METABOLIC DIFFERENTIATION OF MUSCLE FIBRES FROM A HAEMOGLOBINLESS (*Champsocephalus gunnari* Lönnberg) AND A RED-BLOODED (*Notothenia rossii* Fischer) ANTARCTIC FISH

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Abstract. The metabolic differentiation of skeletal muscle-fibre types has been investigated in two species of Antarctic teleost, *Champsocephalus gunnari* Lönnberg (Channichthyidae) and *Notothenia rossii* Fischer (Nototheniidae). The channichthyiids, or "icefish", are unique amongst vertebrates in having no oxygen-transport pigment in the blood plasma. Qualitative histochemical and biochemical determinations have demonstrated the absence of myoglobin in skeletal and cardiac muscle of *C. gunnari*. At the cellular level, compensatory adaptations to a lack of respiratory pigment include extensive capillarization of skeletal muscle. Two main types of fibre type occur in the myotome of both species; these are analogous to the red and white muscles reported in a variety of other teleosts. The pectoral musculature, often well developed in Antarctic fish for low-speed sculling, consists of a complex arrangement of highly oxidative fibres. Adaptation associated with the haemoglobin and myoglobin-free condition in *C. gunnari* are discussed.

Cytochemical and biochemical studies of teleost swimming muscles have mainly considered species showing myotomal and caudal fin propulsion (e.g. Bone, 1966; Webb, 1970, 1971; Johnston and Goldspink, 1973*a*, *b*, *c*). Interestingly, many Antarctic fish show a marked development of pectoral fin locomotion (Robillard and Dayton, 1969; Holeton, 1970, 1975; Twelves, 1972; Lin and others, 1974). Sustained low-speed swimming is accomplished entirely by the action of enlarged fan-shaped pectoral fins, whilst the axial musculature appears to function in turning at low speed and is mainly reserved for short-term burst propulsion (Twelves, 1972; Lin and others 1974).

In the myotome of most teleosts, muscle fibres are organized into distinct anatomical regions. Typically, there is a superficial layer of oxidative fibres adjacent to the lateral line canal. These fibres usually have high concentrations of myoglobin, cytochromes and lipids (Matsuura and Hashimoto, 1954; George, 1962) and have high activities of respiratory chain and tricarboxylic acid cycle enzymes (Boström and Johannson, 1972; Johnston and others, 1977). The bulk of the myotome is composed of larger diameter fibres with low lipid content and having a predominantly glycolytic type of metabolism (George, 1962; Bone, 1966; Hamoir and others, 1972). The comparative biochemistry of the red and white fibre types has been the subject of recent studies (George, 1962; George and Bokdawala, 1964; Bone, 1966; Walker, 1971; Johnston and Goldspink, 1973*a*, *b*, *c*; Bilinksi, 1975; Johnston and others, 1977). Studies of myofibrillar adenosine triphosphatase activities, which parallel the speed of contraction of the fibres (Bárány, 1967), have shown that red and white fibres correspond to "slow" and "fast" fibres, respectively (Johnston and others, 1972). The presence of other kinds of muscle fibre has been reported for several species (George and Bodkawala, 1964; Bone, 1966; Bodkawala, 1967; Johnston and others, 1974; Patterson and others, 1975). In particular, the so-called "pink" fibres have been shown to be of fast twitch type with a metabolism intermediate between red and white muscle (Johnston and others, 1977). Studies on the functional division of labour of fibre types during swimming may have been recently reviewed (Bilinski, 1975). Generally, it appears that red fibres are recruited for sustained low-speed swimming, whilst white fibres provide propulsive effort at speeds above which energy requirements can be met entirely by gas exchange at the gills (Johnston and others, 1977).

Whereas the slow fibres of teleosts are frequently red- or pink-coloured due to concentrations of myoglobin and cytochromes (Hamoir and others, 1972), in Antarctic fish of the family Channichthyidae the skeletal muscle is uniformly white in appearance. The Channichthyidae

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or "icefish" are unique amongst vertebrates in having no haemoglobin present in their erythrocytes (Ruud, 1954; Martsinkevitch, 1958; Hureau and others, 1977). All 16 known species, with the exception of *Champsocephalus esox*, are confined to waters south of the Antarctic Convergence where, in spite of the haemoglobinless condition, icefish are apparently both numerous and successful (Andriashev, 1965). A number of physiological and circulatory adaptations occur which appear to compensate for the lack of oxygen-transport pigment. These include a relatively large blood volume with high cardiac output and low arterial blood pressure relative to other teleosts, and oxygen transport across the skin (Hemmingsen and others, 1969, 1972; Everson and Ralph, 1970; Hemmingsen and Douglas, 1970, 1972, 1977; Holeton, 1970, 1972, 1975; Twelves, 1972; Hureau and others, 1977).

In the present study, the metabolic differentiation of various fibre types has been investigated in two species of Antarctic fish showing marked pectoral propulsion. Particular attention has been given to adaptations in icefish skeletal muscle complementing the haemoglobinless condition.

Materials and Methods

Fish

Specimens of *Notothenia rossii* Fischer, approximately 20 cm in length, were obtained by light trapping from King Edward Point, South Georgia (lat. 54°17′S, long. 36°30′W) during the austral summer of 1975–76. The single live specimen of "icefish" *Champsocephalus gunnari* Lönnberg, length 31 cm, was caught by otter trawl from a depth of approximately 250 m off the coast of South Georgia. The fish were transported to the UK in darkness in tanks of filtered re-circulated sea-water at +1.0° C. This was the first time an icefish had been successfully kept alive outside the Antarctic. Specimens of North Sea cod, *Gadus morhua* L., were obtained from local fishermen at Pittenweem, East Fife, Scotland. Additional specimens of both Antarctic species were freshly frozen and stored at −20° C for biochemical determinations.

Preparation of cytochemical material

Fish were stunned by a blow to the head and killed by decapitation. Muscle samples were dissected from the end and mid regions of the deep pectoral abductor muscle and from several points along the myotome posterior to the anal aperture. Blocks were mounted on cryostat chucks in an artificial embedding medium (OCT compound, Lab-Tek Products Inc., USA) and immediately cooled in isopentane cooled to near its freezing point (−150° C) in liquid nitrogen. The frozen blocks were stored in sealed polythene bags under liquid nitrogen until required. The time taken to prepare the blocks from the start of the dissection was less than 5 min. Before sectioning, blocks were left in a refrigerated cabinet at −25° C for 1 h to equilibriate, and frozen sections approximately 8–10 μm in thickness were cut on a cryostat. Sections were mounted on dry coverslips.

Histochemical methods

All incubations were carried out at +4 °C or at room temperature as appropriate and sections were mounted either in glycerin jelly or dehydrated and mounted in DPX.

Glycogen. Frozen sections were treated for 10 min in 1% aqueous periodic acid. After washing, sections were treated with Schiff reagent (Pearse, 1960) in the dark for 10 min, washed, passed through three washes of 0.5% sodium metabisulphite and finally washed in running tap water for 10 min. Control sections were treated with a diastase solution in phosphate buffer (pH 6.0) for 1 h prior to staining.

Lipid. Sections were stained with Sudan Black B for 30 min. Control sections were digested in acetone prior to staining.

Phosphorylase. Sections were incubated for 3 h in a medium containing 75 mg glucose-1-phosphate, 15 mg AMP, 3 mg glycogen, 0.1 i.u. zinc insulin and either 50 mM sodium acetate buffer (pH 5.8) or 50 mM tris-maleate buffer (pH 6.6) (Takeuchi and Kuriaki, 1956). They were subsequently washed, dried, fixed in absolute alcohol, dried and stained with dilute Lugol's iodine for 3 min. As the colour rapidly faded, iodine staining was repeated before subsequent examination. Control sections were incubated in a medium without glucose-1-phosphate.

Lactate dehydrogenase. Tissue sections were stained using the standard method described by Pearse (1972) except that the intermediate electron carrier phenazine methosulphate (PMS) was added at a concentration of 1 mg ml^{-1} to bypass the diaphorase system and 10 mM sodium azide was added to inhibit the electron transport chain enzymes. Control sections were incubated in a medium from which the specific substrate lactate was omitted.

NADH diaphorase. Sections were stained in NADH diaphorase as described by Pearse (1960).

Succinic dehydrogenase. Sections were incubated in a medium containing sodium succinate and the tetrazolium salt NBT at a concentration of 1 mg ml^{-1} as described by Nachlas and others (1957). Control sections were incubated with either the substrate or NBT omitted, or in which malonate replaced the succinate substrate.

Alkaline phosphatase. Sections were stained for alkaline phosphatase activity which, in muscle, is localized almost exclusively in capillary endothelial cells, by the method of Romanul (1965). Sections were counterstained in 1% aqueous eosin.

Myoglobin. Sections were fixed for 1 h in phosphate-buffered 2.5% glutaraldehyde (pH 7.4) as described by George and others (1971) and stained for myoglobin by the method of James (1968).

Determination of myoglobin

Myoglobin was determined by the method of Reynafarje (1963) as modified by Reis and Wooten (1970). Exactly 1.0 g muscle was homogenized in 5 ml of 40 mM phosphate buffer (pH 6.6) at 0° C. Phosphate buffer was added to a ratio of 19.25 ml per g of muscle tissue. Following centrifugation at $20\,000 \times g$ for 1 h, an aliquot of supernatant was taken for myoglobin assay. Carbon monoxide gas was bubbled through the solution for 8 min, after which a pinch of dry sodium dithionate was added to ensure complete reduction of myoglobin, and carbon dioxide bubbled through for a further 2 min. The solution was transferred to a spectrophotometer cuvette and optical densities measured at 538 and 568 nm. Myoglobin concentration was measured according to the equation (Reynfarje, 1963):

$$C_{M/l}^{Mb} = \frac{OD_{538} - OD_{568}}{E_{538}^{Mb} - E_{568}^{Mb}},$$

where $C_{M/l}^{Mb}$ is the concentration of myoglobin in mole l^{-1}, OD is the optical density and E is the molar extinction coefficient. The extinction coefficients for carboxymyoglobin at 538 and 568 nm were assumed to be 14.8×10^3 and 11.8×10^3, respectively. This technique gives results essentially free from haemoglobin contamination (Reynfarje, 1963).

Estimation of muscle capillary density by alkaline phosphatase assay

In skeletal muscle, alkaline phosphatase is almost exclusively located in capillary endothelial cells. To estimate the capillary content of the different muscles, alkaline phosphatase was determined biochemically.

TABLE I. SUMMARY OF SOME OF THE HISTOCHEMICAL CHARACTERISTICS OF (a) MYOTOMAL, AND (b) PECTORAL MUSCLE FIBRE TYPES IN *Notothenia rossii* AND (c) *Champsocephalus gunnari*

(a)

Enzyme or metabolite	*Species*	*Myotomal fibre types*		
		Superficial (sdf)	*Oxidative*	*Glycolytic*
Myoglobin	*N. rossii*	0	+	0
	C. gunnari	0	0	0
Alkaline phosphatase	*N. rossii*	0	+	trace
	C. gunnari	+	+++	+
Glycogen	*N. rossii*	0	+++	+
	C. gunnari	+	++	+
Lipid	*N. rossii*	0	+++	+
	C. gunnari	0	+++	+
Lactate dehydrogenase	*N. rossii*	0	+	+
	C. gunnari	0	trace	trace
Succinic dehydrogenase	*N. rossii*	0	+++	trace
	C. gunnari	+	+++	trace
NADH diaphorase	*C. gunnari*	+	+++	trace
Phosphorylase	*C. gunnari*	0	+	+++

(b)

Enzyme or metabolite	*Superficial (O1)*	*Mosaic*		*Large oxidative (O2)*
		Small	*Large*	
Myoglobin	++	+	+	+
Lipid	+++	++	+	+
Succinic dehydrogenase	+++	++	+	+

(c)

Enzyme or metabolite	*Superficial (sdf)*	*Oxidative 1 (O × 1)*	*Oxidative 2 (O × 2)*
Myoglobin	0	0	0
Lipid	+	+++	++
Succinic dehydrogenase	+	+++	++
NADH diaphorase	+	+++	++
Phosphorylase	+	+	+

+++ Heavily stained. ++ Intermediately stained. + Lightly stained. 0 Not stained. Abbreviations refer to Fig. 1.

Alkaline phosphatase was extracted by the method of Morton (1954) as modified by Reis and Wooten (1970). Samples of approximately 1.0 g were dissected out, avoiding contamination by extensively capillarized external myocommata, and homogenized 3×40 s at high speed in an Ultra-Turrax homogenizer in 5 ml of ice-cold Young's teleost ringer (pH 7.4). The homogenate was centrifuged at $3\,000 \times g$ for 10 min at +4° C and the precipitate discarded. Aliquots of the supernatant were assayed for alkaline phosphatase activity using the Boehringer (Mannheim) Biochemica Test Combination Kit (catalogue No. 15987) with *p*-nitrophenyl as substrate. Incubations were carried out at +4° C. Alkaline phosphatase activity was expressed in "enzyme units" defined as the amount of enzyme which will liberate 1 μmole *p*-nitrophenyl wet weight of muscle tissue^{-1} h^{-1}.

RESULTS

Cytochemical

Results obtained for the cytochemical localization of various metabolites and enzymes of energy metabolism in the muscle fibres of *Notothenia rossii* and *Champsocephalus gunnari* are summarized in Table I.

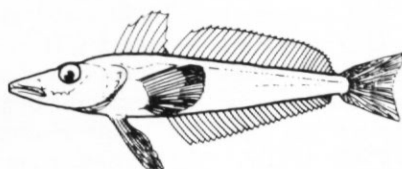
In both species the myotomal fibres consist of two main types (Fig. 1; Table I). Muscle fibres adjacent to the lateral canal system are characterized by high activities of succinic dehydrogenase enzyme (SDHase). The high activities of the mitochondrial enzymes SDHase and NADH diaphorase were correlated with marked staining for glycogen and lipid (Fig. 2a; Table I). Interestingly, unlike the oxidative fibres of other teleost and vertebrate muscle, myoglobin staining was negligible in *Notothenia rossii* and not detectable in *Champsocephalus gunnari* (Table I).

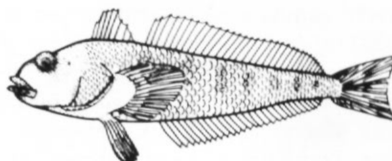
In contrast, the bulk of the myotomal musculature consists of glycolytic fibres characterized by a higher staining for phosphorylase (Table I). Interestingly, in the case of lactate dehydrogenase, no distinct differences in staining intensity were detectable between oxidative and glycolytic fibres.

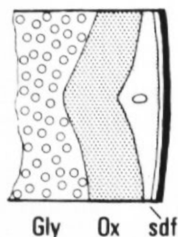
The glycolytic fibres of *Champsocephalus gunnari* were found to contain a number of irregularly distributed glycogen-rich fibres of similar diameter to the majority of glycolytic fibres (Fig. 2b). Additionally, there were occasional intracellular inclusions which stained intense mauve for glycogen with similarly intense diformazan staining for SDHase.

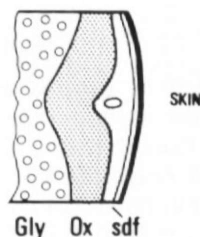
In general, there is a distinct boundary between the two fibre types (Fig. 2c) with no evidence for intermediately staining fibres (Patterson and others, 1975). In both species, a thin layer of small-diameter fibres occurs immediately adjacent to the skin (Fig. 2d). These fibres form a continuous layer of up to five fibres thickness in the region of the lateral line canal, becoming thin and discontinuous towards the anal and dorsal fins. Small-diameter fibres are characterized by negligible or complete absence of staining for the various metabolites and enzymes studied (Table I).

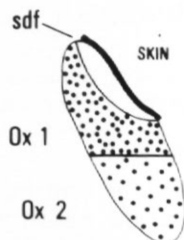
In the deep pectoral abductor muscles, fibre arrangement and differentiation are more complex with some marked differences between the two species. Results obtained for the histochemical localization of various metabolites and enzymes are shown in Table I (b) and (c), and Fig. 1. In contrast to the trunk musculature, all fibres in the pectoral muscles of both species consist of oxidative fibres. The region immediately underlying the epidermis comprises small-diameter fibres (Fig. 1). In *Notothenia rossii*, these fibres (O1) stained more intensely for SDHase and lipid than the corresponding fibres (sdf) in *Champsocephalus gunnari* (Table I). Deeper pectoral fibres showed a progressive increase in fibre size towards the scapula. Staining for glycogen, SDHase and NADH diaphorase was more intense in the smaller-diameter fibres (Table I). In *Notothenia rossii*, fibres were arranged in a distinctive mosaic pattern comprising large fibres with intermediate SDHase, glycogen and lipid staining, surrounded by a group of much smaller, intensely stained fibres (Fig. 2e).











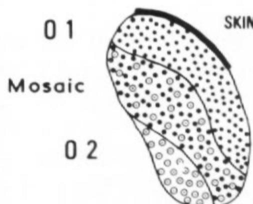


Fig. 1. The distribution of fibres with different staining intensity for various metabolites and enzymes in the myotomal and pectoral muscles of *Champsocephalus gunnari* and *Notothenia rossii*.
Abbreviations used:
(a) Myotomal—Gly, glycolytic "white" fibres; Ox, oxidative "red" muscle; sdf, small-diameter fibres.
(b) Pectoral—sdf, small-diameter fibres; Ox 1, Ox 2, O 1, O 2, various layers of highly oxidative fibres; mosaic, mosaic layer in *Notothenia rossii*. For further details see text.

Of particular interest are the results for the cytochemical localization of alkaline phosphatase activity in the various fibre types of the two species (Table I). In vertebrates, alkaline phosphatase is almost entirely localized in capillary wall endothelial cells. Cytochemical and biochemical determinations of alkaline phosphatase activity may be used as an index of muscle capillarization, which parallels the metabolic differentiation of fibre types. In general, the muscles of *Champsocephalus gunnari* were more heavily capillarized than the corresponding muscles of *Notothenia rossii* (Fig. 2f; Table I). The highest capillary density occurs in the pectoral, intermediate in the oxidative myotomal, and lowest in the glycolytic myotomal muscles (Fig. 2f–h). In contrast to *Notothenia rossii*, oxidative muscles in *Champsocephalus gunnari* contain numerous large-bore capillaries or blood spaces (Fig. 2i and j). Since transverse sections usually contained numerous capillaries running obliquely or at right-angles to the plane of sectioning, no attempt was made to quantify capillary density.

Biochemical

In order to quantify the extent of capillarization in the various muscles, biochemical measurements of alkaline phosphatase activity were carried out (Reis and Wooten, 1970). Table II illustrates alkaline phosphatase activities in various muscles of *Champsocephalus gunnari*, *Notothenia rossii* and *Gadus morhua*. In all species, activities are highest in pectoral, intermediate in oxidative myotomal, and least in glycolytic myotomal fibres. Overall activities were markedly reduced in *Gadus morhua* compared to the two Antarctic species.

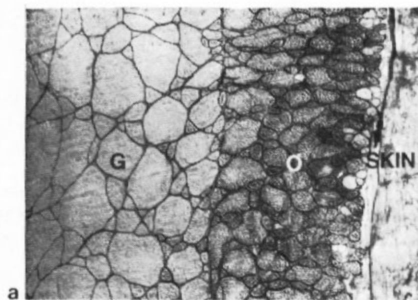
The concentrations of myoglobin in the various muscles of the Antarctic species are given in Table III; data are compared with two species from temperate regions. Interestingly, no myoglobin was detectable in any of the muscles of *Champsocephalus gunnari* (Fig. 3; Table III). In the other species, myoglobin concentrations were found to parallel the alkaline phosphatase series, pectoral>oxidative myotomal>glycolytic myotomal.

Discussion

Previous studies on skeletal muscle of Antarctic fish *Dissostichus mawsoni* and *Trematomus* *[illegible]hgrevinski* (Lin and others, 1974) have suggested that the myotomal musculature is entirely composed of "white" fibres with "red" fibres restricted to the pectoral muscle. Muscle colour in vertebrates substantially results from differing concentrations of myoglobin and, to a lesser extent, cytochromes (Matsuura and Hashimoto, 1954; James, 1968; Wittenberg, 1970; Hamoir and others, 1972). Oxidative myotomal fibres of species examined in the present study have low, or in the case of *Champsocephalus gunnari*, no detectable myoglobin. This undoubtedly contributes to the absence of overt colour differences in myotomal fibre types. However, the present study has shown that, in general, the arrangement and histochemical properties of myotomal fibres in *Notothenia rossii* and *Champsocephalus gunnari* are similar to other teleosts (George, 1962; George and Bokdawala, 1964; Nag, 1972; Patterson and others, 1975) and elasmobranchs (Bone, 1966; Brotchi, 1969; Kryvi and Totland, 1977). In both species, oxidative fibres have characteristically small diameters, high concentrations of lipid and glycogen, extensive capillarization and high activities of SDHase and NADH diaphorase; the converse, to a large extent, applies for the glycolytic fibres. Whilst the majority of glycolytic fibres are characterized by low glycogen content, a number of normal-diameter glycogen-loaded fibres were present in *Champsocephalus gunnari*. Similar glycogen-loaded white fibres have been observed in the muscles of the rohu carp, *Labeo rohita* (George and Bokdawala, 1964). However, the functional significance of these fibres remains obscure. It would appear, therefore, that the oxidative and glycolytic fibres of these Antarctic species are analogous to the "red" and "white" fibres of other teleosts.

The myotome of both Antarctic species contains a thin layer of very small-diameter fibres [illegible]ch show little staining for the enzymes and metabolites studied (Table I; Fig. 1). Similar fibres have been reported in the myotome of *Scyliorhinus canicula* and other elasmobranchs (Bone, 1966) and less commonly in the teleosts *Carassius carassius*, *Gadus virens* and *Chanda ranga* (Patterson and others, 1975). In fingerling *Notothenia rossii* (length 5–8 cm), these fibres are particularly abundant (unpublished observations). It has been suggested that these fibres represent regions of new fibre growth (Patterson and others, 1975).

Previous studies have reported a predominance of the pectoral fin swimming mode amongst Antarctic teleosts (Robillard and Dayton, 1969; Twelves, 1972; Lin and others, 1974; Holeton, 1975). In the present study, the pectoral musculature in both species was characterized by markedly oxidative histochemical profiles (Table I). It is generally accepted that, in fish which mainly swim with the trunk musculature, the slow-contracting "red" fibres are used for sustained low-speed cruising, whilst at higher speeds where the "white" fibres provide the main propulsive force, "red" fibres contract passively (Bone, 1966; Pritchard and others, 1971; Hudson, 1973; Johnston and Goldspink, 1973*a*, *b*; Walker and Pull, 1973; Johnston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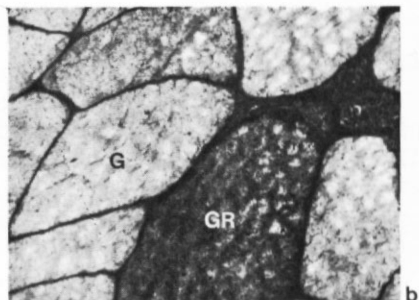
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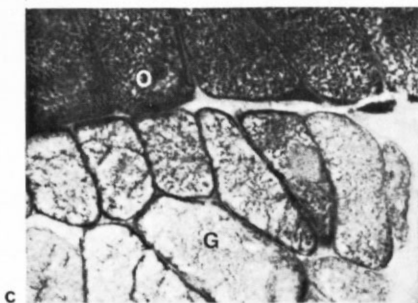
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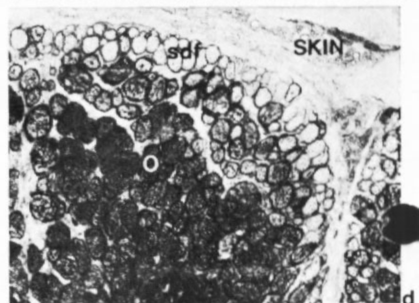
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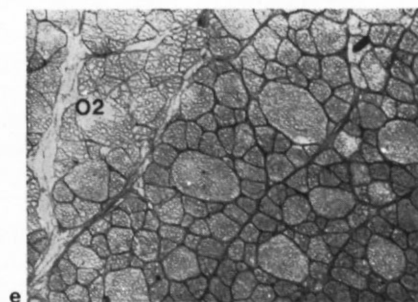
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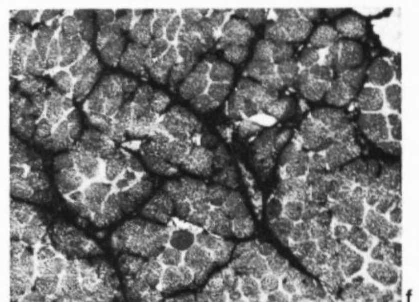
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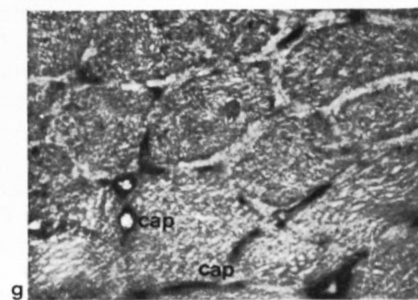
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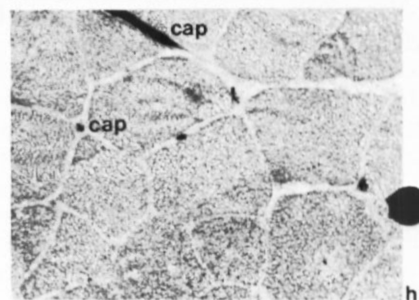
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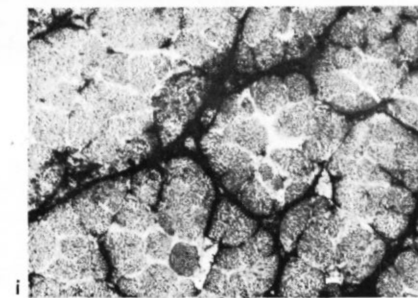
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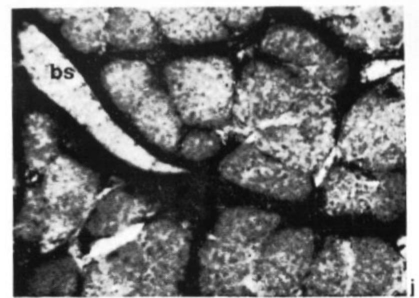
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and others, 1977). Similarly, other continually used muscles, such as the flight muscles of birds (George and Jyoti, 1955; George and Berger, 1966; Crabtree and Newsholme, 1972) and swimming muscles of mackerel (George, 1962), are highly oxidative. In *Notothenia rossii* there is some evidence for further differentiation of pectoral fibres into a mosaic pattern (Fig. 2f). Mosaic fibre patterns have been previously reported in the myotome of various salmonids (Greene and Greene, 1913; Boddeke and others, 1959; Johnston and others, 1974).

In cytochemical studies of muscle, it is most important to ultimately relate the observations to the contractile properties and physiological functions of the different fibre types. The complex orientation of fibres in the teleost myotome associated with short fibre lengths and myoseptal insertions (Alexander, 1969) have largely excluded direct investigations of mechanical properties. However, biochemical studies of vertebrate muscle have shown that the specific activity of the myofibrillar or myosin ATPase closely parallels the contraction speed of the various fibre types (Bárány, 1967). In a number of teleosts, ATPase activity is 2–5 times higher in white (fast-contracting) than in red (slow-contracting) fibres (Johnston and others, 1972; Nag, 1972). Whilst techniques exist for the histochemical demonstration of myofibrillar ATPase activity in fish muscle, observations must be considered in relation to complementary biochemical determinations (Johnston and others, 1974). Further characterization of contractile properties is possible on a comparison of the electrophoretic mobilities of the myosin light chains. These low molecular weight polypeptide chains form part of the enzymatically active head of the myosin molecule and are chemically different for slow and fast muscles (Lowey and Risby, 1971; Weeds and Pope, 1971). Recent studies on the carp myotome have shown that some fibre populations with a mainly oxidative histochemical profile (pink fibres) have light chains characteristic of fast-contracting fibres (Patterson and others, 1975; Johnston and others, 1977). Further studies are in progress in this laboratory on the contractile properties of Antarctic fish muscle fibres.

The absence of myoglobin from the oxidative muscles of *Champsocephalus gunnari* is of particular interest. This finding represents the first record of a myoglobinless oxidative muscle in vertebrates. Myoglobin facilitates the diffusion of oxygen through muscle tissue, enhancing the oxygen supply to the mitochondria, and may increase oxygen fluxes by up to 600% (Hemmingsen, 1963; Cherniak and Longobardo, 1970; Wittenberg, 1970). High

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Fig. 2. a. Transverse section through the myotomal muscle of *Notothenia rossii* stained for lipid, showing regions of oxidative (O) and glycolytic (G) fibres. (Magnification ×75.)

b. Detail from the glycolytic myotomal fibres of *Champsocephalus gunnari*, showing glycogen-rich fibre (GR) surrounded by glycolytic fibres (G). (Magnification ×410.)

c. The junction between oxidative (O) and glycolytic (G) fibres in the myotome of *Champsocephalus gunnari*, showing the absence of any layer of intermediate ("pink") fibres. Stain: NADH diaphorase. (Magnification ×320.)

d. Transverse section near to the lateral line canal of *Notothenia rossii* stained for NADH diaphorase activity, showing the relationship of small-diameter fibres (sdf) to skin and underlying oxidative musculature (O). (Magnification ×95.)

e. Transverse section through pectoral muscle of *Notothenia rossii* stained for lipid, showing detail of the mosaic fibres together with the deep oxidative fibres (O2). Note the large lightly stained fibres surrounded by smaller heavily stained fibres. (Magnification ×340.)

f. Transverse section through the inner pectoral muscle of *Champsocephalus gunnari* stained for alkaline phosphatase activity to demonstrate capillarization. (Magnification ×155.)

g and h. Transverse sections through the oxidative myotomal (Fig. 2g) and glycolytic myotomal (Fig. 2h) muscles of *Champsocephalus gunnari* stained for alkaline phosphatase activity. Note the relative densities of capillaries (cap) between pectoral (Fig. 2f), oxidative myotomal (Fig. 2g) and glycolytic myotomal (Fig. 2i) muscles. (Magnification ×230 and ×190, respectively.)

i. Detail of capillary network in the pectoral fibres of *Champsocephalus gunnari*. Transverse section stained for alkaline phosphatase activity. (Magnification ×320.)

j. Transverse section through pectoral muscle of *Champsocephalus gunnari*, showing one of the blood spaces (bs) supplying the finer capillary network. (Magnification ×410.)

TABLE II. THE ACTIVITIES OF ALKALINE PHOSPHATASE IN THE GLYCOLYTIC MYOTOMAL, OXIDATIVE MYOTOMAL AND PECTORAL FIBRES OF VARIOUS FISH. VALUES ARE ENZYME UNITS g WET WEIGHT OF MUSCLE^{-1} h^{-1}; MEANS±S.E. VALUES IN PARENTHESES ARE NUMBER OF FISH ASSAYED. (FOR FURTHER DETAILS SEE TEXT.)

Species	*Muscle type*		
	Glycolytic myotomal	*Oxidative myotomal*	*Pectoral*
Gadus morhua (North Sea cod)	100.3±19.4 (10)	353.5±65.1 (10)	405.8± 37.5 (10)
Notothenia rossii ("Antarctic cod")	310.0±37.6 (9)	483.2±71.0 (9)	698.1±118.5 (8)
Champsocephalus gunnari (Icefish)	400.0±43.4 (8)	770.0±62.5 (8)	1 185.5±101.4 (7)

TABLE III. THE CONCENTRATIONS OF MYOGLOBIN IN THE PECTORAL AND MYOTOMAL MUSCULATURE OF VARIOUS SPECIES. VALUES ARE mg g DRY WEIGHT OF MUSCLE^{-1}. MEANS±S.E. VALUES IN PARENTHESES ARE NUMBERS FISH ASSAYED.

Species	*Muscle type*		
	Pectoral	*Oxidative myotomal*	*Glycolytic myotomal*
Champsocephalus gunnari (Icefish)	—	—	—
Notothenia rossii ("Antarctic cod")	3.58±0.35 (6)	2.03±0.22 (6)	0.65±0.10 (6)
Gadus morhua (North Sea cod)	1.24±0.15 (6)	2.72±0.29 (8)	0.63±0.10 (6)
*Cyprinus carpio** (Mirror carp)	Not assayed	12.70±1.10 (12)	2.80±0.40 (12)

* Data taken from Johnston and others (1977). — None detectable.

myoglobin concentrations are generally found in muscles capable of continual activity (Wittenberg, 1970). Most vertebrate muscles consist of mixed slow and fast fibres and have a pale pink appearance (Hamoir and others, 1972). In contrast, the separation of these fibre populations into discrete regions in fish (Bone, 1966) and in the tail musculature of salamanders (Totland, 1976) usually imparts a distinctive intense red colour to the oxidative fibres.

It might be anticipated that an absence of myoglobin from oxidative fibres would result in constraints on locomotor activity similar to the lack of an oxygen-carrying pigment in the blood. The compensatory adaptations to the latter condition have been recently reviewed (Holeton, 1975). The existence of an increased cardiac output, low arterial blood pressure and reduced blood viscosity are generally accepted (Hemmingsen and others, 1969, 1972; Holeton, 1970, 1972, 1975, 1976; Hemmingsen and Douglas, 1972, 1977; Twelves, 1972). However, there are conflicting reports as to the role of supplementary cutaneous respiration (Jakubowski and Byczkowska-Smyk, 1970; Holeton, 1975, 1976) and resting oxygen consumption rate relative to other Antarctic species (Hureau, 1966; Ralph and Everson, 1968; Hemmingson and Douglas, 1970; Holeton, 1970, 1975; Hureau and others, 1977). The success of the Channichthyidae can be explained, at least in part, by the oxygen characteristics of the Antarctic marine environment. Low stable temperatures (−2 to +2° C)

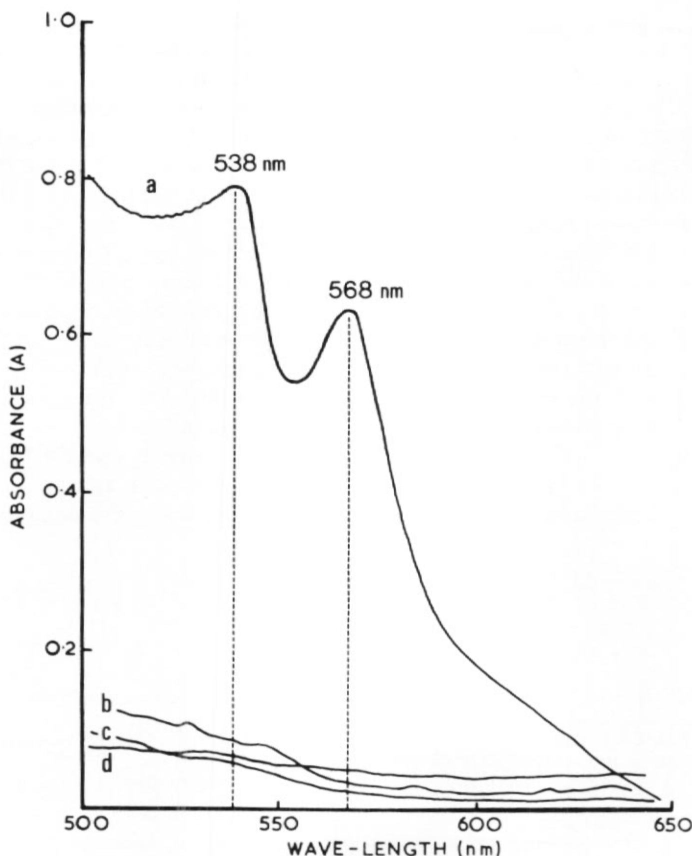

Fig. 3. Difference absorption spectra for carboxymyoglobin from various muscles of icefish, *Champsocephalus gunnari*, and carp, *Cyprinus carpio*.
a. Red myotomal muscle of carp.
b. Cardiac muscle (ventricle) of icefish.
c. Oxidative myotomal muscles of icefish.
d. Glycolytic myotomal muscles of icefish.
Protein concentrations 7.5 mg ml^{-1}. The presence of myoglobin is shown by distinct absorbance peaks at 538 nm and 568 nm wave-lengths. Myoglobin concentrations are calculated from the absorbance difference between the two peaks; for further details see text.

with continual vertical mixing create a highly aerobic external environment leading to high oxygen solubility in the blood plasma and tissues. The persistence of this stable environment over long periods of geological time has facilitated the evolution of the haemoglobinless and myoglobinless conditions. The circulatory adaptations in "icefish" are reflected in a marked susceptibility to hypoxic conditions (Hemmingsen and Douglas, 1970). In the present study, histochemical staining for lactate dehydrogenase was negligible in the glycolytic fibres of *Champsocephalus gunnari*, suggesting only a moderate anaerobic capability or the presence of other mechanisms of maintaining redox balance within the muscle cells during anoxia.

Most studies on compensatory adaptations in the Channichthyidae have considered one representative, *Chaenocephalus aceratus*, a rather sluggish benthic species having a much reduced axial musculature. It has been suggested (Holeton, 1975) that the enlarged blood volume (up to 9% of body weight) reported by various workers (Hemmingsen and Douglas, 1972; Twelves, 1972) might partly result from the reduction in axial musculature which

is usually poorly vascularized (Stevens, 1968). In this context, the present study is of particular interest; *C. gunnari*, unlike many of the "icefish" species (Ruud, 1965), shows no reduction in axial musculature, having a streamlined shape suggesting a moderately pelagic existence. Cytochemical and biochemical determinations of alkaline phosphatase activity (Fig. 2f–j; Table II) indicate massive capillarization throughout the skeletal muscles. The reduced diffusion distances resulting from this capillarization may partly offset the lack of myoglobin.

The lack of haemoglobin in "icefish" is the extreme development in a trend for fish from high latitudes to have reduced haemoglobin levels (Scholander and van Dam, 1957; Tyler, 1960; Hureau, 1966; Everson and Ralph, 1968). Interestingly, *Champsocephalus esox*, the other representative of the genus, is the only species of channichthyiid reported from outside Antarctic waters, occurring in the Falkland and Patagonian Shelf regions off South America (Norman, 1937). Furthermore, this species has a similar body form and pelagic existence to *C. gunnari*. Studies on the gill structure of *Champsocephalus esox* have shown less specialization than in the truly Antarctic counterparts (Hughes, 1966, 1972; Steen and Berg, 1966). Many of the unanswered problems of adaptations in "icefish" centre on their capacity for activity (Holeton, 1975). It is suggested that comparative studies on these two species may provide further insight into the circulatory and muscular adaptations associated with the evolution and persistence of the haemoglobinless/myoglobinless condition.

MS received 10 February 1978

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