Osmoregulation by Vertebrates in Aquatic Environments

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Because the salt concentration of the body fluids of aquatic vertebrates differs from that of their freshwater or marine environment, they face net movements of water and salt across their permeable membranes, most notably the gill. Various organs and transport processes are involved in maintaining internal consistency in the face of these potential osmotic and salt problems.

The Origin of Aquatic Vertebrates and Osmoregulation

The vertebrates arose from marine ancestors nearly 550 million years ago, and many of these early forms apparently entered fresh water soon after they evolved. This transition into an environment that contained far less salt per volume than sea water was associated with a decline in the salt (largely NaCl) concentration of the body fluids that was retained in subsequent vertebrates, with the exception of the hagfishes. (These very primitive, marine parasitic fishes apparently descended from early ancestors that never entered fresh water, and they have retained blood salt concentrations that are essentially equivalent to those in the ancestral marine invertebrates and their surrounding sea water.) Despite this reduction in body fluid salt concentration, these early freshwater vertebrates (fishes) were still hypertonic to their environment. Thus, these fishes faced gradients of both water and salt that were unknown to their marine invertebrate predecessors, relatives of the modern starfish and sea urchins. Water and salt move down their individual concentration gradients, across permeable membranes of cells or organisms, by the process of osmosis and diffusion, respectively. The problem of osmotic and salt gradients is exacerbated by the presence of the gill epithelium in many aquatic vertebrates. This large, thin tissue is modified for gas exchange; unfortunately, the very characteristics that facilitate gas exchange (large surface area, thin, highly supplied with blood) make it a site for passive movements of both water and salt.

Most fish groups re-entered sea water and remain there today, but one group remained in fresh water and emerged on to land as the ancestor of the modern amphibians. As might be expected, water and salt balance problems exist for the semiterrestrial amphibians because of the presence of gills in the larval stage of some species and a relatively thin skin in the adult. Some reptiles, birds and mammals (which succeeded the amphibians as the truly terrestrial vertebrates) have returned to aquatic existence from their terrestrial ancestry, and they may face similar problems of osmosis and diffusion. But these are reduced by the absence of gills and the presence of scaled, feathered or furred outer body coverings. In sea water, however, salt loading may take place because of salty food, whether plant or animal.

In summary, because of these water and salt gradients and permeable gills or skin, freshwater vertebrates face a net osmotic influx of water and net loss of salt by diffusion; marine forms face dehydration and a net influx of salt, the opposite problems.

Freshwater Vertebrates

Fishes

In these descendants of early vertebrates, water influx across the gills must be balanced by water excretion by the kidney, but urinary (renal) salt loss must be minimized at the same time in an attempt to minimize the diffusional loss of salt across the gills (**Figure 1**). In all vertebrates, urine is formed by filtration of blood across the glomerulus in the kidney, driven by blood pressure. Because of the need to rid the body of excess fluid, glomerular filtration is high in freshwater fishes, and the kidney (renal) tubules reabsorb little of the urine. Freshwater existence is associated with the evolution of a distal tubule, specializing in the reabsorption of needed salt back into the blood. Such a terminal, diluting renal segment is also found in some invertebrates that have evolved in fresh water (earth worms, and crustacea, for instance). The diffusional and renal loss of ions is balanced by a relatively small gain from food, but largely by active transport of Na^+ and Cl^- inward, across the gill epithelium. The mechanisms of ionic transport are still debated, but recent evidence suggests that Na^+ is driven into the gills cells by a favourable
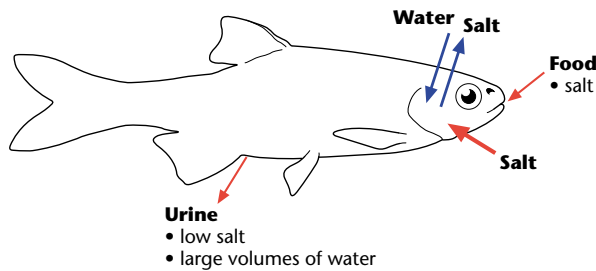
Figure 1 Osmoregulation in a freshwater fish. The net osmotic gain of water and diffusional loss of salt across the gills is balanced by excretion of relatively dilute urine, active uptake of salt across the gill, and possibly some ingestion of salt in the food. Blue arrows represent passive movements of salt and water, and red arrows indicate active pathways of osmoregulation. See text for details. Outline drawing of a goldfish was redrawn from Greenwood HP, *et al.* (1966) *Bulletin of the American Museum of Natural History* **131**: 339–456.

electrochemical gradient produced by the active extrusion of H^+ by a transport protein termed H-ATPase which moves positive charges out of the fish. Cl^- enters the cells from the fresh water in exchange for cellular HCO_3^-. These electrical and biochemical couples could, theoretically, be involved in the net secretion of either H^+ or HCO_3^-. In terrestrial vertebrates, renal loss of acid or base equivalents, coupled with respiratory excretion of CO_2, controls acid–base balance, but in fishes the extrusion of either H^+ or HCO_3^- is the regulatory pathway.

Amphibians

In those species of amphibians that have tadpoles as larvae, the osmotic adaptations to life in fresh water are basically the same as those for fishes. In amphibians (adult frogs, salamanders, etc.) that are gill-less, the skin has evolved salt transport pathways similar to those in the gills of fishes or tadpoles. Indeed, much early work on the biophysical mechanisms of Na^+ transport involved study of the movement of Na^+ across frog skins.

Reptiles

Freshwater reptiles are relatively uncommon; turtles are the only group that has been studied in any detail. In reptiles, net influx of water, which is minimized by the thick skin, is balanced by variable urine flows. Urinary salt loss is minimal (because of salt reabsorption by the distal renal tubule), as is diffusional loss across the skin. What salt is lost is balanced by either salt in the food or, in some species, active extraction of salt from water drawn into either the mouth or anus, but the salt transport mechanisms involved are unknown.

Birds and mammals

In these very impermeable animals (cormorants and otters, for instance), osmotic uptake of water is minimal, balanced by increased urine flows if necessary, and any salt lost in the urine is compensated for by food. There is no evidence for any skin uptake of salt from the environment.

Seawater Vertebrates

Fishes

The freshwater ancestors of modern marine fishes re-entered the marine environment approximately 500 million years ago and radiated into the huge array of marine bony (teleosts) and cartilaginous (elasmobranchs: sharks and relatives) fishes present in sea water today. Because of their freshwater ancestry and attending reduction in the salt concentration of their body fluids, both groups face an osmotic loss of water and diffusional gain of salts. But their solutions to these common problems are quite different. Teleosts balance the net loss of water across the gill epithelium by obtaining water from the only source available: the surrounding sea water (**Figure 2**). They ingest sea water, and absorb the needed fluid across the intestine by transporting the salt across the gut lining, into the blood. This salt transport produces gradients, which osmotically withdraw the water from the intestinal contents at the same time. Marine teleosts limit their renal loss of water by reducing glomerular filtration to near zero, and some species have evolved glomerular-free renal tubules. Marine fishes can tolerate a very small urine flow, because they can excrete unwanted nitrogen as ammonia across the gills. (Terrestrial vertebrates, including mammals, must excrete unwanted, and toxic, ammonia as urea
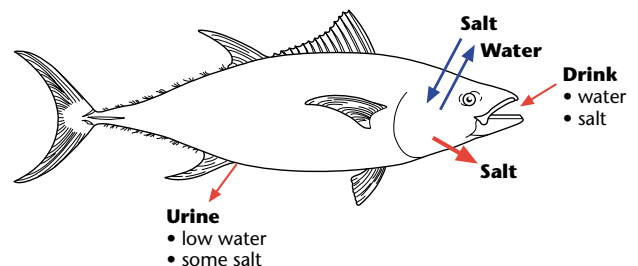

Figure 2 Osmoregulation by a marine teleost fish. The net osmotic loss of water and diffusional gain of salt across the gills is balanced by ingestion of sea water, production of small volumes of urine that contains some salt, and active extrusion of salt across the gill. Blue arrows represent passive movements of salt and water, and red arrows indicate active pathways of osmoregulation. See text for details. Outline drawing of a tuna was redrawn from Bigelow HB and Schroeder WS (1953) *Fisheries Bulletin, Fish and Wildlife Service* **53**: 1–577.

or uric acid via the kidney.) Marine teleosts increase their renal loss of ions (to partially compensate for the net diffusional gain) by increasing renal salt loss, but they are not able to secrete a net amount of salt because they cannot produce a urine that contains more salt per volume than the body fluids. (This requires the evolution of another renal tubule, the loop of Henle, which did not appear in the vertebrates until the birds and mammals evolved from their reptilian predecessors.)

Net salt secretion in marine teleosts is managed by the gill tissue, which uses a suite of transport proteins that are also found in our own kidney tubules. Na^+ enters the gill cell from the blood co-transported with K^+ and Cl^-, driven by the favourable electrochemical gradient for Na^+. Cl^- exits the apical portion of the cell through a channel that is very similar to the defective structure that produces cystic fibrosis in humans. Na^+ is transported back across the basolateral membrane into the blood by Na,K-activated ATPase and then diffuses out into the sea water via the relatively leaky paracellular pathways between adjacent gill cells, driven by a favourable electrochemical gradient. The net result is secretion of NaCl across the gill epithelium, balancing the net influx of salt. It is important to note that these net excretory NaCl pathways are not reversed freshwater uptake pathways; they are quite different. Freshwater ionic uptake pathways for acid–base regulation may be present in marine fish gills, even though they actually move NaCl into the marine fish.

Marine elasmobranch fishes have evolved a different strategy for osmoregulation. To avoid the desiccating effects of sea water, they retain urea (an ammonia detoxification compound) in the blood by reabsorption in the kidney and the maintenance of a low gill permeability to this organic solute. Urea concentrations may approach $500 \, mmol \, L^{-1}$, a concentration that is fatal in humans. Elasmobranch enzymes are not damaged by such high urea concentration because another organic osmolyte, trimethylamine oxide, counters the denaturing effects of the urea when present at a ratio of 1:2 inside the cells. The presence of these two organic solutes raises the total solute concentration of elasmobranch blood slightly higher than that of sea water (these fishes are hypertonic), thus avoiding the osmotic loss of water. The osmotic gradient favours uptake of water across the elasmobranch gill, thereby providing sufficient water for the relatively high urine flows seen in marine elasmobranchs. Despite being hypertonic to sea water, elasmobranch blood still contains less Na^+ and Cl^- than sea water, so these ions diffuse inward across the gill, in the same way as they do across the teleost gill epithelium. Thus, like teleosts, marine elasmobranchs face a salt load, which must be excreted (**Figure 3**). Like the teleost kidney, that of elasmobranchs is incapable of producing a net secretion of salt, so extrarenal mechanisms have evolved. In this group, the gills probably play a role in salt excretion, but this has been largely unstudied. Instead, research attention has focused on the
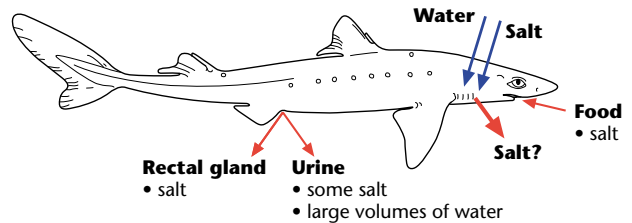

Figure 3 Osmoregulation by a shark. The osmotic gain of water and diffusional gain of salt across the gills is balanced by production of large volumes of urine that contains some salt, active secretion of salt via the rectal gland, and possibly active extrusion of salt across the gill. Blue arrows represent passive movements of salt and water, and red arrows indicate active pathways of osmoregulation. See text for details. Outline drawing of a spiny dogfish shark was redrawn from Bigelow HB and Schroeder WS (1953) *Fisheries Bulletin, Fish and Wildlife Service* **53**: 1–577.

rectal gland, an extension of the terminal intestinal tract. This gland is capable of secreting a concentrated NaCl solution that contains more salt than either blood or sea water. The mechanisms of salt secretion are the same as described for the teleost gill tissue. Importantly, experimental removal of the gland is associated with a slight increase in the plasma NaCl concentration, but the experimental animals (sharks) survive in sea water. This experiment suggests that the gill also must be capable of net salt secretion.

Amphibians

Amphibians are rarely associated with sea water. One exception is the crab-eating frog of southeast Asia, whose tadpole osmoregulates like a marine teleost and whose adult osmoregulates like an elasmobranch, by storing urea. How salt is secreted by these animals is not known.

Reptiles

Marine reptiles are common, and include turtles, snakes, lizards, crocodiles, and even alligators that may have entered the marine environment. In all cases, the desiccating effects of sea water are minimized by their thick skin, but the salt in their food and ingested sea water must be excreted. The absence of a loop of Henle precludes net renal salt secretion, so extrarenal pathways have evolved. In all cases, the secretory tissue is in the head region, with orbital salt glands in turtles and lizards, sublingual glands in sea snakes, and supralingual glands in crocodiles and to a much lesser extent, alligators. The specific mechanisms of net salt secretion by these salt glands is unknown, but assumed to be similar to that described for the teleost gill tissue and shark rectal gland.

Birds

Marine birds have evolved the same strategies as the reptiles, including an orbital salt secretory gland (termed a nasal gland because its secretory fluid drips from the nasal openings). The mechanisms of salt secretion have been better studied in birds than reptiles, and the published evidence suggests that the pathways are identical to those described for the teleost gill tissue and shark rectal gland. In addition to extrarenal salt secretion, bird kidneys are able to elaborate a slightly salty urine because of the origin of the loop of Henle.

Mammals

Like the outer covering of marine reptiles and birds, the relatively impermeable skin of mammals avoids many of the osmoregulatory problems of life in sea water. The salt loading produced by ingestion of sea water and invertebrate food is offset by the presence of a loop of Henle, which allows the production of urine that is 2–3 times as salty as the blood. No extrarenal salt secretory mechanisms have been found, or are necessary.

Summary

Because of the evolutionary history of aquatic vertebrates, the net movement of water and/or salt across their body surfaces must be countered in order to maintain the volume and salt content of their internal tissues and body fluids. A variety of strategies has evolved to maintain this consistency, involving organs as diverse as gills, intestine, kidney, and specialized salt secretory glands. The underlying mechanisms that transport salt across these structures are conserved throughout vertebrate evolution.

Further Reading

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