CHAPTER 6

CHANGES IN CALCIUM AND PHOSPHORUS CONTENTS OF THE RED AND WHITE MUSCLES OF *HILSA ILISHA* AND *HILSA TOLI* DURING DIFFERENT STAGES OF LIFE CYCLE

Phosphorus is known to be an important constituent of numerous intermediate compounds and co-enzymes which are essential for the metabolism of carbohydrates and fats as well as for many oxidation reduction reactions and other intracellular processes. Thus phosphate cycles are vital in regulating tissue metabolism. The significance of phosphorylation and generation of high energy phosphate compounds like ATP and CP in the energetics of muscular contraction is well recognized. Calcium ions, besides their essential role in excitation-contraction coupling in skeletal muscle, serve as activator of many enzymes.

The phosphorus and calcium contents of fish muscle have been shown to exhibit appreciable variation from species to species (Saha and Guha, 1939; Niyogi *et al.*, 1941). In the mackerel the amount of phosphorus ranged from 330 - 740mg./ 100 g. and in palice from 240 - 410 mg./ 100 g. (Van der Velde, 1932). Declercq (1934) showed that Ca/P ratio in herrings decreased from 3.6 to 1.3 between November and January. The freshwater fishes are reported to possess lower amounts of calcium and phosphorus (Basu *et al.*, 1942; Airan, 1950) than the marine ones. These fluctuations in the calcium and phosphorus contents of muscles in different

fishes have been attributed to numerous factors, especially the concentration of calcium in the water, age, sex and sexual maturity (Vinogradov and Odum, 1953). Calcium and phosphorus levels in the serum have been shown to be elevated in female fish during egg laying season(Laskowski, 1936). Bailey (1957) demonstrated that these changes can be induced in either the male or female goldfish by the administration of estradiol. However, Nakano (1960) reported that the variations in phosphorus levels were negligible in different individuals of the same species, but significant depending upon the kind of muscle and its location.

Practically no information is available regarding the changes in the muscle phosphorus levels during fish migration except for the studies of Chang et al. (1960) on the sockeye salmon. Considerable increase of inorganic phosphates following severe muscular exercise have been observed in the muscles of yearlings and adults of steelhead trout (Nakatani, 1956, 1957). Similarly Jones and Murray (1957) found that the ATP levels were rapidly depleted (80%) in the muscles of codlings which were driven to exhaustion.

In view of the importance of phosphorus and calcium in muscle metabolism, it was thought worthwhile to investigate the changes in their contents in the red and white muscles of a migratory fish, H.ilisha at different stages of its life cycle and to compare the results with those of the closely related non-migratory species, H.toli. Hence the justification for the present study.

MATERIALS AND METHODS

The dried red and white muscles from the middle and tail regions of different stages of H.ilisha and H.toli used in the present investigation were obtained as described in chapter 4.

The calcium content was estimated by the flame photometric method using an Eel flame photometer. 200 mg. of each sample was taken in clean crusibles and ashed in a muffle furnace at about 350° to 400°C., till the entire organic matter was oxidized and a constant weight was obtained. The ashed samples were dessolved in 5 ml of 1N analar HCl. The concentration of calcium in the samples were determined by comparing their readings with those of a standard solution containing 2 mg. calcium per 100 ml. (Evans Electroselenium Ltd, Ref. No. 1704).

For the estimation of phosphorus, 100 mg. of the dried red and white muscle from both regions were taken separately and the fat was completely extracted from them using a 2:1 chloroform methanol mixture. The extracted fat as well as the tissue were digested in 2.5 ml of 5N sulphuric acid and subsequently oxidised with 30% hydrogen peroxide. The phosphorus content was estimated by the method of Fiske and Subbarow (Hawk et al., 1954).

After proper dilution of the digested samples with glass distilled water, 1 ml aliquots were taken in graduated test tubes. The blank used was 1 ml of glass distilled water, while the standard solution contained 0.04 mg. phosphorus

in 1 ml. Then 2.5% ammonium molybdate solution (1 ml) followed by 0.4 ml of aminonaphthol-sulphonic acid reagent were added to each test tube, and diluted to 10 ml with glass distilled water. The solutions were mixed thoroughly and allowed to stand for 5 minutes. The optical densities were read on a Klett-summerson photoelectric colorimeter at 660 mμ. The phosphorus content in the samples was determined by comparing the readings with that of the standard.

The phosphorus and calcium contents were expressed as mg./100 g. wet muscle, calculated from the original dry weight and the water content of the muscles. This was found necessary as the fat content was high and variable, but the fat plus water levels were more or less constant.

The red and white muscles from 9 - 10 juveniles were pooled together for the analysis of calcium and tissue phosphorus and only two experiments were conducted.

RESULTS

The values obtained for the calcium content of the red and white muscles from the middle and tail regions of H.ilisha and H.toli and their changes during different stages of life cycle are presented in Table I and II respectively. A regional difference in the calcium content was observed for both types of muscles at all stages, wherein the middle region showed higher values than the tail. The white muscle was found to be richer in calcium as compared to the red muscle in all the stages, except in the juveniles,

where the reverse was the case. In H.ilisha a reduction in calcium took place from both muscles (Table I) upon completion of migration and spawning, but in H.toli no significant difference was noticed among the values obtained for the marine immature, mature and spent fishes. H.toli collected from the river however, showed comparatively lesser values.

Tables III to VI represent the distribution of phosphorus in the red and white muscles from the above mentioned regions and its variation during the different stages of maturity in both species of Hilsa. In both species the white muscle contained higher tissue phosphorus and lower lipid phosphorus content than the red. A marked difference in the tissue phosphorus level between the middle and tail regions were observed only for the white muscle wherein, the former region showed higher values. The lipid phosphorus content was lower in the tail region in both muscles.

In marine H.toli a wide difference in the tissue phosphorus contents of the red and white muscles could not be observed upon maturation and spawning (Tables III and IV). However, the average values obtained were higher in both muscles of mature and spent fishes; but their lipid phosphorus content was lower than in immature ones. The values obtained for the drifted H.toli were very inconsistent.

The changes in phosphorus content of red muscle on migration and spawning (Table V) were different from those of white muscle (Table VI). In the red muscle of

Table I. Calcium content of the red and white muscles of H. ilisha in various stages of maturity.

Place of collection	Stage of Gonad	RED MUSCLE mg Ca/100 g. wet tissue			WHITE MUSCLE mg Ca/100 g. wet tissue		
		Mid.region	Tail region	Average	Mid.region	Tail region	Average
Sea	Immature Group I(3)	6.61±0.52	5.96±0.44	6.28±0.48	7.08±0.46	6.25±0.66	6.67±0.56
	Immature Group II (4)	7.52±0.54	6.77±0.32	7.15±0.43	10.01±0.62	8.53±0.71	9.27±0.66
Bhadbhut (River mouth)	Mature(4)	6.12±0.61	5.32±0.52	5.72±0.56	6.78±0.59	6.03±0.91	6.41±0.75
Makthampore & Zadeswar (F.W.zone)	Mature & Spent(8)	5.45±0.62	4.80±0.49	5.13±0.56	5.97±0.43	5.71±0.39	5.84±0.41
	Juveniles (2 groups)			5.11			4.10

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

Table II. Calcium content of the red and white muscles of *H. toli* in different stages of maturity

Place of collection	Stage of Gonad	RED MUSCLE mg. Ca/100 g. wet tissue			WHITE MUSCLE mg. Ca/100 g. wet tissue		
		Mid.region	Tail region	Average	Mid.region	Tail region	Average
Sea	Immature (4)	7.22±0.48	6.31±0.44	6.76±0.46	9.39±0.76	8.47±0.65	8.93±0.702
	Mature & Spent (6)	6.93±0.62	6.15±0.52	6.54±0.57	9.24±0.65	8.23±0.69	8.74±0.67
Bhadbhut (River)	Mature (3)	6.19±0.36	5.59±0.32	5.89±0.342	8.11±0.59	7.79±0.387	7.95±0.49

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

Table III. Phosphorus content of the red muscle of H. toli in various stages of maturity.

Place of collection	Stage of gonad	Middle region			Tail region			Average		
		mg. P/100 g. wet tissue								
		Total P	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.
Sea	Immature (4)	716.36±	575.92± 16.90	140.44± 10.60	660.20	566.20± 16.03	94.00± 8.06	688.28	571.06± 16.47	117.22± 9.33
	Mature & Spent (6)	706.76	593.30± 17.80	113.46± 13.60	588.42	575.42± 16.15	83.76± 11.30	582.59	584.48± 16.98	98.11± 12.45
Bhadbhut (River)	Mature (3)	691.50	571.60± 22.72	108.30± 12.68	638.83	570.90± 20.42	67.93± 10.80	665.17	577.05± 21.57	88.12± 11.74

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

Table IV. Phosphorus content in the white muscle of *H. toli* in various stages of maturity.

Place of collection	Stage of gonad	Middle region			Tail region			Average		
		mg. P/100 g. wet tissue								
		Total P.	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.
Sea	Immature (4)	662.04	635.84± 17.02	26.20± 8.94	605.78	589.94± 18.84	15.84± 5.47	633.91	612.89± 17.93	21.02± 7.21
	Mature & Spent (6)	668.92	650.30± 16.26	18.62± 6.63	611.67	598.81± 11.53	12.86± 4.12	640.30	624.55± 13.85	15.74± 5.33
Bhadbhut (River)	Mature (3)	657.30	632.36± 18.70	14.94± 5.83	607.36	596.86± 17.12	10.50± 4.84	632.33	619.61± 17.91	12.72± 5.39

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

Table V. Changes in Phosphorus content in the red muscle of H. ilisha during different Stages of maturity

Place of collection	Stage of gonad	Middle region			Tail region			Average		
		mg. P/100 g. wet tissue								
		Total P.	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.
Sea	Immature Group I(3)	702.20	553.10± 14.46	149.10± 9.15	654.10	555.80± 12.83	98.30± 7.67	678.15	554.45± 13.65	123.70± 8.41
	Immature Group II(4)	688.27	504.56± 18.70	183.71± 20.80	623.72	517.52± 15.50	106.20± 14.83	656.00	511.04± 17.10	144.96± 17.80
Bhadbhut (River mouth)	Mature (4)	681.78	525.08± 16.64	156.70± 10.08	620.50	522.90± 13.07	97.60± 8.84	651.14	523.99± 14.86	127.15± 9.46
Mak.& Zad. (Fresh water)	Mature & Spent (8)	667.30	535.50± 15.60	131.80± 13.04	613.86	537.55± 14.14	76.31± 9.04	640.58	536.53± 14.87	104.05± 11.04
	Juveniles (1 group)	-	-	-	-	-	-	570.45	-	

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

Table VI. Changes in Phosphorus content in the white muscle of H. ilisha during different stages of maturity.

Place of collection	Stage of gonad	Middle region			Tail region			Average		
		mg. P/100 g. wet tissue								
		Total P.	Tissue P.	Lipid P.	Total P.	Tissue P.	Lipid P.	TotalP.	Tissue P.	Lipid P.
Sea	Immature Group I(3)	650.30	523.00± 18.05	27.30± 7.60	607.36	592.18± 10.24	15.18± 5.09	628.83	607.59± 14.15	21.24± 6.35
	Immature II(4)	680.94	632.15± 26.83	48.79± 14.07	638.80	609.40± 14.18	29.40± 8.94	659.87	620.78± 20.50	39.09± 11.50
Bhadbhut (River mouth)	Mature (4)	633.52	598.06± 15.97	35.46± 9.12	596.32	576.58± 13.42	19.94± 6.29	614.92	587.32± 14.70	27.60± 7.70
Mak. & Zad. (Fresh water zone)	Mature & Spent (8)	613.00	579.74± 16.50	33.26± 6.55	574.00	560.40± 14.20	13.76± 5.24	593.58	570.07± 15.35	23.51± 5.90
	Juveniles (1 group)	-	-	-	-	-	-	484.50		-

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

Group II marine immature H.ilisha, a drop in tissue phosphorus, but at the same time a rise in lipid phosphorus was observed as against the Group I fishes. The former fishes also differed from the latter in having an enormous store of fat (Chapter 3). During the migratory ascent, a gradual increase in tissue phosphorus, and a corresponding decrease in lipid phosphorus was noted. The decrease of the latter was higher than the increase of the former in mature and spent fishes from the freshwater zone. Moreover as compared to Group I marine immature fishes, the gravid and spent ones from the river revealed a lower tissue as well as lipid phosphorus level.

In contrast to the red muscle, the white muscle showed a gradual decline in both tissue and lipid phosphorus contents as the fishes ascent the river (Table VI). Thus the total phosphorus content was lower in the mature and spent fishes from freshwater as compared to the immature forms. Among immature H.ilisha (marine) in Group II fishes, the lipid as well as tissue phosphorus was higher than in all other stages of the life cycle.

The red muscle of juveniles showed comparatively higher values for tissue phosphorus than those of other stages, but the white muscle exhibited the reverse condition.

DISCUSSION

The values obtained in the present investigation for the calcium level were on the whole much less compared

to those reported for other Indian marine fishes by various workers (Saha & Guha, 1939; Niyogi et al., 1941). Relatively higher values have been obtained for the marine fishes of the Indian seas than the figures published from other parts of the world. Thus fishes from Bombay area were found to have 1136 mg. to 97.5 mg./ 100 g., and majority of the species showed values above 350 mg./ 100 g.(Setna et al., 1944). This higher calcium level according to Causeret (1962) denotes a substantially changed Ca/P ratio, since the level of phosphorus remains normal in the above mentioned fishes. Rose (1933) obtained average calcium contents of 22 mg./ 100 g. in the meat of lean fish and 19 mg./ 100g. in fatty fish, the extreme values ranged between 6 mg. and 120 mg. Similarly in the salmon also a low calcium level has been recorded (2.84 - 2.95 mM/ Kg. tissue water) by Parry (1961). In the mammals calcium content was found to vary between 2.3 to 7.3 meq/ Kg. wet weight of muscle (Widdowson and Dickerson, 1964). Therefore the values for the calcium content obtained for H.ilisha and H.toli are nearer to the figures obtained for fishes and mammals from abroad and do not tally with those of the marine indigenous fishes.

In migrating salmons the level of calcium has been shown to remain more or less constant (Parry, 1961). On the contrary, in the mature and spent H.ilisha from the river a significant reduction in both the types of muscles was obtained in the present investigation (Table I). Studies

of Idler and Tsuyuki (1958) have shown a lowered plasma calcium level in sockeye salmon after the spawning migration. The calcium content of fish muscle is known to be dependent on its concentration in the water in which they live (Usui et al., 1937). Thus the tissue calcium in specimens of Hypomesus olidus taken from two Japanese lakes of different salt levels was found to be nearly proportional to the calcium content of the lake water itself. From an analysis of the water (Chapter 5) it was found that the seawater contained a much higher level of calcium (21.5 to 23 meq/l.) than the water of the river (0.08 to 0.15 meq/l.). This accounts for the reduction in this electrolyte level from the muscles of the freshwater mature and spent H.ilisha.

Another plausible relationship between calcium on the one hand and fat utilization on the other, has been suggested by French (1942). He showed that in growing albino rats, the amount of calcium utilization decreased moderately but consistently, in pace with the increasing fat content of the diet. The Group II marine immature H.ilisha have been shown to possess an enormous store of fat in their trunk muscles (Chapter 3) as well as a higher calcium level, which gradually declines during the fasting migration. Incidentally, the fat content too showed a corresponding reduction.

In an earlier chapter (Chapter 3) it has been shown that the fat content of the red muscle of both the

species studied was higher than that of the white. The present investigation revealed a higher lipid phosphorus content also in the red muscle, therefore, one might expect greater amount of phospholipids in these muscles. The white muscle on the contrary revealed a higher tissue phosphorus level. The white muscle of birds (Kare, 1951) and rabbit (Ogata, 1960) have been shown to possess a greater load of ATP, CP and tissue phosphates. An inverse relationship between energy rich phosphates and myoglobin, cytochrome oxidase activity was demonstrated by Lawrie (1953). In other words the level of energy rich phosphates are inversely proportional to the content of iron in a muscle. Iron content was found to be more in the red muscle of both the species of Hilsa (Chapter 4), with that of H.ilisha having more than in H.toli.The level of phosphorus indicated the reverse, viz. higher in the white muscle and the red muscle of H.toli possessed more as compared to that of H.ilisha, thereby supporting the work of above mentioned author.

The phosphorus content has been shown to be related inversely to fat utilization. Thus the fat content of the carp muscle is known to be increasing as growth progresses, while the phosphorus content to be decreasing in response to the fattening of the fish (Ono et al., 1953). A similar inverse relationship between phosphorus and fat was observed during the development of the rainbow trout by the above authors (1959 a, b). Thus it was concluded by them that during the utilization of fatty substances, an increase in

phosphorus occurred due to its necessity as an activator in the formation of energy and a carrier of it. A higher utilization of fat for energy is evident from the gradual reduction of fat (Chapter 3) and lipid phosphorus during migration. However, the increase in phosphorus observed in mature and spent fishes from the river was not proportional to the reduction in fat, as evident from the lesser phosphorus content in their muscles as compared to Group I marine immature fishes. This finding and the decrease from the white muscle may probably be due to starvation, because a reduction in the concentration of phosphorus per unit weight of the muscle is reported to take place in adult underfed cockerels (Dickerson and McCance, 1960).

Studies of Chang et al.(1960) on the distribution of phosphorus compounds in the different tissues and organs of sockeye salmon, showed that in the muscles the total phosphate per unit weight of muscle remained more or less constant in females during the river migration, but decreased markedly in the males upon arrival at the spawning grounds. In the present investigation also, the total phosphorus level of both muscles decreased gradually during the migratory ascent.

The importance of phosphorus compounds in carbohydrate metabolism is well known. An accumulation of inorganic phosphorus has been associated with an accelerated metabolism of carbohydrate (Kaplan, 1951). The increase in phosphorus in the liver of cold exposed rats according to

Beaton (1963) might reflect an increase in carbohydrate metabolism. Hence a decrease in phosphorus content in the white muscle of H.ilisha during migration when the fish does not feed may be a manifestation of decreased carbohydrate utilization. Further evidence for such an assumption comes from the work of Lardy (1951) and Kachmar and Boyer (1951), who reported a relation between potassium and glycolytic sequence. According to them potassium ions are essential for the transfer of phosphate from phosphopyruvate to ADP in order to form pyruvate and ATP. A decrease in the potassium level was also observed in mature and spent H.ilisha from the freshwater zone (Chapter 5). It may also be mentioned here that eventhough the red fibres of fish muscle contain more glycogen than the white (Bokdawala, 1965; Bone, 1966), yet after strenuous exercise glycogen is depleted from only the white fibres, and not from the red ones (Bone, 1966). This difference in metabolic adaptation may be a factor causing the observed difference in tissue phosphorus changes of the red and white muscles in the migrated mature and spent H.ilisha from the river.

Degeneration of muscle fibres observed in the migrating H.ilisha (Chapter 2) may also influence the phosphorus content. During nutritional muscular dystrophy in various laboratory mammals and chick, an increased incorporation of P^{32} as compared to normal muscle has been reported (Cohen and Warringa, 1951; Fitch and Dinning, 1959; Calvert et al., 1961). The decrease in energy compounds such as ATP and CP

in the dystrophic muscle of the chick while the uptake of total phosphorus increases, indicate that phosphorus metabolism may be specially affected during dystrophy (Calvert et al., 1961).

It is not clearly understood whether the metabolism of calcium and phosphorus in fishes are under hormonal control or not. Srivastava (1960)has shown an increase in the uptake of radiophosphorus from the medium by the yearlings salmon, Salmo salar L. on thyroxin administration. If thyroid has got such a function, its increased activity (unpublished observations from this laboratory) may be responsible for the phosphorus balance during the migration of H.ilisha.

The ultimobranchial body of fishes, which has been considered to be a homologue to parathyroid of higher vertebrates also showed increased activity during migration (unpublished observations from this laboratory). Moreover, parathyroid is known to regulate calcium and phosphorus levels through its secretion, but since hardly any experimental work has been done on the metabolic effects of the secretion of the ultimobranchial bodies, it is not possible to say whether they have a definite control on calcium and phosphorus metabolism in fishes.