# The Metabolism of Myotomal Muscle Fibres of the Rainbow Trout (Salmo Gairdneri Richardson) during swimming.

A thesis submitted to the University of St Andrews for the Degree of Master of Science

by

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## DECLARATION

I hereby declare that the research presented in this thesis is composed by me, the experimental work is original and carried out by me. It has not been presented previously in any form for the purpose of obtaining a higher degree.

CERTIFICATE

I hereby certify that Irvine Alele Wokoma has fulfilled the conditions of General Ordinance No. 12 (Resolution of the University Court No. 1, 1967), and that he is qualified to submit the accompanying thesis for the degree of Master of Science

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## SUMMARY

The metabolism of myotomal muscles in rainbow trout (Salmo gairdneri Richardson) have been investigated in relation to sustained swimming performance. The concentrations of glycogen, lactate and glucose in the whole carcass, slow myotomal and fast myotomal muscles were measured at a series of different swimming speeds.

Prior to experiments fish were pre-conditioned for several days in a flow-chamber at a water velocity equivalent to 1.1 bodylengths $s^{-1}$. At the start of experiments water velocity was rapidly increased ( 25s) to speeds equivalent to between 2.3 and 7.0 bodylengths $s^{-1}$. Following various periods of swimming of up to 24 h, fish were freeze-clamped in liquid nitrogen (-196°C) and metabolites analysed. The resting whole carcass concentration of glycogen for 84g fish was 73 mg, 100 $g^{-1}$. During the first two minutes, swimming glycogen depleted at a rate equivalent to 0.12 and 0.22 mg, $min^{-1}$ g $wt^{-1}$ at 2.8 and 4.0 bodylengths $s^{-1}$ respectively. Rates of glycogen depletion for slow fibre muscle were twice those for fast fibres at both swimming speeds. Total body lactate concentrations (μmoles, $g^{-1}$) were 8.55 and 7.88 in tank rested and exercise conditioned (1.1 body-lengths $s^{-1}$) rainbow trout. In 27g rainbow trout, following 30 minutes swimming at either 3.8 or 7.0 bodylengths $s^{-1}$ lactate was increased by 6.77 and 10.1 μmoles, $g^{-1}$ $min^{-1}$ respectively. In fish of this size lactate concentrations continued to rise for a minimum of 5 minutes following the attainment of the new swimming speed. Peak of lactate concentractions following 5 minutes of steady swimming were 16.9 μmoles, $g^{-1}$ at 3.8 bodylengths $s^{-1}$ and 24.3 μmoles, $g^{-1}$ at 7.0 bodylengths $s^{-1}$. The $\log_{10}$ of net lactate production in the first

five minutes steady swimming (excluding the period of acceleration to a given speed) was found to be linearly related to the swimming speed and of the form y = 0.30 x -0.63. This is equivalent to an ATP yield from anaerobic glycolysis of 0.2 $\mu$moles, $g^{-1}$ bodyweight $min^{-1}$ at 2.3 bodylengths $s^{-1}$ and 1.6 $\mu$moles, $g^{-1}$, $min^{-1}$ at 7.0 bodylengths $s^{-1}$. The initial rate of ATP generation from anaerobic glycolysis during the first five minutes of swimming were 39% and 74% of the total energy requirements calculated from steady state values of oxygen consumption obtained from the literature.

The results provide evidence for a significant anaerobic contribution to activity even at speeds at which the fish remains in oxygen balance. It is suggested that lactate produced during the initial phase of acceleration could constitute an important substrate for slow fibre mitrochondria.

Reoxidation of the cytosolic NADH generated during glycolysis is essential for the continuous provision of NAD. The mechanisms which allow cyclic irreverisble transfer of hydrogen between the cytosol and mitochondria have been investigated. Amino oxyacetate was employed to limit the transport of hydrogen by inhibiting amino asparate transferase activity in isolated fibre bundles incubated in a media (containing 140 mM NaCl, 5mM KCL, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 10 mM sodium phosphate, 20 mM glucose; pH 7.4). Evidence has been presented in favour of the occurrence of the malate-asparate shuttle and a glycerophosphate dehydrogenase shuttle in slow and fast fibres.

Finally, untrastructural studies have been made on the skeletal muscles. Mitochondrial volume fraction, capillarisation and oxygen diffusion distances have been determined. The mitochondrial density (Vv (mit)) was 31.6% for slow and 9.6% for fast fibres. The number

of capillaries per muscle fibre was 1.26 for slow and 0.54 for fast fibres. The percentage of fibre perimeter in direct capillary contact was 5.87 for slow and 2.17 for fast fibres. It is suggested that in rainbow trout the aerobic capacity of the fast fibres is a significant fraction of that of the slow fibres.

# CHAPTER 1. GENERAL INTRODUCTION

The diverse forms of fishes reflect both their ways of aquatic locomotion and their means of making a living. The most successful group of fishes are the teleosts, as their number already exceeds 20,000 species. These inhabit a wide range of habitats, including different salinities, temperatures, pressures and oxygen tensions.

Salmon and trout are teleosts that have been widely cultivated in temperate countries for food and as sport fish. An understanding of the physiology of salmonid muscle is important for aquatic culture industry. In addition studies of fish myotomal muscle may lead to a better understanding of the vertebrate muscular system in general. Early studies of the histology and the structure of salmon muscle had been made by the beginning of this century (Stirling, 1885; Patton, 1898; Greene, 1913). Foremost in the scientific studies of fish musculature was that of Lorenzini (1678), who distinguished red and white muscles in the elasmobranch, Torpedo.

Various suggestions were put forward to account for the presence of the different fibre types. Lankester (1871) and Ranvier (1873) suggested that red muscles are used for prolonged and sustained effort. However, it was Arloing and Lavocat (1875) who first suggested that the slow (red) and fast (white) muscle fibres in fishes are used during different types of swimming.

## Organisation

The bulk of this fish myotome consists of white (fast) fibres (80-90%) (Figure 1). The lateral trunk of most fishes contains a superficial triangular wedge of red fibres (Figure 1), which runs along the lateral line from head to tail. The proportion of this fibre type

varies along the length of the body and with the mode of life of the species (Greer-Walker and Pull, 1975). There is a correlation between the relative proportion of red muscle in the myotomes of most fish and their sustained swimming ability (Tables 1 and 2). For example, Chub mackerel (Scomber colias) which is an active pelagic swimmer contains significantly more red muscle (29.8%) than that of the Angler fish (Lophius piscatorius) (7.2%) which is a sedentary bottom living species. This positive correlation between the amount of myotomal red fibres and swimming ability does not apply to those fishes which use their pectoral fins as the prime means of locomotion. For example, the Antarctic cod (Notothenia rossii) primarily uses enlarged pectoral fins for swimming. Superficial red fibre in the myotomes of N. rossii constitutes 5.5% compared to 85% for the pectoral fin muscles (Walesby and Johnston, 1980).

The different fibre types of the myotomal muscles are anatomically separated into discrete zones (Lorenzini, 1678). The individual fibres of the myotomal muscles insert via the tendons into the myosepta, which consist of connective tissue sheets. The complex shape of the myomeres varies between species. These myomeres are similar to "V" or "W" shaped overlapping cones (Bone, 1978). It has been suggested that the purpose of the complex geometry is to permit each muscle fibre to be inserted in a similar angle with the myosepta. Following studies involving a histological analysis and mathematical modelling, Alexander (1969) has documented two basic patterns of fibre geometry, one for selachians and primitive bony fishes such as anguilla and the other for advanced teleosts.

The superficial fibres run parallel to the longitudinal axis of the body. The deep white fibres are arranged in complex patterns which make angles of up to 30 to 40$^{o}$ with the longitudinal plane of the fish. Alexander (1969) has concluded from his studies that all white fibres

would contract to the same degree for a given body flexure and only minimal sarcomere shortening would result from bending of the body. For example, he calculated that the longitudinally arranged red fibres would only shorten by 10% of their resting lengths during swimming, while the selachians white fibres, by 7-9%, and the advanced teleosts helically arranged white fibres by only 2-3% (Alexander, 1969). This isometric nature of contraction of the myotomal muscles has been supported by indirect evidence provided by endurance exercise training experiments. For example, prolonged enforced swimming in a flume produced hypertrophy of both red and white muscle fibres in Saithe and Brook trout (Greer-Walker and Pull, 1975; Johnston and Moon, 1980a,b).

There is a third type of fibre in fish myotomal muscle. This is located between the red and white fibres (Figure 1). Because of its intermediate colouring, it has been termed pink fibre. This narrow band of pink fibres has reduced myoglobin and mitochondrial content and increased glycolytic enzyme activities compared to slow fibres (Johnston et al., 1977; Johnston and Maitland, 1980). They have been differentiated from other fibre types histochemically, by their staining for myofibrillar ATPase activity following alkaline preincubation at pH 10.4 (Johnston et al., 1974; Mosse and Hudson, 1977). As a result of the aerobic characteristics of these fibres they have recently been termed as fast aerobic fibres to differentiate them from the bulk of the white fibres which are termed fast glycolytic fibres (Johnston, 1980a,b). Aerobic fast fibres are restricted to a few cells in rainbow trout (Johnston et al., 1975a), but constitute up to 10% of the muscle bulk in mirror carp (Davison et al., 1976).

Classification of muscle fibre types

Classification of fibre types based on colour originated from Lorenzini (1678). Terms such as red and white have been abandoned by workers on amphibians, avian and mammalian muscle. These terms are considered unsatisfactory because some red fibres in bat cricothyroid muscle are faster contracting than white fibres (Revel, 1962). Since good correlations have been observed between muscle histochemistry, morphology and speed of contraction in these animals, classification is based on innervation, contraction speed and metabolic characteristics. Terms such as twitch and tonic are used indicating innervation and response to electrical stimulation. These terms are further sub-divided into three broad groups: slow oxidative, fast oxidative glycolytic and fast glycolytic.

The situation is different in fish. Morphologically and histochemically different fibre types are arranged in discrete zones and can therefore be distinguished by their position. However the inconvenient physiological preparation of the myotome has delayed detail investigation until recently. Consequently, no correlations have been established between fibre types and contraction velocity. This situation is further complicated by the fact that both red and white muscles are multiply innervated in the majority of teleosts (Barets, 1961; Bone, 1964). Classification on the basis of innervation would be unsatisfactory and terminology such as red and white are consequently retained. Even in fish groups there is an exception to classification based on muscle pigmentation and aerobic capacity. For example, haemoglobinless icefish, Champsocephalus gunnari, has white slow fibres due to the absence of myoglobin although the fractional volume of the fibre occupied by mitochondria is 45% (Walesby, Nicol and Johnston, 1982).

The different fibre groups are further sub-divided into sub-populations of fibres. Histochemical techniques, ultrastructural features as well as biochemical and physiological criteria are employed in this differentiation. Serial frozen sections are stained for a mitochondrial enzyme, for example succinic dehydrogenase (SDH), a glycolytic enzyme such as myofibrillar ATPase . As glycogen storage levels are usually higher in fish slow than fast fibres, staining with PAS also provides a method of distinguishing the fibre types. There is a good correlation between the myofibrillar ATPase activity, measured biochemically, and the contraction speed in different muscles (Barany, 1967). Biochemical studies have shown that Carp pink fibres have intermediate characteristics in myofibrillar ATPase activity to white and red fibres and a myosin light chain composition characteristic of fast fibres (Johnston et al., 1977a).

These identification procedures have resulted in distinguishing five major types of fibres in the dogfish, two of which are termed slow fibres type I and II (Bone, 1978).

There is considerable heterogeneity in fast fibre sizes. The histological studies of Greene(1912) and the histochemical studies of Boddeke et al. (1959) provided evidence for the variety of aerobic capacities amongst white fibres. Usually larger diameter fibres are surrounded by smaller diameter fibres in a mosaic arrangement. The smaller fibres have a greater succinic dehydrogenase activity and lipid contents than the large fibres. Fast fibres in rainbow trout show a range of sizes from 10-95 μm (Johnston et al., 1975a). These fibres have homogeneous myofibrillar ATPase activity irrespective of size despite the greater aerobic capacity of the smaller fibres (Johnston et al., 1975a; Johnston and Moon, 1980c).

It is suggested that the smaller fibres are in the early developmental stages of the larger fibres.

Division of labour between red and white muscle fibres

The histological studies of Boddeke et al. (1959) provided indirect evidence on the division of labour between red and white fibres during swimming. In these studies the relative proportions of the red and white muscles in the myotome of a number of fresh water teleosts of different habits were examined. It was observed that fishes which were capable of rapid sustained swimming such as salmonids and scombroid fishes had a greater amount of red muscle than 'sprinters' such as pike or perch which are often sedentary but show occasional bursts of speed. The same correlation had been observed in other pelagic and non-pelagic fishes. For example, the glass or silver eel is pelagic and moves actively to the coasts in contrast to the yellow eel which is sluggish and mainly a bottom dwelling animal (Bostrom and Johansson, 1972). Recently, Greer-Walker and Pull (1975) made a systematic study of the percentage of red muscle in 84 species of fish and arrived at similar conclusions (see Table 1).

George (1962) has shown from histochemical studies on the red and white muscles of mackerel (Rastrelliger kanagurta Russell) that succinic dehydrogenase was localized in the mitochondria. These were found to be numerous in the red but scarce in the white fibres. It was found that the red fibres along the lateral line had greater concentration of fat and lipase activity. The red muscle was considered well adapted for an aerobic metabolism and would use fat as the chief fuel while the white muscle, adapted for anaerobic metabolism, would use mainly glycogen.

In a classic paper, Bone (1966) studied the function of red and white muscles in the common dogfish (Scyliorhinus canicula). He observed that red fibres contained greater concentration of glycogen fat and mitochondria than white fibres. Bone (1966) also made

extracellular recordings from the myotomal muscle of spinal fish. He found that during slow swimming movements only the superficial slow fibres are active but during vigorous movements electrical activity could also be recorded in the fast fibres. Bone (1966) observed that after a period of rapid swimming the glycogen content of the fast fibres was reduced significantly but that after a prolonged period of slow swimming it was unchanged. In contrast, the glycogen content of the slow fibres was unchanged during vigorous swimming. However, during prolonged periods of slow swimming the fat content of the slow fibres was reduced and slight decrease of glycogen was evident. Bone (1966) concluded that the two muscle types operate independently and utilize different metabolites. The fatigue resistant red fibres would use aerobic pathways and lipid as fuel for sustained swimming while the fast fibres would almost be entirely dependent on anaerobic glycogenolysis during short bursts of vigorous activity. In the latter situation, glycogen content of the white fibres would only be sufficient for 1-2 minutes as energy source (Bone, 1966). This conclusion has been supported by other investigators (Rayner and Keenan, 1967; Hudson, 1973; Bilinski, 1974; Bone et al., 1978a).

Different lines of investigation have shown that where fast fibres are multiply innervated such as in advanced teleosts, this simple division of labour between slow and fast portions of the myotome is an oversimplification. In many such fish, fast fibres have been shown to be active during continuous swimming at slow speeds. Hypertrophy of both slow and fast fibres has been observed in coal fish (Gadus virens) following prolonged swimming at 2-3 lengths/second (Greer-Walker, 1971; Greer-Walker and Pull, 1973). Various biochemical studies have shown glycogen depletion and lactate accumulation in fast fibres during sustained swimming (Pritchard et al., 1971). For example, an elevation of muscle lactate in Crucian carp (Carassius carassius)

following continuous sustained swimming at 2 lengths/second has shown fast fibres to be active (Johnston and Goldspink, 1973a,b,c).

Finally, electromyographic evidence has been obtained for the recruitment of fast fibres at slow and intermediate speeds (Hudson, 1973; Johnston et al., 1977; Greer-Walker and Emerson, 1978; Bone et al., 1978; Johnston and Moon, 1980a,b). The threshold speed for the recruitment of fast fibres appears to vary amongst teleosts species and is probably dependent on the degree of polyneuronal innervation. For example, fast glycolytic fibres are recruited at 1.4 body lengths/second in rainbow trout, 0.8-2.0 body lengths/second in Saithe (Johnston and Moon, 1980a), but not until 3.2 and 4.5 body lengths/second, respectively, in Striped bass (Morone saxatilis) and Blue fish (Pomatomus sultanix) (Freadman, 1979).

One interesting suggestion is that there is a rotation of fast motor units which depends on swimming speeds (Hudson, 1973; Johnston et al., 1977). Direct evidence was provided in the carp for an orderly recruitment of fibre types: slow > fast aerobic > fast glycolytic (Johnston et al., 1977). It appears that differences in multiply innervated fast fibre aerobic capacity, vascularisation and innervation may all play significant roles in fast fibre recruitment.

Elasmobranchs, dipnoans and some taxonomically primitive teleosts have been shown to have focally innervated fast fibres (Bone, 1970). Electromyographic evidence has shown that in such fish the slow fibre system is entirely reserved for sustained swimming. Bone et al. (1978a) provided evidence that in herring species (Clupea harengus pallasi) (primitive teleost) a 15 cm fish maintained speeds of up to 4 body-lengths/second by recruiting only slow fibres. Higher swimming speeds > 5 body lengths/second resulted in recruitment of fast fibres and then fatigue after 1-2 minutes of burst swimming (Bone et al., 1978). In comparison to advanced teleosts where fast fibres are recruited at low

speeds there is a sharp transition between the levels of activity which can be sustained almost indefinitely and those which lead to fatigue.

Other suggestions on the role of red and white muscles

Some workers after physiological and histological studies of the red and white muscles come to a different conclusion from that presented by Arloing and Lavocat (1875). They maintained that, although the two types of muscles played distinct and different roles in swimming, the red muscle was used in rapid swimming while the white was used for sustained swimming (Gerebtzoff, 1956; Buttkus, 1963; Bergman, 1964, 1967).

A third suggestion was that of Braekkan (1956, 1959). His investigation showed the high concentration of certain vitamins and lipids in the red muscle which is similar to that of the liver. On account of this correlation and the high concentration of oxidative enzymes in the red muscle, he concluded that the slow muscle fibre functions as an organ like the liver, synthesizing metabolites for oxidation in the white muscle. He suggested that the high content of lipid and the anatomical location of the red muscle would prevent it from performing efficient muscular work (Braekkan, 1956, 1959). But George (1962) has shown that the presence of high concentration of lipids in birds, bats and fish provides a feature of adaption for prolonged periods of slow continuous activity. However, Braekkan's hypothesis has received support from the experimental work of Wittenberger and his co-workers (Wittenberger and Deacuic, 1965; Wittenberger, 1973; Wittenberger et al., 1975). These experiments involved the stimulation of strips of carp muscle. Isolated white muscle fibres were incubated separately from muscle pieces comprising a combination of red and white fibres, in a media containing hormones known to stimulate glucose transport or pharmacological agents which affect membrane permeability

(Wittenberger et al., 1975). It was shown that the amount of work obtained on stimulating white muscle to exhaustion was very low, but it increased when strips of muscle consisting of red and white fibres were stimulated. A decrease in glycogen content was observed during the stimulation of white fibre strips; but glycogen was increased when stimulated in strips containing red fibres. It was therefore concluded that this was an evidence of the non-circulatory transfer of metabolites from the red muscle for use in the white muscle. The criticism of this technique has been the use of muscle fibres with an unknown degree of damage and the loss of orientation of these fibres. The demonstration of a contractile function for red muscle and the large diffusion distances between red and white muscle masses rules out this hypothesis at least in its more extreme forms (Johnston, 1981).

Innervation

All fish groups have been shown to have superficial slow fibres which are multiply innervated with small diameter myelinated fibres terminating in en grappe endings (Baret, 1961; Bone, 1978). In the dogfish, two types of slow fibres have been distinguished histochemically. The more superficial slow fibres show a greater staining for succinic dehydrogenase (S.D.H.) and decreased alkaline myofibrillar ATPase activity (Bone, 1978; Bone and Chubb, 1978). These authors have shown that both fibres are innervated in a similar pattern.

The en grappe terminations in the dogfish are supplied from two separate axons which pass into the fibre from the myotomal ends or traverse the surface, innervating a number of different fibres in the process. Nerve terminals in teleosts are usually embedded in sarcolemma and subjunctional folds are absent (Nishira, 1967). In contrast, dogfish has been shown to possess subjunctional folds in the two types

of slow fibres (Bone, 1978). Stanfield (1972) has shown from electrophysiological measurements of membrane space constants that motor terminals on dogfish slow fibres are 150-200 μm apart. The electrophysiology of fish slow fibres is not fully understood. Fish slow fibres are not generally thought to be capable of producing action potentials and are thus similar to the slow or tonic fibres of amphibians (Barets, 1961; Stanfield, 1972). However, in the case of dogfish (Scyliorhinus canicula L.), it has been demonstrated that 30% of the slow fibres examined displayed a large inward sodium current on depolarisation, 22% had no inward sodium current while the remainder showed a small inward sodium current. Thus, although slow fibres are usually activated by non-propagating graded junction potentials, the present evidence suggests that a certain proportion might under certain circumstances be able to produce propagated action potentials.

Acetylcholinesterase has been demonstrated at the terminals on all slow fibres examined (Pecot-Dechavassine, 1961). However, Korneliussen (1973), in a study on hagfish, suggested slow fibres might also have monoaminergenic innervation. But this suggestion has not been supported by formaldehyde-induced fluorescence (Pecot-Dechavassine, 1961).

The separation of slow and fast fibres occurs at the level of the spinal cord (Hudson, 1972). It has been suggested that activation of any of the fibre systems may involve different levels of activations of the neuronal pools or neuronal pathways. White muscle fibres in the dogfish are focally innervated by large diameter axons which pass in the myosepta to produce basket-like en plaque end formations at one end of the fibre (Bone, 1964, 1966). Two axons usually innervate a single fibre. However, the axons fuse together to form a single endplate (Bone, 1964, 1972).

Best and Bone (1973) have shown that the two motor terminals in the dogfish contain vesicles of different sizes of 50 mm and 100 mm. The reason for the dual innervation is not clear because only acetylcholinesterase has been detected in the sub-synaptic folds (Pecot-Dechavessine, 1961). Hagiwara and Takahashi (1967) have demonstrated typical over-shooting spike-potentials in white fibres on depolarization. In this respect, white fibres of elasmobranchs resemble fast twitch fibres.

Fast fibres of advanced teleosts have a different pattern of innervation to those of elasmobranchs, chondrosteans, dipnoans and some primitive teleosts (Bone, 1961, 1964). Instead of a single motor end-plate, a single fibre is overwhelmed by a dense network of innervation which range up to 23 terminations per fibre (Barets, 1961). Multiple innervated fast fibres have a range of fibre sizes and variety of aerobic capacities. The smaller fibres have greater mitochondrial and glycogen content than the larger fibres (Green, 1913; Boddeke et al., 1959). Although the mosaic arrangement of the fast fibres show differences in aerobic capacities, the myofibrillar ATPase staining is homogeneous (Johnston, 1980b). Furthermore, Altringham and Johnston (1981) have shown that branches of a given axon innervate fibres with a spectrum of sizes. Therefore the contractile activity of these fibres would be homogeneous. It is suggested the small fibres are in their growth stages (Johnston, 1980b).

Muscle fibres which are multiterminally innervated by a single axon will give an all or nothing response to suprathreshold stimulation. In polyneuronally innervated fibres each is innervated by a number of different axons, the junction potentials are quantized and consequently each step in amplitude represents an additional axon. Therefore a graded response in contraction of fast muscles is obtained (Hudson, 1967). Isolated polyneuronally innervated fast fibres may require ten

times the stimulation frequencies to elicit maximum tension than fibres with single endplates (Johnston, 1981). Studies by Flitney and Johnston (1979) on Tilapia, adductor operculi muscle show that the rate of rise of isometric tension on multiple stimulation was about seven times faster in white than red fibres. However, since Alexander (1969) has calculated that fast fibres only shorten by 2-3% of their resting length, it seems that the rate of tension development in multiterminally innervated fast fibres is limited by the membrane properties as well as by its intrinsic maximum speed of shortening. In order to obtain full activation of fast fibres simultaneous recruitment of a number of different motor neurones may be necessary (Johnston, 1980).

Ultrastructure

Both fast and slow fibre types have been distinguished using histochemically and ultrastructural criteria (Patterson and Goldspink, 1972; Johnston et al., 1975; Mosse and Hudson, 1977; Bone, 1978a,b). There is a wide species variation in muscle fine structure between homologous fibre types. Ultrastructural studies show that these may be modified by exercise and/or environmental factors (Totland and Kryvi, 1978; Johnston and Maitland, 1980; Walesby and Johnston, 1980).

Slow fibres have been shown to be more homogeneous than fast fibres (Johnston and Moon, 1980a; Egginton and Johnston, 1981a,b). This has been shown in brook trout, anchovy and eel (Johnston and Moon, 1980a; Johnston, 1982b; Egginton and Johnston, 1981a,b). In contrast, slow fibres which are adjacent to the skin have been shown to have significantly higher fractional volumes of subsarcolemmal mitochondria than the deeper fibres (Johnston and Bernard, 1982b). Egginton and Johnston (1982b) have shown that heterogeneity is more marked in fast fibre populations not only with respect to size but also to regional

distribution within the myotome. See Tables 1 and 2 for a selection of some of the quantitative data in the literature on the ultrastructure of fish slow and fast glycolytic muscle fibres. In general, the characteristics of fast aerobic fibres are intermediate between the slow and fast glycolytic fibres (Kryvi, 1977; Johnston and Maitland, 1980; Akster, 1981). The fast glycolytic fibres near the skin have greater concentration of mitochondria and capillaries than those near the vertebral column and have been shown to be smaller (Egginton and Johnston, 1981). Akster (1981) has shown that myosin filament lengths are about 1.6 μm in all fibre types. Actin filament lengths increase in the order slow < fast aerobic < fast glycolytic. The relative contraction velocities would give less sarcomeres to larger actin filament lengths and hence a slower speed of shortening (Akster, 1981).

Comparative electron microscopical studies (Table 2) have shown that fish slow fibres have higher mitochondrial content, abundant capillaries and well developed sarcoturbular system than the multiply innervated slow fibres of amphibia (Johnston, 1980b, 1981a). Mitochondrial volume of fish slow fibres is similar to those of mouse and finch ventricle, 38% and 34%, respectively (Bossen et al., 1978). These values are higher than mammalian slow twitch fibres (Fisenburg et al., 1974).

The aerobic capacity is determined by vascularization and the mitochondrial content of fibres. From Table 2 it is clear that the mitochondrial volume is significantly higher in the fast fibres of advanced teleosts than the focally innervated fast fibres of elasmobranchs, chondrosteans and taxonomically primitive teleosts.

Mitochondrial volume of the slow fibres occupy 18-24% in the elasmobranch, Scyliorhinus canicula (Totland et al., 1981). The mitochondrial volume of the slow fibres is lowest in the elasmobranch, Chimera montrosa, with a value of 5% (Kryvi and Totland, 1977),

Recruitment of slow fibres in the trunk muscles may be associated with movements requiring change of direction. Therefore such fibres may have a postural function like those of amphibia (Kryvi and Totland, 1978). Another exception in the general correlation is that of skipjack tuna (Katsuwonus pelamis). There is a counter-current vascular heat exchanger in this species which maintains elevated brain and muscle temperature (Carey and Teal, 1969; Stevens and Neill, 1978). Under this condition mitochondria appears to function more efficiently because it has been shown to have the highest aerobic capacity (Stevens and Neill, 1978). But the mitochondrial volume of skipjack tuna is only half (16%) that of another scombroid, the Atlantic mackerel (Scomber scomber). The only difference is that this species does not operate muscle temperature as that of skipjack tuna (Bone, 1978). The highest mitochondrial volume of fish slow fibres is that of European anchovy (Engraulis encrasicolus) with a value of 45.5% (Johnston, 1982). Anchovies are highly active pelagic fishes and feed mainly on plankton by filter-feeding technique.

Recruitment of fast glycolytic fibres during slow speed swimming is associated with multiply innervated advanced teleosts. The mitochondrial volume has been shown to be higher than those of focally innervated fast fibres of elasmobranch or primitive teleosts (see Table 2). For example, mitochondrial volume range from 2 to 9% in advanced teleosts while the value is 0.5-1% in elasmobranchs. Even in multiply innervated fast fibres there is considerable heterogeneity. This may be related to the mode of life of the different species. For example, brook trout is an aerobic fish with mitochondrial volume occupying 9.3% of the fibre while carp which is a sluggish species has a mitochondrial volume of 4.6% (Johnston, 1980c; Johnston and Moon, 1981). Smaller fibres have been shown to have greater mitochondrial volume than the larger ones (Figure 2a,b). This may consequently enhance

slow speed swimming.

In the present study, the mitochondrial volume of the slow fibres in rainbow trout is 31.6% as opposed to 9.6% in the white fibres. A previous study by Nag (1972) on rainbow trout obtained a value of 8:3 by number in the red to white fibres. The volume fraction of sarcoplasmic reticulum is 5.1 for slow and 13.7 for fast fibres. These are greater than the tonic or slow twitch muscles of other vertebrates (Johnston, 1980b). It has been shown that the S,R, and the tubular system are concerned with the transmission of impulses in the fibre and the subsequent release of bound calcium ions which then activate muscle contraction (Peachey, 1965).

Myofibrillar volumes have been shown to occupy 80-90% in the white fibres and 40-60% in the red fibres (Johnston, 1980b) (Table 2). The fast glycolytic fibres have been shown to have a more regular packing of the myofibrils probably to allow rapid development of tension for burst swimming. Teleosts are characterised by elongated peripheral myofibrils in white fibres. In the small white fibres the arrangement of the fibrils is such that they appear to diverge towards the periphery from a central point. From high power electron micrograph, hexagonal pattern of arrangement is observed in both red and white fibres, such that each thick filament is surrounded by six thin filaments on the A band (Nag, 1972; Patterson and Goldspink, 1972).

Histochemical and biochemical techniques have shown a higher concentration of glycogen and lipid in the red myotomal muscle than the white fibres (George, 1962; Bokdawala et al., 1967; Johnston and Moon, 1980b).

Metabolism of red and white muscle

The activities of enzymes of energy metabolism have been measured in the muscles of various species of teleosts and elasmobranchs (Crabtree and Newsholme, 1972; Johnston, 1977; Johnston et al., 1977; Guppy et al., 1979; Newsholme and Zammitt, 1979). These studies have shown distinct metabolic differences between the red and white myotomal muscles.

The red muscle of Salmo gairdneri contains 2.6 times as much blood as the white muscle (Stevens, 1968). Oxygen consumption is six times higher in the red than white muscles in tuna (Gordon, 1968). In the carp, freeze-clamped samples of red, pink and white muscle fibres, the concentration of myoglobin was shown to be in the ratio of 5:2:1 (Johnston et al., 1977). In the same fish hexokinase, malate dehydrogenase, succinic dehydrogenase and cytochrome oxidase activities were 4-8 times higher in the red than white muscles (Johnston et al., 1977). The activities of aerobic enzymes in the pink muscle were intermediate between red and white muscles. However, the activities of lactate dehydrogenase and pyruvate kinase were significantly higher than either the red or white fibres (Johnston et al., 1977). It has been established that the slow fibres of fish myotomal muscle are recruited during slow speed swimming. In some species this function has been transferred to the pectoral fins. For example, in the Antarctic Cod, Notothenia rossi, the activities of succinic and malate dehydrogenases, cytochrome oxidase and hexokinase are 2-8 fold higher in slow fibres from the pectoral fins than in slow fibres from the trunk (Walesby and Johnston, 1980).

There are interesting differences in lipid metabolism between elasmobranchs and teleosts. Triacyl glycerol and non esterified fatty acids occur in higher concentration in teleosts than elasmobranchs

(Zammit and Newsholme, 1979). High activities of carnitine transferase and triacyl glycerol lipase have been found in the slow fibres of teleosts. In contrast, these enzymes are absent in elasmobranchs (Zammit and Newsholme, 1979). There are also differences in the sites of storage for lipids. The storage sites for triacyl glycerol in salmonids are viscera, fat pad and adipocytes interspersed in the muscle fibre. In contrast, the storage site in elasmobranch is the liver (Bilinski, 1974). During starvation teleosts rely on fatty acid oxidation for their source of fuel while elasmobranchs depend on the utilization of ketone bodies (Zammit and Newsholme, 1979).

Fish and mammals differ in the mechanism of the regulation and transport of lipids from storage sites to the muscle or sites of utilization (Driedzic and Hochachka, 1978). The mechanism of activation is unknown but it seems that activation does not proceed through a cyclic AMP dependent protein kinase system as in mammals (Farkas, 1969). However, Lin et al. (1977) have provided evidence that in teleosts such as coho salmon and in rats, reducing equivalents for lipogenesis are channelled through the malic enzyme as well as the pentose phosphate pathway. In primitive teleosts such as the American eel, isocitrate dehydrogenase is the major source of reducing equivalents for lipogenesis (Aster and Moon, 1981). As a result of the higher oxygen content (Stevens, 1968) and mitochondria (Nag, 1972), the slow muscle is able to oxidize NADH indirectly by the electron transport system. Under these conditions, pyruvate formed in the metabolic processes (Figure 3) is not reduced to lactate but enters the mitochondria where it is completely oxidized or is available for other reactions (Candy, 1981).

There is a good correlation between the unloaded speed of shortening of a muscle and the myofibrillar ATPase activity (Bárány, 1967). The activities of myofibrillar ATPase are significantly different between

muscle types and the ratio 4:2:1 has been reported for white, pink and red fibres of carp, respectively (Johnston et al., 1977). Although in Brook trout and most teleosts activities of glycolytic enzymes including phosphofructokinase may be 2-5 times higher in the white than the red muscle (Johnston and Moon, 1980b), this is not always the case. For example, the glycolytic potential of the red and white muscles of the mirror carp have been shown to be broadly similar (Johnston et al., 1977). Bostrom and Johansson (1972) have also obtained similar results for the slow and fast muscles of the yellow eel. Activities of enzymes responsible for producing ATP supply during muscular contraction, such as creatine kinase, adrenylate kinase and 5 AMP amino-hydrolase are 2-4 times higher in the fast fibres as are those of myofibrillar ATPase (Johnston et al., 1980b). Newsholme et al. (1978) have shown that the higher the initial ATP/AMP ratio, the greater the AMP and the consequent increase in stimulation of glycolysis. During vigorous activity or at higher cruising speeds increasing proportion of the energy is supplied by anaerobic glycogenolysis resulting in the production of large lactate load (Black et al., 1966; Smith et al., 1971; Edington et al., 1973; Wokoma and Johnston, 1981). Excessive production of lactate from glycogen would lower the pH and retard oxygen delivery to the tissues. It is also known to affect calcium distribution (Seraydarian et al., 1961; Nassar-Gentina et al., 1978), calcium binding to troponin (Fuchs et al., 1970), glycolytic enzyme activities (Trivedi et al., 1966) and calcium activation of myosin-ATPase (Portzehl et al., 1969).

The regulation of glycogenolysis in fish fast muscle has been summarized in Figure 3. The regulatory steps include phosphorylase, phosphofructokinase and pyruvate kinase (Newsholme and Start, 1973). In rabbit, glycogen breakdown depends on enzyme cascade mechanism initiated by the activation of muscle phosphorylase kinase or specific

phosphatases by cAMP. In contrast, the dogfish enzyme depends only on $Ca^{2+}$ for its activity (Fischer et al., 1975). This may be due to the relatively poor capillary supply and consequently low blood circulation in the white fibres of this species. The phosphofructokinase reaction is the first unique step in the control of glycolysis. Phosphofructokinase (EC 2.7.1.11) is activated by various substrates including fructose 6-phosphate (F6P), fructose 1,6-diphosphate, AMP, ADP, Pi and $NH_4$ and inhibited by ATP, citrate and creatine phosphate (Mansour, 1972). Phosphofructokinase from goldfish white fibres has been shown to have similar characteristics (Freed, 1971).

Regulatory pyruvate kinase (EC 2.7.1.40) has interesting feature in control of glycolysis (Driedzic and Hochachka, 1978). It catalyses the conversion of phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP. The integration of the activities of phosphofructokinase and pyruvate kinase involves adenylate coupling since ADP which is a product of PFK reaction serves as substrate for pyruvate kinase (Driedzic et al., 1978). The pyruvate kinase from fish skeletal muscle is further regulated by strong feedforward activation by low concentration of fructose 1,6-diphosphate, a product of PFK reaction. This reaction is temperature independent (Somero and Hochachka, 1968). The mechanism of F 1,6-P activation is by lowering the Michaelis constant (km) of the enzyme for the substrate, phosphoenolpyruvate; hence the specific activity of the enzyme is increased (Somero and Hochachka, 1968). Regulatory pyruvate kinases are associated with muscle in which there is rapid and dramatic increase in glycolysis. In contrast to mammalian skeletal muscle isozyme (Jimenez De Asua et al., 1971), the carp red muscle as well as the white muscles of other teleosts have been shown to be under tight metabolic regulation (Somero et al., 1968; Scrutton and Utter, 1968; Johnston, 1975). The consequence of this phenomenon and the $Ca^{+}$ release from SR during burst swimming produce a rapid generation of

energy anaerobically through increased glycolytic flux. Anaerobic generation of energy is also useful when immediate muscular activity is needed, such as escape from predators or in feeding.

Lactate dehydrogenase (EC 1.1.27) is essential in providing a continuous supply of oxidised $NAD^+$ during anaerobic glycolysis, hence redox balance is maintained. It catalyses the conversion of pyruvate and $NADH^+$ to lactate and $NAD^+$ (Driedzic and Hochachka, 1978). There is evidence that the $M_4$ LDH is the major isozyme in anaerobically metabolizing tissues because it is less sensitive than the $H_4$ LDH to inhibition by high substrate or pyruvate concentration (Kaplan et al., 1964). Several experiments have shown that the relative anaerobic capacities and the metabolism of the red and white muscles can be modified by environmental factors such as seasonal variation, temperature acclimation, fish migration (Hazel and Prosser, 1974), exercise training (Johnston and Moon, 1980), nutritional state such as starvation (Walker, 1971; Moon and Johnston, 1980) and development (Bostrom and Johansson, 1972).

Aim of experiments

The basis of this thesis is to study the physiology and biochemistry of myotomal muscles of rainbow trout. Various studies have been made on fish myotomal muscle fibres following sustained swimming at different speeds, with a view to investigating the division of labour between fast and slow fibres. In these experiments an open top flume was employed to study the time-course of lactate production in the fast and slow fibres as well as the whole body concentration of exercise-conditioned rainbow trout at various sustained swimming speeds. The glycogen and glucose concentrations were also determined.

The shuttle systems which are essential for the reoxidation of cytosolic NADH were investigated in the slow and fast muscles of

rainbow trout. In-vitro techniques were modified and enzymatic methods were employed to examine up to seven metabolites following incubation of fibre bundles inthe presence or absence of transaminase inhibitors, amino oxyacetate.

Finally, ultrastructural studies have been made on slow and fast fibres of the myotomal muscles. Muscle capillary supply, mitochondrial volume density and oxygen diffusion distances have been determined.

Fig. 1

Diagrammatic representation of the organisation, innervation and the relative amounts of A, slow (red) B, fast aerobic (pink) and C, fast glycolytic (white) fibres in fish myotome.

Fig.1

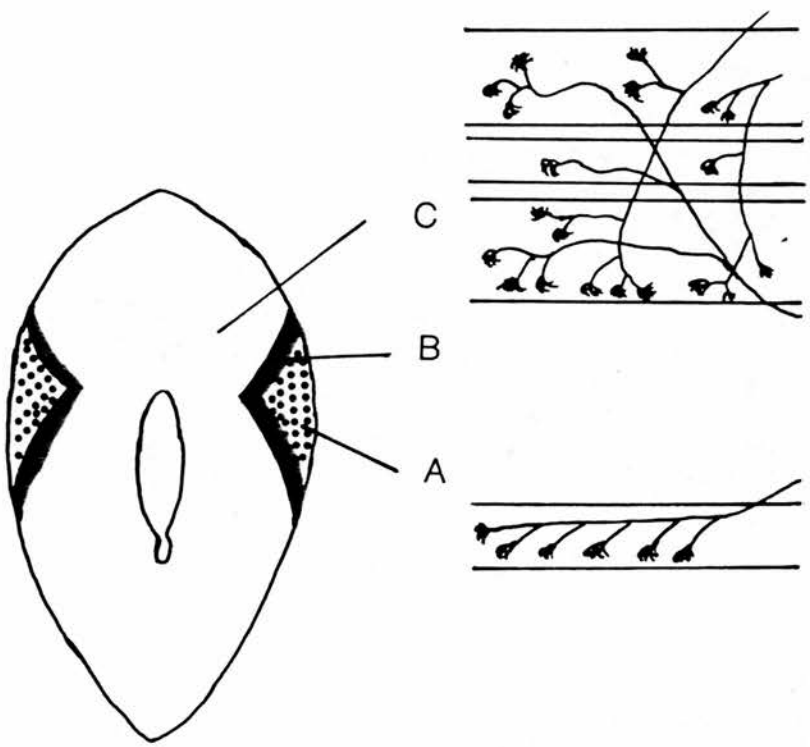


Fig. 2a

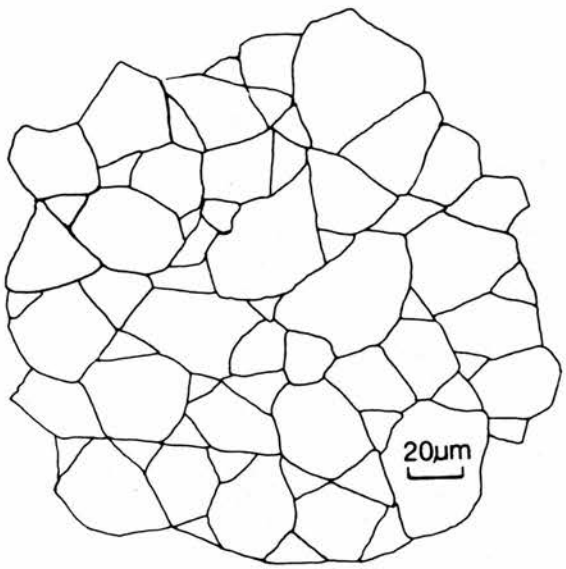
A tracing of a transverse section of a low power micrograph showing the wide range of fibre sizes in rainbow trout fast muscle.

Fig. 2b

Histogram showing frequency distribution (% occurrence) of fast fibre area ($um^2$) in rainbow trout.

Fig.2

a


20µm

b

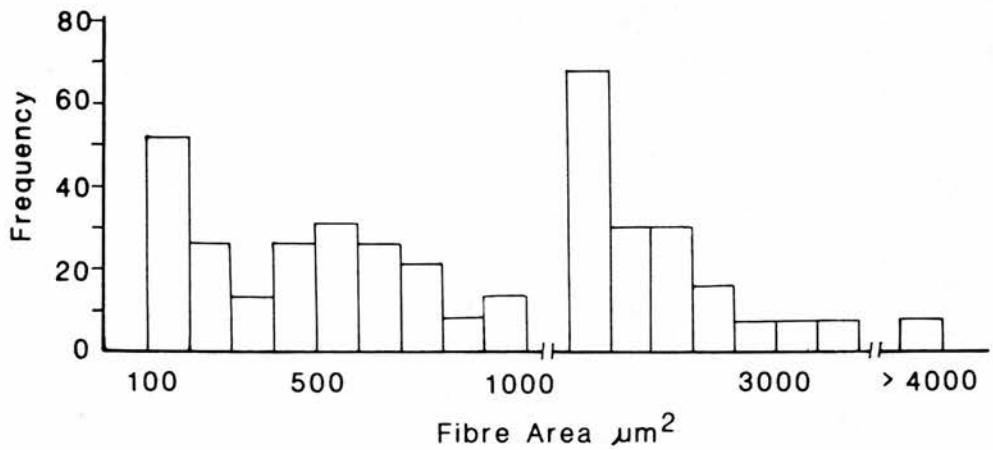

80
60
40
20
0
Frequency
100
500
1000
3000
> 4000
Fibre Area µm2

Fig. 3

The control of anaerobic glycogenolysis of fish fast (white) fibres. Three regulatory steps are shown A, glycogen phosphorylase, B, phosphofructokinase and C, pyruvate kinase.

Abbreviations:

SR, sarcoplasm reticulum; Phos, phosphorylase;

G1P, glucose-1-phosphate; PFK, phosphofructokinase;

FDPase, Fructose diphosphatase; FBP, fructose 1,6-biphosphate;

AMP, adenosine 5' monophosphate; TP, triose phosphate ;

NAD, -nicotinamide adenine dinucleotide; NADH, -nicotinamidé adenine dinucleotide reduced form; 1,3 DPG, 1,3 diphosphoglycerate;

PEP, phosphoenolpyruvate; PYK, pyruvate kinase.

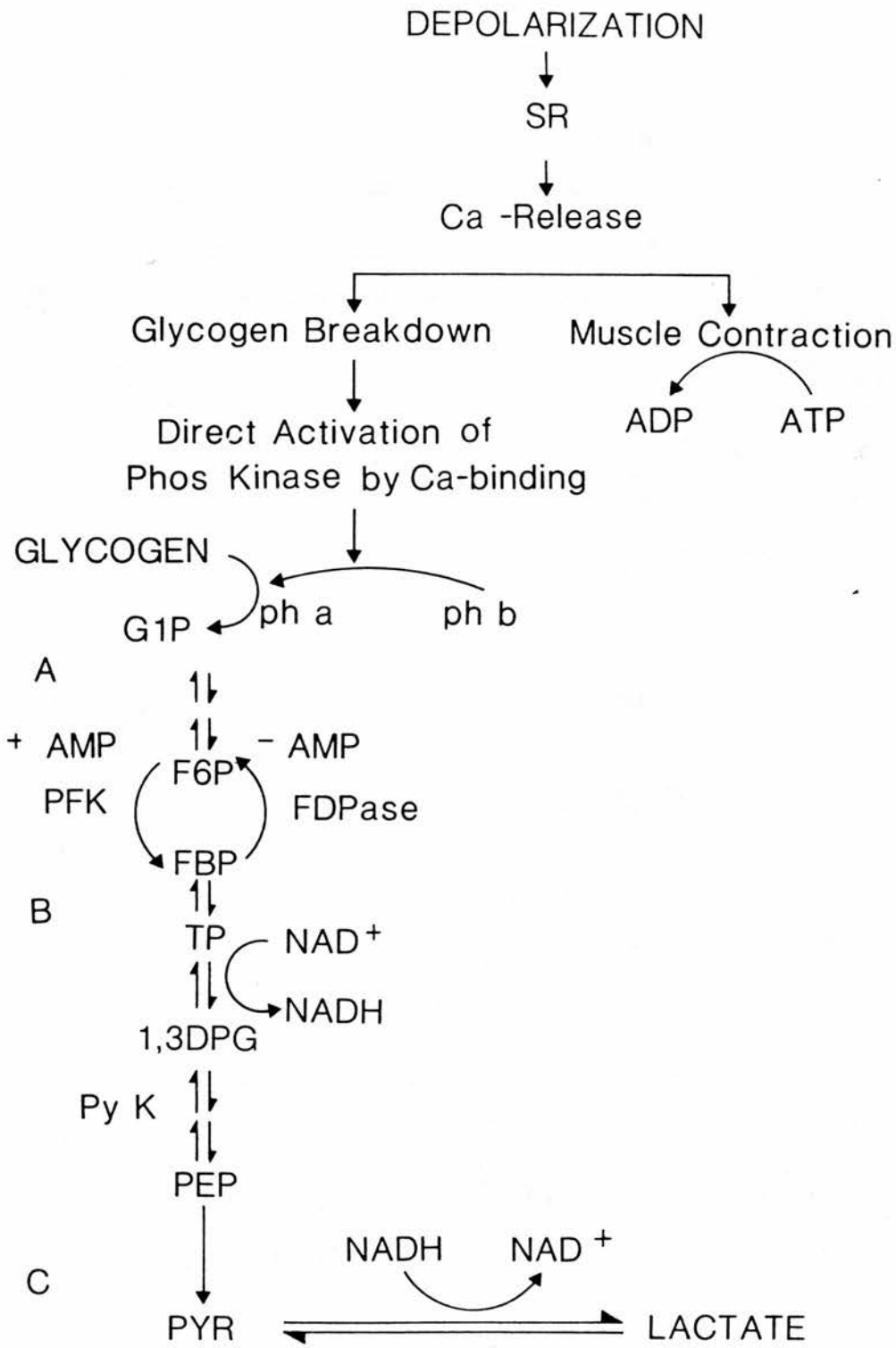

DEPOLARIZATION
SR
Ca -Release
Glycogen Breakdown
Muscle Contraction
ADP
ATP
Direct Activation of
Phos Kinase by Ca-binding
GLYCOGEN
ph a
ph b
G1P
A
+ AMP
- AMP
F6P
PFK
FDPase
FBP
B
TP
NAD+
NADH
1,3DPG
Py K
PEP
NADH
NAD+
C
PYR
LACTATE

Fig.3

TABLE 1: THE RELATIONSHIP BETWEEN THE PROPORTION OF RED OF THE MYOTOMAL MUSCLE AND ACTIVITY IN VARIOUS FISHES

| Species | % of red muscle in the caudal region | Remarks | Source |
|---|---|---|---|
| Elasmobranch<br>*Chimaera monstrosa* | 1 | Sluggish fish | Kryvi and Totland, 1977 |
| Wrasses<br>*Crenilabrus melops* | 2 | Sedentary | Greer-Walker and Pull, 1975 |
| Teleost<br>Antarctic cod<br>*Notochenia rossii* | 5.5 | Primarily uses enlarged pectoral fins | Walesby and Johnston, 1980 |
| Angler fish<br>*Lophius pistatorius* | 7.2 | Sedentary bottom dweller | Greer-Walker and Pull, 1975 |
| Elasmobranch shark<br>*Galeus melastomus* | 10 | Most active, cruise continuously | Totland et al., 1981 |
| Teleost<br>Skipjack tuna<br>*Katsuwonus pelamis* | 26 | Actively migrating open ocean species | Greer-Walker and Pull, 1975 |
| Teleost<br>Chub mackerel<br>*Scomber colias* | 29.8 | Active pelagic swimmer | Greer-Walker and Pull, 1975 |

TABLE 2: ULTRASTRUCTURE OF FISH MYOTOMAL MUSCLES

| Parameter | Muscle Fibre Type | | | |
|---|---|---|---|---|
| | Slow fibres (S) | | Fast fibres (FG) | |
| Z-disc | Thick | | Thin | |
| M-line | Present | | Present | |
| Myofibrillar volume % | 61.0 | Eel[a] | 80.4[a] | |
| | 63.0 | Perch[b] | 78.0[b] | |
| | 43.1 | Tench[c] | 73.1[c] | |
| | 46.0 | Brook trout[d] | - | |
| | 49.3 | Rainbow trout[g] | - | |
| Mitochondria per cent muscle fibre volume | 21.4 | Eel[a] | 1.2 | Eel[a] |
| | 22.0 | Perch[b] | 6.0 | Perch[b] |
| | 22.9 | Tench[c] | 4.5 | Tench[c] |
| | 31.3 | Brook trout[e] | 9.3 | Brook trout[e] |
| | 31.6 | Rainbow trout[g] | 9.5 | Rainbow trout[g] |
| Sarcoplasm reticulum per cent muscle fibre volume | 2.1 | Eel[a] | 6.0 | Eel[a] |
| | 4.4 | Tench[c] | 3.2 | Tench[b] |
| | 5.1 | Rainbow trout[f] | 13.7 | Rainbow trout[f] |
| T-system per cent myofibrillar volume | 0.31 | Eel[a] | 0.38 | Eel[a] |
| | 0.19 | Perch[b] | 0.27 | Perch[b] |
| | 0.29 | Tench[c] | 0.40 | Tench[c] |
| | 0.10 | Rainbow trout[f] | 0.40 | Rainbow trout[f] |

[a] Egginton and Johnston (1981b)
[b] Akster (1981)
[c] Johnston and Bernard (1982 )
[d] Johnston and Moon (1980a)
[e] Johnston and Moon (1981)
[f] Nag (1972)
[g] This thesis

CHAPTER 2.

# A STUDY OF GLYCOGEN, LACTATE AND GLUCOSE IN THE MYOTOMAL MUSCLES OF RAINBOW TROUT (SALMO GAIRDNERI, RICHARDSON) DURING SUSTAINED SWIMMING

## Introduction

The locomotory role of slow and fast fibres of the myotomal muscles is critically dependent on the intensity and duration of activity (Bone, 1966). Wardle (1975) has shown that small fish (~ 10 cm) maintain speeds in excess of 20 body lengths $s^{-1}$ during an initial acceleration (Wardle, 1975). Walesby and Johnston (1980) have suggested that the energy source for such initial bursts of acceleration is derived from the breakdown of phosphoryl creatine stores. Longer periods of swimming results in a shift of the energy source to anaerobic glycogenolysis. In rainbow trout (Salmo gairdneri) about 50% of fast fibre glycogen are catabolized following two minutes maximal swimming (Black et al., 1966). The hexokinase activities of trout fast fibres are comparable to those of the slow fibres of elasmobranchs and primitive teleosts; suggesting the potential for aerobic glucose utilization (Newsholme and Smart, 1973; Johnston and Moon, 1980c).

Salmonids are known to have a wide variation in both maximal metabolic rates and swimming performance (Beamish, 1978). The maximal oxygen consumption rates have been calculated to be in the range of 2 to 4 body lengths $s^{-1}$ (Webb, 1971). Small trout (~ 20 cm) has been shown to maintain speeds of up to 8 body lengths $s^{-1}$ for several hours. Therefore anaerobic metabolism contributes to a major proportion of the energy demands at the higher range of swimming speeds.

Lactate accumulation has been reported in both plasma (Black et al., 1961; Hammond and Hickman, 1966) and muscle (Johnston and Goldspink, 1973) at relatively low swimming speeds (< 4 body lengths $s^{-1}$). Although the fish may be in oxygen balance at these speeds it seems

likely that the fast fibres are operating anaerobically. Few of the previous studies on glycogen utilization and lactate accumulation gave details of swimming speeds studied. Conditioning of fish prior to exercise regime is also essential in order to avoid stress. For example, Johnston and Moon (1980b) showed that in brook trout glycogen and lactate levels were not statistically different between tank-rested and exercise-conditioned fish swimming at 3 L/S for 21 days. Previously measurement of whole-body lactate had been employed to assess the contribution of anaerobic metabolism in amphibia and reptiles (Bennett and Licht, 1972; Bennett, 1978, 1980). This technique has the advantage of eliminating the problems associated with the compartmentalised nature and transport of lactate from specific tissues (Bennett and Licht, 1972). In the present experiments measurement of whole-body metabolites has been employed to determine the time-course of glycogen, lactate and glucose concentrations in exercise-conditioned rainbow trout. In order to follow the specific changes in the slow and fast fibres freeze-clamped samples of these tissues were also analysed.

## Materials and Methods

### Fish:

Rainbow trout (Salmo gairdneri Richardson). Three groups of fish (mean ± S.E. length 13.59 ± 0.24 cm, mean weight 27.17 ± 1.54 g), (mean ± S.E. length 16.00 ± 0.53 cm, mean weight 53.93 ± 4.67 g), and (mean ± S.E. length 19.94 ± 0.73 cm, mean weight 83.77 ± 9.09 g), respectively, were obtained locally from the North East Fife Fish Farm and held in tanks of filtered fresh water. They were fed to excess daily on a proprietary brand of fish pellets.

Swimming tests:

Swimming experiments were carried out in an open-top flume (Figure 4) 150 cm long x 25 cm diameter, through which water could be moved at speeds ranging from 5 to 125 cm/sec previously described by Johnston and Moon (1980). Temperature in both holding tanks and exercise chamber were maintained at 10 ± 0.5°C. Small groups of 6-8 fish were introduced to the swimming chamber for a minimum period of two days before experiments. During this conditioning period the water flow was maintained at 15 cm $s^{-1}$ (1.1 body lengths $s^{-1}$). A resting sample was taken from fish swimming steadily at 1.1 body lengths $s^{-1}$.

The water flow was rapidly increased to between 2.3 and 7.0 body lengths $s^{-1}$ over 25 s and 35 s respectively and an initial exercised sample taken (Figure 5). Other groups of fish were allowed to swim for various periods at 2.3-7.0 body lengths $s^{-1}$ prior to sampling. Only fish that exhibited steady swimming were used for analysis. Fish which fell back to the restraining barrier were immediately removed from the chamber (5-10%) and discarded. Whole fish were freeze-clamped in flasks of liquid nitrogen (-159°C) to arrest metabolism. The lengths and weights of a total of 356 fish were recorded.

Preparation of muscle samples for metabolite analysis:

Fish were allowed to thaw to -20° and fish of group III were carefully dissected into two. The levels of glycogen and lactate has been shown to rise towards the tail in rainbow trout (Black et al., 1962). Hence this region has always been sampled in the present study to follow the specific changes in the red and white muscles. Following removal of the external myocommata, homogeneous samples (~ 100 mg) of these tissues were dissected from the mid-region, immediately underneath the second dorsal fin, of each side of the partially thawed carcass. The whole body and muscle samples from the left hand side were

immediately refrozen separately for glycogen analysis.

Fish of groups I and II were used for whole-body lactate analyses. The weighed muscle samples of the right hand side of group III fish were used for lactate and glucose analyses. These were cut into smaller bits and pulverised to a fine powder in a stainless steel pestle and mortar cooled in liquid nitrogen (-159°C). Metabolites were deproteinised in 5 volumes of 0.6 M perchloric acid ($HCLO_4$), homogenised with an Atomix M.S.E. or Ilado-X10 homogenizer (I.C.A. Gmbtt, Dotinger, West Germany) at 4°C and extracted for 10 min. Homogenates were centrifuged for 15 min at 6000 g and aliquots of the clear supernatant neutralized with 2 M $K_2CO_3$ in the presence of methyl orange indicator.

## Metabolites Assays

### Glycogen:

The glycogen content of the samples of myotomal muscle was determined colorimetrically at a wavelength of 620 nm following digestion of the weighed sample in 30% KOH at 100°C for 15 min. Glycogen was precipitated from an aliquot of the digest (Seifter et al., 1949) the resulting precipitate reacted with anthrone reagent (500 mg anthrone in 720 ml of $H_2SO_4$) as previously described by Carroll, Longley and Roe (1956).

### Lactate:

Lactate was determined enzymatically from the neutralised extract based on the reduction of pyridine nucleotide at 340 nanometers in a medium containing 150 mM hydrazine, 400 mM glycine buffer pH 9.5, 2.5 mM NAD, 20 µg lactate dehydrogenase (Hohorst, 1965).

### Glucose:

The glucose content of the neutralised extract was determined spectrophotometrically at a wavelength of 450 nanometers in a buffer-

enzyme medium containing 120 mM phosphate pH 7.0, 40 µg peroxidase/ml, 250 µg glucose oxidase/ml to which was added 5 mg O-dianisidine hydrochloride (Bergmeyer and Bernt, 1965).

Oxygen consumption of fish was estimated from data in the literature (Rao, 1968), corrected for body weight and temperature (Prosser, 1973).

## Statistical Analysis

Results for non-exercised and exercise conditioned fish were compared using one-way analyses of variance for equal sample numbers.

## Results

The concentrations of glycogen, lactate and glucose for the myotomal muscles of 84 gm rainbow trout are summarized in Figures 9-14. Total concentrations of glycogen (mg/100 g) were 73 and 57 in tank rested and exercise-conditioned (1.1 bodylengths $s^{-1}$) fish, respectively. Acceleration from 1.1 bodylengths $s^{-1}$ to a steady swimming speed of 2.8 or 4.0 bodylengths $s^{-1}$ (Figures 9a and 12a) resulted in glycogen depletion at a rate equivalent to 0.12 or 0.22 mg $g^{-1}$ $min^{-1}$. Glycogen levels continued to fall at these swimming speeds until 2 minutes when the concentrations stabilized. Following 20 minutes of continuous swimming, the glycogen concentrations were not significantly different from those prior to the increase in swimming speed.

Glycogen concentration of the slow fibres of tank rested fish was 805 mg/100 g. This is 3.4 times the concentration of the fast fibres ($P < 0.001$). Acceleration of exercise-conditioned (1.1 bodylengths $s^{-1}$) fish to the new swimming speed of 2.8 or 4.0 bodylengths $s^{-1}$ resulted in different rates of glycogen depletion in the slow and fast fibres. The rate of glycogen depletion in the slow fibres was equivalent to

0.34 and 0.43 mg, $g^{-1}$ $min^{-1}$ at 2.8 and 4.0 bodylengths $s^{-1}$, respectively (Figures 11a and 14a). The rate of decrease of glycogen in the slow fibres is equivalent to 2 - 2.5 times that of the fast fibres ($P < 0.05$) (Figures 10a and 11a). The decrease in glycogen concentration continued for 2 minutes at these cruising speeds after which the level stabilized.

The total glucose concentration (μmole $g^{-1}$) in tank-rested and exercise-conditioned fish were 0.72 and 1.61, respectively. After an initial decrease at the new swimming speed of 2.8 bodylengths $s^{-1}$, the glucose level gradually increased to a peak of 2.89 μmole $g^{-1}$ at 8 minutes (Figure 9c), while the glucose level at 4.0 bodylengths $s^{-1}$ continued to fall (Figure 12c). However, the values of both groups, after 20 minutes' continuous swimming, were not significantly different from the control group swimming at 1.1 bodylengths $s^{-1}$.

Glucose concentration in the fast fibres of tank rested and exercise-conditioned fish were not statistically different, 1.06 and 0.95 μmole $g^{-1}$, respectively. In contrast to the slow fibres, the level of glucose gradually increased for 2 minutes to 3.34 μmole $g^{-1}$ at 2.8 bodylengths $s^{-1}$. This was equivalent to 40% higher than the corresponding level at 4.0 bodylengths $s^{-1}$ (Figure 10c). The concentrations then decreased gradually. After 20 minutes the glucose concentration at 2.9 bodylengths $s^{-1}$ was not statistically different from the control fish at 1.1 bodylengths while that at 4.0 bodylengths $s^{-1}$ was 30% lower (Figure 13c).

The concentration of glucose in the slow fibres of exercise-conditioned fish was equivalent to 3.7 times that of the fast fibres ($P < 0.001$). There was an initial gradual decrease of glucose level to 1.95 μmole $g^{-1}$ in 2 minutes at 2.8 bodylengths $s^{-1}$ (Figure 11c). This was followed by a rise in concentration but the trend at 4.0 bodylengths $s^{-1}$ showed a consistent decrease (Figure 14c). After 20 minutes' exercise regime the value at 2.8 bodylengths $s^{-1}$ was 20% lower than the

zero time while that at 4.0 bodylengths $s^{-1}$ was only equivalent to 30% the value at zero time ($P < 0.001$).

The 24 hour time course of total body lactate concentrations of 54 g trout swimming at 4.0 bodylengths $s^{-1}$ is shown in Figure 5. Total lactate concentrations (μmoles, $g^{-1}$) were 8.55 and 7.88 in tank-rested and exercise-conditioned (1.1 bodylengths $s^{-1}$) rainbow trout, respectively. Acceleration from 1.1 to 4.0 bodylengths $s^{-1}$ resulted in an increase of lactate in the whole carcass of 12.65 μmoles, $g^{-1}$. This represents rate of lactate rise of 4.77 μmoles $g^{-1}$ $min^{-1}$. At this swimming speed total lactate concentrations continued to increase for about 2 minutes and then gradually declined. Following 24 hour continuous swimming lactate level was 10.55 μmoles $g^{-1}$ (Figure 5). This is significantly higher than that observed in fish swimming steadily at 1.1 bodylengths $s^{-1}$ for 48-72 hr (Figure 5) ($P < 0.05$).

Net total body lactate concentrations of 27 g trout following 30 minutes' swimming at either 3.8 or 7.0 bodylengths $s^{-1}$ are shown in Figure 6. Acceleration from 1.1 bodylengths $s^{-1}$ to a new swimming speed of 3.8 or 7.0 bodylengths $s^{-1}$ is associated with a rate of initial lactate production of 6.77 and 10.1 μmoles $g^{-1}$ $min^{-1}$, respectively. In fish of this size the lactate concentrations continue to rise for a minimum of 5 minutes at the lower swimming speed following the attainment of the new speed (Figure 6). The peak of lactate concentration reaches 24 μmoles $g^{-1}$ after 5 minutes swimming at 7.0 bodylengths $s^{-1}$ (Figure 6).

The maximum period at which lactate concentrations continued to rise in 27 g fish between 2.3 and 7.0 bodylengths $s^{-1}$ was 5 minutes (Figure 6). Hence the initial rate of lactate production has been determined at these swimming speeds (Figure 7). Following longer periods of swimming the catabolism of lactate results in a fall of total body lactate (Figures 6 and 7). Figure 8 represents $\log_{10}$ of the net

lactate production during the first 5 minutes. This was found to be linearly related to the swimming speed ($P < 0.001$) and the form $y = 0.30x - 0.63$. The data is plotted in terms of ATP equivalents assuming an ATP yield of 0.016 mmoles ATP per mg lactate (Bennett and Licht, 1972). ATP production from aerobic metabolism (Figure 8, upper graph) was calculated from literature data (Roa, 1968), following appropriate correction for body weight employing the equation $M = kW_B^{0.78}$ and for temperature assuming a Q10 relationship for aerobic metabolism of 2.1 (Prosser, 1973) (Table 4). The initial rate of ATP generation from anaerobic glycolysis during the first 5 minutes of swimming were 39 and 74% of the total energy requirements calculated from steady state values (Table 4).

## Discussion

A major problem associated with studying metabolism in swimming fish is the influence of stress. Many environmental factors cause stress such as the methods of capture (Bouck and Bell, 1966; Wardle, 1972), transportation (Black and Barrett, 1957; Fraser and Beamish, 1969) and handling in the laboratory (Black and Barrett, 1957; Stevens, 1972; Driedzic and Kiceniuk, 1972). Stress produces a haemoconcentration, glycogen depletion, elevated blood lactate and glucose concentration (Soivio et al., 1976). For example, after capture by trawl, hyperglycaemia in plaice (Pleuronectes platessa L.) lasted 2-3 days (Wardle, 1972), and non-release of lactate in muscles of plaice following stress (Wardle, 1978). Handling led to a sharp rise in blood lactate which required 12 h to return to the initial level (Soivio et al., 1976). Therefore when stressed fish are used for swimming exercise studies there is a further rise in lactate or depletion of glycogen concentration which may influence the results. For example, early

studies have shown blood lactate increase from 2-5 fold above resting levels after swimming at sustainable speeds for periods ranging from 30 minutes to 24 hours (Black, 1955, 1957a,b,c; Black et al., 1960, 1962, 1966). These results are in contrast to fish which had been pre-conditioned before the swimming regime. For example, Hammond and Hickman (1966) demonstrated that rainbow trout (Salmo gairdneri) which had been conditioned to flowing water were able to maintain higher levels of blood lactate and remove it from the blood quicker than unconditioned trout. Hochachka (1961) has shown that glycogen levels in trained fish fell by 70% following 5 minutes swimming but only by 40% in untrained fish. Brook trout (Salvelinus fontinalis Mitchill), exercise-conditioned at 3.0 bodylengths $s^{-1}$ for 21 days showed no significant difference in the glycogen and lactate levels from tank rested fish (Johnston and Moon, 1980). Other investigators have used similar procedures for training other fish species (Greer Walker, 1971; Greer Walker and Pull, 1973; Davison and Goldspink, 1977; Greer Walker and Emerson, 1978).

In the present experiments rainbow trout were conditioned to a swimming speed of 1.1 bodylengths $s^{-1}$ for a minimum of two days. The water velocity was gradually increased over a period of 25 seconds and another 35 seconds was required to enable the fish to settle down to a more steady mode of swimming. Fish which could not maintain a steady mode of swimming darted irregularly and were driven to the back of the restraining barrier. These fish were excluded from the experiments. The normal pattern of glycogen utilization in the fast and slow fibres of accelerating fishes may differ from normal cruising fishes. Fish which maintained steady swimming were plunged into liquid nitrogen ($-159^{o}C$) to arrest metabolism and prevent post mortem changes in exercised and non-exercised fish. This reduces any chance of recovery from exercise which may occur if the fish are anaesthetized or killed in any other way.

The concentrations of glycogen in the muscle fibres of tank rested fish is 3.4 times higher in the superficial red than the deep white fibres ($P < 0.001$). Similar results have been reported for other teleosts (Johnston and Goldspink, 1973a,b; Pritchard et al., 1971). In contrast, tuna fast fibres contain a higher concentration of glycogen in the white than the red fibres (Hulbert, Guppy and Murphy, 1979).

The results in this study have demonstrated a total glycogen depletion rate of 0.12 and 0.22 mg $g^{-1}$ $min^{-1}$ at 2.8 and 4.0 bodylengths $s^{-1}$ respectively. The rate of glycogen depletion in the slow fibres was 0.34 mg $g^{-1}$ $min^{-1}$ at 2.8 bodylengths $s^{-1}$ and 0.43 mg $g^{-1}$ $min^{-1}$ at 4.0 bodylengths $s^{-1}$. This is equivalent to 2-2.5 times that of the fast fibres ($P < 0.05$). Similar values have been obtained on the crucian carp (Carassius carassius L.) (Johnston and Coldspink, 1973a,b). The involvement of higher proportion of fast fibres at increasing swimming speed has been demonstrated in Figures 10a and 13a. Following 2 minutes of sustained swimming at 2.8 and 4.0 bodylengths $s^{-1}$ glycogen content reduced by 50 and 67%, respectively. The involvement of both muscle types over a whole range of swimming speeds has been established for a number of other teleost species (Pritchard, Hunter and Lasker, 1971; Smit et al., 1971; Johnston and Goldspink, 1973a,b; Greer Walker and Emerson, 1978). For example, electromyographical evidence has shown that the threshold speed for the recruitment of fast myotomal muscle fibres in rainbow trout (26-34 cm) is 1.3 bodylengths $s^{-1}$ (Hudson, 1973) to 2-2.5 bodylengths $s^{-1}$ (Bone et al., 1978) (17-30 cm fish). This is in contrast to the electromyographical evidence from dogfish (Scyliorhinus canicula) (Bone, 1966) and the Pacific herring (Clupea pallensia) (Bone et al., 1978) which show that slow fibres alone are reserved for sustained activity in these species, while the fast fibres are almost entirely reserved for burst swimming. In contrast to herring and dogfish the more advanced teleosts have multiply innervated

fast muscles. This type of innervation is associated with an involvement of the fast muscle in sustained activity (Totland et al., 1981). It appears that the aerobic capacity of fast fibres in some teleosts is sufficient to support the contraction associated with slow swimming speeds. For example, in the older literature it was thought that in salmonids and carp a proportion of slow fibres were interspersed amongst the deep fast fibres (Green, 1912; Boddeke et al., 1959). This was due to the fact that histological techniques showed a higher glycogen and lipid content of the smaller fibres in the mosaic. However recent studies have shown that these small fibres are in fact fast fibres with greater aerobic capacity than the larger fast fibres. For example, Johnston et al. (1975a) have demonstrated by histochemical techniques homogeneous ATPase and higher succinic dehydrogenase activity, and glycogen content (Johnston, 1981a) in the smaller fast fibres of the mosaic. Nag (1972) has reported that the mitochondrial content of fast and slow fibres in rainbow trout is 3:8 (by number). This indicates that the mitochondrial content of fast fibres is a significant fraction of that of slow fibres. Furthermore, Egginton and Johnston (1981) have demonstrated that fast fibres are heterogeneous, not only with respect to size, but also according to their positions within the myotome. The peripheral fast fibres are smaller and have a higher mitochondrial content or glycogen than the deep fast fibres (Egginton and Johnston, 1981). An assessment of the oxidative enzyme activities such as citrate synthetase and cytochrome oxidase has indicated that fast fibres of brook trout have 15-20% of the activity of the slow fibres (Johnston and Moon, 1980). In this species the hexokinase activities of the fast fibres are twice those of the slow fibres, reflecting a great potential for aerobic glucose utilization. This is in agreement with the findings in the present study. Figures 9c-14c demonstrate glucose mobilization with time. As glucose is in equilibrium with other carbohydrate

sources during swimming no quantitative information on glucose turnover can be obtained. Smaller fast fibres can supply a significant proportion of the energy requirements aerobically at low sustained speeds (< 3 bodylengths $s^{-1}$). Since recruitment of any fast fibre neurone even at low speeds will activate a spectrum of fibre sizes (Altringham and Johnston, 1981), the larger fast fibres are also likely to operate. Therefore in fast fibres with low mitochondrial content (e.g. 0.2-1.5%) it seems probable that this is only sufficient for resting metabolism such as protein turnover and ion pump operation. All energy demands for contractile activity would have to be met by anaerobic glycogenolysis with a concomitant increase in lactate production.

Hudson (1973) has suggested a rotation of firing of fast fibre motor units which depends on the swimming speeds. Davison and Goldspink (1977) have expressed the view that as a result of the close proximity of the smaller aerobic fast fibres of the mosaic to the larger ones, enough oxygen could be reaching the latter to allow oxidation of lipid to occur. It is likely that there is a net production of lactate at speeds below which the fish reaches oxygen balance. This results in transient increase of total lactate concentrations (Figures 5 and 6), followed by a subsequent fall as steady state conditions are attained. At this point lactate produced by anaerobic glycogenolysis in the fast muscle is catabolized at an equal rate probably by oxidative decarboxylation in the peripheral tissues such as red muscle.

Interestingly, the $\log_{10}$ of net lactate accumulation during the first 5 minutes swimming is linearly related to swimming speed (Figures 7 and 8). A similar logarithmic relationship to swimming speed has been previously observed with respect to both oxygen consumption (Beamish, 1978) and power output (see Webb, 1975). This reflects the way in which drag forces on fish increases with increasing speed.

Increase in speed requires proportional increase in performance and effort. This results in the recruitment of an increasing number of fast fibres.

Measurements of whole body lactate do not provide an estimate of anaerobic metabolism during long periods of sustained swimming. Experiments with small mammals and lizards have shown that 1-2 minutes are required for oxygen consumption to stabilize at a new level following an increase in running speed (Gollnick et al., 1973; Bennett, 1980). In salmon, this period is somewhat longer and depends on temperature and the increment between swimming speeds (Brett, 1972; Beamish, 1978). The actual oxygen consumption of fish in the present study is unknown. But steady state values of oxygen consumption can be determined from data in the literature and corrected from differences in body weight and temperature (see Methods). The ATP yield is assumed for anaerobic glycolysis of 0.016 mmoles ATP per mg lactate and that 1 mg $O_2$ yields 0.20 mmol ATP (Bennett and Licht, 1972) it is possible to determine the anaerobic contribution to energy expenditure as a percentage of the steady state level of aerobic metabolism (Tables 3 and 4). This varies from 39% at 2.3 bodylengths $s^{-1}$ to 74% at 7.0 bodylengths $s^{-1}$ (Table 4). It appears that the contribution of anaerobic metabolism to total energy requirements declines with time as oxygen uptake increases and the fish settle down to a more steady mode of swimming.

As increases in net lactate concentration are transient it is expected that a definite time is required to achieve maximal rates of lactate catabolism.

These results show that glycogen replenishment following muscular exercise takes place faster during slow or moderate speeds, of exercise conditioned fish than at vigorous speeds. For example, Black et al. (1962) obtained glycogen depletion in the myotomal muscle at a rate

equivalent to 0.7 mg $g^{-1}$ $min^{-1}$ for rainbow trout swimming strenuously for 2 minutes. A mean rate of glycogen depletion of 1.29 mg $g^{-1}$ muscle has been obtained in Jack mackerel (Trachurus symmetricus) swimming at 9 bodylengths $s^{-1}$ until exhaustion (Pritchard et al., 1971). At these high speeds the replenishment of glycogen takes up to 12-16 hours (Black et al., 1961, 1966). In the present study rainbow trout were conditioned to moderate speeds (1.1-1.3 bodylengths $s^{-1}$). The level of glycogen and lactate were not significantly different from tank-rested trout. Similar results have been obtained on other teleosts species (Johnston and Moon, 1980a,b). This increase in muscle glycogen stores and the capacity to catabolize lactate has been suggested as mainly due to increased activities of fatty acid oxidation enzymes in the fast and slow muscles induced by training. Utilization of lipid fuels is enhanced hence glycogen stores are relatively spared (Johnston and Moon, 1980a). This is likely to be a major factor in improved swimming performance and fatigue resistance in exercise-conditioned fish.

Fig. 4

Swimming chamber employed in the exercise experiments. The inner perspex cylinder (25 cm diameter x 150 cm length) contained in a tank of marine plywood ¾", coated with araldite paint, and an outer box filled with 1½" of plastic foam.

Abbreviations:

a, aeration; cf, activated charcoal filter; s, baffles for reducing turbulence; w, water-tight shaft bearing; p, plastic blade propeller; he, glass heat exchanger; m, high power variable speed electric motor.

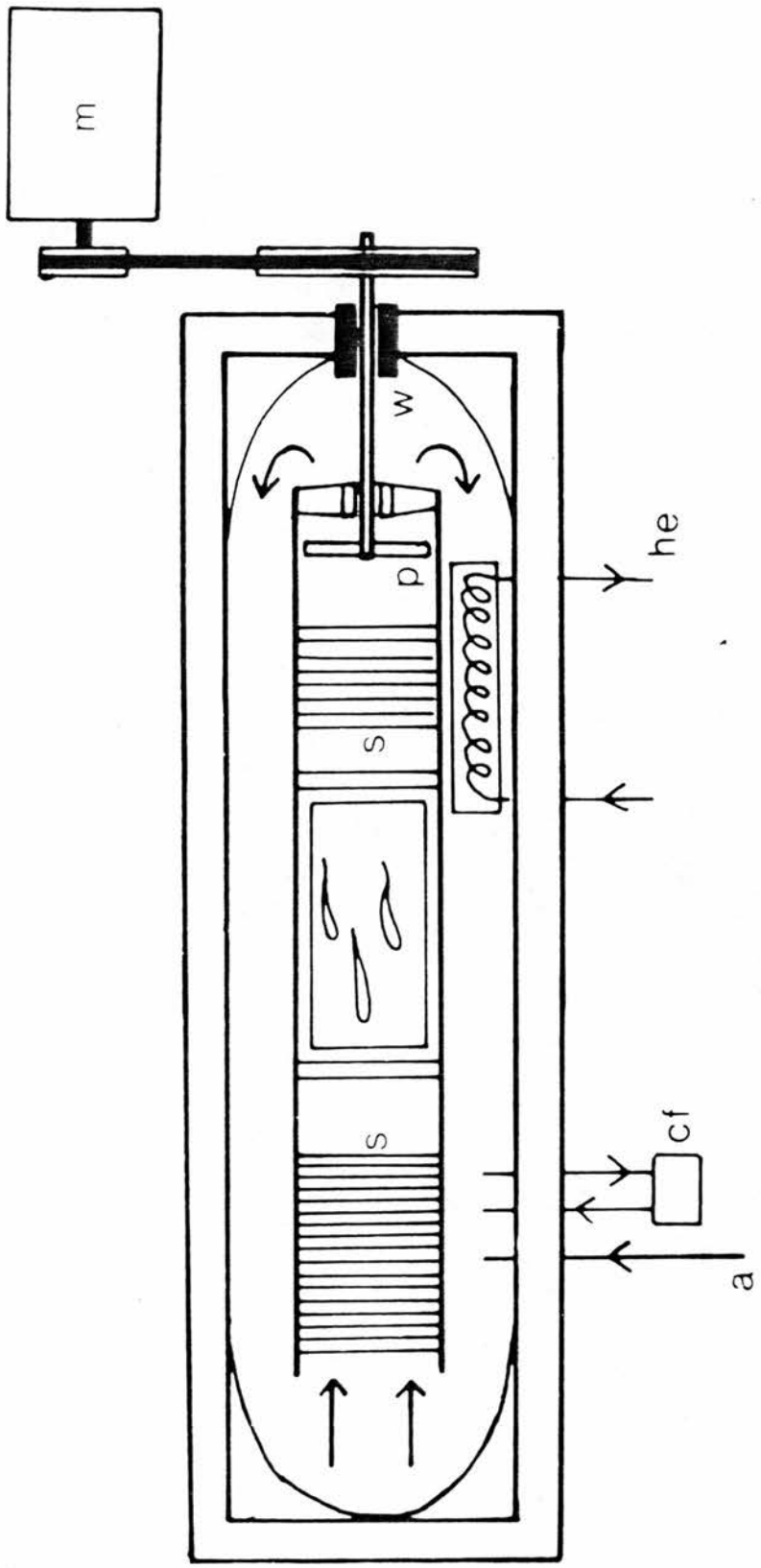



Fig.4

Fig. 5

Total body lactate concentrations (μmoles, $g^{-1}$) in 54 g rainbow trout exercised at 4.0 bodylengths $s^{-1}$ (mean ± S.E., seven fish).

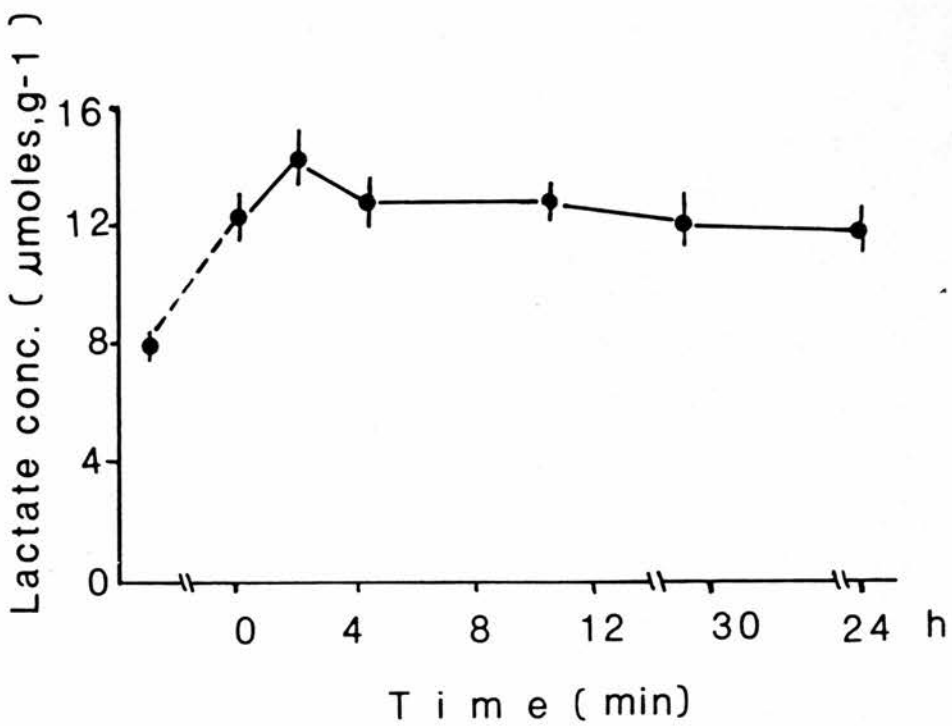



Fig.5

Fig. 6

Total body lactate concentrations (μmoles, $g^{-1}$) in 27g rainbow trout exercised at either 3.8 bodylengths $s^{-1}$ (open circles) or 7.0 bodylengths $s^{-1}$ (closed circles) (mean ± S.E., nine fish).

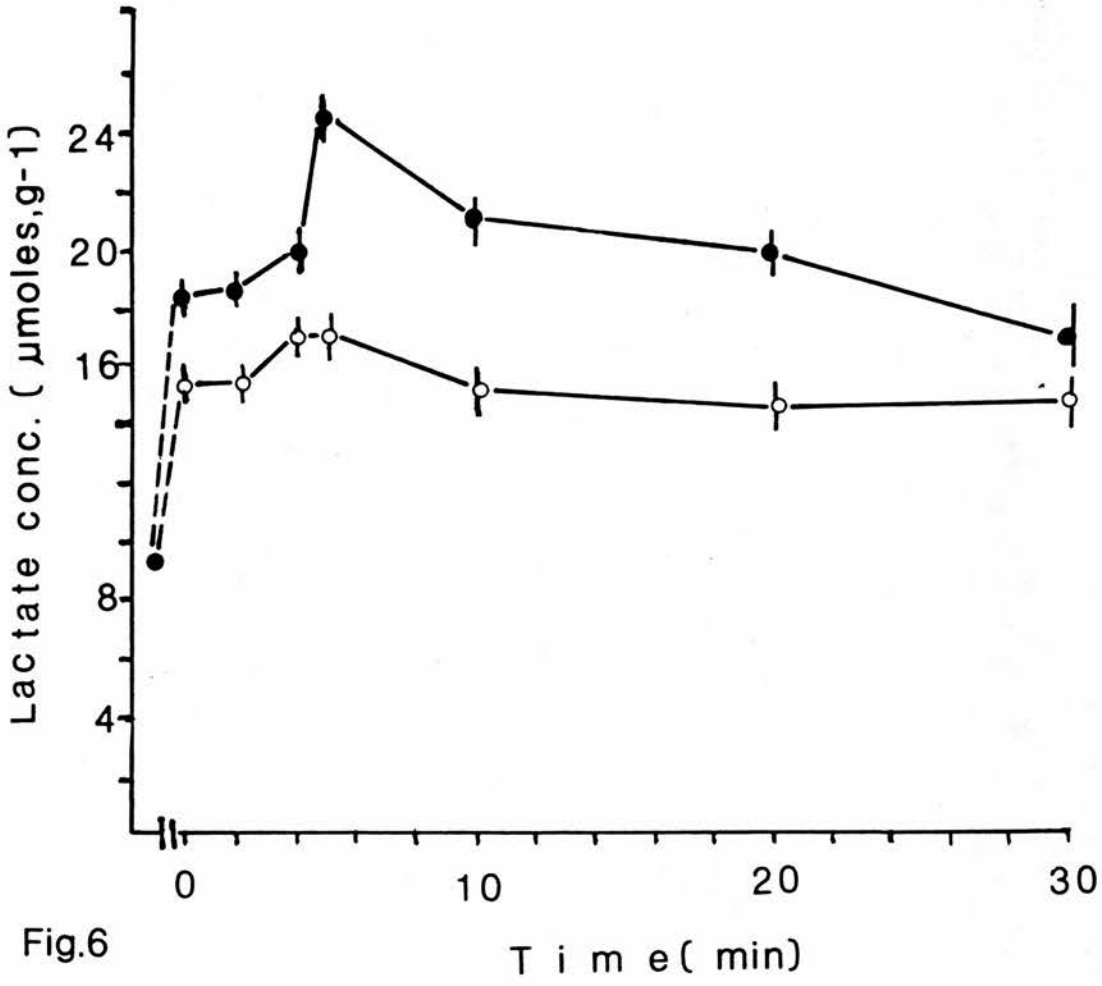



Fig.6

Fig. 7

Total body lactate concentrations (μmoles, $g^{-1}$) in 27 g rainbow trout following acceleration from 1.1 to either, 2.3, 3.8, 5.3, 6.1 or 7.0 bodylengths $s^{-1}$ and after 5 minutes steady swimming at these speeds (mean ± S.E., eleven fish).

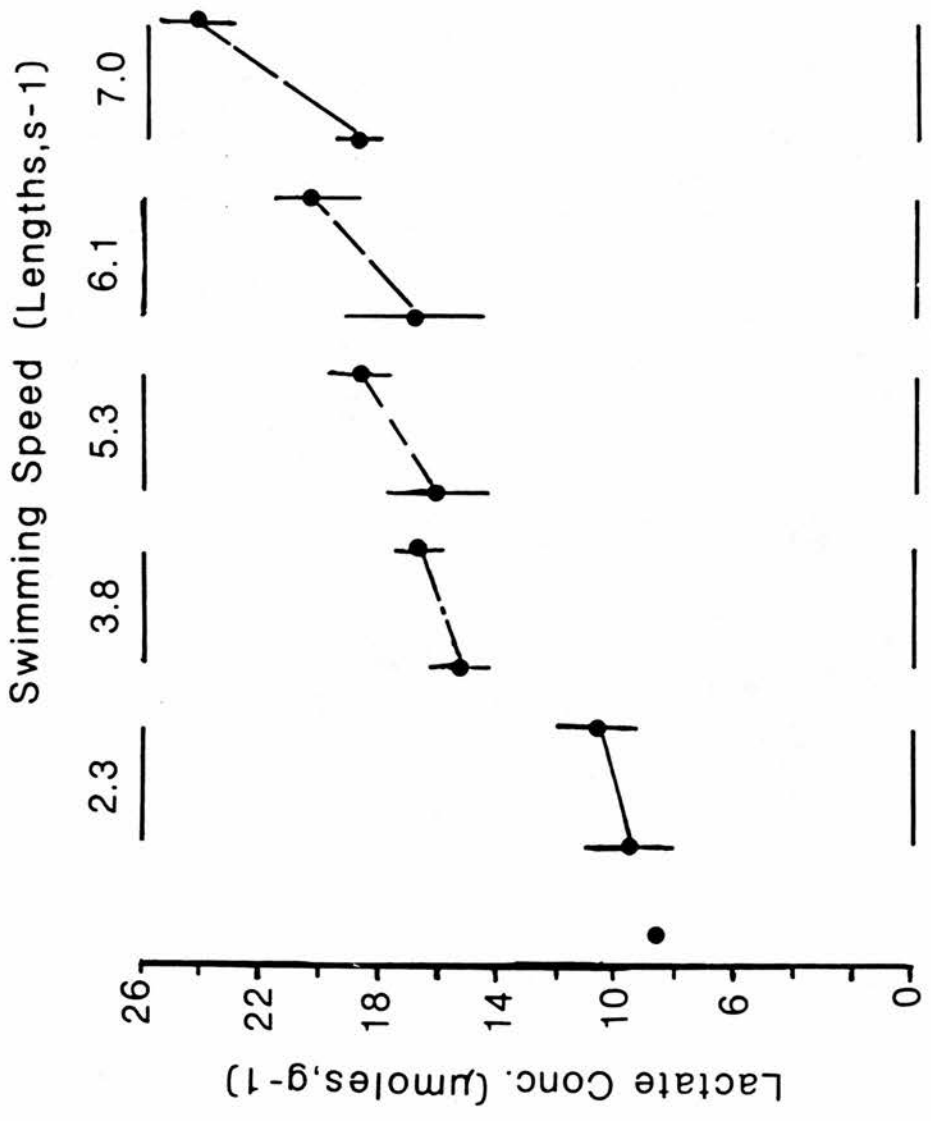

Swimming Speed (Lengths,s-1)
2.3
3.8
5.3
6.1
7.0
Lactate Conc. (μmoles,g-1)
26
22
18
14
10
6
0
0 5 0 5 0 5 0 5 0 5
T i m e ( min)

Fig.7

Fig. 8

Rate of production of ATP equivalents ($\mu$moles, $g^{-1}$, $min^{-1}$) from aerobic (upper graph) and anaerobic (lower graph) metabolism in 27g trout at various swimming speeds. Details of the methods of calculation are given in the text.

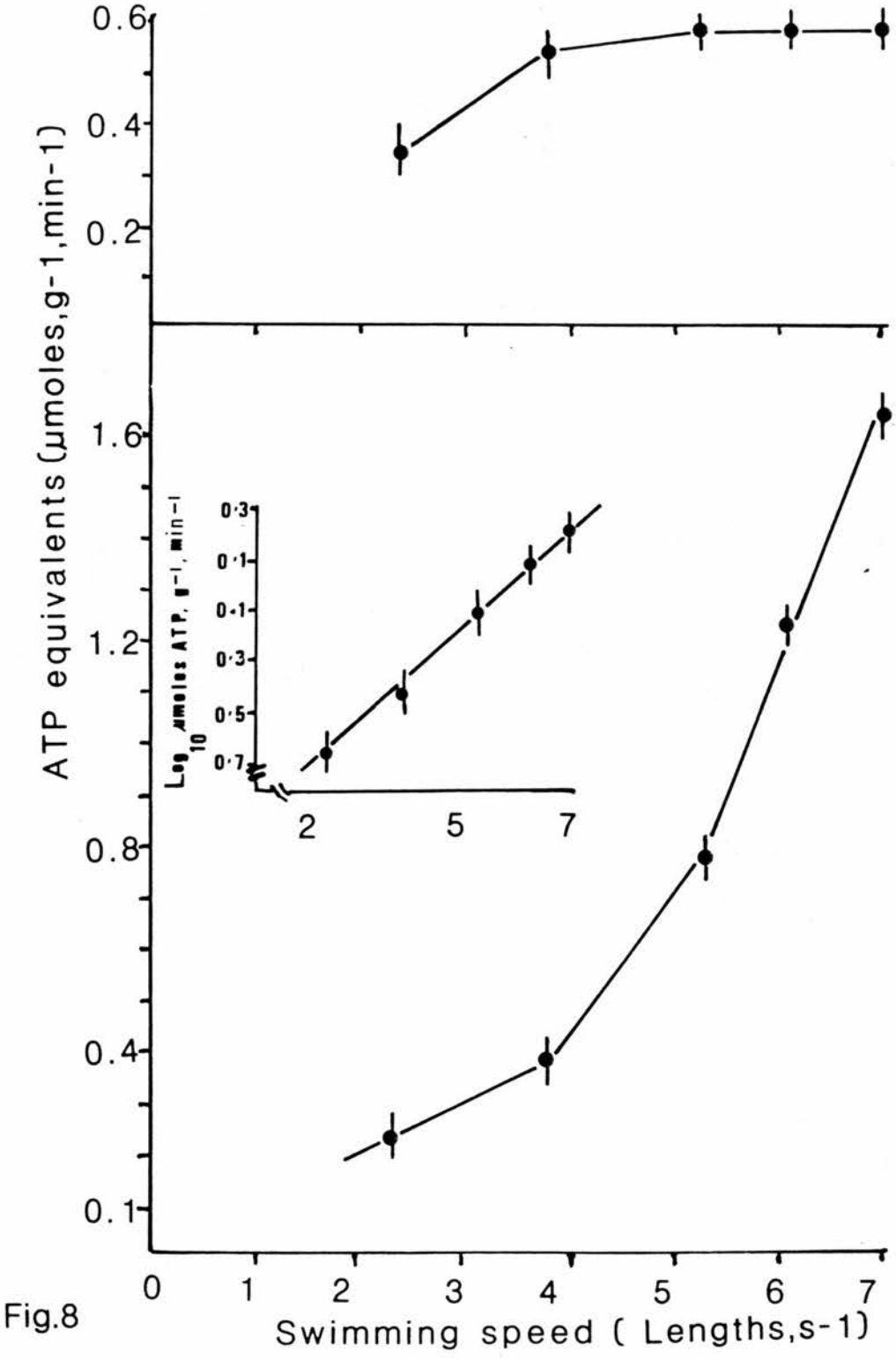

ATP equivalents (µmoles,g-1,min-1)
0.6
0.4
0.2
1.6
1.2
0.8
0.4
0.1
0
1
2
3
4
5
6
7
Swimming speed ( Lengths,s-1)
2
5
7

Fig.8

Fig. 9a,b,c

Concentrations of total body levels of glycogen, lactate and glucose (mg, 100 $g^{-1}$, μmoles, $g^{-1}$, μmoles, $g^{-1}$) in 84 g rainbow trout exercised at 2.8 bodylengths $s^{-1}$ (mean ± S.E., nine fish).

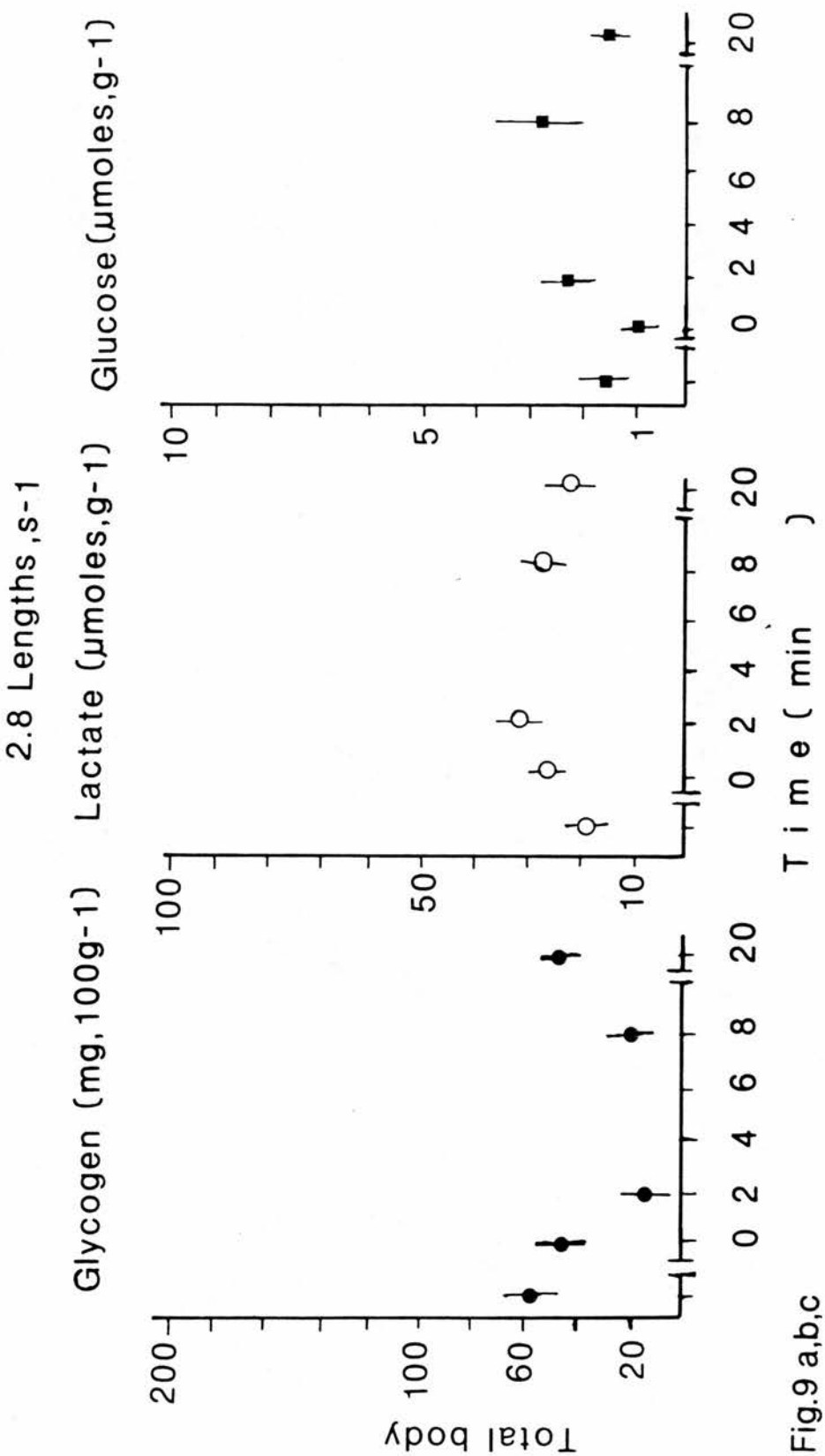

2.8 Lengths ,s-1
Glycogen (mg,100g-1)
Lactate (µmoles,g-1)
Glucose (µmoles,g-1)
Total body
200
100
60
20
100
50
10
10
5
1
0 2 4 6 8 20
0 2 4 6 8 20
0 2 4 6 8 20
T i m e ( min )

Fig.9 a,b,c

Fig. 10a,b,c

Concentrations of glycogen, lactate and glucose (mg, 100 $g^{-1}$, μmoles, $g^{-1}$; μmoles, $g^{-1}$ respectively) in the fast muscles of 84 g rainbow trout exercised at 2.8 bodylengths $s^{-1}$ (mean ± S.E., nine fish).

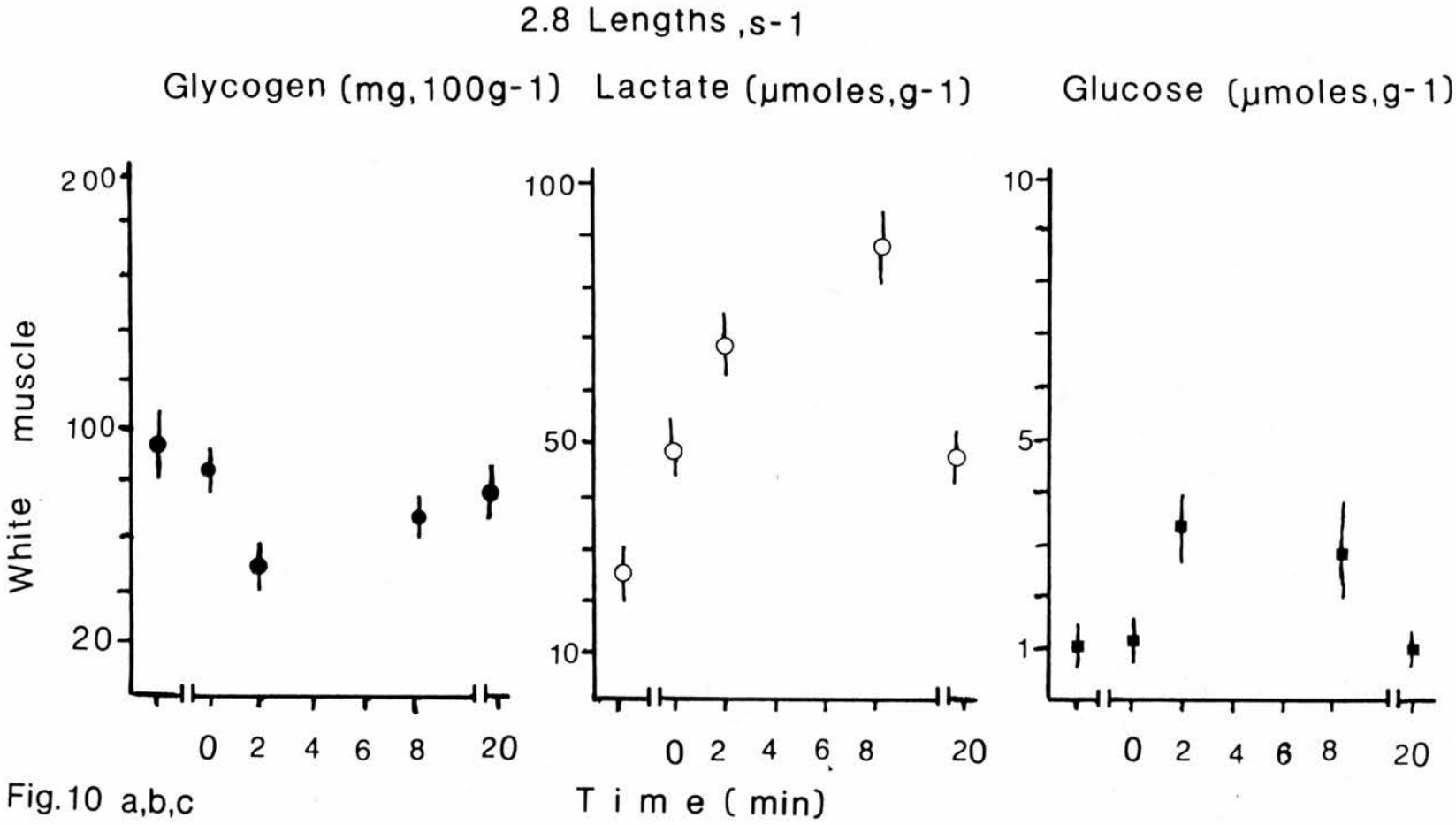

2.8 Lengths ,s-1
Glycogen (mg,100g-1)
Lactate (µmoles,g-1)
Glucose (µmoles,g-1)
White muscle
200
100
20
100
50
10
10
5
1
0 2 4 6 8 20
0 2 4 6 8 20
0 2 4 6 8 20
T i m e ( min)

Fig.10 a,b,c

Fig. 11a,b,c

Concentrations of glycogen, lactate and glucose (mg 100 g, μmoles, $g^{-1}$; μmoles, $g^{-1}$ respectively in the slow muscles of 84 g rainbow trout exercised at 2.8 bodylengths $s^{-1}$ (mean ± S.E., nine fish).

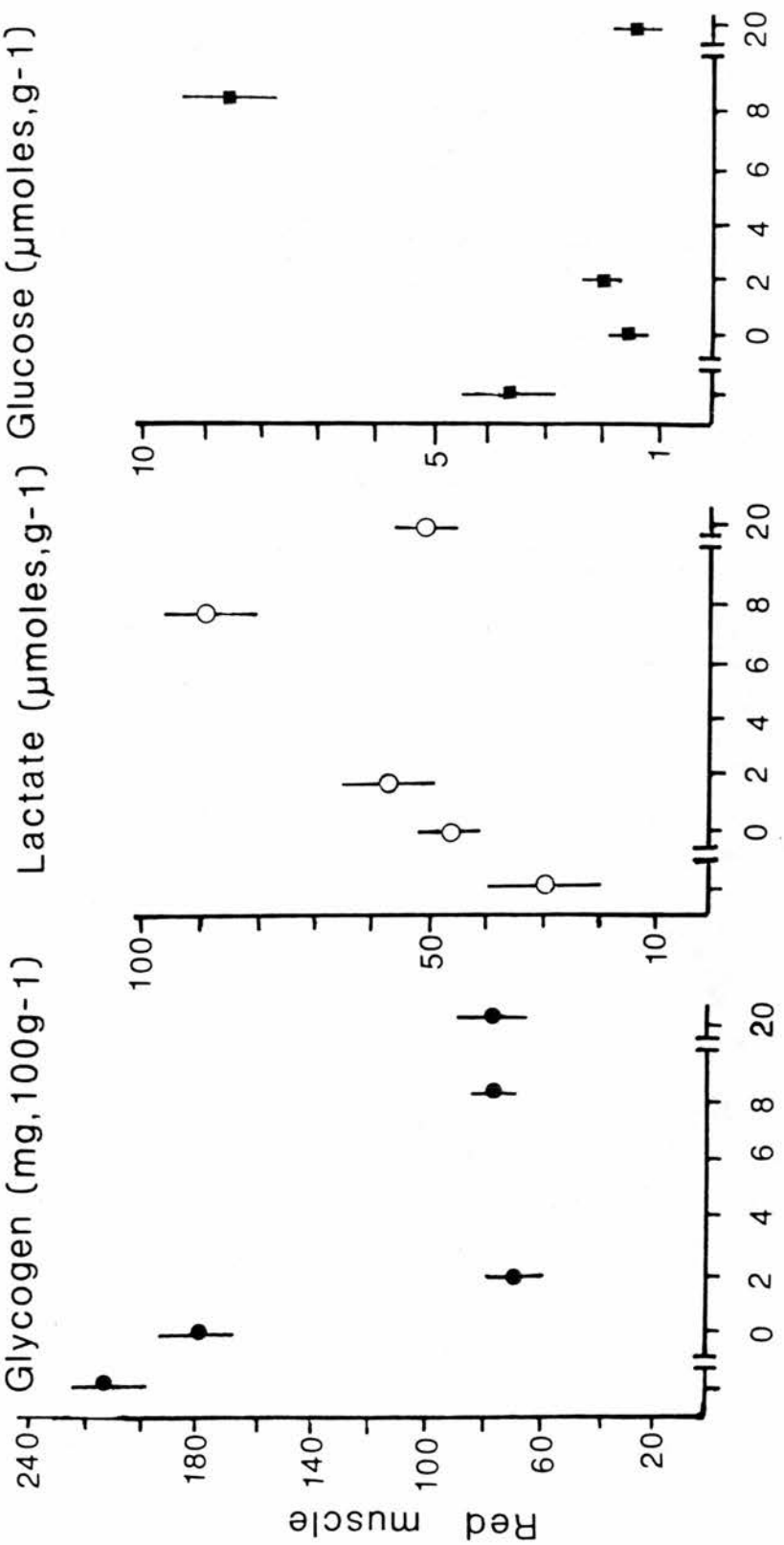

2.8 Lengths ,s-1
Glycogen (mg,100g-1)
Lactate (µmoles,g-1)
Glucose (µmoles,g-1)
Red muscle
240
180
140
100
60
20
100
50
10
10
5
1
0 2 4 6 8 20
0 2 4 6 8 20
0 2 4 6 8 20
T i m e ( min)

Fig.11 a,b,c

Fig. 12a,b,c

Concentrations of total body levels of glycogen lactate and glucose (mg, 100 $g^{-1}$; μmoles, $g^{-1}$; μmoles, $g^{-1}$ respectively) in 84 g rainbow trout at 4.0 bodylengths $s^{-1}$ (mean ± S.E., nine fish).

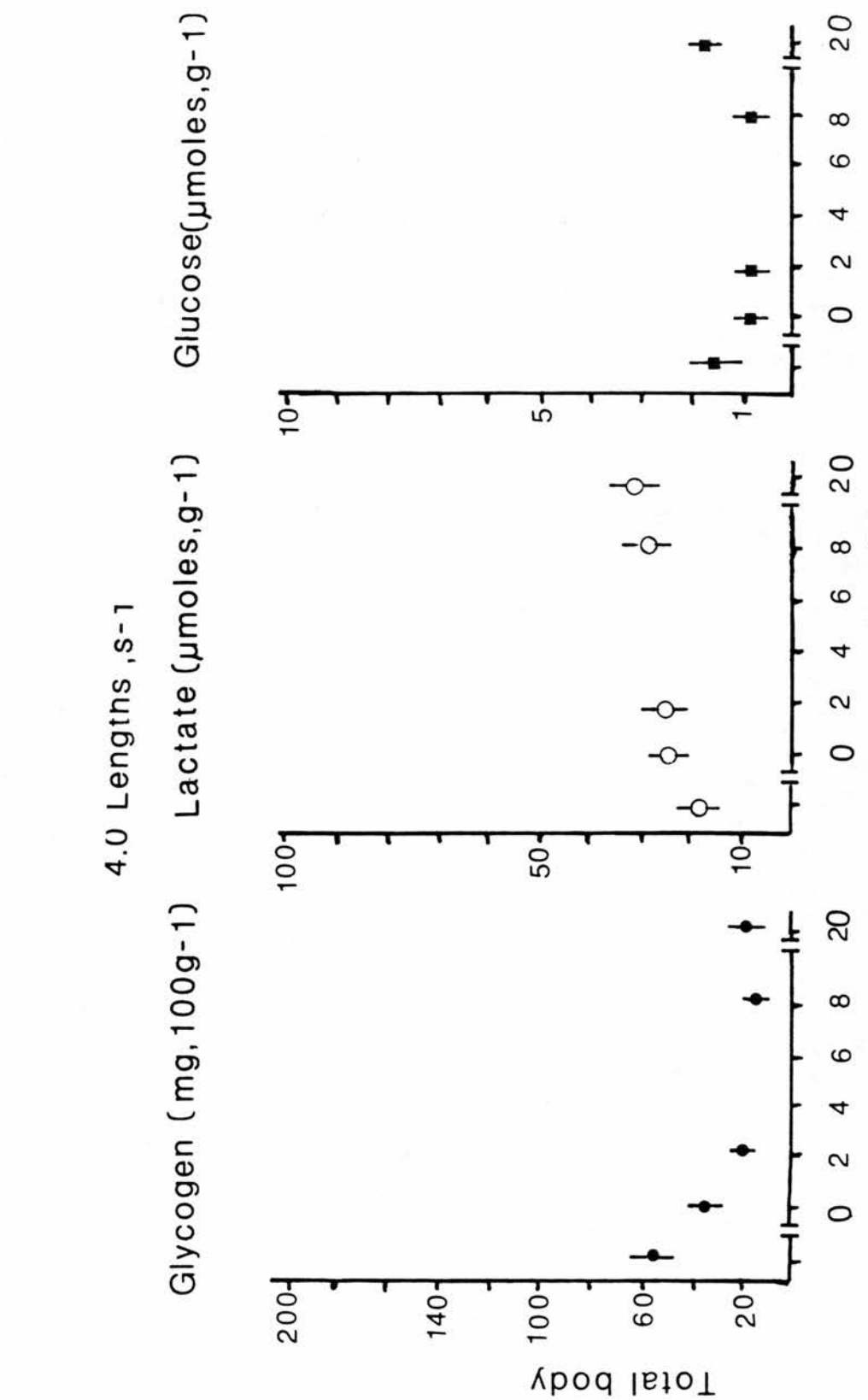

4.0 Lengths ,s-1
Glycogen (mg,100g-1)
Lactate (μmoles,g-1)
Glucose(μmoles,g-1)
Total body
200
140
100
60
20
100
50
10
10
5
1
0 2 4 6 8 20
0 2 4 6 8 20
0 2 4 6 8 20
Time (min)

Fig.12 a,b,c

Fig. 13a,b,c

Concentrations of glycogen, lactate and glucose, lactate and glucose (mg, 100 $g^{-1}$; μmoles, $g^{-1}$; μmoles, $g^{-1}$ respectively), in fast muscles of 84 g rainbow trout at 4.0 bodylengths $s^{-1}$ (mean ± S.E., nine fish).

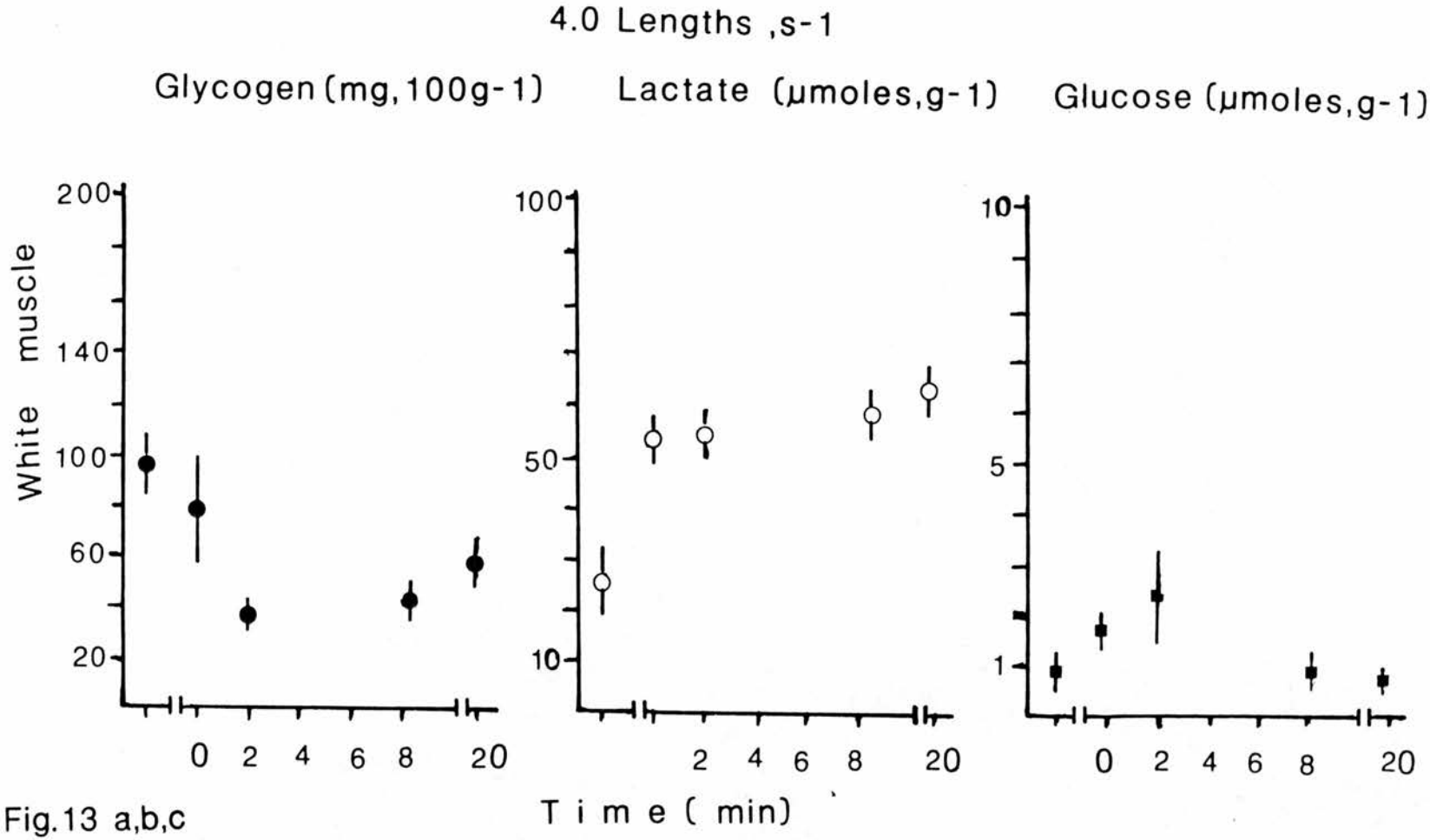

4.0 Lengths ,s-1
Glycogen (mg,100g-1)
Lactate (µmoles,g-1)
Glucose (µmoles,g-1)
White muscle
200
140
100
60
20
100
50
10
10
5
1
0 2 4 6 8 20
2 4 6 8 20
0 2 4 6 8 20
T i m e ( min)

Fig.13 a,b,c

Fig. 14a,b,c

Concentrations of glycogen lactate and glucose (mg, 100 $g^{-1}$; μmoles, $g^{-1}$; μmoles, $g^{-1}$ respectively) in slow muscles of 84 g rainbow trout at 4.0 bodylengths $s^{-1}$ (mean ± S.E., nine fish).

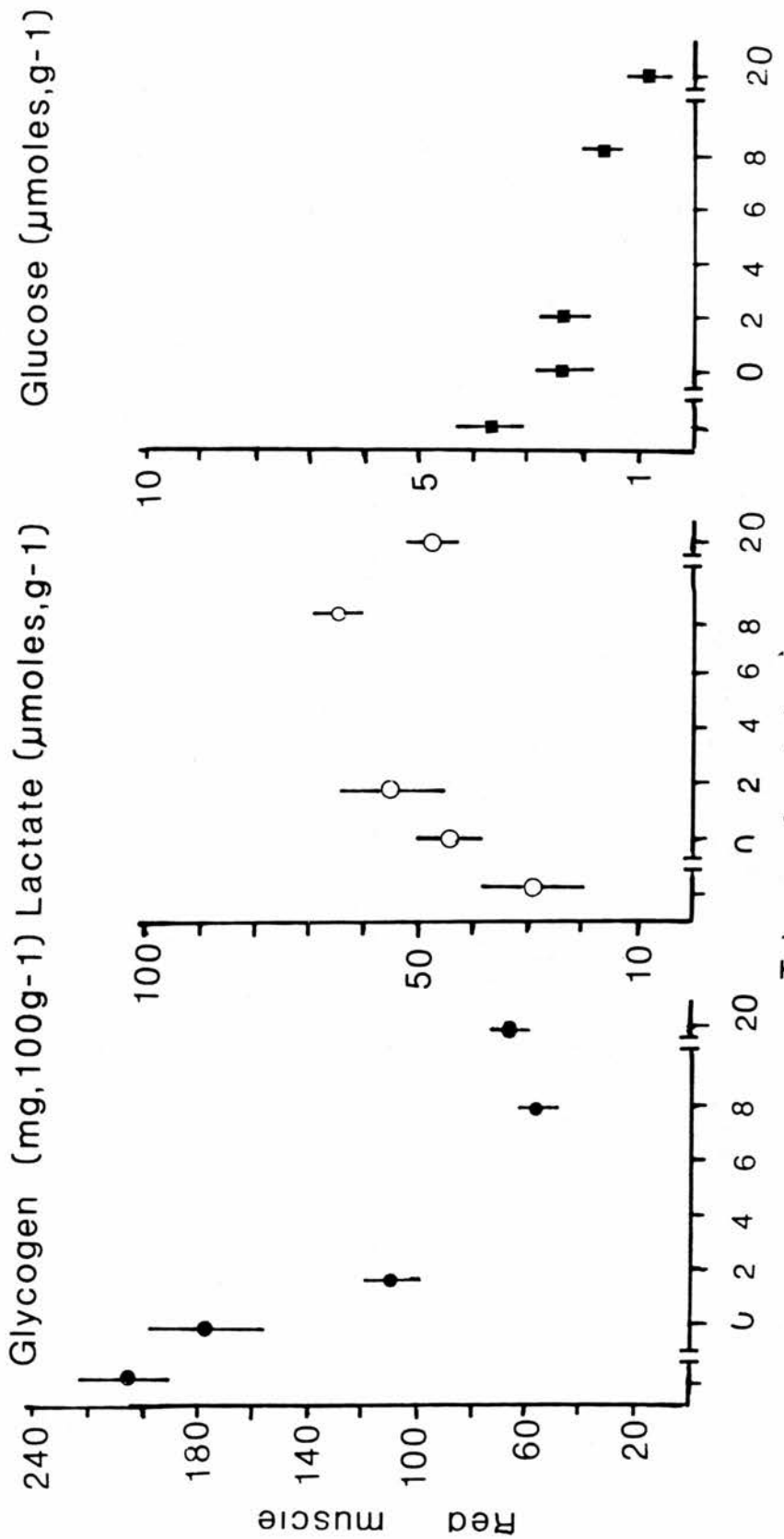

4.0 Lengths,s-1
Glycogen (mg,100g-1)
Lactate (μmoles,g-1)
Glucose (μmoles,g-1)
Red muscle
240
180
140
100
60
20
100
50
10
10
5
1
0 2 4 6 8 20
0 2 4 6 8 20
0 2 4 6 8 20
Time ( min)

Fig.14 a,b,c

TABLE 3: DATA OF LENGTHS AND WEIGHTS OF FISH USED FOR TOTAL BODY LACTATE ANALYSIS (FIGURE 6) AND CALCULATION OF ATP EQUIVALENTS (TABLE 4)

| Body lengths $s^{-1}$ | Exercise time (mins) | Length (cm) Mean ± S.E.M. | Weight (g) Mean ± S.E.M. | Number of fish |
|---|---|---|---|---|
| EXPERIMENT 1 | | | | |
| Tank-resting | | 10.90 ± 0.24 | 12.20 ± 0.97 | 5 |
| 2.3 | 0 | 13.13 ± 0.48 | 21.15 ± 1.32 | 16 |
| | 5 | 13.00 ± 0.32 | 19.00 ± 1.87 | 14 |
| 3.8 | 0 | 13.97 ± 0.39 | 30.50 ± 1.46 | 9 |
| | 2 | 13.50 ± 0.16 | 27.20 ± 0.87 | 5 |
| | 4 | 13.45 ± 0.28 | 26.97 ± 1.97 | 6 |
| | 5 | 13.75 ± 0.21 | 31.75 ± 1.35 | 8 |
| | 10 | 13.30 ± 0.30 | 25.10 ± 1.28 | 5 |
| | 20 | 13.71 ± 0.21 | 29.00 ± 1.45 | 7 |
| | 30 | 13.60 ± 0.24 | 26.70 ± 1.99 | 5 |
| 5.3 | 0 | 13.38 ± 0.22 | 25.50 ± 1.27 | 17 |
| | 5 | 14.06 ± 0.32 | 26.89 ± 2.02 | 9 |
| 6.1 | 0 | 12.90 ± 0.44 | 22.50 ± 2.42 | 6 |
| | 5 | 13.00 ± 0.30 | 23.46 ± 2.10 | 13 |
| 7.0 | 0 | 14.07 ± 0.37 | 27.50 ± 2.05 | 7 |
| | 2 | 14.66 ± 0.30 | 33.58 ± 2.25 | 6 |
| | 4 | 15.28 ± 0.09 | 38.56 ± 1.20 | 9 |
| | 5 | 14.13 ± 0.35 | 26.35 ± 1.00 | 10 |
| | 10 | 15.10 ± 0.24 | 38.67 ± 1.54 | 6 |
| | 20 | 14.92 ± 0.27 | 36.08 ± 2.16 | 6 |
| | 30 | 14.58 ± 0.30 | 36.42 ± 2.20 | 6 |
| | | | 27.17 ± 1.54 | |
| EXPERIMENT 2 | | | | |
| Tank-resting | | 14.00 ± 1.05 | 35.90 ± 8.12 | 10 |
| 4.0 | 0 | 16.86 ± 0.30 | 55.41 ± 3.24 | 11 |
| | 2 | 17.30 ± 0.34 | 59.00 ± 2.93 | 5 |
| | 5 | 17.33 ± 0.28 | 56.78 ± 3.78 | 6 |
| | 30 | 17.08 ± 0.35 | 59.50 ± 4.64 | 6 |
| | 24 hr | 17.03 ± 0.41 | 57.33 ± 4.95 | 6 |
| | | | 53.93 ± 4.67 | |

TABLE 4: ATP EQUIVALENTS FROM AEROBIC AND ANAEROBIC SOURCES IN RAINBOW TROUT AT VARIOUS SWIMMING SPEEDS

| Body lengths $s^{-1}$ | oxygen consumption ($mg^{-1}k^{-1}h^{-1}$) for 100 g fish | oxygen consumption ($mg^{-1}k^{-1}h^{-1}$) for 27.17 g fish | Energy expenditure utilizing aerobic pathway: µmoles ATP, $g^{-1}min^{-1}$ | Energy expenditure utilizing aerobic pathway: $\log_{10}$ ATP, $g^{-1}min^{-1}$ | Energy expenditure utilizing anaerobic pathway: µmoles ATP, $g^{-1}min^{-1}$ | Energy expenditure utilizing anaerobic pathway: $\log_{10}$ ATP, $g^{-1}min^{-1}$ | % contribution of anaerobic pathway |
|---|---|---|---|---|---|---|---|
| 2.3 | 275.0 | 100.5 | 0.34 | -0.4685 | 0.22 | -0.6576 | 39.0 |
| 3.8 | 436.5 | 159.0 | 0.53 | -0.2757 | 0.38 | -0.4202 | 41.8 |
| 5.3 | 479.0 | 174.8 | 0.58 | -0.2366 | 0.77 | -0.1135 | 57.0 |
| 6.1 | 479.0 | 174.8 | 0.58 | -0.2366 | 1.22 | 0.0864 | 67.7 |
| 7.0 | 479.0 | 174.8 | 0.58 | -0.2366 | 1.63 | 0.2122 | 73.6 |

Aerobic metabolism expressed as power function of body size $M = k \times w^{0.78}$

M = Total oxygen consumed per unit time

w = Body weight

0.78 = A constant (literature value obtained from the slope of a plot of the logarithm of $O_2$ consumption against the logarithm of body size)

k is obtained from the intercept

CHAPTER 3.

# THE OPERATION OF THE MALATE-ASPARTATE SHUTTLE IN THE RE-OXIDATION OF GLYCOLYTIC NADH IN SLOW AND FAST MUSCLE FIBRES OF RAINBOW TROUT (SALMO GAIRDNERI, RICHARDSON)

## Introduction

Anaerobic metabolism provides the additional energy required at speeds in excess of that at which oxygen consumption becomes maximal. Evidence has been provided in the previous chapter that in advanced teleosts anaerobic metabolism occurs even below the velocity at which oxygen consumption is maximized. For example, lactate increased by 27% and 38% in slow and fast fibres respectively in exercise-conditioned (1.1 body lengths $s^{-1}$) rainbow trout, following 2 mins swimming at slow speed, 2.8 body lengths $s^{-1}$ (Figures 10b and 11b). After 20 mins continuous swimming at this speed, lactate concentrations returned to levels not significantly different from zero time. This reflects a decrease in reliance on anaerobic metabolism with time. Whatever the pathway involved, it appears that energy production in the muscle involves a partial or complete oxidation of a suitable substrate. The sustained oxidation of some important substrate such as glucose or lactate depends on the provision of a continuous supply of cytosolic nicotimamide adenine dinucleotides ($NAD^+$).

In the glycolytic sequence of the cytosol (Figure 3), glyceraldehyde-3-phosphate dehydrogenase reduces $NAD^+$. Under anaerobic conditions, $NAD^+$ is regenerated by the lactate dehydrogenase (LDH) reaction, pyruvate is converted to lactate and redox is maintained. Under aerobic conditions pyruvate is no longer available since it enters the mitochondria for complete oxidation or it is utilized in some other metabolic reaction. Regeneration of $NAD^+$ is still essential for the continuation of glycolysis. However as the inner mitochondrial membrane

is impermeable to pyridine nucleotides (Lehninger, 1951), cytoplasmic NADH cannot be oxidized directly by the mitochondrial electron transport system. NADH formed in the mitochondrion has direct access to the phosphorylating electron transport chain and its reducing equivalents are transferred rapidly to oxygen. The mechanisms for the oxidation of cytosolic NADH are provided by the operation of cyclic irreversible shuttle systems (Estabrook et al., 1958; Borst, 1963). These involve the NADH-dependent reduction of a suitable substrate in the cytosol, followed by the transfer of the reduced substrate into the mitochondrion. Here it is reoxidized by a second enzyme, so that the oxidation can be linked to the electron transport system. The oxidized substrate is then transported out of the mitochondrion to act as a carrier for further reducing equivalents from NADH (Newsholme and Smart, 1973).

The Malate-Aspartate (Borst, 1963) and Glycerophosphate (Estabrook and Sacktor, 1958) (Figures 15a and b) shuttles have been suggested as the principal pathways of reducing equivalents transport into mitochondria (Meijer and Van Dan, 1974). The Malate-Aspartate shuttle involves NAD-linked malate dehydrogenase (MDH) and glutamate oxaloacetate transferase (GOT) and two membrane bound soluble carriers. This involves a unidirectional influx of cytosolic NADH into most mammalian mitochondria. Oxaloacetate apparently does not penetrate the inner mitochondrial membrane (Borst, 1963). Lardy et al. (1965) have produced evidence that a dual transaminase system may operate such that oxaloacetate is transaminated to aspartate which passes to the cytosol by an energy linked process. Here it is reconverted into oxaloacetate by cytosolic glutamate oxaloacetate transaminase. The system also requires free flow of glutamate and α-oxoglutarate between the compartments. The glycerol 3-phosphate shuttle involves $NAD^+$ and $FAD^+$ linked dehydrogenases located in the cytosol and the outer surface of the inner mitochondrial membrane respectively (Estabrook et al., 1958).

FAD is a flavoprotein and the shuttle generates $FADH_2$ at the expense of NADH with the attendant loss of ATP synthesizing efficiency. The mechanism of operation is that dihydroxyacetone phosphate (DHAP) oxidizes NADH in the cytosol via the cytosolic NAD-linked glycerophosphate dehydrogenase. The resulting glycerol phosphate passes through the permeable membrane where it is reoxidized to DHAP by the mitochondrial flavin-linked glycerophosphate oxidase (Klingenberg, 1970).

Previously transaminase inhibitor, amino-oxyacetate has been considered useful to determine the occurrence of the malate-aspartate shuttle in liver slices from fetal rats (Dani et al., 1977). This method was therefore employed to inhibit flux of reducing equivalents via the malate-aspartate shuttle in slow and fast fibre bundles of rainbow trout.

## Materials and Methods

### Fish:

Rainbow trout (Salmo gairdneri Richardson). A total of 7 fish length mean ± S.E. 33.29 ± 0.04 cm and weight 435.52 ± 10.15 g were obtained in June 1980 from Ceres Fish Farm, Fife, and held in tanks of filtered fresh water at 10 ± 0.5°C and were fed daily on a proprietary brand of trout pellets. No food was given in the 24 hr period before the fish were killed for analysis.

### Dissection of muscles and incubation:

Fish were stunned by a blow to the head. Following removal of the skin and external myocommata small fibre bundles (~ 100 mg) muscle were carefully dissected from the region anterior to the dorsal fin. Superficial red muscle was dissected from an area adjacent to the lateral line region and the white fibres from the underlying deep expaxial muscle. Fibre bundles were incubated for 70 min in 3 ml of

oxygen saturated medium containing 140 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 10 mM sodium phosphate pH 7.4 at 15 ± 0.5°C. Parallel experiment was set up in a medium of the same composition but with further addition of 0.4 mM Amino-Oxyacetate pH 7.4 (transaminase inhibitor). 20 mM glucose was present in all experiments. In addition fibre bundles were dissected, immediately freeze-clamped as control from resting fish.

After the completion of the incubation period the muscle fibres were deproteinised in 0.6 mM cold perchloric acid, homogenised at 0°C with an Ilado-X10 homogenizer (I.C.A. Gmbtt, Dottinger, West Germany) and extracted for 10 min. Homogenates were centrifuged for 15 min at 6000 g and aliquots of the clear supernatant neutralized with a mixture of 0.5 M triethanolamine and 3 M $K_2CO_3$ in the presence of methyl orange indicator.

Determination of glycolytic intermediates:

From the neutralized supernatant all metabolites were measured enzymatically based on the absorbance of pyridine nucleotides at 340 nm.

Malate was assayed by a spectrophotometric method (Hans Mollering, 1974) with slight modification in a volume of 0.5 ml of 50 mM 3-Amino-1-propanol buffer pH 10.0 containing 100 mM glutamate, 2.7 mM β NAD, 20 µl each of glutamate oxaloacetate transaminase and malate dehydrogenase, 0.2 ml neutralized extract.

Dihydroxy acetone phosphate, Glycerol-3-phosphate and Fructose 1,6-diposphate were assayed by the method of Gerhard Michael et al. (1975), using the same medium by sequential addition of 10 µl Glycerol-3-phosphate dehydrogenase, Triose phosphate isomerase and aldolase in a medium containing 250 mM Triethanolamine buffer pH 7.6, 2.5 mM EDTA, 60 µM NADH.

Pyruvate was measured by following the decrease in absorbance of NADH at 340 nm (method of Czcok et al. (1965)) in a medium containing 250 mM Triethanolamine buffer pH 7.6, 2.5 mM EDTA, 60 µM NADH, 5 µg lactate dehydrogenase.

Glycose 6-phosphate and Fructose 6-phosphate were assayed by the method of Gunter Lang et al. (1975) using the same medium containing 250 mM triethanolamine buffer pH 7.6, 2.5 mM EDTA, 0.2 mM NADP and 1 µM $MgCl_2$. Sequential addition of 10 µl Glucose 6-phosphate dehydrogenase and phosphoglycose ismomenase into the medium containing substrate was made. Change in the optical density was recorded following each addition.

Statistical Analysis:

Data of fibre bundles incorporating glucose medium and those with glucose plus amino-oxyacetate have been compared using a students 't' test. The results are presented as mean ± S.E.M.

## Results

The concentrations of some glycolytic intermediates in slow and fast fibres under different experimental conditions are shown in Figure 16. In freeze-clamped muscles from tank rested rainbow trout, the concentration of glucose 6-phosphate (G6P) was 2.20 times higher in slow than the fast fibres ($P < 0.05$). The concentration of fructose 1,6 diphosphate (F16P) was 1.50 times higher in fast than slow fibres ($P < 0.05$). The concentrations of the other glycolytic intermediates measured, malate, pyruvate, dihydroxyacetone phosphate (DHAP), glyceraldehyde-3-phoshoste (G3P), and fructose 6-phosphate (F6P) were not statistically different between both muscle types.

Following incubation of fibres in glucose medium, the concentrations of F6P and F16P were 1.5 and 1.6 times higher in slow than fast

fibres, respectively ($P < 0.05$). Pyruvate concentration was 1.4 times higher in the fast than the slow muscle fibres. The other glycolytic compounds assayed occurred in similar concentrations in both slow and fast fibres.

Incubation of the fibres with transaminase inhibitor amino-oxyacetate in the presence of glucose, resulted in changes in the concentrations of the measured glycolytic intermediates in both the slow and fast fibres. The concentration of malate increased by 96 ($P < 0.001$) and 42% ($P < 0.01$) in the fast and slow fibres, respectively. Pyruvate concentrations decreased by 42 ($P < 0.05$) and 10% (N.S.) in fast and slow muscle fibres, respectively. The concentration of G6P was decreased by 8 and 24% ($P < 0.05$) in the fast and slow fibres, respectively. An increase of 45% in DHAP concentration of the slow fibres is equivalent to 1.8 times that of the fast fibres. G3P concentration showed 58% increase in the slow fibres ($P < 0.001$) and represents 1.2 times that of the fast fibres. F16P concentration increased by 48% in the fast fibres ($P < 0.01$). This is equivalent to 2.8 times higher than the slow fibres ($P < 0.001$). F6P concentrations were increased by 48 and 233% in slow and fast fibres, respectively.

## Discussion

The involvement of pyridine nucleotides in energy metabolism and major biochemical pathways is well established (Edington, 1973; Dawson, 1979, Hochachka, 1980). For example, at low level of NADH, glycerophosphate dehydrogenase functions to reoxidise NADH. In contrast, during high generation of cytosolic NADH, lactate dehydrogenase reaction is the major reoxidative system. Therefore the amount of NADH would profoundly influence the transitions of aerobic/anaerobic glycolysis (Hochachka, 1980). In the glycolytic pathway

which was previously referred to (Figure 3), during the conversion of glyceraldehyde 3 phosphate (G3P) into 1,3-diphosphoglycerate (DPG), $NAD^+$ is also converted to NADH. NADH must be continuously oxidized in order to maintain glycolysis. This is accomplished during the reduction of catalytic amounts of dihydroxyacetone (DHAP) to glycerophosphate or the reduction of oxaloacetate to malate by the GPDH and MDH (malate dehydrogenase) reactions (Hochachka, 1981; Candy, 1980).

The available evidence shows that a feature of the glyceraldehyde phosphate dehydrogenase reaction is that the equilibrium is such that a high $NAD^+$/NADH ratio is required in the cytosol which favours 3-phosphoglycerol phosphate formation (Candy, 1980). Therefore any other reaction coupled through $NAD^+$ to glyceraldehyde phosphate dehydrogenase (GPDH) must favour NADH oxidation in order to maintain the high cytosolic $NAD^+$/NADH ratio (Candy, 1980). For example, Williamson, Lund and Krebs (1967) have reported that in the rat liver the mean cytoplasmic $NAD^+$/NADH ratio was 725 while the mitochondrial value was 8. The physiological significance of the large difference in the two compartments is to maintain a satisfactory rate of glycolysis in the cytosol as well as a satisfactory rate of electron transport in the mitochondria (Newsholme and Start, 1973). This therefore explains why the inner mitochondrial membrane is impermeable to pyridine nucleotides and requires metabolite shuttles to transport hydrogen across the compartments (Newsholme and Start, 1973).

There is evidence that the GPDH activity is higher in the fast than slow fibres (Pette, 1966; Crabtree and Newsholme, 1972; Hochachka et al., 1978, 1981). For example, following a series of biochemical analyses on fast and slow muscles of vertebrates and invertebrates, Crabtree et al. (1972) showed that the activities of the cytosolic GPDH of the fast fibres of trout were 90-180 times higher than the mitochondrial type and were twice as active as the slow fibres

($P < 0.001$). In contrast to the high activities in trout, those of the slow and fast fibres of the dogfish are lower by 2 to 3 times ($P < 0.001$) (Crabtree and Newsholme, 1972). However, Patterson et al. (1975) have demonstrated by histochemical techniques heavy staining of the mitochondrial GPDH in the slow fibres of five species of teleosts. The fast fibres stained variably depending on the species.

The glycerophosphate shuttle is particularly important in insect flight muscles which have a high oxidative capacity. In these muscles, there is an abundant supply of oxygen since the tracheal system delivers oxygen directly to the fibres. The activity of GPDH in insects is the highest in nature. For example, Crabtree et al. (1972) have shown that the activity of this enzyme in the flight muscle of the honey-bee (Alpis mellifera) or bumble-bee (Bombus hortorum) is 14 and 28 times higher, respectively, than that of trout. Hence glycolysis in insect flight muscle is geared to the complete oxidation of substrate and no lactate is formed.

It is proposed by Hochachka (1980) that during aerobic condition and the initial stages of glycolysic activitation cytosolic redox are maintained by aspartate primed NAD function. These result in malate production which on entry into mitochondria would regenerate mitochondrial NADH and oxalacetate. Efflux of oxaloacetate is through the malate-aspartate shuttle, mediated by glutamic oxaloacetate transaminase. GPDH also functions at this aerobic condition until it is inhibited by its own substrate accumulation. This would allow pyruvate levels to accumulate and gradually turn on lactate dehydrogenase (LDH) function. Once anaerobic conditions arose favouring LDH function, further MDH or GPDH function could be curtailed. This shows that in later stages of anaerobic glycolysis, lactate is quantitatively the only important depository of glucose derived hydrogen (Hochachka, 1980). Crabtree et al. (1972) have shown that the activity of GPDH of the white muscle is

not sufficient to account for the major part of the NADH oxidation when glycolysis is maximized, but it could serve a supplementary role.

The findings in the present experiments are compatible with increased production of NADH in the slow and fast fibres of rainbow trout due to the transaminase inhibitor, amino-oxyacetate. A preliminary investigation with amino-oxyacetate has shown an increase in lactate concentration. This result shows a 46% decrease ($P < 0.05$) in the pyruvate concentration of the fast fibres compared to 10% decrease in the slow fibres. Safer et al. (1971) have demonstrated that AOA did not only diminish hydrogen flux through the malate aspartate shuttle but also limited pyruvate entry into the tricarboxylic acid cycle and electron transport system. This indicates that pyruvate is channelled into lactate as an electron acceptor and $NAD^+$ is generated from the increased cytosolic NADH. However, the decreased flux of pyruvate through the tricarboxylic acid cycle will result in lower levels of adenosine triphosphate (ATP) and creatine phosphate. Glycolysis is normally controlled by feedback inhibition mediated by ATP/ADP and/or citrate. According to the nucleotide adenine theory small changes in ATP would result in proportionately larger changes in AMP through the adenylate kinase reaction (Newsholme and Start, 1973). Therefore the decrease in ATP would further activate phosphofructokinase (PFK) as well as the GPDH reaction. However, this would rapidly lead to a build-up of glycerophosphate and (DHAP) dihydroxyacetone phosphate to inhibit this shuttle system. The results obtained are in agreement with the expected scheme. Amino-oxyacetate (AOA) caused an increase of 48% in glyceraldehyde 3-phosphate (G3P) in the fast fibres compared to the control assay ($P < 0.02$) and 58% in the slow fibres. There was an increase of 24% in DHAP ($P < 0.02$) in the fast fibres and 45% increase in the slow fibres ($P < 0.05$).

Substrate cycling between fructose-6-phosphate and fructose diphosphate by the coupling of fructose diphosphatase and phospho-fructokinase is known to increase the sensitivity of fructose 6-phosphate phosphorylation to changes in AMP (Newsholme, 1976). The results are compatible with this since fructose 6-phosphate increased by 233% in the fast fibres ($P < 0.001$) as against 48% increase in the slow fibres ($P < 0.05$). The increase in fructose diphosphate (F16P) was 48% in the fast fibres and 17% in the slow fibres.

Apart from pyruvate, glucose 6-phosphate also showed a decrease. This was found to be 8% in the fast fibres (N.S.) and 24% in the slow fibres. The decrease in glucose 6-phosphate in the presence of an increase in fructose diphosphate or large increase in fructose-6-phosphate indicates that the glycolytic flux is not significantly impaired by the amino-oxyacetate.

The increase in cytosolic NADH has also resulted in an increase in malate production from cytosolic oxaloacetate. NADH is therefore converted to NAD. However since the mitochondrial glutamate oxaloacetate transaminase is inhibited by the amino oxyacetate there would be no transamination and therefore further efflux of aspartate is impeded. The results show an increase of 96% in malate concentration of the fast fibres ($P < 0.001$) and 42% in the slow fibres. In the fast muscle of tuna malate dehydrogenase (MDH) activity is over 90% in the cytosol. It is considered that only a small fraction of this is required for the malate-aspartate function and the purpose of the large amount of excess malate dehydrogenase is unknown (Hochachka, 1980). In conclusion the results show that the malate-aspartate shuttle is operative in both the slow and fast fibres of rainbow trout.

Fig. 15

Diagrammatic representation of a, the Malate-Aspartate shuttle and b, the -Glycerophosphate dehydrogenase shuttle for the reoxidation of cytosolic NADH

Abbreviations: MAL, Malate; PYR, Pyruvate; DHAP, Dihydroxyacetone phosphate; G3P, glyceraldehyde 3-phosphate Fl, 6P, fructose 1,6 - diphosphate; G6P, fructose 6-phosphate; F6P, fructose 6 phosphate; DAA, Oxaloacetate; GLU, glutamate; KG, ketoglutarate; ASP, Asparate; NAD, -nicotinamide adenine dinucleotide; NADH, -nicotinamide adrenine dinucleotide reduced form; -AOA, inhibition by amino oxyacetate; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate.

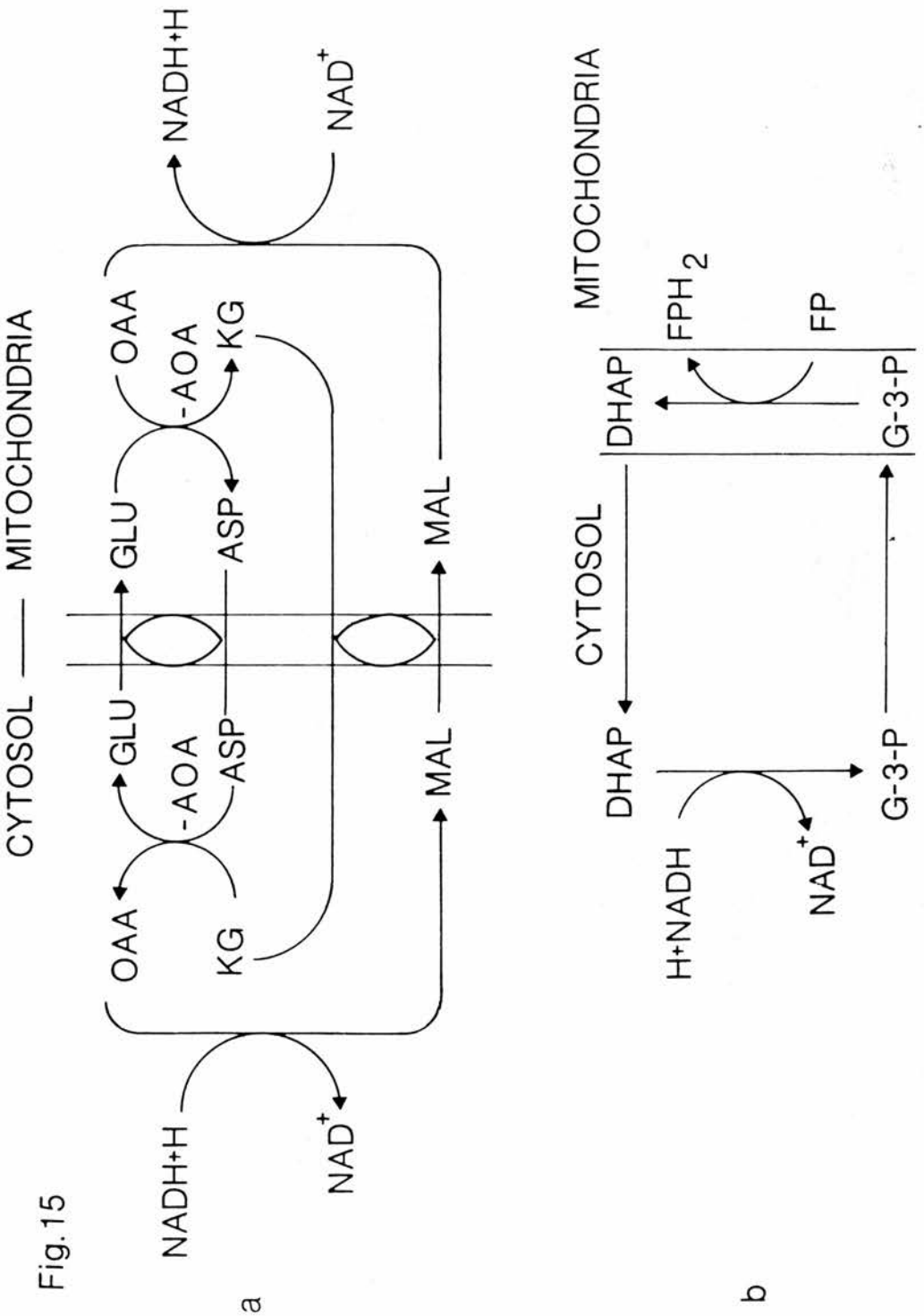

CYTOSOL
MITOCHONDRIA
NADH+H
NAD+
OAA
GLU
-AOA
ASP
KG
MAL
a
CYTOSOL
MITOCHONDRIA
DHAP
G-3-P
FP
FPH2
H+NADH
NAD+
b

Fig.15

Fig. 16

Concentrations of some glycolytic intermediates (umoles, $g^{-1}$ mean ± S.E., seven fish) of isolated fibre bundles, under resting condition  and following incubation at 15°C for 70 minutes in media containing (140 mM NaCl, 5mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 10 mM sodium phosphate pH 7.4), including 20 mM glucose 
or 20 mM glucose plus 0.4 mM aminooxyacetate. 

Abbreviations:

MAL, Malate
PYR, pyruvate
DHAP, dihydroxyacetone phosphate
G3P, Glyceraldehyde 3-phosphate
Fl,6P, fructose 1,6 diphosphate
G6P, Glycose 6 phosphate
F6p, fructose 6 phosphate

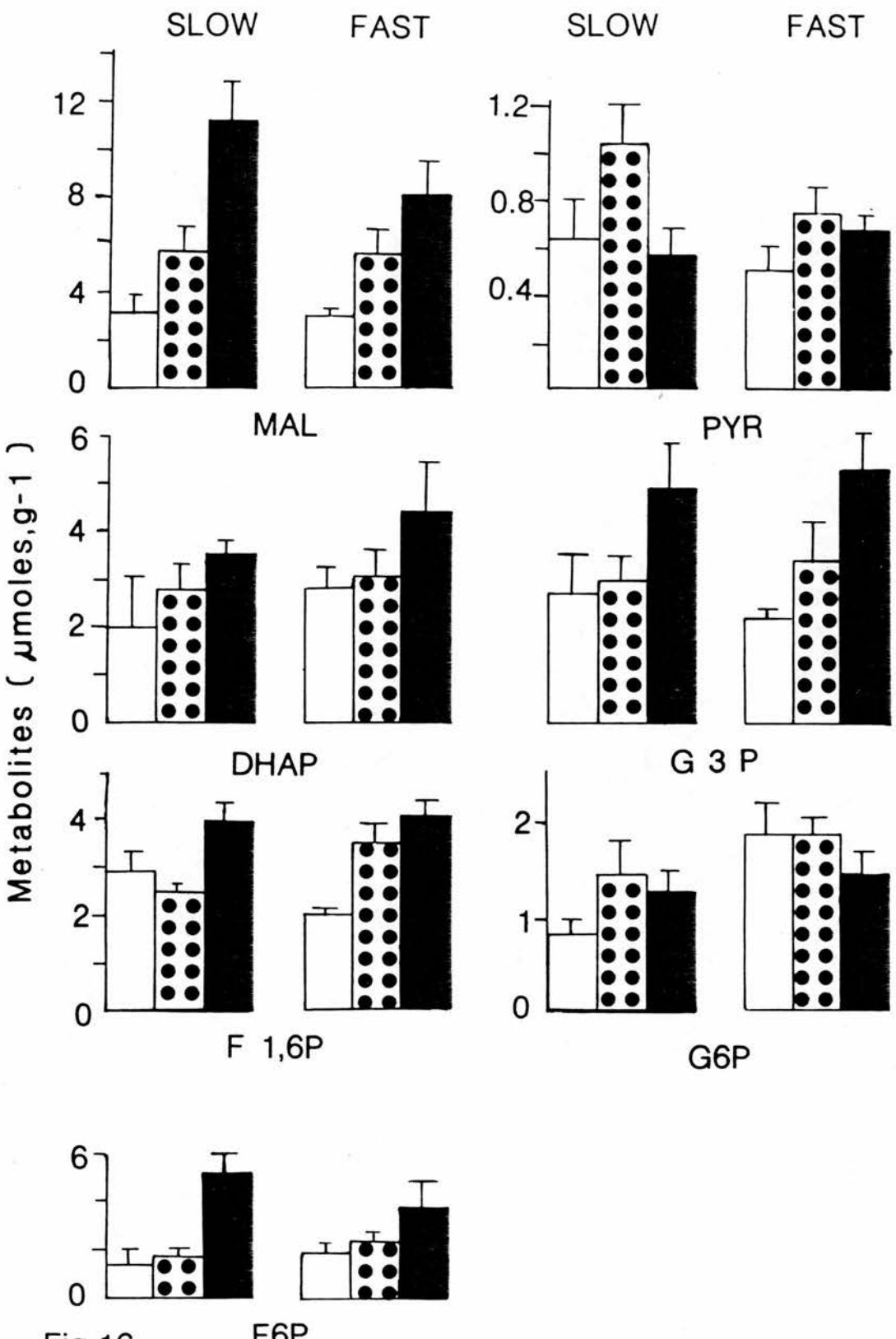

SLOW
FAST
SLOW
FAST
Metabolites ( μmoles,g-1 )
MAL
PYR
DHAP
G 3 P
F 1,6P
G6P
F6P

Fig.16

CHAPTER 4.

# CAPILLARISATION, MITOCHONDRIAL CONTENT AND OXYGEN DIFFUSION DISTANCES OF MUSCLES OF RAINBOW TROUT (SALMO GAIRDNERI, RICHARDSON)

## Introduction

Ultrastructural studies have demonstrated that in rainbow trout the slow fibres are better vascularized than the fast fibres (Nag, 1972; Johnston, 1975b). Following muscular activity lactate and glycogen concentrations return faster to the pre-exercise levels in the slow than the fast fibres (Wokoma and Johnston, 1981). This has been ascribed to the greater aerobic capacity of the slow fibres. However, fast fibres of advanced teleosts have been demonstrated to have more mitochondria than those of elasmobranch (Totland et al., 1981; Johnston, 1981).

In the present investigation, ultrastructural study and morphometric methods have been used to determine the relation between the fibre surface and volume, the size of the mitochondrial compartment, capillary supply and oxygen diffusion distances in slow and fast muscles of rainbow trout.

## Materials and Methods

### Fish:

Rainbow trout (Salmo gairdneri Richardson) of average weight, 400 gm, were obtained from Ceres Fish Farm, Fife, and held in tanks of filtered fresh water at 10 ± 0.5$^{o}$C and were fed daily on a proprietary brand of trout pellets. No food was given in the 24 hour period before fish were sacrificed for analysis.

Fixation of muscles:

Fish were killed by a blow to the head and transection of the spinal cord. Fixative (3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4) was injected into the myotomes adjacent to the sample site which was located posterior to the dorsal fin. During this process the fish was kept on ice with the trunk bent to its point of maximum flexure. In situ fixation of muscle was for an initial period of 1 hr. Using a binocular microscope small fibre bundles were dissected from both the superficial slow fibre layer and the mid-region of the expaxial fast muscle (Johnston and Maitland, 1980). In order to prevent shrinkage fibre bundles were pinned to cork strips at their resting lengths in situ and fixed for a further period of 2-24 hrs in 3% glutaraldehyde, 0.1 M phosphate buffer, pH 7.4 at 4°C. Tissue samples were washed in buffer and post-fixed in 1% osmium tetroxide, 0.1 M phosphate, pH 7.4 for 1 hr. Samples were rinsed in distilled water, dehydrated in a series of alcohols up to 100%, cleared in 1,2 epoxypropane and then allowed to stand in epoxypropane and araldite mixture for 24 hrs to allow penetration by araldite prior to embedding. The samples were then embedded in araldite CY212 resin, the blocks were labelled and cleared at 60°C for 48 hrs. Checking of the orientation of muscle fibres in the embedded material was done by examination of 1 µm semithin sections stained with toluidine blue or p-phenylene diamine in 1:1 isopropanol:methanol (Hollander and Vaaland, 1968) viewed and photographed on a Zeiss optical microscope using Pan F film at 100 ASA.

Sections were cut with a Reichert OMU2 ultramicrotome with glass knives made on an LKB knifemaker. Blocks were prepared by trimming down initially, to a trapezoid cutting face with a razor blade, and then polished with glass knives until all marks had been removed. Ultrathin sections showing deep gold interference colour representing a

thickness of 90 nanometers were collected on Formvar or pyroxylin coated 150 mesh copper grids double stained in 4% uranyl acetate in 50% ethanol with counter staining in 0.4% lead citrate in 0.1 M sodium hydroxide. Sections were examined with a Phillips 301 electron microscope at 60 KV and electron micrographs taken.

Quantitation of capillarisation and mitochondrial volume:

Using a photographic enlarger, the outlines of 300 muscle fibres and adjacent blood capillaries were drawn from either low power electron micrographs (× 1900) of slow fibres, or toluidine blue stained semithin sections (× 820) of fast fibres, by projecting respectively 1/4 plate or 35 mm negative onto cartridge paper.

The fibre areas, perimeter and contact length were determined directly using a summagraphics digitiser coupled to an Olivetti P6060 mini-computer (Walsely and Johnston, 1980). The method of calculating other indices of capillarisation follow those of Flood and co-workers (Flood, 1979; Kryvi, Flood and Gulyaev, 1980). Details of calculating the various indices of capillarisation are given in Table 5.

In order to determine the fractional volumes (%) occupied by mitochondria, myofibrils and lipid the outline of each component structure was drawn on 10" by 8" black and white paper from transverse sections of 100 electron micrographs (× 3800 - 18000), using a point grid method (Weibel, 1967). Lattice grids were constructed such that the spacing was 1.5 times the size of the largest component structure. This was found to be the width of the myofibrillar segment. The grid was placed on the point and a count was compiled of each component structure giving the fractional volume occupied by each structure of the muscle fibre. A minimum of 150 points per micrograph was analysed.

Statistical analysis:

Statistical analysis was carried out using the student's t-test for equal sample numbers.

## Results

Figures 17-22 and Tables 5 and 6 represent results obtained from ultrastructural studies. Superficial slow fibres appear distinctly red in colour and are relatively homogeneous in size (Figure 19). In contrast, fast fibres are white in colour and have a range of fibre sizes. The mean area of the fast fibre is 2-3 times that of the slow fibre ($P < 0.01$) (Table 5; Figure 21). Myofibrils occupied 49.6% of the total volume in slow fibres. Myofibrillar fractional volume is 1.8 times higher in fast than in slow fibres. The fraction of fast fibre volume occupied by mitochondria is 30% of that of the slow fibres ($P < 0.001$) (Table 5). Around 68% of mitochondria in fast fibres are situated in the subsarcolemmal zone, 32% are intermyofibrillar (Table 6). Smaller fast fibres have 2-3 times higher mitochondria and lipid volume fractions than larger ones ($P < 0.01$). In the slow fibres the proportion occupied by the intermyofibrillar mitochondria is 1.4 times higher than the sarcolemmal mitochondria (Table 6). Electron micrograph from the slow fibres have well developed cristae (Figure 22). The lipid volume fractions of the fast fibres are equivalent to 13% for that of slow fibres ($P < 0.001$).

Red cells are present in 80-90% of the capillaries in the slow fibres (Figure 20) suggesting that a high proportion are perfused with red blood even in tank rested fish. Indices of capillarization are shown in Table 5. Both the number of capillaries per fibre and the mean fibre percentage fibre perimeter in direct capillary contact are 2-3 times higher in the slow than fast fibres ($P < 0.01$) (Table 5).

Figures 17 to 18 show that the slow fibres have better capillary supply than the fast fibres. The capillary surface supplying 1 $\mu m^3$ mitochondria is similar in both fast and slow fibres (Table 5). However, the oxygen diffusion distance is 2.24 times higher in fast than slow fibres ($P < 0.01$) (Table 5).

## Discussion

Blood supply to a muscle is an important determinant of muscle function. Histological measurements of vascularization give no indication of physiological blood flow but are useful in determining the potential size of the capillary bed. As a result of different indices of vascularization used by previous workers, only few data are available for comparative analysis. Compatible data have been obtained on some teleosts (Mosse, 1978, 1979), a chondrostean (Acipenser stellatus) (Kryvi et al., 1980), a holocephalan (Chimaera monstrosa) and elasmobranchs (Totland et al., 1981). The average number of capillaries per fibre in the fast fibre muscle in rainbow trout of this study is 0.54 (Table 5). Lower values have been obtained in some species: Australian salmon 0.14-0.27 (Mosse, 1979), Acipenser, 0.2 (Kryvi et al., 1980), Chimaera and Galeus, 0.3 and 0.4, respectively (Totland et al., 1981). The corresponding values for the slow fibres are 1.26, 1.9-4.2, 2.3, 0.3 and 2.5, respectively. The range of values is further demonstrated in the pelagic anchovy with 12.9 capillaries per slow fibre, resulting in vascularization of 51% of the fibre surface (Johnston, 1982b). The results show that the fast fibres in rainbow trout have better vascularization than those of many other species. Figure 17 shows the distribution of capillaries in fast and slow fibres. However the slow fibres are better vascularised than the fast fibres since 42% of the slow fibres made direct contact with 11% of the capillaries

compared to 5% of the fast fibres (Figure 18). The percentage of fast fibre vascularisation compared to the slow fibres is 0.5-1% in Etmopterus, 3% in Galeus, 7.7-13% in Chimaera and 17% in Scyliorhinus canicula (Totland et al., 1981). The value in rainbow trout is 30% (Table 5).

The capillary surface required to supply 1 $\mu m^3$ of mitochondria in slow and fast fibres are similar (Figure 5). Similar results have been obtained in Galeus, Chimaera (Totland et al., 1981), and Carp (Johnston, 1982a). There is a good correlation between capillary density, mitochondria and sustained swimming performance (Johnston, 1981). The fractional volume occupied by mitochondria in the present study is 31.6% in the slow fibres (Figure 5). Similar value has been obtained in brook trout (Johnston and Moon, 1980). In contrast, elasmobranchs are generally shown to have mitochondrial content of 18-24% (Totland et al., 1980). However the value is 34% for an active species of elasmobranch, Etmopterus spinax (Kryvi, 1977).

The mitochondrial volume occupied in the fast fibres of elasmobranchs ranges from 0.5-1%. In contrast, in advanced teleosts this is 2-9% (Johnston, 1981). The mean fractional volume occupied by fast fibre mitochondria in the present study is 9.6%. A value of 9.3% was reported in brook trout (Johnston and Moon, 1980a). There was a decrease in mitochondrial volume with increase in fibre size. Subsarcolemmal mitochondria occupied 6.5% of the fractional volume, equivalent to 2.17 times higher than the intermyofibrillar ($P < 0.01$) (Figure 6). In contrast to that of slow fibre, intermyofibrillar mitochondrial volume was 1.4 times higher than subsarcolemmal mitochondria (Figure 6). The results demonstrated a high degree of intra-membrane transport of metabolites since subsarcolemmal mitochondria are associated with generation of ATP for this process. Intermyofibrillar mitochondria supply the energy for muscle contraction (Romanul, 1965).

Other important factors determining the aerobic capacity of the capillary bed are the rate of utilization of oxygen by mitochondria as well as diffusion distances. The maximum hypothetical diffusion distances in the slow fibres of some species are: 52 μm for Chimaera monstrosa, rat fish, 47.5 μm for Galeus melastomus, 27.4 μm for Scyliorhinus canicula, dogfish (Totland et al., 1981). The value for rainbow trout is 11.48 μm (Table 5) and 8 μm in European anchovy (Engraulis encrasicolus) (Johnston, 1982b).

Greer Walker et al. (1980) indicated that data from 73 species have shown faster swimming fish developed narrower fast fibres with shorter diffusion distances. Maximum hypothetical diffusion distances in fast fibres are (μm) 341, 198, 168 and 53 in Etmopterus, Chimaera, Galeus and Scyliorhinu respectively (Totland et al., 1981) and 27.72 in rainbow trout (Table 5).

In contrast to mitochondrial density being correlated with activity, in skipjack tuna (Katsuwonus pelamis), slow fibre mitrochondrial volume is low (~ 16%) (Bone, 1978). Tuna appears to have a higher aerobic potential than another scombroid, the Atlantic mackerel (Scomber scomber) with a mitochondrial content of ~36%. However, the former is further distinguished by the possession of a counter-current brain and muscle heat exchanger which maintains elevated muscle temperatures (Stevens and Neill, 1978; Bone, 1978). It is not clear whether this contributes to an increased mitochondrial efficiency such that fewer mitochondria are required to achieve an equivalent rate of respiration.

Ultrastructural parameters are not fixed but can be modified by environmental factors. Cold acclimation is associated with an increase in the volume of mitochondrial compartment and capillary density in both fast and slow fibres (Johnston, 1982a). For example, in carp, Carassius carassius, the mean mitochondrial volume in fast fibres is 6.1% and 1.6% for cold and warm-acclimated fish (Johnston, 1982a).

There is evidence that temperature compensation of mitochondrial function is less than perfect and results from an increase in respiratory or tricarboxylic acid enzymes (Wilson, 1973; Hazel and Prosser, 1974). The cellular mechanisms underlying seasonal adjustments in metabolic rate are complex. However, it has been suggested that these may be due to altered gene expression, differential effects of temperature on protein synthesis and degradation, and changes in the micro-environment of key enzyme systems (Sidell, 1977; Sidell et al., 1980; Johnston and Maitland, 1980).

In conclusion, the fast fibres of rainbow trout are likely to have aerobic capacity which is a significant fraction of that of slow fibres.

Fig. 17

Histograms showing the distribution of the frequency distribution of the number of capillaries per fibre in slow (upper) and fast (lower) fibres of rainbow trout.

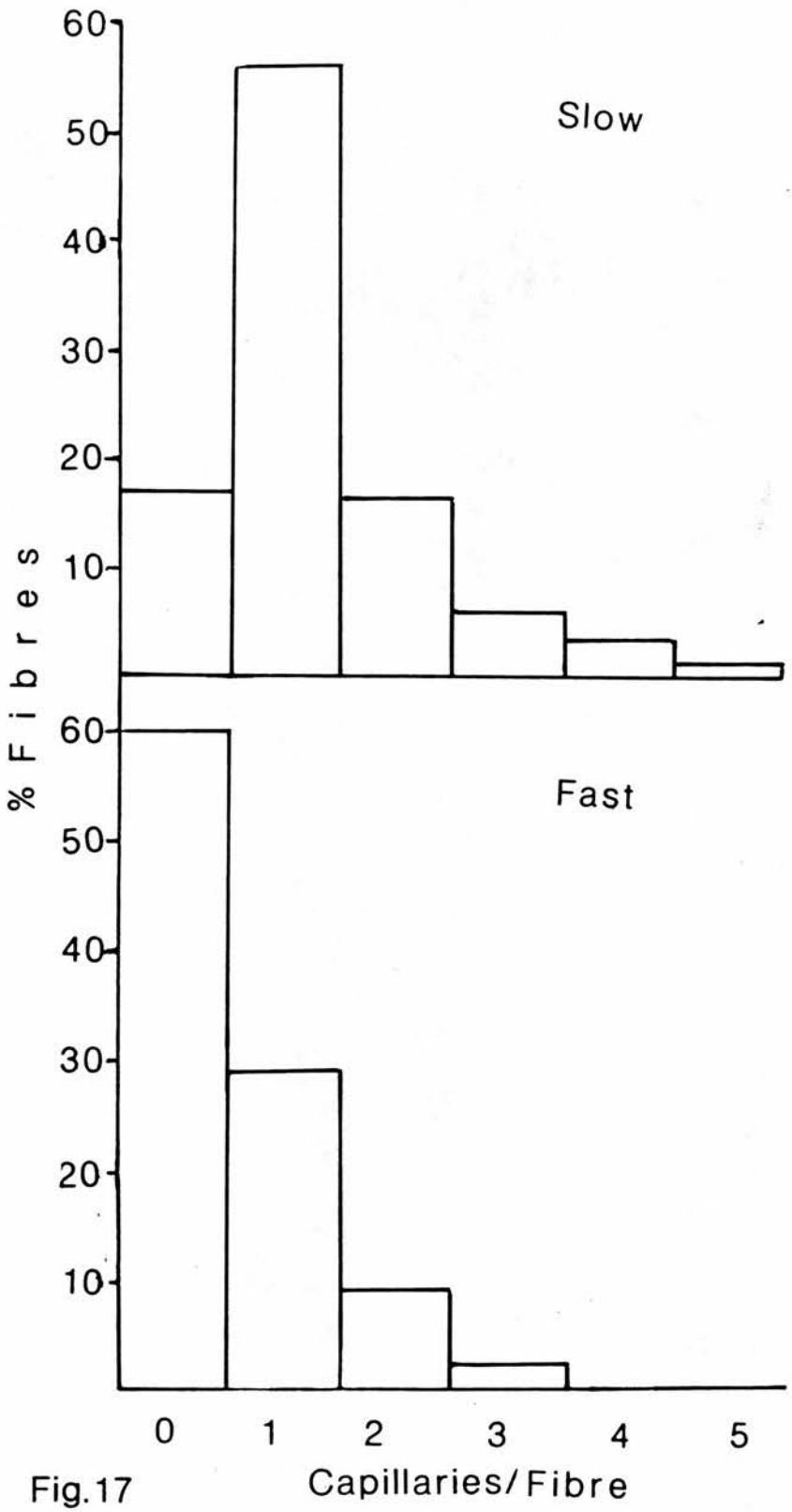

60
50
40
30
20
10
Slow
% Fibres
60
50
40
30
20
10
Fast
0
1
2
3
4
5
Capillaries/Fibre

Fig.17

Fig. 18

Histograms showing the frequency distribution of percentage fibre vascularized in slow (upper) and fast (lower) fibres of rainbow trout.

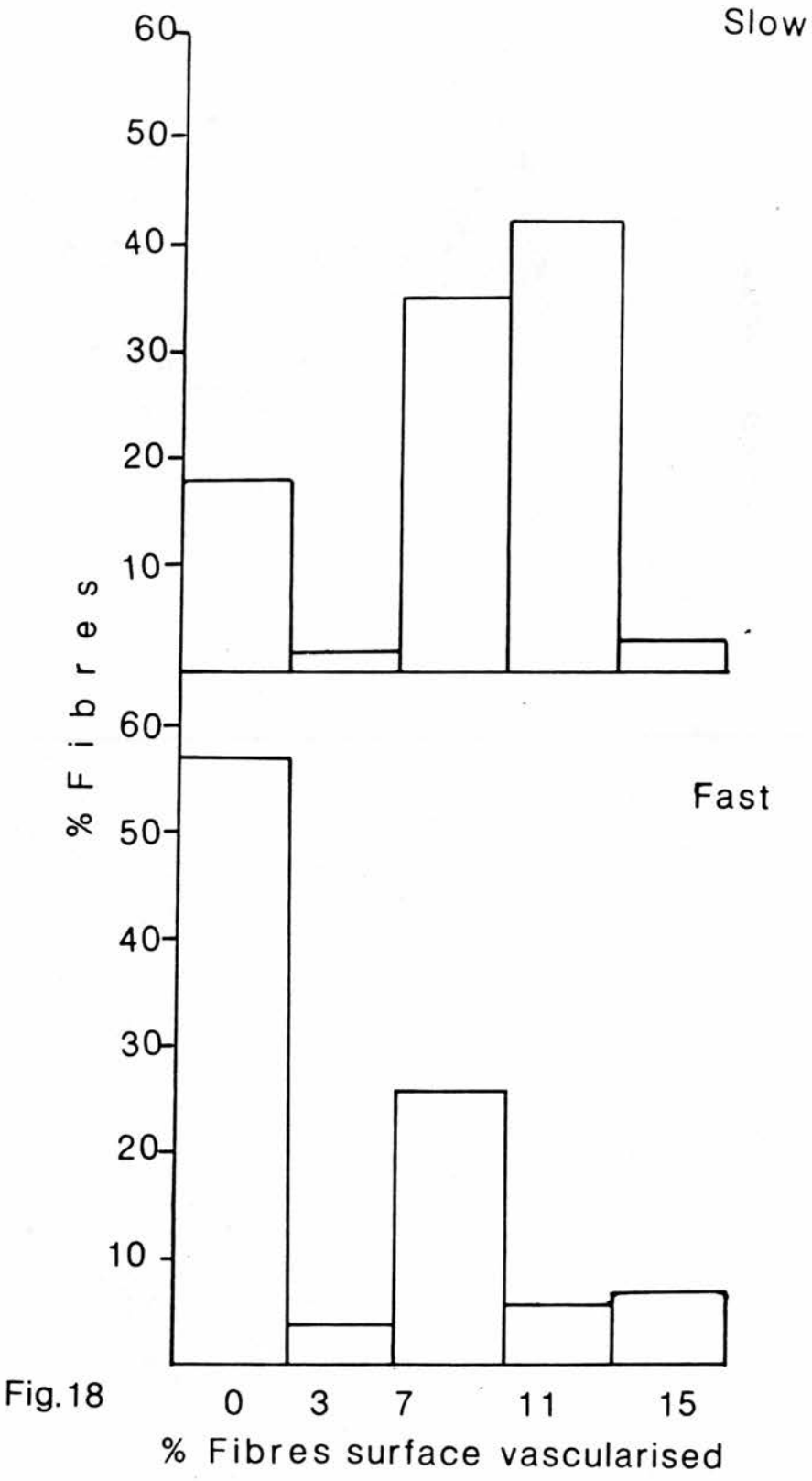

Slow
60
50
40
30
20
10
% Fibres
60
50
40
30
20
10
Fast
0
3
7
11
15
% Fibres surface vascularised

Fig.18

Fig. 19

Low magnification electron micrograph (x900) from slow fibre of rainbow trout showing numerous mitochondria (dark) myofibrils (light) and lipid droplets (L).

Fig. 20

Low magnification electron micrograph (x1950) from slow fibre of rainbow trout showing capillaries (C), myofibrils (MY), mitochondria (M).

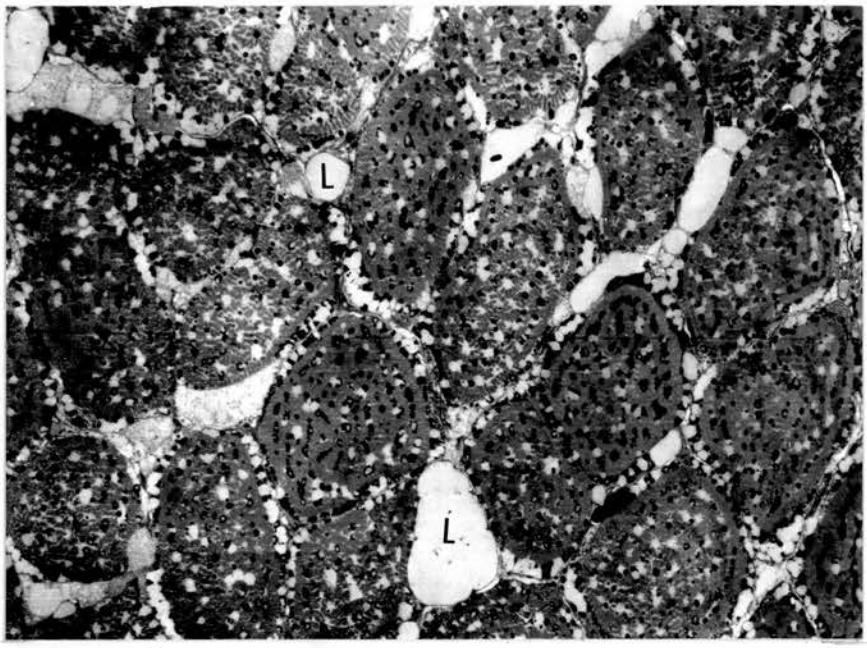



Fig.19

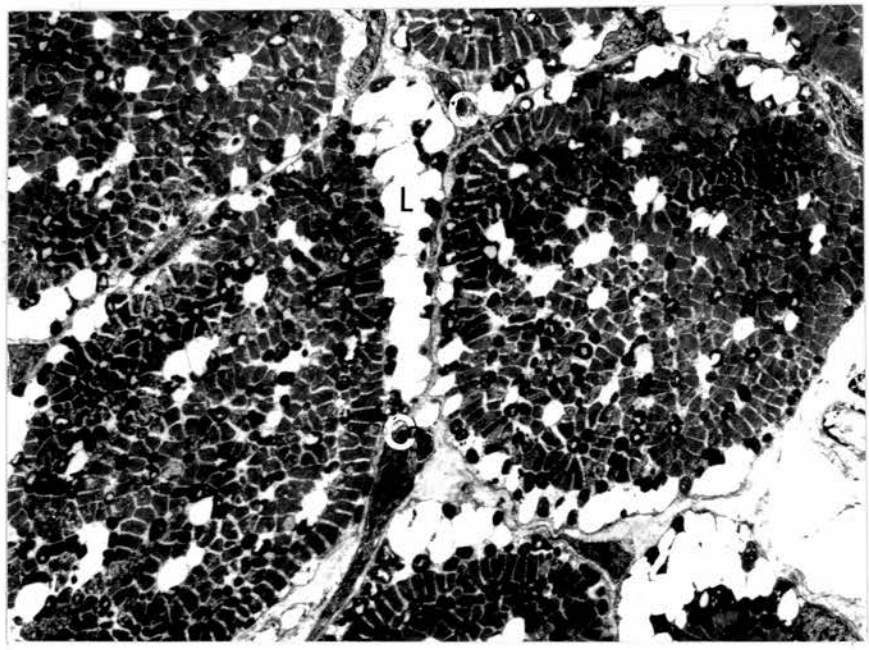



Fig 20

Fig. 21

Transverse section electron micrograph (x2680) from fast fibre of rainbow trout showing mitochondria (M), myofibrils (MY), nucleus (N).

Fig. 22

Transverse section electron micrograph (x4600) from slow fibre of rainbow trout showing mitochondrial cristae structure and lipid.

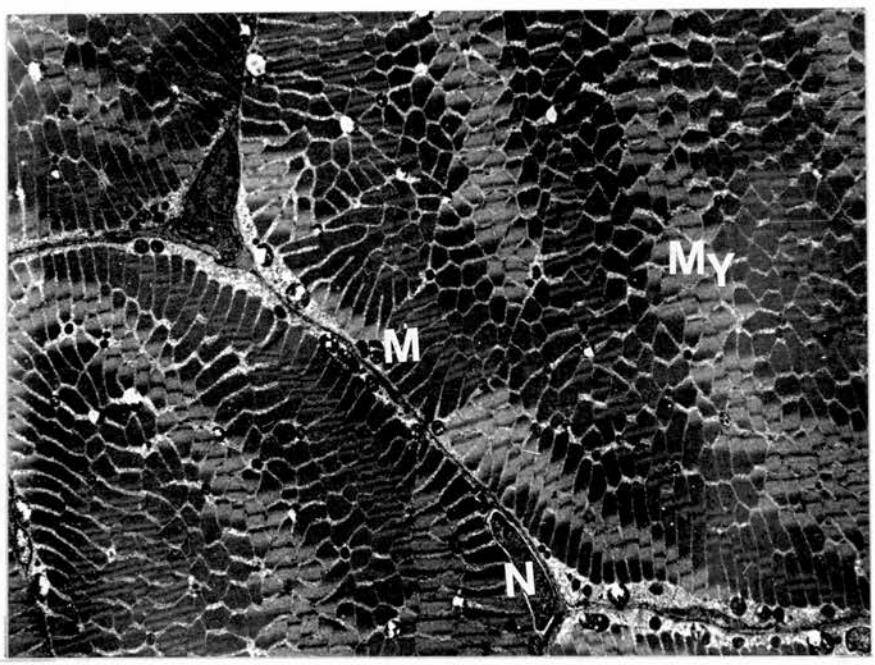



Fig 21

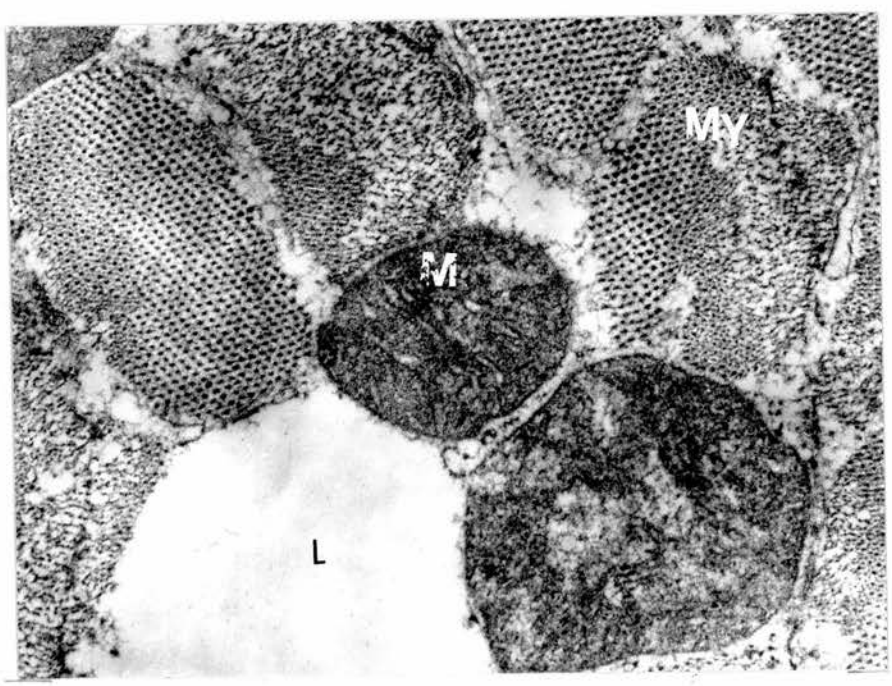



Fig.22

TABLE 5: QUANTITATIVE ANALYSES OF THE VASCULARIZATION OF SLOW AND FAST MUSCLE FIBRES OF RAINBOW TROUT (SALMO GAIRDNERI)

| Parameter† | Unit | Symbol calculation | Slow | Fast |
|---|---|---|---|---|
| Number of fibres | | A | 104 | 105 |
| Number of capillaries | | B | 132 | 57 |
| Fibre per cross-sectional area | $\mu m^2$ | C | 526.41 ± 23.50 | 1129 ± 92.11** |
| Fibre perimeter | µm | D | 91.0 ± 2.11 | 153.87 ± 7.79** |
| Fractional volume occupied by mitochondria | % | E | 31.6 ± 2.25 | 9.6 ± 0.25*** |
| Number of capillaries per muscle fibre | | F | 1.26 ± 0.1 | 0.54 ± 0.08** |
| Capillary contact length per fibre | µm | G | 5.34 ± 0.23 | 3.34 ± 0.31* |
| Percentage of fibre perimeter in direct capillary contact | % | $H = \frac{G \times 100}{D}$ | 5.87 ± 0.22 | 2.17 ± 0.25** |
| Mean perimeter served by one capillary | µm | $I = \frac{D}{F}$ | 72.2 | 284.9*** |
| Capillary contact length per $\mu m^2$ fibre cross-sectional area | $\mu m^2$ | $J = \frac{G}{C}$ | 0.01 | 0.003*** |
| Capillary surface ($\mu m^2$) supplying 1 $\mu m^3$ of mitochondria | $\mu m^2$ | $K = \frac{J \times 100}{E}$ | 0.032 | 0.0312 N.S. |
| Maximum hypothetical diffusion distance | µm | $L = \sqrt{\frac{A \times C}{B.\pi}}$ | 11.48 | 25.72** |

N.S. Not significant at $P < 0.05$

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

† Values represent mean ± S.E. mean

TABLE 6: FRACTIONAL VOLUME OCCUPIED BY MITOCHONDRIA AND LIPID DROPLETS IN SLOW AND FAST MUSCLE FIBRES OF RAINBOW TROUT

| Parameter | Fractional volume (%) | |
|---|---|---|
| | Slow | Fast |
| Total mitochondria | 31.60 ± 2.25 | 9.60 ± 0.25 |
| Subsarcolemmal mitochondria | 13.40 ± 1.00 | 6.50 ± 0.10 |
| Intermyofibrillar mitochondria | 18.20 ± 1.25 | 3.00 ± 0.15 |
| Total lipid | 15.50 ± 2.14 | 2.50 ± 0.50 |
| Subsarcolemmal lipid | 9.00 ± 1.14 | 1.35 ± 0.40 |
| Intermyofibrillar lipid | 6.50 ± 1.00 | 1.15 ± 0.10 |

Values represent mean ± S.E. mean

CHAPTER 5

## GENERAL DISCUSSION

In performing metabolic studies of swimming fish it is necessary to realise the importance of three fundamental requirements. These are the elimination of stress, pre-conditioning of the fish and the inclusion of adequate control fish in parallel at each test velocity or swimming speed. Environmental factors that cause stress in fish are methods of capture, transportation and handling (Black and Barrett, 1957; Wardle, 1972). Stress results in increased lactate or depletion of glycogen. Consequently when such fish are used for exercise studies there is a further rise in lactate and the actual result is masked. For example, early studies have shown blood lactate increase from 2-5 fold than the resting value following swimming at sustainable speeds (Black, 1955; Black et al., 1960, 1966). In contrast, rainbow trout of this study, conditioned to moderate speeds (1.1-1.3 body lengths $s^{-1}$) showed lactate and glycogen levels that were not significantly different from tank rested fish. Similar results have been obtained by Johnston and Moon (1980a,b). In both experiments exercised and non-exercised fish were immediately freeze-clamped in liquid nitrogen ($-159^{o}C$). This is the recommended technique as it arrests metabolism very quickly in contrast to other methods in which post-mortem changes or recovery from exercise are likely to occur.

The biochemical studies on the myotomal muscle fibres of swimming fish in this thesis are consistent with slow fibres being mainly recruited at slow swimming speeds. Following increase in swimming speeds greater proportion of fast fibres are recruited. The threshold speed for the recruitment of fast fibres in rainbow trout (13-20 cm) appears to be around 2-3 body lengths $s^{-1}$. The evidence in support of this is the increase in net lactate of 27% and 38% in slow and fast fibres, respectively, following 2 minutes sustained swimming at

2.8 body lengths $s^{-1}$. This is in close agreement with the electromyographical studies of Bone et al. (1978) on rainbow trout (17-30 cm) which showed recruitment of fast fibres at 2-2.5 body lengths $s^{-1}$. The involvement of both slow and fast fibres over a whole range of swimming speeds has been established for a number of other teleosts species (Pritchard, Hunter and Lasker, 1971; Smit et al., 1971; Johnston and Goldspink, 1973a,b). For example, Smit et al. (1971) used oxygen consumption as a means of muscular activity in goldfish, Carassius auratus, and concluded that both fibre types are used at all velocities. However, the proportion of slow to fast fibres used was higher at the slow swimming speeds. The decrease in this ratio with increasing speed was due to the recruitment of higher proportion of fast fibres. In this species it was observed that there was a significant contribution of fast fibres at speeds in excess of 3.4 bodylengths $s^{-1}$ (Smit et al., 1971). In a study of fibre hypertrophy Greer-Walker and Emmerson (1978) obtained increase in size of slow fibres of rainbow trout and coalfish (Greer-Walker and Pull, 1973) at 1 bodylength $s^{-1}$. There was a decline in slow fibre hypertrophy between 2 and 3 bodylengths $s^{-1}$. In contrast, the fast fibres increased in cross-sectional area at these speeds (Greer-Walker and Emerson, 1978).

The fast fibres of higher teleosts, which are recruited over a wide range of swimming speeds, are multiply innervated (Hudson, 1973; Johnston et al., 1977; Bone, 1978). In contrast, fast fibres of elasmobranch, for example dogfish and primitive teleosts such as herring, are focally innervated. These are only reserved for bursts of rapid swimming and fatigue in 1-2 minutes swimming (Bone, 1966; Bone et al., 1978). Therefore it would appear that multiple innervation has advantage over focal innervation since it allows fast muscle fibres to be recruited at low as well as high swimming speeds. It seems likely that there is a very sharp transition between the levels of effort which

result in almost indefinite sustained activity in higher teleosts and those which lead to fatigue in elasmobranchs. Multiple innervation was derived independently on several occasions during the evolution of teleosts (Bone and Ono, 1982). Both focal and multiple innervation have been found in some orders (Bone and Ono, 1982). The multiple pattern of innervation in stormiformes is suggestive of early transitional phase from focal to multiple innervation in teleosts (Bone and Ono, 1982). It is suggested that the evolution of multiply innervated fast fibres may be related to a requirement to achieve rapid tail-beat frequencies associated with efficient swimming (Johnston, 1982d). In fibres where sustained activity is supported solely by slow (red) fibres, the massive bulk of fast fibres is likely to operate passively. If a certain proportion of these fibres are activated continually the passive resistance would be overcome (Johnston, 1982d).
Hudson (1969) has suggested the rotation of the firing frequency of polyneuronal innervated fast fibres which produce graded response within the myotome.

The recruitment of multiply innervated fast fibres has been related to the degree of multi-terminal innervation (Bone et al., 1978; Johnston, 1981a). For example, electromyographical studies have shown the recruitment of fast fibres at 1.8 bodylengths $s^{-1}$ in brook trout (Johnston and Moon, 1980a), 2-2.5 bodylengths $s^{-1}$ in mirror carp (Johnston et al., 1977). However, this is complicated by the fact that some multi-terminally innervated fast fibres are recruited at 3.2 and 4.5 bodylengths $s^{-1}$ such as found in stripped bass (Morone saxtilis) and bluefish (Pomatomus sultatrix), respectively. These are speeds comparable to those at which focally innervated fast fibres are recruited in primitive teleosts and elasmobranchs. In view of the diversity of multiply innervated fast muscle fibres (i.e. number of endplates/fibre, extent of polyneuronal innervation), it seems unlikely

that a sharp division can be drawn between the two types of behaviour.

There is a good correlation between multiple innervation of the fast fibres and the capacity for aerobic metabolism. The aerobic capacity of multiply innervated fast fibres is greater than that of the focally innervated fast fibres: for example, the mitochondrial volume fraction and capillary densities of trout are 10 times higher than those of Galeus melastomus, chimaera or scyliorhinus (Totland et al., 1981; Johnston et al., 1980a; Johnston, 1982d).

Diverse forms of teleosts inhabit different ecological environments. Various evolutionary adaptations have been developed for living in different habitats. For example, there are different types of swimming activity and body shape among fishes. The three main patterns of body movement are anguilliform, ostraciform and cerangiform with a range of intermediate types of swimming activity. The tunny (Thynnus thynnus L.), for instance, has a number of features adapting it to high-speed cruising. Skipjack tuna (Katsuwonus pelamis) has a stream-lined fusiform shape and double-jointed lunate tail and counter-current blood supply to the slow fibres enabling it to maintain temperatures above ambient (Carey and Teal, 1969; Carey et al., 1971). Pike has reduced caudal fins and swimming is achieved by undulations of their dorsal and pectoral fins. In the holocephalon, rat fish (Chimaera monstrosa) slow speed swimming is achieved by use of pectoral fins. In this species the myotomal slow fibres may only produce rudder-like movements of the trunk associated with change of direction (Totland et al., 1981). This is similar to Notothenia rossii, Antarctic cod, where enlarged pectoral fins have taken over the function of locomotion (Walesby and Johnston, 1980). Nishihara (1967) observed that the pectoral fin muscles of goldfish (Carassius auratus) are mainly slow fibres in contrast to the pelvic fin fibres which are composed of fast fibres. He suggested that

this might relate to difference in function of the two sets of fins (Nishihara, 1967). The relative importance of the fin and body movements is dependent on the species and the swimming speed.

It has been demonstrated by kinematic studies that many free swimming fishes do not maintain a constant mode of swimming at all velocities (Videler and Weighs, 1982). For example, burst speed is alternated with glide in which the fish does no swimming activity. The thrust initiated at the acceleration period or burst speeds keeps the fish in motion and so energy is conserved. It is suggested that at velocities greater than 2.5 bodylengths $s^{-1}$ this type of swimming in cod is 2-3 times more economical hydrodynamically than a continuous mode of swimming (Videler and Weighs, 1982).

It would appear possible to correlate the threshold swimming speed at which the fast fibres start to make a significant contribution to acceleration and the activity pattern of the fish. For example, Greer-Walker and Pull (1973) have suggested that species with anguilliform type of locomotion are likely to have lower thresholds for contraction of fast fibres than species which are adapted for high speed swimming such as tuna (Katsuwonus pelamis) and Atlantic mackerel (Scomber scomber). Grillner and Kashin (1976) have found evidence of electrical activity in the focally innervated fast fibres of adult eels even at low swimming speeds. In anguilliform type of locomotion, the range of contraction times for each segment is less than that for carangiform and sub-carangiform swimming (Webb, 1978). This type of swimming is energetically most efficient at low speeds (Webb, 1978) and primitive teleosts and elasmobranch are able to maintain up to 3 bodylengths $s^{-1}$.

Another important aspect of fish skeletal muscle metabolism is the maintenance of cytosolic and mitochondrial redox potential during swimming. There appears to be a correlation between aerobic metabolism

and the activities of the glycerophosphate dehydrogenase and enzymes of the malate aspartate shuttle. For example, in insect flight muscle where the glycerophosphate dehydrogenase activity is higher by 14-25 times than any vertebrate tissue, glycolysis is geared to complete oxidation of substrate and little or no lactate is formed (Crabtree and Newsholme, 1972). The GPDH activity is 4-5 times higher in the fast than slow fibres of tuna (Guppy et al., 1978; Hochachka, 1980). The activity of the cytosolic GPDH in the fast fibres of trout is equivalent to 2 times that of dogfish (Crabtree and Newsholme, 1972). This study has shown the operation of the GPDH and the malate aspartate shuttles in both the slow and fast fibres of rainbow trout. Amino oxyacetate (transaminase inhibitor) has been used in this study for the determination of reoxidation of cytosolic NADH. Various other inhibitors can be employed to determine other aspects of the electron transport system or other hydrogen shuttle systems. Examples are rotenone, an inhibitor of mitochondrial oxygen consumption and also antimycin. It is essential in such experiments to set up parallel control tests so that the percentage inhibition or stimulation can be assessed.

In contrast to rainbow trout, the fast fibres of carp (Carassius carassius) show little increase in lactate even after intense stimulation or following burst swimming (Prosser et al., 1957). It is probable that this may be due to reduced metabolic rate, the existence of non-circulatory transfer of metabolites between slow (red) and fast (white) fibres (Wittenberger et al., 1975; Johnston et al., 1977) or complex anaerobic pathways (Johnston, 1975; Hughes and Johnston, 1977; Hochachka, 1980). For example, anaerobic end products increased in fish tissues during anoxia in addition to lactate including carbon dioxide (Van den Thillart et al., 1976), ethanol (Shoubridge and Hochachka, 1980), ammonia (Kutty, 1972), alanine (Johnston, 1975) and succinate (Johnston, 1975; Hughes and Johnston, 1978).

Ultrastructural studies have shown that several fish species have developed various adaptations to increased oxygen delivery to mitochondria. For example, in the European anchovy (Engraulis encrasicolus) special adaptations appear to increase the rate of oxygen delivery to the slow fibres. There is an increase in the ratio of perimeter to area and the fibres appear as flattened lamellae in contrast to the normal cylindrical pattern in transverse section (Greer-Walker et al., 1980). Furthermore, each fibre is capillarized by an average of 12.9 capillaries resulting in ~52% of the fibre surface in direct contact with capillary (Johnston, 1982b). In anchovy the maximum distance between any capillary and mitochondria in the slow fibre is 8 μm (Johnston, 1982b). Good correlation between increased mitochondrial volume density, capillary supply and decrease in oxygen diffusion distance would appear to be some of the prerequisites of potential aerobic metabolism. Ultrastructural studies of the skeletal muscles in rainbow trout and biochemical findings are consistent with fast fibres having aerobic capacity which is a significant proportion of slow fibres. Weatherly et al. (1982) have tagged swimming rainbow trout with radiotelemetry apparatus for sensing and transmitting electromyograms. These studies show that EMG values are highly correlated with the oxygen consumption during activity periods. It is therefore possible that swimming fish in the wild, fitted with telemetry packages, can be used to indicate oxygen consumption and the mode of swimming of the fish.

Ultrastructural parameters may be altered by physical changes in environment (such as temperature pressure, pH, ionic concentration, etc.). Many species undergo compensatory physiological and biochemical changes in order to adapt to the different environmental temperatures. These adaptations would include changes in metabolic pathways, enzyme activity and intermediates of metabolism. For example, salmon and

trout are known to undergo seasonal migrations from the low environmental temperatures of the ocean to higher temperatures of the rivers and streams where they spawn (Dean, 1969). In addition to temperature acclimatory changes, there are changes due to evolutionary adaptations which affect the metabolism and division of labour between fast and slow fibres of fish muscle (Dean, 1969). Johnston (1981) has also shown changes in the skeletal muscles of marine flatfish (Pleuronectes platessa) following starvation. Boström and Johansson (1972) have demonstrated changes due to development.

Although anaerobic metabolism provides an inefficient means of generating ATP during sustained activity, it is likely that it supplements that of aerobic metabolism. In support of this is the anaerobic contribution to activity during the first five minutes swimming to total energy production (calculated from literature oxygen consumption measurements) amounting to 39% at 2.3 bodylengths $s^{-1}$ and 74% at 7.0 bodylengths $s^{-1}$. Consequently the fish is able to increase the range of swimming speeds. An interesting possibility is that lactate produced during acceleration constitutes a major substrate for aerobic metabolism by slow muscle mitochondria.

## REFERENCES

AKSTER, H. A. (1981) Ultrastructure of muscle fibres in head axial muscles of the perch (Perca fluviatilis L.). A quantitative study. Cell. Tissue Res., 219, 111-131.

ALEXANDER, C. McN. (1969) The orientation of muscle fibres in the myomeres of fishes. J. mar. biol. Ass. U.K., 49, 263-290.

ALTRINGHAM, J. D. and JOHNSTON, I. A. (1981) Quantitative histochemical studies of the peripheral innervation of cod (Gadus morhua) fast myotomal muscle fibres. J. comp. Physiol., 143, 123-127.

ARLOING, S. and LAVOCAT, A. (1875) Recherches sur fanatomie et la physiologie des muscles striés pales et foncés. Mem. Acad. Sci. et Belles-Lettres Toulouse, 7, 177.

ASTER, P. L. and MOON, T. W. (1981) Influence of fasting and diet on lipogenic enzymes in the American eel, Anguilla rostrata lesueur. J. Nutri., III(2), 346-354.

BÁRÁNY, M. (1967) ATPase activity of myosin correlated with speed of muscle shortening. J. Gen. Physiol., 51, 197-216.

BARETS, A. (1961) Contribution a l'etude des systemes moteur lent et rapide du muscle lateral des teleosteens. Arch. Anat. Morph. exp., 50 (suppl.), 91-187.

BEAMISH, (1978) Swimming capacity. In: Fish Physiology, Vol VII (Ed. W. S. Hoare and D. J. Randall), pp. 101-187. New York, San Francisco, London: Academic Press.

BENNETT, A. F. (1978) Activity metabolism of the lower vertebrates. Ann. Rev. Physiol., 40, 447-469.

BENNETT, A. F. (1980) The metabolic foundations of vertebrate behaviour. BioScience, 30(7), 452-456.

BENNETT, A. F. and LICHT, (1972) Anaerobic metabolism during activity in lizards. J. comp. Physiol., 81, 277-288.

BERG, G. and STEEN, J. B. (1965) Physiological mechanisms for aerial respiration in the eel. Comp. Biochem. Physiol., 15, 469-484.

BERGMAN, R. A. (1964). Mechanical properties of the dorsal fin musculature of the marine teleost, Hippocampus hudsonius. Bull. John Hopkins Hosp., 114, 344-353.

BERGMAN, R. A. (1967) Motor nerve endings of twitch muscle fibres in Hippocampus hudsonius. J. cell Biol., 32, 751.

BERGMEYER, H. U. and BERNT, (1965) Methods of Enzymatic Analysis. Weinhelm/Bergstr.: Verlag Chemie.

BEST, A. C. G. and BONE, Q. (1973) The terminal neuromuscular junctions of lower chordates. Z. Zellforsch. Mikrosk. Anat., 143, 495-504.

BILINSKI, R. (1974) Biochemical aspects of fish swimming. In: Biochemical Perspectives in Marine Biology (Eds. D. C. Malling and J. R. Sargent), Vol. I, pp. 239-288.

BLACK, E. C. (1955) Blood levels of haemoglobin and lactic acid in some freshwater fishes following exercise. J. Fish. Res. Bd. Can., 12, 917-929.

BLACK, E. C. (1957a) Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity. I. Kamloops trout, Salmo gairdneri. J. Fish Res. Bd. Can., 14, 117-134.

BLACK, E. C. (1957b) Alterations in blood level of lactic acid in certain salmonid fishes following muscular activity. II. Lake trout, Salvelinus namaycush. J. Fish Res. Bd. Can., 14, 645-649.

BLACK, E. C. (1957c) Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity. III. Sockeye salmon, Onco rhynchus nerka. J. Fish Res. Bd. Can., 14, 807-814.

BLACK, E. C. and BARRETT, I. (1957) Increase in levels of lactic acid in the blood of cut-throat and steelhead trout following handling and live transportation. Can. Fish Cult., 20, 13-24.

BLACK, E. C., ROBERTSON, A. C., HANSLIP, A. R. AND CHIU, W.-C. (1960) Alterations in glycogen, glucose and lactate in rainbow and kamloops trout, Salmo gairdneri, following muscular activity. J. Fish Res. Bd. Can., 17, 487-500.

BLACK, E. C., ROBERTSON, A. C. and PARKER, R. R. (1961) Some aspects of carbohydrate metabolism in fish. In: Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals (Ed. A. W. Martin), pp. 89-124. Seattle: University of Washington.

BLACK, E. C., ROBERTSON, A. C., CONNOR, A., LAM, K.-C. and CHIU, W.-G. (1962) Changes in glycogen, pyruvate and lactate in rainbow trout (Salmo gairdneri) during and following muscular activity. J. Fish Res. Bd. Can., 19, 409-436.

BLACK, E. C., MANNING, G. T. and HAYASHI, K. (1966) Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate and lactate in venous blood of rainbow trout (Salmo gairdneri) during and following severe muscular activity. J. Fish. Res. Bd. Can., 23, 783-795.

BODDEKE, R., SLIJPER, E. J. and VAN DER STELT, A. (1959) Histological characteristics of the body musculature of fishes in connection with their mode of life. Proc. K. Ned. Akad. Wet., Ser. C, 62, 576-588.

BOKDAWALA, F. D. and GEORGE, J. C. (1967) A histochemical study of the red and white muscles of the carp, Cirrhina mrigala. J. Anim. Morphol. Physiol., 14, 60-68.

BONE, Q. (1964) Patterns of muscular innervation in the lower chordates. Int. Rev. Neurobiol., 6, 99-147.

BONE, Q. (1966) On the function of the two types of myotomal muscle fibres in elasmobranch fish. J. mar. biol. Ass. U.K., 46, 321-349.

BONE, Q. (1970) Muscular innervation and fish classification. Symp. Int. Zoofil. 1st Univ. Salamanca, pp. 369-377.

BONE, Q. (1972) The dogfish neuromuscular junction: Dual innervation of vertebrate striated muscle fibres. J. cell Sci., 10, 657-665.

BONE, Q., KICENUIK, J. and JONES, D. R. (1978) On the role of the different fibre types in fish myotomes at intermediate swimming speeds. Fisheries Bulletin, 76, 691-699.

BONE, Q. (1978) Locomotor muscle. In: Fish Physiology, Vol. VII (Eds. W. S. Hoare and D. J. Randall), pp. 361-424. New York, San Francisco, London: Academic Press.

BONE, Q. and CHUBB, A. D. (1978) The histochemical demonstration of myofibrillar ATPase in elasmobranch muscle. Histochemical Journal, 10, 489-494.

BONE, Q. and ONO, R. D. (1982) Systematic implications of innervation patterns in teleost myotomes. Breviora Museum Comp. Zoo., 470, 1-23.

BORST, P. (1963) Hydrogen transport metabolite transport. In: Funktionelle und Morphologische Organisation der Zelle (Ed. P. Karlson), Vol. 1, p.137. Berlin, Gottingen, Heidelberg: Springer Verlag.

BOSSEN, E. J., SOMMER, J. R. and WAUGH, R. A. (1978) Comparative steriology of the mouse and finch left ventricle. Tissue and Cell, 10(4), 773-784.

BOSTROM, S. L. and JOHANSSON, R. G. (1972) Enzyme activity patterns in white and red muscle of the eel (Anguilla anguilla) at different developmental stages. Comp. Biochem. Physiol., 42B, 533-542.

BOUCK, G. R. and BELL, R. C. (1966) Influence of capture on methods on blood characteristics and mortality in rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 95, 170-176.

BRAEKKAN, D. R. (1956) Function of the red muscle in fish. Nature (London), 178, 747-748.

BRAEKKAN, D. R. (1959) Red muscle as a possible character for the identification of sharks. Nature, 183, 556-557.

BRETT, J. R. (1972) The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Resp. Physiol., 14, 151-170.

BUTTKUS, H. (1963) Red and white muscle of fish in relation to rigor mortis. J. Fish. Res. Bd. Can., 20, 1607-1612.

CANDY, D. J. (1980) Biological Functions of Carbohydrates. Glasgow, London: Blackie and Son Limited.

CAREY, F. G. and TEAL, J. M. (1969) Regulation of body temperature by the blue fin tuna. Comp. Biochem. Physiol., 28, 205-213.

CAREY, F. G., TEAL, J. M., KANWISHER, J. W. and LAWSON, K. D. (1971) Warm bodied fish. Am. Zool., 11, 137-145.

CARROLL, N. V. R., LONGLEY, W. and ROE, J. H. (1956) The determination of glycogen in liver and muscle by use of anthrone reagent, J. Biol. Chem., 220, 583-598.

CRABTREE, B. and NEWSHOLME, E. A. (1972) The activities of phosphorylase hexokinase, phosphrofructokinase, lactate dehydrogenase and glycerol 3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. Biochem. J., 126, 49-58.

CZOK, R. and WALTHER, L. (1965) Determination of: Pyruvate, Phospho-enolpyruvate and D-Glycerate-2-phosphate. In: Method Enzymatic Analysis (Ed. H. U. Bergmeyer).

DANI, A., BARTOLI, G. M. and CALEOTTI, T. (1977) The operation of the malate-aspartate shuttle in the reoxidation of glycolytic NADH in slices of fetal rat liver. Biochem. Biophys. Acta, 462, 781-784.

DAVISON, W., GOLDSPINK, G. and JOHNSTON, I. A. (1976) The division of labour between fish myotomal muscles during swimming. J. Physiol. (London), 263, 185-186.

DAWSON, A. G. (1979) Oxidation of cytosolic NADH formed during aerobic metabolism in mammalian cells. TIBS, August 1979, 171-176.

DEAN, J. M. (1969) The metabolism of tissues of thermally acclimated trout (Salmo gairdneri). Comp. Biochem. Physiol., 29, 185-196.

DRIEDZIC, W. R. and HOCHACHKA, P. W. (1978) Metabolism of fish during exercise. In: Fish Physiology, Vol VII (Eds. W. S. Hoare and D. J. Randall), pp. 503-543. New York, San Francisco, London: Academic Press.

DRIEDZIC, W. R. and KICENUIK, J. W. (1976) Blood lactate levels in free swimming trout (Salmo gairdneri) before and after exercise resulting in fatigue. J. Fish. Res. Bd. Can., 33, 173-176.

EDINGTON, D. W., WARD, G. R. and SAVILLE, W. A. (1973) Energy metabolism of working muscle: Concentration profiles of selected metabolites. Am. J. Physiol., 224, 1375-1380.

EGGINTON, S. and JOHNSTON, I. A. (1981a) Muscle fibre differentiation and vascularisation in the juvenile European eel (Anguilla anguilla L.). Cell and Tissue Res., 222, 563-577.

EGGINTON, S. and JOHNSTON, I. A. (1981b) A morphometric analysis of regional differences in myotomal muscle ultrastructure in the juvenile eel (Anguilla anguilla L.). Cell Tiss. Res., 222, 579-596.

EISENBERG, B. R., KUDA, S. M. and PETER, J. B. (1974) Steriological analysis of mammalian skeletal muscle. I. Soleus muscle of the adult guinea pig. J. cell Biol., 60, 732-754.

ESTABROOK, R. W. and SACKTOR, B. (1958) α-Glycerophosphate oxidase of flight muscle mitochondria. J. Biol. Chem., 233, 1014-1018.

FARKAS, T. (1969) Studies on the mobilization of fats in lower vertebrates. Acta Biochim. Biophys. Acad. Sci. Hung., 4, 237-249.

FISCHER, E. H., BLUM, H. E., BYERS, B., HEIZMANN, C., KERRICK, G. W., LEHKY, P., MALENCIK, D. A. and POCINWONG, S. (1975) Concerted regulation of glycogen metabolism and muscle contraction. In: Metabolic Interconversion of Enzymes (Ed. S. Shaltiel), pp. 1-8. Heidelberg: Springer-Verlag.

FLITNEY, F. W. and JOHNSTON, I. A. (1979) Mechanical properties of isolated fish red and white muscle fibres. J. Physiol., 295, 49-50.

FLOOD, P. R. (1979) The vascular supply of the three fibre types in the parietal trunk muscle of the Atlantic hagfish (Myxine glutinosa L.). Microvasc. Res., 17, 55-70.

FRASER, J. M. and BEAMISH, F. W. H. (1969) Blood lactic acid concentrations in brook trout, Salvelinus fontinalis, planted by air drop. Trans. Am. Fish. Soc., 98, 263-267.

FREADMAN, M. A. (1979) Role of partitioning of swimming musculature of striped Bass, Morone saxatilis, Walbaum and Bluefish, Pomatomus saltatrix L. J. Fish Biol., 15, 417-423.

FREED, J. M. (1971) Properties of muscle phosphofructokinase of cold- and warm-acclimated Carassius auratus. Comp. Biochem. Physiol., 39B, 747-764.

FUCHS, F., REDDY, V. and BRIGGS, F. N. (1970) The interaction of cations with the calcium binding site of troponin. Biochim. biophys. Acta, 221, 407-409.

GEORGE, J. C. (1962) A histophysiological study of the red and white muscles of the mackerel. Am. Midl. Nat., 68, 487.

GERBTZOFF, M. A. (1956) Contribution a la morphologie comparee des appareils cholinesterasiques myo-neural et musculotendineux des vertebres. Ann. Histochim., 1, 145.

GERHARD, M. and BEUTLER, H.-O. (1975) D-Fructose-1,6-diphosphate, Dihydroxyacetone Phosphate and D-Glyceraldehyde-3-phosphate: Determination. In: Methods of Enzymatic Analysis (Ed. H. U. Bermeyer).

GOLLNICK, P. D. and HERMANSEN, L. (1973) Biochemical adaptation to exercise: Anaerobic metabolism. In: Exercise and Sport Science Reviews, Vol. I. (Ed. J. H. Wilmore), pp. 1-43. New York: Academic Press.

GORDON, M. S. (1968) Oxygen consumption of red and white muscles from tuna fishes. Science, 159, 87-90.

GREEN, C. W. (1912) A new type of fat storing muscle in the salmon, Oncorhynchus Tschawytscha. Amer. J. Anat., 13, 175-181.

GREEN, C. W. (1913) An undescribed longitudinal differentiation of the great lateral muscle of the King Salmon. Anat. Rec., 7, 99-101.

GREER-WALKER, M. (1971) Effect of starvation and exercise on the skeletal muscle fibers of the cod (Gadus morhua L.). J. Cons., Cons. Perm. Int. Explor. Mer, 33, 421-427.

GREER-WALKER, M. and PULL, G. A. (1973) Skeletal muscle function and sustained swimming speeds in the coalfish, Gadus virens L. Comp. Biochem. Physiol. A44, 495-501.

GREER-WALKER, M. and PULL, G. A. (1975) A survey of red and white muscle in marine fish. J. Fish Biol., 7, 295-300.

GREER-WALKER, M. and EMERSON, L. (1978) Sustained swimming speeds and myotomal muscle function in the trout, Salmo gairdneri. J. Fish Biol., 13, 475-481.

GREER-WALKER, M., HORWOOD, J. and EMERSON, L. (1980) On the morphology and function of red and white skeletal muscle in the anchovies, Engraulis encrasicolus L. and E. mordax girard. J. mar. biol. Ass. U.K., 60, 31-37.

GRILLNER, S. and KASHIN, S. (1976) On the generation and performance of swimming in fish. Adv. Behavioral Biol. 18, 181-201.

GUPPY, M., HULBERT, W. C. and HOCHACHKA, P. W. (1979) Metabolic sources of heat and power in tuna muscles. II. Enzyme and metabolite profiles. J. Exp. Biol., 82, 303-320.

GUNTER, L. and GERHARD, M. (1975) Determination of: D-Glucose 6-phosphate and D-Fructose-6-phosphate. In: Methods of Enzymic Analysis, Vol. 3 (Ed. H. U. Bergmeyer), pp. 1239-1242.

HAGIWARA, S. and TAKAHASHI, K. (1967) Resting and spike potentials of skeletal muscle fibers in salt-water elasmobranch and teleost fish. J. Physiol. (London), 190, 499-518.

HAMMOND, B. R. and HICKMAN, C. P., Jr. (1966) The effect of physical conditioning on the metabolism of lactate, phosphate and glucose in rainbow trout (Salmo gairdneri). J. Fish Res. Bd. Can., 23, 65-83.

HAZEL. J. and PROSSER, C. L. (1974) Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev., 54, 620-677.

HERMANSEN, L. and GOLLNICK, P. D. (1973) Biochemical adaptation to exercise: Anaerobic metabolism. In: Exercise and Sport Science Review, Vol. I (Ed. T. H. Wilmore), pp. 1-43. New York: Academic Press.

HOCHACHKA, P. W. (1961) The effect of physical training on oxygen debt and glycogen reserves in trout. Can. J. Zool., 39, 767.

HOCHACHKA, P. W. (1980) Living without oxygen. Closed and open systems in hypoxia tolerance, p. 181. Cambridge, Massachusetts: Harvard University Press.

HOLLANDER, H. and VAALANC, J. L. (1968) A reliable staining method for semi-thin sections in experimental neuroanatomy. Brain Res., 10, 120-126.

HOHORST, H. J. (1965) L (+) lactate: Determination with lactic dehydrogenase and $DPN^+$. In: Methods of Enzymatic Analysis (Ed. H. U. Bergmeyer), pp. 266-270. New York: Academic Press.

HUDSON, R. C. L. (1969) Polyneuronal innervation of the fast muscles of the marine teleost, Cottus scorpius L. J. exp. Biol., 50, 47-67.

HUDSON, R. C. L. (1973) On the function of the white muscles of teleosts at intermediate swimming speeds. J. exp. Biol., 58, 509-522.

HUGHES, G. M. and JOHNSTON, I. A. (1978) Some responses of the electric ray (Torpedo marmorata) to low ambient oxygen tensions. J. exp. Biol., 73, 107-117.

HULBERT, W. C., GUPPY, M. and MURPHY, B. (1979) Metabolic sources of heat and power in tuna muscles. I. Muscle fine structure. J. exp. Biol., 82, 289-301.

JIMENEZ DE ASUA, L., ROZENGURT, E., DEVALLE, J. J. and CARMINATTI, H. (1971) Some kinetic differences between the M isozymes of pyruvate kinase from liver and muscle. Biochim. Biophys. Acta, 235, 326-334.

JOHNSTON, I. A. and GOLDSPINK, G. (1973a) A study of the swimming performance of the crucian carp, Carassius carassius (L.), in relation to the effects of exercise and recovery on biochemical changes in the myotomal muscles and liver. J. Fish Biol., 5, 249-260.

JOHNSTON, I. A. and GOLDSPINK, G. (1973b) Quantitative studies of muscle glycogen utilisation during sustained swimming in crucian carp (Carassius carassius L.). J. exp. Biol., 59, 607-615.

JOHNSTON, I. A. and GOLDSPINK, G. (1973c) A study of glycogen and lactate in the myotomal muscles and liver of the coalfish (Gadus virens L.) during sustained swimming. J. Mar. Biol. Ass. U.K., 53, 17-26.

JOHNSTON, I. A., PATTERSON, S., WARD, P. S. and GOLDSPINK, G. (1974) The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. Canad. J. Zool., 52, 871-877.

JOHNSTON, I. A. (1975a) Anaerobic metabolism in the carp (Carassius carassius L.). Comp. Biochem. Physiol., 51B, 235-241.

JOHNSTON, I. A. (1975b) Studies on the swimming musculature of the rainbow trout. II. Muscle metabolism during severe hypoxia. J. Fish Biol., 7, 459-467.

JOHNSTON, I. A., WARD, P. S. and GOLDSPINK, G. (1975 ) Studies on the swimming musculature of the rainbow trout. I. Fibre types. J. Fish Biol., 7, 451-458.

JOHNSTON, I. A., DAVISON, W. and GOLDSPINK, G. (1977a) Energy metabolism of carp swimming muscles. J. comp. Physiol., 114, 203-216.

JOHNSTON, I. A. (1977 ) A comparative study of glycolysis in red and white muscles of the trout (Salmo gairdneri) and mirror carp (Cyprinus carpio). J. Fish Biol., 11, 575-588.

JOHNSTON, I. A. (1980a) Specializations of fish muscle in 'development and specializations of muscle' society. Exp. Biol. Seminar Series Symp. (Ed. D. F. Goldspink). Cambridge: Cambridge University Press.

JOHNSTON, I. A. (1980b) Structure and function of muscles. Zool. Soc. Symp. 'Vertebrate Locomotion' (Ed. M. H. Day), Vol. 45. London: Academic Press.

JOHNSTON, I.A. and MAITLAND, B. (1980) Temperature acclimation in crucian carp: A morphometric analysis of muscle fibre ultrastructure. J. Fish Biol., 17, 113-125.

JOHNSTON, I.A. and MOON, T.W. (1980a) Exercise training in skeletal muscle of brook trout (Salvelinus fontinalis). J. exp. Biol., 87, 177-195.

JOHNSTON, I.A and MOON, T.W. (1980b) Endurance exercise training in the fast and slow muscles of a teleost fish (Pollachius virens). J. comp. Physiol. B, 135, 147-156.

JOHNSTON, I. A. and MOON, T.W. (1981) Fine structure and metabolism of multiply innervated fast muscle fibres in teleost fish. Cell Tiss. Res., 219, 93-109.

JOHNSTON, I.A. (1981) Structure and function of fish muscles. In Symp. Zool. Soc. Lond., 48 (Ed. M.H. Day), pp. 71-113.

JOHNSTON, I.A. (1982a) Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. Cell Tiss. Res., 222, 325-337.

JOHNSTON, I.A. (1982b) Quantitative analyses of ultrastructure and vascularization of the flow muscle fibres of the anchovy. Tiss. and Cell, 14(2), 319-328.

JOHNSTON, I.A. (1982c) Dynamic aspects of fish myotomal muscles. "Fish Mechanics" (Ed. Webb, P. and Weih, D.) Praeger Press (in Press).

JOHNSTON, I.A. (1982d) On the design of fish myotomal muscles. Mar. Physiol. Behaviour (in Press).

JOHNSTON, I.A. and BERNARD, L. (1982) Routine oxygen consumption and muscle capillary supply in the tench (Tinca tinca): the effects of long term acclimation to hypoxia. Cell Tiss. Res. (in press).

KAPLAN, N.O. and GOODFRIEND, T.L. (1964) Role of two types of lactic dehydrogenase. In: Advances in Enzyme Regulation, Vol. 2. Oxford: Pergamon, pp. 203-212.

KLINGENBURG, M. (1970) Localization of glycerol phosphate dehydrogenase to the outer phase of the mitochondrial inner membrane. European J. Biochem., 13, 247-252.

KORNELIUSSEN, H. and NICOLAYSEN, K. (1973) Ultrastructure of four types of striated muscle fibres in the Atlantic hagfish (Myxine glutinosa L.). Z. Zellforsch., 143, 273-290.

KROGH, A. (1919) The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J. Physiol. Lond., 52, 405.

KRYVI, H. (1977) Ultrastructure of the different fibre types in axial muscles of the sharks, Etmopterus spinax and Galeus melastomus. Cell Tiss. Res., 184, 287-300.

KRYVI, H. and TOTLAND, G.K. (1978) Fibre types in locomotory muscles of the cartilanginous fish, Chimaera monstrosa. J. Fish Biol., 12, 257-265.

KRYVI, H., FLOOD, P. and GULJAEI, D. (1980) The ultrastructure of the fibre types in the axial muscles of the sturgeon, Acipenser stellatus. Cell Tiss. Res., 212, 117-126.

KUTTY, M.N. (1972) Respiratory quotient and ammonia excretion in Tilapia mossambica. Mar. Biol., 16, 126-133.

LANKESTER, E.R. (1871) Ueber des Vorkommen von haemoglobin den Muskeln der Mollusken und die verbreitung desselben in den lebendigen organismen. Arch. Ges. Physiol. Pflügers, 4, 315.

LARDY, H.A., PAETKAU, V. and WALTER, P. (1965) Paths of carbon in gluconeogenesis and lipogenesis: the role of mitochondria in supplying precursors of phosphoenolpyruvate. Proc. Nat. Acad. Sci. U.S.A., 53, 1410-1415.

LEHNINGER, A.L. (1951) Phosphorylation coupled to oxidation of dihydrophosphopyridine nucleotide. J. biol. Chem., 190, 345-359.

LIN, H., ROMSOS, D. R., TACK, P. I. and LEVEILLE, G. A. (1977) Effects of fasting and feeding various diets on hepatic lipogenic enzyme activities in Coho salmon (Oncorhynchus kisutch Walbaum). J. Nutr., 107, 1477-1483.

LORENZINI, S. (1678) Observazioni intorno alle Torpedini. Onofri (Italian).

MANSOUR, T. E. (1972) Phosphofructokinase. Curr. Top. Cell Regul., 5, 1-46.

MEIJER, A. J. and VAN DAM, K. (1974) The metabolic significance of anion transport in mitochondria. Biochim. biophys. Acta, 346, 213-244.

MÜLLERING, H. (1974) L-Malate: Determination with phalate dehydrogenase and glutamate-oxaloacetate transaminase. In: Methods of Enzymatic Analysis, Vol. 3 (Ed. H. U. Bergmeyer), pp. 1589-1594.

MOSSE, P. R. L. (1978) The distribution of capillaries in the somatic musculature of two vertebrate types with particular reference to teleost fish. Cell Tiss. Res., 187, 281-303.

MOSSE, P. R. L. (1979) Capillary distribution and metabolic histochemistry of the lateral propulsive musculature of pelagic teleost fish. Cell Tiss. Res., 203, 141-660.

MOSSE, P. R. L. and HUDSON, R. C. L. (1977) The functional roles of different muscle fibre types identified in the myotomes of marine teleosts: a behavioural anatomical and histochemical study. J. Fish Biol., 11, 417-430.

NAG, A. C. (1972) Ultrastructure and adenosine triphosphatase activity of red and white muscle fibers of the caudal region of a fish, Salmo gairdneri. J. Cell Biol., 55, 42-57.

NASSAR-GENTINA, V., PASSONNEAU, J., VERGARA, J. and PAPOPORT, S. (1978) Metabolic correlates of fatigue and recovery from fatigue in single frog fibres. J. Gen. Physiol., 72, 593-606.

NEWSHOLME, E. A. (1976) The role of the fructose 6-phosphate/fructose 1,6 diphosphate cycle in metabolic regulation and heat generation. Trans. Biochem. Soc., 4, 978-984.

NEWSHOLME, E. A. and START, C. (1973) Regulation in Metabolism. New York: Wiley, p. 349.

NEWSHOLME, E. A., ZAMMIT, V. A. and CRABTREE, B. (1978) The role of glucose and glycogen as fuels for muscle. Biochem. Soc. Trans., 6, 512-520.

NEWSHOLME, E. A. and ZAMMIT, V. A. (1979) Activities of enzymes of fat and ketone-body metabolism and effects of starvation and blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem. J., 184, 313-322.

NISHIHARA, H. (1967) Studies on the fine structure of red and white fin muscles of the fish, Carassius auratus. Arch. Histol. Jpn. (Niigata, Japan), 28, 425-447.

PATTERSON, S. and GOLDSPINK, G. (1972) The fine structure of red and white myotomal muscle fibresof the coalfish (Gadus virens). Z. Zellforsch. Mikrosk. Anat., 133, 463-474.

PATTERSON, S., JOHNSTON, I.A. and GOLDSPINK, G. (1975) A histochemical study of the lateral muscles of five teleost species. J. Fish Biol., 7, 159-166.

PATTON, D. N. (1898) The physiology of salmon in freshwater. J. Physiol., 22, 333-356.

PEACHEY, L. D. (1965) The sarcoplasmic reticulum and transverse tubules of the frog sartorius L. Cell Biol., 25, 209-231.

PECOT-DECHAVASSINE, M. (1961) Etude biochemique, pharmacologique et histochemique des cholinesterase des muscles stires chez les poissons, les batraciens, et les mammiferes. Arch. Anat. Microsc. Morphol. Exp., 50 Suppl., 341-438.

PETTE, D. (1966) Mitochondrial enzyme activities. In: Regulation of Metabolic Processes in Mitochondria (Eds., J. M. Tager, S. Papa, E. Quagliariello, E. C. Slater), pp. 28-50. Amsterdam: Elsevier Publishing Company.

PORTZEHL, H., ZAORALEK, P. and CAUDIN, J. (1969) The activation by $Ca^{2+}$ of the ATPase of extracted muscle fibrils with variation of ionic strength, pH and concentration of MgATP. Biochim. Biophys. Acta, 189, 440-448.

PRITCHARD, A. W., HUNTER, J. R. and LASKER, R. (1971) The relation between exercise and biochemical changes in red and white muscles and liver in the jack mackerel, Trachurus symmetricus. U.S. Fish Wildl. Serv., Fish Bull., 69, 379-386.

PROSSER, C. L. (1973) Comparative Animal Physiology. Philadelphia: W. B. Saunders.

PROSSER, C. L., BARR, L. M., PINC, R. A. and LAUER, C. Y. (1957) Acclimation of goldfish to low concentrations of oxygen. Physiol. Zool., 30, 137-141.

RANVIER, L. (1873) Proprietes et structures differentes des muscles rouges et des muscles blancs chez les lapins et chez les Raies. C. R. Acad. Sci., 77, 1030-1034.

RAYNER, M. D. and KEENAN, M. J. (1967) Role of red and white muscles in the swimming of the skipjack tuna. Nature (London), 214, 392-393.

REVEL, J. P. (1962) The sarcoplasmic reticulum of the cricothyroid muscle. J. Cell Biol., 12, 571-588.

ROA, G. M. M. (1968) Oxygen consumption of rainbow trout (Salmo gairdneri) in relation to activity and salinity. Can. J. Zool., 46, 781-786.

ROMANUL, G. C. A. (1965) Capillary supply and metabolism of muscle fibres. Arch. Neurol., 12, 497-509.

SAFER, B., SMITH, C. M. and WILLIAMSON, (1971) Control of reducing equivalents across the mitochondrial membrane in perfused rat heart. J. Mol. Cell. Cardiol., 2, 111-124.

SCRUTTON, M. C. and UTTER, M. F. (1968) The regulation of glycolysis and gluconeogenesis in animal tissues. Ann. Rev. Biochem., 37, 249-301.

SEIFTER, S., DAYTON, S., NOVIC, B. and MONTWYLER, E. (1949) The estimation of glycogen with anthrone reagent. Arch. Biochem., 25, 191-200.

SERAYDARIAN, M. W., ABBOTT, B. C. and WILLIAMS, F. (1961) Studies on the frog's sartorius at different stages of activity. Biochim. Biophys. Acta, 46, 355-363.

SHOUBRIDGE, E. A. and HOCHACHKA, P. W. (1980) Ethanol: Novel end product of vertebrate anaerobic metabolism. Science, 209, 308-309.

SIDELL, B. (1977) Turnover of cytochrome c in skeletal muscle of green sunfish (Lepomis cyanellus R.) during thermal acclimation. J. exp. Zool., 169, 233-250.

SIDELL, B. (1980) Responses of goldfish (Carassius auratus L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fiber types. Physiol. Zool., 53, 98-107.

SMIT, H., AMELINK-KOUTSTALL, J. M., VIGVERBERG, J. and VON VAUPEL-KLEIN, J. C. (1971) Oxygen consumption and efficiency of swimming goldfish. Comp. biochem. Physiol., A39, 1-28.

SOVIO, A. and OIKARI, A. (1976) Haematological effects of stress on a teleost, Esox lucius L. J. Fish Biol., 8, 397-411.

SOMERO, G. N. and HOCHACHKA, P. W. (1968) The effect of temperature on catalytic and regulatory functions of pyruvate kinases of rainbow trout and the Antarctic fish, Trematomus bernacchii. Biochem. J., 110, 395-400.

STANDFIELD, P. R. (1972) Electrical properties of white and red muscle fibres of the elasmobranch fish, Scyliorhinus canicula. J. Physiol., 222, 161-186.

STEVENS, E. D. (1968) The effects of exercise on the distribution of blood to various organs in rainbow trout. Comp. Biochem. Physiol., 25, 615-625.

STEVENS, E. D. (1972) Change in body weight caused by handling and exercise in fish. J. Fish Res. Bd. Can., 29, 202-203.

STEVENS, E. D. and NEILL, W. H. (1978) Body temperature relations of tunas, especially skipjack. In: Fish Physiology, Vol. 7 (Eds. W. S. Hoar, D. J. Randall), pp. 315-359. New York: Academic Press.

STIRLING, W. (1885) Fourth Ann. Rept. Fish Board, Scotland, p. 166.

TOTLAND, G. K., KRYVI, H. and SLINDE, E. (1978) LDH isozymes in the axial muscle of sharks, Galeus melastomus and Etmopterus spinax. J. Fish Biol., 12, 45-50.

TOTLAND, G. K., KRYVI, H., BONE, Q. and FLOOD, P. R. (1981) Vascularization of the lateral muscle of some elasmobranchiomorph fishes. J. Fish Biol., 18, 223-234.

TRIVEDI, B. and DANFORTH, W. (1966) Effect of pH on the kinetics of frog muscle phosphofructokinase. J. biol. Chem., 241, 4110-4111.

THILLART, Van Den G., KESBEKE, F. and WAARDE, A. V. (1976) Influence of anoxia on the energy metabolism of goldfish (Carassius auratus L.). Ph.D. thesis, State University of Leiden, The Netherlands.

VIDELER, J. J. and WEIHS, D. (1982) Energetic advantages of burst-and-coast swimming of fish at high speeds. J. exp. Biol., 97, 169-178.

WALESBY, N. J. and JOHNSTON, I. A. (1980a) Fibre types in the locomotory muscles of an Antarctic teleost, Notothenia rossii: A histochemical, ultrastructural and biochemical study. Cell. Tiss. Res., 208, 143-164.

WALESBY, N. J. and JOHNSTON, I. A. (1980b) Temperature acclimation in brook trout muscle: Adenine nucleotide concentrations, phosphorylation state and adenylate energy charge. J. comp. Physiol., 139, 127-133

WALESBY, N. J., NICOL, C. J. M. and JOHNSTON, I. A. (1980) Metabolic differentiation of muscle fibres from a haemoglobinless (Champsocephalus gunnari) and a red-blooded (Notothenia rossii) Antarctic fish. Br. Antarct. Surv. Bull., 53,

WALKER, M. G. (1971) Effect of starvation and exercise on the skeletal muscle fibres of the cod (Cadus morhua L.) and the coalfish (Gadus virens L.) respectively. J. Cons. int. Explor. Met., 33, 421-426.

WARDLE, C. S. (1972) The changes in blood glucose in pleuronectes platessa following capture from the wild: a stress reaction. J. mar. Biol. Ass. U.K., 52, 635-651.

WARDLE, C. S. (1975) Limit of fish swimming speed. Nature (London), 255, 725-727.

WARDLE, C. S. (1978) Non-release of lactic acid from anaerobic swimming muscle of plaice, Pleuronectes platessa L.: A stress reaction. J. exp. Biol., 77, 141-155.

WEATHERLEY, A. H., ROGERS, S. C., PINCOCK, D. G. and PATCH, J. R. (1982) Oxygen consumption of active rainbow trout (Salmo gairdneri), derived from electromyograms obtained by radiotelemetry, 20, 479-489.

WEBB, P. W. (1971) The swimming energetics of trout. I. Thrust and power output at cruising speeds. J. exp. Biol., 55, 489-520.

WEBB, P. W. (1975) Hydrodynamics and energetics of fish propulsion. Bull. Fish. Res. Bd. Can., 190, 158.

WEBB, P. W. (1978) Hydrodynamics: Nonscombroid fish. In: Fish Physiology, Vol. 7 (Eds. W. S. Hoar, D. J. Randall), pp. 189-237.

WEIBEL, E. C. (1969) Stereological principles for morphometry in electron microscopy catology. Int. Rev. Cyt., 26, 235-299.

WILSON, F. T. (1973) Quantitating changes of enzymes of the goldfish (Carassius auratus L.) in response to temperature acclimation. An immunological approach. Ph.D. thesis, University of Illinois.

WITTENBERGER, C. (1973) Metabolic interaction between isolated white and red muscles. Rev. roum. Biol. Zool., 18, 71-76.

WITTENBERGER, C., COPREAN, D. and MORAR, L. (1975) Studies of the carbohydrate metabolism of the lateral muscles in carp (influence of phlorizin, insulin and adrenaline). J. comp. Physiol., 101, 161-172.

WITTENBERGER, C. and DIACUIC, I. V. (1965) Effort metabolism of lateral muscles in carp. J. Fish Res. Bd. Can., 22, 1397-1406.

WOKOMA, A. and JOHNSTON, I. A. (1980) Lactate production at high sustainable cruising speeds in rainbow trout (Salmo gairdneri Richardson). J. exp. Biol., 90, 361-364.

ZAMMIT, V. A. and NEWSHOLME, E. A. (1979) Activities of enzymes of fast and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem. J., 184, 313-322.

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