Evans DH. Teleost fish osmoregulation: what have we learned since August Krogh, Homer Smith, and Ancel Keys. Am J Physiol Regul Integr Comp Physiol 295: R704–R713, 2008. First published June 4, 2008; doi:10.1152/ajpregu.90337.2008.—In the 1930s, August Krogh, Homer Smith, and Ancel Keys knew that teleost fishes were hyperosmotic to fresh water and hyposmotic to seawater, and, therefore, they were potentially salt depleted and dehydrated, respectively. Their seminal studies demonstrated that freshwater teleosts extract NaCl from the environment, while marine teleosts ingest seawater, absorb intestinal water by absorbing NaCl, and excrete the excess salt via gill transport mechanisms. During the past 70 years, their research descendents have used chemical, radioisotopic, pharmacological, cellular, and molecular techniques to further characterize the gill transport mechanisms and begin to study the signaling molecules that modulate these processes. The cellular site for these transport pathways was first described by Keys and is now known as the mitochondrion-rich cell (MRC). The model for NaCl secretion by the marine MRC is well supported, but the model for NaCl uptake by freshwater MRC is more unsettled. Importantly, these ionic uptake mechanisms also appear to be expressed in the marine gill MRC, for acid-base regulation. A large suite of potential endocrine control mechanisms have been identified, and recent evidence suggests that paracines such as endothelin, nitric oxide, and prostaglandins might also control MRC function.

What Did Krogh, Smith, and Keys Know?

On the basis of published values for teleost plasma ionic concentrations (reviewed in Refs. 72 and 129), Krogh, Smith, and Keys knew that the plasma of marine teleosts was hyposmotic to seawater, with ~60% less NaCl per liter than the marine environment. They also knew that freshwater teleosts, or euryhaline marine teleosts in fresh water, had a significantly lower plasma salt content, but they were still distinctly hyperosmotic to their environment (~300 mOsm/l vs. ~1 mOsm/l), with Na⁺ and Cl⁻ the dominant ions in the plasma. Because of these osmotic and ionic gradients, they knew that marine teleosts were constantly dehydrated and salt loaded (depending upon their gill epithelial water and ionic permeabilities), while freshwater teleosts were overhydrated and salt depleted. Smith, Krogh, and Keys also knew that, as a consequence of these osmotic and ionic gradients, marine teleosts excrete a
small volume of urine that is approximately isosmotic to the plasma, while freshwater teleosts produce large volumes of dilute urine (e.g., 129).

What Did Krogh, Smith, and Keys Discover?

August Krogh, working in Copenhagen, proposed that freshwater teleosts must extract NaCl from the environment to maintain ionic balance (73). Using laborious chemical analyses (think no radioisotopes, flame photometers, or atomic absorption spectrophotometers!), his elegant experiments demonstrated that nonfeeding, freshwater fishes (e.g., catfish, stickleback, perch, trout) could actually reduce the Cl⁻ content of the tank water from concentrations below ~1 mM (in one case from a solution that was only 20 micromolar). Using a divided chamber, Krogh also demonstrated that the Cl⁻ uptake was from the head end, which he suggested was most probably across the gills. Most important, he measured Cl⁻ uptake from a variety of Cl salt solutions (e.g., NaCl, KCl, NH₄Cl, and CaCl₂) and found that the cation made no difference, indicating that Cl⁻ uptake was independent of Na⁺ uptake. He suggested that Cl⁻ uptake was probably in exchange for HCO₃⁻. Likewise, he found that Na⁺ uptake was similar from solutions of NaCl, NaBr, NaHCO₃, and NaNO₃, demonstrating that Na⁺ uptake was independent of Cl⁻ uptake. He suggested that Na⁺ uptake might be coupled to NH₄⁺ excretion, since he measured increases in NH₄⁺ efflux when Na⁺ uptake was stimulated by an increase in external Na⁺ concentrations (73). Krogh performed similar experiments on a variety of freshwater organisms (e.g., annelids, mollusks, and crustacea) and concluded that this ability to extract Na⁺ and Cl⁻ independently from a hypo-osmotic medium was a general phenomenon (72, 74).

Homer Smith was on the medical faculty at New York University but worked during summers on marine teleosts (and elasmobranchs) at the Mt. Desert Island Biological Laboratory in Cambridge, UK, during the same period. Keys knew of Smith’s work and set out to confirm the hypothesis of salt secretion across the marine teleost gill. He invented a complex perfused heart-gill preparation using the eel (67), which has never been duplicated in its stability despite attempts of at least three groups (e.g., 12, 103, 105). By monitoring the Cl⁻ concentration of the perfusate (fish saline) or irrigate (seawater) before and after the gill, Keys showed clearly that Cl⁻ was excreted across the gills (into the seawater irrigation solution) despite the nearly threefold concentration gradient in the opposite direction. In Keys’ words: “It is clear that in this experiment the internal medium became diluted by reason of a concentrated chloride solution being secreted by the gills” (66). In a subsequent paper, Keys goes on to say: “the chloride secretion exhibited by the gills of the eel in seawater is an active process” (68). Surely, these must be some of the earliest suggestions of active transport across an epithelium.

In that third paper, Keys and Willmer (68), described “chloride secreting cells” in the gills of various species of marine teleosts (e.g., eel, conger eel, salmon, plaice). They commented that, given the fact that they were “clearly evident . . . it is rather surprising that [they] were not reported earlier.” Because of their structure (not similar to mucous cells) and position (between the blood and the external medium), they proposed that this provided “a possible and even probable histological basis for the branchial chloride secretion.” They suggested that, because the freshwater species that they examined (roach, dace, bream) had similar cells (albeit fewer in number), the cells also could be involved in salt uptake by freshwater fishes (68).

4 The angler fish (Lophius sp.) became famous during the same era, because E. K. Marshall and Homer Smith found that it was aglomerular and could be used to prove definitively that the vertebrate proximal tubule could secrete salts and water into the nephron (88, 89).

5 The clearest balance sheet for monovalent vs. divalent ion absorption and excretion in marine teleosts remains the work of Hickman (51).

6 A nontechnical discussion of Smith’s work can be found in his famous “From Fish to Philosopher” (127).

7 This may have been due to the fact that these groups did not include the heart in the preparation. Keys found that the presence of the heart was critical to the hemodynamic success of the preparation, and he suggested that “the perfusion medium derives a hormone, or hormones, from the heart, which acts to preserve capillary tone.” This proposition that the heart might produce a vasotoxic substance predates DeBold’s discovery of atrial natriuretic peptides by 50 years (15).

8 Cl− was chosen because of the relative ease (compared to Na⁺) of chemical determination.

9 But’s classic description of active salt transport across the frog skin was published four years later (56).

10 Keys left fish physiology a few years later but went on to a very distinguished career in nutrition, including the formulation of “K rations”
Fig. 1. Mechanisms of osmoregulation by teleost fishes. Freshwater teleosts are hyperosmotic to the surrounding solution, so they face osmotic gain of water and diffusion loss of NaCl across the permeable gill epithelium. These potentially disruptive osmotic and ionic movements are compensated for by excretion of relatively large volumes of a dilute urine, and active uptake of NaCl across the gill epithelium. Marine teleosts are hyposmotic to seawater, so they face osmotic loss of water and diffusion gain of NaCl across the gill. Compensatory mechanisms include ingestion of seawater, intestinal absorption of NaCl and water, excretion of small volumes of blood-isotonic urine (after tubular reabsorption of Na, Cl, and water), and active secretion of NaCl across the gill epithelium. Passive ion movements are denoted by dashed arrows; active by solid arrows. See text for details. Fish outline is used, with permission, from Ref. 119.

Gill salt secretion. The first suggestion of a mechanism for gill salt extrusion came from Frank Epstein’s laboratory [working at the Mount Desert Island Biological Laboratory (MDIBL)], which demonstrated that the newly discovered Na\(^+\)-K\(^+\)-activated ATPase (126) had high enzymatic activity in fish gill tissue and that the activity was higher in marine species (sculpin, killifish, sea raven, flounder, angler fish) than freshwater species (minnow, bass, eel) and increased in the eel gill when that euryhaline species was acclimated to seawater (22, 60). This correlation of Na\(^+\) uptake by the perfused trout gill was correlated with proton, rather than ammonia, efflux. They proposed that, like the frog skin, the gill epithelium of the freshwater fish extrudes proton actively, which draws in Na\(^+\) (via a channel) down the electrochemical gradient across the apical membrane (2). The alternative, obviously, is that Na\(^+\)/H\(^+\) exchange could account for their findings, but they proposed that the apical electrochemical gradients could not support this passive exchange. A similar conclusion, based upon thermodynamic calculations, was reached by Potts (115) and Kirschner (69).

Thus, it certainly can be said that the basic model for teleost osmoregulation was formulated and published in the 1930s, due to the work of Krogh, Smith, and Keys (Fig. 1). In fact, one reviewer of my first National Science Foundation proposal in 1970 wondered why they should fund my proposal, because “Homer Smith has told us what we need to know about fish osmoregulation.”

Since the late 1960s, laboratories in the United States and Europe (and more recently, Asia) have produced a substantial body of literature on the mechanisms of fish osmoregulation that were first described by Krogh, Smith, and Keys. Space limits this review to the pathways of teleost gill salt transport, but the reader can get a much broader perspective of fish osmoregulation by reading some recent reviews (31, 62, 91), as well as a recent review by Grosell (49) on fish intestinal physiology.

Early Mechanistic Studies

Gill salt uptake. Support for Krogh’s hypothesis for the mechanisms of gill Na\(^+\) and Cl\(^-\) uptake by freshwater fishes awaited the advent of radioisotopes after World War II. Jean Maetz’s group confirmed that the Na\(^+\) and Cl\(^-\) uptakes were independent (44) and found that external NH\(_4\)\(^+\) inhibited radioisotopic Na\(^+\) influx, but injection of NH\(_4\)\(^+\) stimulated Na\(^+\) influx. Similar experiments demonstrated a linkage between radiochloride uptake and external or internal HCO\(_3\)\(^-\) (86). Importantly, they also suggested that these mechanisms might be related to acid-base balance, as well as ionic regulation (14). We followed up on this idea, demonstrated what appeared to be Na/NH\(_4\) exchange in marine fishes and euryhaline fishes in fresh water (24, 28), and suggested that the exchanges even might be important in acid-base regulation in marine fishes (26). We also proposed that these exchange mechanisms evolved in the stenohaline, marine hagfishes, before the vertebrates entered fresh water (25). Thus, evolution of the first vertebrates into fresh water (and modern euryhalinity) may not have been limited by the presence or absence of Na\(^+\)/NH\(_4\)\(^+\) and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers but by the affinity of the uptake mechanisms vs. gill ionic permeability (30). These data seemed to confirm Krogh’s hypothesis, but others suggested that the extremely low external Na\(^+\) and Cl\(^-\) concentrations in most freshwaters would limit the rate of these passive exchangers. Work by Ehrenfeld and Garcia-Romeu in Maetz’s laboratory demonstrated that frog skin Na\(^+\) uptake is actually via an apical channel, driven by an electrochemical gradient produced by active proton secretion (21), and other Maetz colleagues, Avella and Bornancin, found that Na\(^+\) uptake by the perfused trout gill was correlated with proton, rather than ammonia, efflux. They proposed that, like the frog skin, the gill epithelium of the freshwater fish extrudes proton actively, which draws in Na\(^+\) (via a channel) down the electrochemical gradient across the apical membrane (2). The alternative, obviously, is that Na\(^+\)/H\(^+\) exchange could account for their findings, but they proposed that the apical electrochemical gradients could not support this passive exchange. A similar conclusion, based upon thermodynamic calculations, was reached by Potts (115) and Kirschner (69).

11 The grant was funded, for the then grand amount of $50,000 for two years.

12 Heisler’s group in Germany had found that acid-base disturbances in freshwater and marine fishes were largely compensated for by transfer of acid or base equivalents across the gills (29, 30).

13 Bill Potts and Len Kirschner were pioneers in comparative animal osmoregulation. Indeed, Potts’s book with Gwyneth Parry (117) started many of us in this subdiscipline.

14 Jean Maetz was one of the leading figures in fish osmoregulation in the 1960s and 1970s (e.g., 84). He was tragically killed in an automobile accident.
then popular, radioisotopic flux methods. Maetz found that the measured K\(^+\) influx was identical to the Na\(^+\) efflux from the flounder, and that the latter was proportional to the external K\(^+\) concentration (85). We found the same linkage of external K\(^+\) with Na\(^+\) efflux, with a \(K_m\) that was equivalent to the \(K_m\) for the K-stimulated Na\(^+\)-K\(^+\)-activated ATPase activity in gill tissue from the fat sleeper (36). Thus, it seemed clear that Na\(^+\) extrusion was via an apical Na/K exchange pump. This hypothesis was falsified three years later by the clear demonstration by Karl Karnaky (also working at the MDIBL) that Na\(^+\)-K\(^+\)-activated ATPase was basolateral, rather then apical, in the gill “chloride cells” in the euryhaline killifish (63).

One of the major problems with studying the mechanisms of transport across the fish gill is that it is a very complicated epithelium, not amenable to the classic Ussing chamber approach, which was proving so important to studying the mechanisms of Na\(^+\) transport across the toad skin and bladder during the same period (e.g., 141); (reviewed in Ref. 61). Karnaky solved this problem by discovering that the epithelium lining the inner surface of the gill cover (operculum) of some species of teleosts (the euryhaline killifish was the prime example) contains high concentrations (60% vs. 10% in branchial epithelium) of “chloride cells.” When mounted in an Ussing chamber, the opercular epithelium produced a short-circuit current that could be entirely accounted for by the net radioisotopic Cl\(^-\) efflux (64). Under short-circuited conditions, there was no net flux of Na\(^+\). Thus, ironically, it appeared that the “chloride cells” were aptly named by Keys 40 years previously. Further work by this group (16) demonstrated that this Cl\(^-\) extrusion was dependent upon oxygenation and serum Cl\(^-\) and Na\(^+\) concentrations but inhibited by serum ouabain (an inhibitor of Na\(^+\)-K\(^+\)-activated ATPase), as well as furosemide [a Cl\(^-\) transport inhibitor in the mammalian kidney; (6)]. During the same time period, Silva et al. (124) (in the Epstein group) demonstrated that injected ouabain inhibited both Cl\(^-\) and Na\(^+\) radioisotopic effluxes across the gill of the eel, and they suggested that the mechanism for Cl\(^-\) extrusion might be a coupled Na\(^+\) + Cl\(^-\) cotransport, similar to that which was being described for a variety of other epithelial tissues (reviewed in Ref. 42). The definitive proof that the “chloride cell” was the site of the Cl\(^-\) extrusion came five years later, when Kevin Foskett showed clear Cl\(^-\) currents when a microprobe was placed over “chloride cells” in the opercular skin of tilapia, which also has high concentrations of these cells (41). Thus, by 1980, the model for NaCl extrusion by the marine teleost gill epithelium was in place and is still accepted today (Fig. 2).

Recent Molecular and Physiological Studies

Gill salt uptake. Molecular techniques have been applied in the study of the mechanisms of gill salt uptake since the early 1990s (for reviews, see Refs. 53 and 57). The fact that known inhibitors of proton transport (e.g., vanadate or decreased external pH) inhibited H\(^+\) extrusion by the trout gill (79) supported the hypothesis of the active, apical proton pump that Avella and Borancin (2) had proposed. Subsequent studies in Randall’s laboratory (80) demonstrated a proton-sensitive ATPase activity in trout gill homogenates, which was inhibited by known proton pump inhibitors (e.g., N-ethylmaleimide; dicyclohexylcarbodiimide, diethylstilbestrol, p-chloromercuribenzenesulfonate, and bafilomycin). Bafilomycin-sensitive Na\(^+\) uptake now has been demonstrated in zebrafish (3), tilapia and carp (39), and trout (118). Randall’s group also showed the apical localization of a V-type H\(^+\)-ATPase in the trout gill, via immunofluorescence and Western blot analysis (78). The apical cellular localization has now been extended to tilapia and colocalized with what appears to be an ENaC-like sodium channel (143). Finally, phenamil, an ENaC inhibitor in mammals (70), reduces Na\(^+\) uptake in the goldfish and trout (102, 118, 120). Interestingly, there is no ortholog or paralog for ENaC in the published genomes of any fish (e.g., zebrfish, fugu, stickleback, medaka), so (despite the immunohistochemical signal, using a heterologous antibody) it is unlikely that the putative channel is homologous to ENaC. On the other hand, a fish V-type H\(^+\)-ATPase has now been cloned from the trout (106), as well as the killifish (65). Somewhat surprisingly, the V-type H\(^+\)-ATPase appeared to be localized to the basolateral membrane in the killifish. Most recently, P.-P. Hwang’s group has demonstrated cells in the skin of zebrafish larvae that express apical V-type H\(^+\)-ATPase and show a bafilomycin-sensitive current when a proton-sensitive ion probe is passed over the cell in the intact tissue (81). Injection of morpholinos containing antisense sequences to V-type H\(^+\)-ATPase mRNA decreased the cellular expression of V-HAT and the acid efflux from the tissue, as well as the Na\(^+\) content of the embryos (55). It is generally assumed that basolateral Na\(^+\)-K\(^+\)-activated ATPase provides the pathway for basolateral transport of Na\(^+\) into the extracellular fluids, but there is some evidence that a basolateral Na\(^+\)+HCO\(_3\) co-transporter may also be involved in the rainbow trout (102, 107) and Osorezian dace (52). The cells that secrete acid via an apical V-type H\(^+\)-ATPase, and Na\(^+\) via an apical channel may be equivalent to a subpopulation of mitochondrion-rich cell (MRC) from the trout gill (isolated by Percoll gradient) that does not bind to peanut lectin agglutinin (PNA-negative) (43, 47, 109). These cells express relatively high V-type H\(^+\)-ATPase activity and a phenamil-sensitive Na\(^+\) uptake (120) and may be equivalent to “cuboidal cells” described in the killifish gill (77).

Parallel to the foregoing studies on the proton pump-Na\(^+\) channel hypothesis, there has been an emerging literature supporting the presence of an apical Na\(^+\)/H\(^+\) exchanger [Image 335x600 to 551x722]
Na\textsuperscript{+}\textsuperscript{(AE1; SLC4)} was immunolocalized to the apical surface of the other hand, a pendrin-like anion exchanger (Cl\textsuperscript{−}low-pH fresh water (52). These authors proposed that Na\textsuperscript{+} uptake via the apical exchanger was thermodynamically possible because of the low intracellular Na\textsuperscript{+} content that was maintained by the basolateral Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase, plus a basolateral Na\textsuperscript{+}−HCO\textsubscript{3}\textsuperscript{−} cotransporter (NBC\textsubscript{1}), which they colocalized with Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase. The H\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{−} may be provided by intracellular carbonic anhydrase, which also was demonstrated by immunohistochemistry (52). Most recently, Perry’s group (59b) has used homologous antibodies to localize apical NHE2/NHE3 in MRC in the trout gill epithelium. Similar data have now been reported for elasmobranchs, where branchial, Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase-containing cells also express apical NHE3 or NHE2 (7, 9, 11).

There is also emerging evidence that an apical Na-CI cotransporter may be involved in ionic uptake in freshwater fishes. Both NKCC-like and NCC-like (59a) have been immunolocalized to apical membranes in the gill of tilapia (57, 144), but there are no physiological data supporting a role in NaCl uptake.

The evidence for an apical anion exchanger is somewhat less conflicting, although relatively scant. A Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchanger (AE\textsubscript{1}; SLC\textsubscript{4}) was immunolocalized to the apical surface of Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase-containing cells in tilapia (143). On the other hand, a pendrin-like anion exchanger (Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−}; SLC\textsubscript{26}) has been immunolocalized in the stingray to cells in the gill that contain basolateral V-type H\textsuperscript{+}-ATPase, distinct from Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase-expressing cells (9, 112). Intracellular carbonic anhydrase (e.g., 52) coupled to basolateral V-type H\textsuperscript{+}-ATPase could provide the cytoplasmic HCO\textsubscript{3}\textsuperscript{−} to drive the apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchanger (140). These base-excreting cells may be equivalent to the isolated trout gill cells that bind to peanut lectin agglutinin (PNA-positive) (43, 47, 109), but the evidence is “scarce and indirect” (140). Surprisingly, Perry’s group has recently demonstrated that these PNA\textsuperscript{+} cells in the trout branchial epithelium also express apical NHEs and that the mRNA for NHE2 is upregulated (59b).

This somewhat conflicting, and incomplete database gives us a rather complex working model for Na\textsuperscript{+} and Cl\textsuperscript{−} uptake by freshwater fishes (Fig. 3). It is clear that the presence and importance of apical proton pumps coupled to Na\textsuperscript{+} channels vs. apical Na/H exchangers in freshwater teleosts (or in acid-base regulating species in any salinity) may vary, and the actual distribution of the proteins on the apical vs. basolateral membranes of one or two cells may be species specific.

Interestingly, Hwang’s group has recently published a cloning and immunohistochemical (IHC) study that demonstrated both apical V-type H\textsuperscript{+}-ATPase and NHE3 in the same, non-NKA cell in the zebrafish gill; V-type H\textsuperscript{+}-ATPase was upregulated during acidosis, and NHE3 was upregulated in a reduced Na\textsuperscript{+} solution (145).

Finally, it is noteworthy that the putative pathways for Na\textsuperscript{+} and Cl\textsuperscript{−} uptake by the gill epithelium of fishes are similar to those described in the mammalian proximal tubule (apical NHE exchanger, basolateral Na\textsuperscript{+}−K\textsuperscript{+}−activated ATPase) (1) and the α- vs. β-type intercalated cells of the mammalian collecting duct and turtle bladder (apical V-type H\textsuperscript{+}-ATPase and basolateral Cl/HCO\textsubscript{3}\textsuperscript{−} vs. apical Cl/HCO\textsubscript{3}\textsuperscript{−} and basolateral V-type H\textsuperscript{+}-ATPase) (e.g., 4, 5, 132, 133).

**Gill salt secretion.** Since the early physiological studies, numerous groups have demonstrated that “chloride cells” in a variety of teleosts express Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase, measured both immunohistochemically for the protein and via in situ hybridization for the mRNA. In the past decade, “chloride cells” have been renamed “mitochondrion-rich cells,” or MRC, and IHC localization of Na\textsuperscript{+}−K\textsuperscript{+}-activated ATPase has been used to visualize the cells in specific regions of the gill of many species of teleosts, as well as in elasmobranchs and agnathans (reviewed in Ref. 37).

Supporting the initial suggestion of Silva et al. (124), modern IHC techniques have localized the Na, K, 2Cl cotransporter (sensitive to furosemide; actually NKCC1) to the basolateral regions of the MRC in the fish gill (e.g., 104, 138) and, as one might expect, the expression of the transporter declines as the salinity is lowered. Fish-specific NKCC1 has now been cloned from a variety of fish species (e.g., eel, brown trout, Atlantic salmon, and striped bass; Ref. 37). Basolateral, recycling of K\textsuperscript{+} may be via an inward rectifying K\textsuperscript{+} channel, which increases in expression in seawater-acclimated eels (136). To complete the extrusion pathway across the MRC, one must propose an apical Cl\textsuperscript{−} conductance, and Marshall’s group (90) discovered a Cl\textsuperscript{−} channel (in cultured killifish opercular MRCs) whose electrical properties and response to relatively specific inhibitors suggested that it is related to the cystic fibrosis transmembrane conductance regulator (CFTR; Ref. 121). Subsequently, the first fish CFTR was cloned (125) and localized...
to the apical membrane of the MRC, after the killifish was acclimated to seawater (92).

Thus, molecular localization has confirmed the model that was first suggested by the Karnaky and Epstein laboratories at the MDIBL, where Homer Smith also did his early experiments. The model is generally accepted, including the assumption that Na\(^+\) is in electrochemical equilibrium across the marine teleost gill epithelium and is excreted by passive transport across the gill (92). In some cases, however, the transepithelial electrical potential measured across intact fishes (and assumed to be across the gill) is quite different from the Nernst equilibrium potential for Na\(^+\) (reviewed in Refs. 27 and 91), suggesting that alternative Na\(^+\) pathways might be discovered in some species in the future.

In summary, modern, molecular techniques have provided an array of data that have extended and clarified the models of gill salt transport that were first proposed by Krogh, Smith, and Keys. The transport mechanisms in the seawater gill are relatively well accepted. We are less certain of the specific mechanisms (or cellular distribution of these mechanisms) that mediate NaCl uptake in freshwater fishes, and in marine fishes for acid-base balance. Certainly, this is an area for future research, using this array of modern techniques that Krogh, Smith, and Keys did not have available.

Control of Gill Salt Transport

Krogh, Smith, and Keys did not study mechanisms of control of fish osmoregulation, but the past 50 years has seen the publication of a large amount of literature on this subject. Early studies demonstrated the importance of prolactin or survival of teleosts in fresh water (87, 110, 116) and cortisol for acclimation to sea water (40, 94). More recent work has shown that cortisol is also important in freshwater osmoregulation, and that circulating hormones such as growth hormone, IGF, angiotensin, arginine vasotocin (fish equivalent of vasopressin), natriuretic peptides, thyroid hormones, glucagon, urea, calcitonin, and calcitonin-related peptide, stanniocalcin, and parathyroid-related protein may be involved in gill transport and gill perfusion. Space does not permit even a cursory discussion, but the interested researcher can read a much broader review in the following papers: (23, 37, 95, 96, 137).

As might be expected, the gill is innervated by adrenergic, cholinergic, serotonergic, and nitricergic neurons, each of which has been shown to affect function (17, 37, 99). Recent evidence suggests that gill neural or epithelial cells may function as local or cellular distribution of these mechanisms) that mediate NaCl uptake in freshwater fishes, and in marine fishes for acid-base balance. Certainly, this is an area for future research, using this array of modern techniques that Krogh, Smith, and Keys did not have available.

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As might be expected, the gill is innervated by adrenergic, cholinergic, serotonergic, and nitricergic neurons, each of which has been shown to affect function (17, 37, 99). Recent evidence suggests that gill neural or epithelial cells may function as local or cellular distribution of these mechanisms) that mediate NaCl uptake in freshwater fishes, and in marine fishes for acid-base balance. Certainly, this is an area for future research, using this array of modern techniques that Krogh, Smith, and Keys did not have available.

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D. H. Evans, unpublished data). Our working model involves some unknown trigger (external salinity, blood osmolality, blood pressure, oxygen tension?) stimulating the production of EDN by the filamentous epithelial cells and pillar cells, which stimulates adjacent MRC (via intracellular production of PGE) to inhibit salt extrusion across the gill epithelium, and stimulates the pillar cells to contract and redirect blood flow to the outer marginal channels of the lamellae.

Perspectives and Significance

We have learned much about fish gill transport (and osmoregulation in general) since August Krogh, Homer Smith, and Ancel Keys published their seminal papers in the 1930s—most specifically, the cellular mechanisms that control the basic epithelial transport steps that they proposed. The physiological studies of the “middle years” (1960–1990) provided some support for their hypotheses, but these studies were ultimately limited by the complex intracellular structure of the transporting, mitochondrion-rich cell, and the variable specificity of putative transport inhibitors. The advent of techniques that allow specific cell protein localization has revolutionized this field. This started with the radiolabeling of basolateral Na+–K+–activated ATPase with tritiated ouabain and has evolved into the routine immunolocalization of all of the transporters thought to play roles in salt uptake and extrusion. Conclusions are sometimes limited by the use of heterologous rather than species-specific antibodies, and there are apparent instances of species differences, but these techniques are powerful and should be extended to an array of species to attempt to get some general pattern, especially for salt uptake. Obviously, the emerging “knockdown” techniques (siRNA, morpholinos, etc.) will be powerful tools to study the role of specific transporting and signaling proteins. We can all look forward to rapid advances secondary to the application of these new protocols, and those that are yet to be published.

ACKNOWLEDGMENTS

My research and academic career has been blessed with excellent mentors (Don Kennedy, Howard Bern, Malcolm Gordon, Bill Potts, and Jean Maetz), superb graduate students (Jeff Carrier, David Moffett, Gregg Kormanik, J. B. Claiborne, Linda Farmer, John Payne, Tes Toop, Peter Piermarini, Keith Choe, and several others) and eager undergraduates (too numerous to mention). For over 25 years, my research has been done at the Mt. Desert Island Biological Laboratory—still a world-class center for marine physiology and functional genomics.

GRANTS

I have been fortunate enough to have had nearly continuous funding from the National Science Foundation since 1970; most recently: IOS-0519579. Without the National Science Foundation, my research (and research in comparative physiology in the United States) would have been very limited.

REFERENCES

Foskett JK, Scheffey C.

Evans DH.

Fenwick JC, Wendelaar Bonga SE, Flik G.

Evans DH, Piermarini PM, Choe KP.

Garvin J, Sanders K.

Hyndman KA, Choe KP, Havird JC, Rose RE, Piermarini PM, Evans DH.

Hoagland TM, Weaver L Jr, Conlon JM, Wang Y, and Olson KR.

Horng JL, Lin IY, Huang CJ, Katoh F, Kaneko T, Hwang PP.

Huf E.

Hirose S, Kaneko T, Naito N, Takei Y.

Hirose S, Kaneko T, Naito N, Takei Y.

Hyndman KA, Evans DH.

Isenring P, Forbusch B.

Ivanis G, Esbaugh AJ, and Perry SF.

Jampol LM, Epstein FH.

Jorgensen CB.

Karnaky KJ Jr.

Karnaky KJ Jr, Kinter LB, Kinter WB, Stirling CE.

Keys AB.

Krogh A.

Krogh A.

Kroschner W, Suthers S, Lewis P.

Krogh A.

Krey M, Eide HD.

Krebs HA.

Krebs HA.

Krebs HA.

Krebs HA.

Kreuzer D, Krex M.

Krevis H.

Krevis H.

Krevis H.

Krogh A.

Krogh A.

Krogh A.

Krogh A.

Krogh A.

Krogh A.

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