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Corticosteroid induction of Na-K-ATPase in the fresh water eel (Anguilla Rostrata)

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Yale University

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DEDICATION

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WHATEVER END MAN AIMS AT IN THIS WORLD IS NOT
THE FINAL END, FOR IT GIVES NOT MAN FULL HAPPINESS.
WHAT WAS AN END BECOMES A NEW BEGINNING ACCORDING
TO THE COURSE THAT MAN CAN UNDERSTAND.

AUZIAS MARCH (1393-1459),

CATALAN POET AND PHILOSOPHER

ACKNOWLEDGEMENT

I WOULD LIKE TO THANK DR. FRANKLIN H. EPSTEIN FOR PROVIDING THE OPPORTUNITY FOR ME TO WORK WITH HIM IN HIS LABORATORY AT YALE UNIVERSITY SCHOOL OF MEDICINE AND AT THE MOUNT DESERT ISLAND BIOLOGY LABORATORY. IT WAS AN INTELLECTUALLY REWARDING INTRODUCTION TO COMPARATIVE BIOLOGY AND A MEMORABLE SUMMER ON THE MAINE COAST.

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INTRODUCTION

Euryhaline teleosts are able to regulate their tissue water and ion concentration in response to changes in the environmental salinity. In freshwater the passive inward diffusion of water and the renal and extrarenal loss of sodium and chloride is compensated by maintaining a copious hypotonic urine flow coupled with feeding and the active uptake of sodium and chloride by the gill epithelium. In a marine environment euryhaline teleosts drink seawater and increase intestinal transport of sodium chloride and water which coupled with extrarenal excretion of sodium chloride by the gill epithelium compensates for the passive outward flux of water (1-4). There is also a decrease in urine flow (isomotic urine) which conserves water. Both salt and water balance are regulated through hormonal mechanisms mediated particularly by the adrenal cortex and the pituitary gland (5-8).

Sodium - and potassium - activated ATPase is a ubiquitous enzyme found in high concentration in many tissues in which active sodium transport plays a prominent role. The function of this enzyme has recently been reviewed by Skou (9) and Glynn (10). It is known that the level of Na-K-ATPase in the gills and intestine of several species of euryhaline teleosts increases markedly when the fish are transferred from a freshwater to a seawater environment (11-14). This increase in the level of Na-K-ATPase parallels the increase which occurs in the net sodium transport by these tissues. In freshwater the teleosts' kidneys transport considerably more sodium chloride than they do in seawater and it would therefore be expected that the Na-K-ATPase levels

should be higher in freshwater than in seawater (15). The outflux of sodium across the gills of Anguilla anguilla is 5 - 10 times higher in seawater - adapted than in freshwater - adapted eels (16,17). Adrenalectomy interferes with this adaptation, and cortisol injected over a 24 hour period rapidly restores the outflux to normal levels (18). The factors which enable freshwater euryhaline teleosts to adapt to seawater are only partially understood. The present work was undertaken to investigate the effects of exogenous cortisol on the Na-K-ATPase activity of the gill epithelium and intestinal mucosa of freshwater eels, and to see whether pretreatment with cortisol would enable freshwater eels to adapt to a seawater environment maintaining better osmoregulation than untreated controls.

GENERAL COMMENTS ON Na-K-ATPase

Sodium-potassium activated ATPase is an enzyme system which may play an integral role in the active transport of Na and K across cell membranes (9,10,19,20). This enzyme has been found in a wide range of tissues from one animal (21,22) and in various tissues from many different species (see review articles 9,10,19). It appears to be present in all tissues in which it was carefully looked for. This enzyme seems to play an important role in the active reciprocal transfer of sodium and potassium across the plasma membrane of individual cells, particularly the erythrocyte. Skou (9) proposed the following requirements for such a transport system; it should 1. be located in the cell membrane, 2. have an affinity for Na that is greater than for K at a site located on the inside of the cell membrane, 3. have an affinity for K that is greater than for Na at a site located on the outside of the membrane, 4. contain an enzyme system that can catalyze the hydrolysis of ATP and thus convert the energy from ATP into a movement of cations, 5. be capable of hydrolyzing ATP at a rate dependent on concentration of Na inside the cell and also on concentration of K outside the cell, and 6. be found in all cells in which active, linked transport of sodium and potassium occurs. A transport system was found which met these criteria and had two additional properties, 7. a close correlation was found between the effect of cardiac glycosides on the cation transport in the intact cell and their effect on this system and 8. this enzyme system has the same quantitative relation to Na and K as the transport system in intact cells (9).

The activity of Na-K-ATPase parallels the level of sodium transport in various tissues (23,24) indicating that this enzyme may play a role in active sodium transport. It is found in various organs in which bulk transport of sodium across an epithelial membrane occurs; kidney (25), gill (11) and the avian salt gland (26,27). Since this sodium transport is not known to be coupled with active K transport in the opposite direction of comparable magnitude, the role of the enzyme is unclear. That Na-K-ATPase may play some role is suggested since it has been shown that increases in cation transport are correlated with increases in enzyme activity, furthermore decreases in active transport are followed by decreases in enzyme activity. The parallels in enzyme activity and cation transport in these tissues will be discussed in some detail subsequently.

Properties of Na-K-ATPase

Requirements for sodium and potassium: This enzyme system hydrolyzes adenosinetriphosphate to adenosinediphosphate and orthophosphate. For maximal activity it requires Mg^{++} usually equimolar with the ATP plus both sodium and potassium. In the presence of Mg^{++} alone, the enzyme system has low activity which increases slightly with addition of sodium but little or not at all with addition of potassium. If both sodium and potassium are present with magnesium there is a marked increase in activity. Potassium can be replaced by ammonium, rubidium, cesium or lithium in order of decreasing efficiency, however, sodium cannot be replaced by another cation (28). The requirement for sodium is of interest since this cation rarely is an enzyme activator. A kinetic analysis of the effect of Na and K on

activity suggests that the system has two sites with affinities for cations - one where the affinity for Na is six to eight fold greater than that for K and where K can competitively displace Na, and a second with high affinity for K and very low affinity for Na (28). When the concentration of K is high relative to the concentration of Na, K competitively displaces Na from the first site and the activity is decreased.

The activity of this enzyme system is strongly inhibited by low concentration of Ca++ which can be reversed to some extent by increasing the Mg++ concentration (28). The inhibition is apparently not caused by ionic calcium but by the Ca-ATP complex which competes with Mg-ATP for enzyme binding (29).

Requirements for ATP as energy substrate: The substrate for this enzyme system is adenosinetriphosphate since there is little if any phosphatase activity when other nucleotide triphosphates such as inosine, guanosine or uridine triphosphate are used as substrate (20,28).

Cardiac glycoside inhibition: Cardiac glycosides in concentrations from less than 10^{-8} M to more than 10^{-4} M inhibit that portion of enzyme activity stimulated by the simultaneous presence of Na and K, whereas, the Mg-dependent activity is unaffected (10). The inhibition of Na-K activated ATPase by low concentrations of ouabain can be prevented by an increase in the concentration of K, however, this effect cannot be explained by simple competitive kinetics and the displacement of K is probably due to an allosteric effect on the K site secondary to glycoside enzyme interaction (30). These glycosides possess an unsaturated lactone ring attached in β configuration to the C17 of a cyclopentanophenanthrene

nucleus. Partial or complete loss of inhibitory activity is caused by saturation of the lactone ring, α configuration at C17, dehydrogenation of the hydroxyl at C3 or epimerization of this hydroxyl from β to α position (10).

Location of enzyme: This enzyme is found in the membranes of the erythrocyte (31) and the sheath of the squid axon (32). Following differential centrifugation of a cell homogenate the highest activities are present in the "nuclear" fraction (sedimenting at 600-1000xg) which contains cell debris and large fragments of cell membranes, and/or in the "microsomal" fraction (sedimenting at 30,000-100,000xg) which contains endoplasmic reticulum and cell membrane fragments. Wallach and coworkers (33-35) concluded that small fragments of plasma membrane sediment with the "microsomes" and are responsible for the bulk of the Na-K-ATPase activity in this fraction. The "mitochondrial" fraction has little or no enzyme activity (36).

CORRELATION OF Na-K-ATPase ACTIVITY WITH SODIUM TRANSPORT

An effective method of examining the physiologic role of enzyme systems is to correlate changes in the amount of enzyme within a tissue and its activity per milligram of protein with changes in the traffic over a metabolic pathway catalyzed by the enzyme system. With an increase in traffic over a pathway the enzyme activity often increases, whereas a decrease in traffic over a pathway frequently results in a decrease in enzyme activity. Enzymes which are rate-limiting are more sensitive to such maneuvers, however, enzymes which are non rate-limiting may also be affected. The lack of adaptation when volume of reaction changes does not exclude a functional role for the particular enzyme system. This approach has been utilized in several tissues (e.g. mammalian kidney, avian salt gland and teleost gill and intestine) in which Na-K-ATPase is thought to play an integral role in sodium transport.

Mammalian Kidney

In the kidney the bulk of the oxygen consumption and energy production is probably related to active sodium transport which in turn is closely related to renal blood flow and glomerular filtration rate. If Na-K-ATPase plays a role in active sodium transport it might be expected that by increasing or decreasing the tubular reabsorptive work, changes in enzyme activity would be effected.

An increase in Na-K-ATPase per milligram of protein in microsomal fraction of rat kidney cortex has been shown to occur following a

chronically increased tubular reabsorptive load of sodium per gram of kidney tissue (36). Increased tubular reabsorptive load follows unilateral nephrectomy (36), a course of methylprednisolone (2.5 mg/day for 4 days) (37) or feeding animals a high protein diet (50 percent protein for 7 days)(38,39). There is a 50 percent or greater increase in Na-K-ATPase activity following unilateral nephrectomy with lesser increases after the other two methods. This observation is of interest since the activity of glucose-6-phosphatase* and Mg-ATPase, two other microsomal enzymes, remained unchanged as did succinic dehydrogenase (a mitochondrial enzyme participating in oxidative metabolism) and glutaminase.

When the net tubular reabsorption of sodium was decreased following bilateral adrenalectomy (40) the Na-K-ATPase activity of a microsomal fraction of rat kidney reaches a level of about one-half that for normal controls by the seventh postoperative day (36,41-43), however, the Mg-ATPase activity does not change significantly. This can be restored to normal levels in 2-3 days with physiologic doses of corticosterone, but cannot be restored with physiologic doses of aldosterone (42). In a microsomal fraction of rat renal cortex two weeks post hypophysectomy there is a 50 percent decrease in Na-K-ATPase activity and a 30 percent decrease in Mg-ATPase activity compared with normal controls (unpublished data).

Glucocorticoids increase the glomerular filtration rate and net tubular reabsorption of sodium, therefore part of the effect of these hormones in stimulating Na-K-ATPase activity may be secondary to these changes rather than to a direct effect upon the cellular production or

*The activity after methylprednisolone increased as a result of corticoid-induced stimulation of renal gluconeogenesis.

degradation of the enzyme. The cellular mechanism of methylprednisolone induced increase in enzyme activity in rat kidney is not understood. This hormone increases the enzyme activity in a whole homogenate of kidney, however, there is no difference between the Na-K-ATPase activity per mg of protein of plasma membrane obtained from treated or untreated rats (44). This finding supports the hypothesis that glucocorticoids increase the Na-K-ATPase activity in the kidney by increasing the amount of plasma membrane per cell, which might be accomplished by proliferation of the infoldings of the limiting cell membrane at the antiluminal border, rather than by an increase in the specific activity of enzyme per unit of plasma membrane.

The sodium pump is thought to be located on the antiluminal surface of the renal tubular cell (45), and as sodium is pumped out, potassium might be reciprocally transferred into the cell, only to leak out again through the basilar membrane, which is considerably more permeable to potassium than is the luminal surface of the tubular cell (46). Saline loaded dogs respond to infusion of strophanthidin into the renal artery with a rapid fall in the glomerular filtration rate and a delayed natriuresis and diuresis (47). Since the diuresis could be blocked by potassium loading and enhanced by potassium depletion it suggests that the glycoside acts by limiting the availability of this ion to the exchange mechanism and therefore limits the transfer of sodium from tubular cell to the peritubular space, which in turn dissipates the gradient for movement of tubular sodium to tubular cell and results in the observed diuresis. Ouabain infused into the renal artery of dogs has produced a natriuresis which could be correlated with a dose-dependent

inhibition of renal cortical Na-A-ATPase, however, there was no consistent effect on enzyme from the medulla (48). Martinez-Maldonado and coworkers (49) found that infusion of digoxin into one canine renal artery resulted in a unilateral natriuresis with impaired concentrating capacity. The Na-K-ATPase activity was found to be greater in medullary tissue than in cortical tissue and both were decreased by about 50 percent after digoxin infusion.

The observation that Na-K-ATPase activity changes in an adaptive way when the renal absorptive load of sodium is increased coupled with the effects of cardiac glycosides on both enzyme and transport systems strengthens the hypothesis that this enzyme is involved in active sodium transport.

Avian Nasal Gland

Marine birds are faced with the necessity of conserving water and excreting salt. The avian kidney is composed of two types of nephrons; the cortical nephron, the more common, lacks a loop of Henle whereas the medullary nephron has a loop of Henle. The avian kidney cannot concentrate urine more than approximately twice the osmolarity of plasma and an extrarenal means of salt excretion has evolved. All birds have paired nasal glands situated along the dorsal margins of the orbit and discharging their excretory fluid, in most species, via ducts into the anterior nasal cavity. In terrestrial species the glands are small, but in birds with an evolutionary history of adaptation to a marine environment the gland has developed a significant capacity to secrete a hypertonic solution of sodium chloride. The capacity of the salt

secreting gland in birds depends on a primary genetic factor and on a secondary phenotypic effect of salt stress (i.e. diet e.g. fresh fish, invertebrates or marine plants).

The nasal gland was long known to be present in various avian species, however, its function in osmoregulation was not appreciated until Schmidt-Nielsen (50) found that cormorants utilized an extrarenal mechanism for salt secretion in response to an oral or intravenous hypertonic saline load and that an intravenous non-electrolyte osomotic load (sucrose) would also stimulate secretion. The stimulus to secretion may depend on volume or stretch receptors rather than on osmoreceptors (51). In subsequent studies Schmidt-Nielsen and coworkers (52-54) found that the main components of the nasal gland secretion were sodium and chloride in nearly equal amounts ranging from 500-1100 meq sodium/liter with small amounts of bicarbonate and potassium, magnesium and sulphate being virtually absent. The concentration of the secretion remains fairly constant for each species.

The gland is innervated by parasympathetic cholinergic stimulatory fibers and sympathetic adrenergic inhibitory fibers. Acetylcholine and its analogues cause secretion whereas epinephrine, atropine and anesthesia block secretion (55,56). The actively secreting salt gland of the herring gull develops a positive potential difference between the ducts of the gland and the blood. Retrograde injection of strophanthin (0.05 mg) through the duct prevented the development of the duct potential in response to stimulation and abolished secretion, however, the vasodilation in response to secretory nerve stimulation was maintained (54). Acetazolamide, a carbonic anhydrase inhibitor, blocks

secretion in an active gland, however, this effect can be reversed with methacholine (55). Since acetazolamide had no effect on the duct potential or secretory response of an electrically stimulated gland the block in secretion in response to an osmotic load which occurs after administration of acetazolamide must be due to an action of the drug in the reflex chain outside the salt gland (54). Figure 1 is a summary of the pathways involved in the regulation of excretion by the nasal gland.

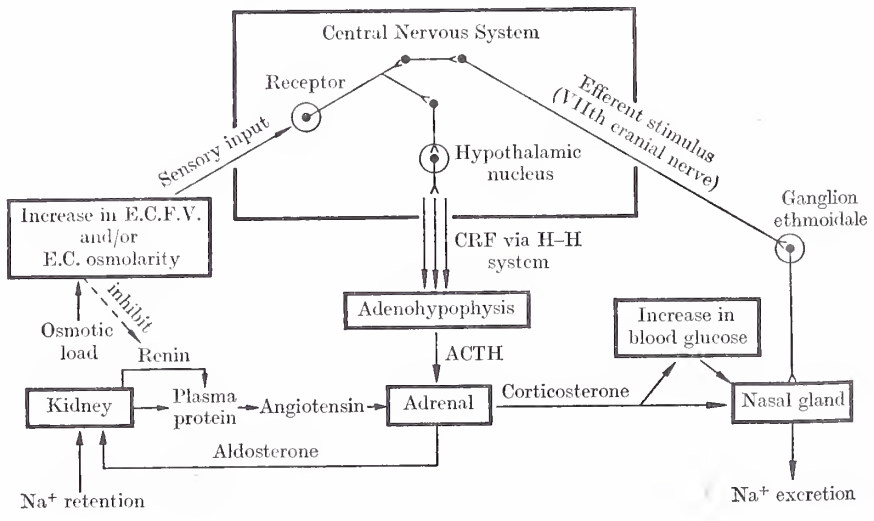


Fig. 1. A schematic representation of the possible pathways involved in the regulation of excretion in marine birds. (See reference 137)

When ducks (Anas platyrhnchos) are raised on one percent saline as drinking water, the salt secreting glands hypertrophy and the extra-renal salt-secreting capacity is increased primarily by increasing the volume of fluid that can be produced per unit time, however, there is also an increase in the sodium concentration of the fluid (57,58).

Both gulls (59) and ducks (60) when reared on salt water have increased absolute and relative adrenal weights when compared to freshwater controls. This suggests that adrenosteroids may be involved in nasal gland activity.

The oral administration of a sodium chloride load to intact ducks results in a diphasic response, beginning with a renal phase of excretion followed by an extrarenal phase of excretion (61). The onset of the extrarenal phase was significantly earlier in birds pretreated with cortisol, cortexone, aldosterone or ACTH and the total extrarenal excretion of sodium and potassium and initial rate of secretion was greater than in control ducks (61).

Bilateral adrenalectomy obliterates the extrarenal excretory phase in saline loaded ducks. Unilateral adrenalectomy resulted in a delay in onset and duration of the extrarenal phase with a decrease in the total excretion of sodium and potassium when compared with intact controls. The extrarenal phase began at a higher serum osmolarity than for control ducks. Maintenance of totally adrenalectomized ducks on cortisol restored their response to saline loads to near normal levels (62).

When the extrarenal mechanism of excretion is studied uncomplicated by effects secondary to the renal phase (63), aldosterone was found to have no effect on the excretion of a hypertonic saline load and to be unnecessary for the normal functioning of the nasal glands of the domestic duck.

In neither ducks (Anas platyrhnchos) nor gulls (Larus argentatus) is there a relationship between plasma corticosteroid levels and the activation or sustained secretion of the nasal gland (64). Phillips

and Bellamy (65) found an increased uptake of 14 C- cortisol and ^{3}H corticosterone in the nasal glands of ducks injected with 0.5 M NaCl as compared to 0.15 M NaCl. They postulated that the role of the neural component in nasal gland function is to cause dilation of the vascular bed which would provide the parenchyma with a greater volume of blood per unit time and therefore a larger amount of corticosterone.

The role of the adenohypophysis in nasal gland function has not been clarified, however, it has been shown that there is a significant depression of the extrarenal response to a hypertonic saline load in adenohypophysectomized ducks (66) and an enhanced output from the minimally stimulated nasal gland of ducks injected with ovine prolactin (67). Glucose administered with a saline load increase nasal gland secretion while insulin-induced hypoglycemia diminishes response to a similar saline load (68).

The presence of Na-K-ATPase in the avian salt gland was first demonstrated by Hokin (26). Using Great Lakes gulls and domestic geese maintained on 1.5% sodium chloride as their drinking water for one week, she was unable to demonstrate a change in Na-K-ATPase levels in a mitochondrial-microsomal fraction. The explanation for this in light of subsequent work is probably that the salt load was not large enough and the duration was too short.

Bonting and coworkers (27) demonstrated that the Na-K-ATPase activity of the nasal glands from gulls maintained on fresh water for seven weeks had only 51% of the activity per gram nasal gland compared to wild birds and the gland weighed only 65% of the control weight. An increase in the Na-K-ATPase activity of nasal gland homogenates from domestic ducks maintained on about one percent sodium chloride as

drinking water for varying time periods has been demonstrated (69-71). Fletcher and coworkers (72) have shown that domestic ducks during the period of adaptation to hypertonic saline (284 mM/liter sodium and 6 mM/liter potassium) undergo changes in the function of nasal glands characterized by increase in Na-K-ATPase activity which follows a similar time course to changes in sodium-excreting capacity, and increases in nasal gland weight and sodium concentration of the excreted fluid. These changes were reversed when ducks were returned to fresh water. Table 1 summarizes these findings.

Table 1

Some Parameters of Nasal Gland Function in Ducks Maintained on Various Fresh Water and/or Saline Regimes
(modified from reference 72)

Group	Number of birds	Relative Nasal gland weight (mg % B.W.) 1	Maximum excretory rates[2] (mμL or mμM/mg/min) Water	Na	Conc. Na in nasal fluid (mM/L)	Pi Release (mμM/mg protein/min) Mg-ATPase Ouabain insensitive	Na-K-ATPase Ouabain sensitive
30 days fresh water	5	15.9 ± 0.9+	209 ± 29	99.0 ± 16	466 ± 17	283 ± 20	149 ± 19
30 days saline	5	28.7 ± 3.4*	668 ± 54*	395 ± 29*	592 ± 8.0*	579 ± 56*	517 ± 17
51 days fresh water	5	17.5 ± 1.0	278 ± 34	132 ± 15	480 ± 11	280 ± 32	194 ± 25
30 days saline + 21 days fresh water	5	17.2 ± 0.6	318 ± 31	151 ± 16	474 ± 16	223 ± 32	158 ± 17
58 days fresh water	5	14.5 ± 1.1	284 ± 15	129 ± 6.0	455 ± 9.0	161 ± 32	154 ± 10
30 days saline + 21 days fresh water + 7 days saline	7	26.9 ± 1.3*	598 ± 37*	344 ± 19*	581 ± 11*	479 ± 53*	419 ± 41*

+ All values are expressed as means ± S.E.M.
* $P < 0.01$ with respect to value for corresponding freshwater-maintained birds
1 Body weights were not significantly different among these groups
2. Maximum extra-renal excretory rates were determined by the intravenous infusion of 10% sodium chloride, and expressed per mg. wet weight of nasal gland tissue

THE EEL

Natural History of The Eel

The life history of the European eel (Anguilla anguilla) is composed of three phases separated by two metamorphoses (73). Spawning occurs in the Sargasso Sea during spring and summer at a depth of 400 - 500 meters. After hatching the larvae (leptocephali) ascend toward the surface to a depth of about 50 meters and are carried by surface currents and dispersed. This period, the marine larval phase, lasts 2 - 3 years. The metamorphosis of the leptocephali into elvers occurs in autumn immediately adjacent to the Continental Slope. The elvers ascend (anadromous migration) to freshwater areas and complete their metamorphosis into young eels. The sexually immature yellow eel lives in a freshwater environment where it feeds and grows slowly for a period of 9 - 18 years depending on food supply, temperature and living space; this is the freshwater larval stage. The second metamorphosis is accompanied by several changes: 1. the eel which was greenish on the back and yellow ventrally now becomes nearly black on the dorsal and lateral aspects and silvery white ventrally due to an increase in malanins and guanine respectively, 2. an alteration of the hormonal environment which enables the eel to adapt to a marine environment more rapidly, 3. the cessation of food consumption with a reduction in the digestive tract and 4. sexual maturation (73,74). In autumn, the silver eel begins the reproductive or catadromous migration to the Sargasso Sea to spawn (74) or die (75), this period is the adult marine phase.

Tucker (75) proposed that: 1. Anguilla rostrata and Anguilla anguilla are not distinct species but eco-phenotypes, their distinguishing features (103 - 110 vertebrae versus 110 - 119 vertebrae) being environmentally determined, 2. the European eels perish in their own continental waters without completing their reproductive cycle and 3. the population of European eels is entirely maintained by reinforcements of larvae of North American parentage.

Although the natural history of the American eel has not been carefully studied, it is likely that it is similar to that of the European eel. The change from a seawater to a freshwater environment during migration requires rapid osmoregulatory readjustment of the gills, intestine and kidneys.

Gills

The gills of euryhaline teleosts play an integral role in osmoregulation in both freshwater and seawater (1,3). Fish are able to compensate for the renal and extrarenal loss of sodium and chloride in freshwater by active absorption of sodium and/or chloride (76-79). The skin and gills of freshwater eels seem to be highly impermeable, there being little passive loss of sodium or potassium, or gain of water (17). When isolated gills of freshwater silver eels were incubated in tap water aerobically they were able to maintain a constant level of sodium chloride against the normal diffusion gradient (80). This steady state appeared to be due to active movement of sodium chloride into the gills balancing the passive outward diffusion. This transport might be a $NH_4+/Na+$ exchange mechanism since intraperitoneal injection of NH_4+ resulted in

an increase in sodium influx (79), no evidence for a HCO_3^-/Cl^- exchange was found.

In a marine environment the gills transport sodium and chloride outward against the osmotic gradient (3). Isolated gills of seawater-adapted eels are able to maintain a steady state concentration of sodium chloride when incubated in seawater, whereas gills from freshwater eels could not transport sodium chloride against the concentration gradient (80,81). During the course of seawater adaptation the gills become capable of actively secreting sodium chloride. When ouabain is injected into seawater-adapted eels the isolated gills lose the capacity to excrete sodium and the sodium content of the gills during incubation in seawater increases as it does in freshwater eels (12,82). This inhibitory effect of ouabain is not observed when it is added to the incubation medium (82). Incubation of isolated gills from seawater-adapted eels in potassium-free seawater causes a complete inhibition of sodium chloride excretion (83).

Intestine

Marine teleosts compensate for the passive outward diffusion of water by drinking seawater and excreting sodium chloride extrarenally (1,4). Eels can adapt to external environments ranging from tap water (containing 0.4 mM sodium, 0.02 mM potassium and virtually no chloride) (84) to double-strength seawater (17). In going from freshwater to double-strength seawater there is an increase in drinking rate, sodium turnover rate and plasma sodium concentration. (see Table 2). The drinking reflex apparently depends upon an increase in tissue osmotic pressure rather than the presence of specific ions in the external medium

since freshwater eels placed in hypertonic sucrose solutions drink from the surrounding medium (85). In seawater there is an initial dilution of the intestinal contents, achieved by a passive flow of water from plasma to lumen, which is necessary before absorption of water is possible. Subsequently, the concentration of sodium chloride in the ingested fluid decreases due to active transport of sodium chloride with "solute-linked water flow". The bivalent ions, Mg and SO_4, are not well absorbed and are retained in the intestine in an isotonic solution becoming several times more concentrated than in seawater (1).

Table 2

Some Parameters of Osmoregulation in Eels Adapted to Various Salinities (modified from reference 17)

Adaptation medium	External Na conc. meq/L	Drinking rate µl/hr/100g b.w.	Na turnover rate % exchangeable Na/hr	Plasma Na meq/L
Freshwater	0.5	135 ± 27.7 (17)++	0.5-1.0+	130 ± 2.43 (25)
Seawater	5.25	325 ± 33.5 (12)*	27.2 ± 3.54 (5)*	147 ± 2.34 (25)
Double-Strength seawater	1040	802 ± 182 (5)*	61.2 ± 7.92 (4)*	176 ± 3.84 (11)

\+ From Garcia Romeu and Motais 1966
++ Mean $\pm$ S.E. number of subjects in parenthesis
* The increase in drinking rate and sodium turnover with increasing external salinity in significant $P < 0.01$

Permeability to water and ionic absorption capacity of the intestine is greater in seawater-adapted eels than in freshwater eels (86-90). As the external salinity increases the osmotic gradient between external and internal media increases and the rate of drinking increases to compensate for the increased water loss across the gills. Skadhauge (90)

studied the absorption of salt and water in vivo by intraluminal perfusion of the intestine of European eels adapted to freshwater, seawater and double-strength seawater. The net absorptive rate of sodium and chloride ions and the rate of water absorption is augmented in relation to the increased osmotic stress of higher external salinities, as is the osmotic gradient against which water absorption becomes possible. Perfusion experiments with impermeant solutes demonstrated that in the absence of active salt movement water movement was essentially a passive process. Following transfer from freshwater to seawater the Japanese eel increases its drinking rate gradually to a maximum on day five and then it decreases to a constant level after three weeks (86). Transport of water and sodium in isolated gut sacs parallels the change in drinking rate. In isolated intestine sacs of the American eel (Anguilla rostrata) following transfer to seawater, the salt and water transport increases to a maximum on the third day and then decreases slowly to a stable level after about two weeks (89). Ouabain (10^{-4} M) decreased water transport in gut sacs from both fresh and seawater-adapted eels. All of the increase in water transport that develops during seawater adaptation is ouabain sensitive and therefore probably represents an increase in sodium transport dependent on Na-K-ATPase (89). A considerable component of water transport, possibly due to transport of solutes other than sodium, was insensitive to ouabain in both freshwater and seawater-adapted eels but was completely inhibited by KCN (10^{-3} M) plus iodoacetic acid (10^{-3}M) suggesting its dependence on metabolic energy.

Kidney

The eel kidney is similar to other teleost glomerular kidneys; having nephrons composed of a glomerulus, a proximal convoluted tubule, a distal convoluted tubule and a collecting system (91,92). The thin segment of the loop of Henle is absent and this limits the teleosts to elaboration of an iso or hypotonic urine. The arterial vascularization accounts for about 30 percent of the total, with the rest arising from the caudal vein giving rise to a renal-portal system (93). In seawater the urinary electrolytes consist primarily of Mg and SO_4 with small quantities of Ca, PO_4, Na, K and Cl. In freshwater only small quantities of Mg and SO_4 are excreted.

Freshwater teleosts combat the passive influx of water by maintaining a rapid flow of hypotonic urine (1,2). In freshwater eels the GFR and urine volume is greater than in seawater-adapted eels, however, the percentage reabsorption of water and sodium is greater in the latter resulting in a slightly greater absolute loss of sodium in freshwater eels (84). The reabsorbed sodium load is about four fold greater in freshwater compared to seawater-adapted eels. These parameters of renal function are illustrated in Table 3. Oide and Utida (94) found that in Japanese eels transferred to seawater both the rate of urine flow and the GFR was reduced markedly within six hours. However, after ten days the urine flow was still slight but the GFR recovered to the level of freshwater eels. In seawater the eel conserves water by increasing the absorption of Na, Cl and water.

Table 3

Changes in Glomerular Filtration and Tubular Reabsorption of Water and Electrolytes in Freshwater and Seawater Eel (Anguilla anguilla) (modified from reference 84)

	Freshwater Eels				Seawater Eels+			
	H_2O++	Na	K	Cl	H_2O	Na	K	Cl
Filtered (GFR)	110	16.5	0.297	11.5	25	4.37	0.077	3.85
Excreted (Urine)	84	1.6	0.055	nil	15	0.098	0.031	1.80
% Reabsorbed	24	90.3	81.5	100	40	97.8	59.7	53.2

+ adapted to seawater for at least 10 days
++ results expressed as ml/kg/24 hr or mM/kg/24 hr

Pituitary Influence on Osmoregulation

Teleosts can be divided into two categories: one in which the pituitary appears to play no essential role in osmotic adjustments, the other in which the fish cannot live in freshwater without the gland (7). Fundulus heteroclitus is unable to survive in freshwater after hypophysectomy (95) due to progressive asthenia secondary to hyponatremia. Ovine prolactin enables hypophysectomized F. heteroclitus to maintain normal serum osmolarity in freshwater (96-98). Osmoregulation in the eel appears not to be closely controlled by prolactin since hypophysectomized yellow eels can survive in freshwater and de-ionized water for long periods of time (99,100). Hypophysectomy induces a slow decline in plasma sodium of freshwater eels which is due to an augmentation of the sodium outflux (101) and to a probable decrease in sodium influx secondary to adrenocortical insufficiency (102,103). Prolactin administered to recently hypophysectomized eels can reduce

the sodium outflux to normal levels but has no effect on sodium influx (101). Chan and coworkers (103) and Butler (102) found that hypophysectomized silver eels in freshwater were unable to maintain water and electrolyte balance, manifested by marked decrease in serum sodium and potassium concentration coupled with increase in percent muscle water and a decline in the muscle sodium and potassium concentration compared with intact and sham-operated controls. Injections of bovine prolactin (2 mg/100 b.w./day) plus cortisol (20 μg/100g b.w./day) returned the above parameters to normal whereas neither alone was adequate (103).

In hypophysectomized seawater adapted silver eels there is an increase in serum sodium and chloride concentration with a decrease in percent muscle water compared to intact controls (102). The rate of sodium exchange between the internal and external environment of seawater-adapted eels involves about thirty percent of the exchangeable sodium per hour and hypophysectomy reduces this rate by about fifty percent (101). Prolactin does not increase the reduced sodium turnover rate in hypophysectomized seawater eels, however, ACTH does increase the rate (101). On transfer from seawater to freshwater the hypophysectomized eel readjusts sodium fluxes in a pattern similar to that of intact eels; however, the sodium influx is reduced and the sodium outflux is maintained at levels above that of freshwater adapted eels resulting in a slightly negative sodium balance (101).

Corticosteroid Influence on Osmoregulation

Cortisol is the major corticosteroid secreted by teleost interrenal tissue (8). In eels the interrenal tissue extends for approximately ten millimeters in the wall of the anterior and posterior cardinal veins. The main nodes are in the walls of both left and right posterior cardinal veins ending posteriorly in the lymphoid head kidney (104). Cortisol has been demonstrated in the plasma of eels (105). In the European eel the plasma cortisol concentration was found to be about 2 - 3µg/100ml in both freshwater and seawater-adapted eels (106). In vitro incubation of interrenal tissue with progesterone-4-C^{14} or pregnenolone-16-H^{3} yields labelled cortisol as predominant product with smaller quantities of cortisone and corticosterone (107-111). Aldosterone has not been conclusively demonstrated in plasma or by in vitro incubation techniques (107,108,112), however, a low-activity NADPH-dependent 18-hydroxylase which transforms exogenous corticosterone to 18-hydroxycorticosterone has been found in eel interrenal tissue (112).

There is evidence for a pituitary-adrenocortical feedback mechanism based on histologic (113,104) and physiologic (114) data. The administration of metopirone (an 11β-hydroxylase inhibitor) to eels results in an activation of the interrenal, with nuclear, nucleolar and cellular hypertrophy with mitotic activity. In hypophysectomized eels the interrenal hypertrophy and hyperplasia is suppressed but the nuclei undergo changes similar to those of intact eels. The epsilon cells (corticotrophic cells), located in the rostral pars distalis along its posterior border with the neurohypophysis, undergo hyperplasia,

hypertrophy, degranulation and vacuolization during treatment with metopirone. The changes in the interrenal cells are presumably secondary to drug induced hypersecretion of ACTH by the pituitary, which is reflected by the changes in morphology of the epsilon cells of the pars distalis.

In the eel, hypophysectomy decreases the in vitro rate of conversion of endogenous precursors in the interrenal gland without altering the composition of secretion. In vivo injection of ACTH restores the corticosteroidogenic activity of in vitro gland preparations. ACTH stimulates in vitro corticosteroidogenesis of the interrenal gland but only after prior hypophysectomy (115). Butler and coworkers (114) have shown that hypophysectomized eels have decreased plasma cortisol levels compared to intact controls, that ACTH (0.20 I.U./100g b.w.) elevated the plasma cortisol level of hypophysectomized eels to twice that of intact controls and that the plasma cortisol level could be decreased significantly by dexamethasone-21 phosphate (4mg/kg b.w.). These findings suggest that both a positive and negative feedback machanism operates between the hypophysis and the teleost interrenal gland.

Effects of Adrenal Hormones on Composition and Distribution of Body Fluids

The changes in plasma and tissue electrolyte composition, the distribution of body fluid between the intra-and extracellular spaces and the level of sodium transport performed by eels after adrenalectomy, hypophysectomy or treatment with exogenous hormones depends on many factors (e.g. completeness of endocrine ablation, schedule and dose of

hormones used, season and phase of eel, specific experimental protocol and the inclusion of appropriate sham-operated controls). The interpretation of data from one experiment to another often gives conflicting results and does not allow generalizations to be made concerning the effects of a specific treatment on the above parameters.

Freshwater eels: Adrenalectomy of freshwater (yellow or silver) eels was accompanied by an increase in body weight and muscle water content, decrease in serum sodium concentration and maintenance of normal serum potassium concentration (116). Butler and Langford (117) found no change in plasma or tissue electrolyte concentration in partially adrenalectomized freshwater yellow eels, however, it is likely that residual interrenal tissue was present since no change in plasma cortisol concentration was observed (118). Subsequently using a more radical technique of adrenalectomy (105) they were able to demonstrate a decrease in serum sodium and chloride concentration but no change in serum potassium concentration or body weight in adrenalectomized freshwater yellow eels compared to intact controls. There was a significant decrease in plasma cortisol levels in the adrenalectomized group. Cortisol (5-10 mg/day for 10 days) given to freshwater yellow eels produced a decrease in serum sodium concentration and the serum potassium level either fell or remained within normal range without change in muscle water content (116). Mammalian ACTH (2 I.U./day for 10 days) in intact freshwater eels did not change the above parameters (116). ACTH administered to hypophysectomized freshwater silver eels elevated serum sodium concentration toward normal levels (103).

Seawater eels: Adrenalectomy in seawater-adapted eels resulted in a decrease in body weight with diminution of muscle water content and an increase in serum sodium concentration with normal serum potassium concentration (116). Mayer and coworkers (18) found that adrenalectomized seawater eels could not survive in seawater for more than 48 hours, however, they were able to survive in one-third seawater.

Effects of Adrenal Hormones on Sodium Transport in Gill and Intestinal Mucosa

Gill: Freshwater adapted silver eels have a net extrarenal uptake of sodium from the surrounding tap water (made up to 600 μM sodium) which can be changed into a net extrarenal loss with cortisol (10 mg/day for 4 days) (119). Distilled water adapted silver eels, which are salt depleted with low serum concentration, have a net extrarenal sodium uptake three times greater than freshwater controls. Hypophysectomy or adrenalectomy decreased the net extrarenal sodium uptake in distilled water adapted eels and this uptake was increased in adrenalectomized eels treated with aldosterone or cortisol (10 μg/500-800g b.w.) (119). Cortisol appears to play a role in the net extrarenal uptake of sodium in freshwater adapted eels probably by increasing the sodium influx rather than by decreasing the sodium outflux (103).

Adrenalectomized seawater-adapted yellow eels have a much lower sodium turnover rate, 6 percent (expressed as percent of internal sodium exchanged/hr), than do sham-operated controls, 27 percent (18). Cortisol (50 μg/100g b.w.) given during the 24 hours prior to flux measurement brought the sodium turnover rate of adrenalectomized eels to a level comparable with that of sham-operated controls. The sodium

outflux in adrenalectomized seawater adapted eels was considerably reduced compared to sham-operated and adrenalectomized cortisol treated controls. On transfer of adrenalectomized freshwater adapted eels to seawater there was an increased lag period before the sodium outflux was augmented, and the level achieved was significantly less than that for sham-operated controls (18). The presence of the adrenals seems to facilitate the increased sodium outflux across the gills which is necessary for seawater adaptation.

Intestine: The increase in sodium and water transport by isolated intestine sacs which occurs after transferring freshwater eels to seawater (86,87,89) can be abolished by hypophysectomy (81,120), and restored toward normal with cortisol (120). ACTH or cortisol produced a significant augmentation in intestinal water transport within 24 hours in freshwater Japanese eels (Anguilla japonica) and the response appeared to be dose dependent (121). Other hypophyseal or adrenocortical hormones were without effect. A single injection of cortisol acetate (2.5 mg) into freshwater eels produced changes in water transport in isolated intestine sacs similar to those occuring after transfer of freshwater eels to seawater (121). Injection of freshwater eels (Anguilla rostrata) with cortisol (400 µg/100g b.w./day for 14 days) or methylprednisolone (0.3 mg/100g b.w./week for 2 weeks) increased intestinal water transport markedly (89). Adrenalectomy reduces the water permeability of everted intestine sacs from freshwater eels and cortisol administration restores it to normal levels (122). The adrenal steroids appear to be closely linked with changes in intestinal transport occurring during seawater adaptation.

Na-K-ATPase Activity in Gill and Intestinal Mucosa of Eels

The activity of Na-K-ATPase in a sodium iodide treated microsomal fraction of gills from seawater-adapted Japanese eels was five times greater than that of freshwater eels, the Mg-ATPase activity remained unchanged (12). Oide (123) found a three fold increase in Na-K-ATPase level in intestinal mucosa from seawater-adapted Japanese eels compared to freshwater controls. Jampol and Epstein (13) observed a two fold increase in the Na-K-ATPase level in both the gills and intestine of seawater-adapted eels (Anguilla rostrata) compared to freshwater controls, however, the Mg-ATPase activity was not significantly different. In the European eel (Anguilla anguilla) and flounder (Platychthys flesus) no difference in the Na-K-ATPase or the Mg-ATPase activity was found in seawater-adapted fish compared to freshwater water controls (124), however, Motais (14) subsequently observed a doubling of the Na-K-ATPase activity in gills of seawater adapted eels. Hypophysectomy of seawater-adapted Japanese eels did not affect either the active excretion of sodium ions from isolated gills (81) or the level of Na-K-ATPase of the gills (125). The failure to show a decrease in Na-K-ATPase activity or active sodium excretion was probably due to the short time interval between hypophysectomy and the measurement of these parameters.

METHODS

Freshwater yellow eels (Anguilla rostrata) were trapped in a freshwater pond (sodium concentration less than 5 mM/L) by the collecting crew of the Mt. Desert Island Biology Laboratory. The eels, both male and female, weighing between 80 and 400 grams were obtained in July and August. They were initially kept in running freshwater tanks at ambient temperature. Those designated as seawater-adapted were preadapted for two days in 50 percent seawater prior to being placed in running seawater tanks (about 475 mM sodium/L at about 15° C).

Eels were adapted to seawater for periods of from several days to three weeks. Some freshwater eels were given intramuscular injections of long-acting methylprednisolone acetate (Depo-Medrol, Upjohn) 300 µg/100 gram body weight (200 µg/0.1 ml) weekly for two or three weeks. Several groups of freshwater eels received intramuscular injections of hydrocortisone hemisuccinate (Solu-Cortef, Upjohn) daily, either 50 µg or 400 µg/100 gram body weight (50 µg or 400 µg/0.1 ml) for seven to fourteen days. All fish were starved for several days prior to use.

Preparation of tissue homogenates: The eels were sacrificed by multiple spinal cord sectioning. The gills were dissected and the gill filaments were removed and placed in tared iced beakers and weighed. The intestine was dissected, then opened longitudinally and washed with water after which the mucosa was scraped with a glass slide and then weighed.

The tissue was homogenized in an ice-cold solution containing 0.25 M sucrose, 30 mM imidazole buffer, 5 mM sodium ethylenediaminetetraacetate and one gram sodium deoxycholate per liter at pH 6.8 in a ratio of 2 ml homogenizing solution to 100 mg of tissue. This was carried out in a glass homogenizer immersed in ice with a Teflon pestle at 1725 rpm and 0.18 mm clearance using 20-25 strokes. The homogenate was filtered through a double layer of gauze and then immediately assayed for Na-K-ATPase activity.

Adenosine triphosphatase assay: One-tenth milliliter of the tissue suspension (containing 2-8 mg protein/ml) was added to 4.6 ml of incubation medium containing 10 mM imidazole buffer, and either 100 mM NaCl and 20 mM K Cl (regular medium) or 120 mM NaCl (no potassium medium) at pH 7.8. This mixture was preincubated at 37°C for 5 minutes in a shaking water bath. The reaction was begun by the addition of 0.3 ml of a solution containing 100 mM disodium ATP (sodium adenosine triphosphate, Sigma Chemical) and 100 mM $MgCl_2$ at pH 7.8 and incubated for 15 minutes at 37°C in a shaking water bath. It was terminated by the addition of 1 ml of ice-cold 35 percent trichloracetic acid to the incubation flasks. After centrifugation the precipitated protein was discarded and the inorganic phosphate in the supernate was determined by the method of Fiske and Subbarow (126), the optical density being read at 660 mμ on a Coleman Junior spectrophotometer. Correction for the spontaneous nonenzymatic breakdown of ATP was made by measuring the inorganic phosphate liberated in the absence of enzyme. This blank, containing homogenizing solution was run along with phosphate standards (0.2, 0.4 and 0.8 mM/L K_2HPO_4) with each set of phosphate

determinations. Each sample was done in duplicate and averaged.

The protein content of the tissue suspensions was determined by the method of Lowry, Rosebrough and coworkers (127) using crystalline human albumin standards. The activity of Na-K-ATPase was defined as the difference between the amount of inorganic phosphate (Pi) released from ATP in the medium containing potassium and that released in the medium without potassium, and is expressed as micromoles of Pi released per hour per milligram of protein. The breakdown of ATP in the medium without potassium is referred to as "Mg-ATPase" or "residual" ATPase. In separate experiments the Na-K-ATPase activity was shown to be equal to the amount of ATP breakdown inhibited by 10^{-4} M ouabain.

Blood was aspirated into syringes, treated with heparin or EDTA, from the caudal vein of unanesthetized restrained eels. The plasma was separated by centrifugation and the chloride was determined by an amperometric method (Cotlove chloridometer).

The statistical significance between mean values was assessed by Student's t test. P values less than 0.05 were considered significant.

RESULTS

ATPase Activity in Gill Epithelium and Intestinal Mucosa (Table 4)

Adaptation to seawater for one to three weeks resulted in a 50 percent increase in Na-K-ATPase activity in the gill filaments, from 4.6 to 7.3 or 6.7 μ moles Pi released / mg protein/hr respectively ($p<0.01$). Cortisol in low-dose (50 μg/100g b.w./day for 7-12 days) and high-dose (400 μg/100g b.w./day for 7-14 days) induced a rise in the Na-K-ATPase activity in freshwater eels. The former raised the enzyme level in the fill filaments to that seen in seawater-adapted eels and the latter to a level almost three times that of untreated freshwater controls. Methylprednisolone (300 μg/100g b.w./week for 2-3 weeks) similarly raised the level of Na-K-ATPase. The Mg-ATPase activity was not changed significantly after transfer to seawater or by the corticosteroid treatment.

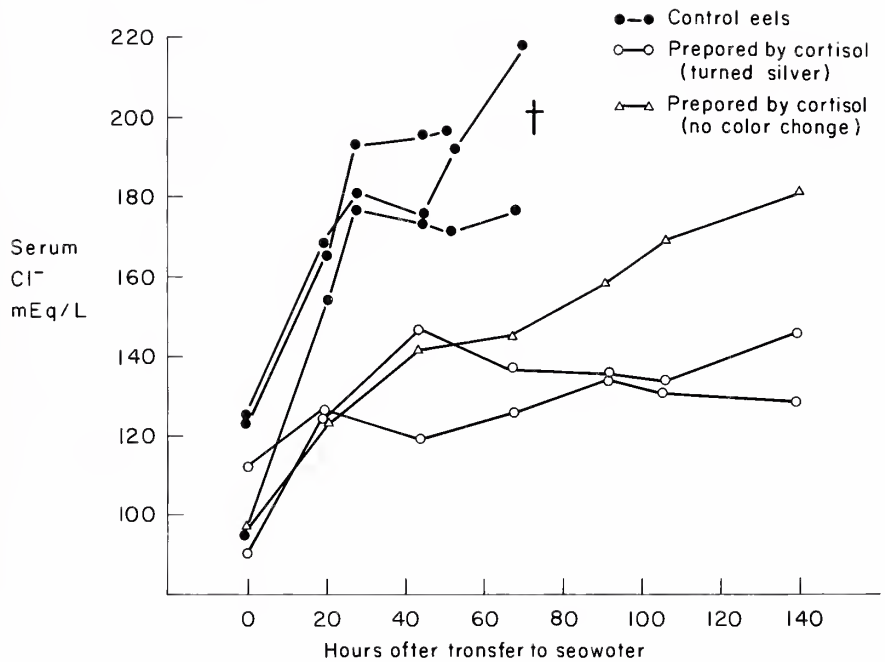
The activity of Na-K-ATPase in intestinal mucosa was increased after treatment with high-dose cortisol from 9.6 in freshwater eels to 18 ($p<0.05$), a level comparable with that found previously in intestinal mucosa after seawater adaptation (13). The Mg-ATPase activity was also augmented by the high-dose cortisol from 11 to 22 ($p<0.01$). Low-dose cortisol treatment increased the level of both enzymes insignificantly.

Effect of Treatment With Cortisol on the Adjustment of Freshwater Eels to Seawater

Seawater adapted European and Japanese eels have serum sodium and chloride levels which are about 20-40 meq/L higher than that of fresh-

water eels (17,94,116,128). Freshwater yellow eels (Anguilla rostrata) can be adapted to seawater but must spend a minimum of two days in 50 percent seawater prior to transfer to full-strength seawater. In the present experiments, the plasma chloride level of six freshwater yellow eels averaged 94.0±6.8 meq/L (mean ± S.E.) (range 76-121 meq/L) and after 2-3 weeks of adaptation to seawater the average plasma chloride level in nine eels was 134±2.5 meq/L (range 121-145 meq/L). When freshwater eels were transferred directly to full-strength seawater, they usually died on the second or third day following transfer, apparently due to inability to readjust their osmoregulatory mechanisms rapidly. This is illustrated in Figure 2 by serial plasma chloride samples obtained from three eels. Plasma chloride levels rose rapidly to levels of 170-190 meq/L during the initial 24 hours and all three eels died by the third day. Pretreatment of freshwater eels with cortisol (400 µg/100g b.w./day) for two weeks prior to and after transfer to full-strength seawater enabled them to survive direct transfer. The plasma chloride levels rose slowly during the first day and then leveled off the second day and remained between 128 and 145 meq/L in the two eels which had turned silver after the cortisol injections. In the third eel, which had not turned color, the adaptation seemed to be partial, since the plasma chloride rose slowly over the six days of the experiment reaching 180 meq/L at 140 hours.

Figure 2

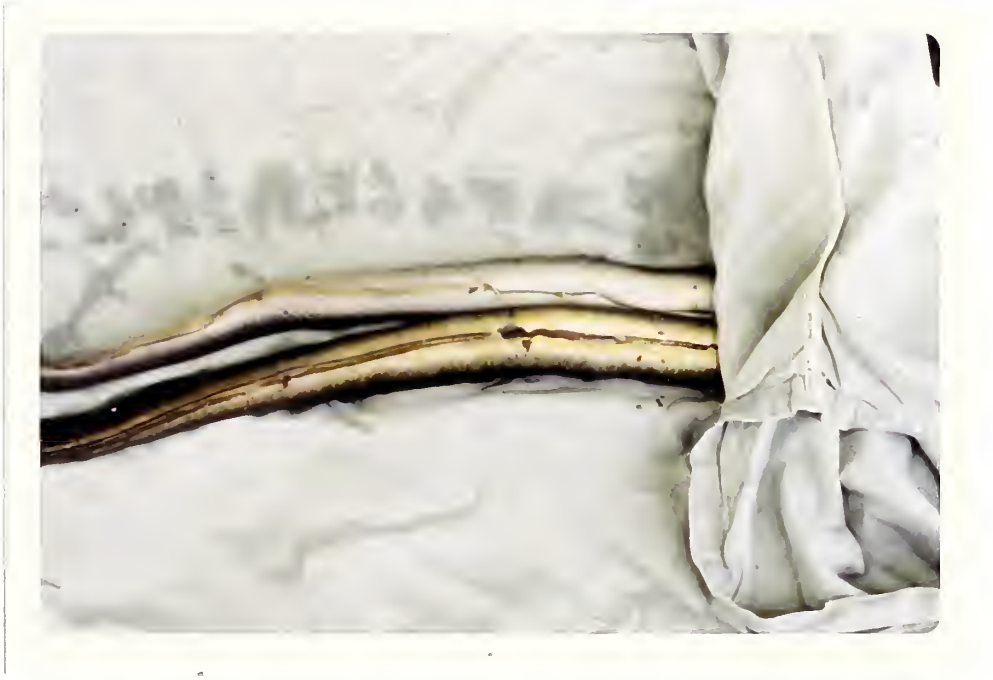


Effect of pretreatment with cortisol (400 μg/100g b.w./day) on plasma chloride level during rapid adaptation to seawater, (modified from reference 129)

Pigmentary Effects of Cortisol and Seawater Adaptation (see Figure 3)

After seven to ten days of cortisol injections the ventral surface of freshwater yellow eels lost its yellow color and turned silver, similar to the silver hue of European eels spontaneously migrating to the sea (130). Freshwater yellow eels adapted to seawater for 2-3 weeks did not undergo a color change.

Figure 3



Freshwater yellow eels. The one above was treated with cortisol (400 µg/100 g b.w./day for ten days), the one below was control.

Table 4

Effect of Seawater Adaptation and Corticosteroids on ATPase Levels in Gill Epithelium and Intestinal Mucosa of Anguilla rostrata

	N	Na-K-ATPase	Mg (residual) ATPase
Freshwater gill	11	4.6 ± 1.2+	11 ± 6.3
Seawater gill (adapted 3 weeks)[1]	10	6.7 ± 1.0**	8.7 ± 3.3
(adapted 1 week)[2]	4	7.3 ± 1.5**	8.0 ± 0.75
Low-dose cortisol gill[3]	5	7.7 ± 2.3*	7.6 ± 2.1
High-dose cortisol gill[4]	11	12 ± 2.8**	8.3 ± 1.2
Methylprednisolone gill[5]	6	6.9 ± 1.7**	9.4 ± 2.8
Freshwater gut	5	9.6 ± 1.9	11 ± 3.6
Low-dose cortisol gut	5	12 ± 3.9	14 ± 2.1
High-dose cortisol gut	5	18 ± 6.6*	22 ± 3.5**

+ mean ± S.D. results expressed as μ moles Pi released/mg protein/hr

* $p < 0.05$ compared with freshwater controls

** $p < 0.01$ compared with freshwater controls

[1] mid July

[2] mid August

[3] received 7-12 daily injections cortisol 50 μg/100g b.w.

[4] received 7-14 daily injections cortisol 400 μg/100g b.w.

[5] received 2-3 weekly injections methylprednisolone 300 μg/100g b.w.

DISCUSSION

Sodium Transport in Freshwater and Seawater Teleosts

The essential features of sodium transport in freshwater and seawater teleosts are summarized in Figure 4. In freshwater the internal sodium turnover rate is small. The net branchial uptake of sodium compensates for the renal salt loss. In seawater the high internal sodium turnover rate involves 10-30 fold more sodium than can be accounted for by drinking, gut absorption and gill net excretion rates. Maetz (131,132) proposed a linkage between sodium outflux and potassium influx through a common exchange carrier, with external sodium and potassium competing for this common carrier. The exchange diffusion mechanism (linkage of sodium influx and outflux) and the high internal sodium turnover rate results from this competitive process. Support for this model is based on the following observations in the seawater-adapted flounder (Platychthys flesus) (131): 1. the net sodium extrusion rate by the gill is similar to the potassium influx, 2. a small but significant reduction of sodium outflux is seen in K-free seawater which results in suppression of net sodium excretion, and 3. in the absence of external potassium the sodium turnover rate remains high. Maetz (131) suggested that Na-K-ATPase might play a central role in this Na-K exchange pump.

Cortisol Induction of Na-K-ATPase in Gill Epithelium and Intestinal Mucosa

The activity of Na-K-ATPase in the gills of stenohaline teleosts is much higher in marine species than in freshwater species (13,133) which is consistent with the known high level of sodium outflux in the gills of marine teleosts compared to freshwater teleosts. The

Na-K-ATPase activity in gills from seawater-adapted; eels (Anguilla anguilla, Anguilla japonica, and Anguilla rostrata)(12-14), killifish (Fundulus heteroclitus) (11), coho salmon (Oncorhynchus kisutch) (134), rainbow trout (Salmo gairdnerii) (133) and the goby (Acanthogobius flavimanus) (133) was several times higher than that of freshwater-adapted controls, however, the Mg-ATPase activity was constant. Hypophysectomy of seawater adapted killifish decreases the Na-K-ATPase activity in the gills compared to intact controls (11). Cortisol replacement therapy in hypophysectomized seawater killifish increases the Na-K-ATPase activity in the gill epithelium and intestinal mucosa compared to hypophysectomized control eels, however, the activity of Mg-ATPase was not changed (135).

Cortisol, the major corticosteroid secreted by the teleost interrenal gland (8) appears to be a salt-excreting factor in seawater-adapted eels and a salt-absorbing factor in freshwater eels (6). The present experiments demonstrate that cortisol as well as methylprednisolone increase the Na-K-ATPase activity in gill epithelium and intestinal mucosa of intact freshwater eels to levels equal to or greater than those of seawater-adapted eels. The magnitude of this effect appears to be dose dependent. It is apparent at a dose of cortisol (50 μg/100g b.w./day) that suffices to restore the sodium turnover rate across the gill of adrenalectomized eels to levels comparable with sham-operated controls (18), and it is more marked when a larger dose of cortisol (400 μg/100g b.w./day) is given. The increase in mucosal Na-K-ATPase activity is accompanied by an increase in ouabain-sensitive sodium transport capacity of isolated intestine sacs in vitro (89).

The failure of the Mg-ATPase activity to change significantly, except in one instance, suggests that this enzyme does not play a role in the active sodium transport system. The plasma cortisol level was not significantly different between the freshwater and the seawater-adapted eels, being less than 3 µg/100 ml, however, the high-dose cortisol treated (400 µg/100g b.w./day) eels had a mean level of 17 µg/100 ml indicating a state of hyperadrenalism (unpublished data).

Hormones may influence the activity of cellular enzymes by a direct action on the target organ to enhance or depress the rate of synthesis and/or degradation of the enzyme, or an indirect action by altering the physiology of an organism so as to change the traffic over the pathway catalyzed by the enzyme. The cortisol induced increase in the Na-K-ATPase activity in gill epithelium and intestinal mucosa of freshwater yellow eels appears to be a direct effect of the hormone on the target tissue since there is no significant difference in the sodium flux across the gills of cortisol treated freshwater eels compared to untreated controls (Epstein, F. H. personal communication).

The augmentation in the enzyme activity following transfer of freshwater eels to seawater may represent an adaptive response of the gill epithelium and intestinal mucosa to increased sodium transport initiated by transfer. It is likely that cortisol has a role in facilitation or induction of enzyme in this instance also, since the plasma cortisol levels, measured by a competitive protein-binding technique, which are not significantly different in freshwater or seawater-adapted eels are elevated from 3-9 µg/100 ml two to three

days following transfer from freshwater to seawater, and return to normal level (2-3 μg/100 ml) by the eighth day (106, Epstein, F. H. personal communication). The stimulus for the increase in interrenal activity, probably mediated through the hypothalamo-hypophyseal-interrenal axis, during the early period of seawater adaptation is not known, however, it may be secondary to an increase in serum osmolarity or a decrease in extra-or intracellular volume.

The cellular mechanism responsible for the increase in Na-K-ATPase activity in gill epithelium or intestinal mucosa of freshwater eels following seawater adaption or corticosteroid treatment is not known. The possibilities are the following: 1. an increase in specific activity per unit plasma membrane, 2. an increase in the amount of cell membrane per cell and/or 3. an increase in the number of cells involved in sodium transport. There is little basis for favoring any one of these mechanisms. The "chloride cell" a mitochondria-rich cell of the gill filament is felt by some workers to play a role in salt transport, however, this is still controversial (5). During seawater adaption intestinal mucosa of eels undergoes mucosal hypertrophy, however, this was not observed to occur in cortisol treated freshwater eels (Epstein, F. H. personal communication).

Eels adapted to seawater for one week during the late summer had a larger increase in gill Na-K-ATPase activity than did eels adapted to seawater for three weeks earlier in the summer. Utida and coworkers (136) found that the rate of water transport in isolated intestine sacs showed a tendency to increase and the sodium penetration into isolated gills incubated in seawater tended to decrease during autumn (when the

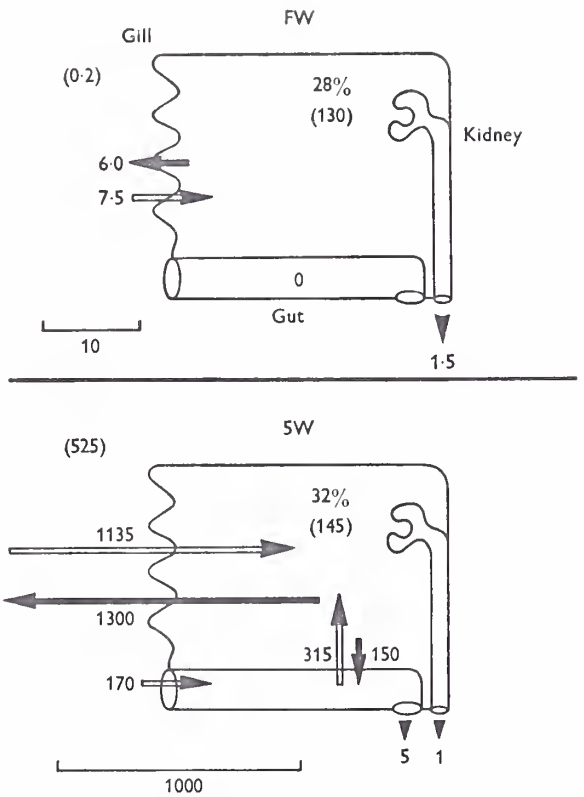
catadromous migration is thought to occur) in freshwater eels. During autumn freshwater eels were better able to resist the osmotic stress of seawater transfer. He suggested that the eel repeats these seasonal variations in adaptability to seawater each year until attaining maximal adaptability and sexual maturity when it migrates to the sea as a catadromous silver eel. Zaugg and McLain (134) monitored the Na-K-ATPase activity in a microsomal fraction of gills from yearling coho salmon (Oncorhynchus kisutch) from February to October and found a doubling of activity during late March as seaward migration begins, and a later decline in this activity if the fish remained in freshwater. The activity of Mg-ATPase remained constant during this period.

There is no consistent difference observed in the activity of Na-K-ATPase of freshwater silver compared to freshwater yellow eels (unpublished data). The freshwater Japanese silver eel has a higher rate of water transport in isolated intestine sacs and a lower rate of sodium penetration through isolated gills incubated in seawater compared to freshwater yellow eels (87). It would be of interest to measure serially the plasma sodium, sodium outflux and Na-K-ATPase activity of seawater adapting silver eels compared to yellow controls.

Motais (14) (see Table 5) found that actinomycin D reduced the Na-K-ATPase activity in gills from seawater-adapted eels (Anguilla anguilla) but had no effect on the enzyme level of the freshwater-adapted eel. The activity of Mg-ATPase was unchanged after treatment of seawater or freshwater adapted fish. He suggested that there were two types of Na-K-ATPase; an actinomycin sensitive form which is involved in the branchial sodium-excreting pump with a short half-life

(2-3 days) and an actinomycin insensitive form with a relatively long half-life which could be directly involved in the branchial sodium-absorption pump of freshwater eels or could be unrelated to the osmoregulatory function of the gill but be associated with the Na-K exchange pump which maintains the high intracellular potassium level in all cells.

Figure 4



Comparative Na balance in the freshwater (FW) and seawater (SW) eel. Note different scales for the fluxes (in -equiv $h^{-1}(100\ g)^{-1}$). External and internal Na concentrations in brackets (in m-equiv $l.^{-1}$). Size of the extracellular spaces in percentage body weight.
(from reference 132)

Table 5

Effects of Actinomycin D[1] on Na-K-ATPase and Mg-ATPase Activity of the Gill of the European Eel (Anguilla anguilla) (modified from reference 14)

	Seawater adapted		Freshwater adapted	
	Control	Actinomycin treated	Control	Actinomycin treated
Na-K-ATPase	10.8 ± 0.85	6.9 ± 0.63*	5.5 ± 0.61	5.1 ± 0.72
	(9)	(16)	(16)	(8)
Mg-ATPase	12.4 ± 0.93	10.9 ± 0.72	9.3 ± 0.76	8.6 ± 1.1
	(9)	(16)	(16)	(8)

[1]Actinomycin D 50 μg/100g b.w. intraperitoneally, given five days prior to sacrifice

Activity expressed in μ moles Pi released/mg protein/hr (mean ± S.E.), the number of fish in parenthesis

* $P < 0.01$ compared with untreated controls

Effect of Pretreatment With Cortisol on Seawater Adaptation of Freshwater Eels

Freshwater yellow eels (Anguilla rostrata) pretreated with cortisol (400 g/100g b.w./day for 14 days) are able to withstand direct transfer to full-strength seawater, whereas, untreated controls die within 2-3 days unless they are placed in 50 percent seawater for two days prior to the transfer to full-strength seawater. The Japanese eel and the European eel are able to withstand direct transfer to seawater. This ability may be due to species differences or may reflect previous exposure of these eels to water of varying salinity, whereas the eels used in these experiments probably had lived wholly within a freshwater environment.

Although pretreatment of freshwater eels induced an increase in Na-K-ATPase activity comparable to that of seawater adapted eels the transfer to full-strength seawater was accompanied by an elevated plasma chloride level for 3-4 days before adequate osmoregulation is achieved. It would seem that some factor in addition to Na-K-ATPase, perhaps induced by osmotic stress, is necessary to increase the sodium outflux from the pretreated seawater adapting eel (132). Epstein and coworkers (personal communication) have found that there is a parallel increase in sodium outflux and Na-K-ATPase activity in the gills of seawater adapting freshwater eels and that pretreatment with cortisol leads to an earlier increase in the sodium outflux.

Pigmentary Effects of Cortisol and Seawater Adaptation

Cortisol (400 µg/100g b.w./day for 7-10 days) produced a change in the ventral pigmentation of freshwater yellow eels to the silver

hue of spontaneously migrating eels. Seawater-adapted yellow eels did not undergo a change in pigmentation. The failure of the seawater-adapted eels to undergo a change in pigmentation might be due to the short duration of the elevated cortisol levels or possibly the cortisol induced pigment change was secondary to feedback inhibition of ACTH or MSH. The seaward migration of eels is thought to be induced by changes in their hormonal environment. Since the alteration in pigmentation and increase in enzymatic (Na-K-ATPase) and functional (sodium transport) activity of the gills and intestinal mucosa induced by treatment of freshwater yellow eels with cortisol are similar to the changes thought to occur in naturally migrating eels, it appears that this hormone may be responsible for the sequence of physiological alterations that facilitate the spontaneous catadromous migration of freshwater eels.

SUMMARY

Adaptation to seawater by freshwater eels (Anguilla rostrata) involves an increase in active sodium transport across gill epithelium and intestinal mucosa which is mediated in part by cortisol secreted by the interrenal gland. Cortisol induces an augmentation in the activity of Na-K-ATPase in the gill filaments and the intestinal mucosa of freshwater eels similar to the changes produced by adaptation to seawater. Freshwater eels pretreated with cortisol (400 µg/100g b.w./day for 14 days) were able to withstand direct transfer. The plasma chloride level of the cortisol treated eels did not rise as high as that of the untreated eels. The ventral surface of freshwater eels injected with cortisol (400 µg/100g b.w./day for 7-10 days) loses its yellow pigmentation and turns silver, resembling the color of eels spontaneously migrating to the sea. The data suggests that cortisol plays an integral role in the adaptive changes associated with the catadromous migration of freshwater eels.

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