CHAPTER 5

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

The salt concentration of aquatic vertebrates, with the exception of myxinoid cyclostomes and elasmobranchs (Robertson, 1954) is about a quarter to a third that of sea water. The regulation of salt and water concentrations in blood and tissues is of importance to all animals living in environments with which they are not in osmotic equilibrium. In migratory fishes the ionic concentration in blood and muscle will be affected by changes in the external salinity which they encounter during migration. In the sea, these fishes have to overcome dehydration and excrete the excess sodium and potassium, while in freshwater they must prevent the influx of excess water into the body, conserve sodium and excrete surplus potassium in order to survive. Drilhon and Pora (1936) suggested two possible responses of muscle tissue to such changes: 1. it can follow the osmotic and ionic changes in the blood more or less closely or 2. it can behave differently and thus act as a reservoir of ions or water to buffer blood changes. Gordon (1959) in a study on the ionic regulation in the brown trout, *Salmo trutta* L., showed that the muscle did not act as storage sites either for sodium or for potassium during or after adjustments to non-fatal osmotic stresses. Parry (1961) has pointed out that in a migratory fish such as salmon, the response of muscle is of great importance to encounter the external salinity changes which it has to confront during migration.

Wide variations in the level of sodium and potassium have been shown to exist in fish muscle. According to Vinogradov and Odum (1953) diverse ecological conditions might partially explain this large range of variations. Studies of McBride and MacLeod (1956 a, b), Thurston and MacMaster (1960) and Thurston (1961 a, b) indicated that variations in composition of the fish muscle depended on a number of factors such as size, sexual maturity, season, area of capture and part of the fish from which the sample was taken.

The present investigation was undertaken with a view to understand how well the sodium and potassium contents in the red and white muscles of *H.ilisha* are maintained at an equilibrium during the various stages of the life cycle from the sea to the river. Studies were also made on the different stages of a closely related non-migratory marine species, *H.toli* for comparison.

MATERIALS AND METHODS

The dried red and white muscles of different stages of *H.ilisha* and *H.toli* used in the present investigation were obtained as described in the previous chapter.

In the case of juveniles of *H.ilisha*, since the fishes were small, complete red and white muscles from the lateral line region of each fish was separated and the tissues from 8 - 10 fishes were pooled together before drying.

Sodium and potassium contents were estimated by the flame photometric method using an Eel flame photometer. Known weights of the tissues were ashed in a muffle furnace at 350° - 400°C., till all the organic matter was oxidized and a constant weight was obtained. The ashed samples thus obtained were dissolved in 10 ml 1N HCl and then brought to proper dilutions using glass distilled water. Inorganic phosphates and sulphates were removed by barium precipitation since they are known to interfere in the flame photometric estimation (Shapiro and Hoaglund, 1948).

Standard curves for sodium and potassium for different concentrations of analar grade chlorides of the elements were prepared. In each analysis, the reading of the maximum concentration used in the standard curve was adjusted to the 100th division of the scale on the instrument while glass distilled water was used to set at zero, and the reading for the samples prepared in the manner described above, were noted. Potassium and sodium contents in mg./100 g. wet weight were calculated from the original dry weight of the muscle and the water content. This was found to be necessary since the fat content in the samples were high and variable, but the fat plus water was more or less constant.

Samples of water collected from the sea, rivermouth, and the freshwater zone at the time of collection of fishes were also analysed for sodium, potassium and calcium by the flame photometric method. Chloride content of the water samples was estimated by the Mohr method (Kolthoff and Stenger, 1947).

RESULTS

Tables I and II show the sodium, potassium and water contents of red and white muscles in different stages of life cycle of H. ilisha and H. toli respectively. Analyses were made of the red and white muscles of the middle and tail regions separately and the average values were taken for each fish.

The potassium content was found to be more than that of sodium in both the types of muscles. White muscles showed much higher values for potassium and slightly higher values for sodium than that of red muscle.

Among the immature H. ilisha from the sea an elevated potassium content was observed in Group II fishes as compared to Group I forms. It may be mentioned here that these fishes (Group II) also showed an enormous storage of fat and better developed gonads(Stage IV). During the migratory ascent a gradual increase in sodium and water and a decrease in potassium was noted. In the case of fishes collected from the rivermouth the sodium content was more

Table I. Na^+, K^+ and Water contents in the red and white muscles of H. ilisha at various stages of maturity

Place of collection	Stage in life cycle	RED MUSCLE mg/100 g.wet tissue Sodium	RED MUSCLE mg/100 g.wet tissue Potassium	RED MUSCLE % water	WHITE MUSCLE mg/100 g.wet tissue Sodium	WHITE MUSCLE mg/100 g.wet tissue Potassium	WHITE MUSCLE % water
Sea	Immature(3) Group-I	83.86± 6.96	265.70± 10.63	66.69± 2.20	94.42± 7.93	315.70± 8.06	75.37± 1.32
	Immature(4) Group-II	73.45± 8.75	282.70± 12.44	55.30± 2.60	85.90± 5.14	329.60± 10.24	62.77± 1.95
Bhadbhut River mouth	Mature (4)	85.12± 6.56	256.70± 8.75	65.90± 1.70	93.17± 9.40	301.49± 8.30	76.31± 1.58
Makthampore & Zadeswar (F.W.Zone)	Mature (6)	94.28± 8.42	245.08± 12.84	70.37± 1.81	101.70± 7.68	284.79± 9.90	75.87± 1.73
	Spent (4)	95.35± 6.63	243.62± 9.20	72.89± 1.40	101.30± 6.40	277.62± 10.61	77.65± 2.21
	Juveniles (3 groups)	70.79± 8.06	252.63± 9.40	77.29± 0.56	69.04± 5.30	259.45± 6.88	81.99± 1.24

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

Table II. Na^+, K^+ and Water contents in the red and white muscles of H. toli at various stages of maturity

Place of collection	Stage of Gonad	RED MUSCLE mg/100 g. wet tissue Sodium	RED MUSCLE mg/100 g. wet tissue Potassium	RED MUSCLE % water	WHITE MUSCLE mg/100 g. wet tissue Sodium	WHITE MUSCLE mg/100 g. wet tissue Potassium	WHITE MUSCLE % water
Sea	Immature (4)	82.83 ± 8.24	280.25 ± 10.67	68.19 ± 2.17	95.16 ± 7.87	324.59 ± 15.50	72.97 ± 1.96
Sea	Mature & Spent (6)	78.67 ± 10.58	288.55 ± 7.21	71.92 ± 1.20	86.92 ± 8.42	335.92 ± 12.20	76.90 ± 1.41
Bhadbhut (River)	Mature(3)	70.70 ± 12.84	279.17 ± 10.14	70.46 ± 1.63	76.50 ± 11.35	321.30 ± 17.80	77.06 ± 1.23

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

Table III. The concentration of various ions in the water samples

Place of collection	Sodium meq/l Range	Potassium meq/l Range	Calcium meq/l Range	Chloride meq/l Range
Sea	590-610	13.5-14.5	21.5-23	650-670
Bhadbhut (River mouth)	165-180	3.5-4	7-8	180-190
Zadeswar (Fresh water zone)	2-3.5	0.1-0.15	0.08-0.15	2.8-3.8

or less similar to that obtained for Group I marine immature fishes, but the potassium content showed a decline. In Table III the concentration of ions such as sodium, potassium, calcium and chloride in the water samples taken from the places of collection of fishes, ie. sea, river-mouth (estuarine zone) and freshwater zone in the river are presented. Data obtained clearly show that the ionic content of the river water is obviously negligible compared to that of sea water. In the mature and spent H.ilisha collected from the freshwater zone a marked decrease in potassium and increase in sodium level was observed in both the types of muscles. The increase in sodium was more significant in the red muscle, while the decrease in potassium was more in the white muscle. In both the muscles the decrease in potassium content exceeded the rise in sodium and hence the sum of sodium and potassium concentrations was less than that recorded in immature fishes from the sea. However, much variation existed among the individual mature fishes analysed from the river. Many fishes showed similar values for sodium and potassium as were obtained for fishes from the estuarine zone.

Comparing the average values obtained for immature (Stage IV), mature (Stage V & VI) and spent H.toli from the sea, in mature and spent fishes a reduction in sodium and a rise in potassium was observed in both the types of muscles. But considering the great variations shown by individual fishes analysed in both groups, the observed changes cannot be considered much significant. In H.toli collected from the river (Bhadbhut) the values showed much inconsistency, but comparing the average values obtained with that of mature fishes from the sea, a decline in both sodium and potassium levels was observed.

DISCUSSION

As has been shown by earlier workers the potassium content was found to be much more than sodium in both the types of muscles. The difference in the ionic composition of red and white muscle was shown by Drahota (1961), who suggested that the various muscles could be grouped under two groups based on their sodium and potassium contents. Since differences in the concentration of intracellular potassium are not compensated by a corresponding change in intracellular sodium, the sum of sodium and potassium ions in the red muscle is lower (Drahota, 1961). Serter and Woo (1963) have reported that the water content and ionic composition of different skeletal muscles of rat are related to the ratio of white to red fibres; as the ratio of red to white fibres increased, sodium increased and potassium decreased proportionately. The present studies revealed a much higher amount of potassium in the white muscle than in the red, while in the sodium content no distinct difference could be noticed between the muscle types. This observation is in agreement with what has been shown by Thurston and Newman(1962) in sockeye salmon, Oncorhynchus nerka.

Disturbance in the ionic concentration of the muscle as indicated by the lowered sodium and elevated potassium was found to occur in marine Group II immature H.ilisha. It is quite probable that it may be a reflection of the changes in hormonal levels associated with migration. In the above mentioned fishes the pituitary and thyroid were found to exhibit markedly increased activity (unpublished obsererervations from this laboratory). The growth hormone is known to enhance the tolerance of brown trout to hypotonic saline solutions(Smith, 1956). Fontaine (1960) suggested that the pituitary growth hormone, by causing an imbalance of ions (especially of K^+) in the tissues may induce the fish to seek new environment and/ or increase the irritability of nerve and muscle tissues.The thyroid has been implicated in the osmoregulatory process and also in the migration of fishes (Hoar, 1958, 1959; Fontaine, 1960). According to Jenkin (1962) since the fishes show a concurrent phase of growth and maturation, it seems more probable that the thyroid may be concerned with those processes than with osmoregulation.

In mature and spent H.ilisha from the freshwater zone along with the lowering in the electrolyte level, a rise in the percentage of water was also observed in both the types of muscles. That the lowered electrolyte concentration is not likely to be due to dilution, is evident from the fact that the increased percentage of water recorded is mainly due to a reduction in the amount of fat. Parry (1961) reported an increased sodium and lowered potassium content in the muscle of salmons transferred from one salinity to other, which according to him could be interpreted as a movement of both water and ions out of muscle fibres into a slightly increased extracellular space.

Studies of McBride and MacLeod on sockeye salmon in 1956 showed high sodium levels in both males and females, while in 1957 the steep rise was recorded only in the males. According to MacLeod *et al*. (1958) this discrepancy observed may be due to the fact that the fishes do not spawn immediately on reaching the spawning grounds, but rather a week after they have arrived. During this week, the final stages in gonad development apparently occur. The same may be the case with *H.ilisha* also and the differences in the stage of physiological development of fishes sampled at the spawning grounds may be the reason for the variations noted in mature fishes collected from the river in the present investigation also.

A rise in the sodium and a drop in the potassium levels in fishes from the spawning grounds (as recorded in the present study also) has been reported by MacLeod *et al*. (1958) and Thurston and Newman (1962) in the sockeye salmon. On the other hand in the Fraser river spring salmon the sodium level dropped to one-half that found in the sea salmon during the first 100 miles, and thereafter, except in one group studied, the sodium content rose to that at the start of migration (MacLeod et al., 1958). In spawning Salmo salar from river, a rise in muscle sodium as well as potassium has been recorded (Parry, 1961). Eventhough much inconsistency exists in the results reported, it is clear from these studies that the levels of these ions in the muscle bear no relation to the content of sodium and potassium in the water from which the fishes were caught.

During the migratory ascent H.ilisha is under starvation. Studies on the effect of starvation on the water and mineral content of muscles of mammals have shown that their amounts per unit weight of muscle increased during starvation (Dicker, 1949; Huth and Elkinton, 1959; Elkinton and Widdowson, 1959). A deficiency of potassium in the diet caused a fall in its concentration in skeletal muscle and an increase in sodium content (Heppel, 1989; Conway and Hingerty, 1948; Cotlove et al., 1951; Muntwyler et al., 1953). In rats, dietary depletion of potassium was accompanied by degenerative changes in the kidney (Fourman et al., 1956; Kennedy et al., 1960). It may be mentioned that the kidney tubules in mature and spent H.ilisha from the spawning grounds also showed degenerative changes (unpublished observations from this laboratory).

The degenerative changes in the muscles of migrating and spawning *H.ilisha* have been described earlier (Chapter 2). It is known that in the various types of muscular dystrophies in man and other animals an increase in sodium and decrease in potassium takes place (Horvath *et al.*, 1955; Williams *et al.*, 1957; Young *et al.*, 1959; Zierler, 1961). The loss of muscle cells and the accompanied increase in extracellular space may be related with the changes observed in potassium and sodium levels in *H.ilisha* also as in the case in muscular dystrophy.

The distribution of water and electrolytes in the different tissues of the body is known to be controlled by the activity of hormones. In the higher vertebrates, aldosterone is known to be mainly responsible for salt balance. But in teleosts it is not known clearly whether aldosterone is secreted or not. However, Philips *et al.*(1959) have reported the presence of aldosterone in the blood of the Pacific salmon. Robertson *et al.* (1961) have suggested the possibility that this hormone may act to prevent a marked drop in plasma sodium, despite starvation and prolonged stay in freshwater. According to Chester Jones *et al.* (1959) if aldosterone or an aldosterone-like compound is not secreted in teleosts, the hydrocortisone or cortisone may be acting in a duel capacity influencing both carbohydrate and electrolyte metabolism. It was proposed by MacLeod *et al.* (1958) that the sharp rise in sodium and drop in potassium observed in salmon at the end of migration may be a result of the increased activities of hormones associated with the final stages of sexual development. Various workers have shown the presence of hyperplastic adrenocortical tissue and high levels of 17-hydroxy corticosteroids, hydrocortisone and cortisone accompanying sexual maturation and spawning in Pacific salmon (Robertson and Wexler, 1959; Hane and Robertson, 1959; Idler *et al.*, 1959). Spalding (as cited by Chester Jones, 1956) found that the injection of ACTH, DCA or cortisone caused a rise in sodium and fall in potassium in the muscles of *Salmo trutta*. It has already been pointed out (Chapter 2) that in mature and spent *H.ilisha* a higher level of adrenal hormones should be expected in the blood, since the adrenal tissue showed hyperplasia and hypertrophy as in Pacific salmons (unpublished observations from this laboratory) and this may be considered to be an important factor in bringing about the changes recorded in the present investigation also.

Besides adrenocortical tissue, the caudal neurosecretory system has also been implicated in osmoregulatory processes. Takasugi and Bern (1962) showed that injected extracts of the terminal spinal cord (including urohypophysis) of eels to *Tilapia mossambica* were capable of decreasing the sodium content of fish kept in hypertonic salt water, but increased the sodium content of fish kept in isotonic or freshwater. It may be mentioned that unlike in marine immature H.ilisha, where the bulb of the urohypophysis was found to be in storage phase; in mature and spent ones from the river their neurosecretory material was fully discharged into the blood (unpublished observations from this laboratory).

In conclusion it may be said that the ionic changes noted in H.ilisha may be mainly due to the changes in hormonal activity accompanying migration and spawning, the catabolic effects of which in turn are influenced by factors such as aging, starvation, muscular activity and the physiological adjustments required in the transition from salt to freshwater.