CHAPTER 4

ON THE IRON CONTENT IN THE RED AND WHITE MUSCLES OF A MIGRATORY AND A NON-MIGRATORY FISH DURING DIFFERENT STAGES OF MATURITY

In the muscle, iron forms an important constituent of various soluble organic compounds of high molecular weight, such as the heme enzymes like the cytochrome system, catalase and of the specific muscle pigment, myoglobin.The latter contains the bulk of the haeminic iron, eg. human muscle has been shown to have an average of 15 - 20 g. of myoglobin per 1000 g. dry weight, amounting to 50 - 68 mg. of iron per 1000 g. (Dreyfus, 1952). Muscle proteins, myosin and actin also have small amounts of iron (Dreyfus and Schapira, 1949), the exact function of which is not well understood.

Although considerable work has been done on the iron content in the whole muscle of marine and freshwater fishes (Parks and Rose, 1933; Ranganathan, 1938; Nilson and Coulson, 1939; Khorana et al., 1943; McCance and Widdowson, 1946; Airan, 1950), yet practically nothing is known about its distribution in the red and white muscles individually. It was observed in these fishes (Peterson and Elvehjem, 1928; Parks and Rose, 1933) that those having dark coloured flesh contained about 75% more iron than the ones with pale colour. They have further shown that the saltwater fishes were richer in iron than the freshwater ones. However, information regarding the changes in the iron content when marine fishes migrate

to freshwater is lacking. Hence, the present investigation was undertaken to assess quantitatively the concentration of iron in the red and white muscles of a migratory and a non-migratory fish.

MATERIALS AND METHODS

The migratory fish, Hilsa ilisha and the non-migratory form, Hilsa toli in different stages of maturity were utilized for the present investigation. They were procured as described in earlier chapters.

The fishes were sacrificed immediately after collection and pieces of the lateral muscle were excised from the middle (below dorsal fin) and tail regions. To prevent the possible leaching of the tissue by melting ice, they were packed individually in polyethylene bags, kept in ice and transferred to the laboratory. The red and white muscles of both the regions were separated, cleared off skin, bones, connective tissue and dehydrated to constant weight in a hot air oven at 100°C.

The samples thus obtained were also used as sources of material for other experimental works besides that recorded here.

The iron content was determined by Kennedy's method (Hawk et al., 1954). A known weight of the dried muscle was digested in a mixture of concentrated sulphuric acid and 60% perchloric acid (5:1 ml) and after appropriate dilution, 5 ml of 20% potassium thiocyanate was added to 10 ml aliquots of the digested material. The standard and blank were run along with the samples. The colour developed was extracted in 10 ml of amyl-alcohol and the intensity was

read on a " Spectronic 20 " Bausch and Lomb colorimeter at 480 mμ. The iron content was expressed as mg / 100g. wet weight, which was calculated from the original dry weight and water content of the muscle. This was found necessary as the fat content of the muscle was exceptionally high and variable which could thus induce gross errors.

RESULTS

The distribution of iron in the red and white muscles from the middle and tail regions of H.ilisha and its changes during different stages of growth and migration are shown in Table I. Table II on the other hand, presents similar data for the non-migratory marine H.toli, as well as for some specimens which had drifted into the river at the time of the highest high tide of the year.

In all the stages of maturity of H.ilisha, the red muscle contained a higher concentration of iron than the white and also greater as compared to the red muscle of H.toli. In the white muscle of both the species, there was however, not much variation. But a regional difference in the the amount of iron was observed in all the stages of both the species, wherein, the red muscle from the middle region was richer in iron than the same muscle from the tail. In the latter region on the other hand, the reverse condition existed, viz. the white muscle of tail region possessed more iron (Tables I & II).

It was clear from the values obtained on dry weight

TABLE I

Distribution of iron in the red and white muscles of *H. ilisha* at various stages of maturity

Place of collection	Stage of Gonad	RED MUSCLE mg. iron/ 100 g. wet tissue			WHITE MUSCLE mg. iron/ 100 g. wet tissue		
		Middle region	Tail region	Average	Middle region	Tail region	Average
SEA	Immature (3) Group I	16.213 ± 0.616	13.245 ± 0.591	14.73 ± 0. 604	1.592 ± 0.26	2.64 ± 0.173	2.12 ± 0.216
	Immature (4) Group II	19.915 ± 1.01	17.305 ± 0.72	18.61 ± 0.87	2.718 ± 0.14	3.34 ± 0.35	3.03 ± 0.25
BHADBHUT (River mouth)	Mature (4)	16.695 ± 0.818	14.72 ± 0.791	15.71 ± 0.804	1.9 ± 0.117	2.56 ± 0.241	2.23 ± 0.178
MAKTHAMPORE AND ZADESWAR (Freshwater zone)	Mature (6)	14.35 ± 1.12	13.95 ± 0.943	14.15 ± 1.03	1.537 ± 0.104	2.128 ± 0.172	1.83 ± 0.14
	Spent (4)	13.607 ± 0.95	13.13 ± 0.888	13.368 ± 0.919	1.411 ± 0.132	2.046 ± 0.218	1.73 ± 0.175

± standard deviation. Figures in parentheses indicate the number of fishes analysed.

TABLE II

Distribution of iron in the red and white muscles of *H. toli* at various stages of maturity

Place of collection	Stage of Gonad		RED MUSCLE mg. iron/ 100 g. wet tissue			WHITE MUSCLE mg. iron/ 100 g. wet tissue		
			Middle region	Tail region	Average	Middle region	Tail region	Average
SEA	Immature	(4)	9.25 ± 0.76	6.45 ± 0.714	7.85 ± 0. 74	1.42 ± 0.17	1.95 ± 0.045	1.69 ± 0.107
	Mature	(5)	10.37 ± 0.57	7.4 ± 0.45	8.88 + 0.51 -	1.24 ± 0.14	1.58 ± 0.086	1.411 ± 0.113
	Spent	(4)	9.89 ± 0.282	6.91 ± 0.114	8.397 ± 0.198	1.152 ± 0.104	1.49 ± 0.054	1.32 ± 0.079
BHADBHUT (River)	Mature and Spent	(3)	9.4 ± 0.56	6.74 ± 0.097	8.07 ± 0.329	1.38 ± 0.038	1.696 ± 0.025	1.54 ± 0.032

± standard deviation. Figures in parentheses indicate the number of fishes analysed.

basis that both muscles from the tail region had a greater level of iron than those from the middle part.

The quantity of iron in the red muscle from different stages of the migratory species of Hilsa was much more as compared to similar stages of the non-migratory fish (Table I & II). Further, an increase in iron content of both muscles was observed in H. ilisha of Group II marine immature fishes. This increase was more prominent in the red muscle. The Group II fishes also possessed better developed gonads, together with higher fat, electrolyte content and cholinesterase level than Group I marine immature fishes (Chapters 3, 5, 6 and 8). The activity of endocrine glands especially the thyroid (unpublished observations from this laboratory), resembled that of migrating salmons from tidal waters. Hence, this stage of H.ilisha (Group II) may be considered to be the one which are on their way to the spawning grounds.

A gradual reduction in iron content took place in both muscle types of H.ilisha (marked change in the red muscle only) during their migratory ascent to the river. Thus the spent fishes from the freshwater zone showed the minimum values, but their red muscle was richer in iron than that of mature and spent H.toli. The fishes from the river mouth showed intermediate iron levels.

Upon maturation, in H.toli there was a slight increase in iron content in the red muscle. The marine spent specimens as well as the mature and spent ones from freshwater were poorer in iron content than the mature fishes from the sea.

DISCUSSION

Extensive studies from this laboratory on a variety of vertebrate and invertebrate skeletal muscles have clearly shown the presence of two basic types of fibres, the red and white. The former possess numerous mitochondria, high concentrations of fat and oxidative enzymes and thus adapted for aerobic metabolism using mainly fat as the energy source whereas, the latter with few mitochondria, low levels of fat and oxidative enzymes but with high concentrations of glycogen and glycolytic enzymes for an anaerobic metabolism (George and Berger, 1966). The red and white fibres of fishes too, have their respective metabolites and fuel as described above (Boddeke et al., 1959; George, 1962; George and Bokdawala, 1964).

The red fibres not only contain more fat and an oxidative machinery for fat utilization, but also a higher content of myoglobin (Drews and Engel, 1961; Chinoy, 1963) than the white, thereby indicating their richer store of iron. Thus the red muscle of Scatophagus argus and Labeo rohita (Alexander, 1955) as well as the red fibres of the pigeon breast muscle (Talesara, 1961) were like wise found to contain more iron than the white. The presence of high levels of iron in the red muscle of all the stages of H.ilisha and H.toli are also in agreement with the above findings.

On the basis of the distribution of myoglobin and cytochrome c in many fishes (mainly Scombroids) (Matsura and Hashimoto, 1954); in different vertebrates (Korzhuev,

1961), it was postulated that higher the myoglobin content of the muscle, the greater would be its capacity for respiratory metabolism. Thus the pigment concentration of muscles of vertebrates adapted to higher altitudes (where respiratory rate is higher due to lower oxygen level) would be higher than their low altitude counterparts (Tappan and Reynafarje, 1957; Anthony et al., 1959; Reynafarje, 1962).Nair (1952) while studying the iron content in the pectoral muscle of some Indian birds, reported a greater concentration amongst the good fliers, thereby suggesting a higher quantity of myoglobin in their muscles. Saito (1955) recorded a higher iron content in the muscles of active fishes than in those of the sluggish ones. In the light of these findings, the presence of higher amounts of iron in the red muscle of migratory fish, H.ilisha as against the non-migratory fish, H.toli, may be considered as an indication of its higher oxidative capacity than that of the non-migratory fish.

Since the amount of myoglobin depends on the extent of muscular activity and exercise, it is important to consider the influence of thyroid hormones on it. Thyroid hormones are believed to activate central nervous system and cause increased locomotor activities in muscles. Hickman (1959) performed several experiments on starry flounder, Platichthys stellatus, and concluded that greater activity of the thyroid gland correlates with greater oxygen demands in seawater. The cytochrome c in tissues have been shown to

be dependent on the activity of the latter gland (Klitgaard and Hart, 1960). Thyroxin is assumed to influence oxygen consumption of tissues by a change in cytochrome levels (Drabkin, as cited by Vest and Wang, 1950). In rabbits thyroidectomy cause a rise of 15 - 25% in serum iron and a slight fall in tissue iron. Thyroxin cause a reversion of these levels to normal (Prina, 1951). It was observed that the thyroid gland was more active in migrating H.ilisha (unpublished observations from this laboratory). Thus the rise in iron content obtained in Group II marine immature H.ilisha could be accounted for.

In the spawning H.ilisha, an increase in iron content could be expected, but on the contrary, as these fishes ascend the river, there was a gradual reduction in both the muscles. The minimal values were obtained for the spent fishes from freshwater zone. Starvation might be a cause for the above result, since nutritional iron deficiency tends to decrease myoglobin levels in both skeletal and heart muscles of growing pups (Gubler et al., 1957). During fasting loss of weight in rats the total amount of iron contained in the gastrocnemius muscle has been shown to be lower than in the controls (Dreyfus and Schapira, 1948).

The reduction in the metabolic rate during the transfer of marine fishes to freshwater has been shown by several workers (Hickman, 1959; Job, 1959). Peterson and Elvehjem (1928) reported that the saltwater fishes contained about 40% more iron than the freshwater ones. It is common knowledge that at an increased salinity, the avail-

ability of oxygen is affected. Thus it is tempting to assume that the lowering of iron content in the muscles of H.ilisha from the freshwater zone may be either due to reduced oxidative metabolism or due to the readily available oxygen there in which case the problem of storage does not arise.

Another factor which may be leading to reduction in iron content in the above mentioned fishes from the freshwater zone may probably be the degenerative changes occurring in their muscles, which in turn is brought about by diverse factors as discussed earlier (Chapter 2). In muscular diseases (eg. muscular dystrophy) the metabolism of this metal has been shown to be disturbed, wherein, it is lowered due to the loss of myoglobin (Dreyfus, 1952).

Based on the present state of our knowledge it is not possible to pinpoint which particular factor is mainly responsible for the changes in iron content of the muscles observed in the different stages of H.ilisha. It may be concluded that an interplay of the various factors discussed above may be the cause for the observed changes.