Corrigendum

Reina, R. D., Jones, T. T. and Spotila, J. R. (2002). Salt and water regulation by the leatherback sea turtle *Dermochelys coriacea*. *J. Exp. Biol.* **205**, 1853-1860.

The final paragraph of the 'Collection of samples' section of the methods (p. 1854) incorrectly refers to dissection of salt glands from hatchling turtles that were killed by chilling and freezing. In fact, no hatchlings were killed, and salt glands were removed from naturally dead hatchlings collected over a period of weeks and kept frozen until dissection.

The results and conclusions are unchanged.

The authors apologise for any inconvenience to readers this may have caused.

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Salt and water regulation by the leatherback sea turtle *Dermochelys coriacea*

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Summary

We measured the salt and water balance of hatchling leatherback sea turtles, *Dermochelys coriacea*, during their first few days of life to investigate how they maintain homeostasis under the osmoregulatory challenge of a highly desiccating terrestrial environment and then a hyperosmotic marine environment. Hatchlings desiccated rapidly when denied access to sea water, with their hematocrit increasing significantly from 30.32±0.54 % to 38.51±1.35 % and plasma Na^+ concentration increasing significantly from 138.2±3.3 to 166.2±11.2 mmol l^{-1} in 12 h. When hatchlings were subsequently put into sea water, hematocrit decreased and plasma Na^+ concentration was unchanged but both were significantly elevated above pre-treatment values. In other hatchlings kept in sea water for 48 h, body mass and plasma Na^+ concentration increased significantly, but hematocrit did not increase. These data show that hatchlings were able to osmoregulate effectively and gain mass by drinking sea water. We stimulated hatchlings to secrete salt from the salt glands by injecting a salt load of 27 mmol kg^{-1}. The time taken for secretion to begin in newly hatched turtles was longer than that in 4-day-old hatchlings, but the secretory response was identical at 4.15±0.40 and 4.13±0.59 mmol Na^+ kg^{-1} h^{-1} respectively. Adrenaline and methacholine were both potent inhibitors of salt gland secretion in a dose-dependent manner, although methacholine administered simultaneously with a subthreshold salt load elicited a transient secretory response. The results showed that hatchling leatherbacks are able to tolerate significant changes in internal composition and efficiently use their salt glands to establish internal ionic and water balance when in sea water.

Key words: ion regulation, sodium, water balance, leatherback sea turtle, *Dermochelys coriacea*, osmoregulation, salt gland.

Introduction

Sea turtles and other marine reptiles live in an osmotically challenging environment where the concentration of salt in their surroundings is approximately three times greater than that of their internal fluids. Although sea turtles show some plasticity in their internal ionic composition (Bolten et al., 1992; Lutz and Dunbar-Cooper, 1987; Reina and Cooper, 2000), they maintain homeostasis in what must be considered a desiccating environment. Excess salt entering the body down the concentration gradient, ingested in the food or by drinking is largely excreted by paired salt glands because the reptilian kidney cannot produce a hypertonic urine. All marine turtles (Lutz, 1997), sea snakes (Dunson et al., 1971), the marine iguana (Dunson, 1969), estuarine crocodiles (Taplin and Grigg, 1981) and many marine birds (Schmidt-Nielsen, 1960) possess salivary, lingual, lachrymal or nasal salt glands that are a remarkable example of convergent evolution.

Salt glands are all composed of specialised, secretory cells that concentrate Na^+ and Cl^- from the blood to the lumen of secretory tubules through an energy-dependent process (Abel and Ellis, 1966; Gerstberger and Gray, 1993) and drain at the corner of the eye, through the nostrils or on the surface of the tongue. The biochemical processes through which ions are concentrated have been extensively studied in birds (e.g. Gerstberger and Gray, 1993; Shuttleworth and Hildebrandt, 1999), but our understanding of reptilian salt glands has not yet reached a similar level (Franklin et al., 1996; Reina and Cooper, 2000; Shuttleworth and Thompson, 1987). However, chelonian salt glands certainly possess the necessary cellular and vascular structures to support the requirements of extensive energy-dependent ion transport (Abel and Ellis, 1966; Ellis and Abel, 1964).

Sea turtle lachrymal salt glands secrete a solution composed almost entirely of sodium chloride at approximately 1500–1800 mosmol l^{-1} (Marshall and Cooper, 1988; Nicolson and Lutz, 1989; Reina and Cooper, 2000) in response to increasing plasma Na^+ concentration, and their activity is regulated by microcirculatory changes in or near the glands (Reina, 2000). Both adrenergic and cholinergic stimulation of the salt gland of the green turtle *Chelonia mydas* stop secretion within 2 min and inhibit its activity for a dose-dependent duration (Reina and Cooper, 2000). Chelonian salt glands are not activated by either adrenergic or cholinergic stimulation,

unlike the typical antagonistic actions of sympathetic and parasympathetic stimulation on avian salt glands (Fänge et al., 1963; Lowy et al., 1989). The reasons for this phenomenon are unclear and require further investigation.

The leatherback turtle *Dermochelys coriacea* diverged evolutionarily from other living sea turtles some 100 million years ago (Pritchard, 1997) and possesses many striking morphological and physiological differences such as growth rate (Zug and Parham, 1996), thermoregulation (Paladino et al., 1990) and type of diet (Bjorndal, 1997; Eisenberg and Frazier, 1983). Leatherbacks subsist on a diet of jellyfish and other gelatinous invertebrate prey that are approximately iso-osmotic with sea water and low in nutritional value (Lutcavage and Lutz, 1986). The large quantities of coelenterates required to support the growth rate must result in an enormous salt load. However, the response of the salt glands to a large salt load has never been measured in leatherbacks of any age.

To reach the sand surface after hatching from the egg, hatchling turtles must spend several days digging vertically through the sand and may lose over 10 % of their hatched body mass (Bennett et al., 1986). In addition to emerging from nests that are deeper than those of other species (Billes and Fretey, 2001), leatherback hatchlings on some beaches must crawl more than 100 m to reach the water. With deep nests, long crawls to the ocean and an invertebrate diet, neonate leatherbacks are probably more osmotically stressed than any other marine vertebrate. Their state of hydration and osmoregulatory effectiveness are probably vital factors influencing their ability to emerge from the nest, reach the ocean and survive. However, we know nothing of the salt and water balance of leatherback hatchlings during these important first few days of life. Previous studies of sea turtle salt gland physiology have shown that salt glands are an essential route of extra-renal salt secretion, but this study focuses on a quantification of water loss and gain by neonates under the specific conditions that they encounter following hatching from the egg. We hypothesised that neonate leatherbacks would experience significant changes in salt and water balance during exposure to sand and sea water and that they would osmoregulate effectively to reach homeostasis. To test this hypothesis, we measured changes in mass, water and Na^+ concentration over time, determined the threshold for activation of their salt glands, quantified the salt-secreting ability of their salt glands and tested adrenergic and cholinergic control of salt gland secretion.

Materials and methods

Animals

We collected hatchling leatherback turtles *Dermochelys coriacea* L. of approximately 35–40 g body mass as they emerged from nests in Parque Nacional Marino Las Baulas, Costa Rica. Hatchlings were transported to the laboratory and maintained at 27 °C either in sea water or on dry sand, depending on the experiment.

Collection of samples

We collected and measured tear samples as described previously (Marshall and Cooper, 1988; Reina, 2000; Reina and Cooper, 2000). Tear samples from the corner of the eye were drawn into 5 µl micropipettes, and we determined flow rate from the time taken to fill the tube. The contents of the micropipettes were absorbed directly onto filter paper discs for immediate measurement of total osmotic concentration (Wescor HR-33T dew-point microvoltmeter), and the discs were then sealed in Eppendorf tubes for subsequent analysis of sodium content by atomic absorption spectrophotometry. We determined the mass-specific rate of tear production ($ml\,kg^{-1}\,h^{-1}$) and tear Na^+ concentration ($mmol\,l^{-1}$) from the right salt gland to calculate the mass-specific rate of removal of Na^+ by the gland ($mmol\,Na^+\,kg^{-1}\,h^{-1}$). The technique does not easily permit simultaneous collection from both salt glands but allows an accurate quantification of the output from a single gland. Although the instantaneous rates differ between left and right glands (Nicolson and Lutz, 1989), we assumed that they balanced over the duration of the experiment (Reina and Cooper, 2000) so the rates of tear production and mass-specific Na^+ removal are presented for the whole animal with both glands operating rather than for the right gland alone.

We took blood samples in insulin syringes (Terumo, 27G×1/2) of approximately 100 µl from the cervical sinus of hatchlings using the method of Owens and Ruiz (1980). We immediately transferred the blood to hematocrit tubes for centrifugation and measurement of hematocrit. We then removed 5 µl of plasma and absorbed it onto a paper filter disc for immediate measurement of blood osmotic concentration as described above. The disc was then sealed for later analysis of sodium content as described for the treatment of tear samples above.

We dissected and weighed salt glands from hatchlings that had not been exposed to sea water (N=5 animals) and from those that had been in sea water for 24 h (N=5 animals). Animals were killed by chilling and freezing. We used the Mann–Whitney rank test to determine whether there was any difference in the mass of the salt glands before and after first exposure to sea water.

Experiments

We measured body mass, hematocrit and plasma Na^+ concentration of hatchlings over time immediately following their emergence from the nest. Hatchlings did not eat during this time because they subsisted on internalised yolk after hatching. We kept one group of animals (sibling turtles, N=10) in a darkened box on damp sand for 12 h at 27 °C, then put them into sea water at 27 °C for 12 h. We measured body mass and took blood samples for measurement of hematocrit and plasma Na^+ concentration at 0, 12 and 24 h. We maintained another group of animals (sibling turtles, N=10) in sea water for 48 h, measured mass and obtained blood samples at 0 and 48 h. We compared mass, hematocrit (arcsine-transformed) and plasma Na^+ concentration over time using a repeated-

measures analysis of variance (ANOVA) or non-parametric Mann–Whitney rank test as appropriate (Sokal and Rohlf, 1981).

We determined the secretory threshold in a dose/response manner by injecting hatchlings turtles that had been exposed to sea water for 12 h with salt loads of 0, 2, 4, 5 or 6 mmol NaCl kg^{-1} (25 ml kg^{-1}, N=6 each group) into the body cavity (Reina and Cooper, 2000). If visible salt gland secretion occurred within 40 min, we took a blood sample and measured plasma Na^+ concentration.

We quantified the secretory response of the salt gland by injecting animals (kept in sea water for 12 h) with a salt load of 27 mmol NaCl kg^{-1} (1.5 mol l^{-1} NaCl, 18 ml kg^{-1}) into the body cavity and measuring the time elapsed to commencement of secretion. Phosphate-buffered saline (20 mmol l^{-1} NaH_2PO_4, 154 mmol l^{-1} NaCl, pH 7.2) was the volumetric control (2.7 mmol NaCl kg^{-1}, N=6). We measured the flow rate, osmotic concentration and Na^+ concentration of tears every 10 min for 80 min for newly hatched (N=6) and 4-day-old turtles kept in sea water (N=6). We compared the secretory responses of the two groups using the Mann–Whitney rank test. We measured secretion of the salt gland from commencement of secretion until cessation following a salt load of 13.5 mmol kg^{-1} (N=6), collected secretions every 30 min and took a blood sample when secretion stopped. A salt load of 13.5 mmol kg^{-1} was used to reduce the duration of the experiment and the possibility of dehydration changing the secretory response of the animals.

We examined the ability of the cholinergic agonist methacholine (acetyl-β-methylcholine chloride, Sigma) and of adrenaline (adrenaline bitartrate, Sigma) to inhibit or stimulate secretion of the leatherback salt gland in a manner similar to that previously shown in the green turtle *Chelonia mydas* (Reina and Cooper, 2000). Methacholine was employed as an exogenous cholinergic agonist because it is more resistant than acetylcholine to degradation by cholinesterases (Cooper et al., 1991). We injected methacholine (10 ng kg^{-1}, 100 ng kg^{-1}, 1 μg kg^{-1}, 100 μg kg^{-1}, 1 mg kg^{-1} and 10 mg kg^{-1}, N=6 for all groups) or adrenaline (1 μg kg^{-1}, 10 μg kg^{-1} and 1 mg kg^{-1}, N=6 for all groups) into the body cavity of animals under three different experimental conditions. We examined the effects of methacholine and adrenaline on (i) non-secreting animals to determine whether the neurochemicals activated secretion, (ii) animals stimulated to secrete by injection of a salt load of 27 mmol NaCl kg^{-1} to determine whether they inhibited the secreting gland, and the effect of methacholine on non-secreting animals when a subthreshold salt load was administered simultaneously. We determined subthreshold salt load from the results of the dose/response experiment described above. All hatchlings were kept in sea water for 12 h before the experiments.

Statistical analyses

We used a Mann–Whitney rank test (Sokal and Rohlf, 1981) to determine significant differences in salt gland secretion rate among groups. The program Statview v5.01 (SAS Institute) was used to conduct all statistical tests. Significance was assumed at P<0.05, and all results are shown as the mean ±1 S.E.M.

Results

Water and Na^+ balance following hatching

Hematocrit and plasma Na^+ concentration increased significantly in hatchling turtles (N=10) kept on moist sand for 12 h after hatching (Table 1). When we subsequently placed these turtles into sea water for an additional 12 h, hematocrit decreased and plasma Na^+ concentration was unchanged, but both were significantly higher than pre-treatment values. Although hatchlings lost approximately 5 % of their body mass on sand, they regained it in 12 h in sea water. Body mass and plasma Na^+ concentration increased significantly in hatchlings kept only in sea water for 48 h (N=10), but hematocrit did not change (Table 2). Neither group of turtles ate during the study period.

Salt gland mass before and after exposure to sea water

There was no significant difference between absolute or relative salt gland mass of turtles exposed to sea water for 12 h (mass of both glands 109.0±5.3 mg, 0.33 % of body mass, N=5) or newly emerged hatchlings that had not been exposed to sea water (mass of both glands 120.6±10.4 mg, 0.35 % of body

Table 1. *Change in body mass, hematocrit and plasma [Na^+] of hatchlings of the leatherback turtle* Dermochelys coriacea *kept on moist sand (from 0 to 12 h) and then in sea water (from 12 to 24 h)*

	Time (h)		
	0	12	24
Body mass (g)	38.21±0.94	36.62±0.94	38.30±0.94
Hematocrit (%)	30.32±0.54[a]	38.51±1.35[b]	33.69±1.21[c]
Plasma [Na^+] (mmol l^{-1})	138.2±3.3[a]	166.2±11.2[b]	159.9±6.1[b]

Values are means ± S.E.M. (N=10).

Different superscript letters indicate significantly different values within a row.

Table 2. *Changes in body mass, hematocrit and plasma [Na^+] of hatchlings of the leatherback turtle* Dermochelys coriacea *kept for 48 h in sea water*

	Time (h)	
	0	48
Body mass (g)	38.11±0.71[a]	42.42±0.56[b]
Hematocrit (%)	30.29±1.16	29.35±1.10
Plasma [Na^+] (mmol l^{-1})	146.3±2.6[a]	165.3±3.6[b]

Values are means ± S.E.M. (N=10).

Different superscript letters indicate significantly different values within a row.

Table 3. *Rate of tear secretion, Na^+ concentration of the tears and total rate of Na^+ secretion by newly hatched and 4-day-old hatchlings of the leatherback sea turtle* Dermochelys coriacea *after injection of a salt load of 27 mmol NaCl kg^{-1}*

	Newly hatched	4-day-old
Rate of tear secretion (ml kg^{-1} h^{-1})	4.64±0.34	4.80±0.54
Tear [Na^+] (mmol Na^+ l^{-1})	887±29	843±41
Rate of Na^+ secretion (mmol Na^+ kg^{-1} h^{-1})	4.15±0.40	4.13±0.59

Values are means ± S.E.M.; *N*=6.

mass, *N*=5). There were no visible differences in salt gland color or gross appearance between the groups.

Secretory threshold

Salt glands of animals injected with 0, 2 and 4 mmol NaCl kg^{-1} did not secrete, four of six turtles secreted after a salt load of 5 mmol NaCl kg^{-1} and all turtles secreted after a salt load of 6 mmol NaCl kg^{-1} (*N*=6 all groups). At the time secretion commenced, plasma Na^+ concentration was 214±8 mmol l^{-1} (*N*=6), significantly higher than that of untreated animals (166.2±11.2 mmol l^{-1}, *N*=6, *P*<0.001).

Difference between day 0 and day 4 hatchlings in secretory ability

Newly emerged turtles began secreting 16.3±2.1 min after being salt-loaded with 27 mmol NaCl kg^{-1}, significantly later than 4-day-old hatchlings, which began secreting after 10.3±1.1 min (*P*<0.05). There were no significant differences in the rate of tear production, tear Na^+ concentration or total rate of Na^+ secretion by newly hatched and 4-day-old hatchlings (Table 3). None of the newly emerged (*N*=6) or 4-day-old hatchlings (*N*=6) secreted following injection of an equal volume of phosphate-buffered saline. Data from both age groups were pooled to determine the parameters of secretion because there were no significant differences between them. When secretion was initiated by salt loading, it reached 70 % of maximum rate in 2 min and peaked at 4.84±0.52 mmol Na^+ kg^{-1} h^{-1} approximately 20 min later (Fig. 1). At the termination of the experiment after 80 min of secretion, the rate had fallen to 70 % of maximum. Approximately 20 % of the injected salt load was removed in the first hour of secretion.

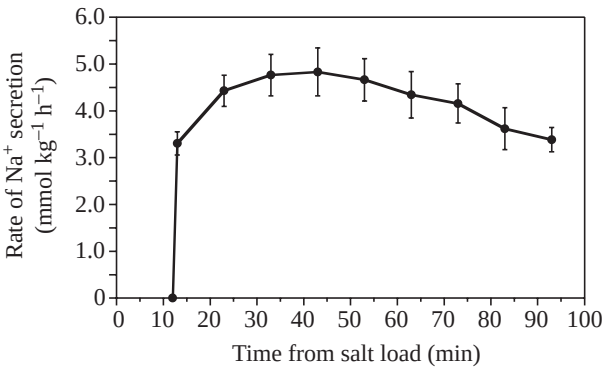

Fig. 1. The mass-specific rate of Na^+ secretion *via* the salt glands of hatchling leatherback turtles *Dermochelys coriacea* (*N*=12) following an injected salt load of 27 mmol kg^{-1}. Data points show mean secretion rate ±1 S.E.M.

Composition of secretions

The osmolarity of secreted tears of hatchlings was 1780±31 mosmol l^{-1} (*N*=120), with 48.6 % of the osmolarity composed of Na^+ (865±25 mmol l^{-1}).

Duration of the secretory response

Hatchlings that were injected with a salt load of 13.5 mmol kg^{-1} (*N*=5) secreted at a rate of 3.67± 0.27 mmol Na^+ kg^{-1} h^{-1} and stopped after 79.3±2.8 min, with 35.8±2.5 % of the injected salt load having been secreted. Plasma Na^+ concentration (190±16 mmol l^{-1}) was significantly higher than that in untreated animals (166.2±11.2 mmol l^{-1}, *N*=6, *P*<0.01) when secretion ceased.

Drug effects

Adrenaline did not stimulate the salt gland to secrete at any of the doses examined (*N*=6 for all groups). Adrenaline did not affect the active salt gland at a dose of 1 μg kg^{-1} but was a potent inhibitor of secretion when administered at a dose of 1 mg kg^{-1}, stopping secretion within 2 min (Fig. 2). There appeared to be a dose-dependent response, with 10 μg kg^{-1} adrenaline reducing but not abolishing secretion within 10 min of injection (Fig. 2).

Methacholine also inhibited secretion from the active salt gland in a dose-dependent manner, with a dose of 10 mg kg^{-1} (*N*=6) inhibiting secretion within 2 min of injection and the inhibition continuing for up to 110 min (Fig. 3). At a dose of 1 mg kg^{-1} (*N*=6), methacholine significantly reduced the secretory output of the active salt gland within 10 min of injection, with maximum inhibition reached 20 min after injection. Secretion returned to the pre-treatment rate 30 min later (Fig. 3). The doses examined below 1 mg kg^{-1} had no effect. Methacholine alone did not visibly affect the inactive salt gland at any of the doses tested (*N*=6 for all groups). However, when simultaneously injected with a subthreshold salt load of 2 mmol NaCl kg^{-1} (determined from the results of the secretory threshold experiment described above), 100 μg kg^{-1} methacholine caused a transient secretion from the salt glands in six of eight of hatchlings. Secretory rate could not be determined from three of the secreting animals because secretion stopped before the collection pipette was filled, but for the remaining three animals the mean secretory rate was 0.40±0.2 mmol Na^+ kg^{-1} h^{-1}, significantly lower than the typical secretion rate measured from salt-loaded animals. The low rate was due to a reduction both in tear flow rate and in tear concentration, and the maximum duration of secretion was

10 min. This transient secretion was not seen at any of the other doses tested with the subthreshold salt load (100 ng kg^{-1}, 1 µg kg^{-1}, 50 µg kg^{-1}, 1 mg kg^{-1} and 10 mg kg^{-1}, N=6 for all groups).

Discussion

Neonate leatherback turtles are subject to a great osmoregulatory challenge following hatching, and their salt and water balances change, but when they have access to sea water they osmoregulate quickly to maintain homeostasis through salt gland secretion. Thus, we accept the hypothesis that neonate leatherbacks are effective osmoregulators in response to desiccating environmental conditions. Other species of sea turtle hatch in similar or slightly less challenging conditions, and these hatchlings can be considered extremely good osmoregulators as a group.

Hatchlings without access to water lost approximately 5 % of their body mass in just 12 h, while they tolerated an increase in plasma Na^+ concentration of over 20 % and an increase in hematocrit of approximately 27 %. Although the mass change in 12 h was not significant, the changes in hematocrit and plasma Na^+ concentration suggest that water was lost, resulting in their blood becoming more concentrated. It seems likely that a significant mass loss would have occurred from dehydration if the experiment had continued without access to water. The total extracellular fluid volume (both plasma and interstitial) in a 38.2 g hatchling must have been approximately 6 ml if the loss of 1.6 g mass as water resulted in an increase in hematocrit of 27 %. The extracellular fluid (ECF) volume calculated from mass and hematocrit changes was therefore approximately 16 %, matching that determined empirically by Thorson (1968) in a number of other sea turtle species.

Hatchling turtles subsist on internalised yolk during the first few days of life, and metabolism of this yolk results in the liberation of 90 % of its mass as pre-formed and metabolic water (Schmidt-Nielsen, 1990). This liberated water helps to replenish some water lost through other routes, but the remaining 10 % of yolk mass is consumed as energy and results in a net loss of body mass in addition to that lost by dehydration. In terms of the response of neonate leatherbacks to the environmental challenge following hatching, the data show that, because neonates cannot drink in the desiccating environment of the nest, their internal salt and water balances change significantly. The hatchlings tolerate a loss of body water and increase in plasma Na^+ concentration rather than mobilising water stored in internalised yolk, thereby preserving that vital energy reserve for the energetic demands of digging, crawling and swimming.

Leatherback hatchlings lost body mass at a higher rate than loggerhead (*Caretta caretta*) hatchlings exposed to similar conditions (Bennett et al., 1986), but unlike the loggerheads did not continue to lose mass on exposure to sea water. They instead regained all lost body mass in 12 h through drinking, with approximately one-third (0.5 ml) of the consumed water entering the ECF, assuming the ECF volume of 16 % of body mass calculated above and no change in the number of red blood cells. The remaining water presumably remained in the gut. The salt glands must have been actively secreting the consumed salt load because there was no increase in plasma Na^+ concentration during the 12 h in sea water.

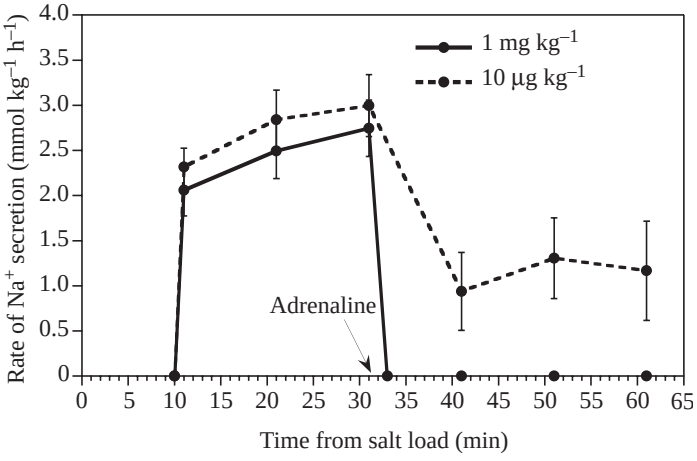

Fig. 2. The effect of adrenaline (10 µg kg^{-1} and 1 mg kg^{-1}) on Na^+ secretion rate by the salt glands of hatchling leatherback turtles *Dermochelys coriacea*. We stimulated hatchlings to secrete by injection of 27 mmol NaCl kg^{-1} and measured secretory rate before and after injection of adrenaline (N=6 for both groups). Data points show mean secretion rate ±1 S.E.M.

Drinking sea water is an extremely effective osmoregulatory strategy used by hatchling leatherbacks to offset the desiccation they experience during emergence from the nest and exposure to air while crawling to the sea. Not only can they regain lost body mass, but they are able to gain mass by continuing to drink water while actively swimming and consuming their yolk energy reserves. Hatchlings unrestrained

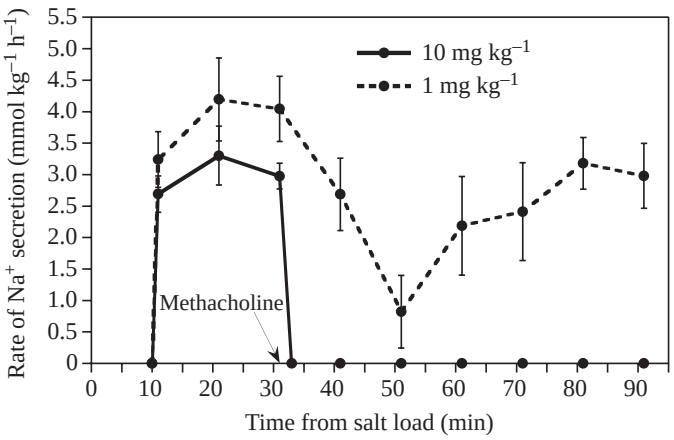

Fig. 3. The effect of methacholine (10 mg kg^{-1} and 1 mg kg^{-1}) on Na^+ secretion rate by the salt glands of hatchling leatherback turtles *Dermochelys coriacea*. We stimulated hatchlings to secrete by injection of 27 mmol NaCl kg^{-1} and measured secretory rate before and after injection of methacholine (N=6 for both groups). Data points show mean secretion rate ±1 S.E.M.

in sea water for 48 h increased in body mass by 12 %. Plasma Na^+ concentration was significantly higher in all turtles kept in sea water than in newly hatched turtles and did not return to the post-hatching concentration in the first 4 days. The data of Bennett et al. (1986) show that loggerhead hatchlings increased their plasma Na^+ concentration to 140 mmol l^{-1} almost immediately after entering sea water, compared with 120 mmol l^{-1} at emergence from the nest, and that the concentration did not fall in the following 2 weeks in sea water. A mechanism by which the effects of dehydration can be offset by sea turtles is to hatch from the egg with a dilute plasma and to reach a new steady-state plasma Na^+ concentration after their first exposure to sea water. Increased hydration at the time of emergence from the nest is correlated with an increased survival time and improved physiological performance in the snapping turtle *Chelydra serpentina* (Finkler, 1999). The data from our study and that of Bennett et al. (1986) are consistent with this strategy. On long-term exposure to fresh water, both hatchling green turtles (Holmes and McBean, 1964) and diamondback terrapins *Malaclemys terrapin* (Dunson, 1970) had steady-state plasma Na^+ concentrations significantly lower than their seawater counterparts, so turtles clearly have some plasticity in plasma ionic composition.

Free water obtained from metabolism of the internal yolk supply can be used to excrete Na^+ from the salt glands as plasma concentration increases with dehydration, but less energy would be required to retain this metabolic water within the body to delay the concentration of plasma over time when coupled with a lower initial plasma Na^+ concentration. The plasma Na^+ concentration of leatherback hatchlings in sea water was approximately 15–20 mmol l^{-1} higher than reported for other species (Bennett et al., 1986; Lutz, 1997; Reina and Cooper, 2000); this may be related to the enormous quantities of gelatinous prey that leatherbacks must eat to fuel their rapid growth rate. Neonates must have a massive dietary salt load because they need to eat approximately their own body mass in coelenterates each day (Lutcavage and Lutz, 1986) so, by maintaining a higher plasma concentration, they reduce the initial work required to maintain homeostasis. Previous studies had not shown that neonate turtles use such a suite of mechanisms to survive the challenges of hatching. By allowing their internal composition to change and then establishing a new homeostatic state when able to drink, neonates maximise the energetic resources available for escaping from the hatching beach.

Leatherback hatchlings emerged from the nest fully prepared to osmoregulate and showed no difference in salt gland mass or performance whether they had been previously exposed to sea water or not. Unexposed turtles took approximately 6 min longer to activate the gland but, once secretion began, they secreted the same amount of salt as previously exposed turtles (4.15±0.4 mmol Na^+ kg^{-1} h^{-1}), and both groups reached maximum secretory rate approximately 20 min after secretion commenced. We suggest that the 6 min difference between the two groups is due to a priming of the stimulus/secretion pathway for first use of the salt glands but that once it had occurred they were identical in all functional respects. The tear concentration of hatchlings after salt loading was approximately twice that of sea water and higher than previously reported for this species (Hudson and Lutz, 1986) but similar to that of other sea turtle species studied under similar conditions (Marshall and Cooper, 1988; Nicolson and Lutz, 1989; Reina and Cooper, 2000). The total mass-specific secretory rate was slightly lower than that reported for green turtle hatchlings of the same age (Reina and Cooper, 2000) because the mass-specific flow of tears was slower per unit time.

Hudson and Lutz (1986) measured spontaneous secretion in young juvenile unfed leatherbacks of approximately half the osmotic concentration reported here, while fed animals secreted at a concentration similar to that in the present study. The spontaneous secretions almost certainly did not represent the true osmoregulatory capacity of the turtles, but salt loading, either experimentally or through the diet, results in the salt glands secreting at maximum Na^+ concentration. We measured a low Na^+ concentration of 285±29 mmol l^{-1} in spontaneous secretions from nesting adult females (N=5, data not shown). Nesting adult green turtles also produce dilute salt gland secretions (P. Cooper, personal communication). These results and the presence of mucocytes lining the canals of sea turtle salt glands (Ellis and Abel, 1964) strongly suggest a dual role for salt glands not previously proposed. The tears of nesting adult females cannot serve an osmoregulatory function because their formation will result in a net water loss. We propose that the earliest hypothesis attributing a lubricative function to these tears was correct (Carr, 1952), although later rejected (Schmidt-Nielsen and Fänge, 1958). We also propose that the salt gland is capable of performing both a lubricative function with the mucocytes and an osmoregulatory function when the principal secretory cells become active, as determined by the homeostatic requirements of the animal at the time.

The threshold salt load required to initiate secretion was between 5 and 6 mmol NaCl kg^{-1}, the same as that reported for hatchling (Reina and Cooper, 2000) and juvenile (Nicolson and Lutz, 1989) green turtles. This amount of salt would be consumed in less than 500 µl of sea water and resulted in an increase in plasma Na^+ concentration of 50 mmol l^{-1} by the time secretion commenced. A greater salt load of 13.5 mmol NaCl kg^{-1} resulted in a sustained salt gland secretion that stopped after approximately 80 min when plasma Na^+ concentration had fallen to 190 mmol l^{-1} but was still significantly higher than in untreated animals. Animals injected with 13.5 mmol NaCl kg^{-1} secreted a lesser proportion of the salt load in the first hour than animals injected with 27 mmol NaCl kg^{-1} because of a lower mean secretory rate, but the Na^+ concentrations of the tears were not different. The lower mean secretory rate was a result of the tailing off of Na^+ secretion that occurs over time as salt is removed, but the two groups secreted an approximately equal proportion of the salt load over the first 30 min. Secretion stopped when less than half of the salt load had been secreted, but the remaining salt in the body could not be removed *via* the kidney without net

water loss because of the concentration of urine and its relative contribution to total Na^+ efflux (Kooistra and Evans, 1976). It seems likely that the salt glands recommenced secretion subsequently, but this was not observed because the animals had since been returned to the seawater holding tank. Measuring the secretion of animals over a longer period following salt loading is problematic because the results of this study show that hatchlings desiccate rapidly when not able to drink, and it will therefore be difficult to isolate the animal's response to the salt load from its response to desiccation.

Exogenous adrenaline and methacholine both had the same inhibitory action on the hatchling leatherback salt gland as on the hatchling green turtle salt gland (Reina and Cooper, 2000) and inhibited the active gland with a delay and for a duration dependent on dose. Systemic injection of methacholine stimulates the salt glands of the crocodiles *Crocodilus porosus* and *C. acutus* (Taplin et al., 1982), the diamondback terrapin *Malaclemys terrapin* (Dunson, 1970) and the herring gull *Larus argentatus* (Fänge et al., 1958) within the range of doses examined in the present study. It is perplexing why there is such a clear inhibitory action in the sea turtles so far examined when dose and route of administration are the same as in other reports. Neither adrenaline nor methacholine stimulated the salt glands to secrete when injected alone, but an intriguing possibility is suggested by the result that a low dose of methacholine ($100\,\mu g\,kg^{-1}$) initiated a transient secretion when injected with a subthreshold salt load of $2\,mmol\,NaCl\,kg^{-1}$ in six out of eight animals. Animals that were injected with the subthreshold salt load alone did not secrete.

We propose that the sea turtle salt gland is downregulated unless Na^+ concentration in the plasma or in some other compartment is elevated above a threshold that requires secretion to maintain homeostasis. If Na^+ concentration is below the threshold at which secretion is inhibited, exogenous stimulation will not result in secretion because the gland is immediately inhibited endogenously in the absence of a need to secrete. Exogenous cholinergic stimulation concurrent with an elevation of Na^+ concentration by a subthreshold salt load satisfies the two conditions required for secretion to begin but, because the amount of salt injected is small and hence Na^+ concentration is elevated only slightly, the secretion is brief. Downregulation of the chelonian salt gland seems to be a plausible control mechanism because the huge osmoregulatory challenge faced by sea turtles will require the salt gland to function for a large proportion of the time.

Applying the estimates of Lutcavage and Lutz (1986) of the mass of jellyfish required daily by leatherback hatchlings and the secretory rate measured in the present study, dietary salt consumption will exceed the capacity of the salt glands to remove it. However, leatherback turtles possess special structures in the esophagus and stomach to squeeze water from their food items and to expel it from the mouth and nostrils, thereby reducing their intake of salt and water from their prey. It is therefore difficult to estimate their total Na^+ intake, but it seems likely that it is of a magnitude that requires almost constant secretion by the salt glands to maintain internal ionic balance. Thus, a system of downregulation would be efficient because it would only be required to inhibit intermittently when salt secretion is not required; in contrast, a stimulatory system would need to stimulate gland activity during the much larger proportion of the time that the glands are active.

A similar dietary salt load is encountered by other species of marine turtles; marine birds eat prey of lower salt content and therefore require less salt gland activity to remove excess salt. This downregulatory system as a consequence of dietary salt load may explain the different responses to exogenous cholinergic stimulation in marine turtles and marine birds. However, we are unlikely to elucidate more fully the salt gland control mechanisms without *in vitro* techniques such as those used by Silva et al. (1987, 1990, 1993) to isolate secretory cells of the elasmobranch rectal salt gland from endogenous inhibition and stimulation. Using cell culture techniques, we may be able to demonstrate changes in ion-transport activity of chelonian salt gland cells in the presence of exogenous modifiers.

This study showed that neonate leatherbacks tolerate the desiccating conditions of the nest by shifting their internal salt and water balances and that they are capable of re-establishing homeostasis once they encounter sea water. They use highly efficient salt-secreting glands that quickly activate after relatively small salt loads and that function intermittently as required. The control mechanism appears to inhibit the activity of the gland when secretion is not required, but the precise role of cholinergic nerves in the glands remains somewhat unclear.

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