CHAPTER 5

CELLULAR ORGANIZATION AND FAT UTILIZATION IN FISH MUSCLE

In a recent study on certain histological characteristics of the body musculature of fishes, Boddeke et al. (1959) observed that the fibres of the red lateral line musculature are narrower, more sudanophilic and have higher sarcomeres than those of the white muscle of the epaxial and hypaxial musculature. Studies carried out on the changes in protein, carbohydrate and fat contents of the body of fishes during migration have been reviewed by Drummond and Black (1960). These studies have shown that fish muscle generally contains little glycogen which could soon get depleted while fat should provide the main bulk of the energy required for muscular activity. George (1962) studied the red and white muscles of the mackerel with regard to their fat content and enzyme (lipase and succinic dehydrogenase) concentrations. The red muscle was found to contain enormous amount of extra- as well as intra- muscular fat, high concentration of lipase and numerous mitochondria showing high succinic dehydrogenase activity. The white muscle on the other hand contained considerably less fat, fewer mitochondria and low enzyme activity. He also observed that the former is well adapted for an aerobic metabolism to use fat as the chief fuel and the latter for an anaerobic metabolism for the use of glycogen as the main fuel.

Miescher-Rusch (1883) was the first to study the change in the quantity of lipid in the body of the Atlantic salmon during its migratory ascent. Paton and his associates concluded that the fat which accumulates outside and within the muscle fibres in the Atlantic salmon during active feeding was later used up either for the development of gonads or as a source of muscular energy (Paton, 1898). Greene (1913) suggested that the very large quantity of fat stored in the muscle cells of the king salmon (Oncorhynchus tshawytscha) prior to the upstream migration is to be regarded as stored fuel. Pentegov et al. (1928) studied changes in the fat content of the chum salmon (Oncorhynchus keta), during spawning migration up the Amur river and arrived at the same conclusion. Recently Idler et al. (1959) have also shown that a considerable amount of fat is reduced during the spawning migration in the sockeye salmon (Oncorhynchus nerka).

In the light of the investigation mentioned above, it became necessary to study the cellular organization of the red and white muscles in fish and the relative capacity of these two types of muscle to metabolize fat.

MATERIAL AND METHODS

A freshwater fish Labeo rohita was used in the present study. They were kept in spacious open-air tanks and maintained on a ration of vitamin supplanted wheat pellets. The fish was killed by decapitation and pieces of the muscle containing the three regions, A, B and C (Text Fig.1) were fixed for the

histochemical studies of fat and glycogen, in Baker's calcium formol (Baker, 1946) and in cold alcoholic picroformol respectively. A piece of the muscle was kept in the deep freeze chamber of a refrigerator and thin frozen hand sections were cut for studying the localization of the enzymes lipase and succinic dehydrogenase. The diameter of the fibres was measured from sections that were stained for fat.

Histochemical localization:-

Fat: The tissue was fixed in Baker's calcium formol for 24 hours, washed in running tap water for the same time and embedded in 20% gelatin. 30 to 40 μ sections were cut on a freezing microtome and stained with Sudan black B.

Glycogen: Thin strips of the tissue were fixed in cold alcoholic picroformol for 24 hours and embedded in paraffin wax of melting point 58 to 60°C. Sections 10 to 12 μ thick were cut and stained for glycogen by the periodic acid Schiff's reagent (Pearse, 1960).

Lipase: Localization of lipase activity was studied by the method described in chapter 7.

Succinic dehydrogenase: Thin frozen sections of the tissue were cut and the demonstration of the enzyme activity was accomplished by the improved method of George and Talesara (1961a) at room temperature (28°C) using neotetrazolium chloride as the hydrogen acceptor.

Fatty acid oxidation:-

A comparative study on the fatty acid oxidation by the

red and white muscles respectively of three fishes viz. Labeo rohita, Labeo fimbriatus and Cirrhina mrigala of the family Cyprinidae was conducted. The red and white muscles along the lateral line were separated, blotted free of blood on a filter paper and separately homogenized in ice cold distilled water.

The capacity for in vitro oxidation of fatty acid by the red and white muscles respectively was estimated by measuring the oxygen uptake in the conventional Warburg manometric system with sodium butyrate as the added substrate (Dixon, 1943; Umbreit et al. 1957). Since fatty acid oxidation is known to be initiated by the presence of a small amount of a sparker, sodium malate was included in all the flasks to spark the reaction. The centre wells of the Warburg flasks contained 0.2 ml of 20% KOH to trap the metabolic carbon dioxide. The main chamber of the reaction flask contained :

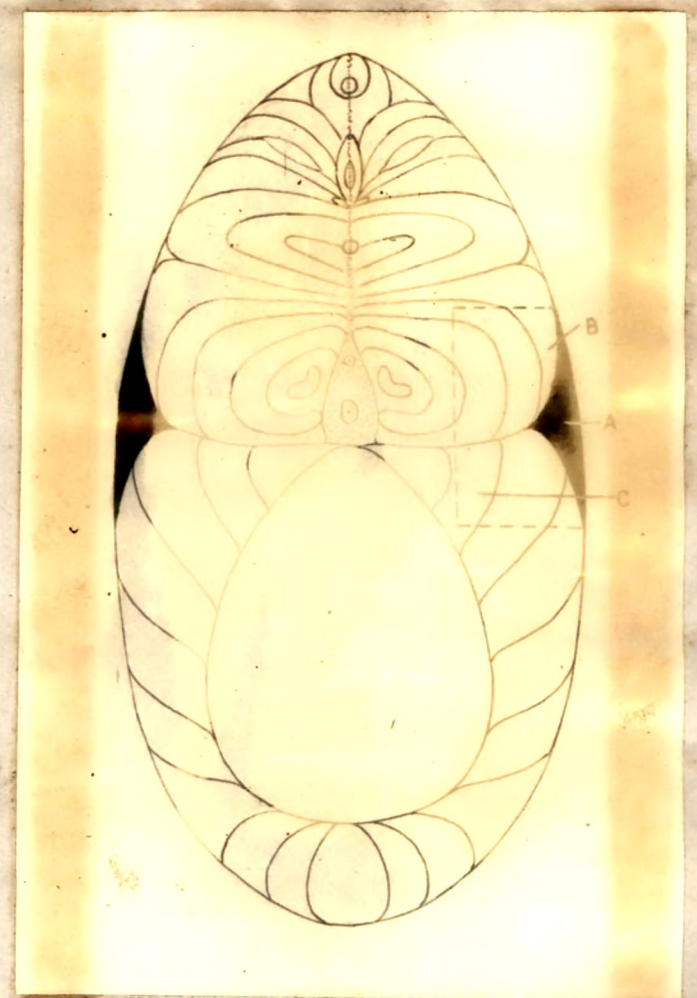
Phosphate buffer	0.1M pH 7.4	0.60 ml
Magnesium chloride	0.15M	0.15 ml
ATP	0.0225M	0.20 ml
Cytochrome c	0.00225M	0.15 ml
DPN	0.015M	0.20 ml
Coenzyme A	0.0075M	0.20 ml
Sodium malate	0.15M	0.20 ml
Sodium butyrate	51 mg/10 ml	0.50 ml
Tissue homogenate		0.80 ml

For both the muscle samples there was a control flask with no substrate added but only the sparker. A roll of filter

paper (3 cm square) was introduced into the central well containing the alkali, thus making up a total fluid volume of 3.2 ml. Incubation was carried out for one hour at 37°C with air as the gas phase. The Warburg system was oscillated at 100 oscillations/minute. After an equilibration period of 10 minutes, the levels were adjusted and the readings on the manometers were taken at an interval of every 10 minutes for an hour. The oxygen uptake is expressed as µl oxygen uptake/ milligram protein/hour at 37°C. The protein content of the homogenate was determined by the Biuret method (Gornall et al. 1949).

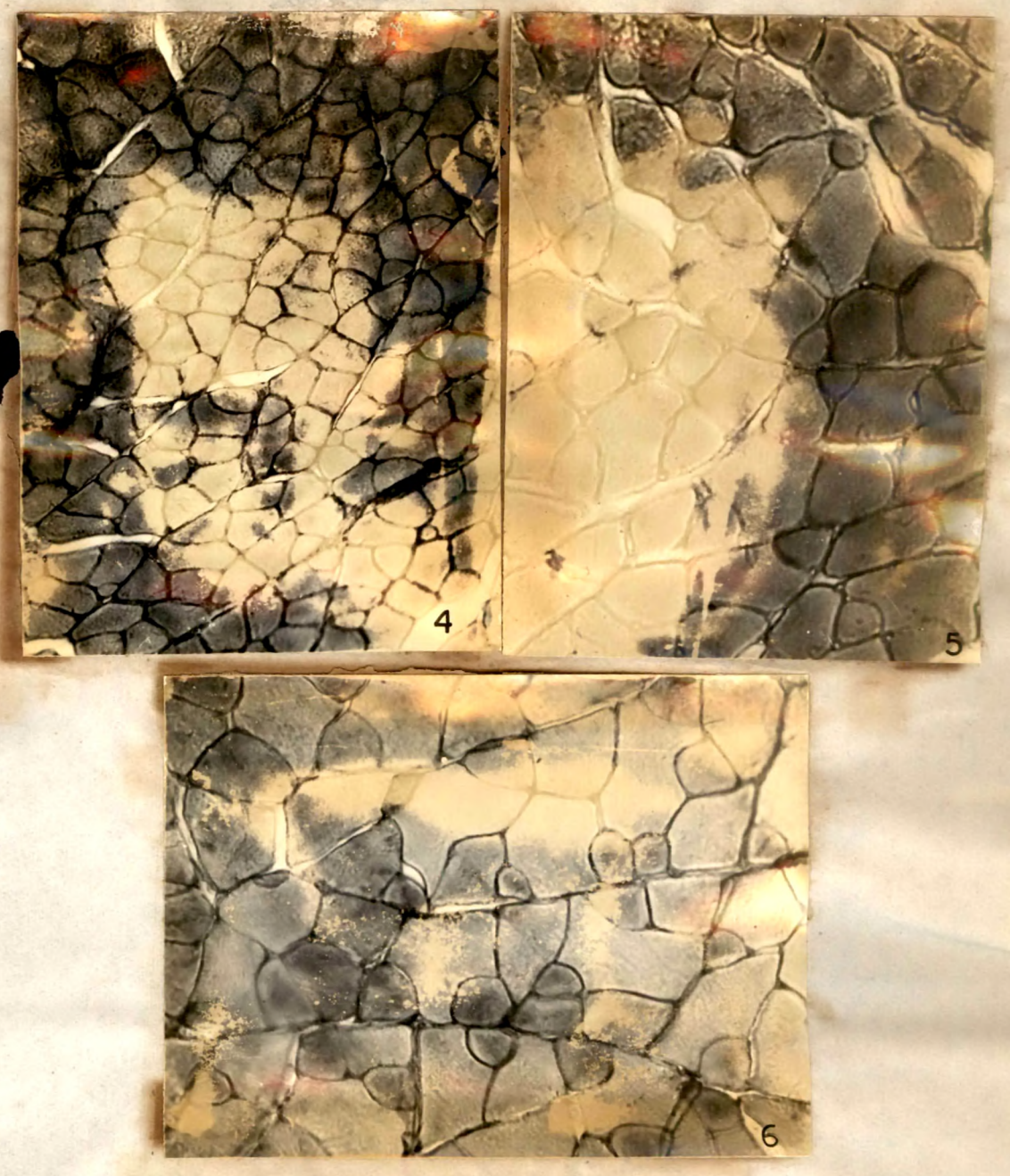
RESULTS

The red muscle (Figs. 1,4 & 7) along the lateral line (Region A, Text Fig. 1) was composed entirely of red narrow fibres of diameter varying between 25 and 45 µ and the white muscle (Region C, Text Fig. 1) mainly of white broad fibres (135 µ) and a few white narrow fibres (45 µ) (Figs. 3, 6 & 9). A number of intermediate sized fibres (45 µ) together with the broad white fibres (85 µ) were found in the region (Region B, Text Fig. 1) where the red muscle merged with the white. This region will be called the intermediate region (Figs. 2, 5 & 8). The staining for fat with Sudan black B showed that a considerable amount of extra- as well as intra-cellular fat was present in the red muscle (Fig. 1). A characteristic feature of the narrow red fibres was that the mitochondria at the periphery of the fibre were found to be

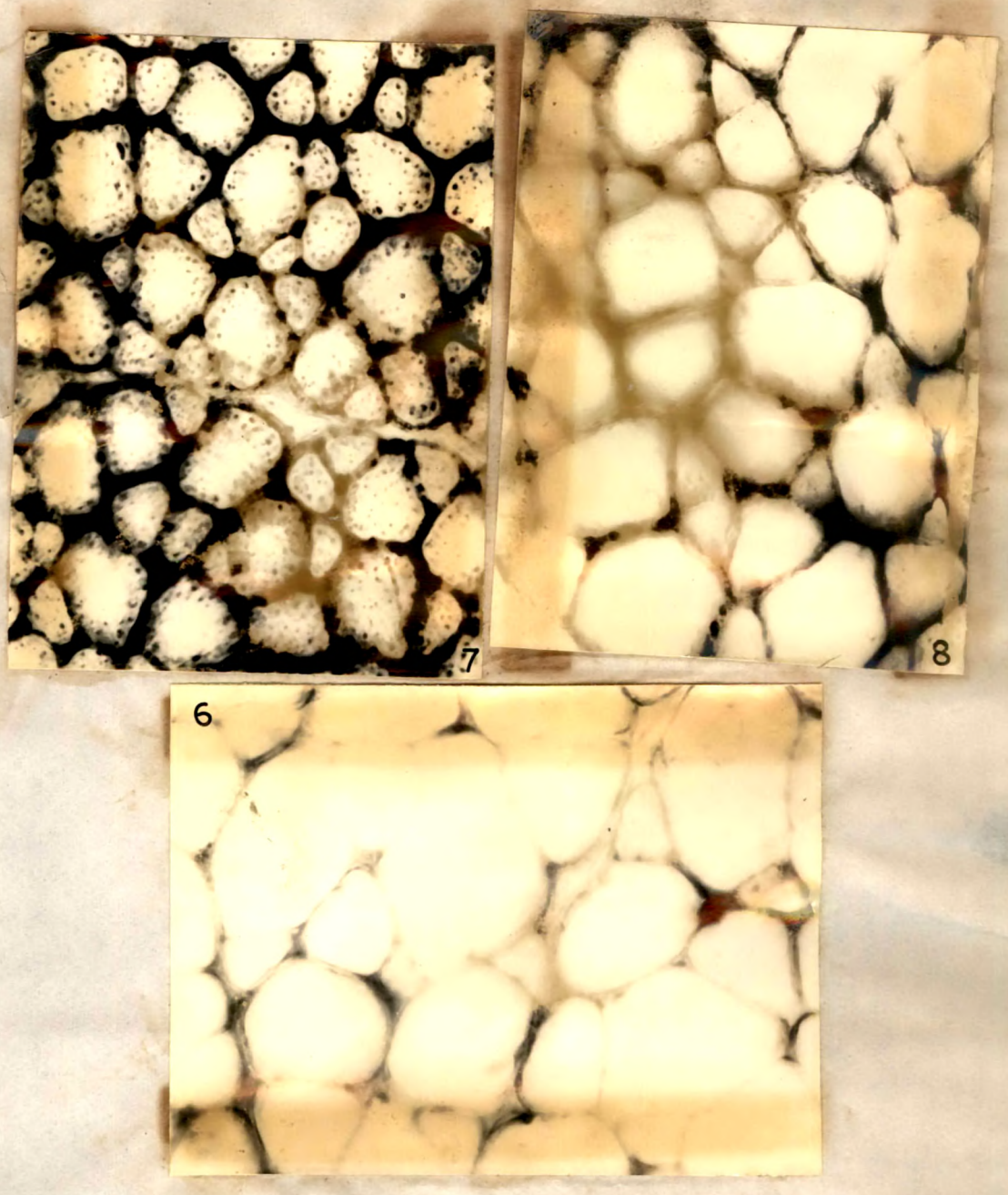


Text Fig. 1. Transverse section of the fish showing the three regions : A, red narrow fibres; B, intermediate region and C, white broad fibres.

Figs. 1, 2 & 3 Localization of lipids in the fibres of the red, intermediate and white muscle regions respectively. X 260



Figs. 4, 5 & 6 Localization of glycogen in the fibres of the red, intermediate and white muscle regions respectively. X 260



Figs. 7, 8 & 9 Localization of succinic dehydrogenase in the fibres of the red, intermediate and white muscle regions respectively. X 260

Table I

In vitro fatty acid (sodium butyrate) oxidation by the red and white lateral line muscles of three fishes of the family Cyprinidae, expressed in terms of O_2 uptake/mg protein/hour at $37^{\circ}C$.

Name of fish	Nature of the muscle	µl O_2 uptake due to the oxidation of butyrate and malate	µl O_2 uptake due to the oxidation of malate	µl O_2 uptake due to the oxidation of added fatty acid
L. rohita	Red	3.271	2.865	0.406
L. fimbriatus	Red	1.469	0.990	0.480
C. mrigala	Red	2.309	2.134	0.175
L. rohita	White	0.727	0.895	-0.168*
L. fimbriatus	White	0.641	0.860	-0.219*
C. mrigala	White	0.428	0.677	-0.249*

The values obtained in each case is the average of three experiments. The results show that with the red muscle there was greater butyrate oxidation, whereas with the white muscle the difference obtained was (*) due to the higher oxidation of malate.

larger than those at the centre. Histochemically no fat could be demonstrated in the broad and narrow white fibres (Fig. 3), although some amount was detectable in the fibres of the intermediate region (Fig. 2).

Sections stained to demonstrate glycogen showed that more glycogen was found in the red narrow than in the broad white fibres. However, in the narrow white fibres of the white muscle more glycogen was present than in the broad white fibres of the same muscle. The fibres of the intermediate region contained more glycogen than the fibres of the region of white muscle but less than the red narrow fibres (Fig. 5).

Lipase activity was found to be localized mainly in the mitochondria of the red muscle (Chapter 7), whereas in the white muscle the enzyme activity was negligible.

Succinic dehydrogenase was found to be localized in the mitochondria which were numerous in the fibres of the red muscle (Fig. 7), few in those of the intermediate region (Fig. 8) and practically undetectable in the white (Fig. 9).

The results obtained for fatty acid oxidation are presented in Table 1.

DISCUSSION

George and Naik (1957, 1959) distinguished two distinct types of fibres, a broad white glycogen-loaded type and a narrow red fat-loaded variety in the pectoralis muscle of the pigeon and a bat. The former is well adapted for an anaerobic

metabolism using glycogen as the chief fuel and the latter for an aerobic metabolism using fat (George and Talesara, 1961 a, 1961 c, 1962 a). Similar observations were made by George (1962) on the white (epaxial and hypaxial musculature) and red muscles (lateral line musculature) of the fish, mackerel. It was shown that the red muscle fibres unlike the white contain numerous mitochondria and high concentrations of fat, lipase and succinic dehydrogenase.

The histochemical observations made in the present study have confirmed the observations made by George (1962) in the mackerel and have also shown that the white muscle fibres contain a considerably lower concentration of glycogen than the red. The higher concentration of glycogen in the red fibres is possibly due to the fact that these fibres utilize mainly fat as fuel thus sparing the glycogen. However, it was noticed that in the region of the white fibres, there were some narrow fibres with smaller diameter which did not stain with Sudan black B for fat, but were found to contain more glycogen than the broad ones. Thus these narrow white fibres which contain more glycogen than the broad white fibres may be compared with the narrow red fibres also having more glycogen than the broad white fibres. These narrow white fibres may then be considered as also capable of utilizing fat possibly in the form of short chain fatty acids and thereby spare the glycogen. This explains the higher concentration of glycogen in these fibres.

Boddeke et al. (1959) have described the white muscle of the salmon as a mosaic of broad and narrow fibres, the latter being more sudanophilic than the former. The difference in Labeo is that both the types of fibres are nonsudanophilic. This shows that the pattern of fibre architecture in the two fishes is the same except for the fact that the salmon being a migratory fish, the muscle fibres have more stored fat and are better adapted for utilizing fat as fuel for sustained muscular activity.

In the mammalian skeletal muscle, a higher concentration of glycogen was observed in the narrow red fibres (Hess and Pearse, 1961). Dubowitz and Pearse (1960) demonstrated higher phosphorylase activity in the narrow red fibres of mammalian as well as fish skeletal muscle. On the other hand it has been shown that there is greater concentration of glycogen (George and Naik, 1959) and phosphorylase (Dubowitz and Pearse, 1960) in the broad white fibres of the pigeon breast muscle. The narrow red fibres in all cases have been shown to possess a high level of the oxidative enzyme, succinic dehydrogenase. In the present study also higher levels of glycogen and succinic dehydrogenase in addition to fat and lipase were observed in the red narrow fibres.

The low concentration of glycogen in the white muscle may be attributed to the fact that glycogen is being constantly utilized by this muscle whereas in the red muscle this metabolite is being conserved while fat is used as the major

fuel. In vitro experiments conducted to assess the capacity of these two types of muscle for fatty acid oxidation have clearly shown that the red muscle oxidized fatty acid (butyrate) in addition to malate while the white muscle oxidized only malate.

Evidence for the utilization of fat by fish muscle is also available from the work of Bilinski (1963). He obtained in the rainbow trout, greater rate of oxidation of Na hexanoate-1-C^{14}, K octanoate-1-C^{14} and K myristate-1-C^{14} by the red muscle than the white, determined by measuring the formation of $C^{14}O_2$.

It was observed that in the red narrow fibres of Labeo, the mitochondria situated at the periphery of the fibre appeared slightly larger than those towards the centre. This is indicative of a higher rate of oxidative metabolism of the periphery of the fibre with the possibility of transport of fatty acids from the intercellular (extracellular) fat store across the cell membrane. The demonstration of intracellular lipase activity also suggests the hydrolysis of fat into fatty acids as the first step in the utilization of fat. That the lipolytic action of lipase on fat is necessary as the first step in the oxidation of fat, has been shown in the pigeon breast muscle (George and Talesara, 1962 b).

However, it should be pointed out that fat is used as the fuel in the white muscle as well, in which there is very little fat. This has been shown in the case of the fowl breast muscle (George and Jyoti, 1957) and the dog muscle (Young and

Price, 1961) during continuous and prolonged activity. Additional supplies could come from the liver and the adipose tissue; In the red muscle which utilizes fat as the chief fuel for energy, fatty acids are oxidized to a greater extent in preference to glycogen, and glycolysis is resorted to only when oxygen is deficient.