exercise induced rigor has been noted in mammals and has been attributed to intracellular acidosis (Guyton, 1981) and/or an increase in water movement into the muscle cells (Bergstrom *et al.*, 1971). Isolated perfused trunks from unexercised fish remained flexible throughout the 180 minute perfusion period.

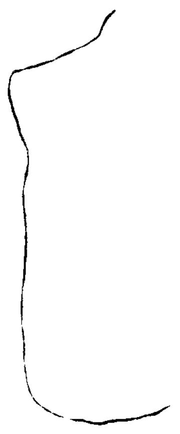
The aerobic viability of the preparation was determined by measuring the oxygen consumption of one isolated trunk. Oxygen consumption as calculated by the Fick principle, from measurements of perfusion flow rate and inflow and outflow perfusate O_2 content, remained constant over the 120 minute experiment and ranged from

Figure 6

Blood acid-base status (pH_a, $[HCO_3^-]_p$ and Pa_{CO_2})($\bar{X} \pm 1$ SEM; n = 8) prior to and following an L-lactic acid infusion in rainbow trout.

R = rest; bar = indicates a 15 minute infusion and mixing period; 0h = immediately post infusion and mixing; 2,4h etc. = time from end of infusion in hours.

(*) indicates a significant difference ($P < .05$) from rest (by paired "t" test).



8.000
7.800
7.600
7.400
pHa

11
9
7
5
3
$[HCO_3^-]_p$ (mM)

5
4
3
2
1
0
$PaCO_2$ (torr)

R 0 2 4 6 8
Time (h)

TABLE 6. Alterations in plasma sodium, potassium and chloride, and mean corpuscular haemoglobin concentration ($\overline{X} \pm 1$ SEM) following an l-lactic acid in rainbow trout. 0 h = immediately post-exercise; 0.5 h, 1 h etc = time after infusion in hours.

ION	REST	0 h	0.5 h	1.0 h	2.0 h	4.0 h	8.0 h
LACTIC ACID INFUSION (n = 8)							
Sodium (mEq L^{-1})	145 ±2.5	148.8 ±2.0	150.8 ±1.4	148.1 ±1.9	149.9 ±1.5	151.4 ±2.5	148.0 ±2.7
Chloride (mEq L^{-1})	129.9 ±0.9	128.5 ±1.3	129.4 ±1.2	129.1 ±0.8	130.4 ±0.7	131.8 ±0.9	132.0 ±0.8
Potassium (mEq L^{-1})	2.14 ±0.13	2.40 ±0.19	2.98* ±0.15	2.68* ±0.13	2.57* ±0.11	2.50* ±0.09	2.53* ±0.08
Mean Corpuscular Haemoglobin Concentration (g 100 ml^{-1})	0.309 ±0.014	0.257* ±0.012	0.303 ±0.021	0.295 ±0.021	0.316 ±0.023	0.319 ±0.024	0.323 ±0.037

* Indicates significant difference ($P < .05$) from resting levels (by paired "t" test).

0.099-0.110 ml $Kg^{-1}min^{-1}$. These values represent approximately 1/6 the oxygen consumption of an intact trout (Cameron and Davis, 1970) and are reasonable since over 90% of the tissue perfused was white muscle, which is known to be predominately glycolytic in nature.

The mean muscle lactate levels (Table 7) which include all the perfused preparations, were not significantly different from in vivo values for the first 30 min of recovery (cf. Figure 5). However, as perfusion continued, the muscle lactate load in the trunk slowly increased with time (Table 7). As the trunks did not move during perfusion, little lactate production from glycolysis would be expected. The apparent production of muscle lactate could be an artifact of anterior-posterior lactate gradients known to exist in trout axial muscle after exercise (Black et al., 1962). However, a single experiment in which these regions were sampled simultaneously failed to show such a gradient.

When exercised trunks were perfused with saline which closely simulated in vivo rest acid-base status (pH = 7.832±0.007, P_{CO_2} = 2.30±0.05 torr and [HCO_3^-] = 5.56±0.06 mM), the net efflux of lactate and protons from the muscle into the perfusate were of similar. In fact, only during the first 30 minutes of recovery was the net lactate efflux significantly higher than that for protons (Table 8). Within this group of eight trunks are included those perfused with adrenaline, chloride-free saline and at a high flow rate in addition to the five control trunks. This grouping was possible as these procedures did not cause the efflux of either lactate or protons to differ from the five control fish. The adrenaline did, however, cause

TABLE 7. Muscle lactate concentration ($\overline{X} \pm 1$ SEM; n = 14) during recovery from six minutes of severe exercise in the isolated, perfused trunk of the rainbow trout

Sample Time During Recovery	Muscle Lactate Load mmol Kg^{-1}	Sig. Level from 0 min	Sig. Level from 30 min
0 min	37.01 ± 3.58	---	P < .05
30 min	40.59 ± 3.67	P < .05	---
60 min	40.52 ± 3.33	P < .05	n.s.
90 min	43.47 ± 5.15	P < .05	n.s.
120 min	44.56 ± 4.09	P < .01	P < .05
150 min	44.72 ± 4.31	P < .01	n.s.
180 min	46.21 ± 4.70	P < .01	P < .01

Significance levels demonstrated by paired "t" test.

TABLE 8. Net lactate and proton efflux ($\mu mol\ min^{-1}\ Kg^{-1}$) ($\overline{X} \pm 1$ SEM) from muscle to blood during recovery from six minutes of severe exercise in the isolated perfused trunk of rainbow trout.

	PERFUSATE pH = 7.832 ± .007 (n=8)		PERFUSATE pH = 7.339 ± .011 (n=4)		SITS $1x10^{-4}$M PERFUSATE pH = 7.745 ± 0.022 (n=2)	
	NET LACTATE EFFLUX	NET PROTON EFFLUX	NET LACTATE EFFLUX	NET PROTON EFFLUX	NET LACTATE EFFLUX	NET PROTON EFFLUX
0-30 min	26.5 ± 4.5	15.7 ± 1.7*	19.7 ± 6.0	6.13 ± 1.5Δ	47.2 (30.7-63.6)	21.4 (16.4-26.3)
30-60 min	19.4 ± 4.3	14.7 ± 2.5	14.2 ± 4.0	5.13 ± 1.4*Δ	51.8 (45.2-58.3)	26.5 (21.9-31.0)
60-90 min	12.4 ± 3.4	13.9 ± 2.4	14.8 ± 2.9	4.55 ± 2.0*Δ	36.8 (30.4-43.1)	20.8 (18.2-23.3)
90-120 min	9.2 ± 2.5	7.6 ± 1.4	13.6 ± 2.6	5.63 ± 1.2	40.3 (33.8-46.7)	27.2 (22.0-32.3)
120-150 min	8.3 ± 2.7	11.3 ± 1.0	12.2 ± 4.8	5.43 ± 2.3	39.0 (32.9-45.1)	22.7 (14.8-30.6)
150-180 min	8.5 ± 3.0	10.3 ± 2.7	15.2 ± 4.9	3.63 ± 2.9	32.8 (19.3-46.2)	23.0 (14.0-32.0)

* Indicates significant difference ($P < .05$) between lactate and proton efflux rates (by paired "t" test)

Δ Indicates significant difference ($P < .05$) between pH = 7.832 and pH = 7.339 efflux rates of protons and lactate (by unpaired "t" test)

extensive vaso-constriction, manifested as a rise in perfusion pressure to over 60 cm H_2O, as seen by Wood (1976).

In four exercised trunks that were perfused with saline which simulated post-exercise acidosis (pH = 7.339±0.011, P_{CO_2} = 6.82±0.27 torr, and [HCO_3^-] = 4.80±0.11 mM (cf. Figure 2), the net proton efflux rates were considerably lower than those for lactate (Table 8). Net proton efflux rates were also significantly lower in this group when compared to proton efflux during normal pH perfusion. This indicates that perfusate acid-base status may affect net proton efflux rates. Lactate fluxes were not affected by alterations in perfusate acid-base status, as net efflux lactate efflux rates were not significantly different between trunks perfused at normal and low pH.

The addition of SITS to the rest perfusate (pH = 7.745±0.022, P_{CO_2} = 2.69±0.11 torr, [HCO_3^-] = 5.28±0.11 mM) caused dramatic increases in net lactate efflux rates. Proton efflux also increased but to a lesser relative extent (Table 8). This observation strongly suggests that some transport mechanism involving lactate exists within the trunk. Further, since SITS enhances rather than inhibits lactate efflux, it would appear that the direction of lactate transport is <u>into</u> the muscle cells. This type of mechanism would tend to reduce net perfusate lactate efflux under normal conditions and would explain increased lactate efflux during SITS inhibition. To clarify and condense the above data, the efflux rates during the six individual flux periods were summated to give the total net efflux of lactate and protons over the experimental period (Figure 7).

Figure 7

Total net lactate and proton efflux ($\overline{X}$ ± 1 SEM; n) (μmol kg^{-1}) over the 180 minute experiment, with exercised, isolated, perfused rainbow trout trunk perfused with three types of saline.

Saline A, pH = 7.832 ± 0.007, [HCO_3^-] = 5.56 ± 0.06 mM and P_{CO_2} = 2.30 ± 0.05 torr

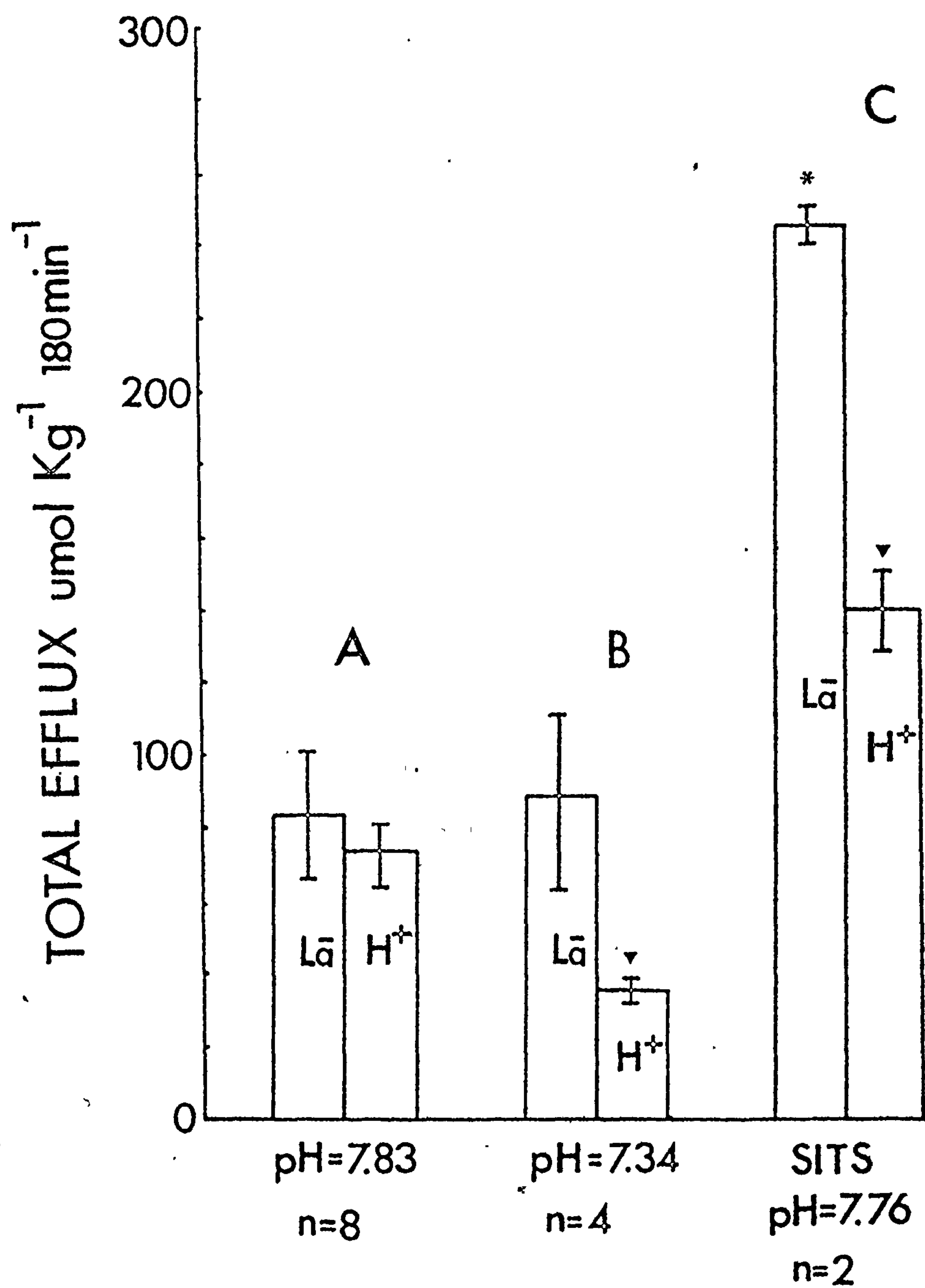
Saline B, pH = 7.339 ± 0.011, [HCO_3^-] = 4.80 ± 0.11 mM, and P_{CO_2} = 6.82 ± 0.27 torr

Saline C, pH = 7.745 ± 0.022, [HCO_3^-] = 5.28 ± 0.11 mM, P_{CO_2} = 2.69 ± 0.11 torr plus 1 x 10^{-4} M SITS

(*) indicates a significant difference (P < .05) in total lactate efflux between experiment C(SITS) and both A and B (by unpaired "t" test).

(▾) indicates a significant difference (P < .05) between the total net efflux of lactate and protons (by paired "t" test).

Note = all total net proton efflux values are significantly different from each other (P < .05, by unpaired "t" test).



TOTAL EFFLUX umol Kg-1 180min-1
300
200
100
0
A
B
C
*
La
H+
La
H+
La
H+
pH=7.83
n=8
pH=7.34
n=4
SITS
pH=7.76
n=2

No consistant inflow-outflow chloride differences were detected in the 11 experimental trunks in which this parameter was measured. Possible reasons for this include the limitations of Cl^- detection by the Radiometer CMT-10. This instrument has a working accuracy of approximately 1 mEq L^{-1} which translates into ±7.0 μmol Kg^{-1} min^{-1}, assuming an average tail wt of 190 g, a 30 min efflux period and a flow of 7 ml Kg^{-1} min^{-1}. Obviously more accurate methods of chloride determination must be utilized to elucidate the role of chloride in this mechanism.

2.00 RECOVERY IN FLATHEAD SOLE

2.10 Blood Parameters Following Exercise

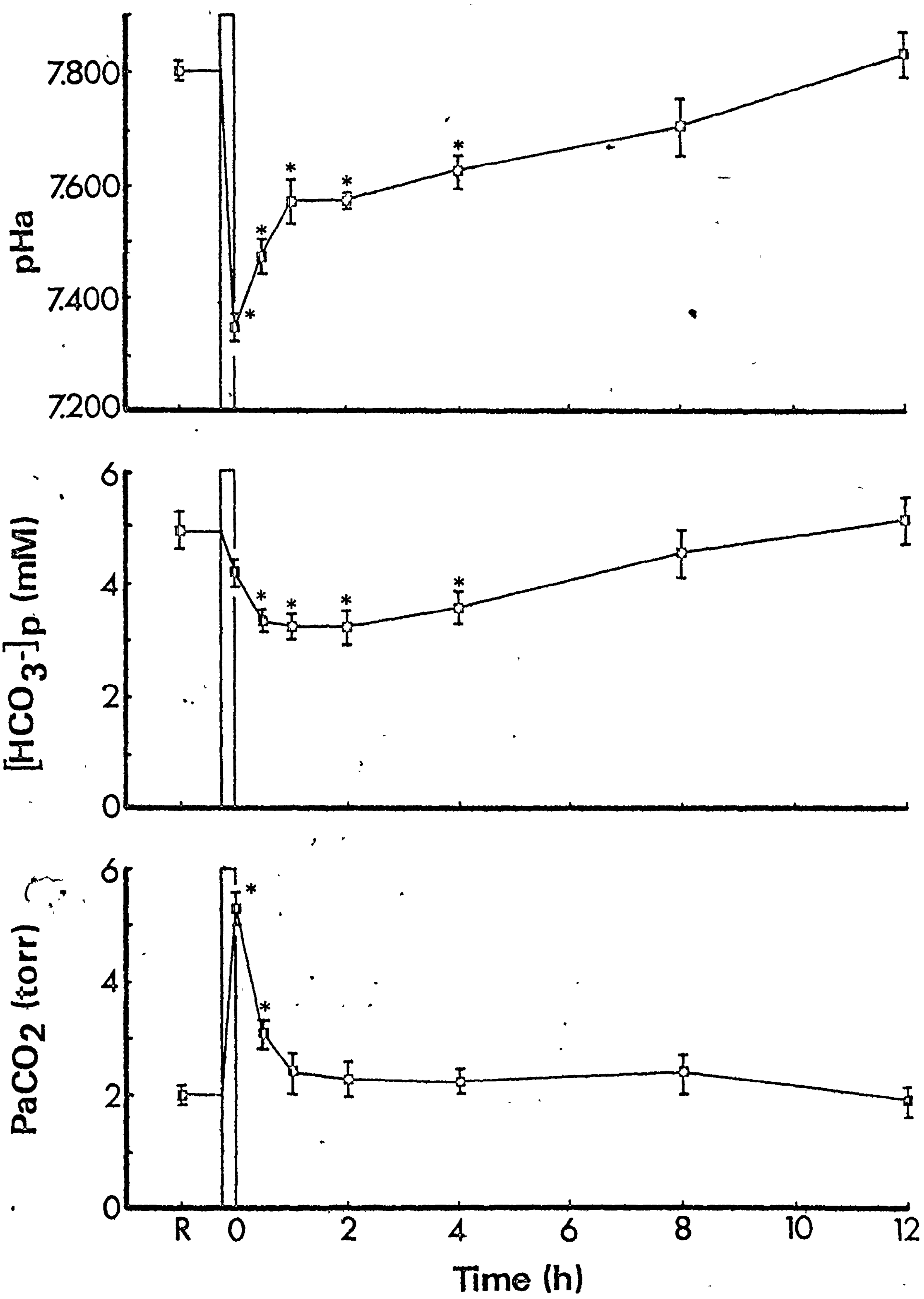
A positive correlation exists in the flathead sole between exercise intensity and the resultant acid-base disturbance. Exhausting exercise caused larger and longer duration disturbances than did moderate exercise (cf. Figure 8,9). Exhausting exercise initiated a prompt reduction in pHa from 7.803±0.016 (5) at rest to 7.352±0.024 (5) at 0 h, which required 8 h to recover. Plasma bicarbonate levels decreased from a resting value of 4.94±0.34 (5) mM to a minimum of 3.22±0.32 (5) mM at 2 h, before recovering by 8 h. Exhausting exercise increased Pa_{CO_2} from 2.02±0.17 (5) torr at rest to 5.31±0.29 (5) torr at 0 h, but was rapidly corrected in less than 1 h. These acid-base disturbances reflect a combined respiratory and metabolic acidosis (Davenport, 1974) (Table 17).

Figure 8

Blood acid-base status (pH_a, $[HCO_3^-]_p$ and Pa_{CO_2})($\overline{X}$ ± 1 SEM; n = 5) in the flathead sole prior to and following exhaustive exercise.

R = rest; bar = indicates six minutes of exhausting exercise; 0h = immediately post-exercise; 2,4h etc. = recovery times in hours.

(*) indicates a significant difference ($P < .05$) from rest (by paired "t" test).



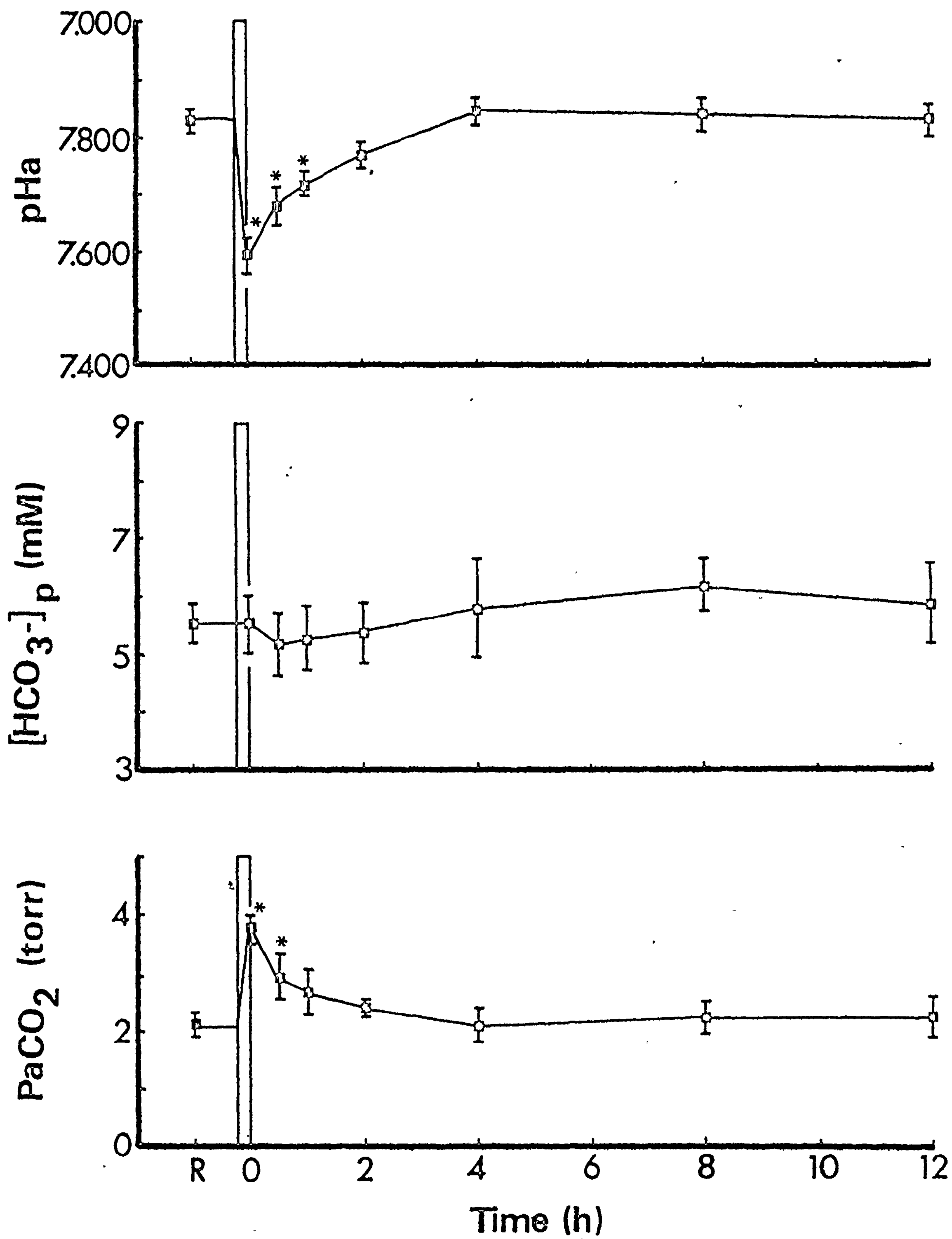
pHa
7.800
7.600
7.400
7.200
$[HCO_3^-]p$ (mM)
6
4
2
0
$PaCO_2$ (torr)
6
4
2
0
R
0
2
4
6
8
10
12
Time (h)

Figure 9

Blood acid-base status (pH_a, $[HCO_3^-]_p$ and P_{CO_2})($\bar{X}$ ± 1 SEM; n = 5) prior to and following moderate exercise in the flathead sole.

R = rest; bar = indicates six minutes of moderate exercise; 0h = immediately post-exercise; 2,4h = recovery time in hours.

(*) indicates a significant difference ($P < .05$) from rest (by paired "t" test).


7.000
7.800
7.600
7.400
pHa
9
7
5
3
$[HCO_3^-]_p$ (mM)
4
2
0
$PaCO_2$ (torr)
R
0
2
4
6
8
10
12
Time (h)

Exercise of moderate intensity, for the same duration as the exhausting exercise caused less severe acid-base alteration. The blood pH_a, dropped moderately from 7.830±0.013 (5) at rest to only 7.587±0.036 (5) at 0 h and was fully corrected within 2 h. Plasma bicarbonate fluctuated insignificantly about 5.58±0.39 (5) mM, except for an unexplained increase at 8 h. Moderate exercise increased Pa_{CO_2} from 2.14±0.17 (5) torr at rest to 3.84±0.19 (5) torr at 0 h; it then returned to resting levels by 1 h. This acidosis was over 70% respiratory in origin and was fully corrected in only 2 h.

During recovery from exhausting exercise, the metabolic acid load (ΔH^+m) was always found in higher concentration in the blood than was lactate (ΔLa^-). The ΔH^+m, reached a maximum of 3.42±0.33 (4) mEq L^{-1} after just 30 minutes of recovery, then slowly decreased to resting levels at 12 h (cf. Figure 10a). Lactate reached a maximum of 0.84± 0.04 (4) mEq L^{-1} at 2 h, before returning to rest at 8 h. Resting lactate levels were 0.11±0.03 (4) mEq L^{-1}.

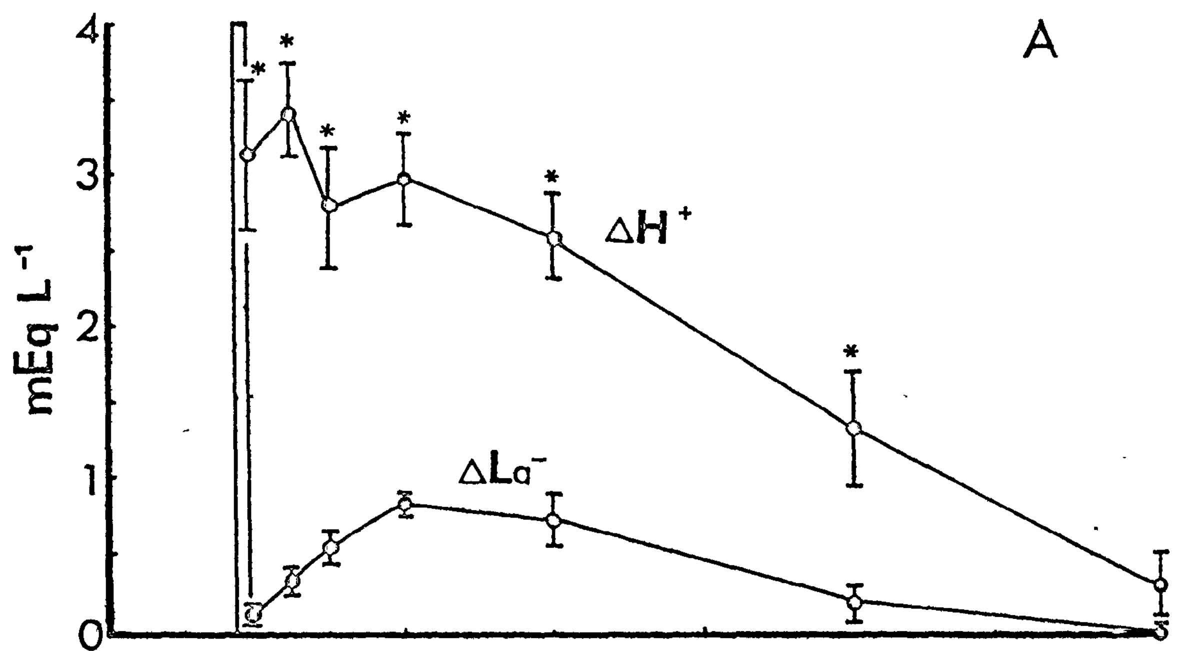
Moderate exercise in the flathead sole produced a much different recovery pattern of lactate and proton accumulation in the blood (cf. Figure 10b). The metabolic acid load reached a maximum of 2.14± 0.47 (5) mEq L^{-1} before returning to resting levels by 2 h. Blood lactate levels were minute, always less than 0.25 mEq L^{-1}. The discrepancy between ΔH^+ and ΔLa^- during recovery from both exhausting and moderate exercise could be explained by a preferential removal of lactate from the blood and/or a slower efflux of lactate from the myotome.

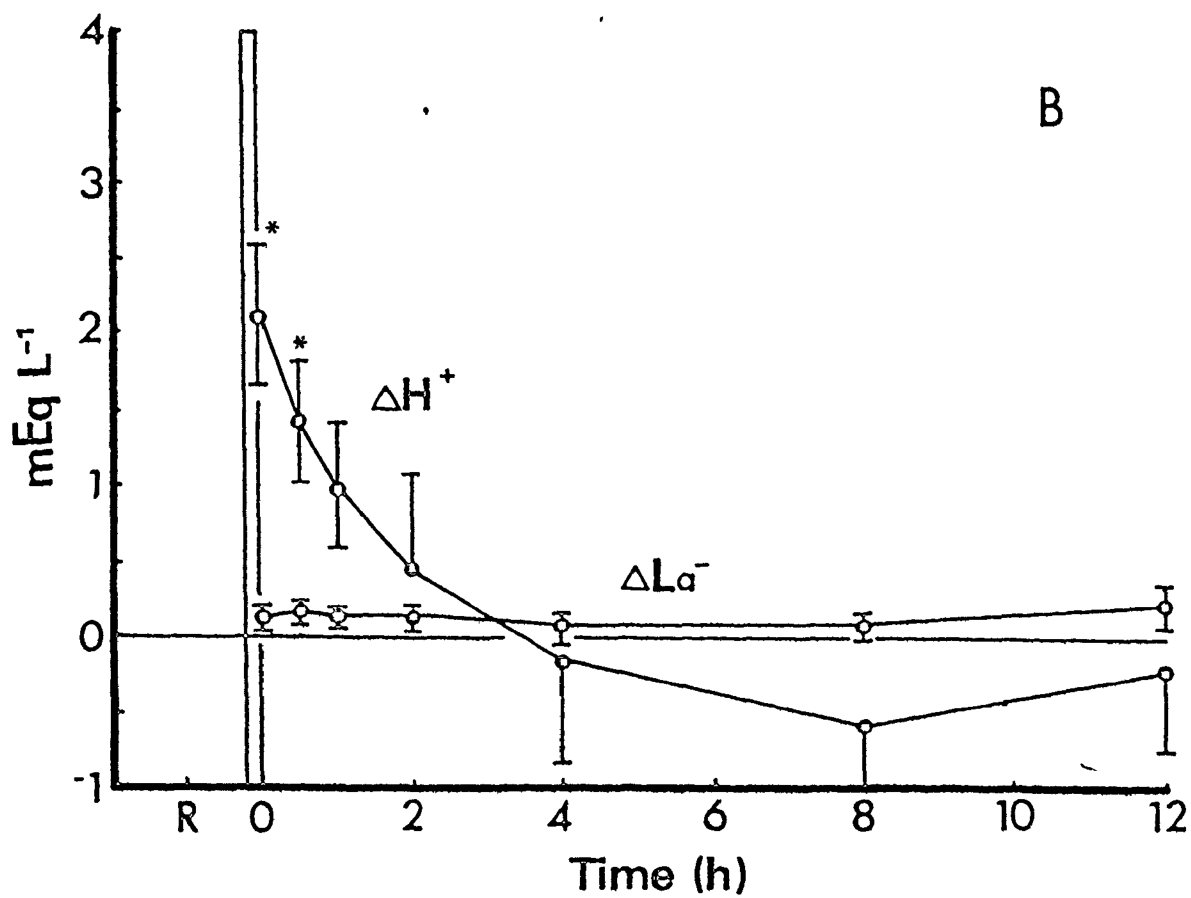
Figure 10

Changes in blood lactate load (ΔLa^-) and metabolic acid load (ΔH^+) ($\overline{X} \pm 1$ SEM), during recovery from exhaustive exercise (A; $n = 4$) and moderate exercise (B; $n = 5$) in the flathead sole.

R = rest; bar = indicates six minutes of either exhaustive or moderate exercise; 0h = immediately post-exercise; 2,4h etc. = recovery time in hours.

(*) indicates a significant difference ($P < 0.5$) between ΔLa^- and ΔH^+ at each sample time (by paired "t" test).


A
mEq L-1
4
3
2
1
0
ΔH+
ΔLa-
*


B
mEq L-1
4
3
2
1
0
-1
ΔH+
ΔLa-
*
R
0
2
4
6
8
10
12
Time (h)

Variations in plasma sodium, chloride, potassium and total osmolality were large between animals. Recovery from exhausting exercise did not significantly alter plasma concentrations of sodium and chloride. However, potassium was increased after exercise and remained elevated until 2 (Table 9). Plasma osmolality was increased by exercise and remained elevated for the duration of the recovery. After moderate exercise, plasma sodium concentration remained constant, while chloride levels were significantly increased at 0.5 h and at 2-8 h, but were fully recovered by 12 h. Plasma potassium levels were increased at 0 h and remained elevated until 8 h. The total osmolality of the plasma was elevated during exercise and remained significantly above resting levels for the duration of the experiment (Table 9).

Blood haemoglobin concentration was not elevated after exercise but slowly decreased with time. This suggests that haemoconcentration had not occurred to any significant extent and that sampling losses have slowly reduced the haemoglobin levels. Mean corpuscular haemoglobin concentration remained unaltered indicating the lack of RBC swelling (Table 10).

2.20 Muscle Biopsy

Exercise of either moderate or exhausting intensity greatly increased muscle lactate concentrations. Resting muscle maintained a lactate load of 2.89±0.51 (6) mmol Kg^{-1} fresh tissue which was increased to 14.91±1.86 (7) mmol Kg^{-1} following exhaustive exercise (Figure 11). Recovery reduced these levels to a 12 h low of

TABLE 9. Changes in plasma sodium, chloride, potassium and total osmolality ($\overline{X} \pm 1$ SEM) during recovery from either six minutes of exhausting or moderate exercise in the flathead sole. 0 h = immediately post-exercise; 0.5 h, 1.0 h etc = recovery time in hours.

ION	REST	0 h	0.5 h	1.0 h	2.0 h	4.0 h	8.0 h	12.0 h
EXHAUSTING EXERCISE (n = 5)								
Sodium ($mEq\ L^{-1}$)	169 5.1	171 6.5	173 8.6	173 8.8	178 9.9	167 7.2	166 10.2	162 11.2
Chloride ($mEq\ L^{-1}$)	152 8.6	167 4.6	172 6.4	170 6.7	168 8.7	163 7.5	158 5.1	162 9.1
Potassium ($mEq\ L^{-1}$)	2.59 0.08	3.61* 0.20	3.00* 0.18	2.84* 0.12	2.84 0.18	2.72 0.11	2.48 0.10	2.60 0.19
Osmolality ($mmol\ Kg^{-1}$)	305 11.6	338* 12.0	337* 15.2	335* 13.6	332* 13.1	326* 14.5	320* 16.4	326* 18.0
MODERATE EXERCISE (n = 5)								
Sodium ($mEq\ L^{-1}$)	173 15.0	178 10.2	180 15.7	178 15.1	179 15.4	183+ 21.5	175 12.5	171 10.7
Chloride ($mEq\ L^{-1}$)	155 9.5	160 9.8	170* 12.3	168 14.1	167* 12.8	174* 12.2	170* 14.0	159 12.1
Potassium ($mEq\ L^{-1}$)	2.52 0.17	3.04* 0.27	2.96* 0.20	2.88* 0.14	2.73* 0.22	2.96* 0.27	2.89 0.29	2.79 0.22
Osmolality ($mmol\ Kg^{-1}$)	329 26.6	350* 26.0	351* 27.0	351* 26.7	351* 26.6	353* 28.8	353* 33.0	360* 34.9

* Indicates a significant difference ($P < .05$) from resting levels (by paired "t" test)

+ indicates n = 4.

TABLE 10. Changes in haemoglobin level and mean corpuscular haemoglobin concentration ($\bar{X} \pm 1$ SEM) during recovery from six minutes of exhaustive or moderate exercise in the flathead sole.

0 h = immediately post-exercise; 0.5 h, 1 h etc = recovery time in hours.

TIME	REST	0 h	0.5 h	1 h	2 h	4 h	8 h	12 h
EXHAUSTIVE EXERCISE (n = 5)								
Haemoglobin ($g\ 100\ ml^{-1}$)	2.75 0.24	3.19 0.34	2.09 0.30	2.09 0.32	1.69* 0.23	1.48* 0.21	1.39* 0.22	0.97* 0.20
Mean Corpuscular Haemoglobin Concentration ($g\ ml^{-1}$)	0.22 0.007	0.22 0.009	0.21 0.022	0.19 0.023	0.19 0.022	0.21 0.025	0.21 0.019	0.20 0.026
MODERATE EXERCISE (n = 5)								
Haemoglobin ($g\ 100\ ml^{-1}$)	3.35 0.58	3.51 0.53	3.28 0.48	2.83* 0.49	2.06* 0.41	2.12* 0.46	1.95* 0.51	1.84* 0.49
Mean Corpuscular Haemoglobin Concentration ($g\ ml^{-1}$)	0.22 0.01	0.21 0.01	0.23 0.01	0.25 0.02	0.20 0.01	0.26 0.04	0.23 0.04	0.23 0.04

* Indicates a significant difference ($P < 0.05$) from resting levels (by paired "t" test).

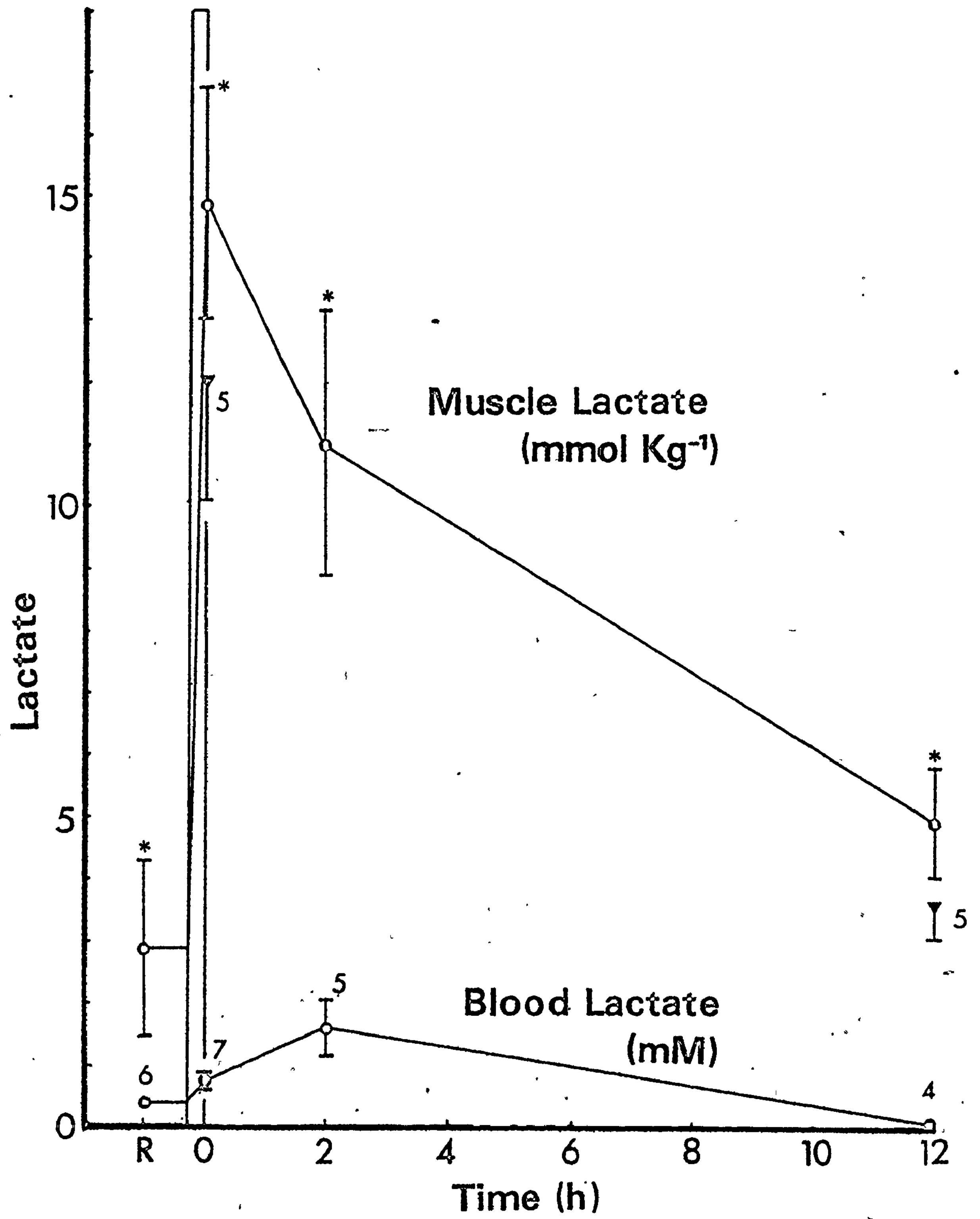
Figure 11

The relationship between muscle and blood lactate concentration ($\overline{X} \pm 1$ SEM; n) in flathead sole prior to and following exhausting exercise (●).

R = rest; bar = indicates six minutes of exhausting activity; 0h = immediately post-exercise; 2,4h etc. = recovery time in hours.

(▼) indicates muscle lactate concentration ($\overline{X} \pm 1$ SEM; n) following six minutes of moderate exercise

(*) indicates a significant difference ($P < .05$) between muscle and blood lactate concentration (by paired "t" test).


Lactate
15
10
5
0
Muscle Lactate
(mmol Kg-1)
Blood Lactate
(mM)
R
0
2
4
6
8
10
12
Time (h)
*
*
*
*
5
5
5
7
6
4

4.97±0.92 (5) mmol Kg^{-1}. One unexpected observation was that immediately following moderate exercise, the muscle lactate load was not significantly different from that of exhausted fish. As in the first series of flathead sole experiments, blood lactate remained very low, below 2 mM during the experiment. At no time were the blood and muscle lactate concentrations anywhere near equilibrium.

Blood pyruvate levels were increased at 0 h and remained elevated above resting concentration for the duration of the experiment. The magnitude of the increase could be related to the severity of the exercise; exhausting exercise increased blood pyruvate over 5 fold, while moderate exercise increased it 3 fold (Table 11).

Plasma ammonia was not altered by moderate exercise but was significantly elevated by exhausting exercise. By 12 h plasma ammonia levels were corrected and significantly below resting levels (Table 11).

The blood acid-base alterations measured during the biopsy experiments were found to be not significantly different from those of the first study. A single point of disagreement was at 0 h following moderate exercise and was due to the sampling of venous blood as opposed to the arterial blood normally sampled. The venous blood values were pH = 7.456±0.03[illegible] (5), $[HCO_3^-]_p$ = 5.75±0.54 (5) mM and Pa_{CO_2} = 5.69±0.69 (5) torr, while the arterial values in the first series were; pH = 7.587±0.036 (5), $[HCO_3^-]$ = 5.58±0.51 (5) mM, Pa_{CO_2} = 3.84±.19 (5) torr.

TABLE 11. Blood pyruvate and plasma ammonia concentrations (mM) ($\overline{X}$ ± 1 SEM; n) prior to and following six minutes of either moderate or exhausting exercise in the flathead sole.

0 h = immediately post-exercise; 2 h, 12 h = recovery time in hours

Recovery Time	Moderate Exercise		Exhaustive Exercise	
	Blood pyruvate	Plasma ammonia	Blood Pyruvate	Plasma ammonia
R	0.049 ±0.005 (6)	0.71 ±0.06 (6)	0.049 ±0.005 (6)	0.71 ±0.06 (6)
0 h	0.156* ±0.016 (5)	0.87 ±0.12 (5)	0.270* ±0.023 (7)	1.17* ±0.04 (7)
2 h	---	---	0.158* ±0.034 (5)	1.01* ±0.07 (5)
12 h	0.080* 0.011 (5)	0.72 0.05 (5)	0.109* 0.024 (4)	0.53* 0.04 (5)

* Indicates a significant difference ($P < .05$) from rest (by paired "t" test).

2.30 Lactic Acid Infusion

L-lactic acid infusion initiated a moderate acidosis which lasted only 30 minutes. Blood pH_a decreased from a resting value of 7.852± 0.040 (5) to 7.579±0.032 (5) at 0 h, but then increased rapidly to resting levels by 0.5 h (Figure 12). Plasma bicarbonate fell from 5.03±0.29 (5) mM at rest to 2.99±0.20 (5) mM at 0 h, then was partially recovered at 0.5 h only to be depressed at 2 h. Complete recovery occurred at 4 h. The P_{CO_2} fluctuated insignificantly around 2.02±0.22 (5) torr throughout the experiment (Figure 12).

Haemoglobin concentrations increased briefly at 0 h, then slowly decreased because of sampling losses. Mean corpuscular haemoglobin concentration fluctuated insignificantly, illustrating the absence of RBC swelling (Table 12).

The infusion placed equivalent quantities of lactate and protons in the blood space; at 0 h lactate concentration was 3.06± 0.41 (5) mEq L^{-1} while ΔH^+ was 3.39±0.51 (5) mEq L^{-1}. Following the infusion from 0 h - 1 h, the rates of ΔH^+ and ΔLa^- removal were not significantly different (Table 13). Between 1 h - 2 h, lactate was removed at a faster rate, but this is of little consequence as the rates were very small, less than 10 μEq L^{-1} min^{-1}. From the experiment it becomes apparent that the descrepancy between H^+ and La^- levels in the blood of recovering flathead sole cannot be explained through the preferential removal of lactate.

The lactate space in flathead sole has been calculated to be 624±64 ml Kg^{-1}.

Figure 12

Blood acid-base status (pH_a, $[HCO_3^-]_p$ and Pa_{CO_2})($\overline{X}$ ± 1 SEM; n = 5) prior to and following an L-lactic acid infusion in flathead sole.

R = rest; bar = indicates a 15 minute infusion and mixing period; 0h = immediately post infusion and mixing; 2,4h etc. = time from end of infusion in hours.

(*) indicates a significant difference (P < .05) from rest (by paired "t" test).

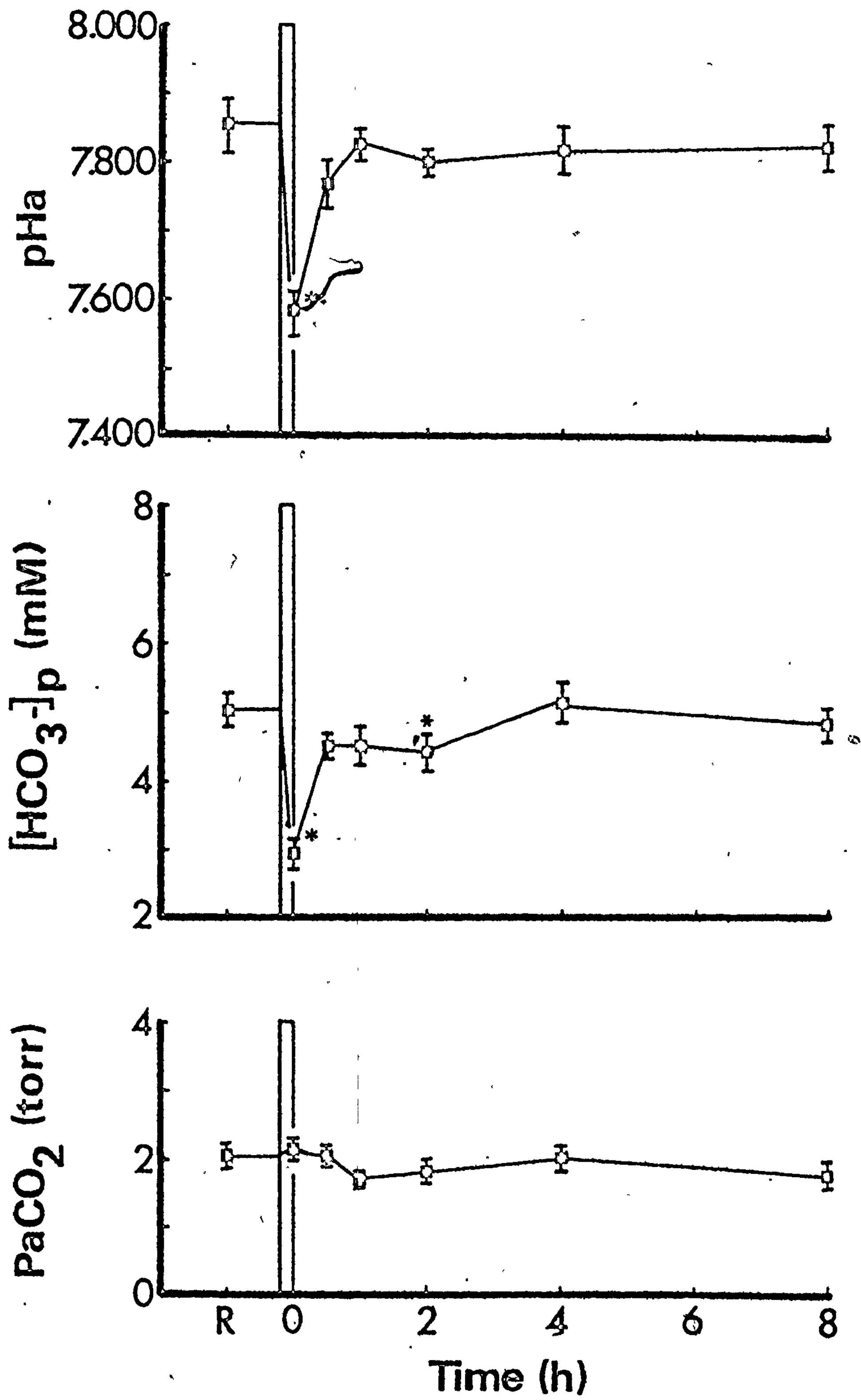

8.000
7.800
7.600
7.400
pHa
8
6
4
2
[HCO3-]p (mM)
4
2
0
PaCO2 (torr)
R
0
2
4
6
8
Time (h)

TABLE 12. Changes in haemoglobin level and mean corpuscular haemoglobin concentration ($\overline{X} \pm 1$ SEM; n = 5) during recovery from a L-lactic acid infusion in flathead sole. 0 h = immediately post-exercise; 0.5 h, 1.0 h etc = recovery times in hours

	REST	0	0.5	1.0	2.0	4.0	8.0
Haemoglobin ($g\ 100\ ml^{-1}$)	2.69 ±0.30	3.32* ±0.21	2.54 ±0.49	2.11 ±0.43	1.80* ±0.33	1.61* ±0.40	1.21* ±0.32
Mean Corpuscular Haemoglobin Concentration ($g\ ml^{-1}$)	0.21 ±0.01	0.22 ±0.01	0.21 ±0.01	0.20 ±0.02	0.21 ±0.01	0.23 ±0.03	0.19 ±0.03

* Indicates a significant difference (P < .05) from rest (by paired "t" test).

TABLE 13. A comparison of the rates ($\overline{X} \pm 1$ SEM; n = 5) of removal of lactate anions and metabolic protons from the blood of the flathead sole during the first 2 h after an L-lactic acid infusion.

TIME	LACTATE $\mu mol\ L^{-1}\ min^{-1}$	PROTONS $\mu mol\ L^{-1}\ min^{-1}$	SIGNIFICANCE LEVEL
0 h - 0.5 h	54.3 ± 10.0	87.9 ± 17.7	n.s.
0.5 h - 1.0 h	18.5 ± 3.7	4.7 ± 6.7	n.s.
1.0 h - 2.0 h	8.9 ± 1.0	-1.6 ± 2.5	$p < .05$

* Indicate significant difference ($P < .05$, by paired "t" test).

IV. DISCUSSION

1. Recovery from Exercise in Rainbow trout

The initial discussion will be devoted to the recovery physiology of the rainbow trout. The reasons of this are two fold: firstly more extensive and detailed experimentation has been performed on this animal in this thesis; and secondly, due to the fact that the rainbow trout is a much studied animal, a considerable body of information is available on its physiology. In a subsequent chapter, the various theories developed for the recovering trout, will be applied to the flathead sole and thereby offer some insight into its physiology during recovery.

As mentioned in the introduction, severe exercise places tremendous demands on the ATP producing machinery of muscle cells. The end products of glycolysis and ATP hydrolysis are lactate and protons. Both species can accumulate in the muscle and blood and may severely disrupt normal cellular function. To regain homeostasis during recovery, these end products must be dealt with either through biochemical or physiological processes. To offer some insight into these complex events, it is necessary to divide recovery into three areas: 1) the production and utilization of lactate by the muscle and blood; 2) the production and utilization of protons in blood; 3) the possible controlling mechanism(s) of lactate movement between body compartments. It should be emphasized that these three

processes are related and have been separated in this context simply to clarify the discussion.

2. Lactate Production and Utilization

The white axial musculature of fish is the body compartment in which lactate is generated and accumulates. Following exercise in the rainbow trout, muscle lactate levels exceed 40 mmol Kg^{-1} (Figure 5). Although this increase in muscle lactate has been observed previously in salmonids (Black, Connor, Lam and Chiu, 1962; Stevens and Black, 1966) and correlated with glycogen depletion, the metabolic fate of lactate remains unknown. It should be noted here that Black's studies employed cardiac puncture for blood sampling, which would no doubt stress the fish and which prevented repetitive blood sampling from the same fish. Muscle samples were taken by excision, a process which is slow because of the time required to cut out and freeze a relatively large muscle sample. In the present study, the fish were fitted with indwelling catheters which permitted repetitive blood sampling with an absolute minimum of disturbance. Furthermore the muscle biopsy technique employed in this study was very rapid (< 5 sec) and produced a small muscle sample (∿ 100 mg) which froze readily. The use of these techniques caused minimal stress to the fish and would reduce error due to sampling artifacts.

Muscle lactate formed during exercise has two possible fates: it may be exported into the blood for use by other tissues and/or it may remain in the myotome to be oxidized or converted to other substrates.

In fish, lactate accumulation in the blood is an extremely slow process (cf. Figure 3, 5), often reaching maximum levels some 2-4 h after exercise. However, simply measuring blood concentrations *in vivo* gives no reliable estimate of lactate flux through the blood space. For example, a rapid release of lactate from muscle and an equally rapid uptake by other tissues is indistinguishable from a slow release rate and reduced uptake. This makes the quantitation of lactate efflux difficult to assess *in vivo*. To circumvent this problem the *in vitro* isolated, perfused trunk preparation was developed which closely approximated *in vivo* conditions. As the perfusate made only a single pass through the muscle and was not modified by other organs, it was possible to determine the rate of lactate release or net efflux. In such preparations, net lactate efflux rates during the first hour following exercise have been observed at 14.7-26.5 μmol Kg^{-1} min^{-1} (Table 8). If these values are compared to the first hour of recovery *in vivo*, where muscle lactate decreases by 13.29 mmol Kg^{-1} or at a rate of 221.5 μmol Kg^{-1} min^{-1} (cf. Figure 5), it becomes clear that the measured lactate efflux *in vitro* could account for only ≈10% of the measured muscle lactate disappearance *in vivo*. This *in vitro* lactate efflux rates seem valid because if these rates are calculated to accumulate in the blood space for 30 min (Table 14), the calculated concentration is very close to measured *in vivo* lactate levels at 0.5 h into recovery. In fact, even if the *in vitro* lactate efflux estimates were in error by as much as 100%, the conclusion remains that and most of the lactate remains in the muscle and that considerable

TABLE 14. A comparison of the measured blood concentrations of lactate and metabolic protons in rainbow trout *in vivo* 0.5 h after severe exercise with values calculated from the measured net efflux rates of lactate and protons from the perfused trunk preparations *in vitro*. It is assumed that blood volume is 55.8 ml Kg^{-1} (Milligan and Wood, 1981) and that there is no excretion or utilization of either species from the blood over the first 30 min post-exercise.

	In Vitro				*In Vivo*
	Measured Efflux rate ($\mu mol\ min^{-1}\ Kg^{-1}$)	Time (min)	Blood Volume ($L\ Kg^{-1}$)	Concentration ($mmol\ L^{-1}$)	Concentration ($mmol\ L^{-1}$)
Lactate	19.70 ± 6.0	30	0.0558	10.59 ± 3.23	11.27 ± 0.71
Protons	6.13 ± 1.5	30	0.0558	3.30 ± 0.81	6.90 ± 0.57

lactate utilization seems to occur in the muscle itself.

This conclusion is not obvious from Figure 5, where it seems that much of the lactate is moving into the blood forming an equilibrium. The confusion here is due to the relative sizes of the blood and muscle compartments. In trout the intracellular fluid volume is 459.1 ml Kg^{-1} while the blood volume is only 55.8 ml Kg^{-1} (Milligan and Wood, 1981). This is why when only 10% of the muscle lactate moves into the blood, large elevations in blood lactate are noted.

It should be mentioned that lactate retention by muscle is not a unique piscine phenomenon,as exercised human leg muscles have been shown to release only about 10% of their total lactate load (Hermansen and Vaage, 1977).

Lactate retention in muscle has certain ramifications. For example, the number of osmotically active particles present in the muscle cells increases as 1 glucose subunit is cleaved into 2 lactate molecules (Introduction, Eq. 4). The net result is a water shift from the blood into the muscle cells. This contracts the blood space and increases the concentration of all blood constituents (Figure 4, Table 3, 4). Milligan and Wood (1981) in trout, have shown that plasma protein is an excellent index of haemoconcentration due to water shifts in and out of the blood space. In trout following exercise, plasma protein levels do infact increase, despite losses due to blood sampling (Table 3). Similar haemoconcentration following exercise has been reported in man (Bergstrom et al., 1971).

The metabolic fate of blood lactate is either oxidation or gluconeogenesis in one of the aerobic tissues. Trout heart has been shown to oxidize lactate in preference to glucose (Lanctin, McMorran and Driedzic, 1980) while Bilinski and Jonas (1972) have demonstrated a high capacity for lactate oxidation in the liver, gills, kidney and red muscle of trout. Evidence also suggests that trout liver cells use lactate for gluconeogenesis (Walton and Cowey, 1979).

As lactate entry into the blood must follow the constraints of electroneutality, its efflux must occur in conjunction with a cation or by exchange for an anion from the blood. In recovering trout, evidence suggests that chloride, the major anionic species in the plasma, is in fact exchanging for the lactate. This assumption is based on the fact that during recovery, plasma chloride does not behave like plasma sodium (Figure 4). Sodium is haemoconcentrated at 0 h and returns to resting levels by 4 h, while chloride is haemoconcentrated less at 0 h, and has fallen well below resting levels by 4 h. This apparent disappearance of 10 mEq L^{-1} Cl^- at 4 h, at a time when blood lactate concentration is elevated by approximately 10 mEq L^{-1}, may indicate a lactate/Cl^- exchange (cf. Figures 3, 4).

Since it seems that insufficient lactate appears in the blood to explain the disappearance of muscle lactate during recovery the possibility of direct utilization within the muscle must be seriously considered. Oxidation of lactate by fish white muscle would be a slow process as this tissue is not geared for extensive aerobic carbon flux (Bilinski and Jonas, 1972). Nevertheless, any oxidation of lactate

would reduce the muscle lactate and proton load and would produce ATP for the recovering muscle. This energy along with ATP from glucose and possibly fat oxidation could be used during glyconeogenesis, which is the other major route of lactate utilization within the muscle (Figure 15).

Although the glyconeogenic capacity of white muscle has been at the center of much controversy (Krebs and Woodford, 1965 vs McLane and Holloszy, 1979; Batty and Wardle, 1979 vs Opie and Newsholme, 1967), two independent lines of evidence suggest its feasibility. Firstly, the essential glyconeogenic enzymes required to by-pass the non-reversible reactions of glycolysis are present with sufficient activity to permit glycogen synthesis. Secondly, radio-labelled and cold lactate can be incorporated into glycogen using *in vivo* and *in vitro* systems.

Glyconeogenesis cannot occur as a simple reversal of glycolysis due to thermodynamic and kinetic considerations (Lehninger, 1970). If glyconeogenesis is to occur *via* typical mammalian biosynthetic pathways, certain enzymes are required. In Table 15, one plausible pathway of lactate utilization in glyconeogenesis is presented along with the muscle tissues in which key enzymes have been found. From this table one can conclude that the white, fast-twitch muscles of a variety of vertebrates, including fish, possess the necessary enzymatic framework in which glycogen synthesis from lactate could occur. Whether lactate utilization actually occurs *via* this pathway and at what rate cannot be predicted by measuring the activities of enzymes.

TABLE 15. Evidence for the Presence of Enzymes Required for Glyconeogenesis in White Muscles

```
lactate
  ↓       1
pyruvate  →  malate
     `4.    ↓ 2
PEP   ←  oxaloacetate
  ↓    3
2PG  →  3PG
         ↓
        DPG
         ↓
DHAP ↔ GAP
  ↓     5
 FDP  →  F6P
          ↓
  G1P  ←  G6P
   ↓
  UDPG  ⇄  glycogen
         6
```

PEP = phosphoenol pyruvate
2PG = 2-phosphoglycerate
3PG = 3-phosphoglycerate
DPG = 1,3-diphosphoglycerate
GAP = glyceraldehyde-3-phosphate
FDP = fructose-1,6-diphosphate
F6P = fructose-6-phosphate
G6P = glucose-6-phosphate
UDPG = uridine diphosphate glucose

ENZYME	WHITE MUSCLE TISSUE	AUTHOR
1. malic enzyme	- teleost axial	Walesby and Johnston (1979)
2. malate dehydrogenase	- frog thigh, hen leg	Opie and Newsholme (1967)
	- rat plantaris	McLane and Holloszy (1979)
3. phosphoenolpyruvate-carboxykinase (PEPCK)	- lamprey axial	Lyzlova and Berhbinskaya (1976)
	- dogfish axial	Crabtree et al. (1972)
	- frog thigh, hen leg	Opie and Newsholme (1967)
	- frog sartorius	Connett (1979)
4. pyruvate carboxylase	- trace in dogfish, axial and frog sartorius	Crabtree et al. (1972)
5. fructose-1,6-bisphospatase	- teleost axial	Batty and Wardle (1978)
	- dogfish axial	Crabtree et al. (1972)
	- leg muscles of frog, rabbit, mouse	Krebs and Woodford (1965) Opie and Newsholme
	- rat plantaris	McLane and Holloszy (1979)
6. glycogen synthetase	- rat superficial vastus lateralis	Conlee et al. (1978)
	- guinea pig superficial quadriceps femoris	Stubbs et al. (1965)

The rate at which radio-labelled and "cold" lactate is incorporated into glycogen can be used to characterize the actual glyconeogenic flux through a tissue. Such experiments with isolated frog and rat muscles have shown that lactate can in fact be used as a substrate for glyconeogenesis (Table 16). The only direct evidence for lactate incorporation into glycogen in fish white muscle is the work of Batty and Wardle (1979). When [1-^{14}C] lactate was injected into the muscles of an exhausted plaice, it appeared to be taken up against a concentration gradient into the cells, and some of the radio-label was found as glycogen. Of additional interest was that [^{3}H] glucose turnover rates from blood were insufficient to account for the white muscle glycogen synthesis rates observed.

Since it can be demonstrated that [^{14}C] lactate is incorporated into glycogen in several fast-twitch, white muscles, both _in vivo_ and _in vitro_, the real possibility of extensive lactate utilization _in situ_ as a substrate for glycogen repletion in fish white muscle exists.

Such a system would be of considerable adaptive significance as lactate and protons would not flux through the blood space causing osmotic and acid-base disturbances, only to be taken up again for glycogen resynthesis. The presence of retention mechanisms, would make large amounts of H^+ and lactate available for glyconeogenesis within the recovering muscle cell. The findings of Batty and Wardle (1979) that blood glucose turnover is too low to provide carbon for glycogen resyn-

TABLE 16. Studies in which lactate has seen shown to be a substrate for glycogen synthesis in white muscle.

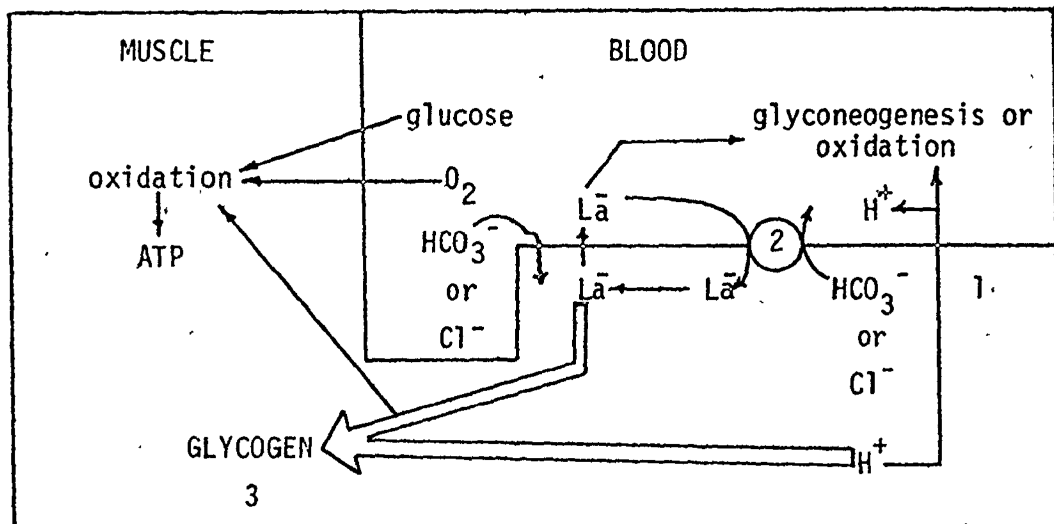
White Muscle Tissue	Precursor	Author
frog sartorius	incubated with cold lactate	Bendall and Taylor (1970)
frog sartorius	superfused with [U-^{14}C] lactate	Connett (1979)
rat plantaris	perfused with [U-^{14}C] lactate	McLane and Holloszy (1979)
rat external oblique (abdomen)	incubated with cold lactate	Bar and Blancher (1965)
fish axial	i.m. injection *in vivo* [1-^{14}C] lactate	Batty and Wardle (1979)

thesis supports this idea. Another piece of evidence is that amino acids and free fatty acids are not used by isolated, perfused, trout trunks for glyconeogenesis but are oxidized readily to CO_2 by this tissue (Moen and Klungsoyr, 1981).

One unexplained detail is the length of time required for lactate removal and glycogen resynthesis in trout white muscle. Black et al. (1962) have shown that muscle glycogen has only recovered by 50% after 24 h of recovery. From the above discussion it would seem that the enzymes required for glyconeogenesis are present and that lactate can be used as a substrate, so why is this process so slow? Once again the answer may be found in the isolated perfused trunk experiments. In this preparation, muscle lactate remains high and relatively constant during 3 h of perfusion (cf. Table 7). The simplest explanation for this observation is that insufficent energy (ATP) is available for the conversion of lactate to glycogen. As the perfusate contained no glucose or other metabolic fuel, only the ATP from oxidation of endogenous lactate would be available. However, during *in vivo* recovery, where muscle lactate is used for glycogen synthesis blood glucose and other metabolic fuels could be taken up and oxidized (Figure 13). As already mentioned, white muscle is not well suited for oxidation as it is hampered by reduced perfusion and subsequent reduced O_2 delivery as well as low titres of aerobic enzymes. These factors limit ATP production and consequently glyconeogenesis would be restricted by ATP availability.

Figure 13

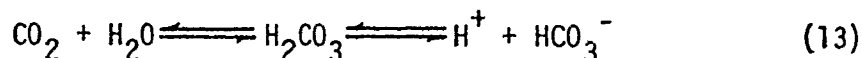
A theoretical model of lactate and proton movements and utilization in fish white muscle during recovery from exercise.



1. Proton release from muscle is modulated by pH and/or P_{CO_2}
2. Active inward lactate transport (SITS sensitive)
3. Glycogen synthesis is the major fate of muscle lactate

3. Proton Production and Utilization

Maintenance of pH between well defined limits is of paramount importance to living organisms. This is because excessive protons can alter the charge characteristics of proteins. For example, during acidosis, the oxygen affinity (Bohr effect) and carrying capacity (Root effect) are reduced when the haemoglobin molecule is allosterically altered (Riggs, 1970). Severe exercise in rainbow trout causes a mixed respiratory and metabolic acidosis (Table 17). The respiratory component is at its maximum at 0 h, contributing to almost 50% of the total acidosis. A respiratory acidosis is formed when CO_2 levels increase in the blood and combine with water to form protons and bicarbonate.



$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad (13)$$

During exercise CO_2 accumulates in the blood as aerobic tissues use more oxygen (Brett, 1972; Jones and Randall, 1978) and produce CO_2. Further, metabolic protons from glycolytic metabolism can combine with HCO_3^- (reversal of Eq. 13), and increase CO_2 levels. In addition to increased production of CO_2 the efficiency of CO_2 excretion is probably reduced during exercise. This is thought to be caused by increased cardiac output during exercise (Jones and Randall, 1978) which abbreviates the blood transit time in the gills, which in turn may prevent adequate HCO_3^- dehydration and CO_2 elimination by the gill (Cameron and Polhemus, 1974). During recovery, P_{CO_2} is rapidly corrected (Figure 2) and the respiratory component of the acidosis is greatly reduced (Table 17).

TABLE 17. The Percent Contribution of Respiratory and Metabolic Components of Acidosis, Induced Through Exercise or L-lactic Acid Infusion in the Rainbow Trout and Flathead Sole.

Rainbow Trout

Severe Exercise

Recovery Time (h)	Δ pH From Rest	% Δ pH Respiratory	% Δ pH Metabolic
0	0.510	48%	52%
0.5	0.398	20%	80%
1.0	0.404	23%	77%
2.0	0.319	10%	90%
4.0	0.184	25%	75%
8.0	Corrected	--	--

L-lactic Acid Infusion

Time After Infusion (h)	Δ pH From Rest	% Δ pH Respiratory	% Δ pH Metabolic
0	0.383	16%	84%
0.5	0.059	5%	95%
1.0	Corrected	--	--

Flathead Sole

Severe Exercise

Recovery Time (h)	Δ pH From Rest	% Δ pH Respiratory	% Δ pH Metabolic
0	0.451	50%	50%
0.5	0.327	25%	75%
1.0	0.230	13%	87%
2.0	0.225	22%	78%
4.0	0.175	25%	75%
8.0	Corrected	--	--

Moderate Exercise

Recovery Time (h)	Δ pH From Rest	% Δ pH Respiratory	% Δ pH Metabolic
0	0.243	67%	33%
0.5	0.152	53%	47%
1.0	0.111	51%	49%
2.0	Corrected	--	--

L-lactic Acid Infusion

Time After Infusion (h)	From Rest	% Δ pH Respiratory	% Δ pH Metabolic
0	0.273	11%	89%
0.5	Corrected	--	--

- Analysis of changes in blood pH as per Davenport (1974), Wood et al. (1977)

The metabolic component of blood acidosis in recovering trout reaches its maximum at 1 h into recovery (Table 17), by which time the respiratory component has almost disappeared. Wood et al. (1977) have postulated that this temporal separation is present so as not to overwhelm the buffering capacity of fish blood, which is low in comparison to mammals (Albers, 1970). The major origin of blood metabolic acidosis is probably the white muscle. During severe exercise the coupled process of glycolysis and ATP hydrolysis (Eq. 4 and 5) produces protons at a rapid rate. The actual intracellular pH of exhausted muscles is difficult to assess, however, as current chemical probes, DMO (5.5-dimethyl-2,4-oxazolidinedione) for example, require extended equilibration periods. Cameron (1980) has determined that DMO mixing and equilibration during in vivo experiments with catfish approaches two hours. This renders this technique unsuitable for measuring dynamic systems as in recovering muscle. No doubt the future use of pH sensitive microelectrodes or ^{31}P nuclear magnetic resonance spectroscopy will add much to what is now conjecture (Roos and Boron, 1981). Trout muscle intracellular buffering capacity (β) is considerable: 60 slykes (Castellini and Somero, 1981). Thus 60 mEq Kg^{-1} acid would reduce intracellular pH (pH_i) by 1 pH unit in the absence of a P_{CO_2} change and biochemical processes. Of course intracellular biochemistry is a complex melange of reactions, many of which use or produce protons. For example, following exercise at 0 h creatine phosphate has been degraded to creatine (Introduction, Eq. 1) which maintains ATP levels and absorbs protons. However, subsequent ATP hydrolysis produces

protons. The net result is no change in proton load. Other reactions such as the coupling of glycolysis and ATP hydrolysis produces many protons. In fact, at 0 h after exercise, muscle lactate levels and probably proton load exceed 40 mEq Kg^{-1}. If this value is divided by the muscle buffer capacity (60 mEq Kg^{-1}), one could predict a drop in pH_i of 0.67 pH units. Estimates via indirect calculation of pHi in human leg muscle following exercise indicates an acidosis of 0.40 pH_i units (Sahlin, Alvestrand, Brandt and Hultman, 1978). As no direct measurements of pHi have been made on exercised muscle it is impossible to accurately assess pHi. One may only assume from indirect calculations, as above, that some significant degree of acidosis occurs in muscle during exercise. Additional evidence that intracellular acidosis occurs during exercise and persists for a long time after exercise in trout is the marked and persistent elevation of plasma potassium (Figure 4). Intracellular acidosis has been shown to correlate well to potassium extrusion (Brown and Goot, 1963; Lade and Brown, 1963). This is in contrast to the time course of changes in sodium, chloride as well as haematocrit and plasma protein (Table 3) which are briefly concentrated at 0 h but recover by 2-4 h in recovery.

Direct pH measurements and metabolic acid load can easily be determined for blood. From such measurements during recovery from exercise, from 0.5 h $\rightarrow$ 12 h, metabolic protons were found to accumulate in the blood to a lesser extent than did lactate (Figure 3). As protons and lactate are produced in equal amounts during exercise, this discrepancy

between blood proton and lactate loads must be a function of: 1) a release effect in which proton movement into the blood is less than lactate and/or 2) a removal mechanism which preferentially extracts protons from the blood.

The existence of a differential release rate for protons and lactate from severely exercised, white muscle has been illustrated with experiments using the isolated perfused trout trunk. When the perfusate used in such experiments closely simulates in vivo post-exercise acidosis (low pH and high P_{CO_2}; Figure 2), the net proton efflux rate is considerably smaller than that for lactate (Table 8, Figure 7). As in the case of lactate efflux, the validity of these in vitro proton release rates become apparent when their accumulation is calculated in the blood space of an intact trout (cf. Table 14). Such calculations for protons yield values which are well below those calculated for lactate and which approximate in vivo proton load at 0.5 h after severe exercise.

Further experiments with the isolated perfused preparation have shown that when perfusate pH and P_{CO_2} levels approximate resting levels (Figure 2), proton efflux increases greatly and is comparable to lactate efflux (Figure 7, Table 8). Therefore, it would seem that extracellular pH and P_{CO_2} have some regulatory function in limiting the efflux of muscle protons. This mechanism may explain the pattern of the discrepancy between H^+ and lactate accumulation in vivo. Immediately after exercise, pH is severely depressed and P_{CO_2} increased, an effect which would reduce proton efflux from the muscle during recovery, while lactate efflux would be unaffected. The result would

be a discrepancy in the blood concentration of these two species. Furthermore, during the short exercise period itself, the pH drop and P_{CO_2} rise are undoubtedly not instantaneous, but probably occur gradually as exercise progresses. Therefore, the inhibition of proton efflux would not be maximal until near the end of exercise so enough protons could efflux into the blood to make the proton load and lactate load comparable at 0 h (Figure 3). Therefore, post-exercise acidosis would ensure development of the discrepancy. Thus, when protons and lactate are found in equal amounts in the blood of recovering fish (eg., 0 h, Figure 3) it would seem to be a function of their respective controlling mechanisms and not a direct relationship between the two species. Another ramification of such a feedback system would be that the respiratory acidosis would always be separated from the metabolic acidosis and overloading of the blood with protons would rarely occur.

The possibility of preferential removal of protons from blood *in vivo* has been examined by means of a L-lactic acid infusion. As this process placed equivalent quantities of protons and lactate in the blood space (see III, 1.30), the existence of a preferential removal mechanism could be detected by a more rapid removal of one species. The results (Table 5) clearly illustrate that no preferential removal mechanism exists, as both lactate and protons were removed from the blood at equal rates. Additional support for this concept of equal removal rates can be drawn from the rate of decrease of blood lactate and protons following exercise (cf. Figure 3). During recovery

from severe exercise, from 2-8 h, the rate of disappearance of ΔLa^- and ΔH^+ were not significantly different at 18.7±4.3 µmol l^{-1} min^{-1} and 21.1±4.0 µmol l^{-1} min^{-1} respectively.

In summary, the discrepancy between lactate and protons in the blood of recovering fish can be explained solely by a release phenomenon in which muscle cells release protons into the blood at a slower rate than lactate when the blood is acidotic and/or has a high P_{CO_2}. The regulating system for proton release seems to be a feedback mechanism which incorporates pH and/or P_{CO_2} as modulators. This mechanism would prevent simultaneous respiratory and metabolic acidosis and would thereby guard the fish from a lethal pH depression. Whether the actual modulators of this system are pH or P_{CO_2} or whether the depression in proton efflux is simply an effect of changes in the transmembrane diffusion gradients for protons requires further elucidation.

4. Regulation of Lactate Movement

The mechanism and control of lactate movement across cell membranes has recently been the subject of intense investigation. The results of this experimentation has determined that two mechanisms exist in which lactate transport may occur: these are the chloride-dependent carrier and the lactate-proton symport mechanism. As noted in Table 18 these mechanisms have many common characteristics, but both possess two unique characteristics. The chloride dependent carrier transports lactate *via* exchange of lactate for HCO_3^- or Cl^-

using the existing Cl^-/HCO_3^- exchange mechanism. This mechanism is nearly completely inhibited by SITS. The lactate symport mechanism seems to be exquisitely sensitive to pH and characteristically immune from SITS inhibition (Table 18). It should be mentioned that the lactate-proton symport is indistinguishable from a lactate/OH^- exchange or antiport mechanism.

Results from isolated perfused muscle preparations of dog gastrocnemius (Hirche, Hombach, Langohr, Wacker and Busse, 1975) and frog sartorius (Mainwood and Worsley-Brown, 1975), have illustrated that lactate transport out of muscle cells exhibits characteristics from both the above transport mechanisms. Increased lactate permeation can be correlated with elevated [HCO_3^-] in the external media, which would make a La^-/HCO_3^- exchange via the chloride dependent mechanism more favorable. Furthermore, lactate efflux is increased when extracellular pH is increased; such increases in transmembrane H^+ gradients causing lactate movement to increase are consistent with a lactate-proton symport mechanisms.

This is not the case with the isolated perfused rainbow trout trunk. In fact, lactate transport in this tissue exhibits few characteristics which are consistent with either the chloride-dependent or lactate-proton symport mechanisms. In the perfused trunk experiments, SITS did not abolish lactate transport, but actually enhanced it by about 300% (Table 8, Figure 7). In addition, a large reduction in external Cl^- levels from 133 mEq L^{-1} to 1.33 mEq L^{-1} did not alter lactate transport (Results, Sec. 1.40). These characteristics are not those

TABLE 18. Mechanism of Lactate Transport Across Membrane

Chloride-Dependent Transport (erythrocytes)	Common Characteristics	Lactate-Proton Symport (erythrocyte and Ehrlich ascites tumor cells)
1) lactate and other monocarboxylates transported by Cl^-/HCO_3^- exchange mechanism. Efflux of carboxylate linked with Cl^- or HCO_3^- as counter-ion.[2]	- carrier mediated[5,3,2,9,4]	1) exquistively sensitive to transmembrane H^+ gradients[3,5,7]
2) SITS (10^{-5}M) severely inhibits Cl^- and other anion transport including lactate. Mechanism of SITS inhibition could be: i) competition with anions for the carrier sites by negatively charged sulfonic acid groups or ii) steric blocking of the transport through covalent or hydrogen bonding to the carrier)[2,6,8,1]	- temperature sensitive[5,3,6,7]	2) SITS and DIDS does not block lactate transport in this system[2,5]
	- exhibit saturation kinetics[3,2]	
	- inhibited by non-transportable analogues, α-cyano-4-hydroxy-cinnamate or iBCLA (isobutyl-carbonyl lactyl anhydride)[3,2,9]	

1. Cabantchik and Rothstein - 1972
2. Halestrap - 1976
3. Spencer and Lehninger - 1976
4. Villereal and Levinson - 1976
5. Dubinsky and Racker - 1978
6. Haswell, Zeidler and Kim - 1978
7. Belt, Thomas, Buchsbaum and Racker - 1979
8. Obaid, Critz and Crandall - 1979
9. Johnson, Belt, Dubinsky, Zimniak and Racker - 1980

of the chloride dependent transporter. Furthermore, results from the perfused trunk experiments also conflict with the lactate-proton symport mechanism. Firstly, large reductions in external pH do not affect lactate efflux from the trunk and secondly, lactate movement may be independent of accompanying protons. Both of these results are in direct opposition to the symport concept which is very sensitive to changes in transmembrane H^+ gradients and possess by definition a 1:1 stoichiometry between protons and lactate. From these results, lactate movement across white muscle cell membranes in trout does not appear to follow either the Cl^- dependent transporter or the lactate-proton symport mechanism. However, before these mechanisms are ruled out unequivocally in the trout context, further experiments are required to evaluate the effects of such parameters as temperature dependence, saturation kinetics, external [HCO_3^-] or inhibition with α-cyano-4-hydroxycinnamate.

A model which does explain the lactate and proton efflux characteristics of trout muscle, involves the <u>active transport of lactate into the muscle cells</u> (Figure 13). This model is consistent with all the results from the isolated perfused trunk work to date. Following exercise, muscle lactate and proton loads would be large (Figure 5). This model proposes that some lactate moves down its electrochemical and concentration gradient into the interstitial space, with either Cl^- and/or HCO_3^- acting as the counter ion. However, much of this lactate is then thought to be actively transported back into the muscle cell against its gradients, again with Cl^- and/or HCO_3^- exchange.

The counter ion used during lactate exchange may partially determine whether a "proton" will move with lactate. If chloride is the counter-ion, lactate would move without a proton, while a lactate/HCO_3^- exchange or a lactate/OH^- exchange would give the 1:1 stoichiometry of "lactic acid" efflux, as HCO_3^- or OH^- influx is functionally equivalent to H^+ efflux.

Evidence for the presence of an active lactate transport mechanism in other tissues is not readily available. In fact, research into the active transport of lactate has been scarce indeed. Craig (1946) illustrated that tubular reabsorption of lactate occurred in the dog kidney. This reabsorption mechanism was independent from glucose transport, exhibited saturation kinetics and dealt with both D and L isomers of lactate. More recent work with rainbow trout by Kobayashi and Wood (1980) has demonstrated that lactate reabsorption against a concentration gradient seems to occur in the kidney of this animal. Uptake of lactate by tissues other than kidney has been demonstrated using isolated rat hepatocytes (Halestrap and Mourao, from Halestrap, 1976). These workers observed that 0.5 x 10^{-4}M SITS severely impaired the utilization of lactate for glyconegenesis. In this case, SITS may be inhibiting the uptake of lactate and consequently affecting lactate utilization.

From this evidence, the active transport of lactate thought to be involved in trout muscle would seem to be at least plausible. Further, the possible similarities between the isolated rat hepatocytes

and trout muscle with regard to their sensitivity to SITS deserves fuller investigation. The apparent paradoxical enhancement of lactate release by SITS seen in isolated perfused trout trunks, can be simply explained by SITS inhibition of the active lactate pump. With uptake blocked, lactate efflux could continue unabated resulting in an increase in net efflux (cf. Figure 14).

The presence of an active transporter of lactate into the cells is consistent with the concept that the major fate of muscle lactate is not release into the blood, but resynthesis *in situ* to glycogen. An active transport mechanism would counteract the passive losses of lactate from the muscle and thereby keep the lactate carbon inside the cell for glycogen synthesis.

Lactate retention and utilization *in situ* obviates the need to ship protons into the blood where they could cause harmful disturbances. The protons can be retained in the muscle and removed along with lactate during *in situ* glyconeogenesis.

5. Summary

Severe exercise in rainbow trout causes a mixed respiratory and metabolic acidosis in the blood and large increases in muscle lactate. The apparent proton release from the muscle is relatively small and modulated by extracellular pH and/or P_{CO_2}. The majority of the protons are thought to remain in the muscle and be reutilized in biochemical reactions. Lactate movement is not necessarily linked to proton movement; further it is not affected by extracellular pH, chloride levels, adrenaline or perfusion limitations. In addition,

lactate efflux is paradoxically enhanced by the anion transport inhibitor SITS, which has led to the hypothesis that a carrier exists which transports lactate into the muscle cells. As the major fate of muscle lactate is thought to be glyconeogenesis, the active transport of lactate into the cell would maintain intracellular lactate levels and minimize net lactate efflux into blood, a process crucial for extensive lactate utilization *in situ*.

V. DISCUSSION

1. Recovery from Exercise in Flathead Sole

Interest in the exercise performance and physiology of flatfish has grown only recently (Wood *et al.*, 1977; Wardle, 1978; Batty and Wardle, 1979; Priede and Holliday, 1980). It is now known that flatfish in general are capable of only brief bouts of activity of moderate velocities (3 L sec^{-1}; Priede and Holliday, 1980). From this observation one could hypothesize that since these fish swim more slowly than trout, their ATP expenditure would be substantially less and that glycolytic activity would be correspondingly less intense. Therefore, one would predict that a severely exercised flatfish would generate less lactate and fewer protons than would a severely exercised trout.

2. Lactate Production and Utilization

Lactate accumulation in the white muscle of the flathead sole reaches only 15 mmol Kg^{-1} following severe exercise (Figure 11). This muscle lactate level remained elevated above resting levels for at least 12 h, while the trout muscle returned to resting levels by 6 h (Figure 5). The fate of muscle lactate in flathead sole is difficult to assess due to the paucity of information in the literature and limited experimental evidence in this study. Whatever the mechanism of lactate removal, it seems to be a very slow process. Circumstantial evidence, including the prolonged presence of elevated muscle lactate levels (Figure 11) and the exceedingly low blood levels (Figures 10,11)

suggest in situ utilization. The work of Batty and Wardle (1979) is consistent with this concept and suggests that glyconeogenesis using lactate is important in glycogen repletion. Additional evidence is the temporal relationship between muscle lactate removal and glycogen synthesis. At 8 h into recovery following severe exercise, muscle glycogen is 50-80% repleted (Wardle, 1978) while muscle lactate has fallen 50-60% (Figure 11). One argument against in situ lactate utilization is the apparent absence of PEPCK activity in plaice white muscle (Johnson and Moon, 1979). Perhaps further investigation will yield a replacement pathway or find activity of this enzyme with some alteration in the assay.

Blood lactate concentrations in recovering flathead sole are very small, always below 2 mM. This observation is consistent with observations made in plaice (Wardle, 1978) and in starry flounder (Wood et al., 1977). Following the model (Figure 13) of lactate movement in the trout after exercise, a low blood lactate load would be indicative of either a lower "leakage" of lactate from the muscle, or an effect produced by a very efficient lactate transporter. An interesting observation in support of the latter idea is the size of the lactate space in flathead sole. The lactate space is the apparent volume of distribution of infused lactate when it is distributed at blood concentration in the entire animal. Lactic acid infusion experiments with rainbow trout yield volumes of 214±16 ml Kg^{-1} in this work and 198±13 ml Kg^{-1} reported by Kobayashi and Wood (1980) (cf. Results, Sec. 1.30). As extracellular fluid volume in rainbow trout is 273.5± 8.8 ml Kg^{-1} (Milligan and Wood, 1981). It would appear that the in-

fused lactate remained in the extracellular fluid. However, lactate space calculations for the sole following lactic acid infusion were 624±64 ml Kg (cf. Results, Sec. 2.30), this distribution volume is much larger than the extracellular space and indicates that some tissue, possibly the white muscle cells are rapidly taking up lactate. An efficient lactate transport system would in fact create such a large lactate space. As in the case of the trout, active transport of lactate into the muscle is consistent with the concept of in situ lactate utilization for glyconeogenesis.

The only worker who has experimentally manipulated lactate release in flatfish in Wardle (1978). Administration of the β-adrenergic antagonist propanolol into exercised plaice resulted in a dramatic increase in blood lactate and subsequent death, but only in about 50% of the animals tested. From this Wardle concluded that muscular lactate retention has a component sensitive to circulating catecholamines. This is interesting, but in light of the fact that catecholamines have no effect on lactate release in isolated perfused trout (cf. Results, Sec. 1.40) and that the release of lactate by propanolol in plaice did not occur in all animals which Wardle (1978) tested suggests that perhaps propanolol is exerting some non-specific effect. Propanolol has many non-specific effects in mammals (Gilman, Goodman and Gilman, 1980), some of which are also found in fish, for example, interaction with α-adrenergic receptors in trout (Wood and Shelton, 1975; Wood, 1976) and a local anaesthetic action on trout cardiac tissue (Wood and Shelton, 1980). Therefore, it is conceivable that propanolol may impose some non-specific inhibition on the lactate transport mechanism. If this inhibition is analogous to SITS inhibition

as seen in trout, then lactate and proton efflux would dramatically increase and could kill the fish. Obviously, further experimentation is required to test the above hypothesis and more fully elucidate the lactate retention mechanism.

3. Proton Production, Utilization and Distribution

During recovery from moderate exercise the acidosis at 0 h is only 0.243 pH units (Figure 9). This acidosis is for the most part of respiratory origin (Table 17) at 0 h which continues to contribute over 50% of the acidosis until recovery of acid-base status at 2 h. Recovery from exhausting exercise produces a much more substantial acidosis (Figure 8) of 0.451 pH units. The magnitude and mixed nature of this acidosis are comparable with that seen in the trout (Figure 2, Table 17). The respiratory component of this acidosis accounts for 50% of the pH depression at 0 h, but by 1 h its contribution is minimal. The metabolic component is much larger following exhaustive exercise. It reaches a maximum between 0.5 - 1 h into recovery when it is contributing 75%-87% of these acidosis. The addition of this large amount of metabolically produced protons explains why recovery is so prolonged after exhausting exercise and 2 h after moderate exercise. Metabolic proton levels are substantially higher than lactate levels (Figure 10). However, the absolute blood proton load after exhausting exercise is smaller than that in the trout (cf. Figure 3). This difference in blood proton load probably reflects differences in the muscle proton loading during exercise between the two fish. Since lactate and protons are produced in coupled reactions (Introduction, Eq. 4 and 5), muscle lactate

concentration can be used as an estimate of muscle proton loading. In trout, immediately after severe exercise, the maximum muscle proton load was 45 mmol Kg^{-1} (cf. Figure 5). The maximum blood proton load was 7.5 mmol L^{-1} (Figure 3) at 1 h. A ratio of these values (muscle proton load/blood proton load) can be calculated to be 6.0 in the recovering trout. In recovering sole, the maximum muscle proton load was 15 mmol Kg^{-1} (Figure 11). If the trout muscle/blood proton loading ratio is applied to the sole, a calculated maximum blood proton load was 2.5 mmol L^{-1}. However, actual measurements are higher, near 4 mmol L^{-1} (Figure 10). In addition, if the alkalinizing effect of 1 mM plasma ammonia seen in sole blood (see Dicussion V. 4) is taken into consideration, the maximum blood proton load would approach 5 mmol L^{-1}. This means that although the trout release a larger absolute amount of protons into the blood, the sole releases a larger fraction of its muscle protons into the blood.

Flatfish in general, compensate for blood proton loading and the resultant effects on oxygen transport in three ways: 1) following exercise the sole remains motionless in the sand and thereby reduces oxygen consumption; 2) flatfish maintain elevated resting cardiac output (Wood et al., 1979) and 3) flatfish have reduced the sensitivity of vital organs to acidosis, for example the heart (Gesser and Poupa, 1979).

The accumulation pattern of lactate and protons in the blood of recovering flathead sole was exactly opposite to that seen in trout (cf. Figure 3, 10). In the sole, the blood proton load is always

greater than the lactate load during the first 8 h of recovery from severe exercise. To determine if this discrepancy could be caused by a preferential removal mechanism, L-lactic acid was infused into resting sole. This process placed equivalent quantities of protons and lactate in the blood space (Results, Sec. 1.30, 2.30) and allowed the measurement of the subsequent removal rates of each species. In sole, the removal rates were not significantly different (Table 13). Therefore, it is unlikely that the observed discrepancy between lactate and protons in the blood of recovering sole could be due to a preferential lactate removal or sequestering. Another process which could be responsible for the discrepancy would be a release mechanism, in which protons were released faster from the muscle cells than was lactate. Unfortunately, the presence of this process remains unknown in sole.

4. Effects of Exercise on Other Blood Constituents

Haemoconcentration did not occur to any great extent during recovery from either moderate or severe exercise in the flathead sole. Although plasma protein was not measured, the absence of consistent increases in haemoglobin concentration (Table 10) or in plasma ions and total osmolality, indicate that little net water movement occurred between body compartments. In fact, since only 15 mmol Kg^{-1} lactate was formed in the muscle, one would not expect much haemoconcentration. Further, since blood lactate levels were always below 2 mEq L^{-1}, the exchange of plasma electrolytes for effluxing lactate would be slight.

Resting blood pyruvate concentrations and the subsequent rise in response to exercise in the flathead sole was very comparable to that observed in trout (cf. Table 4, 11). In both cases blood pyruvate levels were low < .300 mM and were probably increased when muscle pyruvate levels rose during increased glycolytic flux associated with exercise. As pyruvate is an intermediate in glycolysis and is the substrate for a very active lactate dehydrogenase (Driedzic and Hochachka, 1976), it would not be expected to "pile up" excessively during glycolysis. Consequently, muscle pyruvate levels would be expected to rise only slightly during exercise in both fish and the resultant blood pyruvate loads would be comparable. During recovery in the sole, blood pyruvate levels reach a maximum of .270mM at 0 h and were probably in equilibrium with muscle pyruvate, as shown in the trout (Table 4).

The flathead sole maintains high (≈1 mM) plasma ammonia levels (Table 11), which are far greater than those found in the trout (Table 4). Severe exercise in both animals initiates a rise in plasma ammonia which requires some 4-8 h to recover. Ammonia can be generated through at least two processes during exercise: 1) deamination of AMP to IMP and ammonia (Introduction, Eq. 2) or 2) deamination of amino acids. Fish muscle possesses very high activities of 5'AMP deaminase (Fields, 1975) and has been shown to accumulate ammonia during exercise (Driedzic and Hochachka, 1976). The rat gastrocnemius also produces ammonia during exercise but does not deaminate any intracellular amino acid pools (Meyer and Terjung, 1979). The fact that muscle IMP levels are frequently found in excess of ammonia concentration

following exercise indicated that ammonia is lost into the blood (Goodman and Lowenstein, 1977; Meyer and Terjung, 1979). Since flathead sole white muscle could be compared to the rat gastrocnemius, also a fast-twitch white muscle, large amounts of muscle ammonia would be expected to occur along with a concomitant increase in plasma ammonia. The fact that the trout maintains much lower plasma ammonia levels may be indicative of their different muscle composition of red and white fibers or difference in the use of 5'AMP deaminase pathway.

When NH_3 is formed in one of the above reactions, it immediately combines with a H^+ to form NH_4^+. Such a process removes metabolic protons and could cause an underestimate of the metabolic acid load. However, since increases in ammonia levels were small after exercise, errors due to this factor would be minimal.

In conclusion, the flathead sole and rainbow trout are capable of sprinting behavior, through the recruitment of white muscle fibers which in turn produce lactate and protons as metabolic end products. According to experimental evidence and the model presented, the majority of the lactate and protons remain in the muscle cells where they undergo glyconeogenesis at a slow rate. Lactate does follow its electrochemical and concentration gradient out of the cell but is suggested to be actively transported back into the muscle cells. Proton efflux from the muscle is regulated by extracellular pH and/or P_{CO_2}. Such a system has the adaptive significance of preventing simultaneous respiratory and metabolic acidosis which could prove lethal.

5. Ecological Perspective

In light of the very different niches that the flathead sole and rainbow trout exploit, it is interesting to speculate on the adaptive significance of the physiological responses to exercise seen in both animals. The rainbow trout is a pelagic, carnivorous fish capable of continuous cruising or short term very high velocity sprints. This fish has evolved specific red and white muscle to generate thrust for these different swimming speeds. During rapid, anaerobic swimming, lactate accumulates in the white muscle where, during recovery, the majority of it is reconverted to glycogen *in situ*. A small amount of lactate moves into the blood during recovery and can be used as a metabolic fuel for aerobic tissues. Following exercise in trout, sufficient quantities of protons exist in the muscle to fatally depress blood pH if released into this compartment. To prevent such an occurrence, trout muscle has evolved a high buffering capacity and a strict mechanism for proton retention. The proton controlling mechanism is of critical importance to trout as severe acidosis decreases the ability of the blood to transport oxygen. This in turn would limit red muscle activity at a time when white muscle is nonfunctional due to the lack of metabolic fuel. For a pelagic fish, limits on locomotion are a poor strategy. For this reason, trout maintain their aerobic swimming potential by regulating extracellular pH and thereby providing oxygen to the red muscle.

The flathead sole on the other hand spends only a fraction of its time swimming, as it eats small mollusks and other slow moving benthic life. Consequently, it does not require red muscle for extensive cruising. Its primary defensive mode is to burrow in the sand, a task for which its body morphology is well suited. Only when attacked in the sand does this animal use its white muscle to sprint away, escape, then quickly reburrow into the sand (personal observation). By virtue of its excellent camouflage, the sole may then recover from exercise in peace. Like the trout, the flathead sole is thought to use muscle lactate for in situ glycogen synthesis. The sole has compensated for the blood acidosis during recovery from exercise by maintaining a high resting cardiac output and by remaining quiescent, thereby reducing its oxygen consumption. Furthermore, some key organs, the heart for example, have a decreased sensativity to acidosis.

In the final analysis, rainbow trout and flathead sole both use their white muscle for sprinting behavior. During recovery the trout depends on a functioning red muscle for survival while the sole relies on its camouflage while immobilized during recovery.

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