Proc. Indian natn. Sci. Acad. **B51** No. 1 pp 25-32 (1984)

Histochemical and Biochemical Studies of Myotomal Muscle Types in the Pre-adult and Adult Freshwater Carp, *Labeo rohita* (Hamilton)

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(Received 3 July 1984; after revision 10 October 1984)

Three discrete types of muscles—red, white and pink have been recognized in the myotomal musculature of pre-adult and adult fresh water teleost fish *Labeo rohita*. A detailed histochemical and biochemical *p*H-profile for the enzyme m-ATPase was undertaken. Both red and white muscles, have shown characteristics similar to those found in other teleosts. These are classified as slow twitch red-type I and fast twitch white-type IIB, respectively. The pink fibres are distinguished as a separate class with distinctive histochemical and biochemical characteristics and can be classified as fast twitch red-type IIA or C fibres.

Key Words: Myotomal muscles, Myofibrillar-ATPase, Histochemical profile, Freshwater carp

Introduction

Most of the teleost fishes possess two main types of myotomal (Boddeke et al. 1959, Bone 1966, Johnston et al. 1972) as well as respiratory (Ojha 1975, Munshi et al. 1975, Hughes & Johnston 1979) muscles.

However, the myotomal musculature of some teleosts have more than two muscle types (Johnston et al. 1974, Patterson et al. 1975, Bone 1978, Johnston 1981) and apparantly they overlap in function between these different types. The most common fibre type found in addition to red and white is the 'pink' or 'intermediate' situated between the red and white muscle forming a narrow zone of about 3-4 layers of fibres (Johnston et al. 1977, Carpene et al. 1982, Talesara & Shashi Kiran 1982, 1983, Shashi Kiran & Talesara 1984). In the mirror carp these comprise around 1% of the myotomal bulk making them slightly more numerous than the red fibres (Johnston et al. 1977). Little is known about the contractile properties, metabolism or role of these pink fibres in the myotomal muscles probably because of their usual presence in minor proportions.

Additionally, the structure and function of adult teleost propulsive musculature have been the subject of much debate but not much

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attention has been given to the types of muscle fibres in the pre-adult stages. As the different fibre types of the adult fish have been assigned different roles in swimming, the types of fibres present in the pre-adult stages would also have the characteristics to suit the swimming behaviour shown by immature fishes (Proctor et al. 1980).

The present work was undertaken to determine the muscle fibre types in the adult and pre-adult stages of the fresh water teleost *Labeo rohita.*

Materials and Methods

Fish, rohu carp, *Labeo rohita* (Hamilton) were locally collected. Muscle samples were excised from the trunk as well as from the caudal myotomal regions. Small muscle pieces were mounted on cryostat chucks and quickly frozen by immersion in liquid nitrogen. The blocks were placed in the cryostat (IEC-Model CTD) at −25°C. The frozen sections of 10 μm thickness were cut and used for histochemical studies.

Histochemical Methods

Myofibrillar-ATPase (m-ATPase) and succinic dehydrogenase (SDH): Frozen sections were air dried for 60–90 min. and stained using routine m-ATPase method of Padykula and Herman (1955) after preincubating the sections in acid or alkali medium as described by Guth and Samaha (1970). The preincubation period varied from 5 sec to 2 min. The incubation was carried out in every case at 37°C, *p*H 9.4 for about 15 min. The *p*H-profile for m-ATPase staining was done under the same conditions as above at a serial range from *p*H 4.35–10.4 with varying preincubation periods. For the enzyme SDH fresh frozen sections of the muscle were incubated for 15–20 min in the medium described by Nachlas et al. (1957) using Nitro-BT as an electron acceptor.

Biochemical Assays

m-ATPase and SDH: Myofibrils were prepared according to the method of Perry and Grey (1956). The reaction for m-ATPase was carried out at *p*H 7.5 and the detailed procedure was the same as reported earlier (Talesara & Goldspink 1978, Talesara & Shashi Kiran 1982). *p*H-profile for Ca^{++}-activated ATPase enzyme assay was performed on the myofibrils prepared from various muscles under the same assay conditions as described above at a serial range of *p*H from 4.35 to 10.4 in accordance with the work reported earlier (Talesara & Goldspink 1978, Talesara & Shashi Kiran 1982, 1983). The assay procedure for SDH was essentially based on the method given by Nachlas et al. (1960) using INT [2 (p-Iodophenyl) 3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride] as the artificial electron acceptor. Protein concentration was determined by the biuret method (Gornall et al. 1949). All the values for m-ATPase and SDH were expressed as μ moles Pi liberated mg^{-1} protein min^{-1} and μg formazan mg^{-1} protein min^{-1} respectively.

Results

Adult Muscle Fibres

In the myotomal musculature of the fish *Labeo rohita*, three different muscle types could be recognized macroscopically (figure 1).

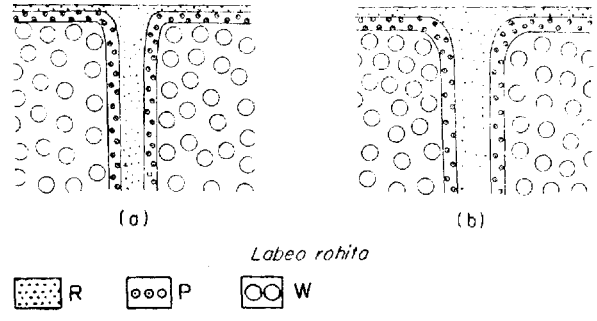


Figure 1 Diagram (reproduced from figures 2–8) showing the distribution and relaive abundance of various muscle types in the trunk (*a*) and tail (*b*) myotomal muscles of the adult fresh water teleost, *Labeo rohita* (R, Red; W, White; P, Pink)

These were seen to have different characteristics by histochemical and biochemical studies.

Histochemically, the three types of muscles (red, white & pink) fibres could be easily distinguished in the adult *Labeo rohita* by their SDH activities. The activity of this enzyme was quite high in all the superficial fibres. The intermediate part also showed some degree of staining whereas the muscle fibres of the deep part had very low SDH activity (figures 2 and 6).

The three muscle fibre types also showed characteristic m-ATPase staining pattern. The alkaline ATPase reactions (preincubation in alkali *p*H) resulted in a dark staining of all the fibre types (figures 3 and 4). In the acidic *p*H-range the staining was very low in the fibre types but increased towards the alkali range (table 1). An increase in *p*H above 10.1–10.2 resulted in complete inactivation of the red fibres (fibures 5, 7 and 13). The next fibres to be inactivated in alkali *p*H were the pink fibres, i.e. the fibres showing intermediate staining of SDH activity (figure 8). The white fibres were the most resistant to alkali *p*H, showing some staining even at 10.4 where all the other fibres are completely inactivated (figure 8 and table 1).

Biochemically, the red muscle showed quite high levels of SDH activity from the trunk as well as tail regions. The pink muscle showed intermediate activity levels while the white muscle had the lowest levels for this enzyme (table 2).

Biochemical levels for the enzyme m-ATPase revealed about 3-4 times higher activity in the white muscles as compared to the red. The activity levels of the pink muscle was found to be intermediate between red and white muscles (table 2). Biochemical *p*H-profile for m-ATPase revealed that the red muscles are the first ones to be inactivated in the alkali *p*H-range as observed by histochemical *p*H-profile. The pink and white

Table 1 *Histochemical* p*H-profile for m-ATPase in the trunk muscles of the* Labeo rohita

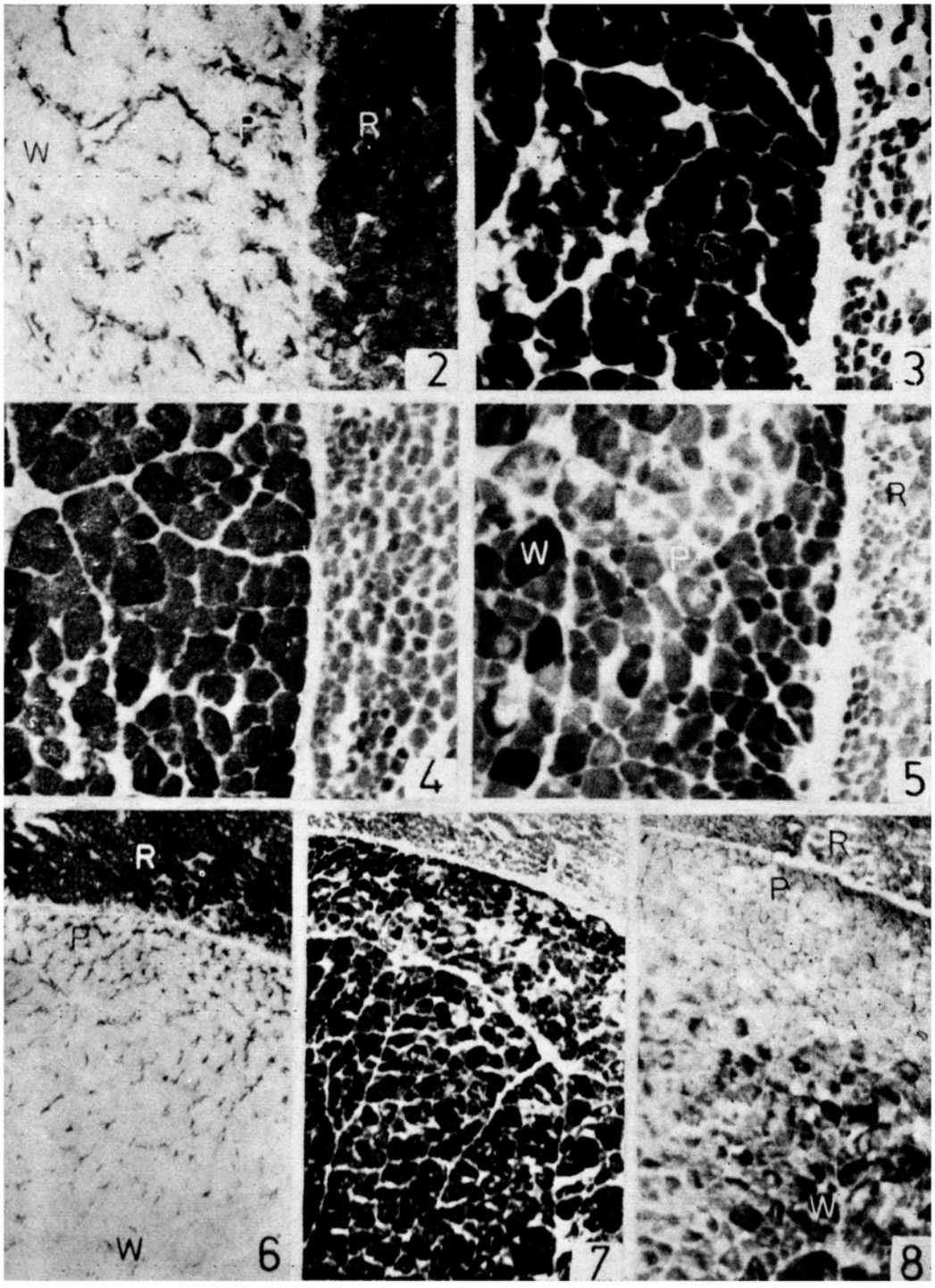
Preincubation		m-ATPase activity		
		Fibre types		
*p*H	Time	Red	Pink	White
4.35–5.5	5–10 sec	±	±	±
5.6–7.9	5–10 sec	1+	1+	1+
8.0–9.6	5–10 sec	1+	2+	2+
9.7–9.8	5–10 sec	2+	3+	3+
9.9–10.0	5–10 sec	3+	4+	4+
10.0	1–2 min	2+	4+	4+
10.1	5–10 sec	2+	4+	4+
10.1	1–2 min	1+	3+	3+
10.2	5–10 sec	±	3+	3+
10.2	1–2 min	—	1+	2+
10.3	5–10 sec	—	—	1+
10.3	1–2 min	—	—	±
10.4	5–10 sec	—	—	±
SDH	—	4+	2+	±

4+, Intensive staining; 3+, Good staining; 2+, Moderate staining; 1+, Poor staining; ±, Traceable staining; —, No staining

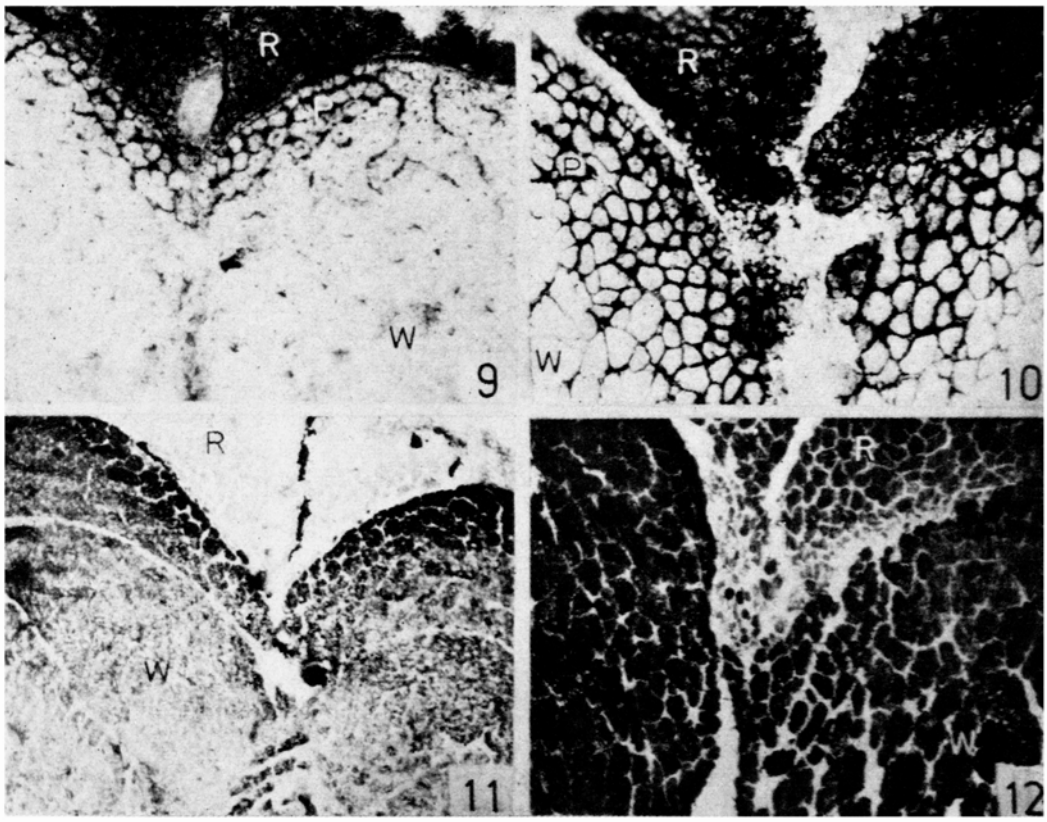
Table 2 *m-ATPase and SDH activity* in the trunk and tail muscles of the fresh water teleost,* Labeo rohita

Particulars	Myotomal region	
	Trunk	Tail
m-ATPase		
Red	0.218±0.011	0.260±0.015
White	0.807±0.020	0.889±0.018
Pink	0.578±0.046	0.686±0.029
SDH		
Red	2.221±0.217	2.176±0.136
White	0.560±0.049	0.682±0.064
Pink	1.092±0.232	1.022±0.080

*Values are Mean ± SE
m-ATPase : μ moles Pi. mg^{-1} protein. min^{-1}
SDH: μg formazan. mg^{-1} protein. min^{-1}



Figures 2–8 Transverse sections of the myotomal muscles of adult *Labeo rohita* showing Red (**R**), White (**W**) and Pink (**P**) fibres. **2–5**, Trunk muscles (× 120); **6–8**, Tail muscles (× 45). **2** and **6**, stained for SDH; **3–5** and **7–8**, Incubated for m-ATPase after varying preincubation (PI) *p*H and time. **3**, 10.0 (5–10 sec.); **4**, 10.1 (1-2 min.); **5** & **7**, 10.2 (1-2 min.) and 10.3 (5–10 sec.), respectively. **8**, 10.4 (5–10 sec.)



Figures 9–12 Transverse sections of the myotomal muscles of pre-adult (**9** & **11**, 1 1/2 month) and (**10** & **12**, $3\frac{1}{2}$ months) *Labeo rohita* showing Red (**R**), White (**W**) and Pink (**P**) fibres (× 120). **9** & **10**, stained for SDH; **11** & **12**, Incubated for m-ATPase at *PI-p*H 10 0 (5–10 sec.)

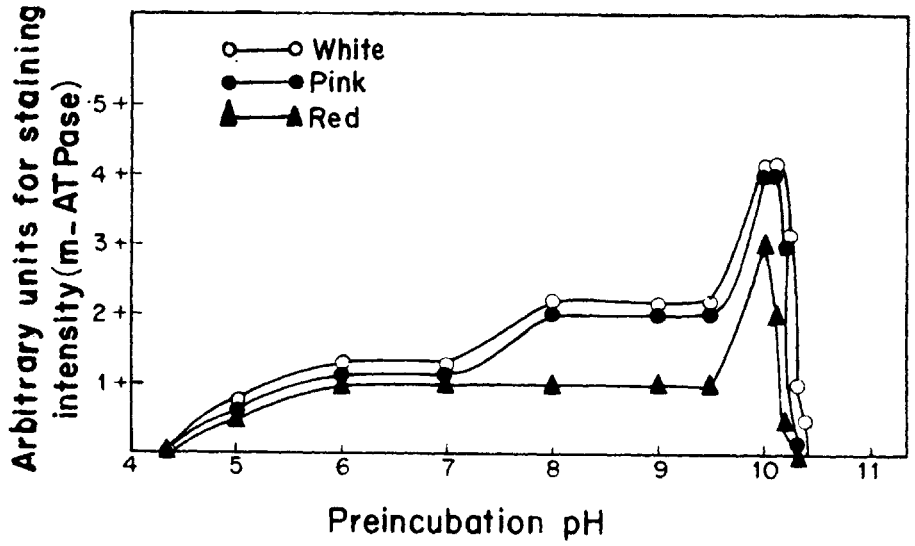


Figure 13 Histochemical *p*H-profile for the various muscles of adult *Labeo rohita* (based on observations as shown in table 1). Muscle sections preincubated at varying *p*H (4.35–10.4), PI time (5–10 sec.) and incubated routinely at *p*H 9.4 for m-ATPase

muscles are more resistant in these *p*H media (figures 13 and 14). The mean diameter of the fibre types have been presented in table 3.

Pre-Adult Muscle Fibres

Histochemically, in the 1½-month-old young fish the fibre types were found to be differentiated into red and white regions with a very narrow zone of pink fibres showing intermediate staining for SDH (figure 9). The m-ATPase reaction showed quite low intensity in the red zone whereas the white and pink fibres had shown good intensity of staining (figure 11). In the 3½-month-old fish, the red and pink fibre zones were quite well developed (figure 10) and were found to stain intensively for m-ATPase in the alkali *p*H 10.0 (5–10 sec.) as shown in the adult muscle fibres (figures 3 and 12).

Table 3 *Fibre diameter* in the various muscle fibre types in the trunk and tail myotomal regions of the freshwater teleost* Labeo rohita

Fibre types	Myotomal Region	
	Trunk	Tail
Red	15.41±1.01	19.73±0.90
White	54.28±2.06	45.55±1.82
Pink	41.00±2.34	37.35±1.59

*Values are Mean ± SE of the mean for 25–50 fibres in each case

Discussion

In *Labeo rohita* the red, pink and white muscles are segregated to such an extent that the three can be separated easily for individual type analysis.

An important point here to be observed is that the alkaline stability of pink muscle is found to be inbetween red and white which is different from what has been observed earlier in other teleosts (Johnston et al. 1974, Patterson et al. 1974, Talesara & Shashi Kiran 1982, 1983, Davison 1983). In *Labeo rohita*, pink muscle is not most resistant in the alkaline preincubation *p*H. This extreme kind of variation in the pink fibres demonstrated with the m-ATPase reaction probably points to a high polymorphism of those myosins

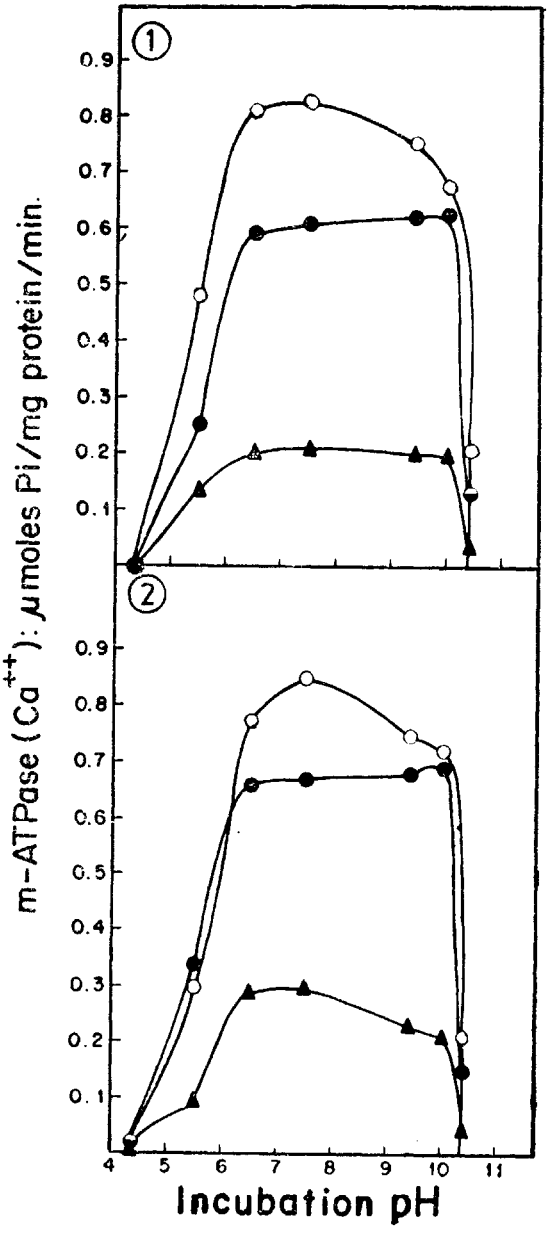


Figure 14 Biochemical *p*H—profile for m-ATPase in the various muscles of the trunk (1) and tail (2) myotomal regions of adult *Labeo rohita* (0——0—White; ●——●—Pink; ▲——▲—Red)

that react differently to alkaline treatment. These variations in the kind and amount of the pink muscle in different teleosts, leads to the assumption that they are less specialized than the white muscle and are, therefore, capable of performing a wider range of functions.

Barany (1967) has demonstrated for a wide range of vertebrate muscles that myofibrillar-ATPase activities parallel the unloaded speeds of shortening. On this basis white muscle of *Labeo rohita* is around 3-4 times as fast as red muscle. The pink muscle is found to be having m-ATPase slightly lower than white muscle, i.e. around 2-3 times higher than red muscle suggesting it to be a fast twitch muscle with an intermediate speed of shortening between the red and white muscles.

The arrangement of the fibre types in the pre-adult stages is found to be basically like the adult ones. The predominance of white muscle fibres in the early free-living stage ($1\frac{1}{2}$ month) reflects the burst type of swimming behaviour of the young ones. As the fish grows, there is a gradual increase in the size and 'red like' character of the myotomal population correlating with a gradual transition from rapid burst type activity to a steady, sustainable swimming behaviour as observed in the rearing tanks. This basically agrees with the earlier reports (Waterman 1969, Nag & Nursall 1972).

To conclude, the present investigations of the myotomal musculature of the teleost fish, *Labeo rohito*, reveal three types of muscle fibres i.e. red, white and pink. The superficial red fibres are slow twitch red-type I fibres suitable for slow, sustained swimming. The white fibres are classified as fast twitch white-type IIB, suitable for rapid burst swimming for short periods. The third fibre type, is the pink which can be fast twitch red-type IIA or C probably best suited for activity ranges otherwise too high for red muscle.

Acknowledgements

This work was supported by a grant from the University Grants Commission, New Delhi. Thanks are due to Mr E A Daniels, for assistance in photomicrography.

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