CHAPTER 4

A QUANTITATIVE STUDY OF FAT, GLYCOGEN, LIPASE AND SUCCINIC DEHYDROGENASE IN FISH MUSCLE

It is well known that in fishes with a well developed lateral line system, the muscle strip is always red because of the presence of myoglobin. In fishes with a poorly developed lateral line system, this strip is generally white having little or no myoglobin (Boddeke *et al.* 1959). The red muscle consists of narrow fibres, quite distinct from the rest of the muscle tissue which is white. Braekkan (1959) suggested that the red muscle provides a storehouse of potential energy in the form of fat, glycogen and other metabolites in close proximity to the white muscle. He further proposed that this tissue might have a metabolic function in fish similar to that of the liver. Love *et al.* (1959) studying the composition of the red muscle in a number of species of fish found that this muscle contains more total fat, fatty acids lecithin, cytochrome *c*, myoglobin and vitamins than the white muscle. Recently, studies on the red and white muscles of the mackerel, which is a migratory fish showed that the fat content and enzyme (lipase and succinic dehydrogenase) concentrations are considerably higher in the red muscle and that this muscle is well adapted for an aerobic metabolism to use fat as the chief fuel for muscular activity and the white for an anaerobic metabolism to use mainly glycogen (George, 1962).

It was therefore thought desirable to investigate quant-

itatively the metabolite load and the concentrations of the enzymes (lipase and succinic dehydrogenase) in the red and white muscles taken separately from three different regions of the body of the fish, with a view to throw more light on their general nature and behaviour and also to see if any variation exists in the red and white muscles from the different regions of the body of the fish.

MATERIALS AND METHODS

Freshwater fishes, Labeo rohita and Labeo fimbriatus were used in the present study. They were kept in spacious open air tanks and maintained on a ration of vitamin supplanted wheat pellets. The fishes were killed by decapitation and skinned. Pieces of the red and white muscle were separated from the anterior (A), middle (B) and posterior (C) regions (Text Fig. 1) and then used for the quantitative estimations of fat, lipase and succinic dehydrogenase. For glycogen however, the red, intermediate and white regions were separately taken from the regions A and C.

Fat : The tissue was dehydrated in a hot air oven at 100°C. The total fat content was then estimated by extraction in a Soxhlet apparatus with a 1 : 1 alcohol-ether mixture.

Glycogen : Glycogen was estimated by the anthrone method of Seifter et al. (1950) using hot 30% KOH for digestion. The intensity of the green colour was measured on a Bausch and Lomb "Spectronic 20" colorimeter at 620 mμ. The glycogen

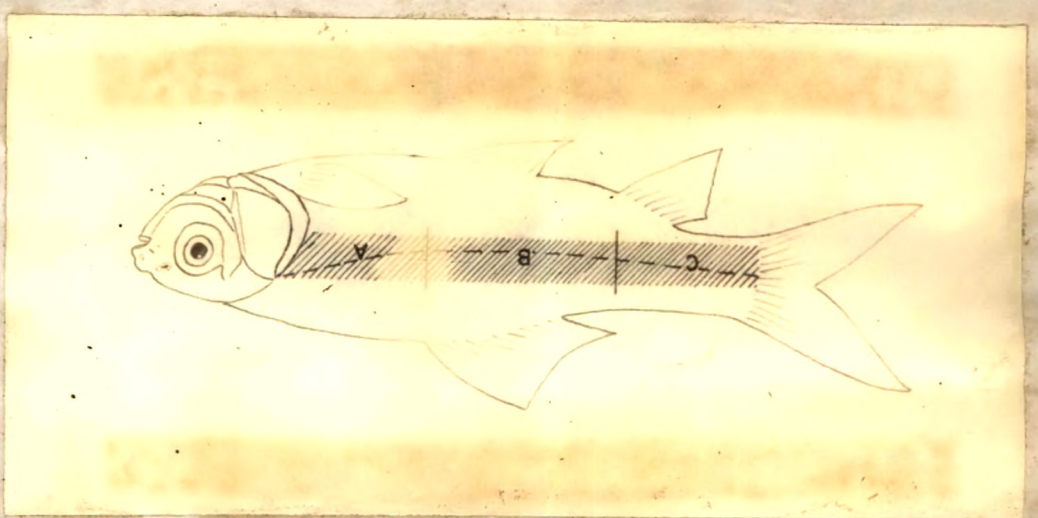
content in the muscle was expressed as per cent of glycogen on wet weight of the muscle.

Lipase : Lipase activity was determined according to the manometric method of Martin and Peers (1953), using a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C in the Warburg apparatus. Tributyrin was used as the substrate. The enzyme activity was based on the mg protein content of the tissue. The protein content was estimated by the Biuret method (Gornall et al. 1949). The enzyme activity is expressed as µl Co_2/mg protein/hour.

Succinic dehydrogenase (SDH) : The quantitative estimation of SDH was done colorimetrically by the method of Kun and Abood (1949). The results are expressed as µg formazan formed/mg protein/hour at 37°C. The protein content was determined as above.

RESULTS

The results are summarized in Tables 1, 2 and 3. From Table 1 it appears that a variation in the quantity of fat present is seen to occur from the anterior to the caudal end of both the fishes. The anterior region (A) contains the largest quantity of fat in the red as well as the white muscles, while the posterior region (C) contains the least. The amount of fat in the red and white muscles of the middle region (B) is intermediate between that of the anterior and posterior regions.



Text Fig. 1 Showing the three different anatomical regions (A, B & C) along the body of the fish from which samples were taken for the various quantitative estimations.

Table I

Showing the fat content in red and white muscles of the anterior (A), middle (B) and posterior (C) regions of L. rohita and L. fimbriatus

Name of Fish	A. red	A. white	B. red	B. white	C.red	C. white
L. rohita	20.37	3.07	13.58	2.19	10.76	1.49
(Range)	(20.09-20.86)	(2.34-3.84)	(10.62-17.26)	(1.09-3.23)	(6.94-17.03)	(1.14-1.87)
L. fimbriatus	26.60	3.34	21.85	2.65	17.44	2.47
(Range)	(23.53-29.17)	(2.30-4.18)	(13.01-33.38)	(1.99-3.24)	(13.87-23.44)	(2.18-2.98)

Figures presented are the average values of 5 sets of experiments.

Table II

Showing the percentage of glycogen in the red, intermediate and white muscles of the anterior (A) and posterior (C) regions of L. rohita and L. fimbriatus.

Name of Fish	A. red	A. int.	A. white	C. red	C. int.	C. white
L. rohita	3.02	1.37	0.48	2.88	1.52	0.40
(Range)	(2.01-3.78)	(0.99-1.74)	(0.30-0.66)	(2.06-3.51)	(1.16-1.74)	(0.25-0.56)
L. fimbriatus	0.85	0.46	0.05	0.86	0.45	0.05
(Range)	(0.64-1.09)	(0.25-0.76)	(0.02-0.08)	(0.59-1.15)	(0.28-0.64)	(0.02-0.08)

Figures presented are the average values of 8 sets of experiments. There was no difference in glycogen content between regions A & B.

The most striking aspect of the data in Table 2 is the large and consistent difference in the glycogen levels between the two fishes. However, no significant difference for glycogen is seen between the anterior (A) and posterior (C) regions of both the fishes. Nevertheless, the greatest concentration is obtained in the red muscle, next in the intermediate and least in the white.

The concentrations of lipase and SDH as represented in Table 3 are more or less equal in the red and white muscles of the anterior (A) and posterior (C) regions. The red muscle of the middle region (B) however, shows higher results for lipase and SDH than the A and C regions.

DISCUSSION

From the quantitative data obtained in the present study it is clear that the red, tonic muscle of the fish is more fatty, glycogen-loaded and has higher concentrations of both lipase and succinic dehydrogenase than the white tetanic muscle. These metabolic differences between the red and white muscles are in agreement with the functions attributed to these two types of muscle. George (1962) has reported that the white muscle of the mackerel is organized for quick and fast contractions and the red muscle for continuous and slow contractions.

Several investigators have estimated the glycogen content in the fish muscle as a whole, without separating the different regions (red, intermediate and white) (Tarr, 1950; Amano *et al.* 1953; Noguchi and Yamamoto, 1955; Miller, 1959; Suyama *et al.* 1960; Black, 1961; Hochachka and Sinclair, 1962; Jones, 1962).

The data obtained in the present study on the glycogen content of the different regions of the fish muscle tally with the qualitative assessment made for the histochemical preparations (Chapter 2). The fact that the red muscle which is adapted for fat metabolism contains high concentrations of glycogen suggests the possibility of an increased rate of glycogen synthesis in these fibres resulting from a sparing action on glycogen when fat is ~~being~~ continuously being used. This finds support in the observation that there is a high concentration of UDPG glycogen synthetase in the red muscle (Chapter 2). The white muscle on the other hand should utilize glycogen constantly and hence its low glycogen content. Revel et al. (1960) found that the sarcoplasmic area of the red muscle of the lingcod contained a very much higher density of glycogen than that of the white muscle.

It is only in recent years that emphasis has been laid on the variations in the different sections of the muscle of the same fish (Brandes and Dietrich, 1953; Thurston, 1958; Thurston and MacMaster, 1960). Mannan et al. (1961) noted considerable differences in composition among samples of the flesh obtained from different regions of the body of the Atlantic halibut. In the present study, the fat content was found to vary considerably from the anterior to the caudal end of the fish. A higher percentage of fat was noted in the anterior region with a gradual decrease towards the tail end, so that the latter had the lowest percentage of fat. This is

particularly interesting in view of the fact that the anterior region does not take much part in the swimming movement of the fish, whereas the tail region has an active role in this process. Thus, despite the prominent red muscle in the caudal region, the fat present therein is utilized to a greater degree to bring about the quick lashing movements of the tail. On the contrary, in the anterior region at which there is little active movement, fat is not much utilized and therefore stored. The middle region however, is the most active in the light of the fact that lipase and SDH activity is highest there. This is in conformity with the effort of the fish in the snake-like movement of the body for forward progression. The effort of the lashing movement of the tail comes next in steering the body for changing the direction of travel. Thurston (1958) obtained comparable results with regard to fat content in the red muscle of Alaska pink salmon. Ono et al. (1959) have also shown that although fat is present in large quantities in the red muscle cells, it is richer in the cephalic portion and decreases gradually towards the tail region. So also, the presence of considerable amounts of fat in the red muscle indicates that fat is the major fuel for energy and fatty acids are oxidized here at a higher rate than in the white muscle. That lipids are utilized to a larger extent in the red muscle of the fish was shown by Bilinski (1963) in rainbow trout and in the present study (Chapter 5) in three fishes of the Family Cyprinidae.

More recently Bilinski (1964) found that in the salmon,

the fatty acid oxidizing system is more complex in the red lateral line muscle than in white muscle. He also noted that the red muscle has a very pronounced ability to metabolize acetate. This is in accordance with the fact that a higher concentration of succinic dehydrogenase was found in the red muscle than in the white in the present study. The high SDH activity indicates a high oxidative metabolism involving a rapid oxidation of fatty acids by the red muscle via the Krebs cycle. In the white muscle SDH activity was rather low. It is quite obvious therefore that oxidative metabolism should be low in this muscle. It may be mentioned here that Umemura (1951), Fukuda (1958) and George (1962) found SDH activity to be considerably more in the red than in white muscle, which is in agreement with the present findings.

That the enzyme lipase has a significant role in the utilization of fat by muscle has been pointed out (George and Talesara, 1962 b). They have shown that the pigeon pectoralis can metabolize fat only after the hydrolysis of fat into fatty acids and glycerol. Consequently, the high concentrations of lipase in the red muscle where fat also predominates is to be expected. George and Scaria (1957) correlated the lipase concentration of the muscle with the extent of fat utilization depending on the activity of the muscle. They showed that there is a significant relationship between the lipase activity of the flight muscle and the bird's ability for sustained flight. In the case of the

developing chick heart, lipase activity was found to be directly proportional to the rate of heart beat (George and Iype, 1959).

The high fat content and lipase activity in the red muscle denotes a predominance of fat metabolism wherein fat forms the chief fuel for muscular contraction. The purpose of the high glycogen content of the red muscle however remains to be explained. It is possible that during periods of low activity of the animal, there is a shift from oxidative metabolism to glycolytic metabolism.