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PROLACTIN AND OSMOREGULATION IN TELEOST FISHES

by

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A CRITICAL ESSAY

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## INTRODUCTION

Teleosts as a group live in a wide range of aquatic media and maintain their osmotic pressure and electrolyte concentration at levels largely independent of the composition of their environment. In order to maintain hydromineral balance, teleosts are equipped with specialized ion and water-transporting epithelia situated in the gills, skin, kidney, intestine, and urinary bladder. In Euryhaline fishes, the function of these osmoregulatory organs must change according to environmental demands.

Comparative physiologists have studied the endocrine control of osmoregulation in fishes for several decades. One of the findings from a large body of results is the demonstrated effect of prolactin on electrolyte and water metabolism. Interest in prolactin as an osmoregulatory hormone in fishes started when Burden (1956) reported the failure of hypophysectomized killifish, Fundulus heteroclitus to survive in freshwater. In further work, Pickford and Phillips (1959) indicated that only prolactin is effective in promoting the freshwater survival of hypophysectomized Killifish. Since these early investigations, mammalian prolactin has been shown to restore

freshwater survival in several other hypophysectomized teleosts: Poecilia latipinna (Ball and Olivereau, 1964), Xiphophorus maculatus (Schreibman and Kallman, 1960), Tilapia mossambica (Dharmamba et.al., 1967).

In this paper, I will attempt to review briefly the current status of the role of prolactin in hydromineral regulation in teleost fishes.

#### EVIDENCE OF PROLACTIN INVOLVEMENT

Burden (1956) described the inability of the hypophysectomized Fundulus heteroclitus to survive in freshwater. The importance of prolactin in the freshwater survival of numerous other species has been documented (Pickford and Phillips, 1959; Ball and Olivereau, 1964; Schreibman and Kallman, 1966; Dharmamba et.al., 1967; Utida et.al., 1971). Although the plains Killifish, Fundulus kansae and the eel, Anguilla anguilla are able to live in freshwater after hypophysectomy nevertheless they suffer some osmoregulatory impairment, which may be corrected by prolactin (Stanley and Fleming, 1966; Maetz et.al., 1967). Lam and Leatherland (1969) showed that the inability of Stickleback, Gasterosteus aculeatus, to survive in freshwater in winter can be restored by prolactin treatment. In the plains Killifish, Fundulus kansae hypophysectomy decreases urine flow and increases urine sodium concentration; prolactin has the opposite

effect (Potts and Fleming, 1970). Prolactin (eta) cells are stimulated in Cichlasoma biocellatum from freshwater and are inactivated when the fish are in 25% seawater (Mattheij et.al., 1971). Abraham (1971) found three different granule sizes in eta cells of the grey mullet, Mugil cephalus, collected from three different salinities. These observations were confirmed by studying prolactin cells in Teleosts at the light and electron microscopic levels in response to changing environment salinity. Leatherland (1972) reported that mitochondria increase in size and develop more cristae and polyribosomes are apparent in active prolactin cells compared to inactive cells in the goldfish.

#### MODE OF ACTION OF PROLACTIN

Prolactin has been known to promote survival of hypophysectomized fish in freshwater, by maintaining normal plasma electrolyte level. The basic mechanism of action of prolactin appears to involve different osmoregulatory sites: gills, intestine, kidney, urinary bladder, and the skin. These sites of action will be considered one by one.

#### EFFECT ON GILLS

Gills of teleosts, occupying more than 60% of the body surface, provide a susceptible osmotic membrane and a main site of water and electrolyte fluxes.



### Effect on Water Permeability

Gill movement of tritiated water indicates that almost all water exchange by eel, flounder, and goldfish occur at the gills (Motaïs et.al., 1969). Chan et.al. (1968) showed that prolactin reduced water influx via the gills of the eel, Anguilla anguilla in freshwater. Prolactin reduces the permeability of the gills to water in the stickleback, Gasterosteus aculeatus (Lam, 1969). Hypophysectomy did not change water influx in the goldfish, Carassius auratus, but prolactin injection of the hypophysectomized fish significantly reduced water influx (Ogawa et.al., 1973). Later Ogawa (1974, 1975) obtained similar results from prolactin with the isolated gills of the rainbow trout, Salmo gairdneri and the Japanese eel, Anguilla japonica. However, Lahlou and Giordan (1970) reported that hypophysectomy causes a reduction of water permeability in gills of the goldfish, and prolactin is able to correct this defect. The same effect of prolactin in Fundulus kansae has been suggested by Potts and Fleming (1970).

On the other hand, stress, ACTH, or Cortisol increased water permeability of the gills of goldfish (Lahlou and Giordan, 1970). The same effect of Cortisol in isolated gills of the Japanese eel has been reported by Ogawa (1975).

### Effect on Electrolyte Flux

Potts and Evans (1966) first demonstrated that prolactin reduces sodium loss in hypophysectomized Killifish, Fundulus heteroclitus, in freshwater. Later, Lam (1969) reported that prolactin reduces the net loss of sodium via the head region in the stickleback. Prolactin treatment of hypophysectomized Tilapia mossambica in freshwater reduced the sodium outflux, although it did not affect sodium influx (Dharmamba and Maetz, 1972). Sodium turnover rate and branchial efflux are inhibited by prolactin in Tilapia mossambica in seawater (Dharmamba et.al., 1973). Prolactin induced a marked increase of plasma sodium in intact or hypophysectomized eel, Anguilla anguilla, transferred to seawater (Olivereau and Lemoine, 1973). MacFarlane and Maetz (1974) reported that the impaired sodium conservation in hypophysectomized euryhaline flounder, Platichthys flesus, in freshwater is due solely to a high branchial efflux; the sodium absorption pump and water permeability are unaffected. Prolactin treatment considerably reduced the sodium turnover rate, but it does not affect water permeability.

### Effect on Chloride Cells

A considerable volume of work has been done on the role of the chloride cells (ionocytes) in electrolyte



transport. Mizuhira et.al. (1970) showed that the Na, K -ATPase activity is mainly localized in the chloride cells in the gill epithelium of the Japanese eel. Evidences indicate that the ionocytes are the probable site of active electrolyte transport in seawater (Maetz, 1971). Utida et.al. (1971) demonstrated that the chloride cells are always larger both in number and size and stronger in stainability to acid fuchsin in marine teleosts than in freshwater teleosts. Pickford et.al., (1970-b) reported that prolactin reduced the gill Na, K -ATPase activity of hypophysectomized Fundulus heteroclitus on transfer from seawater to freshwater. Kamiya (1972) found that when seawater-adapted eels were returned to freshwater, the enzyme activity decreased gradually. Prolactin injection into hypophysectomized eels promoted this decrease, although it was without effect on seawater eels.

On the other hand, Cortisol induces a rise in the activity of Na, K -ATPase in the gills of Fundulus heteroclitus and Anguilla anguilla adapted to seawater (Pickford et.al., 1970-a; Milne et.al., 1971). Cortisol also induces an increase in the enzyme activity in the gills of intact Anguilla rostrata and Anguilla japonica maintained in freshwater (Epstein et.al., 1971; Kamiya, 1972).

### Effect on Mucous Cells

A second branchial epithelial site at which prolactin may act is the mucous cells.

Burden (1956) noted the atrophy of the mucous cells in the gills of hypophysectomized killifish, Fundulus heteroclitus. Blum and Fiedler (1965) found that prolactin increased the number of mucous cells in cichlid fishes. Prolactin stimulates the branchial mucous cells in intact Gasterosteus aculeatus and Anoplichthys jordani (Leatherland and Lam, 1969; Mattheij and Sprangers, 1969).

### EFFECT ON INTESTINE

It is generally accepted that teleosts in freshwater scarcely drink water; whereas in seawater, they drink continually and absorb water together with monovalent ions from the intestine in order to replace water which is lost osmotically. It was shown by Oide and Utida (1967) that in isolated intestines of eels, a larger amount of water passes through the intestine of seawater-adapted eels than freshwater eels. Further, Hirano et.al. (1967) reported that when the hypophysis is removed, the enhancement in water movement of the seawater-adapted eel is no longer observed. A single injection of ACTH or Cortisol into freshwater eels produced a significant augmentation of the intestinal water movement (Hirano and Utida, 1968).

Adrenalectomy produced a significant drop in water movement which was restored to normal values by Cortisol injection (Gaitskell and Chester Jones, 1970). Hirano and Utida (1971) observed that only a short term (2-4 hr.) increase in circulating Cortisol during the course of seawater adaptation, while the increase in intestinal water movement was observed after a latent period of about 10 hours and lasts for more than a week. This latent period may reflect some metabolic process. Jampol and Epstein (1970) reported that intestinal Na, K -ATPase activity is higher in seawater-adapted fish than in freshwater fish. Na, K -ATPase activity increased in intestines of Cortisol-injected killifish in seawater (Pickford et.al., 1970-b). Prolactin does not affect intestinal ATPase activity in hypophysectomized freshwater killifish (Pickford et.al., 1970-a). Oide (1970), on the other hand, noticed that the adaptation of the eel to seawater enhances the production of alkaline phosphatase in the intestinal mucosa.

Utida et.al. (1969) demonstrated that prolactin treatment of seawater eels decreases the rate of water absorption in the intestine. When seawater is introduced into the gut of a freshwater eel, sodium is absorbed but water is not; both are absorbed following Cortisol injection (Utida et.al., 1972).

## EFFECT ON KIDNEY

The teleost kidney plays a major role in hydromineral balance. In freshwater fishes, the renal tubules are nearly impermeable to water (Hickman, 1965). In euryhaline fishes, glomerular filtration rate is increased in hypotonic and decreased in hypertonic environments (Lahlou, 1967). Oide and Utida (1968) reported that glomerular filtration rate and urine flow are higher in freshwater Japanese eels than in seawater fish. The action of prolactin on renal mechanisms has not been as well studied as the extrarenal mechanisms, which is mainly because of the technical difficulties involved (Lam, 1972).

In plains killifish (Stanley and Fleming, 1966, 1967), stickleback (Lam, 1969-c), and goldfish (Lahlou and Sawyer, 1969; Lahlou and Giordan, 1970) hypophysectomy reduces urine flow and increases sodium excretion. Prolactin treatment reverses both effects. Prolactin injections caused structural changes in the kidney. Lam and Leatherland (1969-b) demonstrated that after transfer to freshwater, the prolactin-injected stickleback showed a significant percentage increase in size of the glomerular tuft. Olivereau and Lemoine (1968, 1969) found in the hypophysectomized eel, prolactin has morphological stimulatory effect on the renal tubules, although its effect is not as marked as in intact fish.

Wendelaar and Veenhuis (1974) reported that stickleback kidney cells are stimulated by prolactin. The stimulatory effects of prolactin on kidneys are supported by the work done on kidney ATPase enzyme. Epstein et.al. (1969) reported that the activity of kidney Na, K -ATPase in killifish increased significantly following transfer from seawater to freshwater. Prolactin strongly increases ATPase in the kidney of hypophysectomized killifish (Pickford et.al., 1970). In a survey of a number of freshwater and marine teleosts, Jampol and Epstein (1970) found that kidney Na, K -ATPase activity in freshwater fishes was about twice that of marine fishes. McCartney (1976) reported that Na, K -ATPase activity declined in the kidney during the transition of the Atlantic Salmon, Salmo salar, from a freshwater to a seawater environment.

Cortisol may play a role in kidney function. Chan et. al. (1969) found that interrenalectomy of freshwater eel reduces glomerular filtration rate which can be restored to normal with Cortisol.

#### EFFECT ON URINARY BLADDER

The osmoregulatory role of the urinary bladder in teleosts has been neglected. However, recent investigations have suggested that urinary bladder provides another important surface for ion and water transport. Lahlou (1967) reported that the bladder urine of flounder,



Platichthys flesus, differs significantly from the free flow urine. Johnson et.al. (1970) found that bladders isolated from concentrated (133%) seawater adapted flounder, Platichthys stellatus, have a higher rate of water movement than those from freshwater adapted fish. Prolactin injected into concentrated seawater adapted starry flounder decreases the rate of water movement in the urinary bladder to the level seen with bladders from freshwater fish (Hirano et.al., 1971). Only prolactin treatment and transfer to hypotonic media decrease water absorption and increase sodium influx in the bladder of seawater starry flounder (Johnson et.al., 1972). In general, euryhaline fishes modify their bladders from a permeable state in seawater to a relatively impermeable state in freshwater. Prolactin seems to be involved in this modification (Hirano et.al., 1973). In bladders of the seawater flounders, Platichthys stellatus, Kareius bicoloratus, and Platichthys flesus prolactin decreases water permeability and stimulates sodium and chloride reabsorption (Hirano, 1974, 1975; Johnson et.al., 1974; Foster, 1975). Prolactin treatment or transfer from seawater to freshwater increases Na, K -ATPase activity in Platichthys stellatus bladder, but not Na, K -ATPase activity in Kareius bicoloratus, which does not survive transfer to freshwater (Utida et.al., 1974). Doneen and Nagahama (1973) suggested that Cortisol may act on the



euryhaline Gillichthys mirabilis to stimulate bladder ion movement, whereas the action of prolactin may be confined to controlling water permeability upon transfer to freshwater. Organ-cultured bladders from Gillichthys indicated that Cortisol increases bladder water permeability and that prolactin antagonizes this Cortisol effect, causing a reduction in water permeability (Doneen and Bern, 1974). Doneen (1974) reported that Cortisol stimulation of water permeability in cultured bladders is inhibited by prolactin in a dose-response relationship. Recently, Doneen (1976) confirmed the bihormonal regulation of water and ion movement in Gillichthys urinary bladder. Cortisol can elevate water permeability and stimulate sodium and chloride reabsorption in seawater-adapted fish, whereas prolactin lowers bladder water permeability.

#### EFFECT ON SKIN

The skin is another epithelial site at which prolactin may act. Schreibman and Kallman (1965) reported that hypophysectomy reduces the activity and number of mucous cells in the skin of Betta splendens, and prolactin (Blum and Fiedler, 1965) increases the number of these cells in the skin of the cichlid fish, Symphysodm aequifasciata. In hypophysectomized goldfish, Carassius auratus, prolactin injections caused an increase in both number and size of

the epidermal mucous cells (Ogawa, 1970). In the cichlid, Cichlasoma biocellatum, the number of skin mucous cells could be correlated with the activity of the prolactin producing cells in adenohypophysis (Mattheij and Stroband, 1971). In the eel, Anguilla anguilla, the Sialic acid, a component of mucous, concentration is significantly reduced after hypophysectomy and is restored by prolactin injections (Olivereau and Lemoine, 1971). On the other hand, neither hypophysectomy nor prolactin affect mucous cells in Poecilia latipinna (Ball, 1969-b) and Anoptichthys jordani (Mattheij and Sprangers, 1969).

#### CONTROL OF PROLACTIN SECRETION

The mechanisms controlling prolactin secretion in teleosts has been the subject of many investigations in recent years (Ball et.al., 1972; Leatherland and Ensor, 1973, 1974; Nagahana et.al., 1974, 1975; Peter and McKoewn, 1974; Zambrano et.al., 1974).

Hypothalamic regulation of prolactin cells in teleost fishes has received particular attention. Peter (1970) found that in the goldfish, Carassius auratus, neurosecretory fibers passing from the hypothalamus to the adenohypophysis. Following pituitary transplantation in Gasterosteus aculeatus (Leatherland, 1970), Anguilla anguilla (Olivereau, 1971-a), and Ictalurus melas (Chidamberam et.al., 1972), the prolactin cells appear

more active than in the normal pituitaries. Chambolle (1973) reported that hypophysectomized Gambusia with an autotransplanted rostral pars distalis survive in fresh-water is suggestive of prolactin secretion by prolactin cells independent of hypothalamic influence. Leatherland and Ensor (1973) found no differences in the appearance of prolactin cells between goldfish with autotransplanted pituitaries and sham-operated control fish. Leatherland and Ensor (1974) also provided evidences for a prolactin release-inhibiting factor in the goldfish hypothalamus, judging from the decrease in plasma sodium after treatment with a hypothalamic extract. Peter and McKeown (1974) demonstrated that hypothalamic lesions caused a marked increase in prolactin secretion in the goldfish which suggests that the nucleus lateralis para lateralis is the origin of a factor that normally inhibits prolactin release from the pituitary. Recently, Nagahama et.al. (1975) reported that the grafted prolactin cells of seawater Gillichthys mirabilis exhibited obvious activations with a significant elevation of plasma sodium concentration, strongly indicative of the presence of prolactin inhibitory factor from the hypothalamus.

Another mechanism which may control prolactin secretion in some teleosts is the changes in osmotic pressure of the surrounding medium (Nagahama et.al., 1975). Hirano et.al. (1971) and Ingleton et.al. (1973) found that in

Poecilia latipinna prolactin secretion is stimulated by reduced electrolyte concentrations. Schreibman et.al. (1973) studied the morphology of the teleost pituitary and have demonstrated that prolactin cells are more active in freshwater than in seawater. Bell and Ingleton (1973) reported a 75% decrease in prolactin content of Poecilia pituitaries after transfer from seawater to freshwater. Nagahama et.al. (1975) also reported that the pituitary prolactin content in Gillichthys dropped to 40% after transfer to freshwater. Zambrano et.al. (1974) showed that Tilapia prolactin cells respond strongly to a seawater-freshwater transfer after their aminergic innervation has been disrupted. In another study on Tilapia pituitaries, Zambrano et.al. (1974) indicated that dilution of the medium directly stimulated both synthesis and secretion of prolactin as judged by the proliferation of rough endoplasmic reticulum and greater incorporation of tritiated leucine, and reduction in prolactin content and an increase in the amount of bioassayable prolactin in the medium. Transfer of Gillichthys mirabilis bearing pituitary autografts from seawater to freshwater caused exocytosis of granules from the prolactin cells (Nagahama et.al., 1974). More recently, Nagahama et.al. (1975) demonstrated that in Gillichthys, prolactin release is directly stimulated by low osmotic pressure of the incubation medium.

## PROLACTIN AND OSMOREGULATION IN OTHER FISHES

Cyclostomes (Myxinidae), Elasmobranchs, and Latimeria should have no major problems of hydromineral balance, because their osmotic concentrations are either the same as or slightly above seawater.

Cyclostomes

Cyclostomes are divided into two groups: Hagfishes and Lampreys. The hagfishes (Myxinidae) are strictly marine. Their body fluids with a salt concentration similar to that of seawater. In fact, their plasma sodium concentration slightly exceeds sodium concentration of the sea (Schmidt-Nielsen, 1975). Chester Jones (1962) demonstrated that injections of mammalian prolactin increased muscle water content, and decreased muscle electrolytes in the hagfish, Myxine. Aler et.al. (1971) have used fluorescent antibody techniques and found that rabbit antibodies to ovine prolactin do not cross-react with any protein in the adenohypophysis of the hagfish, Myxine glutinosa. Through assaying of prolactin in different vertebrate pituitaries by its dispersion of xanthophores in the goldfish, Gillichthys mirabilis, Sage and Bern (1972) found a very small activity in the pacific hagfish, Polistotrema stouti, pituitary. This low or absent activity in the hagfish pituitary may be due to either the absence of the prolactin



hormone or to differences in the molecule such that it does not function on a teleost receptor.

Lampreys (Petromyzontidae) are euryhaline. Having an osmotic concentration of about one-third of seawater, they behave osmotically like teleost fishes (Schmidt-Nielson, 1975). Aler et.al. (1971) have found no prolactin activity in the lamprey, Lampetra fluviatilis, pituitary. No further studies along this line have been reported.

#### Elasmobranchs

Sharks, skates, and rays are almost with<sup>out</sup> exception marine. The total osmotic concentration of their blood equals or slightly exceeds that of seawater, because of the addition of urea and trimethylamineoxide to the salt concentration of their body fluids. Although elasmobranchs are isosmotic, they still suffer some ionic impairments due to sodium diffusion from sea into them. This extra sodium is excreted by the kidney and rectal gland (Schmidt-Neilsen, 1975). The elasmobranch pituitary contains a teleost-type prolactin (de Vlaming et.al., 1975; Grant, 1961; Sage and Bern, 1972). Relatively little is known about the involvement of that prolactin in osmoregulation. Payan and Maetz (1971) found that hypophysectomy in three elasmobranchs, Scyliorhinus canicula, Raja montagu, and Torpedo marmorata, decreased both branchial water permeability and urine flow. The branchial water permeability



was restored to normal by both ACTH and prolactin treatments. Only ACTH is able to restore normal kidney function. Payan et.al. (1973) found no change in branchial water permeability in both intact and hypophysectomized euryhaline stakes, Raja erinacea and R. radiata, adapted either to seawater or to 50% seawater. de Vlaming et.al. (1975) reported that in the euryhaline stingray, Dasyatis sabina, hypophysectomy caused a significant increase in plasma osmolarity, urea, and sodium concentrations which were reversed by mammalian prolactin injections. From these findings, one can suggest that prolactin may increase water influx by acting on the gills.

#### Coelacanth

*See Schmidt-Nielsen (1975)*

The coelacanth Latimeria is essentially isosmotic with seawater. No information is available about prolactin effect on salt and water metabolism in Latimeria, because no living specimen has been kept long enough to perform physiological experiments.

#### DISCUSSION AND CONCLUSIONS

Fishes live in a wide range of salinities. Some are stenohaline, limited to one environment, others are euryhaline and can tolerate or migrate from one salinity to another. Marine and freshwater teleosts maintain their osmotic concentration within the same range, although

marine fishes tend to have somewhat higher blood concentrations (Schmidt-Nielson, 1975).

Marine bony fishes are hyposmotic, and their osmotic problem is to conserve water and exclude salt. In order to maintain their body volume in the hypertonic sea, marine teleosts drink large quantities of seawater. As the seawater passes down the intestine, water is absorbed, as well as some salts. Sodium absorbed in the intestine and that entering the body by inward diffusion across the gills must be eliminated via the gills by active transport. The excretion of sodium in the urine is of minor importance because the urine is usually in a small quantity and more dilute than the body fluids. However, some divalent ions are lost in the urine and the gut (Schmidt-Nielson, 1975).

Freshwater teleosts are osmotically more concentrated than the medium, therefore their major problem is the osmotic water influx, mainly through the gills, and loss of solutes through the gills and in the urine (Schmidt-Nielson, 1975). In order to maintain their plasma concentration, freshwater teleosts drink little water, if any, and filter large quantities of water through the kidneys. As the filtrate passes down the kidney tubules, most of the solutes are absorbed, leaving a dilute urine which is temporarily held in the urinary bladder for more salt reabsorption. In freshwater teleosts, serum sodium is also

maintained by reducing outflux of sodium through the gills.

Considerable evidence seems to point to prolactin as the major factor in regulating hydromineral balance in teleosts in freshwater. Gills, intestine, kidney, urinary bladder, and the intergument are the potential sites of prolactin action.

Most of the action of prolactin on salt and water metabolism seems to be at the branchial level. Water permeability of a number of teleosts is greater in freshwater than in seawater (Potts et.al., 1967); and nearly all water turnover is at the gill. Chan (1968), Lam (1969), and Ogawa et.al. (1973) concluded that prolactin may reduce the permeability of the gills to water in freshwater teleosts. The results obtained with both the Japanese eel and rainbow trout by Ogawa (1974, 1975) confirmed the above effect of prolactin. However, work on the goldfish and plains killifish (Lahlou and Giordan, 1970; Potts and Fleming, 1970) gave contradictory results in that prolactin appeared to increase water permeability of the gills. These directly opposite effects of prolactin on branchial permeability are hard to explain. They may be related to species differences and technical difficulties.

Lahlou and Giordan (1970) and Ogawa (1975) suggested that Cortisol may also regulate gill function acting antagonistically to prolactin; Cortisol increases water influx of the gills. The information concerning the effect

of Cortisol on branchial water permeability is scanty, and further experiments are necessary to clarify this suggestion.

The ability to reduce sodium permeability is essential to a freshwater habitat. In general, gill efflux in freshwater is less than 1% of the seawater rate (Bentley, 1971). It has been suggested that in freshwater teleosts, prolactin appears to act by reducing branchial sodium permeability (Dharmamba and Maetz, 1972; MacFarlane and Maetz, 1974). In seawater fishes, prolactin inhibits sodium extrusion pump (Dharmamba et.al., 1973; Olivereau and Lemoine, 1973). In hypophysectomized killifish in freshwater, prolactin reduced the branchial Na, K -ATPase activity, an effect correlated with the decrease in branchial sodium efflux (Pickford et.al., 1970). These results suggest that prolactin alters the activity of the sodium extrusion pump normally operating in seawater rather than influencing passive sodium exchange. The elevated levels of Na, K -ATPase in the gills of seawater teleosts seem to be triggered by Cortisol (Milne et.al., 1971; Pickford et.al., 1971). Thus the coordination of prolactin and Cortisol activity in euryhaline fishes results in the reduction of sodium efflux by prolactin in freshwater, and enhancement of sodium outflux by Cortisol in seawater.

Osmotically the gut is more important in seawater fishes than in freshwater fishes, simply because of the

negligible drinking rate of the latter. It is known that water absorption is linked to active transport of sodium and chloride ions (Hirano, 1974). It has been reported that only ACTH and Cortisol are effective in increasing the rate of sodium and water transport in the gut of freshwater eel (Hirano and Utida, 1968; Utida et.al., 1972). The latent period of Cortisol effect may reflect the metabolic process of increasing Na, K -ATPase activity. An increase in intestinal Na, K -ATPase activity has been reported in seawater-adapted and Cortisol-injected fishes, suggesting that sodium transport and Na, K -ATPase enzyme are directly related (Jampol and Epstein, 1970; Pickford, et.al., 1970-b). Gut levels of Na, K -ATPase in hypophysectomized freshwater killifish are unaffected by prolactin, suggesting that prolactin does not affect sodium transport in intestine (Pickford et.al., 1970-a). However, the important enzyme here may not be Na, K -ATPase, but alkaline phosphatase (Oide, 1970). On the other hand, prolactin treatment inhibits water permeability of the eel intestine (Utida et.al., 1969). From the accumulating evidence, it seems that intestine is under bihormonal control (Cortisol and prolactin); Cortisol increases both the rate of water and sodium transport, whereas prolactin acts to limit the amount of water accompanying the ions. Thus, prolactin acts on the intestinal epithelium as an antagonist to Cortisol. Cortisol is essential in seawater,



and prolactin plays an important role in freshwater.

The kidney of euryhaline teleosts must shift from water excretion and nonvalent ion conservation in freshwater to divalent ion excretion and water conservation in seawater. It has been demonstrated that in hypophysectomized freshwater fishes, prolactin increases urine flow to normal levels, and reduces urinary sodium concentration (Lahlou and Sawyer, 1969; Lahlou and Giordan, 1970; Lam, 1969; Stanley and Fleming, 1966, 1967). The increased urine flow is due to prolactin stimulation of glomerular filtration rate and inhibition of tubular water absorption (Lam and Leatherland, 1969). Wendelaar and Veenhuis (1974) reported that kidney cells are better developed in freshwater than in seawater fishes. It has been also reported that prolactin stimulates kidney cells in the hypophysectomized eel (Olivereau and Lemoine, 1969). The findings that transfer of fish from seawater to freshwater or prolactin treatment increase the activity of kidney Na, K -ATPase, reflect the stimulatory effect of prolactin on the kidney to reabsorb sodium from the large volume of glomerular filtrate (Epstein et.al., 1969; Jampol and Epstein, 1970; McCartney, 1976; Pickford et.al., 1970). The fact that the same renal prolactin effects on hypophysectomized eel were produced in intact fish to a greater degree suggests some synergism between other pituitary factors and the injected prolactin (Olivereau and Lemoine, 1969).



The possibility of an osmoregulatory role for the urinary bladder has been suggested as a result of its modification of the ionic composition of the urine (Johnson et.al., 1970; Lahlou, 1967). By inhibiting osmotic permeability and stimulating sodium reabsorption in freshwater, the urinary bladder of euryhaline fish may supplement and functionally mimic the osmoregulatory function of the distal kidney tubule (Foster, 1975). Two concepts have been suggested regarding the hormonal regulation of water and ion movements in the urinary bladder. The first is unihormonal: prolactin stimulates both sodium uptake and Na, K -ATPase activity while inhibiting water movement across the urinary bladder (Foster, 1975; Hirano, 1974, 1975; Johnson et.al., 1972, 1974). Whereas the second concept is bihormonal: Cortisol increases sodium reabsorption and osmotic permeability, while prolactin limits water movement across the urinary bladder favoring freshwater adaption (Doneen, 1974, 1976; Doneen and Bern, 1974; Doneen and Nagahama, 1973). The differences between those two concepts appear to spring from the differences among teleost species. However, insufficient investigation has been done to allow conclusions regarding taxonomic positions and bladder osmoregulatory properties (Bern, 1975). Any generalizations must await further investigations of more euryhaline teleosts.

An examination of the data presented above leads to the general conclusion that prolactin~~g~~ is the dominant factor in regulating teleost hydromineral balance in freshwater. It maintains ionic balance by inhibiting sodium loss in the gills, and stimulating active transport in the kidney and urinary bladder. Prolactin also maintains water balance through inhibition of osmotic water influx via the gills and limiting water absorption from the gut together with its action of increasing urine flow via an increase in glomerular filtration rate and a reduction in water reabsorption from the urinary bladder. The effect of prolactin and other hormones are not well understood in the problem of adaptation of fish to seawater and freshwater. The role of prolactin in regulating hydromineral balance in marine teleosts awaits further investigation.

The presence of prolactin release-inhibiting factor (PIF) in mammals is well documented (Turner and Bagnara, 1971). From the cumulative evidence, it seems certain that prolactin release in teleosts is regulated by at least two mechanisms: an inhibitory factor from the hypothalamus presumably mediated by aminergic fibers, and a direct stimulation due to low osmotic pressure of the surrounding medium. Moreover, Nagahama et.al. (1974, 1975) indicated that negative feedback by either prolactin itself or elevated plasma sodium level may be another

possible mechanism controlling prolactin secretion in teleosts.

If prolactin exists in cyclostomes, it has antigenic properties differing from those of mammalian and teleostean prolactin. The whole question of the existence of prolactin in cyclostomes is complicated and needs more investigation.

What little is known about the role of prolactin in elasmobranch osmoregulation does not allow any conclusion. However, further research is needed in this field.

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