5-2008

Response of the Salt Gland of the Insectivorous Lizard Novoeumeces schneideri to Ionic and Osmotic Challenges

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Response of the salt gland of the insectivorous lizard *Novoeumeces schneideri* to ionic and osmotic challenges

by

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A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Molecular Biology

May 2008

College/School: College of Science and Mathematics

Department: Molecular Biology

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ABSTRACT OF THE DISSERTATION

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May 2008
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The reptilian kidney can only produce isoosmotic waste. To supplement renal ion excretion and maintain ionic and osmoregulation, some reptile species rely on extrarenal salt glands that can secrete a hyperosmotic solution. Extrarenal glands among reptile taxa include the cranial gland in lizards, lacrimal gland of marine turtles, and sublingual gland of the estuarine crocodile and sea snakes. Secretion composition varies among these taxa, depending on diet, osmotic load and phylogeny. The salt glands of lizards are unique among reptiles in their ability to vary composition of the secreted fluid, secreting cations (potassium and/or sodium) and anions (chloride and/or bicarbonate). Composition of the secreted fluid depends on the ion load incurred: marine and intertidal lizards secrete primarily sodium chloride, while herbivorous lizards secrete mainly potassium chloride.

Most studies on lizard salt glands have been performed on herbivorous and marine species that have high ion loads from their diet. Many insectivorous species also possess salt glands, but their function and contribution to ion regulation have been minimally studied. Compared to marine or desert lizards, the ionic load incurred from the habitat and diet is decreased in insectivorous, arid-adapted lizards. The gland of these species, however, may still be important in maintaining ionic and osmotic balance. My

goal in this study was to examine initiation of gland secretion, secretion composition and range, and the importance of the salt gland in ion regulation of an arid-adapted, insectivorous species. To evaluate these questions, I examined the response of *Novoeumeces schneideri*'s (Scincidae) salt gland to ionic and osmotic loads.

Novoeumeces schneideri subjects were treated to different combinations of cations (sodium, potassium, and histidine control) and anions (chloride and acetate control) and salt gland secretions were examined. Lizards were injected with ion solutions (sodium chloride, potassium chloride, histidine chloride, sodium acetate, potassium acetate, histidine acetate) or controls (saline and sham injected) daily for 4 days. Secreted salt, feces and urine were collected daily and analyzed for sodium, potassium, and chloride. Daily and total cation and anion secretion rates were calculated, as well as ion secretion budgets among the salt gland, feces, and urate.

The salt glands of *N. schneideri* secreted only in response to chloride, regardless of the accompanying cation. Higher secretion rates were seen when histidine or potassium were accompanied with chloride. Both cations and anions were secreted in narrow ranges among all treatments, indicating limited secretion flexibility. Sodium-treated skinks showed a decreased secretion rate, suggesting a possible inhibitory role of sodium. Secretion composition contained a relatively constant mixture of potassium and sodium, regardless of the cation load.

The response of this insectivorous species differs from herbivorous and marine lizard species. Considerable plasticity in the physiology of the salt gland exists across lizard taxa, depending on physiologic needs, indicating ecologic effects on the evolutionary physiology of vertebrates.

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A THESIS

Submitted in partial fulfillment of the requirements

for the degree of Master of Science in Molecular Biology

by

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Montclair, NJ

2008

ACKNOWLEDGEMENTS

This thesis would never have been completed without the help, support and guidance of many people. My advisor, Dr. Lisa Hazard, who helped instill and guide my interest in physiology for the last few years. Her immense patience, encouragement, and friendship has been vital to this thesis coming into fruition. It's been really great having her to look up to and continue to challenge me. Dr. Bonnie Lustigman, who was always a refreshing dose of realism, humor and knowledge. She was very instrumental in both key and mundane decisions I've made throughout my scholastic career and continues to be missed greatly. Stephani Zilinskis, who immensely helped in getting this research done. I would not have been able to finish this research without her proactive help in the lab. To my (hesitant) best friend Jason Rubley, who was pivotal in my return to school and throughout the last few years. It's difficult to imagine the last few years without his support, tolerance, patience and humor; without him, I wouldn't have been able to accomplish any of this. To my mom, sisters and inherited brother in laws who have provided so much (long-distance) support and love. I've always been able to rely on each of you for guidance, conversation and unconditional love and support. It's so wonderful having an amazing foundation with all you. To Augie, who has been a (welcome) distraction during times when I wanted to slow down, yet challenged me to continue. My life perspective has been re-adjusted and fueled by your optimistic outlook on life, tenderness, and joie de vivre.

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Introduction

Physiological Importance of Salt Gland

Animals that can secrete hyperosmotic urine compared to blood plasma are able to conserve body water while eliminating excess ion intake. For these animals, such as mammals and birds, the kidney is able to concentrate the urine via the presence of collecting ducts arranged parallel to true-looped nephrons (Dantzler and Braun, 1980). In contrast, non-mammalian vertebrate kidneys lack parallel-arranged looped-nephrons with collecting ducts (no counter-current exchange system) and are unable to secrete a hyperosmotic fluid compared to plasma (Dantzler and Braun, 1980). At best, reptiles can secrete isoosmotic urine by possibly diluting fluid in discrete nephron sections, then equilibrating the fluid between the tubules and interstitium of a distal nephron segment (Dantzler and Braun, 1980).

Increased ion intake in reptiles, therefore, presents a problem for water conservation and elimination of excess salts. Osmoregulation is further exacerbated in species with increased ion intake, either from a diet with high salt content (such as the Marine Iguana, *Amblyrhynchus cristatus)* (Nagy and Shoemaker, 1984), or limited availability of water (such as the terrestrial desert lizard, *Dipsosaurus dorsalis*) (Hazard, 2001). Birds and reptiles have the ability to excrete concentrated insoluble nitrogenous waste as urate salts or uric acid (Minnich, 1972). This method of excretion still poses a problem for reptiles with limited access to water since only cations, not anions, are able to bind as insoluble urate salts (sodium or potassium urate) without the need for solution.

Two general ways reptiles cope with increased salt loads are through tolerance and regulation (Hazard, 2004). Desert tortoises (*Gopherus agassizii*) have a high-

potassium diet with low-water content. During the dry-season, desert tortoises will tolerate a significantly increased plasma osmolality until rainfall permits the excretion of these excess ions (Peterson, 1996). Most *Amphibolorus* species live in arid environments and have a sodium-rich diet. During periods of water deprivation, sodium plasma levels can increase approximately 46% in osmolality (Bradshaw and Shoemaker, 1967). Due to the inability of these species' kidneys to produce a hyperosmotic fluid and avoid excessive water loss, these animals tolerate hypernatremia until conditions allow the secretion of these excess ions.

Other species have a secondary mechanism of dealing with increased ion intake: regulation of ions through an extrarenal salt gland. The presence of these glands was first noted in several marine birds since the late 1940's (Stebbins, 1948) but their function was not studied until secretion from nasal glands in the marine birds were analyzed (Schmidt-Nielson et al., 1957; 1958). Since their discovery in marine birds, several taxa have also been found to possess salt glands: the rectal gland of the elasmobranch (Burger and Hess, 1960), the sublingual gland of the estuarine crocodile (Taplin and Grigg, 1981), the sublingual and premaxillary salt gland of sea snakes (Dunson, 1968; Dunson and Taub, 1967), and the nasal salt gland of reptiles (Templeton, 1964). Table 1 summarizes these four main classes of salt glands found in various taxa and reptiles.

The ions secreted vary among taxa. In marine birds, the salt gland secretes mainly sodium chloride due to the increased salt content of their diet (Schmidt-Nielsen et al., 1958; Schmidt-Nielsen, 1960). Marine elasmobranches and estuarine crocodiles also regulate high salt loads via a rectal gland and sublingual gland, respectively, that secretes a similar composition (Hammerschlag, 2006; Taplin and Grigg, 1981). Studies on glands

of reptiles have focused mainly on marine/estuarine populations or desert species. Marine reptiles (marine iguana and sea turtles) mostly secrete sodium chloride due to a high salt content diet (Peaker and Linzell, 1975). In contrast, the nasal salt gland of terrestrial herbivorous lizards secretes (mostly) potassium as well as sodium and chloride (Peaker and Linzell, 1975). The desert iguana (*Dipsosaurus dorsalis*) and chuckwalla (*Sauromalus obesus)* are able to secrete very high amounts of potassium and chloride, as well as sodium (in lesser amounts) ingested, via the salt gland (Minnich, 1970; Nagy, 1972). The high concentration of potassium excretion is due to the mainly herbivorous diet with high potassium levels in plant products (Minnich and Shoemaker, 1970). Chloride, unlike sodium or potassium, cannot be secreted as an insoluble urate salt (Minnich, 1972). It can only be released from the kidney in solution or via the nasal salt gland. Since kidney secretion would allow an isoosmotic solution at best, chloride is most efficiently secreted in these species from the nasal salt gland with minimal water loss.

Of note, most lizard studies on salt gland function, secretion composition, or secretion stimulation have focused on marine or herbivorous lizards (i.e. lizards with an increased ion load in their diet). No studies (of notice to this author) have focused on the salt gland function and composition of secretion in arid-adapted insectivorous lizards. Two insectivorous species have been studied, *Uta tumidarostra* and *Ameiva quadrilineata*, but mostly because of their intertidal habitat and increased salt load (Hazard et al. 1998; Hillman and Pough, 1976). This research presents the first extensive look at salt gland function and secretion composition in response to osmotic loads and the possible physiological function in insectivorous arid-adapted species.

Regardless of ions secreted, salt glands are essential to osmoregulation and ionic regulation in vertebrates with increased salt loads due to environment or diet. By circumventing the limitations of kidney function and secreting concentrated ions with virtually no water loss, salt glands allow these vertebrates to secrete excess ions and avoid hyper-natremia, kalemia, or chloremia.

Evolution of Salt Gland in Reptiles

Several vertebrate groups have evolved a modified organ for dealing with increased ion intake and osmoregulation (Table 1). The evolutionary sequence of this extrerenal organ is highly speculative and normally begins with the development of the nasal gland in reptiles. Believed to have first appeared in terrestrial amphibians as a means of moisturizing and cleansing the nasal passages, the unspecialized nasal gland likely secreted an isotonic fluid (Peaker and Linzell, 1975). Assuming histology of current exocrine glands that secrete from the alveoli and revise secretions in the duct system, this nasal gland likely consisted of two main cell types: one secreting mucous, the other ions and water. It is probable that secretion of the nasal gland eventually became more specialized for salt and water balance as terrestrial-oriented changes were made (Bang and Bang, 1959; Peaker and Linzell, 1975). Gills and skin permeability, for example, were major ionic and osmoregulation avenues that were lost in very early reptiles. As early reptiles became more terrestrial and developed reptilian skin for water conservation, only those that had an advantage in ionic regulation could benefit from the potassium-rich terrestrial plants or sodium rich marine diet (if they returned to sea). This enabling advantage could be achieved in three ways: 1. a decrease in cells secreting

mucous and water, 2. an increase in cells secreting ions and 3. a modified ion-transport mechanism to produce a hypertonic solution (Dunson, 1969; Peaker and Linzell, 1975).

Support of this evolutionary theory is seen in the nasal salt gland of two lizard species, *Sauromalus obesus* and *Uromastix acanthinurus*. The nasal glands of these herbivores contain a heterogeneous population of cells in the tubular parenchyma (Barnitt and Goertemiller, 1985; van Lennep and Komnick, 1970). The terminal segment contains agranular cells (principal ion-secretory cells) and granular cells (mucous cells as determined by mucin content in the cytoplasmic granules by van Lennep and Komnick (1970)). A greater proportion of principal-like cells over mucous-cells are found in the terminal segment of the tubular parenchyma (Barnitt and Goertemiller, 1985). These mucous-secreting granular cells are believed, therefore, to be evolutionary vestiges of the transition between amphibian mucous nasal gland to reptilian salt gland. As reptiles adapted to their change in habitat, this proportion of principal ion-secreting cells increased at the expense of mucoid granular cells.

As early reptiles journeyed towards more terrestrial life, it is hypothesized the change in cell ratio and formation of a specialized ion-secreting salt gland is associated with the transition into an herbivorous diet rich in potassium (Sokol, 1967). Since reptilian skin was developed for water conservation, the salt gland's main function could have been to regulate sodium and potassium rather than water. The archetypal salt gland found in early reptilian evolution is believed to have mainly regulated ions (including potassium and bicarbonate) not water (Peaker and Linzell, 1975). This versatile sodium, potassium, and bicarbonate-secreting gland was ideal for the increased ion content in plant diet. The primitive salt gland, resembling that of the modern lizard, maintains this

versatility. It is able to secrete sodium, potassium, or a combination of both alongside chloride or bicarbonate in different proportions, dependent on which ion is being ingested excessively. Under natural conditions, most lizards with salt glands secrete mainly potassium chloride (Hazard, 2004), although long-term exposure to NaCl loads in field and lab studies of the desert lizard *Dipsosaurus dorsalis* will cause an increase in sodium output by the gland (Shoemaket et al. 1972).

As other reptiles returned to a sea-habitat throughout evolution, marine reptiles must have adapted to also secrete sodium-chloride (to cope with increased sodium load in the water and diet), and lost the ability to secrete potassium and bicarbonate. This could have been achieved through the loss of metabolic activity in cells lining the duct system of the glands, development of a permeable barrier to ion and water movement, and the retention of some mucous cells (Peaker and Linzell, 1975). Through these changes, the gland of these marine reptiles became more a mechanism of osmoregulation than ionic regulation in their limited free-water environment. Support of this adaptation is seen in the NaCl secreting gland of marine lizards and those feeding on intertidal foods; the gland mainly secretes sodium.

If nasal salt glands were present in early reptiles, it is likely the gland was passed on to birds during the late Paleozoic period (Fernandez and Gasparini, 2000) and would be present in archosaurs, the reptilian ancestors of birds. Fossil records, in fact, support this presence. Cretaceous period fossils of birds *Ichthyornis* and *Hesperornis* show clear impressions on the skull, in the same location as modern-day marine birds, where a large gland was located (Marples, 1932). Similar depressions are seen in Charadriiform skull frontal bones (Fernandez and Gasparini, 2000). Additionally, all modern-day bird groups

believed to have retained the most ancestral traits also contain members with salt glands, including ostrich and penguins (Schmidt-Nielsen, 1960). Marine birds developed further basal membrane infolding of secretory cells (in addition to the interdigitation and lateral folding seen in reptile salt glands) (van Lennep and Komnick, 1970) to allow the enhanced ability of solute secretion via the nasal salt gland.

The presence of nasal salt in glands in extinct bird species and modern-day bird groups further suggests nasal salt gland evolution from amphibians to early reptiles. This method, however, only explains the current cranial nasal salt gland found in some (not all) modern terrestrial lizards (located laterally) and in birds (located medially). It does not explain the presence of the other specialized reptilian cranial glands (Table 1) found in chelonians (lachrymal gland), sea snakes (sublingual and premaxillary gland), or crocodilians (lingual glands) that have adapted the same purpose of ion secretion. Explaining the presence of this gland requires grouping these glands as mainly NaCl adapted due to the reptile's return to marine habitats throughout evolution (Peaker and Linzell, 1975). This secondary-adapted marine habitat led to the loss of the versatile potassium and bicarbonate-secreting archetypal gland to one primarily sodium-secreting. It is possible the archetypal gland was lost in these groups in different stages of evolution (Peaker and Linzell, 1975). Snakes may have passed a fossorial stage in their early evolution and later modified a posterior sublingual gland (Peaker and Linzell, 1975). Similarly, most crocodilians are fresh-water carnivores and could have lost nasal gland function in evolution due to lack of ionic regulation requirements. Chelonians may have lost archetypal nasal gland function during their terrestrial or freshwater periods in history. A return to estuarine habitats in crocodilians and marine life for chelonians and

sea snakes would have required a secondary adaptation of a separate cranial gland for ionic secretion and osmoregulation (Peaker and Linzell, 1975).

Previous taxonomy of salt glands among lizard taxa suggests salt glands appeared independently in several different lizard families (Minnich, 1979). Recent studies suggest an early nasal salt gland evolution in squamates and archosaurs, common ancestors of all diapsids (Fernandez and Gasparini, 2000). Neither phylogenetic nor molecular analyses based on salt gland distribution or function have been performed in lizards. Gross and microscopic anatomy studies based on salt gland presence (Braysher, 1971; Crowe et al., 1970; Norris and Dawson, 1964; Philpott and Templeton, 1964; Templeton, 1964; van Lennep and Komnick, 1970) have allowed a listing of salt gland distribution among lizard taxa (Hazard, 2004). Table 2 lists the salt glands found in all members of Iguanidae, Scincidae, Varanidae, and Xantusidae; also in some members of Agamidae, Cordylidae, Lacertidae, and Teiidae. In all these families, the lateral nasal gland is the salt gland used for secretion, although secretion mechanisms are still unknown in mostly all of them; secretion mechanisms have only been well studied in the Iguanidae family. No examined members of the Anguidae, Gekkonidae, Helodermatidae, and Pyggopodidae families have nasal salt glands present.

While many reptile groups across many different habitat and diet-types use nasal salt glands for ion secretion and osmoregulation (Tables 1 and 2) and the evolutionary presence of salt glands indicate they may have occurred independently among taxa (Minnich, 1979), there is still strong conservation of histology, secretion regulation and ion transport mechanisms among the salt glands of reptiles. The nasal salt gland of lizards, for example, has been studied in many species and is similar in all in regards to

anatomy and histology, with variation seen in gland size proportional to body size (Barnett and Goertemiller, 1985; Dantzler and Braun, 1980; Stebbins, 1948; Templeton, 1964; van Lennep and Komnick, 1970). Many of the hormones involved in salt and water balance regulation (for kidney and salt gland function), as well as acute and acclimated regulatory responses, are common in reptiles and other vertebrates. However, their precise function and target tissues may have changed throughout evolution (McCormick and Bradshaw, 2006). This relationship further implies a coevolution of hormones and their receptors.

Similarities in ion transporters and channels in secretory cells also show strong conservation. The secondary active co-transporters of sodium, potassium, and chloride (NKCC1) are also conserved among many different reptiles and secretory epithelia across diverse conditions (Shuttleworth, 1988). Interestingly, so have the co-transporter's molecular isoforms, binding affinities, their inhibitors, and phosphorylation requirements been conserved (Haas and Forbush, 1998, 2000; Isenring et al. 1998; Isenring and Forbush, 2001; Russell, 2000; Shuttleworth, 1988; Toop and Donald, 2004). While only lower-order variation effects are seen among diet and habitat of reptiles, it is ultimately phylogenetic history that resonates in conservation of gland structure and function (Siegel-Causey, 1990).

Gland structure and histology in terrestrial lizards

General gland structure and histology

The nasal salt gland of lizards has been studied in many species and is similar in anatomy and histology (Barnett and Goertemiller, 1985; Dantzler and Braun, 1980; Stebbins, 1948; Templeton, 1964; van Lennep and Komnick, 1970). In all examined

cases, the lateral nasal gland is used for secretion and is enclosed within a hyaline cartilage capsule (concha) lateral to the nasal cavity. An abundance of unmyelinated nerve fibers and blood vessels are present. The glands contain a single short and wide duct that branch into overlying secretory tubules that form a vestibule just below the nostril. Hyperosmotic secretions will pool in this vestibule where most of the water content is re-inhaled and retained. The resulting secretion contains mostly dry crystals that can accumulate on the nostril. They can be expelled by sneezing or falling off.

The most ultra-structural study of lizard salt gland histology was performed on *Uromastix acanthinurus* (van Lennep and Komnick, 1970). Secretory tubules of the nasal gland contain two types of cells, principal secretory cells and mucus-secreting cells they are contained in different regions and arrangements. The initial regions of the secretory tubules contain principal secretory cells; terminal regions contain mostly mucous-secreting cells interspersed among principal secretory cells. The apical membranes of the secretory tubules have either a true brush border or few microvilli. Adjoining cells are lined by typical junctional complexes of zonulae occludentes and zonulae adherens. Infolding of cells is also seen among neighboring cells, but not along the basal membranes. Both cell types contain an abundance of mitochondria located mainly towards the cell base. Small golgi apparatus with vesicles are also present near the lateral membranes; their function is believed to be secretion of substances. Other ultra-structural studies of salt glands in birds show the same cell arrangement, abundance and placement of both mitochondria and golgi, and junctional relationships (Thompson, 1979).

While nasal gland histology is consistent among lizard taxa, slight variation in structure is seen due to differences in head structure. These variations have been studied in many lizard families (Stebbins, 1948), but I will only focus on describing the family that *Novoeumeces schneideri* belongs to: Scincidae.

***Gland structure in Novoeumeces schneideri* (Scincidae)**

The closest relative of *Novoeumeces schneideri* whose nasal gland structure has been analyzed is *Eumeces skiltonianus* (Stebbins, 1948). Both lizards were classified under the genus *Eumeces* (Daudin, 1802) but *N. schneideri* was later separated under the new genus *Novoeumeces* proposed after molecular genetic studies were performed (Griffith, 2000). Similarities between the two species still remain (body structure and size, habitat range and diet) to merit the description of salt gland structures interchangeably.

The vestibule of *E. skiltonianus* has a very short vestibule that extends very little into the naris. Its wall is thin at the dorsal and ventral ends and thickest towards the median near the nasal opening. Tissue disposition suggests the vestibule diameter can be reduced by elevation of the medial and ventral surfaces level to the posterior region of the nasal opening to alter secretion flow.

The principal cavity of *E. skiltonianus* is similar to that of a Teiidae family member, *Cnemidophorus tesselatus*. The primary cavity is almost fully composed of the hyaline cartilage concha attached to the lateral wall in an almost continuous framework. The respiratory duct is located beneath the concha and opens into the anterior region of the nasal chamber. The nasal gland, contained within the concha, drains anteriorly into the vestibule and principal cavity juncture. An extensive amount of mucus-secreting

cells along the principal cavity and nasal gland suggest *Eumeces* nares are adept at air humidification.

Mechanism of Salt Gland Secretion in Reptiles

As previously described, a hypertonic solution is created from secretions entering secretory tubules and branching into vestibules below the nares where water will be re-inhaled and retained (Crowe et al., 1970). The crystallized product can be expelled through the nares by sneezing or falling off. The cellular mechanism by which secretion and ion-transport occurs has been studied in elasmobranch and avian salt glands (Shuttleworth, 1987, 1989; Silva et al., 1990; Torchia et al. 1992; Schlatter and Greger, 1988), but very minimally in reptiles (Ellis and Goertemiller, 1974). The shark rectal gland is the most studied due to its permissiveness to *in vivo* catherization and *ex vivo* perfusion (Silva et al., 1990), success at isolation and cloning of cotransporter protein receptors (Bewley et al., 2006), and isolation and molecular analysis of co-transporter protein (Nöel and Pouysségur, 1995).

While there are macrostructural differences among the reptilian and avian salt glands, many microstructural cellular and hormone similarities persist that suggest conservation of cotransport mechanism for secretion. For example, $(Na^{+}+K^{+})$-ATPase activity drives excretion in sea snakes and elasmobranches, and is also localized at the lateral infoldings of secretory cells in the lizard *Dipsosaurus dorsalis* (Shoemaker et al., 1972; Ellis and Goertemiller, 1974), marine birds (Torchia et al., 1992), and the marine turtle *Chelonia mydas* (Nicolson and Lutz, 1989). Similarly, chemical analysis of intracellular sodium and potassium in sea snake and bird salt glands are within ranges of each other (Dunson and Dunson, 1979), indicating similar mechanism and capacity.

Secretion inhibition by ouabain and loop diuretics (furosemide and bumetanide) is seen in both the avian nasal gland and sea turtle *Malaclemys* lachrymal gland (Ernst and Mills, 1982; Shuttleworth, 1987, Shuttleworth and Thompson, 1987). Molecular homology and functional comparison of the Na-K-Cl cotransporters (NKCC1 and NKCC2) found in the secretory epithelia of the shark rectal gland, avian nasal gland, *Xenopus* oocytes, and mouse kidney (among others) have shown at least 45-60% molecular homology among isolated cotransporters of its family, and highly conserved transmembrane regions and isoforms (Russell, 2000; Haas and Forbrush, 1998, 2000; Suvitayavat et al. 1994). All this previous work suggests the mechanism of secretion is similar in all these tissues and that the transporter for each of these secretory tissues has not been isolated from terrestrial lizards due to lack of research, not its molecular absence. For this reason, I will discuss the mechanisms of the isolated Na-K-Cl cotransporter (NKCC1) in this study, which I believe to be a (main) cotransporter also responsible for ion secretion in lizards.

Na-K-Cl Cotransporter

The Na-K-Cl cotransporter (NKCC) is a member of the cation-coupled chloride cotransporter superfamily that have been isolated from a wide range of secretory epithelia (Haas and Forbrush, 1998; Isenring and Forbrush, 2001; Russell, 2000). The other two members of the cotransporter superfamily include the Na-Cl cotransporter (NCC) and K-Cl cotransporter (KCC). The prototype model of NKCC1 is the shark rectal gland cotransporter and is distributed along the basolateral membrane of secretory tissues, although northern blot analysis also shows its presence in salivary glands, stomach, lungs, trachea, and pancreas (Delphire et al., 1994; Payne et al., 1995; Xu et al., 1994).

NKCC1's most similar related protein is the Na-K-Cl cotransporter 2 (NKCC2) found in the kidney cortex and medulla of the prototype rat and rabbit organisms (Haas and Forbrush, 1998; Isenrish and Forbrush, 2001).

All three members of the cation-coupled chloride transporter superfamily share three common properties of ion transport: 1. an electrically silent (electroneutral) process (with a stoichiometry of 1Na: 1K: 2Cl in the NKCC group) that requires all ions to be on the same side, 2. net transport can occur either into or out of the cell, and 3. cotransporter function is limited by loop diuretics (Russell, 2000). Overall transport of the three ions (Na, K, and Cl) is not driven by cellular transmembrane electrochemical gradient differences nor does it produce an electrochemical gradient. Furthermore, NKCC1 ion transport requires that all three ions bind to the basolateral *cis*-side of the membrane in order for their translocation to the *trans*-side. The number of ions required to bind to NKCC1 are not all necessarily translocated so stoichiometry analysis requires both the number of ions that bind and the number of ions that get translocated (Russell, 2000). NKCC1 translocates in the stoichiometry of $1Na^+$:$1K^+$:$2Cl^-$ (Geck et al., 1980). In a single turnover of the cotransporter, therefore, the sum of $Na^+ + K^+$ (cations) will equal the sum of Cl^- (anions) crossing the membrane.

Although ion translocation is electroneutral and is considered a secondary active transport process, the cotransporter still requires cellular ATP. Studies of ATP depletion in avian salt glands results in inactive NKCC1 cotransporters (Russell, 2000; Torchia et al, 1992). This suggests that ATP mediates NKCC phosphorylation into an active state, although the kinase responsible has not been identified (Krebs, 1986). Studies of NKCC1 in the shark rectal gland and avian salt gland have further shown that activation of

NKCC1 occurs by direct phosphorylation of the protein, not from changes in ion gradient concentrations (Lytle and Forbrush, 1992; Torchia et al., 1992). Furthermore, phosphorylation has been shown to occur on specific serine and threonine (Thr-184, Thr-189 and Thr-202), not tyrosine, residues (Lytle and Forbrush, 1992).

Normal NKCC1 functioning requires binding of the required ions on the *cis*-side of the membrane allosterically cooperative. Binding affinity for each of the cotransported ions increase as extracellular co-ion concentrations also increase (Hannafin and Kinne, 1985). Studies establishing binding order have produced a glide symmetry model which describes the first ion bound on the *cis*-side as the first ion released on the *tans*-side (Lytle and McManus, 1986; Lytle et al., 1998). This glide symmetry model has been tested on simulations of elasmobranch rectal glands and avian epithelial tissue (Benjamin and Johnson, 1997; Lytle and McManus, 1986; Lytle et al., 1998). It lists ion binding order as: Na^+:Cl^-:K^+:Cl^- with each sequential ion bound increasing the binding affinity of the following ion and each Cl- having different binding properties (Russell, 2000).

Control of Secretion

Salt gland secretion is under the influence of various control levels that initiate, regulate, and determine composition of excreted matter. Initiation of secretion is normally a response to dietary or environmental stimuli. Once secretion has been initiated, hormones and neuronal signals will regulate the secretion rate. In some species, hormone presence without the dietary/environmental stimuli has initiated secretion during lab studies. Secretion composition is then controlled by the ion transporters (and their mechanisms) located in the secretory cells.

Gland secretion of sea snakes, birds, turtles, and elasmobranch is initiated by changes in plasma osmotic concentration that are detected by plasma volume and/or osmoreceptors (Haas and Forbrush, 2000; Lionetto and Schettino, 2006). These osmoreceptors trigger the parasympathetic nervous system to stimulate gland secretion when increased osmotic loads occur (Peaker and Linzell, 1975). In lizards, however, salt gland secretion is not triggered by osmoreceptor detection of changes in plasma osmotic concentration, but rather by the presence of specific ions. Secretion in the desert lizards *Dipsosaurus dorsalis* and *Uromastix dispar* is only stimulated by the presence of either potassium or chloride (Hazard, 2001, 2007; Shoemaker et al., 1972). In *D. dorsalis*, treatment loads of potassium chloride, potassium acetate, and sodium chloride all stimulated secretion in the gland. Osmotic loads of sodium, acetate, sucrose and mannitol injections had no effect on secretion. Similarly, *U. dispar* increased gland secretion when treated with chloride or potassium. When treated with both ions (as potassium chloride), however, gland secretion increased substantially above the secretion sum of both individual ions. Osmotic loads of sodium also had no effect on secretion.

Once dietary or environmental stimuli are detected, secretion is regulated through hormones and nervous system signals. Many of the hormones involved in parasympathetic control of salt gland secretion are common to all vertebrates although, throughout evolution, their function and target tissues may have changed (McCormick and Bradshaw, 2006). These hormones can be grouped according to their response time to changes in ionic load and water balance as acute responders (fast acting) or acclimated responders (slowly/long term acting). Acute responders generally increase secretion by: increasing blood flow to the salt organs, activating existing transport proteins (i.e. via

phosphorylation), and opening ion and water channels and pumps. Acute responding hormones seen in lizards (Hazard, 1999; Shuttleworth and Thompson, 1987) and sea turtles (Schmidt-Nielsen and Fange, 1958) include acetylcholine (stimulates O_2 consumption in salt gland), its mimic methylcholine, and mimic calcium ionophase (creates channels for Ca^+ entry from extracellular membrane). Bumetanide and ouabain will inhibit stimulation of methylcholine in these lizards (Shuttleworth and Thompson, 1987).

Osmoreceptor detection of plasma changes in birds stimulates the release of vasoactive intestinal peptide (VIP; functions as both a neurotransmitter and a hormone) and carbachol to activate gland secretion (Torchia et al., 1992). In the shark rectal gland, the artificial cAMP stimulator forskolin will activate the NKCC1 cotransporter in the secretory cells of the gland (Lytle and Forbrush, 1992). In terrestrial lizards, where secretion seems to be under parasympathetic nervous system control, acetylcholine (and its analogs) can initiate gland secretion, even when not salt-loaded (Shuttleworth et al., 1987). Methylcholine injections can stimulate gland secretion comparable to rates when chloride-treated in *Sauromalus obesus*, *Dipsosaurus dorsalis*, and *Ctenosaura pectinata* (Templeton, 1964). Hormones may also act to inhibit gland secretion, as seen in several studies with *Dipsosaurus dorsalis* (Shoemaker et al., 1972; Templeton, 1964; Templeton et al., 1972 (I and II)). Aldosterone and dexamethasone both completely inhibit gland secretion of sodium, while corticosterone and ACTH minimally reduce sodium secretion in the desert lizard. Potassium secretion rates were not affected by these hormones, thus, K/Na ratios increased.

Further control of secretion and its composition occurs in regulation of ion transporters in the secretory cells. NKCC1 function, for example, can be regulated by chloride concentration and loop diuretics such as bumetanide and furosemide. (Russell, 2000). The loop diuretics will inhibit cotransporter function by binding to one of the Cl^- binding sites on the protein. Increased chloride concentration allows competitive binding between chloride and bumetanide (or other loop diuretics) to limit bumetanide's inhibition.

Proposed mechanism of secretion in terrestrial lizards

Several methods have been proposed for the mechanisms of secretion that could apply to various salt glands, including those of the desert iguana (*Dipsosaurus dorsalis)* (Hazard, 1999), teleost and sea turtle (Shuttleworth, 1989), elasmobranch (Shuttleworth, 1987, 1989), and bird (Hughes, 2003; Lowy et al., 1989; Shuttleworth, 1989). Each of these mechanisms propose the same overall movements of ions from blood plasma to the secretory lumen, and require the presence of the Na-K-Cl cotransporter moving ions in the stoichiometry of 1:1:2. They differ slightly, however, in ion gradient generation, paracellular transport of ions, and ion channels used. I will describe the two proposed mechanisms generated by Greger (1996) and Hazard (1999) for terrestrial lizards that secrete either NaCl (adapted from Greger, 1996) or NaCl + KCl (depicted in Figures 1 and 2).

The secretion mechanism for sodium chloride (Figure 1) is as follows: sodium, potassium, and chloride move from the extracellular blood plasma (serosal side) into the secretory cells via the Na-K-Cl (NKCC1) cotransporter in the stoichiometry of 1:1:2. Sodium them gets pumped into the intercellular space by the $(Na^+$+-$K^+)$-ATPase located

near the basolateral membrane. Chloride exits the cell and travels down the electrochemical gradient to the secretory lumen (mucosal side) via apical chloride channels. Increased chloride on the luminal side creates a negative electrochemical gradient, causing sodium to follow passively down the electrochemical gradient via a paracellular route. Potassium will then exit the cell into blood plasma through potassium channels located basolaterally in order to maintain membrane potential. The secreted ions will continue to flow through converging canals and ducts located superior to the palate for eventual secretion through the nares.

The secretion mechanism for sodium chloride and potassium chloride (Figure 2) developed for the desert iguana (*Dipsosaurus dorsalis*) by Hazard (1999) describes the same process as for sodium chloride secretion, but with the addition of an apical potassium channel. The added apical potassium channel transports potassium down its electrochemical gradient into the secretory lumen. The secretion mixture contains potassium alongside sodium and chloride, as seen in secretion analysis of the desert iguana. While its presence has not been tested for in lizards, the apical potassium channel is present in other secretory tissues as well as the kidney (Schulman and Bastl, 1991).

Other routes of ion excretion: function and capacity of feces and urate

Reptiles can also secrete ions in the feces or urate (or liquid urine), but at a loss of water. Next to evaporation, the feces account for the highest route of water loss in the terrestrial lizards *Sauromalus obesus*, *Uma scoparia*, and the marine iguana *Amblyrhunchus cristatus* (Minnich, 1976; Shoemaker and Nagy, 1984). Ion secretion via the feces can only account up to 30% of total ion intake in these species. For lizards with

limited free-water access, therefore, fecal excretion of ions is both an expensive and non-beneficial means of ion regulation.

Sodium and potassium can also be secreted from the cloaca as urate salts with less water loss than through fecal excretion. Terrestrial lizards (i.e. *Dipsosaurus dorsalis)* can secrete abundant quantities of cations (sodium and potassium) as monobasic urate salts bound to urate molecules (Minnich, 1970). Cloacal secretion studies in the intertidal lizard *Uta tumidarostra* (Hazard et al, 1998) show that kidney secretion could possibly account for total ingested ions, yet at least 7% of total water intake would be lost (Hazard, et al., 1998). In terrestrial lizards the kidneys cannot produce urine hyperosmotic to plasma and efficiently secrete total ingested ions. It is more economic for these species to reabsorb ions at the cloaca, retain water, and then secrete the reabsorbed cations via the salt gland.

Goals of this study

While many lizard families possess nasal salt glands, only a few species have been extensively studied. The desert iguana *Dipsosaurus dorsalis* and the marine iguana *Amblyrhynchus cristatus* have been model species of study due to their significant ion intake and range of salt gland secretion. Most lizard osmoregulatory studies have been performed on these species in regards to secretion stimulation, composition of secretion, and control of secretion. Other species whose salt gland has also been studied, such as *Uromastix dispar*, *Sauromalus obesus*, *Uma scoparia*, and *Uma tumidarostra*, are also marine or desert herbivores with increased ion intakes from their diet. In these species, gland secretion can, therefore, be greatly attributed to ecological and dietary factors.

In this study, I examined the response of an arid-adapted and insectivorous species' salt gland to ionic and osmotic loads. The species used in this study was *Novoeumeces schnedieri.* In comparison to marine and desert lizards, *N. schneideri* 's diet and habitat present a decreased amount of ions and an increased amount of available free-water. The pressure on its salt gland to maintain ionic and osmotic balance may, therefore, be decreased compared to marine and desert lizards. Its role in maintaining homeostasis may, however, still be significant.

My goals for the study were as follows:

1. Determine what stimulates secretion in the gland of *N. schneideri*
2. Determine the secretion composition and range of *N. schneideri*'s salt gland
3. Determine the importance of *N. schnedieri*'s salt gland in ion regulation compared to other excretion routes
4. Propose a method of ion secretion in *N. schneideri*'s salt gland

To examine these goals, I treated *N. schneideri* subjects to ionic and osmotic loads daily for 4 days. Excretions from the nasal salt gland, feces, and urate were collected and examined for ion content. The response of *N. schneideri*'s salt gland to ionic and osmotic loads provided information regarding its function, including factors stimulating secretion, ion secretion and composition range, importance of salt gland function in ion regulation for the species, and a possible mechanism for ion secretion. Comparisons to previously studied species were also made to determine relationships and variability of salt gland function among lizard taxa.

Background on *Novoeumeces schneideri*

Novoeumeces schneideri (formerly *Eumeces schneideri*; common name Schneider skink) belongs to the Scincidae family of lizards. It is a terrestrial lizard native to the arid-habitat regions of North Africa and Western Asia. It is an insectivorous species with a diet consisting mainly of arthropods and crickets. Average size ranges from 13-18 inches in length (330.2-457.2mm) and up to 100 grams in weight.

The Schneider skink was phylogenetically grouped in the genus *Eumeces* (and *schneideri* group) by Taylor (1935), along with other species occurring in regions of West Asia, Africa, and Cyprus based on shared color patterns and scalation features. While other phylogenetic changes and regroupings occurred within the genus *Eumeces* throughout the rest of the 20th century, taxonomy of the Schneider skink remained unchanged until the year 2000. Griffith et al. (2000) performed 928 base-pair gene sequence comparisons of mitochondrial 16S and 12S ribosomal RNA to devise new cladograms of the genus. They proposed a new name "*Novoeumeces*" for the *schneideri* species-group. The Schneider skink thus became *Novoeumeces schneideri*.

Experimental Methods

Maintenance of lizards

Novoeumeces schneideri (n=13, 33-73g) were obtained from commercial suppliers and maintained in glass terraria in groups of two to three in cages with sand substrate. Heat lamps and UV lighting (Reptisun 5.0, ZooMed) were provided for 10-12 h/day. Temperature ranges from 20°C to 30°C were maintained for night/day cycles. Lizards were fed crickets dusted with vitamin/mineral supplements (RepCal) 3-4 times/week. Water was provided ad libitum. All lizards had been maintained in captivity

at least 6 months before experimentation; only healthy lizards were used in all experiments.

Experiments

N. schneideri were placed into individual collection chambers (8.87cm H x 17.14 cm W x 30.4 cm L) containing a wire mesh bottom that raised the surface approximately 2cm. The collection chambers containing lizards were placed within a temperature cabinet at 30°C with day and night cycles of 12 hours each.

To test the effects of ionic loads on secretion, all lizards were fasted 2-3 days prior to trials. Individual weight (in grams) was measured daily. A 24-hr pre-treatment collection was obtained on Day 0. Throughout Day 0-3, subjects received daily salt loads of one of the following treatments (n=7): sodium chloride, sodium acetate, potassium chloride, potassium acetate, histidine acetate, histidine chloride, saline and sham treatment. Salt load treatments were 2.5μmol/g day at 0.01 mL/g of 0.5M solution concentration, except for Saline (0.01 mL/g of 0.15 M NaCl). *N. schneideri* received treatments orally via a 14-gauge feeding needle.

Salt gland excretions, feces, urate and liquid urine were collected daily and placed in plastic 20ml vials. Time of collection (in 15 minute increments) was recorded. Feces and urate were collected with tweezers; nasal salt secretions were thoroughly swept from the collection chamber. Liquid urine was pipetted. Most samples were easily distinguishable and separated. Any samples that were contaminated or could not be easily separated (i.e. urine in feces) were omitted from analysis. Secretion collections were obtained until Day 6.

Sample preparation and analysis

Salt gland secretion samples were hydrated with 1.0 mL deionized water. Feces samples were crushed and dried at 65°C for 24 hours and then hydrated with 1.0 mL deionized water. Urate and liquid urine samples were crushed and dried at 65°C for 24 hours. Urate samples were treated with 0.5 mL concentrated Nitric Acid for 3-5 minutes and hydrated with 1.5 mL deionized water.

Hydrated samples were analyzed for chloride using a LabConCo chloridometer and for sodium and potassium using a Cole-Parmer dual-channel flame photometer. Measured sample concentrations were converted to total μmol of sodium, potassium and chloride excreted per day for each sample

Data entry, analysis, and statistics

JMP v.6.1 (SAS Institute) was used for statistical analyses of ANOVA and Tukey's t-tests ($p>0.05$ considered statistically significant). Means ± standard errors are presented.

Secretion values were computed as the amount of individual ion (sodium, potassium, or chloride) secreted per gram of animal (based on initial weight) for each day. Total secretion rate was the addition of sodium and potassium secreted per gram of animal for each day. To maintain charge neutrality of the secreted solution, total anion secretion must equal total cation secretion. Total anion output ($Cl^- + HCO_3$) is assumed to equal total cation output (Na+K). Since secretion for bicarbonate was not measured, total anion secretion is based on chloride secretion only, and total cation output (Na+K) was used as a substitute for ($Cl^- + HCO_3$). Where chloride secretion did not equal total cation secretion, the remaining anion was presumed to be bicarbonate (Hazard, 2001).

Flexibility of cation and anion secretion was examined in two ways: 1. calculating cation (K/[Na+K]) and anion (Cl/[Na+K]) ratios for each observation, and 2. regressing

potassium and chloride secretion against total cation secretion. Ratio of cation and anion secretion (Table 3) was computed from the sum of ions secreted, based on treatment and lizard identification number during days 1-6 per gram weight of animal. Potassium secretion ratio of total cation secretion was determined by the mean percent of potassium secretion from total cation (sodium + potassium) of all subjects for each treatment. Chloride secretion ratio of total secretion was determined by the mean percent of chloride secretion from total cation (sodium + potassium) of all subjects for each treatment. Mean average potassium and choride secretion were regressed against total cation secretion for Day 4 secretion only. Day 4 values represent the maximum secretion values after 4 days of ionic loads (days 0-3) and highlight any adjustments the gland has made in response to the ion load incurred.

Rates for individual ions were determined as indicators of excretion route of ions and the importance of salt gland secretion (Figure 7). To compare the relative roles of the different routes for ion excretion, and because feces, urine, and urate were not necessarily produced by each lizard each day, the sum total of each ion (chloride, sodium, and potassium) excreted via each route on days 1-6 was measured for each individual. Average secretion values were plotted based on ion and treatment.

Results

Initiation of secretion: response to ionic loads in *N. schneideri*

Total cation secretion

Secretion was only initiated by chloride treatments (histidine chloride (6.08 μmol cations/gram) and potassium chloride (4.99 μmol cations/gram)) (Table 3; Figure 3). Maximum cation secretion for potassium chloride treatments occurred on day

3 (after 3 ion loads), while occurring on day 4 for histidine chloride treatments (after 4 ion load injections) (Figure 3). The other chloride treatment (sodium chloride), however, did not stimulate secretion in the salt gland as much as potassium chloride or histidine chloride. In comparison, total secretion rates in potassium chloride and histidine chloride were 227% and 276%, respectively, higher than sodium chloride. Sodium chloride did, however, show similarities to histidine chloride in secretion trends and maximal secretion on day 4. The lowest total ion secretion was seen in sodium acetate and histidine acetate treatments (0.38 μmol cations/gram in each). Other treatments (potassium acetate, saline injections, and sham injections) showed minimal total secretion rates (0.66-1.91μmol cations/gram.

Secretion comparisons among chloride treatments indicate treatments with sodium were significantly less versus treatments without sodium. Compared to histidine chloride and potassium chloride treatments, sodium chloride treatment decreased total secretion rate by 66% and 74%, respectively.

Osmotic load treatments (histidine acetate) did not stimulate secretion in the gland. Total secretion values for histidine acetate treatments were similar to sham injected individuals.

Cation to anion interactions and ANOVA statistics indicate chloride has a significant effect (among anions) on total secretion (Figure 4). Treatments containing chloride secreted significantly higher amounts of ions. Among cations, histidine and potassium have a significant effect on total ion secretion. Histidine and potassium secreted similar amounts of ions, and both secreted higher amounts of ions than sodium. Chloride further showed a significant interaction with histidine and potassium.

Treatments with histidine or potassium showed increased secretion rates, but only if chloride was accompanied. High secretion of ions occurs only when chloride is coupled with either potassium or histidine.

Flexibility of ion secretion by *N. schneideri* salt glands

Range of cation secretion

Cation ratio secretion (Percent K/(Na+K)) in the *N. schneideri* nasal salt gland falls within a very narrow range for all treatments (Table 3; Figure 5). Potassium secretion ranged from 45-75% of total cation secretion for days 1-6 among all treatments. The similarities between percent K secretion, across all treatments, indicate no significant treatment effects on cation ratio secretion.

It further indicates that *N. schneideri* secretes a mix of potassium and sodium, regardless of the ion load incurred. While the gland mostly secretes potassium, it also continues to secrete a constant amount of sodium, even when sodium is not administered. Potassium chloride treated animals, for example, secreted large amounts of sodium alongside potassium during all 6 days (Figure 5). Similarly, potassium acetate treatments increased their potassium secretion ratio until day 3, but constantly secreted sodium throughout (Figures 5). Sodium acetate treatments secreted most cations as potassium (60%) at the onset of the experiments. This ratio of cations secretion decreased as the sodium loads continued. At the experiment's end, the cation ratio switched to 40% potassium and 60% sodium of total cation secretion, although total secretion rate was still very low (Figure 5).

Cation ratio secretion did not vary for day 4 values (after 4 ion load treatments) among all treatments (Figure 6). Potassium comprised most of the cations secreted by

the nasal salt gland. Overall, range of cation secretion is very narrow and not affected by the type of ion load. There is some flexibility on cation secretion, as seen in sodium acetate treatment, but it is very minimal.

Range of anion secretion

Chloride secretion ratio is higher across 6-day values for all treatments (Table 3). Percent chloride secretion ranged from 54-90%. There is limited flexibility for anion secretion among all treatments. The chloride treatments (histidine chloride and potassium chloride) had the highest anion secretion percentage over 6 days (85% and 90%, respectively). The other treatments also showed narrow ranges of total anion secretion, but at lower secretion. Potassium acetate treatments had the lowest choride ratio, but still excreted a significant amount of chloride ions (54% chloride).

Day 4 anion secretion values show an increased ratio of anion:chloride secretion for all treatments except histidine chloride, compared to mean secretion for 6-day total values (Figure 7). Saline and sham injected skinks secreted the highest levels of anions on day 4. Histidine chloride was the only treatment to secrete more cations than anions on day 4, although total secretion was still very low (Figure 7).

Ion secretion by *N. schneideri* salt glands

Sodium secretion

Sodium excretion increased in all treatments as overall secretion increased, even when no sodium was administered (Table 4; Figure 8). Chloride treatments (histidine chloride, potassium chloride, and sodium chloride) secreted the largest amount of sodium via the salt gland. Histidine chloride treatments secreted the majority of sodium via the salt gland (72%) compared to urate (24%) and feces excretion. Similarly, potassium

chloride treatments secreted 70% of total sodium via the salt gland. Sodium acetate and histidine acetate treatments excreted most of their sodium via urate (69% and 75%, respectively). Sodium excretion by the salt gland was the same for potassium acetate treatments (0.27-0.28 μmol/g), yet its total sodium secretion was the lowest among all treatments.

Potassium secretion

A large fraction of potassium is secreted via the gland when chloride loads are administered (Table 4; Figure 8). Histidine chloride, potassium chloride, and sodium chloride treatments excreted 85%, 48%, and 59% potassium, respectively, via the salt gland. Similarly, saline injected skinks also excreted 60% potassium via the salt gland. Large fractions of potassium are excreted in the urate, as seen in acetate treatments (sodium acetate (78%), potassium acetate (73%), and histidine acetate (65%)), sham (76%), and potassium chloride (50%) treatments. Sodium acetate, sodium chloride, and saline treatments all secreted approximately the same amount of total potassium (1.85-1.94 μmol K/g), yet their secretion distribution varies. Sodium chloride and saline treatments secreted the majority of potassium via the salt gland (59-60%) and the rest mainly through urate (27-28%). In contrast, sodium acetate treatments secreted the most potassium via urate (78%), while only minimally via the gland (11%).

Chloride secretion

Chloride is most efficiently excreted and almost solely excreted via the salt gland (Figure 8). The majority of chloride output measured was excreted via the salt gland (76-99%) among all treatments. Potential chloride in urate or urine excretion was not measured. Fecal excretion in acetate (histidine acetate, potassium acetate, and sodium

acetate) and sham treatments contained negligible amounts of chloride. Chloride (potassium chloride, histidine chloride, and sodium chloride) and saline treatments contained approximately 10% of total measured chloride secreted in the feces. Salt glands secreting the highest amount of chloride were seen in skinks receiving chloride treatments: histidine chloride, potassium chloride, and sodium chloride.

Secretion of administered ions

In all treatments, total ion output was less than the 10 μmol/g administered; in most treatments, output was significantly less. Potassium chloride-treated skinks excreted the largest amount of potassium ions (7.26 μmol K/g), yet still below the administered amount (Figure 8). Maximum sodium secretion of a sodium-treatment was only 35% of administered sodium (by sodium acetate treatment); the highest chloride secretion was 52% of administered chloride in histidine chloride treatment.

Discussion

Control of secretion

Studies of elasmobranches and marine vertebrates (birds and turtles) with salt glands that secrete mainly sodium chloride have shown gland secretion is initiated by osmotic challenges (Minnich, 1979; Lowy et al., 1989; Hammerschlag, 2006). In these taxa, salt gland secretion increases when plasma volume or osmoreceptors detect an osmotic load and stimulate gland secretion. Neuronal stimulation by these receptors will, in turn, initiate and regulate salt gland secretion.

Studies of the desert iguana *Dipsosaurus dorsalis* indicate this doesn't occur in terrestrial lizards. The desert herbivore *D. dorsalis* eats a potassium-rich plant diet, and its gland predominantly secretes potassium chloride. When given an osmotic load, such

as sucrose or mannitol, *D. dorsalis* does not increase gland secretion (Shoemaker et al., 1972). Salt gland secretion in this species only responds to chloride or potassium (in any of the following forms: potassium acetate, K_2 succinate, or $KHCO_3$). Further studies on *D. dorsalis* confirm salt gland secretion responds to only ionic loads of either potassium or chloride, but not sodium (Hazard, 2001).

Additive effects of ions stimulating gland secretion have also been seen in the herbivorous desert lizard *Uromastix dispar* (Hazard et al., 2007). The salt gland of this species will increase secretion treated with chloride or potassium alone, above control levels. Treatment of both ions (as potassium chloride), however, increased gland secretion above the sum of both individual ions. Sodium or any osmotic loads did not initiate secretion for this gland, either.

Control of secretion in *N. schneideri* salt gland also seems to be under ionic control, but differs from these other two terrestrial species regarding which ion stimulates secretion. Chloride treatments (histidine chloride and potassium chloride) significantly increased total secretion of cations and anions, above all other ion and osmotic loads. Histidine chloride treatments, compared to potassium chloride treatments, secreted a larger anion:cation ratio and a lower potassium:cation ratio. Potassium acetate treatments resulted in secretion rates similar to sham injected controls. This suggests secretion was stimulated by the presence of chloride, not potassium. Osmotic loads (histidine acetate) did not increase secretion rate. Similar to other terrestrial lizards studied, and unlike the glands of birds and marine reptiles, initiation of salt gland function is stimulated by specific ions, not osmotic loads.

While other chloride treatments increased secretion rate, sodium chloride treatments showed secretion rates comparable to saline injected skinks. Similarly, sodium acetate treatments had the lowest secretion rate among all treatments, including sham-injected lizards. This suggests the inhibitory role of sodium in salt gland function. Secretion comparisons among chloride treatments indicate sodium inhibited total secretion rate from 65% (compared to potassium chloride treatments) to 70% (compared to histidine chloride treatments (Table 3). Furthermore, sodium appears to exert more inhibition on gland secretion of cations, not anions (Table 3; Figure 5). The majority of cations secreted (53-70%) were potassium instead of sodium. Sodium chloride-treated skinks showed increased anion:cation secretion ratios compared to all other treatments, while cation secretion remained comparable to histidine chloride and sham injected treatments. Skinks receiving both chloride and sodium (as sodium chloride) continued to secrete large amounts of chloride via the gland, yet decreased their cation secretion. Between the two cations, sodium treatments inhibited gland secretion of potassium the most, suggesting a higher importance of potassium secretion for cation regulation in the salt gland. Sodium acetate decreased the potassium:sodium ratio of gland secretion as the experiment progressed (Figure 5). After day 5, sodium was the main cation being secreted by the gland of sodium acetate-treated skinks. Sodium has also inhibited gland secretion of potassium in two omnivorous terrestrial lizards, *Sauromalus obesus* and *Ctenosaura pectinata* (Templeton, 1964). Subjects in these two species receiving sodium chloride loads showed a decrease of potassium and chloride secretion from the gland, compared to other loads. The inhibition of sodium on gland secretion, therefore, may be a physiological adaptation seen in terrestrial omnivore and insectivore lizards.

In skinks, sodium inhibition of gland secretion exposed significant plasticity between salt gland and urate excretion. Excess cations (both potassium and sodium), when inhibited from nasal gland excretion, were instead excreted via the cloaca as insolute urate salts. Sodium acetate inhibited the secretion of both potassium and sodium via the gland, well below sham secretion levels. Urate secretion of these two ions increased significantly in those subjects. Sodium acetate and sodium chloride treatments both showed the same amount 6-day potassium secretion values (1.85 μmol K/g), yet their secretion routes differed. Sodium chloride treatments excreted potassium mostly via the salt gland (59%), while sodium acetate treatments excreted potassium mostly via the cloaca (78%). The differences between the two treatments highlight the possible roles of both sodium and chloride in salt gland function of the insectivorous lizard: chloride stimulates gland secretion while sodium inhibits it. The plasticity between urate and salt gland excretion demonstrates the ability of the lizard to maintain ionic balance despite inhibition of secretion at the gland.

The differences in ions stimulating gland function among *D. dorsalis*, *U. dispar* and *N. schneideri* highlight the roles that diet and environment have on salt gland function. Diet, in each species, imposes different ion and osmoregulatory challenges. Terrestrial herbivores eat potassium-rich plants with very low energy content. To obtain enough nutrients, terrestrial herbivores require more plant material ingested, imposing a greater potassium load. Marine lizards, whether herbivore or carnivore, have an increased sodium intake. The function of their salt gland reflects the challenges posed by their diet. Terrestrial carnivores, however, do not have the same narrow ion challenges posed by marine or herbivorous diets. The decreased sodium or potassium challenges of

N. schneideri suggest the salt gland is regulated by a completely different ion: chloride. These physiological and regulatory differences between salt glands, despite cellular and macroscopic homology, can be maintained among various species and even within species. Hertz (1980 and 1981) studied physiological differences in osmoregulation among *Anolis gundlachi* species living at different altitudes. Differences in evaporative water loss rates were attributed to the effects their respective diets placed on the populations, allowing physiological differences to be seen despite structural and microscopic homology. Gland structure and function among lizards, therefore, can be maintained, while its physiological regulation can vary.

Besides habitat, body size also affects diet. Herbivorous lizards and reptiles tend to be larger than carnivores. Even though plants contain lower nutrient content and implicate ion regulation, a larger body mass allows for a decreased metabolism. Full herbivory, however, is rare in lizards (50-60 of the approximate 3400 species known). Among other members of the Scincidae family, skinks <100 grams in weight are more prevalently carnivorous, whereas those 300+ grams are herbivores. Like size, gland function of *N. schneideri* may, therefore, be an adaptation to their insectivorous diet.

Range and flexibility of secretion

Most ions excreted via the gland were anions (chloride) rather than cations (sodium or potassium). Another anion (either bicarbonate alone or a mixture of bicarbonate and carbonate) is excreted from the glands of the desert iguana *Dipsosaurus dorsalis* (Hazard, 2001) and its close relative, *Sauromalus obesus* (Nagy, 1972). Values for excretion, however, are very minimal compared to total secretion (0.6 and 1.1 μmol/g/d for *D. dorsalis* and *S. obesus*, respectively) so bicarbonate excretion was not

determined in this study. Similar to *D. dorsalis* and *S. obesus*, *N. schneideri* appears to secrete nearly all anions as chloride, not bicarbonate, regardless of the anion load given. This suggests anion secretion is not very flexible and consists of mainly chloride among lizard species

As with total secretion rate, histidine chloride and potassium chloride treatments excreted the greatest amount of chloride (5.08 and 4.32 μmol/g, respectively). Additionally, all treatments secreted a larger ratio of anions to cations via the gland (54-90% chloride) with no significant difference among treatments. Schmidt-Nielsen et al. (1963) had proposed that since anions are mainly organic acids, they could potentially get metabolized instead of excreted. This doesn't seem to occur in *N.schneideri*, since anion:cation secretion ratio is slightly higher, and the metabolism of anions would also affect the retention of cations. Increased anion secretion, therefore, seems to result from increased chloride loads, as shown by histidine chloride, potassium chloride, and sodium chloride treatments (85%, 90%, and 81% chloride ratios, respectively).

If, as proposed, sodium inhibits cation secretion at the gland, the inhibition would occur on the luminal surface of secretory cells, and mainly acts on sodium, not potassium. Conversely, potassium secretion by the potassium channels could be slightly increased, resulting in increased potassium excretion in the gland. Increased potassium secretion by the lumenal potassium channels seems more plausible since it requires the least amount of energy input. Regardless of method, the result is a slightly higher potassium excretion rate among all treatments, although at insignificant value differences. The slight variance, however, suggests a possible physiological importance potassium secretion has on ionic balance. Studies of intracellular potassium concentrations in

vertebrate muscles show a narrow range of osmolar concentration that must be maintained for proper cell function (Schmidt-Nielsen, 1975). Abnormal intracellular potassium concentrations can lead to improper enzyme function, and disruption of metabolic processes, DNA duplication and transcription of RNA. Abnormal extracellular potassium concentrations can also affect muscle excitability and inhibit muscle function. Potassium excretion, therefore, is physiologically important for function in *N. schneideri* and other vertebrates; its intracellular and extracellular concentrations are highly regulated. The slight increase in gland secretion of potassium secretion (between cations) among all treatments suggests this importance. Skinks treated with potassium chloride excreted large amounts of potassium via the gland. The salt gland in *N. schneideri*, therefore, can play a potentially significant role in potassium regulation, especially when stimulated by chloride.

In contrast to the narrow range of potassium tolerated for physiologic function, increased sodium concentration in blood plasma is better tolerated in the desert lizards *Dipsosaurus dorsalis*, *Sauromalus obesus*, and *Uma scoparia* (Minnich, 1976), as well as in most *Amphibolorus* species (Bradshaw and Shoemaker, 1967). When dehydrated, plasma and body sodium concentrations increase in these desert lizards, causing a shift of water from within the cell to the extracellular fluid. *Amphibolorus* species don't possess a nasal salt gland, so during low periods of rainfall, they will tolerate up to a 46% increase in plasma sodium concentrations. Between the two cations, regulation of potassium is more important for physiologic function.

Excretion of cations and anions were each within narrow ranges and showed very little differences among treatments. While the main cation excreted was potassium, a

constant amount of sodium was also excreted (Table 3). Even skinks that didn't receive sodium treatments (i.e. potassium chloride and potassium acetate) excreted a constant amount of sodium via the gland (~30-40%). Potassium chloride-treated skinks, for example, excreted a 0.7 ratio of potassium:sodium throughout the experiment. It is more plausible, therefore, that the rate of sodium movement from within the secretory cell to the lumen is relatively unchanged, despite the ion load incurred.

Although constant (yet reduced) excretion of sodium occurs during overall gland secretion and potassium plasma concentrations appear to be more physiologically important, a decrease in plasma sodium can also inhibit total secretion rate. Skinks treated with potassium chloride secreted a large amount of ions until day 3, when total secretion rate suddenly dropped. Total secretion rates are normally expected to drop after day 4, when the ion loads have ceased. The constant loss of sodium on days 0-3 could have resulted in hyponatremia, prompting the early decrease of total excretion rates in potassium chloride-treated skinks. To assess whether hyponatremia can inhibit gland secretion, plasma concentrations would need to be assessed for skinks undergoing ionic and osmotic loads.

Only sodium acetate-treated skinks showed differences in cation secretion, highlighting the potential range and flexibility of gland secretion (Figure 5). Secretion levels in these skinks maintained higher potassium excretion (~55-60% potassium), despite the absence of potassium. After day 3, potassium excretion decreased, resulting in a switch to predominantly sodium secretion on days 5 and 6 (~60% sodium). While overall secretion values were still minimal to fully attribute any effects, these results

suggest potential flexibility in the gland's response to the ion load incurred by adapting the secretion composition.

Since all treatments secreted a higher anion:cation ratio via the glands, increased anion secretion could result from ion movement by the sodium-potassium-chloride cotransporter (NKCC1) into the secretory cell. NKCC1 transport results in two chloride ions, along with one ion of sodium and potassium each, moving from the blood plasma into the cell. The stoichiometry of ion movement, coupled with a slight increase of either cation being retained, could account for the overall increased secretion of anions to cations. Increased movement of ions by NKCC1 and slight inhibition of cation secretion by lumenal transporters, therefore, results in increased anion secretion by the gland.

Importance of salt gland

Composition of excretion and importance of the salt gland for ion regulation varies among the lizard species with functional glands. The salt glands of the marine iguana *Amblyrhynchus cristatus* can secrete most of its ingested ions solely via the gland with very little contribution from the cloaca (Shoemaker and Nagy, 1984). Gland secretion can further vary within the same species depending on osmotic and ionic balance. Salt gland secretion in the desert iguana *Dipsosaurus dorsalis*, for example, contributes minimally to total ion secretion in water-balanced subjects in their natural habitat (Minnich, 1970). In these subjects, most ions are excreted as urate. In contrast, non-water balanced *D. dorsalis* will excrete equal amounts of ions via the salt gland and urate (Minnich, 1976).

While secretion rates of insectivorous lizard species has been minimally studied, diet comparisons between lizards with functional salt glands can infer the importance of

ion secretion through the gland. Potassium content of a carnivorous diet contains only up to 23% of potassium found in an herbivorous diet (Minnich, 1972). Applying the same logic, a marine diet would contain larger amounts of sodium than a carnivorous diet. These comparisons aren't meant to relate the ability of glands between taxa and infer superiority, but instead highlight that gland function and importance is only relative to that species' needs.

Like the glands of other terrestrial lizards, the salt gland of *N. schneideri* mainly functions to regulate ions, not water. Despite its reduced dietary ion loads compared to other terrestrial lizards, *N. schneideri*'s salt gland can also excrete relative amounts of all three ions ingested (sodium, potassium, and chloride), especially during increased chloride loads. Sodium secretion by the gland increased as overall secretion rates increased in all treatments even when sodium was not administered (Figure 8). As previously explained, sodium excretion may be minimally regulated at the gland; the amount of sodium transported via the apical chloride channels may be solely dependent on NKCC1 transport of sodium into the cell. The gland is important, therefore, in secreting sodium whenever increased ions loads are incurred; as overall NKCC1 function increases, so does the amount of sodium excreted via the gland.

Potassium excretion via the gland appears to only be stimulated by the presence of chloride (Figure 8). Potassium chloride, histidine chloride, sodium chloride, and saline treatments all increased potassium excretion in the gland. If the NKCC1 cotransporter moves two chloride ions per every sodium and potassium ion into the cell and chloride then moves to the lumenal side via the active chloride channel, this creates an increased negative electrochemical gradient on the mucosal side of the cell. Since sodium

movement via the apical sodium channel is maintained at a steady ratio of total secretion, potassium must exit into the mucosal side to account for the increased negative electrochemical gradient. Increased potassium secretion via the gland is only accompanied during increased chloride loads because the chloride needs to be present to create a large electrochemical gradient. Chloride excretion can only be efficiently excreted through the nasal salt gland (Figure 8).

The nasal salt gland of *N. schneideri* is essential for complete chloride (and possibly anion) regulation, as well as limited potassium and sodium regulation. When sodium and potassium excretion via the gland becomes limited, cations are released in significant amounts as urate salts, either as an alternative to salt gland secretion or because of limitations to salt gland secretion. Sodium was predominantly excreted via the cloaca in sodium acetate and histidine acetate treatments (69-75%, respectively). Potassium was similarly excreted in potassium chloride and all acetate treatments. The extent of *N. schneideri* water loss through urate salt excretion is unknown, so efficiency of excretion via the salt gland and cloaca cannot be compared.

The sum total of secreted ions, whether via the salt gland, urate, or feces, was significantly less than the total ions administered in all treatments (Table 4). At their highest output level, total sodium, potassium, and chloride values excreted were 3.66, 7.26 and 5.19 μmol/g, respectively. Experimental margin of error in the collection of feces, salt, and urate samples is a possibility, but not significant enough to account for the differences between ion intake and output seen. Therefore, while salt gland and urate excretion help maintain ionic balance in *N. schneideri*, a great proportion of ions are retained in blood plasma and tissues. Blood plasma concentrations of subjects

undergoing ion and osmotic loads would account for the discrepancy in administered versus excreted ions, and possibly highlight the effects retained ions have on homeostasis.

Proposed mechanism of secretion in *N. schneideri*

The salt gland of *N. schneideri* represents the primitive salt gland of terrestrial lizards that is able to secrete simple ions (sodium, potassium, and chloride) in varying proportions for ion, not water, regulation. The proposed secretion mechanism of this gland is similar to the proposed mechanism in terrestrial lizards, albeit with minor modifications representing differences in secretion composition. Like the gland of herbivorous desert lizards, potassium regulation must be maintained within a narrow range in the extracellular and intracellular regions for proper cell function. Like marine lizard salt glands, sodium is constantly secreted, although in skinks total secretion rate is initiated by chloride. I've devised this proposed method of secretion using elements from the proposed methods in both sodium chloride secreting glands and potassium, chloride and sodium secreting glands. I've further applied the results of total secretion composition and range to suggest a possible mechanism that accounts for the data. Lastly, I've maintained ion channels that are present in other types of secretory epithelia (i.e. salivary glands) and are proposed to be present in other lizard salt glands, but have not been identified yet in the salt glands of lizards. As with other previously proposed secretory methods in lizard salt glands, further research to isolate and identify the ion transport channels is necessary.

The proposed mechanism of salt gland secretion in *N. schneideri* (Figure 9) is as follows: one sodium, one potassium, and two chloride ions move from the extracellular

blood plasma into the secretory cells via the NKCC1 cotransporter. Sodium is then pumped into intercellular spaces by the (Na^++-K^+)-ATPase located near the basolateral membrane. Chloride exits the cell via an apical chloride channel down its electrochemical gradient into the secretory lumen (mucosa side). Increased chloride loads further increase chloride movement to the luminal side, creating a hyper-negative electrochemical gradient. Sodium will follow passively down the electrochemical gradient via a paracellular route, but only at a constant rate and proportional to total ion secretion. Potassium will be transported via a basolateral potassium channel back to into the blood to maintain membrane potential, but at minimal rates. Most potassium will move into the lumen via an apical potassium channel to compensate for the increased chloride-initiated electrochemical gradient. Final secretion composition contains a mixture of all three ions. Sodium will constantly be excreted into the lumen via the paracellular route, proportional to total ion secretion. Increased chloride loads will create large electrochemical gradients in the lumen, stimulating increased potassium secretion via the apical potassium channel.

Summary

Some consistencies between the salt glands of *N. schneideri* and previously researched lizard species are present. Secretion by the gland, for example, is stimulated by the presence of ions, not osmotic loads. Potassium, like in other species, is constantly excreted, highlighting its deleterious effects on physiological processes if plasma levels increased. However, differences in salt gland function between *N. schneideri* and other previously studied lizard species are present. Chloride, for example, stimulates total gland secretion in *N. schneideri*. Histidine or potassium also increased total secretion

rate, but only when chloride was present. A possible inhibitory role of sodium on total gland secretion is also suggested in *N. schnedieri*.

These differences between *N. schnedieri* and other lizard species suggest a considerable amount of plasticity exists across lizard taxa. Depending on physiologic needs, the salt glands of lizard taxa appear to vary in stimulators of secretion, composition of excreta, and possibly in the molecular mechanisms of secretion. These variances in salt gland function could be further attributed to the variances in ecological factors that dictate the importance of particular ions to stimulate gland excretion. Further research on the ecological effects of salt gland function among lizards, and more extensive knowledge of salt gland secretion among lizard taxa would be required to further assess this relationship.

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TABLE 1: Vertebrate groups (or species) with extrarenal salt gland

Reptile	**Gland Location**	**Ion(s) Secreted**
Marine birds	Nasal	Sodium Chloride
Elasmobranchs	Rectal	Sodium Chloride
Estuarine crocodile, *Crocodylus porosus*	Sublingual	Sodium Chloride
Homalopsid snake, *Cerberus rhynchops*	Premaxillary	
Hydrophid and Achrochordon snakes	Sublingual	
Marine Turtle, Chelonia and Emydid Genus	Lacrimal	Sodium Chloride
Marine lizards	Nasal	Sodium Chloride
Terrestrial lizards	Nasal	Potassium, Sodium, Chloride, Bicarbonate

TABLE 2: Salt Gland Distribution among Lizards

Family	Salt Glands Present	Salt Glands Absent
Iguania		
Chamaeleonidae		
Leiolepidinae	*Uromastix* spp.	
Agaminae		Some (9)
Iguanidae	All	
Phrynosomatidae	All	
Crotaphytidae	All	
Polychridae	*Anolis carolinensis*	
	Liolaemus spp.	
Gekkota		
Gekkonidae		Some (9)
Pygopodidae		*Lialis burtonis*
Scincomorpha		
Teiidae	*Ameiva quadrilineata*	*Tupinambis teguixin*
	Cnemidophorus spp.	
Lacertidae	*Acanthodactylus* spp.	*Lacerta viridis*
	Eremias guttulata	
	Aporasaura	
Xantusidae	*Xantusia vigilis*	
Scincidae	Some (11)	
Cordylidae	*Angolosaurus skoogi*	*Cordylus cataphractus*
Anguimorpha		
Anguidae		*Elgaria multicarinatus*
		Anniella pulchra
Amphisbaenia		*Bipes biporus*
Helodermatidae		*Heloderma suspectum*
Varanidae	*Varanus* spp.	

Adapted from Hazard (2004)

TABLE 3: Composition of salt secreted by *N. schneideri* glands

Treatment (n=7)	Total Cations Secreted (μmol	Cation Ratio (K/[Na+K] x 100%)	Anion Ratio (Cl/[Na+K] x 100%)
Histidine acetate	0.63 ± 0.20	65 ± 5.2	78 ± 6.8
Histidine chloride	6.08 ± 1.04	57 ± 3.3	85 ± 2.2
K acetate	0.66 ± 0.53	61 ± 4.1	54 ± 14.2
KCl	4.99 ± 0.53	70 ± 4.4	90 ± 11.8
Na acetate	0.63 ± 0.24	45 ± 5.9	68 ± 11.3
NaCl	2.20 ± 1.00	56 ± 7.3	81 ± 21.2
Saline injection	1.91 ± 0.39	61 ± 4.0	85 ± 5.7
Sham injection	0.89 ± 0.25	61 ± 3.8	77 ± 15.6

Total secretion during days 1-6 (mean ± SE)

TABLE 4: 6-day total ion output in *N. schneideri*

	Treatment							
Ion Route of excretion	Histidine acetate	Histidine chloride	Potassium acetate	Potassium chloride	Sodium acetate	Sodium chloride	Saline	Sham
Sodium								
Urate	0.92^{B} (75%)	0.87^{B} (24%)	0.28 (44%)	0.45^{B} (21%)	2.42^{A} (69%)	1.04^{B} (36%)	1.09^{B} (49%)	0.90^{B} (57%)
Feces	0.12^{C} (10%)	0.14^{C} (4%)	0.09^{C} (14%)	0.18^{BC} (8%)	0.87^{A} (25%)	0.74^{AB} (26%)	0.41^{ABC} (18%)	0.34^{ABC} (22%)
Salt Gland	0.18^{C} (15%)	2.65^{A} (72%)	0.27^{BC} (42%)	1.49^{AB} (70%)	0.22^{C} (6%)	1.11^{BC} (38%)	0.74^{BC} (33%)	0.33^{BC} (21%)
total µmol ion/g	*1.22*	*3.66*	*0.64*	*2.12*	***3.51***	***2.89***	*2.24*	*1.57*
Potassium								
Urate	1.07 (65%)	0.51 (13%)	1.53 (73%)	3.65 (50%)	1.45 (78%)	0.51 (28%)	0.53 (27%)	2.01 (76%)
Feces	0.13 (8%)	0.11 (3%)	0.17 (8%)	0.11 (2%)	0.20 (11%)	0.25 (14%)	0.24 (12%)	0.09 (3%)
Salt Gland	0.45^{B} (27%)	3.43^{A} (85%)	0.39^{B} (19%)	3.50^{A} (48%)	0.20^{B} (11%)	1.09^{B} (59%)	1.17^{B} (60%)	0.56^{B} (21%)
total µmol ion/g	*1.65*	*4.05*	***2.09***	***7.26***	*1.85*	*1.85*	*1.94*	*2.66*
Chloride								
Feces	0.03 (6%)	0.11 (2%)	0.03 (5%)	0.07 (2%)	0.09 (24%)	0.10 (4%)	0.11 (6%)	0.01 (1%)
Salt Gland	0.47^{C} (94%)	5.08^{A} (98%)	0.55^{C} (95%)	4.32^{AB} (98%)	0.29^{C} (76%)	2.43^{BC} (96%)	1.66^{C} (94%)	0.85^{C} (99%)
total µmol ion/g	*0.50*	***5.19***	*0.58*	***4.39***	*0.38*	***2.53***	*1.77*	*0.86*

Ion µmol/g output (percentage of total secretion). Treatments sharing the same letter are not statistically different from one another (ANOVA and Tukey's *t*-tests). Bold denotes totals for treatments where subjects received that particular ion.

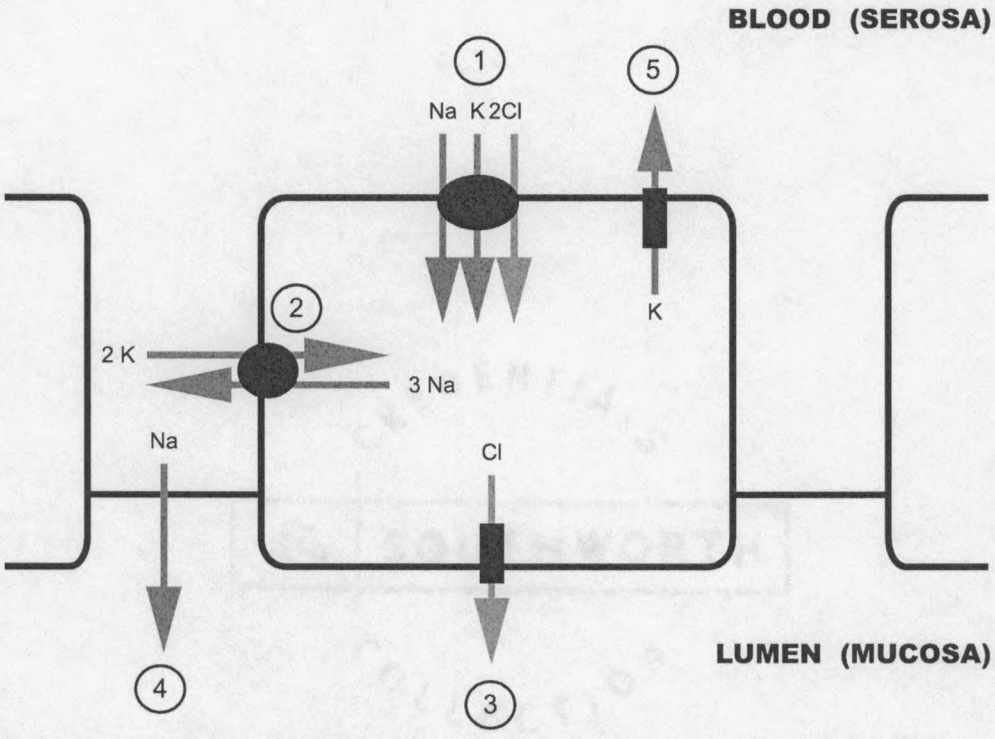


Figure 1: Model for NaCl secretion by nasal salt gland of marine lizards (Taken from Greger, 1996).
Sodium, potassium, and chloride move from the extracellular blood plasma into the secretory cells via the Na-K-Cl cotransporter in the stoichiometry of 1:1:2. Sodium gets pumped into the intercellular space by the $(Na^+ + K^+)$-ATPase. Chloride exits the cells against an electrochemical gradient to the secretory lumen via apical chloride channels. Increased lumenal chloride allows sodium to follow paracellularly down an electrochemical gradient. Potassium exits the cells via basolateral potassium channels. The resulting secretion contains sodium and chloride.

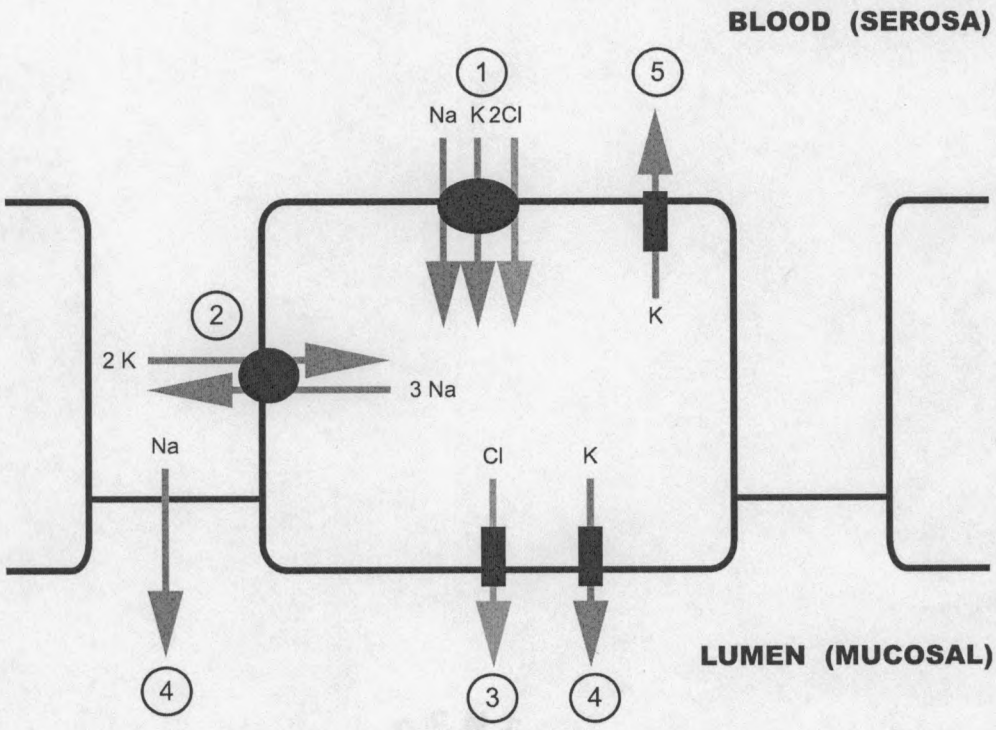


Figure 2: Proposed model for ion secretion by desert iguana (*Dipsosaurus dorsalis*) nasal salt gland (Taken from Hazard, 1999)
Sodium, potassium, and chloride move from the extracellular blood plasma into the secretory cells via the Na-K-Cl cotransporter in the stoichiometry of 1:1:2. Sodium gets pumped into the intercellular space by the $(Na^+ + K^+)$-ATPase. Chloride exits the cells against an electrochemical gradient to the secretory lumen via apical chloride channels. Increased lumenal chloride allows sodium to follow paracellularly down an electrochemical gradient. Potassium exits the cells via basolateral and apical potassium channels. The resulting secretion contains a mixture of sodium, chloride and potassium.

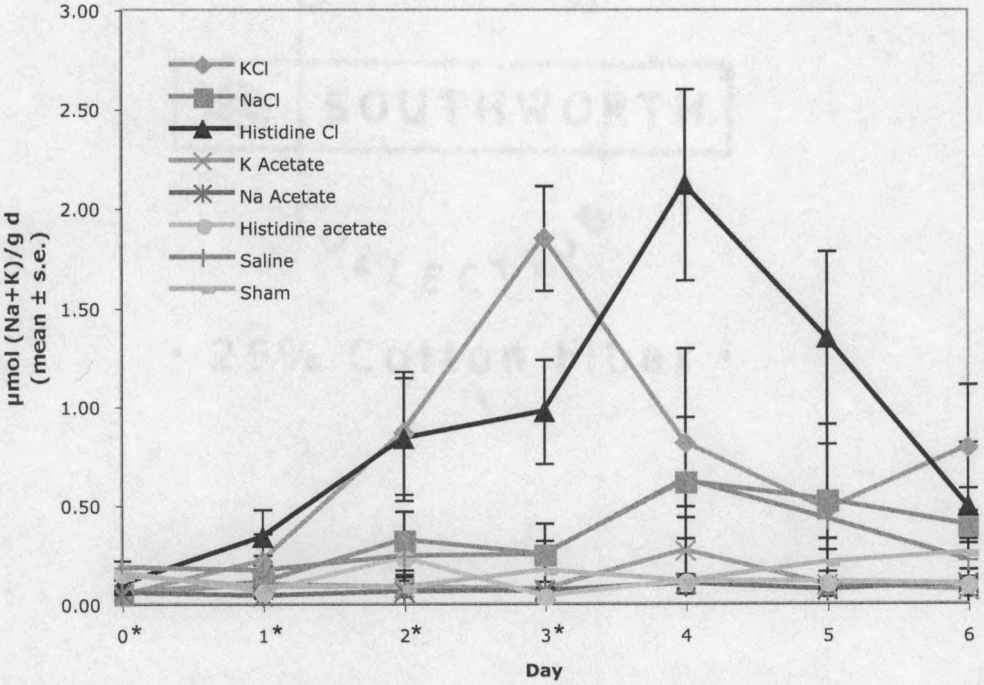
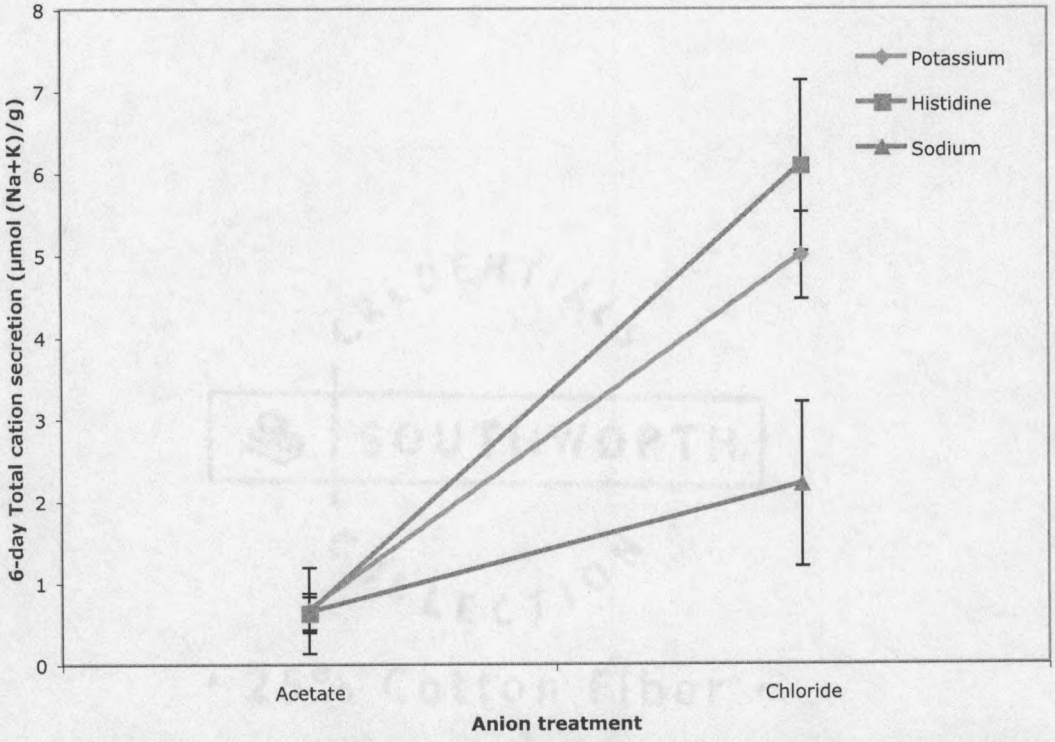


Figure 3: Total cation secretion rate per treatment in *N. schneideri*
Only chloride treatments (potassium chloride and histidine chloride) stimulated cation secretion by the gland. Sodium chloride treatments stimulated secretion to a lesser degree, possibly indicating sodium inhibition of secretion. Potassium acetate, sodium acetate, histidine acetate, saline, and sham-injected subjects secreted minimal amounts of ions.
(means ± s.e.)



Source	D.F.	S.S.	F ratio	P	Tukey's HSD results
Cation	2	19.8	4.6	0.019	K = Hist > Na
Anion	1	117.8	55.2	<0.0001	Cl > Ac
Cation x Anion	2	115.4	3.6	0.042	

Figure 4: Cation and anion interaction in total secretion per treatment in *N. schneideri*

Among anions, chloride has a significant effect on total secretion. Treatments containing chloride secreted higher amounts of ions. Among cations, histidine and potassium have a significant effect on total secretion. Histidine and potassium excreted similar amounts of ions, and both had higher secretion than sodium. A significant interaction between chloride and potassium or histidine causes an increase in total secretion rate.

(means ± s.e.)

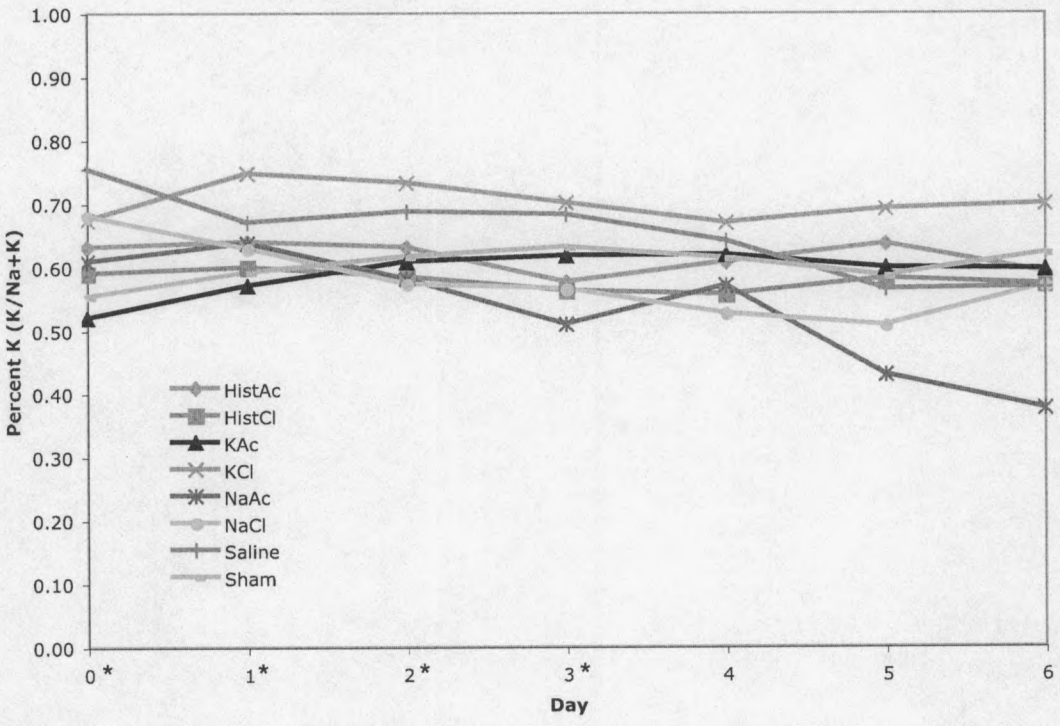


Figure 5: Cation ratio secretion in *N. schneideri*
Mean percent potassium secretion (K/(Na+K)) per gram day. The narrow range of secretion among all treatments indicates no significant treatment effect.
* denotes treatment administered

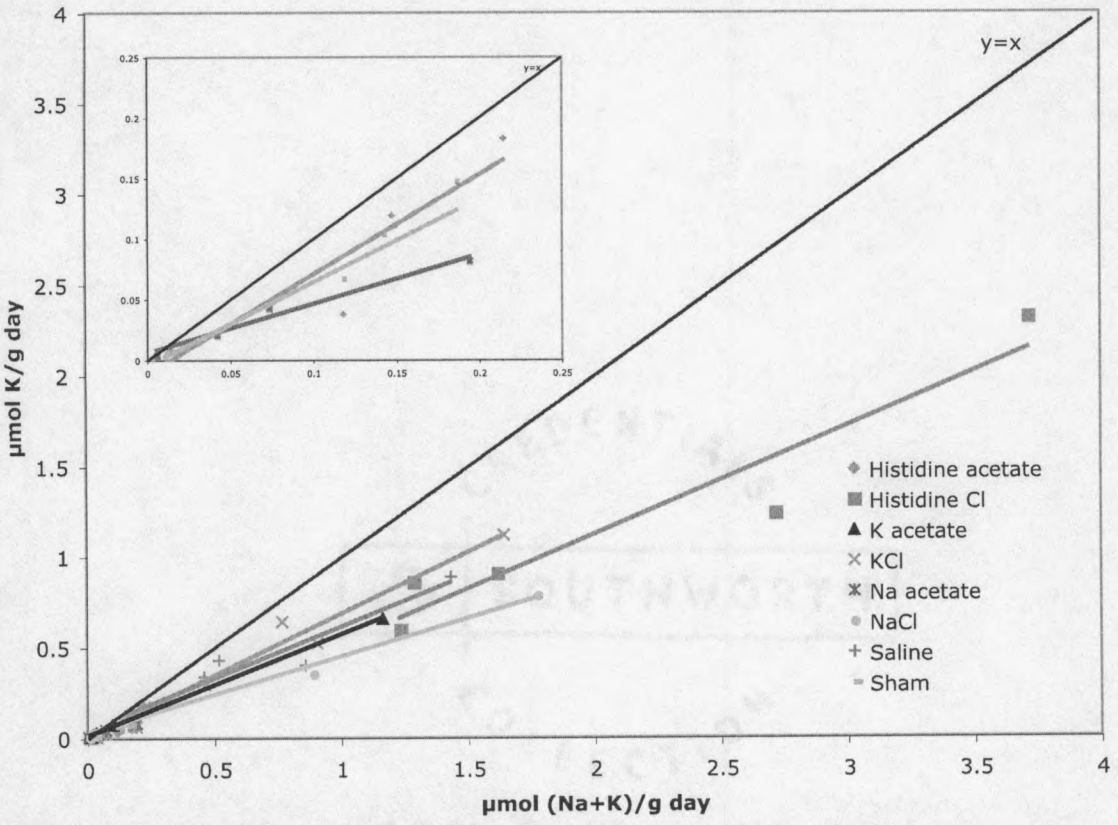


Figure 6: (K/(Na+K)) µmol/gram on Day 4 in *N. schneideri* for all treatments. Increased potassium (versus sodium) secretion is seen in all treatments. Sodium was constantly secreted in all treatmets, even when sodium was not administered.

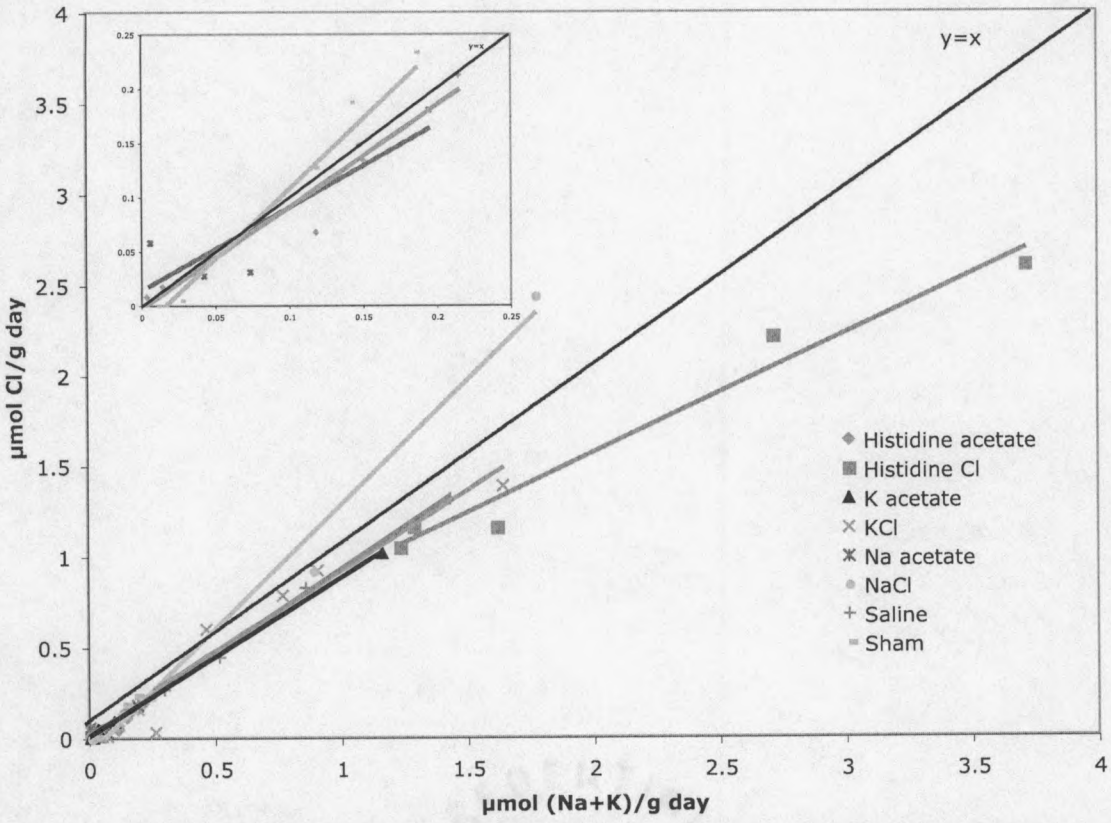


Figure 7: (Cl vs (Na+K)) µmol/gram on Day 4 in *N. schneideri* for all treatments Saline and sham treatments secreted the largest amount of chloride on day 4 and a higher anion:cation ratio than mean values for the experiment's duration. Only histidine chloride-treated skinks secreted more cations than anions on Day 4. Inset: Close-ups of histidine acetate, histidine chloride, potassium acetate, and potassium chloride treatments.

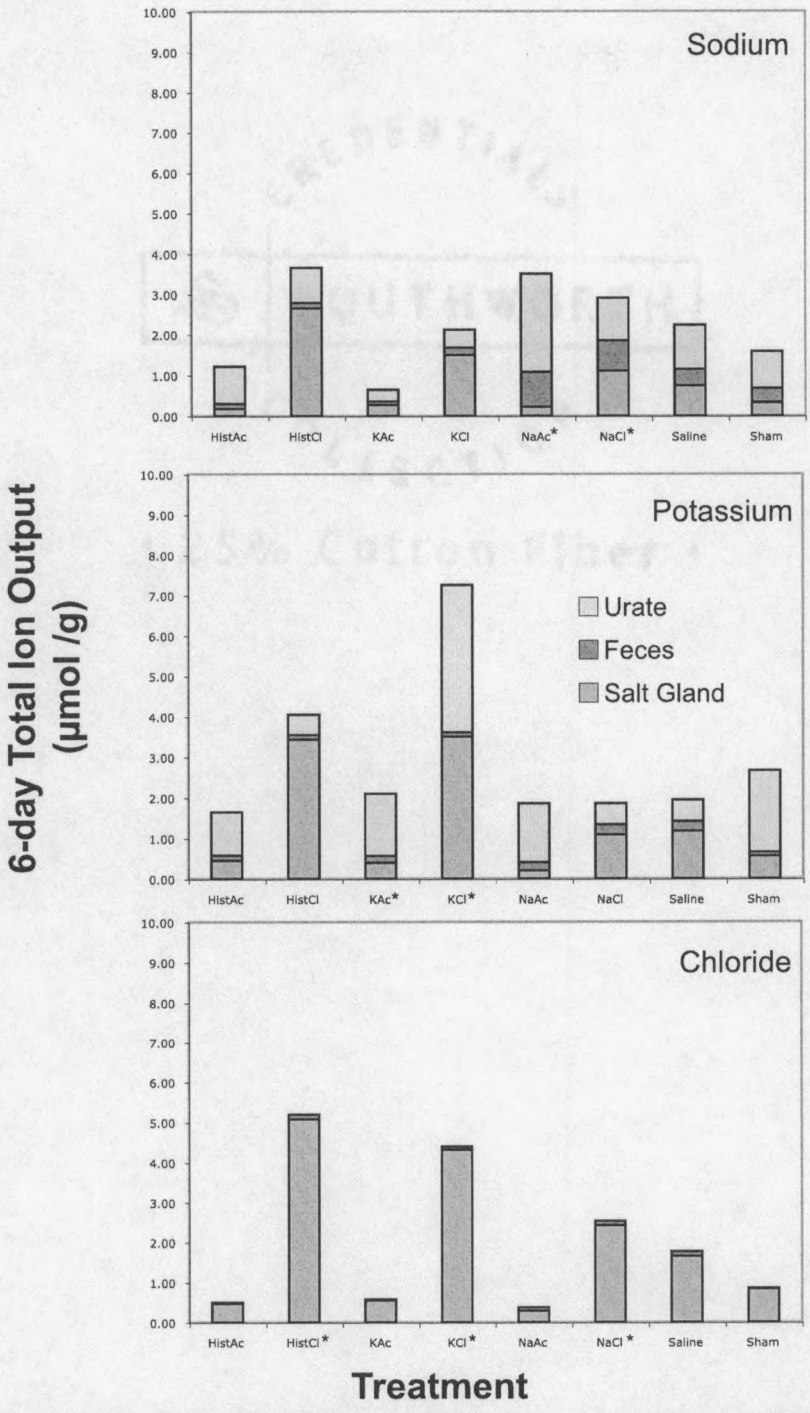


Figure 8: 6-day Total output of sodium, potassium, and chloride in µmol/gram Total sodium secretion as overall secretion increased, even when no sodium was administered. Large quantities of potassium can be excreted by the salt gland, but only when chloride is administered. Excess sodium and potassium are excreted via the cloaca as insoluble urate salts. Essentially all measured chloride was excreted via the salt gland among all treatments. * denotes treatment of that particular ion.

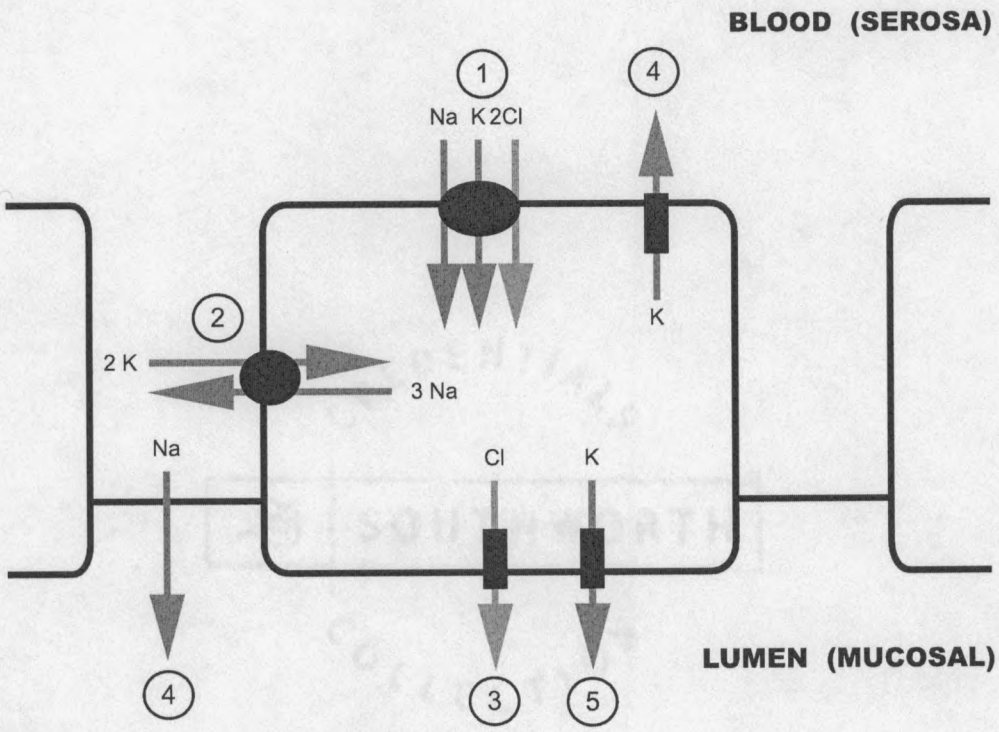


Figure 9: Proposed model for ion secretion by *N. schneideri* nasal salt gland Sodium, potassium, and chloride move from the extracellular blood plasma into the secretory cells via the Na-K-Cl cotransporter in the stoichiometry of 1:1:2. Sodium gets pumped into the intercellular space by the $(Na^+ + K^+)$-ATPase. Chloride exits the cells against an electrochemical gradient to the secretory lumen via apical chloride channels. Increased lumenal chloride (seen in chloride treatment loads) creates a larger negative gradient on the lumenal side. Sodium will follow paracellularly down an electrochemical gradient, but at a constant rate proportional to the ion load incrred (and to total ion secretion). Some potassium will reabsorbed through the apical potassium channel. Most potassium (during increased chloride loads) will exit the cell via basolateral potassium channels in response to the increased negative gradient on the lumenal side. The resulting secretion contains a mixture of chloride, potassium, and sodium.