

STUDIES ON CHLORIDE REGULATION IN *TILAPIA MOSSAMBICA*

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INTRODUCTION

IT is well known that migratory forms like eels, salmon and estuarine forms like *Fundulus heteroclitus* show a tolerance to wide fluctuations in salinity of the medium, ranging from freshwater to 100% sea water. Similar tolerance to variations in salinity is also met with in freshwater fishes such as the guppies (Vickers, 1961) and *Etroplus maculatus* (Virabhadhrachari, 1961) which could successfully be adapted to sea water. It has been claimed by the previous workers (Keys 1938, Burden 1956, Houston, 1959) that in eels, Salmon-and *Fundulus*, the ability to survive in hypertonic and hypotonic media is dependant on the possession of chloride regulatory mechanism. Just like the fishes outlined above, *Tilapia mossambica*, a freshwater fish, is also able to survive in sea water and shows a very wide range of salinity tolerance, but it is not known whether this fish also possesses a mechanism for regulation of chlorides. The present investigation is an attempt to find out whether *Tilapia mossambica* regulates the chloride level of th& blood, if so, it is desirable to know the mode of regulation.

METHODS AND RESULTS

Acclimatisation studies were carried out to determine the total range of salinity tolerance. *Tilapia* weighing approximately 35 to 45 gms., (the size available in abundance) were collected from Chetput or Adyar in Madras and maintained in tap water in glass tanks. Acclimatisation to salinities higher than that of tap water was achieved by replacing measured amounts of tap water (2 litres) every day, with equal amounts of sea water. Distilled water was used to bring down the salinities lower than that of tap water.

Acclimatisation studies showed that *Tiiapia* is able to withstand wide fluctuations in the chloride content of the medium ranging from as low as 28 mM/litre to as high as 1800 mM/litre.

Blood chloride levels were estimated to determine whether *Tilapia* maintains a constant level of chloride against the salinity fluctuation of the environment. *Tilapia* acclimatised to 10% fresh water (28 mM/litre chlorides) 33% sea water (48 mM/litre) and 100% sea water (1800 mM/litre) were used for blood chloride analysis. Methods used for acclimatisation for fishes to the above salinities are similar to those reported by the previous workers. The fishes were not fed during the period of acclimatisation. Once the required salinity was reached in the medium (i.e. either 10% fresh water or 100% sea water) the fish were left undisturbed for 7 days before the blood was collected. This period of 7 days was arbitrarily chosen because unlike the guppies, *Tilapia* do not show changes like darkening to differentiate the acclimatised from the non-acclimatised ones. Key's (1938) work

revealed that eels get acclimatised to sea water, and attain a steady state in two days.

The usual method of collecting blood for analysis by exposing the heart and drawing the blood was to be abandoned, for, in *Tilapia* the blood was seen to clot very quickly and wastage of blood by cutting open the animal had to be avoided because only very small amount of blood could be obtained from this organism. The following procedure was adopted to obtain the blood sample. The heart and the ventral aorta were located by lifting the gill arches and the operculum, and the blood drawn, by introducing the needle into the ventral aorta directly. Oxalate-coated 1 cc. syringe was used. Even this procedure failed in fishes which happened to possess a comparatively thin aorta and small heart. In such cases the blood was collected by cutting the tail-end of the fish, which had been washed thoroughly in distilled water and wiped with a clean towel. The scales from the hind-end were shaved off prior to cutting the tail. Since the blood clots as soon as it oozes out of the blood vessel, the cut-end should be kept in close contact with the oxalate-coated surface of the collecting dish. Chloride values of the blood procured by cutting the tail and from the ventral aorta did not differ markedly.

Sendroy's method modified by Vanslyke and Hiller was used (Hawk, Oser and Summerson, 1954) for the estimation of chloride in the blood. Since fish of the size range used in this investigation did not yield more than 0.2 cc. of blood, this quantity of blood was made up to 1 cc. with 0.8 cc. of distilled water, tested to be free from chlorides, and was used for each estimation.

Blood chloride levels in *Tilapia* adapted to media containing 28 mM/litre, 483 mM/litre, and 1800 mM/litre of chlorides, are given in Table I. The blood chloride range of 151 to 161 mM/litre agrees well with the range obtained by Gordon (1957) for fresh water char and by Selvarajan (1961) for *Cirrhina reba* adapted to 40% sea water and for marine teleosts in general, by Graffiin (1935).

In order to get a complete picture of the mode of chloride regulation, the following experiments were carried out.

A. *Permeability studies.*

Permeability studies using methylene blue showed that the gills were both inwardly and outwardly permeable in both fresh water and sea water adapted *Tilapia*. However, this experiment is of a preliminary character and needs confirmation by studies using isotopes.

B. *Demonstration of swallowing habit.*

Experiments using phenol red was carried out with reference to both fresh and sea-water adapted *Tilapia*. It was found that in fresh water *Tilapia*, phenol red solution does not appear in the intestine even after 13 hours, thereby showing that it does not drink water; while in the sea water adapted *Tilapia* the intestine is seen filled with phenol red solution even after 1 hr. Thus in this respect sea water-adapted *Tilapia* resembles marine teleosts.

C. *Urine collection and estimation of chlorides in the urine.*

Urinary papilla was tied with a fine silk thread, and after 12 hours, the thread was removed and the collected urine was drawn into the syringe. Chlorides were estimated by Sendroy's method. Urine could be collected from fresh water *Tilapia*. Repeated attempts to collect urine from the sea water adapted ones failed.

Chloride estimations showed that the urine is hypotonic in freshwater *Tilapia* containing only 30 to 35 mM/litre of chlorides.

D. *Histological studies of the permeable surfaces.*

Histological studies of the gills, fins, buccal membranes and skin of both freshwater *Tilapia* and seawater adapted ones showed that there are no chloride cells in these regions.

E. *Histochemical studies.*

Gomori's method using frozen sections was used to demonstrate chlorides in tissues. By this method the respiratory epithelium alone stained black in the gills

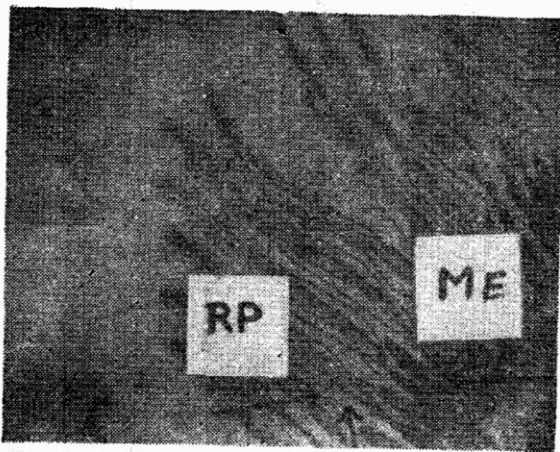


Fig. 1. Section of gill filaments tested for chlorides. RP—Respiratory epithelium. ME—MUSCULATURE stained red with eosin.

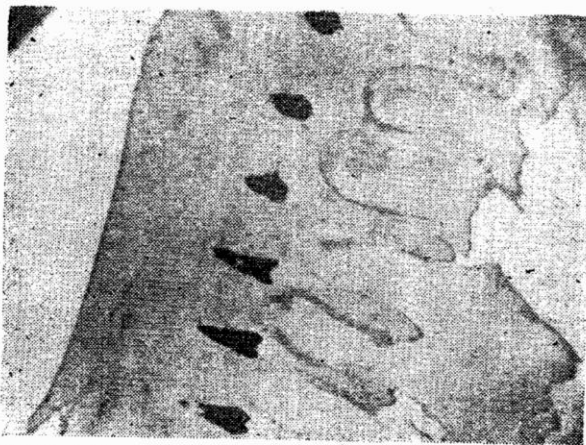


Fig. 2. Central core of mucous tissue of the arch, showing the presence of chloride. (Seawater-adapted *Tilapia*).

of freshwater *Tilapia* (Fig. 1.) while the respiratory epithelium as well as the mucous cells of gills reacted strongly (Fig. 2) in the seawater adapted *Tilapia*.

F. Chloride regulation at tissue level.

Ramsay has pointed out, that the maintenance of blood concentration alone is not enough to ensure ionic regulation in an animal (see Lockwood, 1962) and chloride regulation at the tissue level is also necessary in forms which withstand wide fluctuation in the salinities of the medium. Hence an estimation of the chloride level in muscle of fresh water and sea water adapted *Tilapia* was made to see whether this fish could regulate the chlorides at the tissue level also.

Previously, Selvarajan (1962) working on *Cirrhina reba* estimated the chloride in the tissues by pressing the muscle fragments and collecting the juice. By the above method muscle of *Tilapia* did not yield sufficient quantity of fluid. Hence the following method was adopted. 1 gm. of muscle (wet wt.) taken from the epaxial muscle band was rapidly freed from skin, scales, and cartilage bits and transferred immediately to the hot air oven. The muscle was dried at 80°C. till, the three consecutive weighings yielded the same value. From the wet wt. and dry wt. percentage of water content was calculated. The dried muscle was then powdered, made into a paste with 1 cc. of double distilled water, 25 cc. of phosphotungstic acid, and the total chloride estimated by Sendroy's method as applied for blood.

The results of muscle chloride estimations and percentage of water content are given in Table I. An increase in water content and a marked decrease in muscle chloride, are observed in sea water adapted *Tilapia*,

G. Amino acid levels (total free amino acid) in the muscle of fresh water and sea water adapted *Tilapia* were estimated, since in crustaceans like *Eriocheir sinensis* and *Carcinus* the adjustment of amino acid level of the tissues is reported to be responsible for keeping the tissue chloride level constant. (Lockwood, 1962) Amino acids were estimated by the titrimetric method of Sorenson. (Koch and

Hawk, 1953) 100 mg. (wet wt.) of muscle (taken from the same region as for chloride estimation) weighed accurately in a Torsion balance, was made into a fine paste with 1. cc. of distilled water treated with 8 cc. of N/12 sulphuric acid and 1 cc. of sodium tungstate, centrifuged and the supernatant liquid filtered. The filtrate was made up to 10 cc. mark with distilled water and titrated.

The results are shown in Table I. There is a slight increase in the amino acid level of the muscle in sea water adapted *Tilapia*. Such an increase in the amino acid content has been encountered in a few crustaceans showing ionic regulation (Lockwood, 1962).

TABLE I

Details	fresh water 28.3mM/litre	33 % s. water 483 mM/litre	100 %s. water 1800mM/litre
Blood chloride	mM/litre 151.4±4.5	mM/litre 156.5±3	mM/litre 161.4±4.2
Muscle chloride	mM/kgm. 46.13±1.5	mM/kgm.	mM/kgm. 28.5 ±3.2
Total free amino acid in muscle	ml/100mg. 0.71 ±05	ml/100mg. ••	ml/100mg. 1.67 ±0.45
Water content of the muscle ..	73.8 %2.0	••	78.05%±3

DISCUSSION

* Results outlined above show that *Tilapia* is able to maintain the chloride content of the blood at a fairly constant level (range-151.4 to 161.47 mM/litre) against wide fluctuations in the chloride content of the medium (range- 28 mM/litre to 1800 mM/litre). A slight rise in the chloride level of the blood, encountered in sea water adapted *Tilapia*, though negligible when compared to the enormous rise in the chloride content of the medium, shows that *Tilapia*, like the salmonids (Houston, 1959) and char (Gordon, 1957) maintains the blood chlorides at two different levels, one characteristic of fresh water medium and the other slightly higher level characteristic of concentrated medium like sea water.

Fresh water *Tilapia*, like stenohaline freshwater fish, forms hypotonic urine, and the respiratory epithelium of the gills reacts strongly to chloride test, suggesting that the respiratory epithelium may be concerned with the absorption of chlorides from the medium. Like the marine teleosts which are capable of maintaining the blood hypotonic to the medium, sea water adapted *Tilapia* swallows sea water to prevent water loss. Marine teleosts are shown to be incapable of producing hypertonic urine and hence they cannot excrete much of salts by way of kidney. Since repeated attempts to collect urine from the sea water adapted *Tilapia* resulted in failure, the role of kidney in *Tilapia* in forming isotonic or hypertonic urine as a means of excreting salts is not known. In sea water adapted *Tilapia* in addition to the respiratory epithelium, the mucous tissue of the gills shows the presence of chlorides, hence it is probable that the epithelial cells and to some extent the mucous cells are concerned with the excretion of salts.

Tilapia differs from the other teleosts like eels, salmon, *Fundulus*, *Europlus* and guppies, where the so called 'chloride cells' situated in the interlamellar area of the gills are considered to be concerned with the absorption of chlorides and excretion of chlorides in that, the gill filaments of *Tilapia* seem to be free from such cells. However, it may be pointed out that the role of chloride secretion ascribed to these so called chloride cells has not been established beyond doubt. (See Straus, 1963.)

An alternative suggestion was made by Bevelander (1935) and Krough (1939) that the thin respiratory epithelium is better suited for the absorption and excretion of salts, for the epithelium is extensive and is in immediate contact with the blood on one hand and the environment on the other hand, a condition necessary for facilitating movement of ions from the blood to the medium and vice versa. The result reported above for *Tilapia* rather supports this view. In this respect *Tilapia* resembles the crustacean *Artemia salina*, in which Croghan (1950) has proved that the role of ionic regulation is to be attributed to the gill epithelium.

As has been pointed out earlier, chloride regulation at the tissue level is also necessary to enable animals to tolerate wide fluctuations in the salinity of the medium. *Cirrhina reba*, a freshwater fish fails to survive in salinities higher than 40% sea water, because the chloride level in the muscle goes on increasing with the increase in blood chloride (Selvarajan 1962). A comparison of muscle chloride values in fresh water and sea water adapted *Tilapia* shows that with a fall in the chloride content there is a concomitant increase in the water content and amid free amino acid level in the sea water adapted *Tilapia*. Such a decrease in chloride content and an increase in water content are contrary to the results obtained by Houston (1962) for steelhead trout. However, the rise in the free amino acid level

of the muscle may perhaps be attracting water and decreasing the chloride content of the muscle. In *Eriocheir sinensis* and *Carcinus* the adjustment of the amino acid level of the tissues has been shown to be the mechanism for regulation of the ion level of the tissues (Lockwood, 1962), and it is possible that such a mechanism exists in *Tilapia* also.

SUMMARY

Present investigation indicates that *Tilapia mossambica* is an euryhaline fish, possessing a mechanism for hyper-regulation as well as hypo-regulation of chlorides. It differs from the teleosts already shown to be capable of such regulation in that it lacks the so called chloride cells in the gill filaments, considered to be concerned with the absorption of chlorides. But in *Tilapia*, as in some crustaceans the respiratory epithelium is perhaps capable of absorption and excretion of chlorides. It is also possible that the chlorides are secreted along with the mucus. Presence of chloride regulation at the tissue level is also indicated.

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TABLE I
Showing the rate of infection in different length-groups of host fish.

Length-groups in mm.	No. offish in each group	No. infected	% of infected host.
25-28 \	406	nil	nil
29-32			
33-36 \			
37-40/			
41-44)	31	5	16.12
45-48	23	15	65.22
49-52 53-	33	21	63.64
56	7	5	71.43
57-60			

DESCRIPTION OF THE SPECIES : FEMALE (Figs. 1-8)

External characters : The head is situated almost at right angles to the thorax, with two postero-dorsal horns which are shorter than the head. On the dorsal side of the head in front are situated together the first and second antennae, the first turned backwards and the second forwards. An extensible proboscis is situated in an oral depression bounded by cushions of attachment swellings. This oral depression is continued ventrally as a groove. The free thoracic segments are indicated by four pairs of appendages and the incomplete lines of fusion between them. This region is followed by the long ' neck' which tapers slightly posteriorly only to enlarge at its end to form the thrice wider genital segment or what is called ' trunk' by Leigh Sharpe (1927). A pair of egg strings, about twice the length of the ' abdomen' arises from the hind end of the genital segment. The genital segment after a marked constriction on the ventral side is continued further as the narrower tapering ' abdomen'. This clear demarcation into neck, genital segment or trunk and abdomen is not so evident in the immature specimens. At the hind tip of the abdomen is a pair of rounded prominences with a pair of setae. Annulations on the cuticle are discernible under high magnification, especially on the genital segment. A tough cuticular tunic covers the buried part of the body except the oral region.

Appendages: The first antenna which is turned backwards is indistinctly marked into 5 segments and bears no less than 22 setae. The second antenna is three-jointed, arises close behind the base of the first but extends forwards pressed on to the surface of the head. It is stout and subchelate. The rim of the proboscis bears an inner ring of curved spines and an outer radially striated fringe. Inside the proboscis are the curved conical mandibles, each with a proximal spiniform process. Regarding the identity of the two pairs of maxillae opinions differ. Gnanamuthu (1953) has figured two pairs of maxillae in front of the mandible and* calls a pair of appendages found posteriorly at the base of the proboscis, ' Wilson's maxillae \ as the maxillipedes. The maxillipede is three-segmented with rows of spiny prominences on the basal segments and the distal segment transformed into a strong claw.

The first two pairs of thoracic legs are biramous and the remaining two, uniramous. The basipods are broad plates and the rami, two-segmented. The