# CHAPTER 1

## A HISTOCHEMICAL STUDY OF FAT IN THE RED AND WHITE FIBRES OF FISH SKELETAL MUSCLE

Studies on the pigeon pectoralis muscle which is a mixed muscle, have revealed that it consists of two distinct types of fibres : the narrow red fat loaded type of fibres having numerous large mitochondria and the broad white glycogen-loaded type of fibres with relatively fewer and smaller mitochondria. The red fibres are well adapted for aerobic metabolism using fat as fuel for sustained muscular activity and the white fibres for anaerobic metabolism using glycogen as fuel for short spells of activity (George and Naik, 1959). In a poor flier like the domestic fowl, a third type of fibres viz. intermediate type has also been noted (Chandra-Bose et al. 1964). In similar studies on the heterogeneity among the fibres of the fish muscle, Boddeke et al. (1959) from their observations on the body musculature of 21 different Dutch freshwater fishes, distinguished two types of muscle fibres viz. 1. broad white fibres without myoglobin and fat, with low sarcomeres and poor vascularization. 2. narrow fibres with fat, with high sarcomeres and a large number of capillaries. These narrow, myoglobin containing fibres occur in the region of the lateral line. In some fishes, the lateral line strip is poorly developed in as much as it consists of fibres lacking myoglobin. George (1962) in his studies on the mackerel muscle demonstrated a higher concentration of fat and oxidative enzyme (succinic dehydrogenase) in the red muscle which consists of narrow fibres than in the white in which the fibres are all of the broad variety.

In certain fishes (e.g. Labeo rohita) in between the red and white muscle regions, an intermediate region has been described (Chapter 5) Text Fig. 1. The fibres in the region of red muscle contain high concentrations of fat and glycogen, those in the white have only a negligible amount of these metabolites, but the fibres of the intermediate region come in between, with regard to their metabolite content. In the red muscle fibres the mitochondria that are at the periphery are larger than those in the interior of the fibre. Since mitochondria are the principal sites of fat staining in the fibre it was thought worthwhile to examine the localization of fat in the muscles of a few other fishes with a view to having a better understanding of the nature of the fibres as well as the distribution pattern of mitochondria in them in order to correlate these structural features with function. Accordingly, the present histochemical study of the muscle in a number of marine and freshwater fishes, was undertaken.

## MATERIALS AND METHOD

The following fishes were caught from their natural environments and studied for the demonstration of fat.

| Family | Name of species |
|---|---|
| Percidae | Therapon jerboa |
| Squammipinnes | Scatophagus argus |

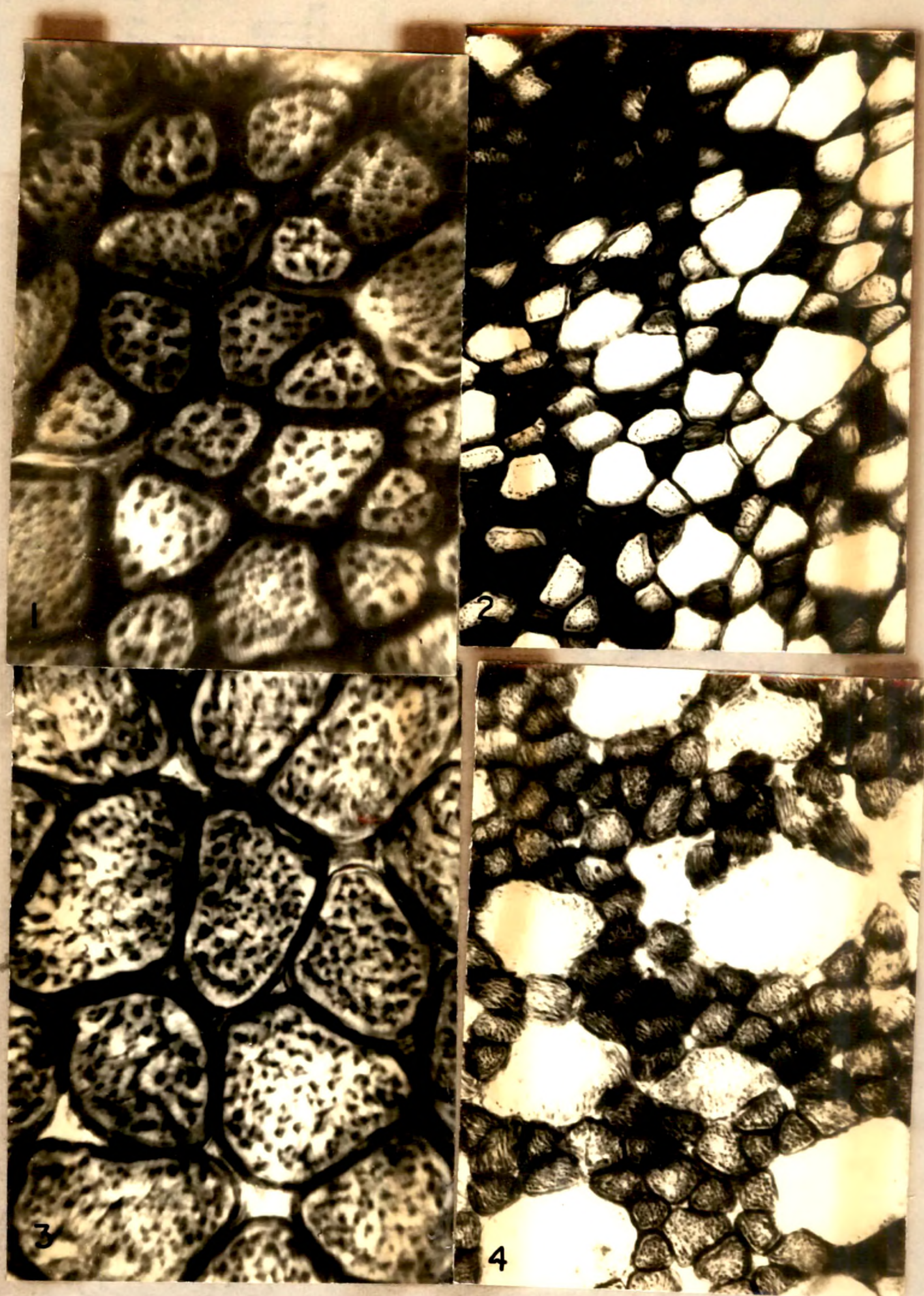
| Family | Name of species |
|---|---|
| Polynemidae | *Polynemus tetradactylus* |
| Carangidae | *Caranx hippos* |
| Mugilidae | *Mugil dussumieri* |
| Ophiocephalidae | *Ophiocephalus punctatus* |
| Pleuronectidae | *Cynoglossus* sp. |
| Scombresocidae | *Belone strongylurus* |
| " | *Hemiramphus cantori* |
| Siluridae | *Saccobranchus fossilis* |
| Cyprinidae | *Labeo rohita* |
| " | *Cirrhina mrigala* |
| " | *Barbus sarana* |
| Clupeidae | *Hilsa ilisha* |
| Chirocentridae | *Chirocentrus dorab* |
| Scyllidae | *Chiloscyllium indicum* |

Histochemical demonstration :

A piece of the muscle from the lateral line of each fish was fixed in Baker's calcium formol (Baker, 1946) for 24 hours, washed thoroughly in water and embedded in 15% gelatin. Sections 15 to 20 μ thick were cut and stained for fat with Sudan black B.

## RESULTS

From the results obtained it is evident that the intermediate region of muscle is present in some fishes and lacking in others. A classification based on the fibre types as indicated by differences in diameter, and the size, number and distribution of mitochondria in them, was possible, with regard to the three (red, intermediate and white muscle regions. The photographs presented (Figs. 1 to 21) show the nature of the fibres and the localization and distribution of fat in them. The quantitative data on fibre diameter, relative distribution of the fibre types and intensity of staining are presented in Tables 1, 2 & 3.

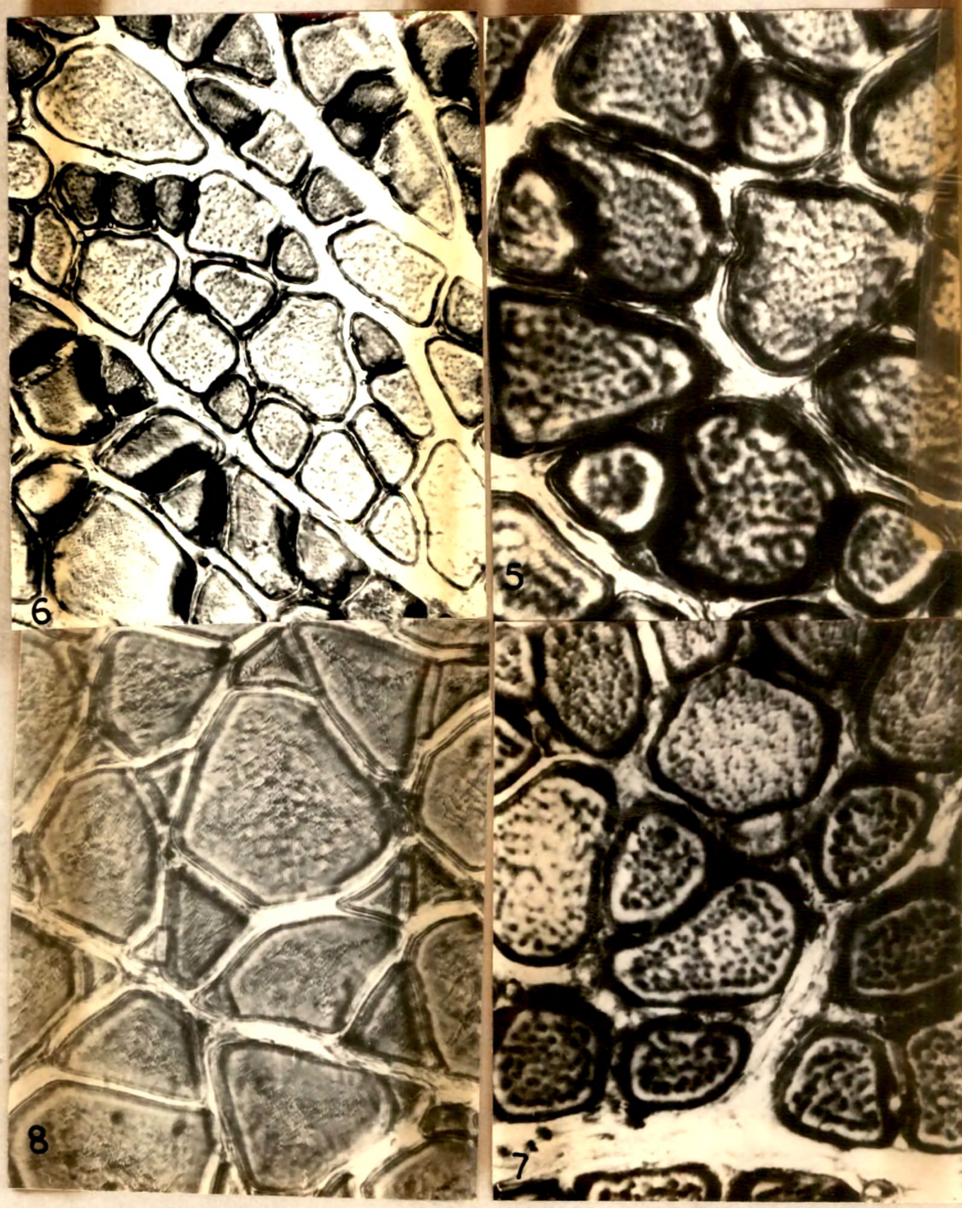



Microphotographs showing the localization and distribution of fat in the red and intermediate muscle regions respectively.

Figs. 1 & 2 *Scatophagus argus*

Figs. 3 & 4 *Mugil dussumieri*

Figs. 1 & 3 X 756; Figs. 2 & 4 X 192.




Microphotographs showing the localization and distribution of fat in the red and intermediate muscle regions respectively.

Figs. 5 & 6 <u>Therapon jerboa</u>

Figs. 7 & 8 <u>Polynemus tetradactylus</u>

Figs. 5 & 7 X 756; Figs. 6 & 8 X 300.


Microphotographs showing the localization and distribution of fat in the red muscle region.

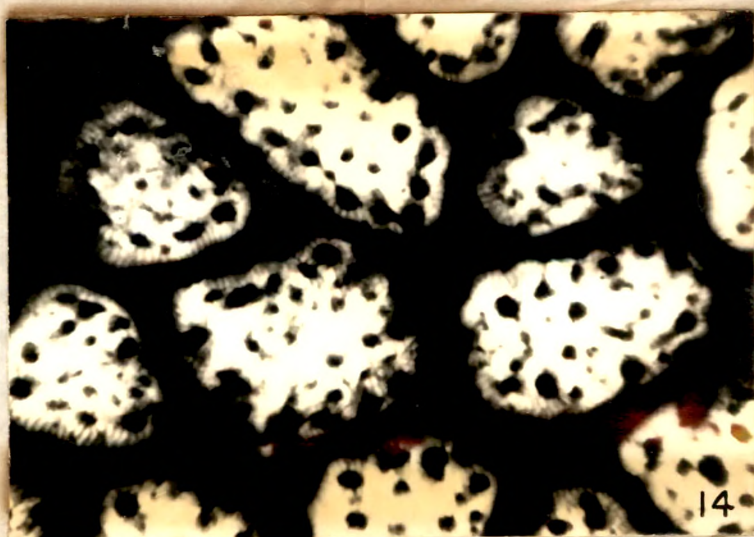
Fig. 9 <u>Belone strongylurus</u>

Fig. 10 <u>Hemiramphus cantori</u>

Fig. 11 <u>Saccobranchus fossilis</u>

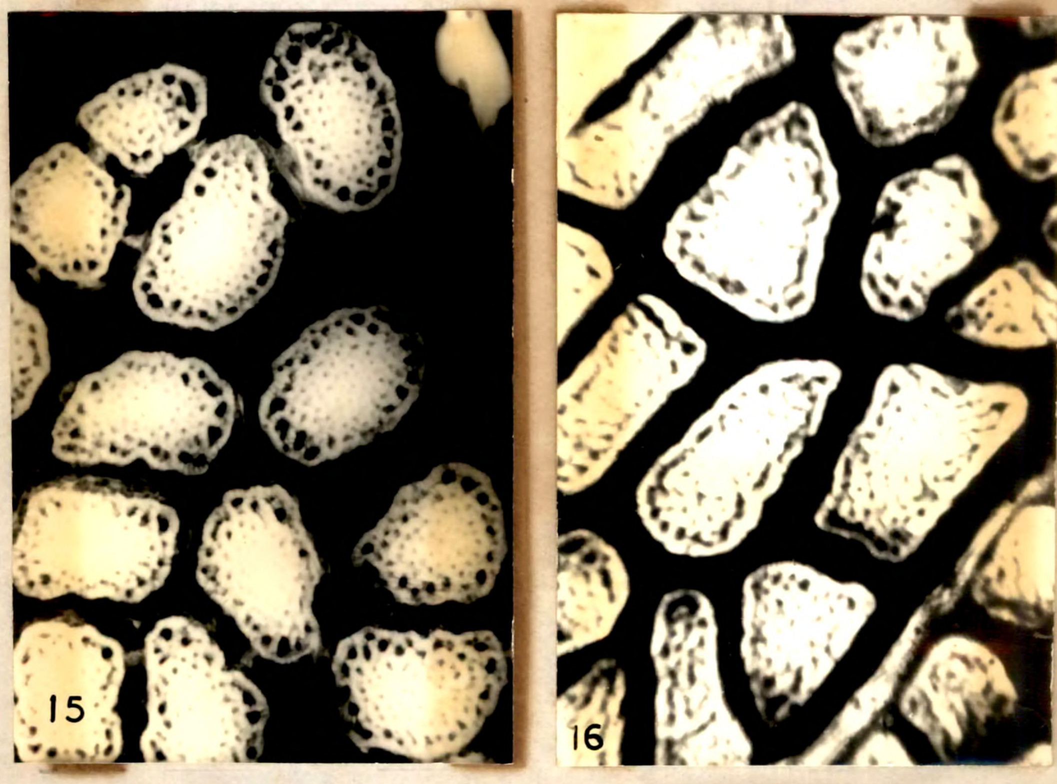
Figs. 9, 10 & 11 X 756.






Figs. 12 & 13 Microphotographs showing the localization and distribution of fat in the red and intermediate regions respectively of Barbus sarana. Fig. 12 X 756; Fig. 13 X 192.

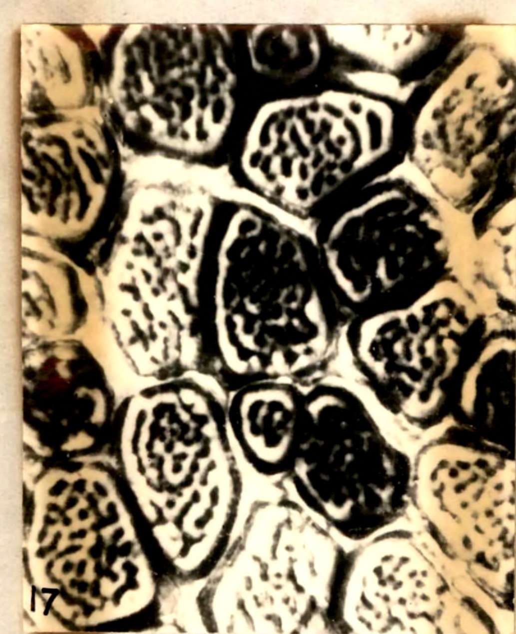
Fig. 14 Microphotograph showing the localization and distribution of fat in the red muscle region of Cirrhina mrigala. X 756.



Microphotographs showing the localization and distribution of fat in the red muscle region.

Fig. 15 Hilsa ilisha X 756.

Fig. 16 Chirocentrus dorab. X 756.

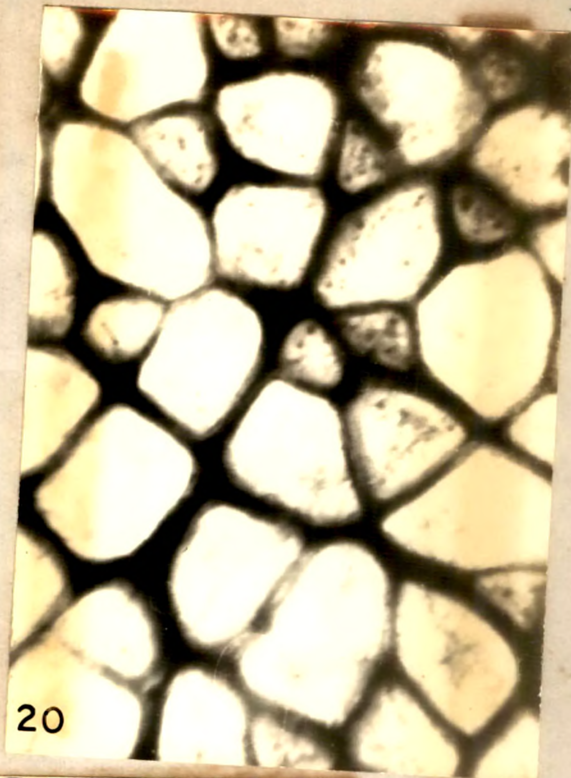


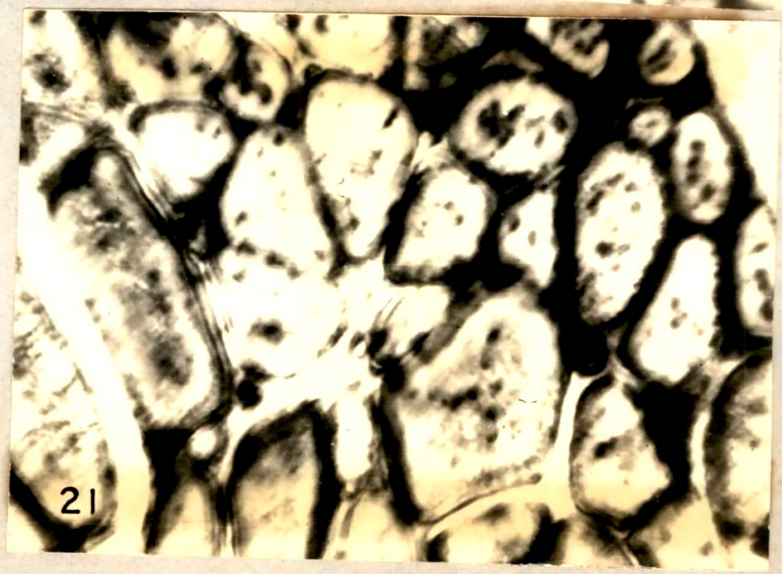


Microphotographs showing the localization and distribution of fat in the red muscle region.

Fig. 17 Caranx hippos X 756.

Fig. 18 Chiloscyllium indicum X 756.


Figs. 19 & 20 Microphotographs showing the localization and distribution of fat in the red and intermediate regions respectively of <u>Ophiocephalus punctatus</u> X 300.

Fig. 21 Microphotograph showing the localization and distribution of fat in the red muscle region of <u>Cynoglossus sp</u>. X 756.

Table I

The relative size and distribution of the fibre types and the concentration of fat in the intermediate muscle region of some fishes

| Name of Fish | Diameter of fibres in μ | | | Relative distribution of the three types of fibres (%) | | | Concentration of fat | | |
|---|---|---|---|---|---|---|---|---|---|
| | Narrow | Intermediate | Broad | Narrow | Intermediate | Broad | Narrow | Intermediate | Broad |
| | $I_1$ | $I_2$ | $I_3$ | $I_1$ | $I_2$ | $I_3$ | $I_1$ | $I_2$ | $I_3$ |
| *Labeo rohita* | 40 | 80 | 120 | 40.0 | 33.3 | 26.7 | - | - | - |
| *Cirrhina mrigala* | 45 | 70 | 95 | 40.0 | 40.0 | 20.0 | - | - | - |
| *Barbus sarana* | 40 | 60 | 85 | 49.3 | 26.6 | 24.0 | - | - | - |
| *Polynemus tetradactylus* | 20 | 50 | 75 | 62.5 | 25.0 | 12.5 | + | + | + |
| *Therapon jerboa* | 20 | 35 | 45 | 44.8 | 36.8 | 18.4 | ++ | + | + |
| *Ophiocephalus punctatus* | 30 | 45 | 65 | 40.0 | 40.0 | 20.0 | ++ | + | + |
| *Scatophagus argus* | 25 | 50 | 80 | 59.2 | 33.3 | 7.4 | ++++ | ++ | + |
| *Mugil dussumieri* | 25 | 50 | 75 | 90.9 | 4.5 | 4.5 | ++++ | ++ | + |

++++ Maximum staining
+++, ++ Medium "
\+ Minimum "
\- Negative "

Table II

The relative size and distribution of the fibre types and the concentration of fat in the red muscle of some fishes

| Name of Fish | Diameter of fibres in µ | | | Relative distribution of 3 types of fibres(%) | | | Concentration of fat | | |
|---|---|---|---|---|---|---|---|---|---|
| | Na. $R_1$ | In. $R_2$ | Br. $R_3$ | Na. $R_1$ | In. $R_2$ | Br. $R_3$ | Na. $R_1$ | In. $R_2$ | Br. $R_3$ |
| B. strongylurus | 15 | 30 | 45 | 40.3 | 40.3 | 19.4 | + | +‡+ | ++++ |
| H. cantori | 20 | 30 | 40 | 43.9 | 35.1 | 21.0 | + | +‡+ | ++++ |
| S. fossilis | 30 | 50 | 70 | 40.0 | 44.0 | 16.0 | + | +‡+ | ++++ |
| Cynoglossus sp. | 25 | 40 | 60 | 37.0 | 55.6 | 7.4 | + | + | + |
| O. punctatus | 15 | 30 | 45 | 25.0 | 50.0 | 25.0 | ++ | + | + |
| C. hippos | 10 | 20 | 30 | 33.6 | 48.7 | 17.7 | +++ | ++++ | ++ |
| P. tetradactylus | 10 | 20 | 30 | 30.0 | 50.0 | 20.0 | +++ | +++ | ++ |
| T. jerboa | 20 | 30 | 40 | 30.0 | 40.0 | 30.0 | +++ | +++ | ++ |
| L. rohita | 25 | 40 | 55 | 30.8 | 38.4 | 30.8 | ++++ | ++++ | ++++ |
| C. mrigala | 25 | 40 | 50 | 23.5 | 58.8 | 17.7 | ++++ | ++++ | ++++ |
| B. sarana | 30 | 45 | 60 | 45.5 | 36.4 | 18.1 | ++++ | ++++ | ++++ |
| H. ilisha | 25 | - | 45 | 89.8 | - | 10.2 | ++++ | - | ++++ |
| C. indicum | 30 | - | 50 | 71.6 | - | 28.4 | ++++ | - | ++++ |
| S. argus | 15 | - | 30 | 83.7 | - | 16.3 | ++++ | - | ++ |
| M. dussumieri | 15 | - | 30 | 75.0 | - | 25.0 | ++++ | - | +++ |
| C. dorab | 20 | - | 35 | 83.7 | - | 16.3 | +++ | - | +++ |

++++ Maximum staining
+++,++ Medium staining
+‡+ Medium staining in some fibres and minimum in others
\+ Minimum staining
\- Absence of fibres

Na. = Narrow fibre, In. = Intermediate fibre, Br. = Broad fibre

Table III

The relative size and distribution of the fibre types and the concentration of fat in the white muscle of some fishes

| Name of Fish | Diameter of fibres in µ | | | Relative distribution of the 3 fibre types (%) | | | Concentration of fat | | |
|---|---|---|---|---|---|---|---|---|---|
| | Na. $W_1$ | In. $W_2$ | Br. $W_3$ | Na. $W_1$ | In. $W_2$ | Br. $W_3$ | Na. $W_1$ | In. $W_2$ | Br. $W_3$ |
| B. strongylurus | 40 | 70 | 110 | 20.0 | 40.0 | 40.0 | - | - | - |
| H. cantori | 60 | 95 | 150 | 14.3 | 28.5 | 57.2 | - | - | - |
| S. fossilis | 45 | 85 | 110 | 57.2 | 28.5 | 14.3 | - | - | - |
| Cynoglossus sp. | 45 | 75 | 105 | 28.5 | 28.5 | 43.0 | - | - | - |
| O. punctatus | 55 | 75 | 100 | 33.4 | 33.3 | 33.3 | - | - | - |
| C. hippos | 25 | 55 | 90 | 44.4 | 44.4 | 11.2 | - | - | - |
| P. tetradactylus | 30 | 55 | 75 | 40.0 | 40.0 | 20.0 | - | - | - |
| T. jerboa | 40 | 60 | 95 | 40.0 | 40.0 | 20.0 | - | - | - |
| L. rohita | 50 | 85 | 135 | 37.5 | 30.0 | 32.5 | - | - | - |
| C. mrigala | 45 | 80 | 105 | 33.4 | 33.3 | 33.3 | - | - | - |
| B. sarana | 50 | 70 | 90 | 40.0 | 40.0 | 20.0 | - | - | - |
| H. ilisha | 40 | 65 | 130 | 40.0 | 40.0 | 20.0 | - | - | - |
| S. argus | 35 | 70 | 115 | 48.6 | 37.1 | 14.3 | - | - | - |
| M. dussumieri | 45 | 75 | 95 | 44.4 | 33.4 | 22.2 | - | - | - |
| C. dorab | 35 | 70 | 90 | 40.0 | 40.0 | 20.0 | - | - | - |
| C. indicum | 90 | | 125 | 20.0 | | 80.0 | - | | - |

\- Negative staining

Na. = Narrow fibre, In. = Intermediate fibre, Br. = Broad fibre

## DISCUSSION

It has been mentioned earlier that in skeletal muscle generally two types of fibres - the narrow red fibres and the broad white fibres could be recognized. The red fibres are believed to be slow (tonic) and capable of sustained activity and the white fibres to be fast (phasic) indulging in contractions of short durations. In the fish body, a region of red muscle is distinctly demarcated from another of white muscle, each having its specialized function. In the present study it was observed that in some fishes, in between the red and white muscle regions, there exists an intermediate region which gradually merges on either side with its adjoining muscle regions. Furthermore, it was seen that the red and white muscle regions, each comprised of three types of fibres ($R_1$, $R_2$ & $R_3$; $W_1$, $W_2$ & $W_3$) respectively in the case of some fishes, and two types of fibres ($R_1$ & $R_3$; $W_1$ & $W_3$) in other fishes. The intermediate region on the other hand, was composed of three types of fibres ($I_1$, $I_2$ & $I_3$) in those fishes in which this region was present. (Tables 1, 2 & 3).

In these different regions the concentration of fat was found to be both extra- as well as intracellular. Intracellularly, fat was localized at sites corresponding to the mitochondria. The fat content was highest in the red muscle where the mitochondria are larger and more numerous, and lowest in the white muscle where the mitochondria could not be demonstrated by fat staining. That the red muscle contains more fat than the white muscle, was shown quantitatively in some fishes

(Braekkan *et al.* 1956, 1959; Alexander, 1958). In some freshwater fishes, Boddeke *et al.* (1959) demonstrated histochemically that the fat content of the red fibres is more than in the white fibres. In a histophysiological study of the red and white muscles of the mackerel which is a migratory fish, it was shown cytochemically and quantitatively that the fibres in the red muscle contain considerably more fat than the fibres in the white muscle (George, 1962). Greater emphasis has therefore been laid on the red muscle region in the present study.

With respect to the concentration of fat in the different fibre types of the red muscle, great diversity was observed in the different fishes studied. Accordingly, the fishes have been classed into two groups.

Group 1 : Fishes in which the fat content varies in the three fibre types ($R_1$, $R_2$ & $R_3$) of the red muscle. This is indicated by the characteristic differences in mitochondrial size, density and distribution in these different types. e.g. *Therapon jerboa*, *Polynemus tetradactylus*, *Belone strongylurus*, *Hemiramphus cantori*, *Saccobranchus fossilis* and *Caranx hippos*. In *T. jerboa* and *P. tetradactylus*, the fibres with smallest diameter ($R_1$) in which the mitochondria are clumped together a little away from the periphery of the fibre had the highest fat content, and those with the largest diameter ($R_3$) had the least. The reverse was seen to be the case in a few other fishes viz. *B. strongylurus*, *H. cantori*, and *S. fossilis* where fibres with the largest diameter ($R_3$) had the largest mitochondria well loaded with fat and those with the smallest diameter ($R_1$) had very small mitochondria containing less fat. The fact that the $R_3$ fibres in the latter case are red fibres indicates that they are well adapted for sustained activity. At the same time, the larger diameter of these fibres shows that they are fast fibres and thereby also capable of quick contractions. A similar condition has been observed by Nene and George (1965) in the supinator muscle of the pigeon and fowl. It is likely that these are by and large phasic fibres but are in a state of hypertrophy since they contract for long periods. In Group 1 could also be included Caranx hippos, in which the fibres of medium diameter ($R_2$) have numerous mitochondria with higher concentrations of fat than the $R_1$ and $R_3$ fibres.

Group 2 : Fishes in which the concentration of fat is more or less uniform in the different fibre types of the red muscle. This is indicated by the uniform distribution of mitochondria throughout the fibres. e.g. Labeo rohita, Cirrhina mrigala, Barbus sarana, Hilsa ilisha, Chirocentrus dorab, Chiloscyllium indicum, Scatophagus argus, Mugil dussumieri, Ophiocephalus punctatus, and Cynoglossus sp. Of these, in the first five fishes, it was observed that the mitochondria containing fat are numerous and unusually large, especially those at the periphery of the fibres. High concentrations of fat were also present between the fibres. Since the red muscle in the fish is known to metabolize fat as fuel for energy (Chapter 5), in H. ilisha which is known to ascend rivers against strong currents for spawning, these fat reserves should provide energy for the ascent. In the freshwater fishes, L. rohita, C. mrigala, and B. sarana, the mitochondria which are larger at the periphery of the fibre indicate a higher rate of oxidative metabolism at these sites with the possibility of transport of free fatty acids from the intercellular (extracellular) fat store across the cell membrane. Owing to the presence in these fibres of an intermediate muscle region between the red and white, their movements are comparably slower than H. ilisha and C. dorab in which the intermediate muscle region is absent. The two types of fibres ($R_1$, $R_3$) seen in the red muscle of H. ilisha and C. dorab, may be compared to the two types of fibres present in the pectoralis muscle of a starling, Sturnus roseus and the giant fruit bat, Pteropus giganteus (George, 1965). In C. indicum, S. argus and M. dussumieri, the fat-loaded mitochondria of the red fibres are uniformly small but densely packed in the entire cytoplasm. These fibres are thus well adapted for an aerobic metabolism to utilize fat for sustained activity. Such examples are to be regarded as convergence in the evolution of sustained muscular activity. A more or less uniform pattern of fat distribution was also found in the different fibre types of the red muscle of O. punctatus and Cynoglossus sp. However, the mitochondria containing fat are very few and scattered in these fibres. The presence of such mitochondria may be considered as a reason for the relatively sluggish movements not involving the use of much energy in these fishes.

Slautterback (1965) reported that some sort of correlation exists between mitochondrial organization and the speed of muscle contraction and has indicated the degree to which mitochondrial elaboration in heart muscle can be correlated with heart rate. He has shown that the heart of the canary which beats very fast has extraordinarily large mitochondria with complex arrays assumed by their cristae in comparison to the slow goose heart which has fewer mitochondria with less complicated cristae. The present observations in the fish muscle also indicate a similar functional significance to mitochondrial distribution and structure.

Boddeke et al. (1959) grouped the freshwater fishes investigated, with regard to the nature and development of their musculature into different groups. 1. Fishes e.g. the Salmon, the Rainbow Trout and the Carp, in which there is present a well developed lateral line strip as well as the other musculature consisting of both the broad and narrow varieties of fibres. These fishes have been considered by them as typical "stayers" which must have a large staying power for their migratory travel (Salmon) or because they have to go long distances food hunting (Rainbow Trout and Carp). 2. Fishes e.g. the Dace, the Ide, the Roach, the Rudd, the Bream, the Gudgeon and the Tench in which the lateral line muscle strip is well developed but the other musculature consists of only the white type of fibres. These fishes showed certain characters of the stayer without being pronounced stayers. With regard to their mode of feeding and the way in which they escape from their enemies, they have been referred to as crawlers". 3. Fishes e.g. the Pike, the Perch, the Stickleback, the Bullhead, the Stone Loach, the Burbot and the Eel, in which the lateral line musculature consists exclusively or nearly exclusively of the broad white type of fibres. From their mode of life, these fishes have been credited with the qualities of the sprinter. However, the Eel and the Burbot show neither the characteristics of the sprinter nor those of the stayer, and therefore have been called as the "sneakers".

Of the fishes examined in the present study, S. argus, M. dussumieri and T. jerboa would come under the first group of Boddeke et al. (1959). It is generally believed that M. dussumieri is migratory. An intermediate region of fibres which occurs in these three fishes presents a mosaic picture with regard to the heterogeneity of the component fibres. These fishes are all well adapted for sustained activity and therefore may rightly be placed in this group. Fishes like L. rohita, C. mrigala, B. sarana, P. tetradactylus, H. ilisha, C. dorab, C. indicum, C. hippos, O. punctatus, Cynoglossus sp. B. strongylurus, H. cantori, and S. fossilis in which the red and white muscle regions are present but the latter lacking the mosaic fibre composition, may be included in Group 2 of Boddeke et al. None of the fishes studied had only the broad white type of fibres and so the third group of Boddeke et al. is not represented.

George and Jyoti (1955) in their studies on the pectoral muscles of the pigeon and some other birds and a bat, concluded that fat in the red fibres is the main source of energy for sustained activity during flight. According to Braekkan (1956) the red muscle in the fish with high fat content is not an efficient system for movement and suggested that it acts as a supplementary liver. Nevertheless Buttkus (1962) in his studies on rigor mortis in fish muscle reported that the red muscle of the lingcod and trout possessed unusual contractile properties which seem to indicate that it is an active component of the muscular equipment designed for efficient movement. George (1962) has pointed out that the red muscle of the fish is organized for continuous and slow contractions which help the body to move forward in water and the white muscle for short and sudden contractions in order to escape from enemies, avoid obstacles or overcome powerful currents.