Structure and Function of Fish Muscles

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SYNOPSIS

Fish swim using a combination of paired and unpaired fins and undulations of the segmental myotomal muscles. Although there is a simple anatomical separation of fibre types within the myotome, the myoseptal organization and orientation of fibres is complex. The number of distinct fibre types described varies from two to five depending on species. Slow red fibres form either a thin superficial or an internalized strip which constitutes between 0.5% and 29% of the total muscle. Red fibres are multiterminally innervated, being activated by local junction potentials. Characteristically, the fraction of red fibre volume occupied by mitochondria (25–38%) is comparable to mammalian heart muscle. The bulk of locomotory muscle consists of larger diameter fast fibres which have a highly developed glycogenolytic capacity. In elasmobranchs, holosteans, chondrosteans and some primitive teleosts fast fibres are innervated by a single basket-like end-plate formation at one myoseptal end. The available electromyographical evidence suggests that in such cases red fibres alone support sustained activity and the fast muscle is reserved exclusively for short periods of burst swimming. In contrast, most teleosts recruit fast fibres for higher sustainable as well as burst swimming speeds. Fast muscles in such fish are unusual among vertebrates in having extensive polyneuronal innervation. Isolated polyneuronally innervated fast fibres require much higher stimulation frequencies (200–300 Hz) to elicit maximum tensions than fibres with single end-plates (15–20 Hz). Full activation of polyneuronally innervated muscles probably requires simultaneous and perhaps asynchronous activity of a number of different motor neurones. This may give additional flexibility to polyneuronally innervated fast muscles allowing their recruitment at a wider range of swimming speeds than fibres with single end-plates. Finally, special features of the regulation of contractility and energy metabolism in fish muscles are discussed in relation to locomotion.

INTRODUCTION

Adaptations for aquatic locomotion account for many of the specialized features of fish muscle. The energy expenditure of locomotion in water is not linearly related to speed as it is for many terrestrial
vertebrates (see Bennett, 1978). In general, the power requirements of swimming are thought to rise as a function of body size and velocity. This reflects the way drag-forces on the body increase with speed (see Webb, 1975). Thus burst speeds require proportionally more effort than sustained swimming. This explains the predominance of fibre types that can develop power rapidly and essentially independently of the circulation. Typically, 90% of fish skeletal muscle is composed of anaerobic white fibres giving the flesh both its characteristic white colour and culinary importance. The possession of a large muscle mass reserved for burst activity does not constitute a serious weight penalty as it would in a terrestrial animal since most fish preserve neutral buoyancy.

In the older fish groups (elasmobranchs, dipnoans, primitive teleosts) the different energetic requirements of sustained and burst swimming have led to a complete anatomical and functional division between fast and slow motor systems (Bone, 1966). Indeed the physical separation of red and white fibres in the swimming muscles of Torpedo led to one of the earliest descriptions of fibre types in vertebrates (Lorenzini, 1678). Thus, in dogfish, sustained swimming is entirely supported by slow red fibres which account for only 5–10% of the myotomal musculature. In contrast, the fast motor system which consists of phasically active white fibres is reserved for burst activity (Bone, 1966). The red and white muscles of elasmobranchs have somewhat similar innervation and physiological properties to, respectively, the slow tonic and fast twitch muscles of other vertebrate groups (Bone, 1964, 1966, 1978a).

However, in most teleosts there is not a simple division of labour between red and white muscles and both fibre types are recruited at sustainable swimming speeds (Hudson, 1973; Johnston, Davison & Goldspink, 1977; Bone, Kicznuik & Jones, 1978). The fast muscles of advanced teleost groups are unusual among vertebrates in having multiple innervation. For example, each fast fibre in the scorpaeniform fish, Myoxocephalus scorpius, is innervated by two to five separate axons from each of four spinal nerves (Hudson, 1969). Characteristically isolated polyneuronally innervated fast muscles require much higher stimulation frequencies to elicit maximum tensions than fibres with single end-plates (Flitney & Johnston, 1979; Johnston, 1981). It seems likely that full activation of polyneuronally innervated muscles requires simultaneous and perhaps asynchronous activity of a number of different motor neurones. This may well give additional flexibility to power development by the musculature over and above that offered by the hierarchical recruitment of motor units of different sizes. The control of contractility and the regulation of

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1. Lorenzini, 1678
2. Flitney & Johnston, 1979
metabolism in multiply innervated fast muscles remains one of the outstanding problems for physiologists and biochemists interested in fish muscle.

LOCOMOTORY MUSCLES

A wide variety of body forms and modes of swimming have been described among the fishes (see Webb, 1975; Lindsey, 1978). Forward swimming is usually achieved by lateral undulations of the segmental myotomal muscles passing backwards along the trunk. However, a large number of fish rely to some degree on the paired and unpaired fins to provide forward motion (e.g. rays, trigger fish, etc.). In many cases, for example the surf perch *Cymatogaster aggregata*, as swimming speed increases the main propulsive thrust switches from the enlarged pectoral fins to oscillations of the trunk and caudal fin (Webb, 1973). The boxfish *Ostracion* provides an extreme example of the use of fins for locomotion in that the trunk is totally inflexible and the fish swims by fan-like oscillations of the caudal fin.

The overwhelming majority of papers published on fish muscle have dealt exclusively with the segmental myotomal musculature. In teleosts and elasmobranchs the myotomes have a complex W-shape and form a series of overlapping cones. The muscle fibres in each myotome insert into a connective tissue sheath or myocommata. Functional aspects of the shape and arrangement of the myotomes have recently been reviewed both in different groups of fishes and in development (Bone, 1978a). The orientation of fibres within the myotome is complex and varies both along the body and with distance from the vertebral column (see Alexander, 1969). In general, superficial fibres run parallel to the surface whereas deeper fibres make angles of up to 40° with the long axis of the body (Alexander, 1969). The significance of this arrangement is thought to be to allow similar degrees of sarcomere shortening at different body flexures. Thus optimal overlap between thick and thin filaments and hence maximum tension generation is achieved at all depths within the myotome.

FIBRE TYPES

Nomenclature

Although classifications based on muscle colour have lost favour with workers on other vertebrate groups, they are still widely applied to
FIG. 1. A diagrammatic representation of the distribution of fibre types in fish segmental myotomal muscle. (A) Dogfish (*Scyliorhinus canicula*) after Bone & Chubb (1978). S, superficial; OR, outer red; IR, inner red; OW, outer white; and IW, inner white muscle fibres. (B) Brook trout (*Salvelinus fontinalis* Mitchill) after Johnston & Moon (1980b), R, red; and W, white muscle fibres. (C) Common carp (*Cyprinus carpio* L.) after Johnston, Davison *et al.* (1977). R, red; P, pink; and W, white muscle fibres. (D) Skipjack tuna (*Katsuwonus pelamis* L.) after Rayner & Keenan (1967) and Bone (1978b). SR, superficial red; DR, deep red; and W, white muscle fibres. The intensity of shading represents the relative histochemical staining reactions for succinic dehydrogenase, a mitochondrial marker enzyme.
fish muscle. In other vertebrates, locomotory muscles contain a heterogeneous mixture of different fibre types. However, in fish, fibre types are largely present in different positions within the myotome (Fig. 1). Thus problems of classification which arise due to the presence of both fast and slow red fibres are not as serious in fish as in other animals. In addition, the complex orientation of myotomal fibres has made it technically difficult to obtain information about relative contraction speeds. The similar innervation of fast and slow muscles in most teleosts also makes this an inappropriate way of distinguishing between fibre types. Since most studies are based entirely on histochemical or ultrastructural criteria it is often safer to retain the rather unsatisfactory nomenclature based on colour than to adopt unsubstantiated physiological criteria such as fast or slow, tonic or phasic. Thus in this review the terms red and white have been used except where data exist to allow classification according to physiological properties.

Histology and Histochemistry

Most investigations have concerned the segmental myotomal musculature, although there have been a few studies of the paired and unpaired fins (Bergman, 1964; Kryvi & Totland, 1978; Nishihara, 1967; Walesby & Johnston, 1980a). In a survey of 84 species of marine fish Greer-Walker & Pull (1975) reported that red fibres constituted between 0.5 and 29% of the myotomal muscle mass. The proportion of red fibres is highest in active pelagic families such as Scombridae and Clupeidae and lowest in bottom dwelling predators, deep-sea fishes and those species which use their fins as a primary means of locomotion.

Cartilaginous fishes which have been studied include Chimaera monstrosa (Kryvi & Totland, 1978), dogfish Scyliorhinus canicula L. (Bone, 1966, 1978a) and the sharks Etmopterus spinax and Galeus melastomus (Kryvi, 1977). Bone (1978a) has described five fibre types in the dogfish on the basis of differences in innervation, ultrastructure and histochemical staining characteristics (Fig. 1A). In dogfish, the outer border of the myotome consists of a single interrupted layer of large diameter superficial fibres which show negative histochemical staining for succinic dehydrogenase (SDHase) and Ca²⁺-activated myofibrillar ATPase activity (Bone & Chubb, 1978). Superficial fibres are characterized by an intense staining for glucose-phosphate-isomerase. The functional significance of these fibres is unclear. They are unlikely to represent growth stages of the underlying red fibres since they appear relatively late during post-embryonic
development (Bone, 1978a). Two types of red fibre can be distinguished on the basis of size, aerobic capacity and myofibrillar ATPase activity (Bone & Chubb, 1978). Outer red fibres have a higher SDHase and lower myofibrillar ATPase activity than the somewhat larger diameter red fibres adjacent to the white muscle. The outer few layers of white fibres are differentiated from deep white fibres by having a higher proportion of mitochondria, more abundant capillary supply, and a somewhat different myofibrillar ATPase activity (Fig. 1A) (Bone, 1978a; Bone & Johnston, in press). Biochemical measurements of myofibrillar ATPase of the five fibre types of dogfish are presented in Table I and correlated with the histochemical observations.

**TABLE I**

Mg$^{2+}$-Ca$^{2+}$-stimulated myofibrillar ATPase activities of fish skeletal muscle fibre types. Activities expressed as μmol P$_i$ released, mg myofibrillar protein$^{-1}$ min$^{-1}$

<table>
<thead>
<tr>
<th>Fibre types</th>
<th>Dogfish$^a$ (15°C)</th>
<th>Trout$^b$ (15°C)</th>
<th>Carp$^c$ (25°C)</th>
<th>Tuna$^d$ (25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer red</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
<td>0.51</td>
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<tr>
<td>Inner red</td>
<td>0.18</td>
<td></td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Pink</td>
<td></td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer white</td>
<td>0.55</td>
<td>0.73</td>
<td>1.09</td>
<td>0.99</td>
</tr>
<tr>
<td>Inner white</td>
<td>0.40</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data from Bone & Johnston (in press)$^a$, Johnston & Moon (1980b)$^b$, Johnston, Davison & Goldspink (1977)$^c$ and Johnston & Tota (1974)$^d$. Numbers of fish used and standard errors of the mean are given in the original publications. Assay temperatures are shown in brackets.

The arrangement of fibre types of some representative teleosts is shown in Fig. 1B–D. The simplest case is exemplified by species such as brook trout (*Salvelinus fontinalis* Mitchell) (Johnston & Moon, 1980b: fig. 2) and the Atlantic mackerel (*Scomber scombrus*) (Bone, 1978b). In these fish the myotome is differentiated into two distinct fibre types on the basis of histochemical staining for aerobic enzymes and myofibrillar ATPase (Fig. 1B; Johnston & Moon, 1980b). Biochemically the myofibrillar ATPase activity of the white fibres is around three times that of the red (Johnston, Frearson & Goldspink, 1972: table 2). White fibres are SDHase negative and form a heterogeneous group with respect to fibre size. In the case of rainbow...
trout there is a continuous distribution of white muscle fibre diameter between 15 and 90 μm, overlapping that of red fibres, 5–40 μm (Johnston, Ward & Goldspink, 1975). Most workers have considered the range of fibre size in teleost white muscle to represent different stages in growth rather than distinct fibre types (Johnston, Ward et al., 1975; Korneliussen, Dahl & Paulsen, 1978; Johnston & Moon, 1980b). Greer-Walker (1970) has shown that fibre number continues to increase throughout life in many species of fish. This contrasts with the situation found in most other vertebrates where fibre number is fixed at or shortly after birth.

There have been relatively few studies of capillary supply and blood flow to fish red and white muscle fibres (Boddeke, Slijper & van der Stelt, 1959; Bone, 1978b; Stevens, 1968; Mosse, 1978). Capillary density is related to the aerobic capacity of the muscle. In a study of three teleost species Mosse (1978) found a mean capillary fibre ratio of 0.2–0.9 for white and 1.9–2.5 for red muscle. In *Platycephalus bassensis* 49% of white fibres have no peripheral capillaries (Fig. 2). Interestingly, the capillarization of white muscles in certain pelagic species is more highly developed. The white fibres of yellow tail scad (*Trachurus maccullochi*), pilchard (*Sardinops neopilchardus*) and mackerel (*Scomber australasicus*) have fewer fibres with no peripheral capillaries (17.8%, 16.5% and 23% respectively) (Mosse, 1979).

![FIG. 2. Percentage of red and white muscle fibres surrounded by a given number of capillaries. The data is for the flathead (*Platycephalus bassensis*) and counts were made by direct visualization of capillaries in semi-thin (1 μm) durcupan sections. From Mosse (1978: table 3).](image-url)
In some species another type of fibre occurs in an intermediate position between the red and white muscle layers (Bokdawala & George, 1967; Johnston, Patterson, Ward & Goldspink, 1974; Mosse & Hudson, 1977). These so-called pink fibres can be distinguished histochemically from other fibre types on the basis of their stability to alkaline (pH 10.3–10.4) preincubation prior to staining for myofibrillar ATPase activity (Johnston, Patterson et al., 1974). In carp (Cyprinus carpio), pink fibres constitute a larger proportion of the myotome than red fibres (~10%) (Fig. 1C) (Davison, Johnston & Goldspink, 1976). Biochemical studies of the fibre types in carp have shown pink fibres to have intermediate aerobic enzyme and Mg²⁺ Ca²⁺ myofibrillar ATPase activities between red and white fibres (Johnston, Davison et al., 1977). The light chain compositions of vertebrate myosins have been shown to be characteristic of fibre phenotype (Lowey & Risby, 1971). Carp red fibres have two, and pink and white fibres, three species of light chain, characteristic of slow and fast muscle myosins respectively (Focant, Huriaux & Johnston, 1976; Johnston, Davison et al., 1977).

Some fishes of the family Scombridae have internalized red muscle masses (Fig. 1D). This feature is associated with a counter-current vascular heat-exchanger which enables elevated red muscle and brain temperatures to be maintained over a wide range of ambient temperatures. The extent to which capacities for regulating body temperature have developed within the tunas varies considerably between species (see Sharp & Pirages, 1978). In the skipjack tuna (Katsuwonus pelamis) the superficial wedge of red muscle is differentiated from the internalized red muscle by a larger fibre size and a significantly lower histochemical staining for SDHase activity (Bone, 1978b). Indeed, quantitative ultrastructural studies by Bone (1978b) have shown ten times as many mitochondria in deep red fibres (Table II) and a more highly developed capillary bed (~4.7 capillaries/fibre). Mitochondria in superficial red and white muscle differ from those of the deep red fibres in showing a reduction of cristae development and the presence of lamellar figures interrupting the cristae array (Bone, 1978b). Mg²⁺ Ca²⁺ myofibrillar ATPase activities of Atlantic bluefin tuna (Thunnus thynnus) red muscles are twice that of white muscle and somewhat higher than analogous red muscles of less active species (Table I) (Johnston & Tota, 1974). In tuna muscular effort associated with ventilation has been transferred from the buccal to myotomal muscles (ram ventilation) (Dizon, Brill & Yuen, 1978). This feature is associated with continuous high-speed swimming. Significantly it has been suggested that both tuna red and white muscles have a significant capacity of aerobic glucose utilization (Guppy, Hulbert & Hochachka, 1979).
<table>
<thead>
<tr>
<th>Species</th>
<th>Red</th>
<th>Muscle Type Intermediate</th>
<th>White</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ELASMOBRANCHS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sharks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Galeus melastomus</em></td>
<td>34</td>
<td>16</td>
<td>1</td>
<td>Kryvi (1977)</td>
</tr>
<tr>
<td><em>Etmopterus spinax</em></td>
<td>30</td>
<td>7</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><strong>CHONDROSTEANS</strong></td>
<td></td>
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<tr>
<td>Sturgeon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acipenser stellatus</em></td>
<td>30</td>
<td>3.7</td>
<td>0.7</td>
<td>Kryvi, Flood &amp; Guljaev (1980)</td>
</tr>
<tr>
<td><strong>TELEOSTS</strong></td>
<td></td>
<td></td>
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<tr>
<td>Coalfish</td>
<td></td>
<td></td>
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<tr>
<td><em>Gadus virens</em></td>
<td>25</td>
<td>1</td>
<td></td>
<td>Paterson &amp; Goldspink (1972)</td>
</tr>
<tr>
<td>Atlantic mackerel</td>
<td>95.5</td>
<td></td>
<td>2</td>
<td>Bone (1978a)</td>
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<tr>
<td><em>Scomber scombrus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skipjack tuna</td>
<td>16.4</td>
<td>3.3 (SR)</td>
<td>2.2</td>
<td>Bone (1978b)</td>
</tr>
<tr>
<td><em>Katsuwonus pelamis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crucian carp</td>
<td>25</td>
<td>20</td>
<td>4</td>
<td>Johnston &amp; Maitland (1980)</td>
</tr>
<tr>
<td><em>Carassius carassius</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaice</td>
<td>25</td>
<td></td>
<td>2</td>
<td>Johnston (1981)</td>
</tr>
<tr>
<td><em>Pleuronectes platessa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antarctic 'cod'</td>
<td>30</td>
<td></td>
<td>2</td>
<td>Walesby &amp; Johnston (1980a)</td>
</tr>
<tr>
<td><em>Notothenia rossii</em></td>
<td></td>
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</table>

Abbreviations: DR, deep red muscle; SR, superficial red muscle. Note that in *Notothenia rossii* the deep pectoral adductor muscle provides the main propulsive thrust for sustained swimming. Red fibres in this muscle have even higher mitochondrial densities (~38%) than the myotomal muscle above.
ULTRASTRUCTURE

There have been a number of recent quantitative studies of the fine structure of fish myotomal (Patterson & Goldspink, 1972, 1973; Kryvi, 1977; Kryvi & Totland, 1978; Bone, 1978b; Walesby & Johnston, 1980a; Johnston & Maitland, 1980) and pectoral muscles (Kryvi & Totland, 1978; Walesby & Johnston, 1980a).

The striking feature of fish red muscle is the high mitochondrial density, which often exceeds that found in the most active mammalian muscles. For example, the fractional volume occupied by mitochondria in red myotomal muscles of various cold-water species is comparable to their volume in the ventricular muscle of the mouse.

**FIG. 3.** (A) Brook trout (*Salvelinus fontinalis*) red muscle fibres. Transverse frozen section (10 µm) stained for succinic dehydrogenase as a mitochondrial marker enzyme. Note lipid droplets between red fibres (R) and the higher SDHase staining in red than in white fibres (W) (bottom left). (B) Brook trout red fibres, semi-thin (1 µm) araldite embedded section stained with p-phenylene diamine (PPDA). Note the abundant capillary supply (C) and the high proportion of fibre volume occupied by mitochondria (M) and lipid (L). (C) Longitudinal section of plaice red fibre from a four-month-starved fish. Note loss of cristae from mitochondria (C) also the presence of a distinct M line (ML) and capillary (CL) containing a nucleated red blood corpuscle (RBC). (D) Plaice (*Pleuronectes platessa* L.) red fibre. Electronmicrograph showing high mitochondrial density (M) and numerous lipid droplets (L). This fish has been food deprived for several weeks. Note the depletion of lipid deposits (DL).
(34%) and finch (37%) (Bossen, Sommer & Waugh, 1978) (Table II; Fig. 3). In contrast, approximately 0.5–8% of white fibre volume is occupied by mitochondria, depending on position within the myotome and species (Table II). An interesting exception to this correlation between muscle pigmentation and respiratory capacity occurs in icefishes of the family Channichthyidae. In this group all muscles are pure white in colour owing to the absence of both haemoglobin (Rudd, 1954) and myoglobin (Hamoir, 1978; Walesby, Nicol & Johnston, in press). The superficial myotomal fibres in the pelagic icefish Champsocephalus gunnarii have a mitochondrial density of around 45% and are almost entirely surrounded by capillaries (Walesby, Flitney & Johnston, unpublished results).

Another characteristic of fish red or slow fibres concerns the highly developed sarcoplasmic reticulum (SR) and T-tubule system. The fractional volume occupied by SR in red muscle is greater than for tonic and slow twitch muscles in other vertebrates and approaches that of fast twitch fibres (Johnston, 1980b). Quantitative data from studies of fish sarcotubular systems are summarized in Table III. In most myotomal muscles studied T-tubules are located at the junction

### TABLE III

*Fractional volume (%) occupied by sarcoplasmic reticulum and T-system in some representative vertebrate muscles*

<table>
<thead>
<tr>
<th>Species</th>
<th>Sarcoplasmic reticulum</th>
<th>Muscle Type</th>
<th>T-system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Red</td>
<td>White</td>
</tr>
<tr>
<td><strong>ELASMOBRANCHS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etmopterus spinax</td>
<td>4.9</td>
<td>6.0</td>
<td>0.33</td>
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<tr>
<td>Galeus melastomus</td>
<td>4.6</td>
<td>6.8</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>TELEOSTS</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Salmo gairdneri</td>
<td>5.1</td>
<td>13.7</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>AMPHIBIANS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rana pipiens</td>
<td>4.5</td>
<td>9.1</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>MAMMALS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3.2</td>
<td>4.6</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Data is taken from the following: aKryvi (1977); bNag (1972); cMobley & Eisenberg (1975); dEisenberg & Kuda (1975); eEisenberg, Kuda & Peter (1974); fPeachey (1965); gFlitney (1971) (Rana temporaria).
of the Z disc (Franzini-Armstrong & Porter, 1964; Kilarski, 1966; Nag, 1972; Patterson & Goldspink, 1972; Kryvi, 1977). A similar location of the tubular system is found among the locomotory muscles of lampreys (Teravainen, 1971), urodeles (Totland, 1976) and anurans (Peachey, 1965). In fish extraocular (Kilarski, 1966) swim bladder and drum muscles (Eichelberg, 1976) and in hagfish (Komeliussen & Nicolaysen, 1973) and mammalian muscles the T-tubules are situated at the A-1 boundary. The functional significance of these different positions is unknown.

The fractional volume occupied by myofibrils varies from 80–96% in white muscles to 40–60% in red muscles (see Johnston, 1980b, for a review). Myofibrillar packing is more regular in white muscles. White fibres of teleosts are characterized by elongated peripheral myofibrils (Fig. 4). In small fibres the arrangement of fibrils resembles

![Image](https://example.com/image1)

**FIG. 4.** (A) Transverse section of a pink fibre from the myotomal muscle of the crucian carp (*Carassius carassius* L.). Note intermediate mitochondrial density (M) and irregular myofibrillar packing (My). In this species lipid droplets are not common in any fibre type; the principal stored fuel is glycogen. (B) Brook trout white fibres. Transverse frozen section (10 μm) stained for succinic dehydrogenase. Dark spots within the fibres correspond to lipid and mitochondrial accumulations. Note the wide range of fibre size. (C) Brook trout white fibres. Semi-thin (1 μm) araldite embedded section stained with p-phenylene diamine (PPDA). Note the peripheral ribbon-like myofibrils (PM), the dense myofibrillar packing (My) and the accumulations of mitochondria (M) and lipid (L). (D) Longitudinal section of crucian carp white fibre. Note distinctive M-line (ML), mitochondria (M) with highly developed cristae array and glycogen rosettes (G).
Structure and Function of Fish Muscles

a cart-wheel with spokes radiating to a small central hub of sarcoplasm. This feature is not observed in elasmobranchs, holocephali or higher vertebrates and may be related to the pattern of fibre growth in teleosts.

In contrast to higher vertebrates, both red and white fibres have a distinctive M-line (Figs 3 & 4). Other ultrastructural criteria for distinguishing between fibre types include a somewhat thicker Z-line in white than in red fibres (Patterson & Goldspink, 1972) and differences in the deposition of stored metabolites (Bone, 1978a). Glycogen particles are present either as rosettes or small chains in all fibre types but are particularly abundant in red fibres. With the exception of tuna Euthynnus pelamis which has higher glycogen stores in white fibres (Guppy et al., 1979) this is a reversal of the typical vertebrate pattern (Johnston, 1980b). Biochemical determinations of glycogen concentrations vary from 350–3600 mg/100 g in red to 9–1440 mg/100 g in white fibres depending on activity level, nutritional status, and species (Love, 1970). Lipid droplets are usually more abundant in red than white fibres constituting 11% of fibre volume in brook trout (Salvelinus fontinalis Mitchell) and plaice (Pleuronectes platessa) (Johnston & Moon, 1980b; Johnston, 1981) (Fig. 3). In fatty fish such as anchovy (Johnston, unpublished) a layer of fat cells occurs between the skin and red muscle layer, while in other species, for example mackerel (Bone, 1978b) and eel, adipocytes are widely distributed among the white muscle fibres. The extent to which the muscle lipid reserves of such fish represent metabolic stores, or function as part of a buoyancy mechanism, is unknown.

There have been relatively few ultrastructural investigations of the fibre types other than red or white. Intermediate/pink fibres of the carp (Johnston & Maitland, 1980) and sharks (Galeus melastomus and Etmopterus spinax) (Kryvi, 1977) have an intermediate mitochondrial density and myofibrillar packing to red and white fibres. The dogfish superficial fibres described by Bone (1978a) are distinct from either red or white fibres in containing an inconspicuous M-line, virtually no lipid, simple mitochondria, and a relatively poorly developed T-system and SR.

INNERVATION AND ELECTROPHYSIOLOGICAL PROPERTIES

In dogfish, red fibres receive a number of terminations of the en-grappe type derived from two separate axons. The motor axons either pass into the fibre from the myoseptal ends or run across the surface of the muscle innervating a number of different fibres (Fig. 5).
FIG. 5. A diagrammatic representation of the different patterns of motor innervation (left) and modes of activation (right) of fish muscle fibres. Traces of electrical activity show stylised responses to depolarizing pulses or nerve stimulation. Vertical calibration bars represent 20 mV, and horizontal bars 5 ms. Zero potential is indicated with a horizontal line. (A) All fish red fibres examined are multiply innervated and are activated by junction potentials (see Barrets, 1961). (B) White fibres in elasmobranchs, dipnoans and certain teleosts (see Fig. 6) are focally innervated at one myoseptal end. Motor terminations are of the en-grappe type. Focally innervated fibres show a propagating action potential overshooting zero potential by around 20 mV (see Hagiwara & Takahashi, 1967). (C) Multiply innervated white fibres are found in all acanthopterygians and certain other fish groups (see Fig. 6). Each muscle fibre is innervated by several different motor axons. Stimulation of the nerve supply results in two distinct kinds of electrical response: (1) junction potentials; and (2) spike potentials (see Hudson, 1969).

From electrophysiological measurements of membrane space constants Stanfield (1972) concluded that motor terminals were around 150–200 μm apart. Subjunctional folds are present on terminals of both superficial and outer and inner red fibres of *Scyliorhinus* (Bone, 1972). Typically, red fibres do not produce action potentials in response to depolarizing pulses (Stanfield, 1972). Thus in this respect and in relation to their innervation dogfish red fibres resemble the true slow or tonic fibres of amphibians. However, Stanfield (1972) found that eight out of 27 red fibres examined showed a significantly large inward sodium current on depolarization to suggest they were capable of propagating an action potential. Of the remaining fibres six showed no inward Na current and the others only a small inward Na current. Thus although it is likely that red fibres are normally
activated by junction potentials the electrophysiology of fish slow fibres warrants further investigation. The pattern of innervation of red muscles in other elasmobranchs and indeed in dipnoans, holosteans, chondrosteans and teleosts would appear to be broadly similar to that described from dogfish (Bone, 1964).

White muscle fibres in dogfish are innervated by large diameter axons which run in the myosepta and give rise to basket-like en-plaque end-formations at one end of the fibre (Bone, 1964, 1966). Each muscle fibre is innervated by two separate axons which fuse to form a single end-plate (Bone, 1964, 1972). In dogfish, the two motor terminals contain vesicles of different size ranges, 50 nm and 100 nm (Best & Bone, 1973). The significance of this dual innervation is unknown since only acetyl cholinesterase has been demonstrated in sub-synaptic folds (Pecot-Dechavassine, 1961). Bone (1978a) has calculated that each motor unit in dogfish white muscle contains around 50-100 fibres. In a study of several species of tropical stingray Hagiwara & Takahashi (1967) found that white fibres showed typical over-shooting spike-potentials on depolarization. Thus white fibres of elasmobranchs resemble frog fast twitch fibres. In some elasmobranchs, for example Torpedo, the more superficial white fibres receive basket-like endings to the mid-region rather than the ends of the fibre (Bone, 1964).

The innervation of white muscles in chondrosteans, holosteans and dipnoans has been less studied but appears to resemble that of elasmobranchs (Fig. 6) (Bone, 1964). However, among teleosts another type of innervation occurs and focally innervated white muscles are only found among the more taxonomically primitive groups (Fig. 6). Indeed, Bone (1970) has suggested that the type of innervation may even serve as a taxonomic character. No acanthopterygians have focal innervation and of the orders which are considered to be primitive on other grounds, for example the Salmoniformes, only the Salmonidae are multiterminally innervated (Bone, 1970). Interestingly, orders such as the Osteoglossiformes and Ostariophysii also contain families with both types of innervation. For example within the Osteoglossiformes which comprise freshwater butterfly fish (Pantodontidae), feather backs (Notopteridae) and mooneyes (Hiodontidae), only Hiodon has terminal innervation (Bone, 1978a).

Thus in the majority of the 20,000 teleosts, including the Perciformes and Cypriniformes which together account for around four-fifths of all the species, the white fibres are multiply innervated. Branches of the spinal nerves run in the myosepta and fan out across the surface of the myotome to form a diffuse network such that each fibre receives numerous nerve terminals (Barets, 1961;
FIG. 6. Distribution of focal and multiterminal innervation among fast muscles of vertebrates. Only representatives of the teleosts have multiterminal innervation. Taxonomically primitive teleosts (lower box) have focal innervation. The middle box contains orders such as the Osteoglossiformes and Salmoniformes containing families with both focal and multiply innervated white fibres. This list is not intended to be comprehensive. See Bone (1964, 1970). Teleost orders with taxonomically advanced features have exclusively multiple innervation (upper box). Land vertebrates have single end-plates as do dipnoans, elasmobranchs, chondrosteans, holosteans, lampreys and hagfish.
Bone, 1964) (Fig. 5). The end-plate formations have been variously described as consisting of small rings or loops as in the tench (Barets, 1961) or as being multiple and made up of numerous separate neuro-fibrillar annuli, e.g. *Myxocephaulus scorpius* (Hudson, 1969). Nerve terminals are usually embedded in the sarcolemma and subjunctional folds are absent (Nishihara, 1967). In *Myxocephaulus*, the short-horned sculpin, Hudson (1969) found that each fibre receives innervation from as many as five axons from each of four spinal nerves. The number of terminals on each fibre ranges from eight to 22 with an average of 14 about 0.7 mm apart. Hudson considered that each end-plate is derived from a separate axon while others have considered that fibres are also multiply innervated by the same axon as is the case with red fibres (Barets, 1961). Hudson (1969) calculated that each abdominal myotome in the sculpin consists of 12 motor units all of a considerable size.

There have been relatively few studies of the electrophysiological properties of multiply innervated fast muscles (Barets, 1961; Hidaka & Toida, 1969; Hudson, 1969). Hudson (1969) found that electrical stimulation of spinal nerves elicits two kinds of electrical response, namely spike potentials resulting in a fast twitch, or junction potentials leading to a graded local contraction by the muscle. The typical response to supramaximal nerve stimulation is an all-or-none spike potential oversooting zero-potential by as much as 20 mV. Depolarization of the membrane occurs in two phases. Duration of the fast phase of the spike potential is of the order of 1.5—2.5 m s⁻¹ at 12°C (Hudson, 1969). Variation in stimulus intensity below maximal results in junction potentials the amplitude of which can be quantitized in discrete steps providing evidence for innervation by several different motor axons. Generally, Hudson found that junction potentials did not exceed 35 mV and that in fresh preparations spike potentials often arose from a single junction potential. However, in a proportion of cases, particularly in older preparations, only junction potentials could be elicited. At present, it is not possible to conclude whether white fibres can be activated *in vivo* in the absence of spike potentials. Clearly, the electrophysiological properties of polyneuronally innervated myotomal muscles are worthy of further study.

The few multiterminally innervated fin muscles that have been investigated appear to be similar to myotomal muscles (Bergman, 1964; Nishihara, 1967). Fin muscles may well provide a convenient nerve—muscle preparation for investigating the combined electrical and mechanical responses of polyneuronally innervated fibres.
MECHANICAL PROPERTIES

In spite of the separation of fish fibre types into different positions within the myotome there have been very few studies of their contractile properties. The complex fibre orientation and myoseptal insertion of fish trunk muscles make such studies technically difficult. In order to obtain preparations in which the fibres run parallel it is necessary to utilize either single or small bundles of fibres. Fin, jaw and sound-producing muscles provide more suitable whole muscle preparations and have been the subject of a limited number of studies (Bergman, 1964; Hidaka & Toida, 1969; Yamamoto, 1972).

Bone & Johnston (in press) have compared the properties of bundles of red and white fibres from the dogfish myotome. Both fibres respond to a single supramaximal stimulus; white fibres giving a fast twitch (half-time peak tension: \( t_f \approx 20 \text{ ms} \)) and red fibres a slow contraction (\( t_f \approx 100 \text{ ms} \)). On multiple stimulation, fused tetani are produced at frequencies above 8 Hz. Twitch-tetanus ratios of around 0.5 occur at 5 Hz and 10 Hz for red and white fibres respectively. Very different stimulation characteristics have been reported for multiply innervated teleost muscle. Flitney & Johnston (1979) studied red and white fibre bundles isolated from adductor operculi muscles of *Tilapia mossambica*. Red fibres from *Tilapia* only respond at stimulation frequencies in excess of 5–10 Hz. Both fibre types produce graded, fused tetani, reaching a maximum at 250–300 Hz. Unloaded speeds of shortening are 2.6 L s\(^{-1}\) for white and 1.5 L s\(^{-1}\) for red fibres at 18°C (Flitney & Johnston, 1979). However, rate of rise of tension during supramaximal stimulation at 200 Hz was 6.5 times greater for white than for red fibres.

The responses of fibre bundles to different stimulation frequencies for (a) the focally innervated white fibres of the cuckoo ray *Raja naevus* and by (b) the polynervronally innervated myotomal fibres of the cod *Gadus morhua* L. are shown in Fig. 7. Typical tetanic fusion frequencies on multiple stimulation are 5–10 Hz for skate and 40–50 Hz for cod. Maximal tensions require stimulation in excess of 200 Hz for cod and only 20 Hz for skate. The rate of tension development is also frequency-dependent. Alexander (1969) has calculated that the complex geometry of white fibres results in very little shortening (\( \sim 3\% \text{L}_0 \)), even at maximum body flexure. It seems likely that the rate of tension development for multiply innervated fibres *in vivo* may be limited as much by the muscles membrane properties as by its maximum speed of shortening.
A full understanding of these differences in the modes of activation of fast fibres from different groups of fishes must await mechanical studies with innervated single fibre preparations.

REGULATION OF MUSCLE CONTRACTILITY

Some features of the regulation of contractility in fish skeletal muscle are illustrated in Fig. 8. Tension development rises as a function of
FIG. 8. The control of contractility in fish muscle. Abbreviation: CNS, central nervous system; SR, sarcoplasmic reticulum; CBP, cytoplasmic calcium binding proteins; CT, calcium transient; PK, Ca\(^{2+}\)-dependent protein kinase. Factors modulating force production include: (1) The number and frequency of motor units activated. (2) Neurotransmission. (3) The muscle action potential (or distributed depolarization) is transmitted to the SR via the T-tubule system causing release of bound calcium. (4) This results in a transient increase in free calcium from 0.1 to 10\(\mu\)M. (5) The duration of the calcium transient is thought to be reduced in fast muscles by the presence of high concentrations of cytoplasmic calcium binding proteins (parvalbumins), see Fig. 9. (6) Calcium ions activate (a) muscle contraction and (b) glycogen breakdown by direct binding effects to troponin C and phosphorylase kinase respectively. There is also evidence that force production can be modulated by myosin phosphorylation through a specific Ca\(^{2+}\)-activated myosin light-chain kinase. (7) In slow mammalian muscles calcium uptake into the SR is modulated by a cytoplasmic factor thought to be a Ca\(^{2+}\)-dependent protein kinase. Slow muscles of salmonid fishes do not appear to have such a mechanism (McArdle & Johnston, in press). The extent to which this is true of other species is unknown. Incubation of Tilapia red and white muscles in ringers containing zero calcium or verapamil (5 \(\times\) 10\(^{-5}\)) leads to a rapid and reversible loss of tension (Flitney & Johnston, 1979). It appears, therefore, that fish muscles have a requirement for extracellular calcium. Whether Ca\(^{2+}\) crosses the sarcolemma or remains bound to sites on the outside is unknown.
myoplasmic free calcium concentration within the range 0.1 to 10 μM. In multiply innervated fibres the release of calcium from the SR is dependent on the degree of depolarization of the muscle membrane which is in turn determined by the number of motor neurones activated and their firing frequency. In fish white but not red fibres cytoplasmic calcium binding proteins (parvalbumins) play an important role in regulating free calcium concentrations (Gerday & Gillis, 1976; Pechère, Derancourt & Harech, 1977).

Parvalbumins are found in all vertebrate fast muscles but occur in particularly high concentrations in fish white fibres (~15% soluble proteins) (Le Peuch, Demaille & Pechère, 1978). Characteristically they are acidic proteins of low molecular weight (11,000–12,000 daltons) which can bind 2-μg atoms Ca²⁺/mole (Pechère, Capony & Demaille, 1973). Sequence studies have shown strict conservation of the amino acid residues at the Ca²⁺-binding sites and a high degree of homology with other muscle calcium binding proteins, troponin C, myosin P light chain and calmodulin (Collins, 1976; Perry, 1979). In the metal-free form parvalbumins are able to inhibit Mg²⁺ Ca²⁺ myofibrillar ATPase activity by chelating Ca²⁺ ions bound to troponin C (Pechère, Derancourt et al., 1977). Calcium bound to parvalbumins can be exchanged and accumulated by SR vesicles (Gerday & Gillis, 1976). The relative binding constants of Ca²⁺ for troponin C and parvalbumins are Kd’s 10⁻⁶ M and 10⁻⁷ M respectively (Benzonana, Capony & Pechère, 1972; Potter & Gergely, 1975). The current view is that parvalbumins function in aiding rapid relaxation (Gerday & Gillis, 1976; Pechère, Derancourt et al., 1977). Since parvalbumins occur in molar excess over TNC in fish muscles Ca²⁺-released by the SR causes only a transient activation of crossbridges as Ca²⁺ rapidly becomes bound within the cytoplasm. Thus relaxation occurs before all the Ca²⁺ is resequestered within the SR (see Fig. 9).

In some fish the sarcoplasmic reticulum occupies a somewhat similar proportion of fibre volume in both fast and slow fibres (e.g. Patterson & Goldspink, 1972; see also Table III).

Evidence has been obtained that Ca²⁺-uptake in mammalian slow but not fast muscles is regulated by a Ca²⁺-dependent protein kinase which phosphorylates components of the pump necessary for transport. Although sarcoplasmic reticulum isolated from red and white muscles of rainbow trout has a somewhat different protein composition and it does not appear to require protein kinases or cAMP for full activation (McArdle & Johnston, in press). The extent to which this is true for other species and represents a phylogenetic difference between fishes and mammals is unknown.
Activation of myosin crossbridges in fish muscle is achieved through direct effects of Ca$^{2+}$-binding to troponin C as in other vertebrates (Lehman & Szent-Györgyi, 1975). In addition, phosphorylation of myosin through a specific Ca$^{2+}$-activated myosin light-chain kinase may lead to a modulation of force production (see Perry, 1979).

FIBRE RECRUITMENT DURING SWIMMING

The recruitment of different fibre types with increasing speed has been studied in dogfish (Bone, 1966), rainbow trout (Hudson, 1973), carp (Johnston, Davison et al., 1977), herring (Bone et al., 1978), skipjack tuna (Rayner & Keenan, 1967; Brill & Dizon, 1979), bass (Freadman, 1979, saithe (Johnston & Moon, 1980a) and brook trout (Johnston & Moon, 1980b). This represents only a small proportion
of the 20,500 species of living fishes. However, at present the results are consistent with a division in the pattern of fibre recruitment between different groups of fishes according to the type of innervation of the fast motor system. Focally innervated fast fibres appear only to be recruited for burst activity. For example, electromyographical studies in the Pacific herring show that only red fibres are recruited at speeds that can be sustained for several hours. Higher speeds (5 body lengths/s) result in the recruitment of white fibres and fatigue of the fish within a further 1–2 min swimming (Bone et al., 1978). Qualitatively similar results have been obtained for the dogfish (*Scyliorhinus canicula*) which also has focally innervated white fibres (Bone, 1966). In contrast a number of studies have shown that both red and white fibres are recruited at sustainable swimming speeds in species with multiply innervated fast muscles (Fig. 10) (Hudson, 1973; Johnston, Davison et al., 1977; Bone et al., 1978).

![Diagram](image)

**FIG. 10.** Recruitment of fibre types of the brook trout (*Salvelinus fontinalis* Mitchill) during steady swimming. (Top) Shows insertion of EMG electrodes in red (r) and white (w) muscles. (Bottom A) Top two traces, EMGs recorded from red and white muscle regions during steady swimming at 1 length/s. Note potentials are only recorded from the red muscle layer. Lower trace, a burst of electrical activity in white muscle associated with a change of position in the swimming chamber during the same experiment. (Bottom B) Recordings of EMGs from red and white muscles at a steady swimming speed of 3 lengths/s. Brook trout are able to maintain this speed indefinitely. Horizontal scale represents 500 ms. Vertical scale represents 1 mv. (From Johnston & Moon, 1980b.)
For example the threshold speed for recruitment of white fibres is 0.8–1.9 lengths/s in saithe (Johnston & Moon, 1980a), and 2.0 lengths/s in carp (Johnston, Davison et al., 1977). Somewhat higher speeds for recruitment of white fibres have been reported in striped bass (*Morone saxatilis*), 3.2 lengths/s, and bluefish (*Pomatomus saltatrix*), 4.5 lengths/s (Freadman, 1979). Thus in some species with multiply innervated fast muscles there may be a secondary reversion to the more primitive pattern of fibre recruitment such as is found in the dogfish. Recent studies have shown a wide range of aerobic capacities for teleost white muscles between species, presumably reflecting different degrees of involvement of this fibre type in sustained swimming (Johnston & Moon, 1980b).

Only in carp have electromyographs (EMGs) been recorded from the pink muscle layers. In this study it was shown that the order of recruitment of fibre types with increasing speed is red muscle > pink muscle > white muscle (Johnston, Davison et al., 1977).

**METABOLISM**

**Enzyme Activities of Red and White Muscle Fibres**

Determinations of maximal *in vitro* activities of enzymes can be used to assess the metabolic capacities of different muscles. In the case of non-equilibrium enzymes such information gives a useful semi-quantitative estimate of maximal metabolic flux (see Newsholme & Start, 1973; Newsholme, Zammit & Crabtree, 1978). Enzymes catalysing non-equilibrium reactions can be identified by measurements of mass action ratios and they usually have among the lowest activities in the pathway, being regulatory steps in metabolism. Enzymes catalysing equilibrium reactions only provide qualitative information about the relative importance of particular metabolite pathways and the principal fuels supporting activity. Enzyme studies of fish muscle have recently been reviewed (Zammit & Newsholme, 1979; Johnston, 1980b). Table IV gives the maximal *in vitro* activities of some enzymes of energy metabolism from the fast and slow muscles of the brook trout measured under optimal conditions of pH, substrates and co-ions (Johnston & Moon, 1980b). Typically white muscles have a higher glycolytic potential (phosphorylase, PFK activities) and lower oxidative capacity (citrate synthetase) than red muscles.

Bárány (1967) has demonstrated for a wide range of vertebrate muscles that myofibrillar ATPase activities parallel the unloaded
TABLE IV

Activities of some key enzymes of energy metabolism and metabolite concentrations in the red and white muscles of brook trout (Salvelinus fontinalis Mitchill). Data from Johnston & Moon (1980b) and Walesby & Johnston (1980b)

<table>
<thead>
<tr>
<th>Enzyme Activities (μmol substrate/g dry wt./min)</th>
<th>Red Muscle</th>
<th>White Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg$^{2+}$ Ca$^{2+}$ myofibrillar ATPase$^+$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'AMP aminohydrolase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
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<td></td>
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<tr>
<td>Hexokinase</td>
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<tr>
<td>Phosphofructokinase</td>
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<tr>
<td>Pyruvate kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td></td>
<td></td>
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<tr>
<td>Citrate synthetase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OH Acyl coA dehydrogenase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolite concentrations (μmol/g dry wt.)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>2.9</td>
</tr>
<tr>
<td>ADP</td>
<td>0.4</td>
</tr>
<tr>
<td>AMP</td>
<td>0.2</td>
</tr>
<tr>
<td>Pi</td>
<td>3.8</td>
</tr>
<tr>
<td>Phosphoryl Creatine</td>
<td>9.3</td>
</tr>
<tr>
<td>ATP</td>
<td>6.1</td>
</tr>
<tr>
<td>ADP</td>
<td>0.6</td>
</tr>
<tr>
<td>AMP</td>
<td>0.2</td>
</tr>
<tr>
<td>Pi</td>
<td>7.1</td>
</tr>
<tr>
<td>Phosphoryl Creatine</td>
<td>25.7</td>
</tr>
</tbody>
</table>

Activity expressed as μmol Pi released/mg myofibrillar protein/min.

speeds of shortening. On this basis, white muscles of the trout and other species studied (see Table I) are around two to five times as fast as red muscle. Activities of enzymes responsible for maintaining ATP supply during contraction such as creatine kinase, adenylate kinase and 5'AMP aminohydrolase parallel those of myofibrillar ATPase. 5'AMP aminohydrolase is particularly important in fast muscles since it catalyses the deamination of AMP to IMP thus shifting the equilibrium of adenylate kinase in favour of ATP production. It has been calculated that in fatigued muscles up to half of the ATP for contraction is supplied by this pathway.

In a study of a large number of invertebrate and vertebrate species Beis & Newsholme (1975) found that muscles with the lowest ATP/AMP ratios usually have the lowest rate of ATP utilization and the least variation in energy requirement. The ATP/AMP ratios in the red
and white muscles of brook trout (R, 18.6; W, 38.3) and tuna (R, 26.3; W, 61.1) reflect the relative activity levels of these muscles and lend support to this concept (Table IV).

Both metabolite concentrations (Freed, 1971; Walesby & Johnston, 1980b) and enzyme activities can be modified by environmental factors such as temperature, activity and nutritional status (see Johnston, 1980b).

Burst Swimming

The type of locomotory activity best understood is burst swimming. Maximum swimming speeds can only be maintained for relatively few tail-beat cycles and are approximately 26 lengths/s for 10 cm fish and 4 lengths/s for 1 m fish (Wardle, 1975). Burst swimming is accompanied by an extremely rapid activation of glycolysis in white muscle. In trout this results in the utilization of around 50% of muscle glycogen stores in 15 s which is equivalent to a flux of 40 μmol glycogen derived glucose/kg/s (Stevens & Black, 1966). There is near-quantitative conversion of muscle glycogen to lactate which may be retained in the muscle for several hours following the cessation of exercise (Wardle, 1978). Lactate levels of 59 (trout) and 69 mmol/kg (skipjack tuna) have been recorded in white muscles following 1–2 min burst swimming (Black, Robertson & Parker, 1961; Guppy et al., 1979). Recovery of white muscle lactate to pre-activity states may require up to 18 h in some species (Black, Robertson et al., 1961) but is usually complete within 30–60 min in red muscle (Johnston & Goldspink, 1973a).

Changes in metabolite concentrations between rest and maximal activity in tuna white muscle are illustrated in Fig. 11. Burst swimming is associated with a large decrease in phosphoryl creatine (90%) and ATP (53%) and a small increase in AMP concentrations (22%) (Guppy et al., 1979: table 2).

In the carp Driedzic & Hochachka (1976) observed a 48% decrease in the total adenylate pool and an essentially 1:1 increase in IMP and NH₄ due to the deamination of AMP by 5' amino-hydrolase (see above). It is possible that this is one source of the anaerobic NH₄⁺ production which has been frequently observed following enforced exercise in fish (Kutty, 1972).

The regulation of glycogenolysis in fish white muscle is illustrated in Fig. 12. Non-equilibrium or regulatory steps in anaerobic glycolysis include phosphorylase, phosphofructokinase and pyruvate kinase (Newsholme & Start, 1973). There is an interesting phylogenetic difference in the control of glycogen breakdown between fish and
mammalian muscles (Fischer, Blum et al., 1975). While rabbit muscle phosphorylase kinase is rapidly activated or inhibited by cAMP dependent protein kinases or specific phosphatases, the dogfish enzyme depends only on Ca$^{2+}$ for its activity (Cohen, Duewer & Fischer, 1971; Fischer, Blum et al., 1975; Fischer, Alaba et al., 1978). Indeed, there is no production of cAMP in response to noradrenaline in dogfish white muscle (Fischer, Blum et al., 1975). The lack of a hormonal mechanism for activating glycogen breakdown in dogfish white muscle may be related to the relatively poor circulation through this tissue during maximal activity. In vitro phosphofructokinase is known to be activated by substrates, AMP, ADP, Pi and NH$_4$ and inhibited by phosphoryl creatine, ATP and citrate (Mansour, 1972). Substrate cycling between F-6-P and FDP by the coupling of the PFK and FDPase reactions is thought to provide a mechanism for increasing the sensitivity of F-6-P phosphorylation to changes in AMP (Newsholme, 1976) (Fig. 12).

Increases in AMP concentration activate PFK and inhibit FDPase resulting in a proportionally larger increase in glycolysis than is possible in the absence of substrate cycling. According to this scheme
FIG. 12. Control of anaerobic glycogenolysis in fish white muscle. Regulatory steps include (A) glycogen phosphorylase, (B) phosphofructokinase and (C) pyruvate kinase (see text for details). Abbreviations: Phos, phosphorylase; GIP, glucose-1-phosphate; PFK, phosphofructokinase; FBP, fructose 1,6-biphosphate; AMP, adenosine 5' monophosphate; TP, triose phosphates; NAD, β-nicotinamide adenine dinucleotide; NADH, β-nicotinamide adenine dinucleotide reduced form; 1, 3 DPG, 1, 3 diphosphoglyceric acid; PEP, phospho(enol) pyruvate; PYK, pyruvate kinase. Other abbreviations are given in the legend to Fig. 11.
small changes in ATP lead to proportionately larger changes in AMP through the adenylate kinase reaction. Thus, in general, the higher the initial ATP : AMP ratio the greater the increase in AMP for a given breakdown of ATP and the greater the stimulation of glycolysis (Newsholme et al., 1978). An interesting feature of the control of glycolysis in fish muscles is the presence of a regulatory pyruvate kinase. In vitro pyruvate kinase is inhibited by ATP and alanine and activated by substrates and FDP (Somero & Hochachka, 1968; Johnston, 1975). Regulatory pyruvate kinases are associated with muscles in which there is a rapid and dramatic increase in glycolysis between standard and burst activity. For example modulator-sensitive pyruvate kinase occurs in the locomotory muscles of diving mammals such as whales but not the limb muscles of terrestrial mammals (Hochachka & Storey, 1975). The observed changes in metabolites and Ca²⁺ release from SR during burst swimming are sufficient to cause a rapid activation of glycolysis through the known regulatory mechanisms described above (Figs 11, 12).

Steady Swimming

The metabolism of muscles during steady swimming is much less well understood. One recurring problem with studies of metabolism of sustained swimming has been the failure of many investigators to control the activity state of fish either before or during the exercise period. It is essential to know the exact swimming speed in lengths/s and to control as far as possible the condition of the fish prior to its introduction to the swimming chamber (e.g. temperature, nutritional status, level of stress etc.). In addition the adaptive variation observed within fish populations and particularly between species means that it is often impossible to reach general conclusions about metabolism.

In general, fish can increase their oxygen uptake by a factor of 10–15 times between rest and maximal activity (Bennett, 1978). Values for maximum oxygen consumption are comparable to the basal levels for birds and mammals and are in the range 190–644 ml kg⁻¹ h⁻¹ (see Jones & Randall, 1978). Unfortunately, only fragmentary data are known for tuna which might be expected to have aerobic activity levels more comparable to homeotherms. It seems likely that red muscle receives a significant proportion of the cardiac output during steady swimming. This is particularly true for species in which red muscles alone support sustained activity (e.g. elasmobranchs, dipnoans, primitive teleosts). Since red fibres constitute a small fraction of the trunk musculature (5–10%) they undoubtedly possess a highly active aerobic metabolism. Indeed, the mitochondrial densities of fish red fibres are greater than those of similar fibres in
mammalian limb muscles (see Table II). Little is known about the regulation of the tricarboxylic acid cycle in fish (for a review see Driedzic & Hochachka, 1978). Lipid is known to be an important fuel during steady swimming particularly in those species which undertake a spawning migration (see Bilinski, 1974). There are indications that both lipolysis and the transport of fat differ significantly between fish and mammals (see Bilinski, 1974). For example, it has been reported that the activation of lipolysis in fish does not proceed via cAMP-dependent protein kinases (Farkas, 1969). There are also differences in the sites of storage and metabolism of lipid between different fish groups. In elasmobranchs triacyl glycerol is stored in the liver, whereas in teleosts a significant proportion is deposited either in discrete stores in the viscera or dispersed throughout the muscle fibres in adipocytes. Red muscles of teleosts contain high activities of carnitine palmitoyl transferase and triacyl glycerol lipase but not 3-hydroxybutyrate dehydrogenase. The opposite situation is found in elasmobranchs which have high activities of enzymes of ketone body but not fatty acid oxidation (Zammit & Newsholme, 1979) (see Table V). Triacyl glycerol and non-esterified fatty acids also occur in much higher concentrations in the plasma of teleosts than elasmobranchs (Zammit & Newsholme, 1979). These differences are augmented during starvation. Thus it seems likely that the most important fat fuels are ketone bodies in elasmobranchs and fatty acids in teleosts. Red muscles in salmonids can oxidize long chain saturated fatty acids to CO$_2$ at around ten times the rate of that of white muscles (Jonas & Bilinski, 1964; Bilinski, 1974). Similarly, carnitine palmitoyl transferase activities of teleost white muscles are generally only 5% of that of red muscles (Crabtree & Newsholme,

<table>
<thead>
<tr>
<th>Species</th>
<th>Hydroxybutyrate dehydrogenase</th>
<th>Carnitine palmitoyl transferase</th>
<th>Triacylglycerol lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.01</td>
<td>0.07</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>&lt; 0.01</td>
<td>0.75</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>&lt; 0.01</td>
<td>0.60</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>&lt; 0.01</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>0.37</td>
<td>&lt; 0.01</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>0.64</td>
<td>&lt; 0.01</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data from Zammit & Newsholme (1979).
Thus it seems likely that oxidation of fats as fuels is much more important in red than in white muscles even when the latter are recruited for sustained activity.

Numerous studies have shown glycogen depletion during sustained swimming in both the red and white muscles of teleosts (Pritchard, Hunter & Lasker, 1971; Johnston & Goldspink, 1973a,b). However, the pathways of carbohydrate metabolism utilized by multiply innervated white fibres at sustained speeds are not known for any species. Two basic possibilities exist: ATP generation could be aerobic using either local glycogen stores or blood glucose, or largely anaerobic resulting in the production of lactic acid. For the latter case, a number of schemes have been proposed involving lactate-exchange with other tissues to keep the fish in overall aerobic balance.

In a quantitative study of glycogen utilization in crucian carp Johnston & Goldspink (1973c) found that glycogen utilization at three lengths/s was two to three times higher in red than in white muscles. However, since red fibres only comprise 7% of the trunk musculature they only account for 15–20% of the total glycogen utilized at this swimming speed.

Crabtree & Newsholme (1972) have demonstrated a good correlation between hexokinase activities in various muscles and calculated maximum capacities for glucose oxidation. Table VI gives values for red and white muscle hexokinase activities in various species. It can be seen that the importance of aerobic glucose utilization is likely to vary considerably between species for both myotomal muscle types. White fibres in two species of trout and the skipjack tuna have high hexokinase activities comparable to those of red muscle (Table VI). Other evidence for a significant aerobic capacity in the white muscles of these species is as follows. In rainbow trout the ratio of mitochondria between red and white muscles is 8:3 by number (Nag, 1972). Activities of citrate synthetase and cytochrome oxidase in brook trout white muscle are 25–35% of that of red muscle (Johnston & Moon, 1980b). Thus in at least trout, and possibly tuna, the aerobic capacity of the white muscle is probably sufficient to support sustained activity and blood glucose may be an important fuel.

A number of early studies reported lactate accumulation two to three times resting levels in various salmonids forced to swim at sustained swimming speeds (Black, Bosomworth & Docherty, 1966). These results may well reflect the presence of a generalized stressed reaction in acutely exercised fish (see Wardle, 1978). Studies of trout trained to swim in an exercise chamber for several weeks prior to experimentation show no net accumulation of lactate over rested
<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature</th>
<th>Red muscle</th>
<th>White muscle</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogfish (Scyliorhinus canicula)</td>
<td>10°C</td>
<td>0.5</td>
<td>0.1</td>
<td>Crabtree &amp; Newsholme (1972)</td>
</tr>
<tr>
<td>Herring (Clupea harengus)</td>
<td>10°C</td>
<td>0.1</td>
<td></td>
<td>Newsholme et al. (1978)</td>
</tr>
<tr>
<td>Rainbow trout (Salmo gairdneri)</td>
<td>20°C</td>
<td>1.8</td>
<td>0.6</td>
<td>Johnston (1977)</td>
</tr>
<tr>
<td>Brook trout (Salvelinus fontinalis)</td>
<td>15°C</td>
<td>0.3</td>
<td>0.6</td>
<td>Johnston &amp; Moon (1980b)</td>
</tr>
<tr>
<td>Bass (Dicentrarchus labrax)</td>
<td>10°C</td>
<td>0.2</td>
<td>0.2</td>
<td>Newsholme et al. (1978)</td>
</tr>
<tr>
<td>Coal fish (Pollachius virens)</td>
<td>15°C</td>
<td>0.7</td>
<td>0.1</td>
<td>Johnston &amp; Moon (1980a)</td>
</tr>
<tr>
<td>Plaice (Pleuronectes platessa)</td>
<td>15°C</td>
<td>0.4</td>
<td>0.06</td>
<td>Moon &amp; Johnston (1980)</td>
</tr>
<tr>
<td>Common carp (Cyprinus carpio)</td>
<td>20°C</td>
<td>1.2</td>
<td>0.1</td>
<td>Johnston (1977)</td>
</tr>
<tr>
<td>Skipjack tuna (Katsuwonus pelamis)</td>
<td>25°C</td>
<td>1.2</td>
<td>0.8</td>
<td>Guppy et al. (1979)</td>
</tr>
<tr>
<td>Red mullet (Mullus surmeletus)</td>
<td>10°C</td>
<td>2.1</td>
<td>0.1</td>
<td>Newsholme et al. (1978)</td>
</tr>
<tr>
<td>Antarctic cod (Notothenia rossii)</td>
<td>4°C</td>
<td>0.8*</td>
<td>0.1</td>
<td>Walesby &amp; Johnston (1980a)</td>
</tr>
<tr>
<td>Sculpin (Myoxocephalus scorpius)</td>
<td>15°C</td>
<td>1.8</td>
<td>0.4</td>
<td>Fitch &amp; Johnston (unpublished results)</td>
</tr>
</tbody>
</table>

Values are expressed as µmol substrate utilized, g wet weight muscle$^{-1}$ min$^{-1}$. Details of numbers of animals used and standard errors of the mean values are given in the original publications. Unpublished results for Myoxocephalus scorpius represent determinations for six fish, red muscle $1.84 \pm 0.27$ (SE) white muscle $0.39 \pm 0.02$ (SE). Assay temperatures are given above.
fish for speeds up to at least four body lengths/s (Johnston & Moon, 1980b).

However, in many species it would appear that anaerobic pathways are important in providing ATP for sustained activity. For example, Smit et al. (1971) have calculated that goldfish (Carassius auratus) obtain 80% of their energy requirements anaerobically during high speed sustained swimming. Further evidence for the importance of anaerobic pathways at sub-maximal swimming speeds comes from measurements of key enzyme activities following endurance exercise training (see Johnston & Moon, 1980a,b). In contrast to mammals, endurance exercise training in fish leads to an increase in glycolytic enzyme activities with little change in overall aerobic capacity of the muscles (Johnston & Moon, 1980a,b).

Metabolic Fate of Lactate Produced by the Swimming Muscles

The metabolic fate of lactate produced during sustained and following burst swimming, has been the subject of a large number of papers. It is likely that a major part of the lactate produced during exercise is oxidized to pyruvate (Bilinski, 1974). Bilinski & Jonas (1972) have demonstrated that gills, liver, red muscle and kidney are all able to oxidize $^{14}$C-lactate at high rates in vitro. Bone (1975) has suggested that fish may be able to maintain overall aerobic balance by transferring lactate produced by anaerobic glycogenolysis in the white muscle to the gills and other peripheral sites for subsequent oxidation. The extent to which fish liver can utilize lactate as a gluconeogenic substrate is unclear. Black, Bosomworth et al. (1966) concluded that the Cori cycle is of little importance in trout since liver glycogen levels are not restored to normal even 24 h following burst swimming. However, recently it has been demonstrated that eel hepatocytes convert lactate to glucose at high rates in vitro (Renaud & Moon, 1980).

Wittenberger and co-workers have suggested that red muscle operates a direct glucose–lactate exchange with white muscles (Wittenberger & Dicauic, 1965; Wittenberger, 1973; Wittenberger, Coprean & Morar, 1975). This idea developed from a suggestion by Braekkan (1956) that red muscle in fish is homologous to the liver in higher vertebrates. 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. However, on the basis of a variety of indirect evidence it has been proposed that red muscle does have a significant role in synthesizing glucose from circulating lactate (see Driedzic & Hochachka,
FIG. 13. Activities of glycolytic and gluconeogenic enzymes in red and white muscles and liver of fed and four-month-starved plaice (*Pleuronectes platessa*) (from Moon & Johnston, 1980). (Top) Activities (μmol min⁻¹ g dry weight⁻¹) of hexokinase, glucose-6-phosphatase (G-6-Pase) and p-nitrophenyl phosphatase (pNPPase). (Centre) Activities (μmol min⁻¹ g dry weight⁻¹) of phosphofructokinase (PFK) and fructose-1,6 bisphosphatase (FDPase). (Bottom) Activities (μmol min⁻¹ g dry weight⁻¹) of pyruvate kinase (PyK). Phosphoenol pyruvate carboxykinase (PEP Ck) activities are given for the soluble (left) and bound (right) fractions of liver (LV) and red muscle (RM). Note the very low activities in red muscle. No activities of PEP Ck were detected in white muscle. In each case bars represent enzyme activities in liver (left), red muscle (centre) and white muscle (right). Data represent mean ± SE of six fish.
Structure and Function of Fish Muscles

1975; Wittenberger et al., 1975; Hulbert & Moon, 1978; Batty & Wardle, 1979). For example, Batty & Wardle (1979) have calculated, on the basis of in vivo $^3$H-glucose turnover experiments in plaice, that the transport of glucose into the muscle following recovery from exercise is insufficient to account for the observed rates of glycogen formation. However, a significant gluconeogenic role for red muscle is not supported by measurements of key enzyme activities (Johnston & Moon, 1979; Moon & Johnston, 1980). Activities of pyruvate carboxylase, PEP carboxykinase and glucose-6-phosphatase in plaice are either absent or present at very low levels in red muscle compared to liver, even under conditions of starvation which would be expected to augment gluconeogenesis (Fig. 13). Whether gluconeogenesis occurs in the red muscle of other species or whether the very low enzyme levels in plaice red muscle are sufficient to account for some glycogen synthesis during a long recovery period (12 h) remains to be seen.

Utilization of Muscle Proteins

Many species of fish undergo periods of seasonal starvation linked to fluctuations in food supply (see Love, 1970). In fish that do not store large quantities of lipid, white muscle myofibrillar proteins serve as an important source of metabolic precursors during starvation. For example, in plaice, after four months starvation white muscle water contents increase from around 75 to 95% with a corresponding loss of contractile proteins (Johnston & Goldspink, 1973b; Johnston, 1981). It seems likely that amino acids released from the muscles during starvation are converted to glucose via gluconeogenic pathways in the liver and hence constitute an important fuel for muscular activity under these conditions (Moon & Johnston, 1980).

Finally a number of authors have suggested that fish muscles are a major site of amino acid oxidation. For example, glycine and histidine together constitute around 50% of the free amino acid pool in the muscles of salmonid fishes (Fontaine & Marchelidon, 1971). The initial enzyme in histidine degradation is histidase, which is found in high titres in the muscles of some fish but not mammals and has a Km for histidine which is five times lower than the liver analogue (see Driedzic & Hochachka, 1978). Wood, Duncan & Jackson (1960) have suggested that histidine is oxidized in situ as an energy source by salmon muscle during spawning migration. There is, however, as yet little direct evidence for amino acid oxidation in fish muscles (for a review see Driedzic & Hochachka, 1978).
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