J Comp Physiol B (1988) 157:821–827

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology
© Springer-Verlag 1988

Secretory capacity of the lachrymal salt gland of hatchling sea turtles, *Chelonia mydas*

A.T. Marshall and P.D. Cooper*

Analytical Electron Microscopy Laboratory, Department of Zoology, La Trobe University, Bundoora (Melbourne), Victoria 3083, Australia

Accepted August 15, 1987

Summary. The lachrymal salt glands of *Chelonia mydas* were functional when hatchlings emerged from the nest. Osmotic concentrations up to 720 mosmol kg^{-1} were recorded in spontaneously produced tears (salt gland secretions). When injected with a Na^+ load (1500–2700 μmol $(100\ g)^{-1}$) newly emerged hatchlings produced tears ranging in osmotic concentration from 1000–1900 mosmol kg^{-1} with Na^+ secretion rates from single glands of 200–475 μmol $(100\ g \cdot h)^{-1}$. In these circumstances the rate of sodium excretion, via the salt glands, was equivalent to the sodium content of 0.2 to 0.5 ml of sea water per hour. Since the apparent drinking rate of hatchlings within the first two days of entering sea water was approximately 0.5 to 1.7 ml per day, the excretion of Na^+ imbibed by drinking is well within the secretory capacity of the lachrymal salt glands.

In feeding hatchlings extraordinarily high Na^+ secretion rates were induced by Na^+ loading. Hatchlings which were loaded with Na^+ by injection (1500–5400 μmol $(100\ g)^{-1}$) produced tears having osmotic concentrations between 1500 and > 2000 mosmol kg^{-1}. The Na^+ secretion rates from single glands were 750–4185 μmol $(100\ g \cdot h)^{-1}$ with extremely high short term rates of 10700 μmol $(100\ g \cdot h)^{-1}$ (50 μmol min^{-1} for 28 g hatchlings).

In terms of gland mass the highest long term secretion rate translates into 21 mmol of Na^+ per gram of salt gland per hour and is the highest secretion rate yet recorded for a reptilian salt gland. This rate is almost three times the highest rate recorded for sea snakes (8 mmol $g \cdot h^{-1}$) and is similar to rates commonly observed in avian salt glands (25 mmol $g \cdot h^{-1}$).

Secretion by the lachrymal salt glands was initiated by increased blood concentrations of Na^+ or K^+, K^+ being as effective as Na^+ but with the composition of the tears being virtually unchanged compared to tears from Na^+ stimulated hatchlings. Preliminary experiments indicated that secretion was not initiated by increased Cl^- concentration in the blood or by increased volume or osmotic concentration of the blood.

Introduction

It is generally presumed that the lachrymal salt gland is the principal route by which sodium balance is regulated in marine turtles, although there is little direct evidence from cannulation of the gland (Schmidt-Nielsen and Fange 1958; Dunson 1976; Minnich 1979). Fluid collected from the orbital region of the eyes or the head region, and presumed to be produced by the salt gland, has been shown to be hyperosmotic to blood in a number of species over a range of different physiological conditions (reviewed by Prange 1985).

Holmes et al. (1963) and Holmes and McBean (1964) have suggested that marine turtles may drink sea water in order to obtain osmotically free water. They were led to this suggestion by their observation that juvenile turtles (*Chelonia mydas*), which had been fed on shrimp, excreted more sodium than could be accounted for by their food intake. They reported that even unfed juvenile turtles ingested 1.33 ml of sea water per 100 g·d and in addition postulated that turtles imbibed 0.5 ml of sea water for every gram of food eaten. The notion that osmotically free water could be obtained by drinking, in conjunction with salt gland secretion, has also been alluded to by Prange (1985) who

Abbreviation: *O.P.* osmotic pressure

*Present address: Department of Zoology, Australian National University, Canberra, A.C.T., Australia

calculated that a 50 kg turtle could drink sea water at the rate of 1.48 ml $(100\ g \cdot d)^{-1}$. Dunson (1985) has introduced the concept of "incidental drinking" whereby sea water is taken in with food in significant quantities. Incidental drinking may be of prime importance once hatchlings start to feed. However, Bennett et al. (1986) have shown that hatchlings of *Caretta caretta* gain mass during the first two weeks after emergence as a result of drinking sea water at a rate close to 4 ml $(100\ g \cdot d)^{-1}$. In these animals at least part of the drinking must occur prior to commencement of feeding.

We have previously investigated mechanisms of salt secretion by avian salt glands by means of x-ray microanalysis and stereology (Marshall et al. 1985, 1987). As a prelude to similar investigations on turtle salt glands we have sought to address the question of how effective the turtle salt gland might be in relation to the drinking of sea water. The secretion rates observed by Holmes and McBean (1964) are quite low, approximately 100 μmol $(100\ g \cdot h)^{-1}$. Excretion at this rate, even if continued over 24 h, would be barely sufficient to eliminate all the Na^{+} ingested as sea water. We have therefore investigated the effect of salt loading on salt gland function in hatchlings of *C. mydas* and have shown that the hatchling salt gland has a remarkable capacity for salt excretion when adequately stimulated. Whereas it has been thought previously that reptilian salt glands in general have low secretion rates compared to avian salt glands (Dunson 1976), we show that the salt glands of hatchling *C. mydas* turtles will secrete at rates comparable to avian salt glands when they are appropriately stimulated.

Materials and methods

Animals. Hatchling green sea turtles (*Chelonia mydas*) were collected upon emergence from nests. Turtle collection was performed under permits No. IS 27, IS 46 and IS 63 from Queensland National Parks and Wildlife Service. Field work was undertaken at Heron Island, at the southern end of the Great Barrier Reef. Laboratory analyses were performed either at Heron Island Research Station, University of Queensland, or in the Department of Zoology, La Trobe University. Because of permit restrictions on the number of hatchlings which could be used and the limited time available each year, a number of different protocols were used over a number of years.

Turtles ranged in size from 18–35 g after emergence from the nests. After capture, turtles were returned to the research station laboratory and held in large aquaria supplied with running sea water for up to 7 weeks. During the first week, animals were not fed since they were still subsisting on the contents of the yolk sac, thereafter they were fed on fresh reef fish.

A record of changes in mass was kept over 82 h of hatchlings placed in sea water immediately after emergence and of hatchlings which were kept dry and in the dark for periods up to 48 h prior to being placed in sea water. During the period over which weighings were made the turtles were actively swimming when in water, and not feeding.

Salt gland secretion. The capacity and rate of salt gland secretion was studied following different protocols for salt loading: 1) stomach loading with salt solutions, 2) injection of salt load via the cervical sinus, and 3) intraperitoneal injection of the salt load at the junction of thorax and front right leg.

(1) Stomach loading was performed by introducing 1.5 mol l^{-1} NaCl (18 ml kg^{-1} wet weight) directly into the stomach using an oesophageal catheter connected to a 1 ml syringe. Animals were rinsed in distilled water, then placed in 500 ml beakers containing 50 ml distilled water. After three hours, animals were removed and samples of the bathing water were taken for the measurement of Na^{+} and K^{+} concentrations.

(2) Individual animals were removed from aquaria, weighed and placed in a beaker. A paper towel, saturated in sea water, was placed over the animals' plastron to minimize the effect of dehydration. The face and back of the neck were washed with distilled water and dried. Sodium chloride (1.5 mol l^{-1}, 10 ml kg^{-1}) or KCl (1.0 mol l^{-1}, 5–7 ml kg^{-1}) was injected by means of a Lo-Dose insulin syringe in equal parts into the cervical sinuses on either side of the cervical vertebrae. After 30 min, tear samples from both eyes were collected on small filter paper discs at various times for up to 3 h. Osmotic pressure was measured immediately and the wet disks were stored in plastic vials for later ion analysis. Two animals were examined for gland functioning immediately after emergence from the nest using this same protocol. Instead of holding the animals for 1–2 weeks following capture, hatchlings were injected immediately with 1.5 mol l^{-1} NaCl as previously and tear gland secretion was collected before the turtles experienced any exposure to sea water.

(3) Turtles were handled as in section (2) above, but NaCl (1.5 mol l^{-1}, 18 ml kg^{-1}) was injected intraperitoneally by means of a Lo-Dose insulin syringe into the body cavity near the junction of the thorax and right front leg. The position of the micro-fine needle was checked in dissected specimens and found to lie between the lung and thoracic wall. Copious tears were produced within 10 min and samples were collected every 10–15 min over 1 h. The rate of secretion was determined from the time in seconds for a 5 μl micropipette to fill from right eye secretions. These samples were transferred to filter paper discs for osmometry and the wet discs were then placed in marked vials for ion analyses at a later date. Samples of tears were also taken by partially filling 20 μl micropipettes, which were either sealed by flame or with Critiseal®, labelled and refrigerated until analysed for ion concentrations. The same procedure was carried out on two hatchlings immediately after emergence from the nest and before they experienced exposure to sea water.

Within 15 min of injection, blood samples were removed from the cervical sinus (approximately 0.1 ml) (Bennett 1986) using an insulin syringe (Lo-Dose), then centrifuged to separate plasma and blood cells. Plasma osmolarity was determined immediately, and the remaining plasma was sealed in hematocrit tubes with Critocaps® and refrigerated until analysed for ion concentrations. Another set of blood samples was taken 18 h later and treated in the same way. Essentially the same procedure as above was carried out on a further 5 hatchlings except that NaCl at 3.0 mol l^{-1} (18 ml kg^{-1}) was injected. Copious tears were produced in 5–10 min and samples were collected every 5 min.

A number of preliminary experiments were carried out in order to determine whether salt gland secretion was initiated by increased osmotic or ionic concentration of the blood. Permit limitations on the number of turtles which could be collected

Table 1. Osmotic pressures and ion concentrations of hatchling turtle tears under various treatments. Salt loads injected into cervical sinus

Treatment	Body mass (g)	Osmotic pressure (mosmol kg^{-1})	Na^+ (mmol l^{-1})	K^+ (mmol l^{-1})	Cl^- (mmol l^{-1})	Na/K
Fed	30.9±2 (5)	1379±98 (5)	602±63 (4)	21±3 (3)	740±22 (3)	29
Unfed	33.0±0.6 (3)	1470 (1)	455 (1)	19 (1)	647 (1)	24
NaCl loaded	25.4±0.8 (10)	[a]1570±42 (12)	743±66 (10)	23±3 (10)	844±47 (7)	32
KCl loaded	30.3±1.5 (5)	1512±44 (5)	635±50 (5)	27±2 (5)	754 (1)	24
P value		<0.05	NS	NS		

Mean±SE; n = number of animals sampled

[a] OP of NaCl loaded animals is significantly different from fed but not KCl loaded animals. OP of KCl loaded animals is not significantly different from fed animals. Unfed animals and Cl results were not included in the statistical analysis

restricted the use of replicates for these experiments. Hatchlings were observed for at least 2 h after injection and all treatments were well tolerated except for choline chloride injection. The hatchling injected with choline chloride died approximately 5 h after injection.

To check whether the injection of fluid alone stimulated tear flow, 3 turtles were injected with 0.15 mol l^{-1} NaCl. A further two groups of 2 turtles were injected variously with 3 mol l^{-1} sucrose and 1.5 mol l^{-1} sodium gluconate. One turtle was injected with 1.5 mol l^{-1} choline chloride. All treatments were made at a dose of 18 ml kg^{-1}. Animals were checked every 10 min in the same way as those given a high salt load.

Sample analyses. Osmotic pressure was measured by vapour pressure osmometry (Wescor 5100). Paper discs and fluid samples were returned to La Trobe University, where following appropriate dilution, sodium and potassium were measured using an atomic absorption spectrophotometer (Pye-Unicam SP90 or Varian Techtron) operated in the emission mode. The method of determining ion concentrations in fluid absorbed by the filter paper discs was that described by Hyatt and Marshall (1977). Chloride was measured by coulometric titration (Buchler). Sodium and potassium concentrations in the water bathing hatchling turtles were measured using flame photometry (EEL) at Heron Island. Statistics reported are mean±standard error, except where noted. Statistical analyses were carried out where appropriate using a oneway analysis of variance. Significant differences in the latter test were determined from Bonferroni confidence intervals.

Results

Newly emerged hatchlings

Two hatchlings (mass 22.7 and 24.5 g) which were examined immediately after emergence from the nest secreted tears spontaneously with osmotic concentrations of 721 and 522 mosmol kg^{-1}, respectively. After injection of 1.5 mol l^{-1} NaCl into the cervical sinus (1500 μmol $(100\ g)^{-1}$), these animals produced a copious secretion within 15–20 min, the osmotic concentration of which varied between 1080–1900 mosmol kg^{-1}. A further two hatchlings which were injected intraperitoneally with 1.5 mol l^{-1} NaCl (2700 μmol $(100\ g)^{-1}$) produced tears with osmotic concentrations within the same range and with maximum flow rates of 2.0 μl min^{-1} and 4.8 μl min^{-1} from a single gland. The secretion rates of Na^+ for a single gland were 200 μmol $(100\ g \cdot h)^{-1}$ over 1 h and 475 μmol $(100\ g \cdot h)^{-1}$ over 1.5 h, respectively. This rate of sodium secretion is equivalent to excreting the sodium content of approximately 0.2 to 0.5 ml of seawater per hour per turtle.

Stomach loading and excretion rates

After stomach loading, the rate of total body sodium loss over three hours was 592±78.3 μmol $(100\ g \cdot h)^{-1}$ ($n = 4$). This accounted for 66.6% (57.2–74.4%) of the introduced sodium load. During this period potassium loss was insignificant (0.02 μmol $(100\ g \cdot h)^{-1}$).

Osmotic concentration of tears following loading with NaCl or KCl via the cervical sinus

The results of these experiments are shown in Table 1. Total osmotic pressures of secreted tears did not change markedly with treatment. Two categories of control animals were used. These were fed and unfed hatchlings. Fed hatchlings all produced tears spontaneously. However, of three unfed hatchlings only one produced tears. Hatchlings loaded with NaCl produced tears at a slightly higher concentration than unloaded fed hatchlings. Loading with KCl also stimulated tear secretion and osmotic pressures of the tears were similar to those of other treatment groups. The decrease in the Na/K ratio following KCl treatment appears to be the result of a slight increase in K^+ concentration. Osmotic concentrations differed between the two eyes of individual animals, with differences up to 200 mosmol kg^{-1} being recorded. Occasionally only one eye at a time secreted during a period of secretion,

Table 2. Plasma and tear osmotic and ion concentrations of hatchling (28–33 g) turtles injected intraperitoneally with 1.5 mol l^{-1} NaCl at 18 ml kg^{-1}

	O.P. (mosmol kg^{-1})	Na^+ (mmol l^{-1})	K^+ (mol l^{-1})	Cl (mmol l^{-1})	Na/K
Plasma 1	387 ± 1 (5)	192 ± 6 (5)	5 ± 0.3 (5)	135 ± 11 (4)	43
Tears	1854 ± 39 (5)	827 ± 42 (5)	17 ± 1.0 (4)	983 ± 37 (5)	51
Plasma 2	339 ± 3 (6)	172 ± 2 (6)	5 ± 0.3 (6)	116 ± 5 (6)	38

Plasma 1 sampled at injection, Plasma 2 sampled 18 h after injection. Body mass of hatchlings 29.0 ± 1.1 g ($n = 5$). Means ± SE; n = number of animals

although secretion would frequently continue for 3 h. Injection of KCl had no apparent effect on the turtles other than to initiate tear secretion.

Effect of loading with 1.5 mol l^{-1} NaCl via intraperitoneal injection

Osmotic pressures and salt concentrations of the plasma and tears are shown in Table 2. Plasma osmotic concentration was 40 mosmol kg^{-1} higher following injection (10–15 min) than 18 h later, and Na and Cl plasma concentrations were 20 mmol l^{-1} higher than 18 h after injection. The osmotic pressure of tears was approximately 4–5 times higher than that of plasma, as were tear Na^+ and K^+ concentrations. Tear chloride concentrations, however, were approximately 8 times higher than plasma values.

Maximum osmotic concentration was reached between 10 and 20 min after injection. Tear secretion rates increased following injection but after the first 15 min were constant during the next 30 min, then began to decrease (Table 3). The rate of sodium excretion, assuming both salt glands functioned, in the first 45 min was 1500 μmol·$(100\ g \cdot h)^{-1}$ for the 810 μmol (2700 μmol·$(100\ g)^{-1}$) load. Approximately 57 percent of the load was excreted in the first hour of secretion.

Effect of loading with 3.0 mol l^{-1} NaCl via intraperitoneal injection

In hatchlings injected with 3.0 mol l^{-1} NaCl, secretion commenced approximately 5 min after injection. The time course of secretion was more varied than in the previous experiment (Table 4). The time course of secretion for a single turtle is given in Table 5 in which the absolute quantities of Na^+ excreted are calculated. Osmotic and ion concentrations, pooled over the first 25 min of secretion were: O.P. 1749 ± 45 (5), Na^+ 771 ± 22 (5), K^+ 26 ± 1.5 (5), and the Na/K ratio was 30 (n = number of animals).

Table 3. Tear secretion rates (right eye only) after injection with 1.5 mol l^{-1} NaCl at 18 ml kg^{-1}. Hatchling turtles of 28–33 g weight

Time period (min)	Secretion rate (μl min^{-1})	n
0	1.1	(1)
0–15	4.7 ± 2.8	(3)
16–30	4.5 ± 0.7	(3)
31–45	4.8 ± 1.3	(2)
46	1.6 ± 0.1	(4)

Mean ± SE; n = number of animals sampled

Table 4. Tear secretion rates (right eye only) after injection with 3.0 mol l^{-1} NaCl at 18 ml kg^{-1}. Hatchling turtles 22–28 g

Time period (min)	Secretion rate (μl min^{-1})	n
0– 4	0	(4)
5–10	30 ± 15	(4)
11–15	21 ± 3	(4)
16–20	36 ± 16	(4)
21–25	27 ± 17	(4)
26–30	54 ± 48	(3)
31–35	7 ± 2	(2)
36–40	1 ± 2	(2)

Mean ± SE; n = number of animals sampled

Table 5. Tear secretion rates (right eye only) after injection with 3.0 mol l^{-1} NaCl at 18 ml kg^{-1}. Recorded from one individual of 28 g

Time period	Secretion rate (μl min^{-1})	Volume (μl)	Na^+ excreted (μmol)
0– 4	0	0	0
5–10	11	44	40
11–15	26	104	102
16–20	50	200	200
21–25	15	60	60
26–30	9	36	35
31–35	5	20	15
36–40	2	8	4

The mean value of Na^+ excreted by 4 hatchlings 25 min after injection was 436 ± 37 μmol. Over the first 25 min following injection the mean Na^+ excretion rate, from both glands, is calculated to be 8370 μmol $(100 g \cdot h)^{-1}$ for the approximately 1500 μmol load (5400 μmol $(100 g)^{-1}$). Approximately 65 percent of the load was secreted in the first 25 min after being administered.

The maximum secretion rate of Na^+ for a single gland was 50 μmol min^{-1} (10700 μmol $\cdot (100 g \cdot h)^{-1}$) and the maximum flow rate for a single gland was 75 μl min^{-1}. The osmotic concentration reached a maximum for more than 2000 mosmol kg^{-1}, beyond the measuring range of the osmometer. The mean volume of fluid secreted per gland in the first 25 min was 513 ± 35 μl.

Initiation of secretion

Tear secretion was not initiated by injection of a volume of isosmotic NaCl (0.15 mol l^{-1}) equivalent to that used for loading with 1.5 mol l^{-1} NaCl. Injection of an equivalent volume of 3.0 mol l^{-1} of sucrose also did not result in tear secretion. The osmotic concentration of the blood was 451 mosmol kg^{-1} 20 min after injection with sucrose compared with the pre injection value of 380 mosmol kg^{-1}. Similarly, injection of an equivalent volume of 1.5 mol l^{-1} of choline chloride did not initiate tear secretion. However, sodium gluconate at 1.5 mol l^{-1} resulted in tear secretion within 5 min of injection and continued at a mean rate of 4.1 μl min^{-1} over a period of 1 h. The maximum rate was 8.6 μl min^{-1} (1905 μl $(100 g \cdot h)^{-1}$) and the maximum osmotic concentration was 1493 mosmol kg^{-1}.

Changes in mass of hatchlings – apparent drinking rates

Hatchlings placed in seawater immediately after emergence from the nest showed variable increases in mass over an 82-hour period. The mean increase ($n = 10$) was 0.46 $g \cdot d^{-1}$ over the 82-hour period. Hatchlings which were kept dry and inactive after emergence from the nest showed a variable reduction in mass over a 48-hour period. The maximum reduction was 0.9 $g \cdot d^{-1}$. After immersion in seawater the increase in mass was again variable with the maximum increase being 1.7 $g \cdot d^{-1}$ over a 44-hour period. This presented an 18 percent increase in body mass. The increase in mass seen in these two experiments is presumed to be due to drinking since they had not yet started to feed. Thus apparent drinking rate was approximately 0.5 to 1.7 ml d^{-1}.

Discussion

The lachrymal salt gland of newly emerged hatchlings of *Chelonia mydas* appears to be fully functional on emergence from the nest, as suggested by Kooistra and Evans (1976). Some newly emerged hatchlings secreted spontaneously, perhaps in response to dehydration which they may suffer prior to emergence as has been suggested for loggerhead turtles (Bennett et al. 1986). They were also capable of producing copious and highly concentrated tears (1000–1900 mosmol kg^{-1}) at a Na^+ excretion rate of 400–950 μmol $(100 g \cdot h)^{-1}$ in response to an injected salt load. These hatchlings were capable of easily excreting the Na^+ content of approximately 0.2 to 0.5 ml of sea water per hour. They would seem, therefore, to be potentially able to obtain osmotically free water immediately on entering the sea by a combination of seawater drinking and salt excretion via the salt gland.

Hatchlings increased in mass prior to starting to feed and inspite of swimming continuously for some 36 h. It is assumed that this increase was due to the imbibition of sea water. The rate of increase appeared to be related to the degree of dehydration before entering sea water. The apparent drinking rate, calculated from the increase in mass, varied from 2.3 ml $(100 g \cdot d)^{-1}$ to 8.5 ml $(100 g \cdot d)^{-1}$

It seems probable that 'incidental drinking' (Dunson 1985) occurs in feeding hatchlings. This is indicated by the finding that unfed hatchlings tended not to produce tears spontaneously whereas all recently fed hatchlings produced tears. This observation is consistent with the findings of Holmes et al. (1963) and Holmes and McBean (1964). These authors accounted for the excess Na^+ excretion, compared with Na^+ intake in shrimp-fed 70 g turtles, by postulating an intake of 0.5 ml of sea water per gram of food eaten. They also observed that unfed turtles (90 g) excreted much less sodium than fed turtles over the experimental period but also noted in addition that even unfed turtles drank 1.33 ml $(100 g \cdot d)^{-1}$ of sea water. The experiments described here on hatchlings which had been stomach-loaded with Na^+, suggested that the absorption of Na^+ from the alimentary tract and the subsequent excretion of Na^+, presumably via the salt gland, can occur without any apparent difficulty.

In feeding hatchlings the total secretion rate, assuming both glands secrete at the same rate, obtained for an injected 810 μmol load of Na^+ was 460 μmol h^{-1} measured over a 45-min period. This corresponds to the Na content of approximately 1.0 ml of sea water. Clearly then the salt glands of

Table 6. Comparison of Na^+ secretion rates and Na/K ratios as a function of Na^+ load in young turtles

Load (μmol 100 g^{-1})	Secretion rate (μmol (100 g·h)$^{-1}$)	Na/K	Mass (g)	Duration of measurement (min)	Method of loading
[a] 500	104–114	23	103–114	60	Intramuscular
1500	–	32	25	180	Cervical sinus
2700	592	–	33	180	Stomach
2700	1530	51	25	45	Intraperitoneal
5400	8370	30	30	25	Intraperitoneal

[a] Holmes and McBean (1964)

30-g hatchlings are capable of dealing with any salt load which might be imposed on the hatchling by drinking sea water in significant quantities. If the salt glands were active continuously at this rate a hatchling could almost drink its own mass in sea water over a 24-hour period and excrete the salt load! This is, of course, an unlikely circumstance. The maximum total secretion rate observed was with a 1500 μmol load of Na^+ in 25-g turtles. This was 2090 μmol h^{-1}, measured over a 25-min period, which corresponds to the Na^+ content of approximately 4.5 ml of sea water. Stomach-loaded hatchlings also eliminated most of the NaCl load within three hours, presumably via the salt gland.

The secretion rates for Na^+ found here are much higher than those obtained by Holmes and McBean (1964) but it should be noted that the Na^+ loads for the former turtles were much greater than the latter. These data are summarised in Table 6 and suggest that as the load increases so does the secretion rate. The rates obtained over very short periods were extremely high, e.g. up to 3000 μmol h^{-1} (10700 μmol·(100 g·h)$^{-1}$). These remarkably high rates may be overestimates due to the pooling of tears in the eye. However, this would probably lead to overestimations by not more than a factor of two or three times.

Although reptilian salt glands are generally considered to secrete Na^+ at a much lower rate than avian salt glands (Peaker and Linzell 1975; Dunson 1976) hatchling turtle lachrymal glands, when maximally stimulated in the experiments reported here, secreted, in terms of gland mass, a rate (21 mmol g·h^{-1}) closely approaching that of stimulated duck nasal glands (25 mmol g·h^{-1}, Holmes and Phillips 1985). This was calculated using a value for the lachrymal gland mass of 300 mg (100 g)$^{-1}$ (Holmes et al. 1963; Marshall et al., unpublished observations). This Na^+ secretion rate is the highest yet recorded for a reptilian salt gland being almost three times the highest rate recorded for sea snakes (8 mmol g·h^{-1} of Cl^- with Na^+ assumed to be similar) (Dunson and Dunson 1974). Unlike the left and right nasal glands of birds, secretion from lachrymal glands of left and right eyes appears to be independent. In loaded hatchlings one side sometimes secreted whilst the other did not and the osmotic concentrations of tears from the two eyes when both glands were secreting differed considerably on occasions. Similar observations on differences between right and left glands have been made on dehydrated sub-adult green turtles (Prange and Greenwald 1980).

The secretory capacity of the hatchling salt gland is clearly adequate to deal with a substantial Na^+ load incurred through drinking sea water. The sea water drinking rates reported in the literature and here would present no difficulties for salt secretion via the salt gland. Since the osmotic concentration of the tears is 1.5 to 2.0 times that of sea water there is a substantial gain in osmotically free water.

It is noteworthy that KCl stimulated secretion. Although the gland is stimulated to secrete by an injected K^+ load the secreted fluid is similar in composition to that from Na^+ stimulated glands. The concentration of K^+ in the secreted tears is only very slightly elevated compared with tears from glands of Na^+ loaded turtles. The injection of KCl had no other observable effect on the turtles. Secretion was stimulated by NaCl and sodium gluconate but choline chloride and an equivalent osmotic load of sucrose did not stimulate secretion. Neither did an equivalent increase in blood volume with isosmotic saline. It would appear that secretion occurs in response to an increase in blood Na^+ or K^+ concentration but not Cl^- although it must be noted that choline chloride had a pharmacological effect. Secretion does not appear to be a response to an increase in blood volume or blood osmotic pressure as it is thought to be in birds (see Holmes and Phillips 1985).

It is interesting to compare the secretory performance of the salt gland of *Chelonia mydas*, a marine turtle, with that of a brackish water terrapin *Malaclemys terrapin*. Lachrymal salt secretions follow-

ing salt loading in 200–300 g *Malaclemys terrapin* (Cowan 1981) have several parameters similar to our observations for hatchling green sea turtles. Following a 2 mmol·$(100\ g)^{-1}$ salt load, plasma osmotic pressure and sodium concentration increased by 60 mOsm·kg^{-1} and 30–40 mmol·l^{-1}, respectively, in *M. terrapin*, approximately the same increase observed in hatchling green sea turtles. Salt loading with sodium chloride resulted in a rapid increase in sodium concentration in tears (from less than 100 up to 600 mmol·l^{-1}) and in rate of fluid secretion. Loading with a 2:1 mixture of sodium chloride-potassium chloride increased sodium but not potassium secretion. The lachrymal gland of *M. terrapin* thus appears to respond to salt loads by increasing sodium secretion in much the same manner as the gland of hatchling green turtles, even to the apparent inability to secrete a high concentration of potassium. One difference between these species, however, is that the levels of secretion in *M. terrapin* declined well before the load was secreted (Cowan 1981), while *C. mydas* hatchlings were able to remove the salt load via lachrymal gland secretion. Much of this observed difference results from the higher rate of secretion in *C. mydas* compared to *M. terrapin* (1000–5600 vs 50 μl $(100\ g \cdot h)^{-1}$, respectively), which is associated with the relatively larger gland in the former turtle (300 mg 100 g^{-1}) (Holmes and McBean 1964) vs 28 mg 100 g^{-1} (Cowan 1974). Hatchling green sea turtles are able to secrete sodium at a considerably greater rate than that calculated for *M. terrapin* (21 and 1.1 μmol/mg gland·h, respectively).

Acknowledgements. We should like to thank C. Limpus and E. Gyuris for assistance in turtle collection and handling and I. Lawn for use of facilities at Heron Island Research Station. We also appreciate the comments of A. Wright, G. Grigg and C. Limpus on earlier drafts of the paper and we are indebted to W.A. Dunson for critically reviewing the manuscript. This work was supported by a grant from the Australian Research Grants Committee to A.T.M.

References

Bennett JM (1986) A method for sampling blood from hatchling loggerhead turtles. Herp Rev 17:43

Bennett JM, Taplin LE, Grigg GC (1986) Sea water drinking as a homeostatic response to dehydration in hatchling loggerhead turtles *Caretta caretta*. Comp Biochem Physiol 83A:507–513

Cowan FBM (1974) Observations on extrarenal excretion by orbital glands and osmoregulation in *Malaclemys terrapin*. Comp Biochem Physiol 48A:489–500

Cowan FBM (1981) Effects of salt loading on salt gland function in the euryhaline turtle, *Malaclemys terrapin*. J Comp Physiol 145:101–198

Dunson WA (1976) Salt glands in reptiles. In: Gans C, Dawson WR (eds) Biology of the Reptilia, vol 5, Physiology A.C. Academic Press, New York London, pp 413–445

Dunson WA (1985) Effect of water salinity and food salt content on growth and sodium efflux of hatchling diamondback terrapins (*Malaclemys*). Phys Zool 58:736–747

Dunson WA, Dunson MK (1974) Interspecific differences in fluid concentration and secretion rate of sea snake salt glands. Am J Physiol 227:430–438

Holmes WN, McBean RL (1964) Some aspects of electrolyte excretion in the green turtle, *Chelonia mydas mydas*. J Exp Biol 41:81–90

Holmes WN, Phillips JG (1985) The avian salt gland. Biol Rev 60:213–256

Holmes WN, Phillips JG, Chester Jones I (1963) Adrenocortical factors associated with adaptation of vertebrates to marine environments. Rec Prog Horm Res 19:619–672

Hyatt AD, Marshall AT (1977) Sequestration of haemolymph sodium and potassium by fat body in the water-stressed cockroach *Periplantea americana*. J Insect Physiol 23:1437–1441

Kooistra TA, Evans DH (1976) Sodium balance in the green turtle, *Chelonia mydas*, in sea water and fresh water. J Comp Physiol 107:229–240

Marshall AT, Hyatt AD, Phillips JG, Condron RJ (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

Marshall AT, King P, Condron RJ, Phillips JG (1987) The duct system of the avian salt gland as a transporting epithelium: structure and morphometry in the duck *Anas platyrhynchos*. Cell Tissue Res 249:179–188

Minnich JE (1979) Reptiles. In: Maloiy GMO (ed) Comparative physiology of osmoregulation in animals, vol I. Academic Press, London New York San Francisco, pp 391–641

Peaker M, Linzell JL (1975) Salt glands in birds and reptiles. Cambridge University Press, London

Prange HD (1985) Renal and extra-renal mechanisms of salt and water regulation of sea turtles: a speculative review. Copeia 1985:771–776

Prange HD, Greenwald L (1980) Effects of dehydration on the urine concentration and salt gland secretion of the green sea turtle. Comp Biochem Physiol 66A:133–136

Schmidt-Nielsen K, Fange R (1958) Salt glands in marine reptiles. Nature 182:783–785