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EXERCISE TRAINING IN SKELETAL MUSCLE OF BROOK TROUT (*SALVELINUS FONTINALIS*)

BY IAN A. JOHNSTON AND THOMAS W. MOON*

Department of Physiology, University of St. Andrews, St. Andrews, Fife, Scotland, U.K.

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SUMMARY

1. The differentiation of myotomal muscles in the brook trout (*Salvelinus fontinalis* Mitchill) has been investigated using p-phenylene diamine stained semi-thin sections and cytochemical and quantitative determinations of enzyme activities.

2. Evidence is presented that the range of fibre size in white muscle represents stages in growth rather than distinct fibre types.

3. Electromyography shows that both red and white muscles are recruited for sustained swimming. The threshold swimming speed for recruitment of white fibres is around 1·8 body length/s (L/s).

4. White muscle citrate synthetase and cytochrome oxidase activities are 25–35% that of red muscle. Hexokinase, phosphorylase and phosphofructokinase activities are 2, 4 and 2 times higher in white than red muscles. It is considered that the aerobic capacity of white muscle is sufficient to support sustained swimming, and that blood glucose could be an important fuel source.

5. Endurance exercise training has been investigated in fish swimming, continuously, for 21 days at 3 L/s. This training regime restricts spontaneous high-speed swimming activity and resulted in a general decline of white muscle glycolytic enzyme activities. Red fibres underwent hypertrophy relative to non-exercised controls (530 ± 64 μm^2 non-exercised, 901 ± 63 μm^2 trained). Aerobic enzyme activities in red muscle and the fraction of fibre volume occupied by mitochondria ($30{\cdot}2 \pm 0{\cdot}8\%$) did not change in response to the training programme, but glycolytic enzyme activities were elevated. 3-OH Acyl CoA dehydrogenase activities increased in both red and white muscles indicating an enhanced capacity for fatty acid catabolism with training.

6. Plasma and muscle lactate levels were not statistically different between tank-rested and trained fish swimming at 3 L/s.

7. Adaptations of fish muscle to endurance training are discussed and compared with results for other vertebrates.

* Permanent address: Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6NS

INTRODUCTION

Two distinct responses to repetitive exercise have been described for mammalian muscle (see Holloszy & Booth, 1976; Goldspink, Howells & Ward, 1976). Endurance exercise, for example, running, cycling or swimming, increases maximal oxygen uptake and also the aerobic capacity of the locomotory musculature. The results obtained in experiments of submaximal exercise depend on the nature of the training regime and on the fibre composition and relative involvement of the muscles under investigation. However, in general, endurance training increases both the size and number of muscle mitochondria together with the activities of enzymes of aerobic metabolism, particularly in red fibres (Gollnick & King, 1969; Baldwin *et al.* 1973; Hoppeler *et al.* 1973; Gollnick *et al.* 1973; Holloszy *et al.* 1975). In contrast, isometric exercise (for example, weight-lifting) results in muscle hypertrophy and increases in muscle strength without corresponding changes in respiratory capacity (Gollnick *et al.* 1972; Goldspink *et al.* 1976). The metabolic adaptations underlying improved muscular performance in man and animals following training have been the subject of extensive research (Holloszy *et al.* 1975; Holloszy & Booth, 1976). In general animals adapted to submaximal work loads utilize lipids as an energy source to a greater extent than untrained individuals (Hermansen, Hultman & Saltin, 1967). This relative 'sparing' of muscle glycogen stores results in improved endurance to fatigue and lower lactate production following training (Mole, Oscai & Holloszy, 1971; Gollnick & Hermansen, 1973; Fitts *et al.* 1975).

There have been relatively few investigations of exercise training in vertebrates other than mammals. However, such studies are of interest since it is likely that differences in the aerobic scopes and capacities for sustained locomotion, both within and between vertebrate groups, would lead to somewhat different responses to training. For example, both reptiles and amphibians are highly dependent on anaerobic metabolism for activity and generally have limited capacities for sustained locomotion (Bennett & Dawson, 1976; Bennett, 1978). A recent study of the iguanid lizard (*Sceloporus occidentalis*) exercised daily to exhaustion on a treadmill revealed no significant increases in either time to fatigue, maximal oxygen consumption or muscle enzyme activities with training (Gleeson, 1979). A wide spectrum of maximal oxygen consumption rates and levels of activity have been described among the fishes (Brett, 1972; Bennett, 1978). Studies of fish swimming in flumes and respirometers have documented higher maximal attainable speeds, improved endurance to fatigue and muscle fibre hypertrophy with training (Bainbridge, 1962; Hammond & Hickman, 1966; Walker, 1971; Davison & Goldspink, 1977). However, little is known about the metabolic responses of fish muscle to exercise training.

In the present study, the recruitment of fast and slow muscles and the effects of training on activities of enzymes of energy metabolism and muscle fine structure have been investigated in the brook trout *Salvelinus fontinalis*.

METHODS

Fish

Brook trout (*Salvelinus fontinalis*) were obtained from a local fish farm and maintained in tanks of filtered fresh water at 15 °C. Fish in the tank-rested (non-exercised) and trained experimental groups were of similar size at the time of sacrifice (17 non-exercised fish, length (mean ± S.E.) 17·8 ± 0·3 cm; body weight 55·8 ± 2·8 g; 18 trained fish, length 17·8 ± 0·4 cm, body-weight 50·0 ± 3·0 g). They were fed daily to satiety on a proprietary brand of trout pellets.

Training regime

Fish were exercised in a swimming chamber described in detail by Johnston & Moon (1980). Basically, it consists of a perspex cylinder (25 cm diameter × 150 cm length) through which water is drawn by a five-blade propeller driven by a 3 hp variable-speed electric motor. Water speed is continuously variable from 5 to 125 cm/s. Turbulence is reduced by a series of honeycomb baffles placed at either end of the tube. The swimming chamber is contained in a tank of filtered fresh water maintained at 15 °C by a flow heater–cooler refrigeration unit. Fish are introduced into the chamber by means of a perspex hatch. The rate of water flow was calculated using a calibrated Miniflow velocity probe (George Kent (Stroud) Ltd) and by introducing dye particles through a syringe and measuring the time taken for the particles to travel through a known length of the chamber. The two methods were found to agree within 5% and a mean value was taken. Fish were acclimatized to the chamber at a low swimming speed (~ 5 cm/s) for 1 week prior to the start of the training programme. Water-speed was subsequently increased to 54 cm/s over a period of several days. This is equivalent to a mean swimming speed of three body lengths/s (L/s). Training was continuous for a further period of 3 weeks except for two periods of 15 min each day when the water flow was turned off to allow for feeding. Fish were fed to excess on a similar diet to the tank-rested group. Interestingly, the training regime itself did not appear to be stressful to the fish; within a week of introduction to the chamber the fish became exceedingly tame and would readily take food from the hand.

Electromyography

Trout were lightly anaesthetized in a solution of MS222 (Sandoz Ltd). Extracellular recordings of muscle activity were made using pairs of 40 s.w.g. insulated copper-wire hook electrodes. The electrodes were bared of insulation 1 mm from the tip and glued 1·0–1·5 mm apart. Pairs of electrodes were threaded into the barrel of a hypodermic syringe and implanted into the red and white muscle regions (Fig. 1) through small cuts in the skin. The wires were securely attached to the fish by sewing them to the base of the dorsal fin rays using surgical thread. E.m.g.s were recorded via an a.c.-coupled differential amplifier (Iselworth Electronics Ltd) using a gain of 1000 and a 10 kHz filter and displayed on an oscilloscope or chart recorder. Recording sites were mapped at the end of the experiment by dissecting out the probe tracts.

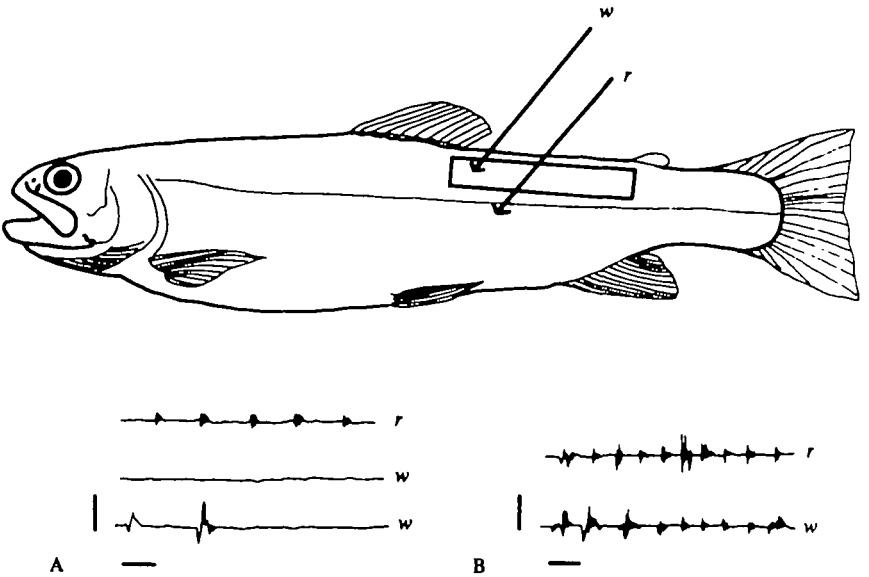

Fig. 1. Top, diagram of brook trout showing sites of sampling of white dorsal muscle and points of insertion of e.m.g. electrodes. *r*, Red muscle; *w*, white muscle.

Bottom. (A) Top two traces e.m.g.s recorded from red and white muscle regions during steady swimming at 1 L/s. Note potentials are only recorded from the red muscle layer. Lower trace, a recording from white muscle associated with a change of position in the swimming chamber during the same experiment. (B) Recordings of electrical activity from red and white muscles at a steady speed of 3 L/s. Note both muscle types are active at the swimming speed employed in subsequent training experiments. Horizontal scale represents 500 ms. Vertical scale represents 1 mV.

Histochemistry

Tissue was prepared by rapidly dissecting small cubes ($\sim 2{\cdot}5$ mm^3) of red and white muscle from the trunk (Fig. 2). Samples were mounted on cryostat chucks in OTC embedding compound (Ames Co. Inc.) and rapidly frozen by immersion in 2-methyl butane cooled to its melting point in liquid nitrogen (-159 °C). Blocks were equilibrated at -20 °C and serial 10 μm sections were cut on a cryostat and stained for succinic dehydrogenase and myofibrillar ATPase as described previously (Johnston, Ward & Goldspink, 1975).

Preparation of semi-thin sections

Fish were killed by a sharp blow to the head followed by transection of the spinal cord. Muscle samples were initially fixed *in situ* for 1 h by injecting 3 % glutaraldehyde, 0·15 M phosphate buffer, pH 7·4, into the region of the myotome posterior to the

Fig. 2. Histochemical sections stained for Myofibrillar ATPase (A, B) and (C, D) succinic dehydrogenase activity. The sites from which these sections were prepared are indicated in the inset on the left. (*a*) Region containing largely small-diameter red fibres which stain darkly for succinic dehydrogenase and lightly for myofibrillar ATPase. (*b*) Deep white muscle region consisting of a spectrum of fibre size overlapping with that of red muscle. Note that both small- and large-diameter fibres show a similar staining intensity for myofibrillar ATPase (dark) and succinic dehydrogenase (pale).

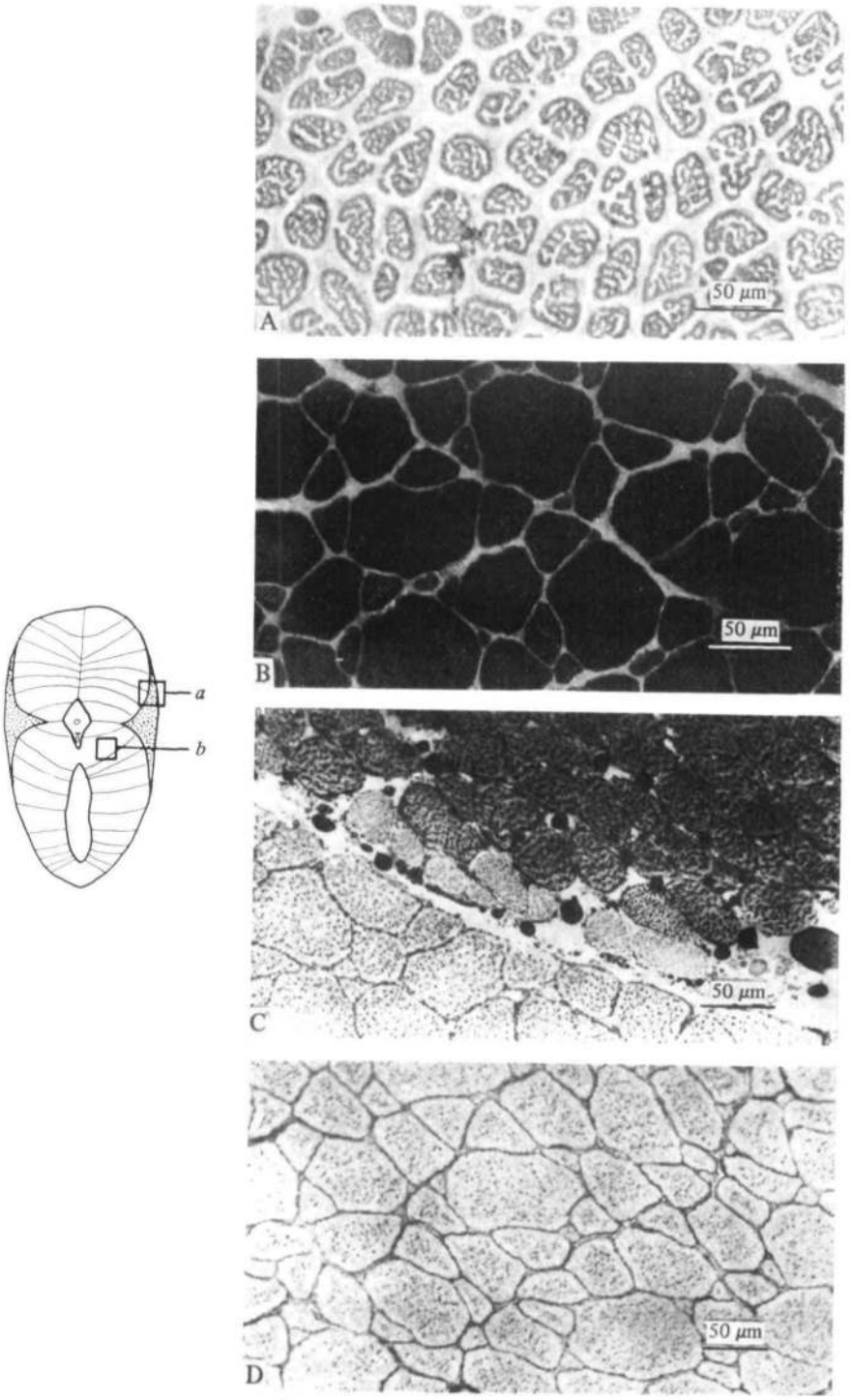

Fig. 2. For legend see opposite.

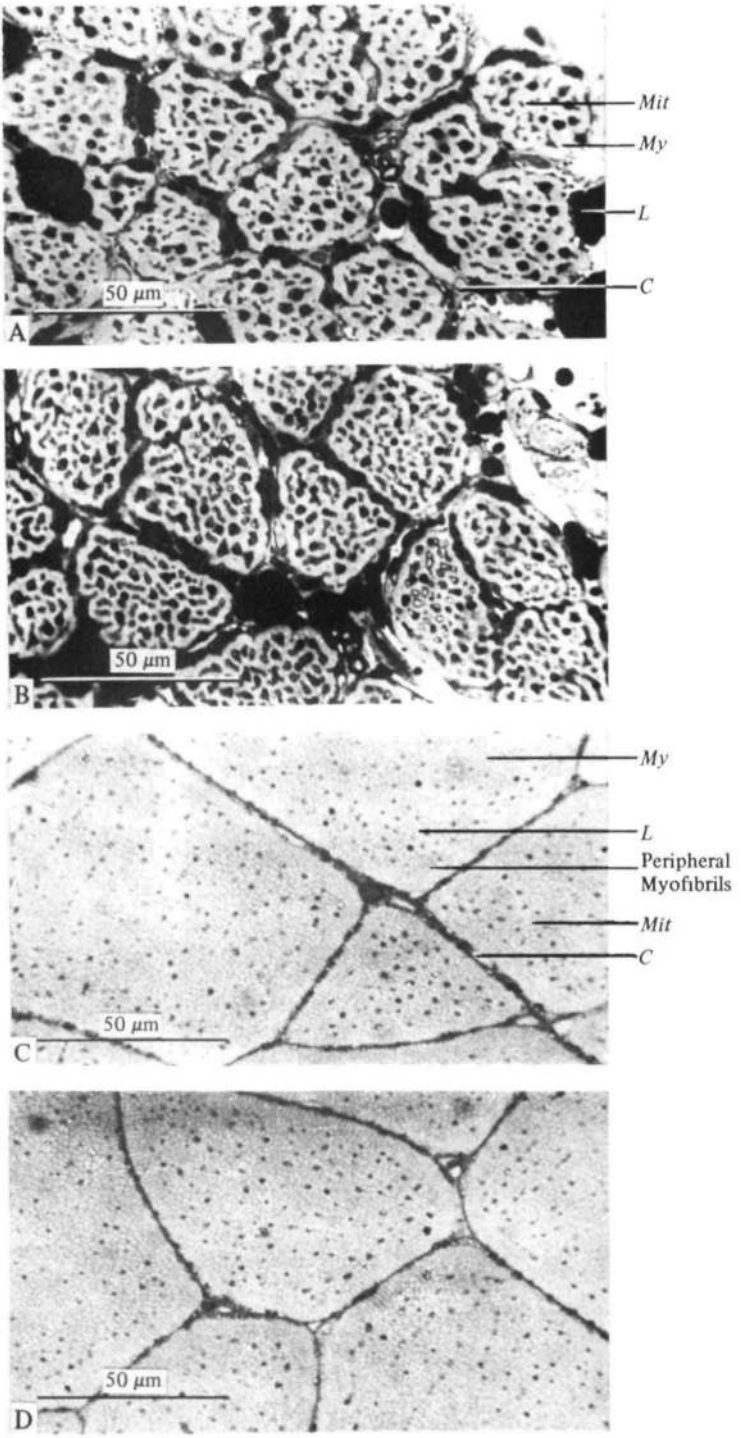

Fig. 3. For legend see opposite.

dorsal fin. During this time fish were kept on ice with the fish bent to its point of maximum flexure. Small fibre bundles of each muscle type were subsequently dissected attached to skin and were fixed at their resting length in the fish for a further 2–24 h in 3% glutaraldehyde, 0·15 M phosphate buffer pH 7·4. Tissue samples were post-fixed in 1% osmium tetroxide in 0·15 M phosphate pH 7·4, dehydrated in a series of alcohols up to 100% and embedded in araldite. Semi-thin sections 1 μm thick were cut on a Reichart Ultramicrotome and stained in 1·5% solution of p-phenylene diamine (PPDA) (Hollander & Vaaland, 1968).

Morphometric methods

Total fibre area and the areas occupied by mitochondria, lipid droplets and myofibrils were determined by tracing from micrographs (× 600) on to uniform-density paper and weighing the cut-out paper profile. A total of ten red fibres were analysed at random from each of six fish from both non-exercised and trained trout. Capillaries were identified directly from PPDA stained semi-thin sections at high magnification (× 600).

Preparation of blood and muscle samples for metabolite analyses

Approximately 1 ml of fresh blood was withdrawn from the caudal vein in a heparinized syringe. Whole blood was immediately centrifuged (3500 *g*) at 4 ° to remove the cells. An aliquot of the plasma was deproteinized by addition of 2 vols of 0·6 N-$HClO_4$. Following centrifugation, aliquots of the clear supernatant were neutralized with 2 M-K_2CO_3 in the presence of methyl-orange indicator and stored in liquid nitrogen (− 158 °C) until subsequent analyses.

In order to minimize post-mortem changes in tissue metabolite concentrations, the tail region of the fish was cut off within 20 s of death and freeze-clamped in liquid nitrogen (− 158 °C). Subsequent dissection of red and white fibres was carried out in partially thawed tissue at about − 10 °C. Weighed samples of muscle (0·5–1·0 g) for lactate and glucose analyses were pulverized to a fine powder in a stainless steel pestle and mortar cooled in liquid nitrogen. Metabolites were extracted in 5 vols of 0·6-$HClO_4$ for 5 min at 4 °C with continous stirring. Tissue debris was removed by centrifugation and an aliquot of the supernatant neutralized and stored as above.

Metabolite assays

Glycogen was determined by an anthrone method following digestion of the muscle in 30% KOH at 100 °C for 15 min as previously described (Johnston & Goldspink, 1973). Glucose was assayed using the glucose oxidase and peroxidase method (Bergmeyer & Bernt, 1965). Lactate was determined enzymatically in a medium of 150 mM hydrazine, 400 mM glycine buffer pH 9·5, 2·5 mM NAD, 20 μg lactate dehydrogenase (Hohorst, 1965).

Fig. 3. Semi-thin sections (1 μm thick) stained with 1·5% p-phenylene diamine. Top: red fibres from non-exercised (A) and trained (B) brook trout. Bottom: white fibres from non-exercised (C) and trained (D) brook trout. Note the increase in fibre size in red fibres following training. *L*, Lipid droplet; *Mit*, mitochondria; *My*, myofibrils; *C*, capillaries.

Measurements of enzyme activity

Superficial red fibres were rapidly dissected from both sides of the entire length of the body. White muscle samples of about 2 g were dissected from the dorsal trunk musculature in the region between the dorsal and adipose fins (Fig. 1). Muscle was minced with scissors and homogenised at 0 °C with an Ultraturrax blender for three periods of 25 s with cooling in 5–8 vols of preparation medium. The following media were used for individual enzymes. Phosphorylase (E.C.2.4.1.1): 100 mM phosphate buffer, pH 6·8, 20 mM-NaF, 1 mM EDTA, 0·5 mg/ml bovine serum albumin, 10 mM dithiothreitol. Adenylate kinase (E.C.2.7.4.3), creatine kinase (E.C.2.7.32), Mg^{2+}-Ca^{2+} myofibrillar ATPase: 0·25 M sucrose, 15 mM Tris-HCl, pH 7·0. 5′-AMP amino hydrolase (E.C.3.5.4.6): 180 mM-KCl, 50 mM phosphate buffer, pH 6·5. Hexokinase (E.C.2.7.1.1), phosphofructokinase (E.C.2.7.1.11), pyruvate kinase (E.C.2.7.1.40), lactate dehydrogenase (E.C.1.1.1.27), 3-OH-Acyl CoA dehydrogenase (E.C.1.1.1.35), citrate synthetase (E.C.4.1.3.7) aspartate amino-transferase (E.C.2.6.1.1) and cytochrome oxidase (E.C.1.9.3.1): 50 mM Tris-HCl, 75 mM sucrose, 200 mM mannitol, 5 mM EDTA, 2 mM-$MgCl_2$, 1 mM-dithiothreitol, pH 7·5.

Homogenates were centrifuged at 600 *g* for 20 min and filtered through glass wool and, with the exception of myofibrillar ATPase, enzyme activities were determined in the supernatant. Activities are expressed in terms of μmoles substrate utilized per g dry weight muscle per min. Water content was determined by drying muscle samples to constant weight at 60 °C. Myofibrils were prepared from the 600 g pellet by differential centrifugation as described previously (Johnston & Tota, 1974).

Measurements of enzyme activity were performed at 15 °C with appropriate controls (usually substrate deletion). Preliminary experiments were carried out to determine the optimal conditions of substrates co-ions and pH required for the measurement of maximal enzyme activities. Assay procedures for the individual enzymes were as follows:

Adenylate kinase

Adenylate kinase was assayed spectrophotometrically in a medium of 100 mM triethanolamine buffer, pH 7·6, 0·2 mM NADH, 0·5 mM PEP, 10 mM-KCl, 4 mM-$MgCl_2$, 2 mM AMP, 2 mM ATP and excess lactate dehydrogenase and pyruvate kinase.

5′-AMP aminohydrolase

5′-AMP amino hydrolase was assayed in a medium of 50 mM imidazole HCl, pH 6·5, 100 mM-KCl, 2 mM AMP, 1 mM 2-mercaptoethanol. The reaction was started by addition of AMP and the increase in optical density at 265 nm wavelength was followed.

3-OH Acyl CoA dehydrogenase

3-OH acyl CoA dehydrogenase was assayed in a medium containing 110 mM phosphate buffer, pH 7·6, 5 mM 5-acetoacetyl *N*-acetyl cysteamine, and 0·15 mM NADH.

Aspartate aminotransferase

Aspartate aminotransferase was assayed spectrophotometrically in a medium of 100 mM tris-HCl, pH 8·0, 0·35 mM NADH, 10 mM α-ketoglutarate, 25 mM L-aspartate and excess dialysed malate dehydrogenase.

Citrate synthetase

Citrate synthetase was assayed in a medium of 100 mM Tris-HCl, 0·5 mM oxaloacetic acid, 0·3 mM acetyl CoA, 0·1 mM 5,5′-dithiobis- (2-nitrobenzoic acid) in 40 mM phosphate, pH 8·0. The reaction was started by addition of oxaloacetic acid and the increase in extinction at 412 nm wavelength monitored ($E_{mM}^{412} = 13{\cdot}1$).

Creatine phosphokinase

Creatine phosphokinase was determined in a medium containing 50 mM Tris-HCl, pH 7·4, 5 mM ADP, 10 mM cysteine, 1 mM-$MgCl_2$, and 5 mM phosphorylcreatine. Following preincubation, the reaction was started by addition of 20 μl of homogenate and stopped at intervals by addition of 0·5 ml of 50 mM phenyl mercuric acid in 50% (w/v) aqueous dioxan. Precipitated protein was removed by centrifugation and free creatine was determined in an aliquot of the supernatant by the method of Eggleton, Elsden & Gough (1943).

Cytochrome oxidase

Cytochrome oxidase activity was assayed by following the oxidation of reduced cytochrome C in 50 mM phosphate buffer pH 7·6 at 550 nm ($E_{mM}^{550} = 19{\cdot}1$).

Hexokinase

Hexokinase was assayed using an ATP regenerating system in a medium containing 85 mM Tris-HCl, pH 7·5, 8 mM-$MgCl_2$ 0·8 mM EDTA, 1 mM glucose, 2·5 mM ATP, 0·4 mM NADP, 10 mM phosphoryl creatine, 100 μg creatine phosphokinase, and 100 μg glucose-6-phosphate dehydrogenase. Control assays contained the above medium with glucose omitted.

Lactate dehydrogenase

Lactate dehydrogenase was assayed spectrophotometrically in a medium of 50 mM phosphate buffer pH 7·5, 1 mM sodium pyruvate and 0·27 mM NADH. This pyruvate concentration was found to be optimal for both muscle enzymes.

$Mg^{2+}Ca^{2+}$ myofibrillar ATPase

The $Mg^{2+}Ca^{2+}$ activated ATPase of myofibrils was determined in a medium of 40 mM Tris-HCl pH 7·5, 5 mM disodium ATP, 5 mM-$MgCl_2$, 0·1 mM-$CaCl_2$ at a myofibril concentration of 0·4–0·5 mg/ml and an ionic strength of 0·10 (adjusted with KCl). The reaction was performed in a volume of 1·0 ml and started by addition of ATP to preincubated myofibrils and terminated with 1·0 ml of 10%(w/v) trichloracetic acid. Precipitated protein was removed by centrifugation and P_i measured in an aliquot of the supernatant by the method of Rockstein & Herron (1951).

Table 1. *Fibre size and percentage muscle fibre volume occupied by lipid droplets, mitochondria and myofibrils in the red myotomal muscle of non-exercised and trained brook trout.*

(A total of 60 muscle fibres were analysed from 6 fish. Values represent mean ± S.E.)

Parameter	Non-exercised fish	Trained fish
Fibre area (μm^2)	471 ± 32	907 ± 45**
Total lipid (%)	10·9 ± 0·4	10·5 ± 0·4
Subsarcolemmal lipid (%)	4·7 ± 0·5	5·1 ± 0·4
Interfibrillar lipid (%)	6·2 ± 0·5	5·4 ± 0·4
Total mitochondria (%)	31·2 ± 0·8	30·3 ± 1·0
Subsarcolemmal mitochondria (%)	10·7 ± 0·7	9·2 ± 0·7
Interfibrillar mitochondria (%)	20·5 ± 0·8	21·1 ± 0·7
Myofibrils (%)	46·0 ± 0·9	51·2 ± 1·2*

* Significant at $P < 0.05$ level.
** Significant at $P < 0.01$ level.

Phosphofructokinase

Phosphofructokinase was assayed spectrophotometrically in a medium of 50 mM Tris-HCl pH 7·4, 4·5 mM fructose-6-phosphate, 3 mM ATP, 25 mM-KCl, 6 mM-$MgCl_2$, 0·15 mM NADH and excess aldolase, triose phosphate isomerase and glycerol-phosphate dehydrogenase.

Phosphorylase

Phosphorylase was assayed in the direction of glucose-1-phosphate formation in the following medium: 50 mM phosphate buffer, pH 7·0, 1 mM EDTA, 9 mM-$MgCl_2$, 10 mM-NaF, 0·2 mg/ml fructose, 1,6-bisphosphate, 0·4 mM NADP, 0·3% BSA, 0·5% purified muscle glycogen, 1 mM AMP and excess phosphoglucomutase and glucose 6-phosphate dehydrogenase.

Pyruvate kinase

The change in extinction of NADH at 340 nm wavelength was measured spectrophotometrically in a medium containing 100 mM imidazole-HCl, pH 7·4, 70 mM-KCl, 4 mM-$MgCl_2$, 2 mM phosphoenol pyruvate, 2 mM ADP, 0·16 mM NADH and excess lactate dehydrogenase.

Statistical analyses

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

RESULTS

Muscle fibre types

The composition of muscle fibre types in the brook trout myotome is typical of many teleosts (Fig. 1). A superficial strip of red muscle is composed of uniformly small diameter fibres (Fig. 2A). Compared to other fibre types in the myotome, red fibres are characterized by a large mitochondrial compartment (~ 30% muscle fibre

Table 2. *Number of capillaries in the red muscle of non-exercised and trained brook trout (mean ± S.E.)*

Fish	Fibres analysed	No. capillaries/ fibre
Non-exercised	46	3·42 ± 0·20
Trained	47	3·80 ± 0·14

Table 3. *Activities of some enzymes of adenine nucleotide metabolism (μmole substrate utilized/g dry weight.min) in the red and white muscles of non-exercised and trained brook trout.*

Myofibrillar ATPase activities are expressed as μmole Pi released/mg myofibrillar protein.min. Values represent mean ± S.E. for eight individual fish. Assays were performed at 15 °C.

	Red muscle		White muscle	
Enzyme	Non-exercised	Trained	Non-exercised	Trained
$Mg^{2+}Ca^{2+}$ myofibrillar ATPase	0·25 ± 0·08	0·34 ± 0·07	0·73 ± 0·14	0·81 ± 0·11
Creatine phosphokinase	166 ± 14	217 ± 17*	538 ± 30	470 ± 43
Adenylate kinase	145 ± 16	197 ± 21	380 ± 43	222 ± 15
5′-AMP amino hydrolase	38·1 ± 2·2	48·6 ± 5·5	175·7 ± 36·5	283 ± 61

* Significant at $P < 0·05$ level.

Table 4. *Activities of some enzymes of energy metabolism (μmole substrate utilized/g dry weight.min) in the red and white muscles of non-exercised and trained brook trout*

(All assays were carried out at 15 °C. Values represent mean ± S.E. of 8 individual fish.)

	Red muscle		White muscle	
Enzyme	Non-exercised	Trained	Non-exercised	Trained
Phosphorylase	3·3 ± 0·2	5·0 ± 0·3*	12·0 ± 0·8	9·3 ± 1·8
Hexokinase	1·1 ± 0·1	1·9 ± 0·3*	2·8 ± 0·6	1·4 ± 0·3
Phosphofructokinase	39·0 ± 8·2	43·3 ± 7·2	66·7 ± 3·8	65·3 ± 4·3
Pyruvate kinase	271 ± 8	410 ± 58*	701 ± 107	538 ± 75
Lactate dehydrogenase	687 ± 58	713 ± 85	1651 ± 233	1280 ± 262
Cytochrome oxidase	7·9 ± 0·6	9·5 ± 1·0	1·8 ± 0·4	1·7 ± 0·4
Citrate synthetase	10·5 ± 0·5	10·5 ± 0·3	3·2 ± 0·2	2·3 ± 0·3
3-OH acyl CoA dehydrogenase	0·29 ± 0·01	0·58 ± 0·06**	0·051 ± 0·004	0·13 ± 0·00
Aspartate amino-transferase	161 ± 11	236 ± 17**	86 ± 15	128 ± 35

* Significant at $P < 0·05$ level.
** Significant at $P < 0·01$ level

volume) (Table 1, Fig. 3), a well-developed capillary network (Table 2), high glycogen storage levels (Table 6), and a relatively low $Mg^{2+}Ca^{2+}$ myofibrillar ATPase activity (Table 3). The bulk of the trunk musculature is composed of white fibres which have a $Mg^{2+}Ca^{2+}$ myofibrillar ATPase activity around three times that of red fibres (Table 3). White fibres show a large range of fibre size which overlaps that of the red fibres (Fig. 2). Histochemically, both large and small diameter white fibres show a similar and high staining reaction for myofibrillar ATPase activity (Fig. 2). Phosphofructokinase activities are around two times higher and glycogen phosphorylase four times higher in white than red fibres indicating a higher glycolytic potential in white

muscle (Table 4). The aerobic capacity of white muscle would appear to be around 25–35% of red muscle as evidenced by activities of TCA cycle and electron transport chain enzymes (Table 4). Interestingly, hexokinase activities were found to be two times higher in white than red fibres suggesting a high capacity for aerobic glucose utilization in this species (Table 4). Although, less well vascularized than red fibres, brook trout white fibres nevertheless have a significant proportion of mitochondria and lipid inclusions (Fig. 3). Characteristically, the peripheral myofibrils of white fibres are highly elongated in cross-section (Fig. 3). Frozen sections were also pre-incubated for a series of times between 1 and 15 min in a solution containing 100 mM 2-amino-2-methyl 1-propanol (pH 10·4) prior to staining histochemically for myofibrillar ATPase activity (Johnston *et al.* 1974). No evidence was found for fibres with alkaline stable myofibrillar ATPase activity in the region between the red and white fibre zones as has been found for some other teleost fish (pink fibres) (Johnston *et al.* 1974; Mosse & Hudson, 1977).

Fibre-recruitment during swimming

Electromyographical recordings (e.m.g.) were made from both red and white muscles to determine the fibre types active at the swimming speeds employed in the training experiment (3 L/s). E.m.g.s could be recorded from the red muscle at all swimming speeds up to 5 L/s, the highest tested (Fig. 1). With increasing swimming speed, red muscle potentials became more synchronous and increased in both frequency and amplitude ($\sim$200 μv peak to peak at 1 L/s to 600 μV peak to peak at 5 L/s). Larger spikes around 1 mV amplitude were occasionally recorded from the red muscle and were associated with changes in position of the fish in the swimming chamber. This activity presumably represents potentials recorded from the underlying fast motor system. The threshold speed for recruitment of white fibres was 1·5–1·8 L/s. E.m.g.s recorded from white muscle were of similar amplitude and appearance to those recorded from red fibres at the same swimming speed. It is concluded that *both* red and white muscle fibres provide the power for swimming at the speed chosen for subsequent training experiments (Fig. 1).

The effect of endurance exercise training

Muscle enzyme activities

The effect of 21 days' continuous swimming at 3 L/s on the activities of some enzymes of energy metabolism is shown in Tables 3 and 4. In general, training was associated with decreases or no significant changes in white muscle enzyme activities (Table 3 and 4). In contrast, the glycolytic enzymes phosphorylase (+52%), hexokinase (+73%) and pyruvate kinase (+51%) all increased significantly in red muscle of exercised compared to tank-rested fish. (Table 4). It is likely that the glycolytic potential of white muscle is already sufficiently high to meet all its power requirements at this swimming speed. Indeed, enforced exercise at 3 L/s restricts high speed swimming activity and may account for the decline in certain enzyme activities. A notable exception is the threefold increase in 3-OH acyl CoA dehydrogenase activity, indicating an enhanced capacity for fatty acid catabolism in trained white muscle (Table 4). Significant increases in this enzyme were also found in red muscle following training (+120%) (Table 4). In contrast, cytochrome oxidase and citrate synthetase activities were not significantly different between non-exercised and

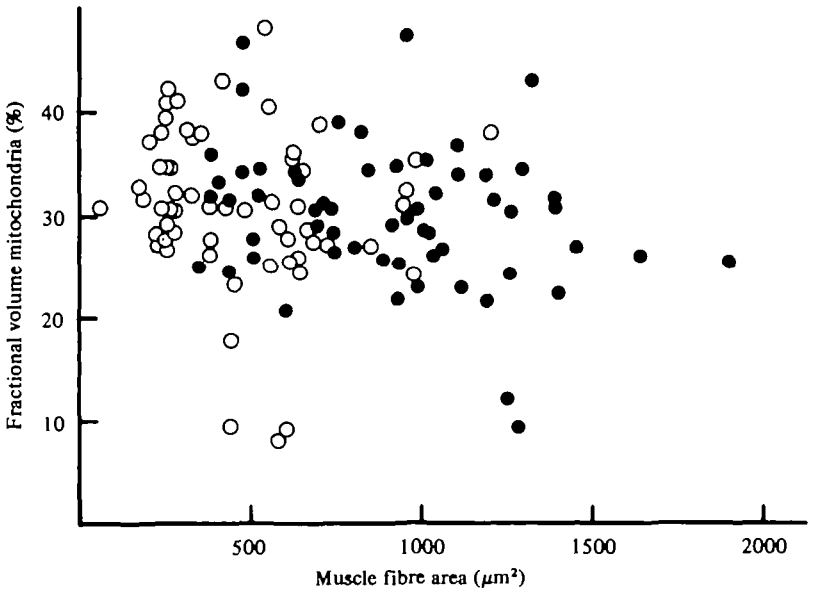

Fig. 4. Fractional volume of red fibres occupied by mitochondria (%) plotted against fibre size. Non-exercised fish (open circles) trained fish (filled circles). Note increase in fibre size with training. There is no evident relationship between fibre size and mitochondrial content at either activity level.

trained fish. This suggests that the total aerobic capacity of red fibres is unaltered by training (Table 4).

Fibre size, capillarization and ultrastructure

During training mean fibre size in red muscle almost doubled (Table 1). There was also a significant increase in the proportion of fibre volume occupied by myofibrils (Table 1). The 10% increase in myofibrillar material was associated with a decrease in interfibrillar space. In agreement with the enzyme activity measurements, the fractional volume occupied by mitochondria (%) did not change following training in the red fibres. The fraction of fibre volume occupied by mitochondria was not correlated with fibre size in either non-exercised or trained fish (Fig. 4). This suggests there is a constant relation between fibre size and mitochondrial density which is independent of activity levels. These results do not reveal the presence of subpopulations of red fibres with different aerobic capacities at least within the portion of red muscle sampled (Fig. 4). However, red fibres adjacent to the white fibres (Fig. 1) were not taken for analyses (see Methods).

The average number of capillaries surrounding each fibre was not significantly altered by training; however, the number of capillaries per unit fibre area actually decreased (6452 to 4217 capillaries/mm^2).

It was not possible to undertake a similar quantitative analyses of white fibres from semi-thin sections due to the smaller and more dispersed nature of mitochondrial and lipid inclusions. However, apart from a noticeable increase in fibre size the ultrastructure of white muscle appeared qualitatively similar in non-exercised and trained fish (Fig. 2).

Table 5. *Plasma glucose and lactate levels in non-exercised and trained brook trout*

(Values represent mean ±S.E. of eight fish)

Substance (mg/ml)	Non-exercised	Trained
Glucose	7·50±0·68	10·69±0·41**
Lactate	4·04±0·15	3·54±0·15

** Significant $P < 0.01$ level.

Table 6. *Glycogen, lactate and water content of muscles from non-exercised and trained brook trout*

(Values represent mean ±S.E. of eight fish)

	Red muscle		White muscle	
Parameter	Non-exercised	Trained	Non-exercised	Trained
Water content (%)	70·8±2·2	70·6±2·5	79·1±0·6	78·5±0·9
Glycogen content (mg %)	422±104	228±35	156±20	164±16
Lactate concentration (mg %)	27·6±5·8	15·6±2·0	73·9±8·4	62·0±8·2

Metabolite levels

The water content (Table 6) of both muscle types was unaltered following training, indicating that the food supply to the exercised group was sufficient to prevent muscle depletion (see Love, 1970). Similarly, liver weight and appearance remained constant between the exercised (1·4±0·2 g) and tank-rested (1·5±0·3 g) fish.

Plasma glucose levels were 43% higher in exercised than non-exercised fish (Table 5). Interestingly, both plasma and muscle lactate levels were *lower* in trained than tank-rested fish, even though the former showed a much higher time-averaged level of locomotory activity (Tables 5 and 6). Glycogen levels were not statistically different in either muscle type with training (Table 6).

DISCUSSION

Studies on the segmental myotomal musculature of elasmobranchs have shown a distinct division of labour between the fast and slow motor systems during swimming (Bone, 1966). For example, in the dogfish myotome, the most numerous fibres are the large diameter, focally innervated, white fibres which are almost entirely dependent on anaerobic glycogenolysis for their energy supply (Bone & Chubb, 1978). White fibres are electrically silent during sustained locomotory activity and are only recruited during bursts of high speed swimming. Only a few minutes' activity is required to deplete glycogen stores and fatigue the white muscle system (Bone, 1964, 1966). Sustained locomotion is supported entirely by multiply innervated red fibres, which form a superficial strip of highly aerobic tissue along each side of the trunk (Bone, 1966). Dogfish red and white fibres differ considerably in their membrane properties (Stanfield, 1972), contraction speeds (Bone & Johnston, 1979), ultrastructure and metabolism (Bone, 1978). Certain taxonomically primitive teleosts also have focally innervated white fibres (Bone, 1964). Although less studied, the division of labour between red and white fibres in these species would appear to be broad

similar to that described for dogfish. For example, in the Pacific herring (*Clupea harengus pallasi*) only red fibres are recruited at speeds of up to 4–5 L/s (Bone, Kiceniuk & Jones, 1978). Herring are able to cruise at these speeds for many hours. However, higher performance requires the recruitment of white fibres and can only be sustained for a few minutes (Bone *et al.* 1978).

In contrast, the white myotomal fibres of the majority of teleosts species are multiply innervated as are the red fibres (Barets, 1961; Bone, 1964; Hudson, 1969). Red and white fibres in at least some of these species are more similar in their innervation (Bone, 1964), contractile properties (Flitney & Johnston, 1979), ultrastructure (Patterson & Goldspink, 1972), metabolism (Johnson, 1977) and function than the corresponding fibre types from elasmobranchs (Bone, 1978). There is now overwhelming evidence that both red and white fibres are active during sustained locomotory activity in these species (Hudson, 1973; Johnston, Davison & Goldspink, 1977; Bone *et al.* 1978; Johnston & Moon, 1980). For example, electromyographical studies have shown that the threshold swimming speed for recruitment of white fibres is 2·0 L/s in carp (Johnston *et al.* 1977), 0·8–1·9 L/s in coalfish (Johnston & Moon, 1980) and 3–3·6 L/s in rainbow trout (Hudson, 1973). In the present study e.m.g.s were consistently recorded from the deep white muscle of brook trout at speeds in excess of 1·8 L/s (Fig. 1). This value is somewhat higher than reported for rainbow trout by Hudson (1973) but similar to the observations in rainbow trout made by Bone *et al.* (1978). Such differences are no doubt influenced by the sites of recording within the myotome and also the condition of the fish. However, in all these studies, white muscle fibres are recruited at speeds which are capable of being maintained almost indefinitely.

Evidence for polyneuronal innervation of teleost white fibres has been obtained for snakefish (Takeuchi, 1959), tench, catfish (Barets, 1961) and short-horned sculpin (Hudson, 1969). In the sculpin (*Myoxocephalus scorpius*) each white fibre is innervated by two to five axons from each of four spinal nerves. Two kinds of electrical response have been observed in these fibres (*a*) all-or-none spike potentials and (*b*) quantized distributed junction potentials (Hudson, 1969). Only the former mode of activation has been reported for elasmobranch white fibres (Hagiwara & Takahashi, 1967). It is tempting to relate this difference in the mode of activation of teleosts and elasmobranch white fibres to their respective roles in locomotion. Dogfish white fibres respond to a single shock with a large twitch (~0·35 Po), which on repetitive stimulation summates to give a smooth maximal tetanus at around 15 Hz (Bone & Johnston, 1979). In contrast, the multiply innervated white fibres of *Tilapia* opercular adductor muscle only give a small twitch on single stimulation (~0·05 Po). On multiple stimulation they produce a series of graded fused tetani reaching a maximum at 250–300 Hz (Flitney & Johnston, 1979). In this respect *Tilapia* red and white fibres are somewhat similar. However, the rate of rise of tension and maximal velocity of shortening are 6·5 times and 1·7 times greater for red than white fibres respectively (Flitney & Johnston, 1979). It would appear, therefore, that tension development is limited by the degree of activation of the fibre to a much greater extent for red than white muscle. A full understanding of the significance of these differences in modes of activation of fast fibres in different groups of fishes must await simultaneous recordings membrane potential and mechanical responses in innervated fibre preparations.

In the older literature it has been queried whether the large range of fibre size observed in teleost white muscle (particularly salmonids) represents the presence of distinct fibre types (Greene & Greene, 1913; Boddeke, Slijper & Van der Stelt, 1959). The present study does not support this view since both small and large white fibres show identical staining for myofibrillar ATPase and succinic dehydrogenase activity (Fig. 2). Similar results have been obtained for another salmonid species *Salmo gairdneri* (Johnston *et al.* 1975). In this study, it was also shown that apparently small and large diameter white fibres actually represented a smooth distribution of fibre sizes between 10–95 μm diameter. It seems likely that the range of fibre size observed in teleost white muscle represents different stages of growth. Unlike mammals, fibre number increases throughout life in many species of fish paralleling increases in body size (Greer-Walker, 1970).

Many authors have assumed that the energy supply to teleost white muscle is almost entirely supplied by anaerobic glycogenolysis (Bilinski, 1974; Driedzic & Hochachka, 1978). While this is undoubtedly true for high-speed swimming, it is likely that alternative pathways of energy production are used for other types of locomotory activity. White muscle in brook trout if relatively highly vascularized compared with that of dogfish (see Bone & Johnston, 1979), each fibre being surrounded by 1–5 capillaries (Fig. 3). Although the mitochondrial compartment is greatly reduced compared to red fibres (Fig. 3) it probably represents an aerobic capacity adequate to supply the energy demands of slow speed swimming. In support of this, activities of tricarboxylic acid cycle and electron transport chain enzymes are around 25–35% of that in red muscle, and the high hexokinase activities of white muscle indicate a significant potential for aerobic glucose utilisation (Table 4).

A swimming speed of 3 L/s was found to be close to the highest speed that brook trout could maintain without depletion of body reserves. Trout exercised at 4 L/s for a further week showed a significant increase in muscle water content and reduction in liver size, indicating starvation in spite of being fed to satiety twice daily (see Love, 1970). It would appear that food conversion rates are not sufficient to meet all the energy demands of continuous swimming at this velocity. Similar results have been obtained for brown trout under comparable experimental conditions (Davison & Goldspink, 1977). Thus there is little doubt that 3 L/s represents a significant increase in work load relative to tank-rested fish. Indeed, there was hypertrophy of both muscle types following training (Table 1, Fig. 3). Telemetric data from wild populations of brown trout fitted with ultrasonic tags indicate low mean swimming velocities ($> 0{\cdot}16$ L/s) in lake fish (Holliday, Tytler & Young, 1974).

In mammals, endurance exercise training involving swimming or treadmill running leads to a large increase in the aerobic capacity of the skeletal musculature, particularly in red fibres. For example, submaximal training regimes ($\leqslant 60\%$ maximal oxygen uptake) result in an increase in both the number and size of muscle mitochondria and the activities of TCA cycle enzymes (see Holloszy & Booth, 1976). In contrast, endurance training in brook trout results in no appreciable increase in the aerobic capacity of the red swimming muscle as judged by these criteria (Tables 1, 4). Somewhat similar results were obtained for a marine teleost (*Pollachius virens*) subject to three weeks' enforced swimming at 2·1 L/s (Johnston & Moon, 1980).

One explanation of these results is that, compared to mammals, the relatively low metabolic rate and efficiency of respiration of fishes (see Bennett 1978) places a lower limit on the extent to which skeletal muscles can adapt their aerobic capacities to meet increases in work load. Thus an increase in energy demand induced by training must be met largely anaerobically. In support of this hypothesis both trout (Table 4) and to a greater extent coalfish (Johnston & Moon, 1980) show significant increases in glycolytic enzyme activities in red muscle following training. However, other factors than gross oxygen uptake at the gills may be limiting the respiratory capacity of fish muscle. For example, the proportion of fibre volume occupied by mitochondria in fish red fibres is greater than in mammalian slow twitch fibres and approaches that of cardiac muscle (McCallister & Page, 1973; Goldspink & Howells, 1974). Most studies have found that training does not alter respiratory enzyme activities in rodent cardiac muscle (Gollnick & Ianuzzo, 1972; Baldwin, Cooke & Cheadle, 1977; Baldwin & Terjung, 1975) in contrast to its effects on skeletal muscle. Studies of the effects of training on fish with higher aerobic scopes, such as tuna, may be useful in deciding between these possibilities.

Lactate production by salmonids following severe exercise has been the subject of a large number of studies (Black, 1957; Black *et al.* 1960; Black, Robertson & Parker, 1961). A few minutes' strenuous exercise can result in white muscle lactate concentrations of the order of 59 mM/kg (Black *et al.* 1961). Recovery of lactate levels following burst swimming requires an extended period of recovery, particularly in white muscle (see Bilinski, 1974). For example, following 15 min severe exercises in lake trout, *Salvelinus namaycush*, blood lactate levels required 12–16 h to return to the pre-exercised state (Black, 1957). Unfortunately, there have been very few studies on lactate production in fish at submaximal work loads (Black *et al.* 1961; Miller, Sinclair & Hochachka, 1959). Most of these earlier studies are semi-qualitative and it is not possible to relate tissue lactate concentrations to particular swimming speeds. However, several studies have reported a 2- to 3-fold increase in blood lactate levels in trout subject to moderate exercise levels. Interestingly, in the present study on trained trout, blood and white muscle lactate concentrations were not significantly higher in fish swimming at 3 L/s than in tank-rested fish (Table 6). The differences in lactate accumulation during submaximal exercise between the present and previous studies (e.g. Black *et al.* 1961) may reflect in part (a) the presence of a generalized stress reaction in acutely exercised fish (see Wardle, 1978) and/or (*b*) changes in the relative importance of anaerobic and aerobic pathways with time. Thus, although white muscle is recruited at sustainable swimming speeds, there is no net lactate accumulation. This could be regarded as evidence for sustained swimming being entirely supported by aerobic metabolism or for an enhanced transport and catabolism of lactate from white muscle following training.

The metabolic fate of lactate in fishes is still a matter of some debate (see Driedzic & Hochachka, 1978). A major part of lactate produced during exercise is probably oxidized to pyruvate (Bilinski, 1974). Indeed, gills, liver, red muscle and kidney are all able to oxidize [^{14}C]lactate at high rates *in vitro* (Bilinski & Jonas, 1972). The extent to which fish liver can utilize lactate as a gluconeogenic substrate is unclear. Black, Bosomworth & Docherty (1966) concluded that the Cori cycle is of little importance in trout since liver glycogen levels are not restored to normal even 24 h after severe exercise.

Wittenberger and co-workers have suggested that red muscle is involved in a direct glucose–lactate exchange with white muscle (Wittenberger & Diacuic, 1965; Wittenberger, 1973; Wittenberger, Coprean & Morar, 1975). However, the large diffusion distances between the red and white muscle masses and the lack or low activities of gluconeogenic enzymes (Johnston & Moon, 1979) argue against a significant synthesis of glucose from lactate in red muscle.

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