J. exp. Biol. 144, 171–184 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

SALT GLAND FUNCTION IN THE GREEN SEA TURTLE *CHELONIA MYDAS*

BY SARAH W. NICOLSON AND PETER L. LUTZ

Division of Biology and Living Resources, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149, USA

Accepted 2 February 1989

Summary

When salt-gland-cannulated green sea turtles were stimulated by the intravenous injection of various salt loads the secretion osmolality rose quickly from a basal value of 300–400 mosmol kg^{-1} to a plateau of 1680–2000. The height of the plateau and the duration of the response were dependent on the amount of salt given. An increase in flow rate accompanied the initial rise in concentration, but the flow rate thereafter declined while the plateau concentration was held. The fluid was protein-free, and was mainly composed of Na^+ and Cl^-, in similar relative concentrations to those in sea water, but had substantial amounts of K^+, Mg^{2+} and HCO_3^- and negligible amounts of glucose. Urea concentrations did not change with osmolarity, and were similar to that of plasma, suggesting that the walls of the salt gland ducts are permeable to urea. The composition of the tears was uninfluenced by the concentration of the injected solutions, and its ionic content did not directly depend upon the identity of the added salts. The two eyes of individual turtles frequently did not function in synchrony. The average amount of the salt load remaining in a turtle at the end of salt gland activity, 4·5 mmol ion kg body $mass^{-1}$, was very similar to the median load required to stimulate the salt gland (4·0 mmol ion kg body $mass^{-1}$). This suggests that there is a threshold salt load level of around 4–5 mmol ion kg body $mass^{-1}$ that determines salt gland on/off activity.

The flow/concentration relationships of the salt gland fluid are consistent with water reabsorption from a primary isosmotic secretion being the major mechanism for concentrating the tears. A high maximal secretory rate is indicated for the primary fluid, 0·32 ml g salt $gland^{-1}$ min^{-1}, equivalent to that of the mammalian kidney. Calculations suggest that 83 % of the initial fluid is recovered, Na^+ is passively concentrated, Cl^- and Mg^{2+} concentrations are enhanced, and that the duct walls are either impermeable to glucose or that glucose is actively taken up.

Introduction

Marine vertebrates exist in a medium that is three times the concentration of their internal fluids, and so face the problems of water loss and ion gain. With the apparent exception of the marine mammals, the kidneys of these animals are

Key words: osmoregulation, salt excretion, water balance, ion regulation.

insufficient to handle the salt influx and, therefore, all marine vertebrates have extrarenal mechanisms for excreting salt. In elasmobranchs, birds and reptiles, discrete salt glands are present (Holmes & Phillips, 1985; Burger & Hess, 1960). In marine turtles, the salt glands have been shown to be the major route for sodium and potassium excretion (Holmes & McBean, 1964; Evans, 1973; Kooistra & Evans, 1976).

The organs used as salt glands in different organisms are nonhomologous and have apparently evolved independently (Peaker & Linzell, 1975). Ion secretion occurs in the rectal glands of sharks (Burger & Hess, 1960), the sublingual glands of snakes (Dunson *et al.* 1971), the nasal glands of birds and lizards, and the post-orbital glands of turtles (Peaker & Linzell, 1975). Despite their nonhomologous origins, the salt glands of these different organisms are all characterized microscopically by principal cells with large quantities of mitochondria and convoluted lateral, and sometimes basal, membranes (Ellis & Abel, 1964; Peaker & Linzell, 1975). The similarity in the structure of the salt glands of different species suggests that there may be similarities in salt gland operation as well. There is evidence of hypertrophy in response to long-term increases in salt loading in both birds and turtles (Peaker & Linzell, 1975; Cowan, 1969, 1971).

The diet of marine turtles consists of a variety of marine invertebrates or, in the case of the green turtle (*Chelonia mydas*), various marine grasses and algae. Since marine invertebrates and plants are generally similar in salt content, and contain three times as much salt kg body water^{-1} as sea turtles (Holmes & McBean, 1964), the salt burden for feeding turtles could be considerable. The importance of the gland for hatchling turtles was suggested by the work of Bennett *et al.* (1986), who found that hatchling loggerhead turtles (*Caretta caretta*) dehydrated rapidly in sea water unless allowed to drink. Since cloacal loss of ions was found to be negligible, the salt glands were assumed to be responsible for maintaining osmotic balance.

Salt glands appear to play an integral part in the adaptation of turtles to the marine environment, yet surprisingly little is known about the functioning of these organs. Primary data on lachrymal fluid composition, on the rate and pattern of flow, or on salt gland response to stimulation are either limited or non-existent, especially in comparison to birds or sharks. The purpose of this study was to describe the parameters of a typical salt gland response, to determine how a sea turtle controls salt gland output and to examine the importance of the glands in the salt economy of the sea turtle. The parameters examined included tear content and concentration, the duration of the salt gland response, the flow rate of fluid (tears) from the gland during the response and the percentage of a given salt load excreted.

Materials and methods

Eight juvenile green turtles ranging in mass from 6 to 19 kg were kept outdoors in large 1·2 m × 2 m fibreglass tanks with a flow-through, sand-filtered seawater system. Turtles were fed Purina Turtle Chow daily or every other day.

Animals were not fed for at least 24 h prior to an experiment, and were not used again for at least 14 days following experiments involving cannulation, or 7 days following experiments not involving cannulation. Animals were allowed to acclimate to the laboratory for at least 1 h before an experiment was begun.

Cannulation of the salt gland was accomplished using the technique of D. Hudson (personal communication). PE-90 tubing was positioned under the posterior corner of the turtle's eyelid and gently moved into the duct of the salt gland. The tubing was taped to the head of the turtle to prevent slippage. Throughout the secretory period, samples of salt gland fluid were collected into polyethylene microcentrifuge tubes attached to the cannula. When secretions were too viscous to move through the tube of their own accord, collection was aided by the application of suction up to 70 kPa using a peristaltic pump or a hand-held vacuum pump.

Because the cannulae were unable to collect all of the secretions, total salt gland output was measured by placing a funnel under the chin and capturing all the secreted fluid in graduated tubes positioned under the funnel. Flow was measured by recording how much fluid was collected during each 15 min sampling period. In some cases the sampling periods were 30 or 60 min if the flow was low.

The duration of the salt gland response was measured from the sampling period in which tear concentration was observed to begin rising until it fell to below 500 mosmol kg^{-1}. The sample-collecting containers attached to each cannula were replaced every 15 min if flow was sufficient or every 30 or 60 min. Samples were measured immediately for osmolality using a Wescor vapour pressure osmometer (model 5100B) and were sealed and stored frozen until other analyses could be performed. Only tears collected by the cannulae were used for analyses to avoid the possibility of contamination by other fluids.

Sodium and potassium were measured by flame photometry (Coleman model 21), chloride by an Aminco chloride titrator and for magnesium a Perkin-Elmer 403 atomic absorption spectrophotometer was used. Urea was measured using Sigma urea–nitrogen kit no. 66-UV and glucose by the use of Sigma glucose kit no. 115. Protein was measured using the Bradford (Biorad) and Lowry (Lowry *et al.* 1951) assay methods.

To measure bicarbonate and pH, a radiometer blood microsystem (BMS3 Mk 2) was used. Fresh, anaerobically collected tear samples were injected directly from the cannula into the sample chamber and pH and the partial pressure of carbon dioxide (P_{CO_2}) were measured with electrodes. Bicarbonate concentration was calculated using the Henderson–Hasselbalch equation: $pH = pK' + ([HCO_3^-]/\alpha P_{CO_2})$. The solubility coefficient of carbon dioxide was estimated by extrapolating the change in α with chlorinity at 20°C (values obtained from Riley & Skirrow, 1965) to the approximate concentration of the tears. Likewise, pK′, the first apparent dissociation constant of carbonic acid, was approximated by interpolating between known values for pK′ at chlorinities higher and lower than tears at 20°C (values obtained from Riley & Skirrow, 1965). Appropriate standards were run with all measurements.

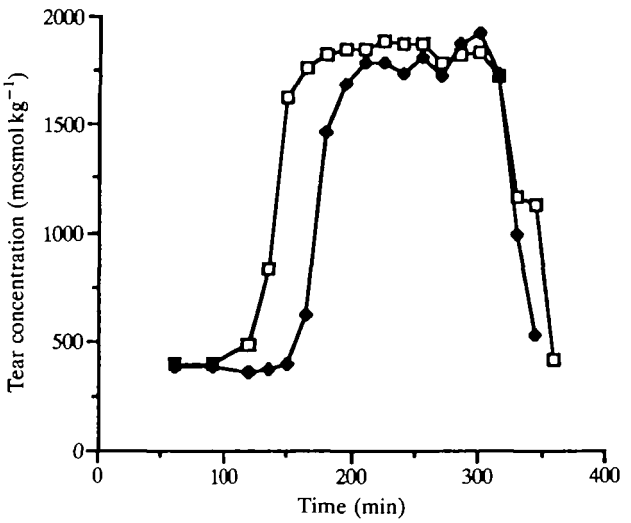

Fig. 1. The concentration of the tears from the left (□) and right (◆) eyes of a salt-gland-cannulated turtle during the salt gland response.

Injection solutions of 3 mol l^{-1}, 1·5 mol l^{-1} and 0·75 mol l^{-1} NaCl were made by dissolving the appropriate amount of NaCl in deionized, distilled water and then filtering this mix through a 0·45 μm filter. Next, the solution was autoclaved and finally transferred to sterile bottles in a clean room. The bottles were sealed with autoclaved rubber stoppers. Injectable seawater solutions were prepared by filtering sea water through a 0·45 μm filter and then autoclaving it in a Teflon container to prevent silica crystallization. The solution was transferred to bottles as described above. 'Isosmotic' sea water was prepared by filling a syringe with one part of sea water and two parts of sterile distilled deionized water.

The salines were injected by syringe directly into the venous blood system *via* the dorsal cervical sinus.

Since different amounts of salt in solutions, ranging from one-third sea water to 3 mol l^{-1} NaCl, were used in this work; loads are presented as mmol ion kg body mass^{-1}.

Student's *t*-test was used to determine the significance of the data where appropriate.

Results

Tear concentration and content

Concentration is the defining feature of a salt gland response. When the gland was not stimulated, the concentration of the tears was 300–400 mosmol kg^{-1} (i.e. isosmotic with plasma). When the gland was active, tear concentration rose rapidly to a plateau at 1680–2000 mosmol kg^{-1} (average 1900 mosmol kg^{-1}; Fig. 1). This plateau was maintained for the duration of the response period, afte

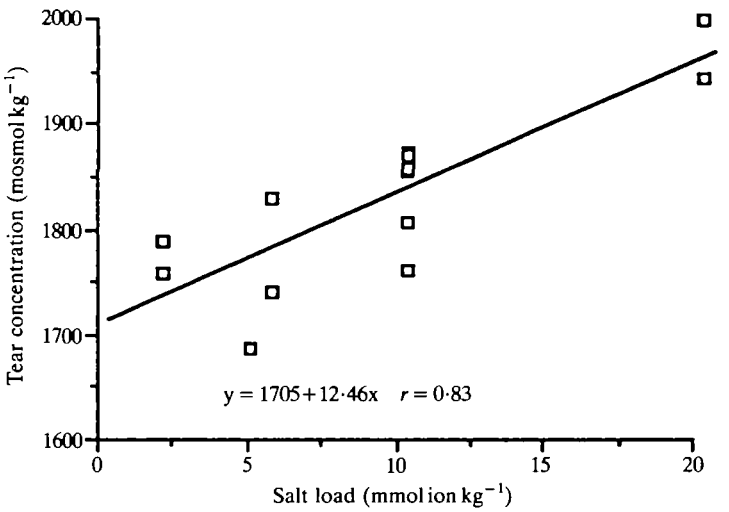

Fig. 2. The change in the average plateau tear concentration with different salt loads (mmol ion kg body mass^{-1}). Each point represents the average of 9–60 measurements from a single turtle.

which it rapidly declined. An exception was seen in only one of 13 experiments where the concentration failed to rise quickly to the plateau level.

The changes in tear concentrations from the two eyes were not synchronous and typically showed a pattern in which one salt gland remained active longer than

Table 1. *The plateau concentrations of ions and urea in the tears of individual turtles given different salt loads*

Salt load (mmol ion kg body mass^{-1})	Load composition	Cl^-	Na^+	Mg^{2+}	K^+	Urea	HCO_3^-
10	3 mmol l^{-1} NaCl	957	902	38·1		8·1	
10	3 mmol l^{-1} NaCl	958	932	30·4		5·6	
10	3 mmol l^{-1} NaCl	927	778	39·8	32·3	2·6	
10	3 mmol l^{-1} NaCl	998	821	44·4	28·6	9·3	
10	3 mmol l^{-1} NaCl						5·4
10	1·5 mmol l^{-1} NaCl	864	894		28·8		
4·75	1·5 mmol l^{-1} NaCl	812	941	26·4			
5·5	Sea water	912	814				
5·5	Sea water	894	794				
5·5	Sea water	870	902				
5·5	Sea water	951	893				
Mean	Sea water	914	867	35·8	29·9	8·9	5·4
±S.D.		±55	±59	±7·3	±2·1	±2·9	

Each value (mmol l^{-1}) is the average of 4–31 tear samples.

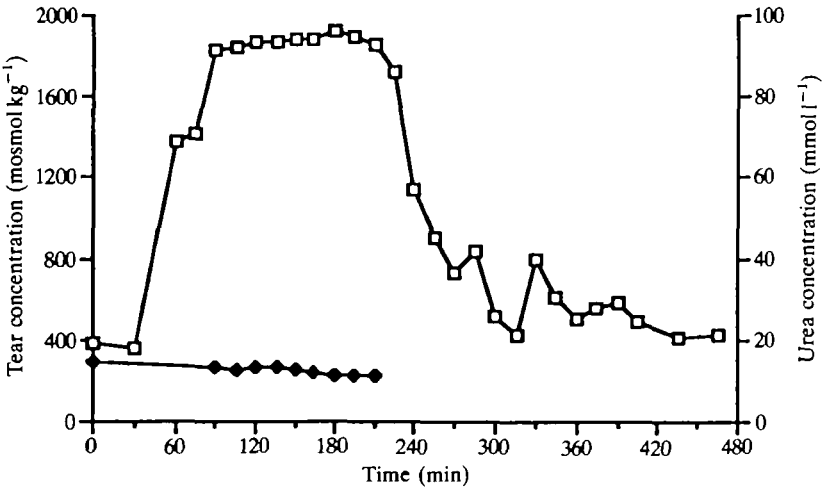

Fig. 3. The change in total concentration (□) and urea concentration (◆) during the salt gland response from the left eye of one turtle.

the other (Fig. 1). The eye exhibiting the subordinate response could either switch on at the same time as the eye with the dominant response, or be delayed by 15–30 min. One turtle did not initiate lachrymation from the second eye until almost 3 h after the first eye had switched on. Three turtles were observed to give a salt gland response in one eye only. No consistent pattern of dominance was seen and individual turtles appeared equally able to give a dominant response from either eye.

The exact level of the concentration plateau seemed to be a function of the amount of salt given to the animal (Fig. 2). The more salt a turtle was given, the higher its average plateau tear concentration. The increase in tear concentration was relatively minor, however, and probably did not have a large effect on salt output.

Chloride and sodium were the major constituents of green turtle tears, but there were also substantial amounts of potassium and magnesium ions and lesser quantities of urea and bicarbonate (Table 1). The composition of the salt gland fluid was fairly constant and the presence of significant amounts of potassium and magnesium in the tears of animals loaded with NaCl solution suggests that the tear ionic composition does not depend directly upon the identity of the added salts.

Interestingly, the urea content of the tears, unlike the ion content or the osmolality, did not vary during the salt gland response (Fig. 3) and averaged 8·9 mmol l^{-1} (Table 1). Tear urea was almost always slightly lower in concentration than initial plasma urea. Glucose was virtually absent in the tears (0·5 mmol l^{-1}) compared to its concentration in the plasma (8·0 mmol l^{-1}). The Bradford and Lowry *et al.* protein assays revealed less than 0·01 mg ml^{-1} of protein in the tears.

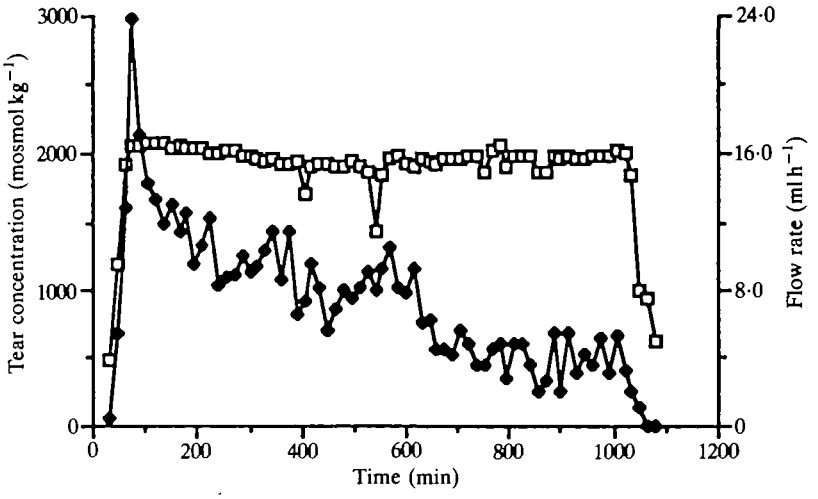

Fig. 4. Relationship between the changes in flow rate (◆) and concentration (□) of the salt gland fluid during a single secretory bout.

Tear flow

Unfortunately, owing to the intermittent viscosity of the salt gland fluid, flow could not be reliably measured using cannulae.

Measurement of total (or cumulative) flow rates using the collecting funnel appeared to be quite variable during the salt gland response period, although a pattern was discernible (Fig. 4). Characteristically, flow rose rapidly to an initial peak and then declined to a lower level where it fluctuated but gradually dropped to zero. The initial increase in flow coincided with the rise in tear concentration, and the sharp decline in concentration at the end of the secretory bout corresponded to the terminal fall in flow. However, once plateau levels had been reached, variations in flow appeared to have no effect on concentration (Fig. 4). It appeared that flow and concentration were interdependent during response initiation and termination, when concentrations were below the plateau levels, but were independent once the plateau concentration had been reached.

The flow rates appeared to be unaffected either by the amount of salt given to an animal or by the amount of water given with the salt load. The maximum recorded total flow rate was 27 ml h^{-1} for a 17 kg animal.

Response duration

No significant difference was found between the duration of the salt gland response in turtles receiving a fourfold difference in water load (1·7 and 6·7 ml H_2O kg body mass^{-1}, with the same salt load); therefore response duration was apparently not affected by water load.

In contrast, the duration of the response was affected by the salt load. Salt loads of 2·5–10 mmol ion kg body mass^{-1} resulted in response durations of 3–8 h. Salt loads of 20 mmol ion kg body mass^{-1} resulted in response durations of 15–18 h.

Table 2. *The amounts of salt and water excreted and salt remaining in individual turtles given different salt and water loads*

Salt load (mmol ion kg body mass^{-1})	Salt load concentration (mol l^{-1})	Salt excreted (%)	Water excreted (%)	Unexcreted salt (mmol ion kg body mass^{-1})
10	3·0 NaCl	72	227	2·8
10	3·0 NaCl	29	102	7·1
10	0·75 NaCl	45	39	5·5
10	0·75 NaCl	59	47	4·1
20	3·0 NaCl	84	257	3·2

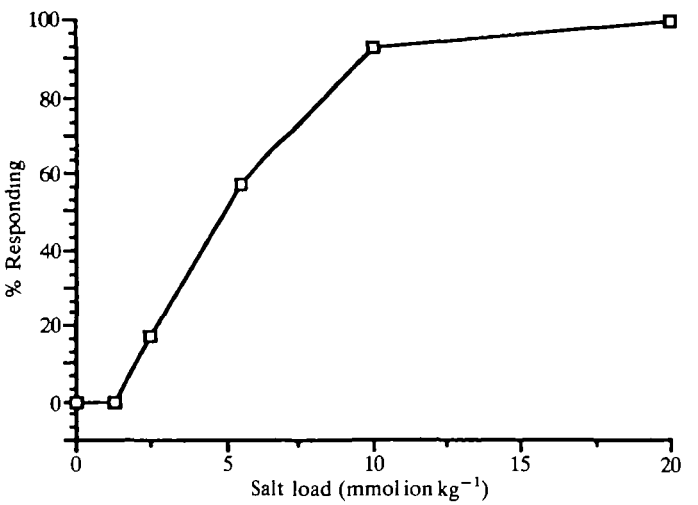

Fig. 5. The dose–response curve for salt-loaded green turtles. A response is defined as the production of tear fluid with a concentration greater than 800 mosmol kg^{-1} within 2 h of salt loading. $N = 2$ for the 20 mmol kg^{-1} dose, $N = 15$ for the 10 mmol kg^{-1} dose, $N = 7$ for the 5·5, 2·5 and 1·25 mmol kg^{-1} doses and $N = 4$ for the 0 mmol kg^{-1} dose.

Durations of less than 3 h were never observed, suggesting that there may be a minimum lachrymation period once the glands have been stimulated.

Salt gland activity and salt load

Table 2 shows the percentage of the given salt and water loads which were excreted by different turtles, and the amount of salt remaining in turtles after salt gland secretion ceased. The animals with higher salt load concentrations excreted more water than they received. None excreted their entire salt load. The average unexcreted load (initial salt load minus salt excreted during response) was 4·5 ± 1·8 mmol ion kg body mass^{-1}.

A dosage–response curve is shown in Fig. 5. Response is defined as the production within 2 h of salt loading of salt gland fluid with concentrations greater

than 800 mosmol kg^{-1}. The dosage–response curve is sigmoid, which suggests that an ED_{50} (the amount of salt that stimulates a salt gland response in 50% of the population) can be calculated. To estimate the median effective salt dose, the equations and tables in Weil (1952) were employed, resulting in an ED_{50} of 4·0 mmol ion kg^{-1} (95% confidence range: 2·6–6·1 mmol ion kg body $mass^{-1}$). So, one would expect half the turtles given 4 mmol ion kg body $mass^{-1}$ to exhibit a salt gland response.

Discussion

Tear content

The salt gland of the green sea turtle produces a protein-free fluid with approximately twice the concentration of sea water and with similar relative concentrations of the major ions Na^+ and Cl^- (Na^+/Cl^-, tears = 0·89, sea water = 0·86). This ratio is quite different from that of plasma ($Na^+/Cl^- = 1·30$), indicating that an adjustment of the ion ratios must occur during the process of tear formation by the salt gland. The relative constancy of the ionic composition of salt gland fluid, regardless of the salt load given (Table 1), suggests that once stimulated the salt gland produces a fluid of fixed composition. Some lizards, however, can vary the composition of their salt gland secretions, in particular the Na^+/K^+ ratios, according to the size and nature of the salt load (Shuttleworth *et al.* 1987). This inflexibility in the sea turtle salt gland composition may reflect a more uniform salt 'insult' for marine turtles compared to lizards. The similarity of the salt gland urea concentration to that of the plasma, and the observation that it does not change with tear osmotic pressure, suggests that this osmolyte is in passive equilibrium across the salt gland duct and is not being actively added to or removed from the salt gland fluid. Conversely, the negligible quantities of glucose in the tears indicate that it is either not entering the salt gland fluid or is actively removed from it.

Interestingly, the avian salt gland fluid differs from that of the turtle in that it is almost pure NaCl, containing less than 1 mmol l^{-1} magnesium and calcium combined (Schmidt-Nielsen, 1960), whereas the green turtle has magnesium concentrations of 35·8 mmol l^{-1} and calcium values of 15–25 mmol l^{-1} (Hudson & Lutz, 1985). This suggests that the salt gland has a much greater role in handling the divalent ions in the turtle than in the bird.

Tear flow pattern

The typical flow pattern following salt loading shown in Fig. 4 has also been reported in birds. McFarland (1964), working with sea gull species, observed that the salt gland secretion rate peaked rapidly following salt loading and then dropped to a much lower level where it levelled off and eventually ceased. In the turtle the response duration, which lasted 3–18 h, was dependent on the salt load. A similar dependency between salt load and duration of salt gland secretion has also been noted in the sea snake *Pelamis* (Dunson, 1968). In the hatchling green

turtle, however, Marshall & Cooper (1988) found that, once initiated, high secretion rates appeared to be maintained throughout the secretory period, although the duration was very much shorter (less than 46 min) and did not appear to be influenced by salt load.

The flow pattern exhibited by different organisms may relate to the salt loads they experience. The leatherback (*Dermochelys coriacea*) sea turtle, which is soft-skinned and feeds on jellyfish, appears to cry continuously (Hudson & Lutz, 1986), suggesting that it has a much higher salt influx than other turtle species.

Unilateral salt gland response

The difference in the salt gland response between the left and right eyes of individual turtles is an interesting feature that has been reported several times. Subadult green turtles (Prange & Greenwald, 1980), hatchling green turtles (Marshall & Cooper, 1988) and leatherback turtles (Hudson & Lutz, 1986) all exhibit left eye/right eye variations in tear flow and concentration. Unilateral differences in flow from the nasal salt glands have also been reported in the desert lizard *Uromastyx acanthinurus* (van Lennep & Komnick, 1970), so the phenomenon is not limited to chelonians. No comparisons of the salt gland secretions from the two eyes of any avian species have been reported, presumably because avian nasal glands empty into the nostrils where the tears mix across an orifice in the nasal septum (Peaker & Linzell, 1975).

Though the function of the unilateral variation in salt gland response is unclear, its occurrence, together with the variability of flow, indicates that estimations of total salt output based on measurements from one eye only, or on flow for a subperiod of the response, must be viewed with caution.

Salt excretion

The maximum rate of Na^+ loss found in this study (137·8 μmol $100\,g^{-1}\,h^{-1}$) is similar to average values for cephalic Na^+ loss in hatchling green turtles found by Holmes & McBean (1964) and Kooistra & Evans (1976) (119 and 118 μmol $Na^+\,100\,g^{-1}\,h^{-1}$, respectively), and is within the range for other reptiles with salt glands, 1–255 μmol $Na^+\,100\,g^{-1}\,h^{-1}$ (Dantzler & Holmes, 1974). It is, however, very much smaller than the maximal values obtained by Marshall & Cooper (1988) for hatchling green turtles (10 700 μmol $100\,g^{-1}\,h^{-1}$). The reasons for this 80-fold difference between hatchlings and juveniles are not clear. Hatchlings have proportionately larger salt glands than adults: hatchling 0·3 % body mass (Holmes & McBean, 1964), adults 0·05 % body mass (Dantzler & Holmes, 1974), and more active salt glands may be necessary since, to maintain body mass, hatchlings must drink sea water when they first enter the sea (Bennett *et al.* 1986). But it is also possible that the technique used by Marshall & Cooper (1988) is not fully reliable. The time to fill a 5 μl micropipette with tears would be strongly influenced by the presence and size of a tear reservoir, and by the horizontal angle of the collecting micropipette.

In the green turtle, salt gland fluid flow and concentration are positively related

at low concentrations but are independent at the plateau high concentrations. An increase in tear concentration with flow is also seen in the dehydrating green turtle (fig. 1, Prange & Greenwald, 1980). This relationship is strikingly different from that found for the goose, where the salt gland fluid flow is negatively correlated with fluid ion concentrations during most of the salt gland response (Hanwell *et al.* 1971). The difference suggests that the secretion mechanisms employed by the nonhomologous salt glands of these organisms may differ, or that increasing flow rates in the turtle salt gland do not outstrip the concentrating capacity of this organ.

In these experiments no turtle was observed to excrete its entire salt load, and the amount of salt excreted did not appear to be related to the salt load concentration (Table 2). It appeared that the salt gland continued to function until a minimum load was left in the body so that proportionately greater amounts of water were excreted at higher salt load concentrations, even to the extent of voiding more water than was given in the salt load (Table 2). It is unlikely that water conservation takes priority over salt excretion (Prange & Greenwald, 1980). In the wild, a turtle is unlikely to encounter any salt load that is more concentrated than its tears, so the need to utilize body water probably never occurs.

The good agreement between the estimated median stimulatory load (4·0 mmol ion kg body mass^{-1}) and the average load remaining in the body after response termination (4·5 mmol ion kg body mass^{-1}) suggests that the salt gland responds to a salt load of around 4–5 mmol ion kg body mass^{-1}. During salt loading, when this threshold is reached the salt gland is activated, and gland activity ceases when the salt content falls below this level. The kidneys probably handle the remaining salt load. The threshold triggering signal is unknown. The suggestion of Marshall & Cooper (1988) that the salt gland is stimulated by increased blood concentrations of Na^+ and K^+, but not of Cl^-, must be regarded as inconclusive since the lack of response to the injection of choline chloride may be more concerned with the lethal effect of this drug on the single hatchling tested.

Tear formation

The concentration mechanism employed by chelonian salt glands is unknown. In birds there is evidence of transepithelial Cl^- secretion (Ernst & van Rossum, 1982), although the initial fluid at the blind end of the salt gland tubules appears to be isotonic with blood (Marshall *et al.* 1985), and it is suggested that the fluid is concentrated, as it passes along the duct, by water absorption across the duct wall under the influence of a standing osmotic gradient, and that further ionic modifications in the fluid composition are brought about by ion pumps (Ellis *et al.* 1977; Marshall *et al.* 1985). Osmotic extraction of water across a standing gradient would explain the independence of concentration and flow at high concentrations seen in the turtle, since once the luminal fluid came into equilibrium with the standing gradient no further water would be taken up, and increasing the flow would merely displace the position along the duct where equilibrium is achieved.

If salt gland fluid concentration is mainly the result of an osmotic transfer of

Table 3. *Comparison between the actual concentrations of sea turtle tear constituents and those calculated for water absorption from a primary isosmotic fluid*

	Cl^-	Na^+	K^+	Mg^{2+}	Urea	Glucose
Actual	914	867	29·9	35·8	8·9	0·5
Calculated	634	857	22·6	12·4	38·9	47·3
Ratio	1·44	1·01	1·32	2·89	0·23	0·01

Concentrations are given in $mmol\,l^{-1}$.
Calculated data from loggerhead sea turtle values (Lutz & Dunbar-Cooper, 1987).

water across the duct wall, then the secretion rate of isosmotic fluid into the salt gland tubules can be calculated from the formula, [B]F1 = [SG]Fsg, where [B] is the osmotic concentration of the blood, [SG] is the osmotic concentration in the salt gland fluid, F1 is the flow of isotonic fluid into the salt gland tubules and Fsg is the flow of fluid out of the salt gland. Using average values for the osmotic concentrations and taking the maximum observed tear flow of $1{\cdot}59\,ml\,kg^{-1}\,h^{-1}$, we get a maximum secretory rate into the tubules of $9{\cdot}41\,ml\,kg^{-1}\,h^{-1}$, indicating that 83 % of the water from the initial isosmotic fluid has been reabsorbed. On a salt gland basis, the maximal initial secretory rate is $0{\cdot}32\,ml\,g\,salt\,gland^{-1}\,min^{-1}$, comparable to a human glomerular filtration rate of $0{\cdot}5\,ml\,g\,kidney^{-1}\,min^{-1}$ (Rose, 1977).

Comparing the actual concentrations of the salt gland constituents with what would result from an osmotic concentration alone (Table 3), we see that the Na^+ values are similar to what would be produced passively, K^+ is somewhat higher, Cl^- and Mg^{2+} concentrations are substantially enhanced, and urea and glucose are much lower. The composition of salt gland fluid is therefore modified as it moves down the duct, with Cl^- and Mg^{2+} concentrations probably being supplemented by active pumping, urea remaining in equilibrium with the blood and moving with the water, and the salt gland tubules must either be impermeable to glucose or they must actively reabsorb it from the initial fluid.

In summary, the salt gland of the sea turtle is stimulated by a salt load of 4–5 $mmol\,kg\,body\,mass^{-1}$. The duct secretion is twice as concentrated as sea water, and its composition (mainly Na^+ and Cl^- but also with substantial amounts of other ions) is uninfluenced by the salt make-up of the load. Increasing salt loads result in more highly concentrated secretions and, more importantly, longer secretion times. The flow/concentration relationship of the salt gland fluid is consistent with a mechanism of water reabsorption from a primary isosmotic secretion as it passes down the salt gland duct. Calculations suggest that the sea turtle salt gland has a high volume-handling capacity, equivalent to that of the mammalian kidney.

References

BENNETT, J. M., TAPLIN, L. E. & GRIGG, G. C. (1986). Sea water drinking as a homeostati

response to dehydration in hatchling loggerhead turtles *Caretta caretta*. *Comp. Biochem. Physiol.* **83**A, 507–513.

BURGER, J. W. & HESS, W. N. (1960). Function of the rectal gland in the spiny dogfish. *Science* **131**, 670–671.

COWAN, F. B. M. (1969). Gross and microscopic anatomy of the orbital glands of *Malaclemys* and other emydine turtles. *Can. J. Zool.* **47**, 723–729.

COWAN, F. B. M. (1971). The ultrastructure of the lachrymal 'salt' gland and the harderian gland in the euryhaline *Malaclemys* and some closely related stenohaline emydines. *Can. J. Zool.* **49**, 691–697.

DANTZLER, W. H. & HOLMES, W. N. (1974). Water and mineral metabolism in reptilia. In *Chemical Zoology*, vol. IX (ed. M. Florkin & B. T. Scheer), pp. 277–336. New York: Academic Press.

DUNSON, W. A. (1968). Salt gland secretion in the pelagic sea snake *Pelamis*. *Am. J. Physiol.* **215**, 1512–1515.

DUNSON, W. A., PACKER, R. K. & DUNSON, M. K. (1971). Sea snakes; an unusual gland under the tongue. *Science* **173**, 437–441.

ELLIS, R. A. & ABEL, J. H. (1964). Intercellular channels in the salt-secreting glands of marine turtles. *Science* **144**, 1340–1342.

ELLIS, R. A., GOERTEMILLER, C. C., JR & STETSON, D. L. (1977). Significance of 'leaky' cell junctions in avian salt gland. *Nature, Lond.* **268**, 555–556.

ERNST, S. A. & VAN ROSSUM, G. D. V. (1982). Ions and energy metabolism in duck salt-gland: possible role of furosemide-sensitive co-transport of sodium and chloride. *J. Physiol. Lond.* **325**, 333–352.

EVANS, D. H. (1973). The sodium balance of the euryhaline marine loggerhead turtle, *Caretta caretta*. *J. comp. Physiol.* **83**, 179–185.

HANWELL, A., LINZELL, J. L. & PEAKER, M. (1971). Salt gland secretion and blood flow in the goose. *J. Physiol., Lond.* **213**, 373–387.

HOLMES, W. N. & MCBEAN, R. L. (1964). Some aspects of electrolyte excretion in the green turtle, *Chelonia mydas mydas*. *J. exp. Biol.* **41**, 81–90.

HOLMES, W. N. & PHILLIPS, J. G. (1985). The avian salt gland. *Biol. Rev.* **60**, 213–256.

HUDSON, H. D. & LUTZ, P. L. (1985). Salt gland function in the Atlantic green turtle. *The Physiologist* **28**, 343.

HUDSON, H. D. & LUTZ, P. L. (1986). Salt gland function in the leatherback sea turtle *Dermochelys coriacea*. *Copeia* **1986**, 247–249.

KOOISTRA, T. A. & EVANS, D. H. (1976). Sodium balance in the green turtle, *Chelonia mydas*, in seawater and freshwater. *J. comp. Physiol.* **107**, 229–240.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

LUTZ, P. L. & DUNBAR-COOPER, A. (1987). Variations in the blood chemistry of the loggerhead sea turtle *Caretta caretta*. *Fish. Bull.* **85**, 37–43.

MCFARLAND, L. D. (1964). Minimal salt load required to induce secretion from the nasal glands of sea gulls. *Nature, Lond.* **204**, 1202–1203.

MARSHALL, A. T. & COOPER, P. D. (1988). Secretory capacity of the lachrymal salt gland of hatchling sea turtles, *Chelonia mydas*. *J. comp. Physiol.* B **157**, 821–827.

MARSHALL, A. T., HYATT, A. D., PHILLIPS, J. G. & CONDRON, R. G. (1985). Isosmotic secretion in the avian nasal salt gland: X-ray microanalysis of luminal and intracellular ion distributions. *J. comp. Physiol.* B **156**, 213–227.

PEAKER, M. & LINZELL, J. L. (1975). *Salt Glands in Birds and Reptiles*. New York: Cambridge University Press. 307pp.

PRANGE, H. D. & GREENWALD, L. (1980). Effects of dehydration on the urine concentration and salt gland secretion of the green sea turtle. *Comp. Biochem. Physiol.* **66**A, 133–136.

RILEY, J. P. & SKIRROW, G. (1965). *Chemical Oceanography*, vol. 1, pp. 650–651. New York: Academic Press.

ROSE, B. D. (1977). *Clinical Physiology of Acid–Base and Electrolyte Disorders*. New York: McGraw-Hill Book Company.

SCHMIDT-NIELSEN, K. (1960). The salt secreting gland of marine birds. *Circulation* **21**, 955–967.

SHUTTLEWORTH, T., THOMPSON, J. L. & DANTZLER, W. H. (1987). Potassium secretion by nasal salt glands of desert lizard *Sauromalus obesus*. *Am. J. Physiol.* **22**, R83–R90.

VAN LENNEP, E. W. & KOMNICK, H. (1970). Fine structure of the nasal salt gland in the desert lizard *Uromastyx acanthinurus*. *Cytobiologie* **2**, 47–67.

WEIL, C. S. (1952). Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions for their use. *Biometrics* **8**, 249–263.