

## Effects of Salt Loading on Salt Gland Function in the Euryhaline Turtle, *Malaclemys terrapin*

F.B.M. Cowan

Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 5A3

Accepted May 22, 1981

**Summary.** The estuarine turtle, *Malaclemys terrapin* is able to ionregulate when acclimated to fresh water, 55% sea water or 100% (full strength) sea water, but when in 100% sea water it does not volume regulate successfully. Orbital gland secretions collected by a new eye cup method are very low in animals from all three salinities without salt load. After salt loading the animals from all three groups produce an orbital gland secretion with a sodium concentration greater than sea water. The concentration of ions and kinetics of the response are similar in all three groups. Orbital gland secretion returns to control pre-load levels well before the injected load is excreted. There is no correlation between the plasma sodium concentration and any of the parameters of the orbital gland response. There is also no correlation between the concentration of sodium in the tear fluid or the rate of sodium excretion and the level of  $K^+$ -stimulated *p*-nitrophenylphosphatase activity in the gland. Some of these unexpected results may relate to the estuarine habitat occupied by *Malaclemys*.

### Introduction

Schmidt-Nielsen and Fänge (1958) suggested that an orbital gland of the euryhaline *Malaclemys* was involved in salt secretion. Morphological evidence indicated it was the lachrymal gland and not the Harderian gland which performed this function (Cowan 1969). Because *Malaclemys* can survive in sea water for long periods (Cowan 1970), it was assumed that this salt gland can secrete a sodium chloride solution at a concentration greater than sea water. However the only quantitative evidence for this has been ob-

tained by a head rinse technique, a method which measures only the absolute amount of sodium chloride secreted from the head region. The actual source of the collected secretion is obscure, and there are several other serious errors. Using this technique rates of secretion have been estimated to be about  $20 \mu\text{eq h}^{-1} 100 (\text{g body weight})^{-1}$  (Dunson 1970; Cowan 1970). Although there is very little else known about the physiology of the lachrymal gland in *Malaclemys*, there is more knowledge about osmoregulation (see Discussion). One pertinent fact is that *Malaclemys* progressively lose weight when in sea water for short term (Bentley et al. 1967) or for long terms (Cowan 1970). This might indicate that the animal is not osmoregulating perfectly, and that salt gland secretions are not hypertonic to sea water.

To study the function of the salt gland and its role in osmoregulation, an eyecup method was devised which avoids some of the problems of the earlier methods (see Discussion) and which allows measurement of ion concentrations in the secretions.

### Materials and Methods

*Malaclemys terrapin* obtained from a commercial source off Chincoteague, Virginia, were all acclimated to 55% sea water. (All sea water values are expressed as percentages of full strength or 100% sea water. Instant Ocean artificial sea water was used according to the suppliers instruction to make 100% sea water.) After initial acclimation the animals were randomly divided into three groups, and placed in 100% sea water, 55% sea water or fresh tap water and left for 2 months.

For the slow deacclimation experiments a group of animals acclimated to 100% sea water were weighed and returned to 100% sea water for several days. They were then weighed again, placed in 95% sea water and left for several days. This procedure was continued with a gradual dilution of the sea water at each step until the animals showed an abrupt increase in weight.

The head rinse was used in some cases to collect salt gland secretion as a check on the earlier work. This technique has been described previously (Cowan 1974a). The ions collected by this method can originate from many parts of the head and there

*Abbreviations:*  $K^+$ -NPPase potassium stimulated *p*-nitrophenylphosphatase; *Na*-*K*-ATPase sodium, potassium stimulated adenosine triphosphatase

is usually complete evaporation of the fluid so measurements of ion concentrations of the secreted fluid are not possible.

For the eye cup technique a 3–5 mm section was cut from a rubber Pasteur pipette bulb. These conical sections were washed for several days in many changes of distilled water. The diameter of the base of the rubber section was about 2 mm greater than the diameter of the orbit. A very light coat of 'Superglue' was put on the lower rim of the eye cup and the eye cup was affixed around the orbit. When the glue had dried a water-proof seal was built up around the rim of the cup. The top of the eye cup could be sealed by a variety of methods, all of which worked equally well. The easiest method was by cutting another section of the rubber pipette bulb slightly larger than the eye cup. The top end of this section was sealed by gluing a coverslip over it, and the whole assembly could be slipped over the installed eye cup. Tests for the suitability of each eye cup are described in the Results.

All salt loads were administered as sub-cutaneous injections into the hind limb. The legs were taped in fixed position to keep them stationary, and prevent attempts to remove the eye cup. The usual salt load was  $3.2 \text{ mmol } 100 \text{ (g body weight)}^{-1}$  unless otherwise specified.

The animals were weighed to the nearest gram on a top-loading balance.  $\text{Na}^+$  and  $\text{K}^+$  concentrations in lachrymal gland secretions and in plasma obtained from blood in the carotid artery were determined in an IL 143 flame photometer; osmolalities were measured in the Advanced Osmometer. The enzyme *p*-nitrophenylphosphatase was analyzed by standard methods modified slightly by Cowan (in press). The glands were homogenized in  $0.1 \text{ mol l}^{-1}$  Tris buffer at pH 7.2 at  $4^\circ\text{C}$  using a Teflon pestle and glass tube. Five passes of two seconds each gave adequate homogenization. Further homogenization did give a finer suspension but also caused considerable inactivation of the enzyme. Cowan (in press) has shown that filtering through layers of cheese cloth or very low speed centrifugation results in significant loss of enzyme, thus these steps were omitted. Quadruplicate samples were used however and this gave adequate assessment of inhomogeneity. The homogenates were analyzed within 15 min of killing the animal. Mercaptoethanol was not used. The assay medium was  $0.1 \text{ mol l}^{-1}$  Tris buffer (pH 7.4 or 8.6),  $10 \text{ mmol l}^{-1}$  disodium *p*-nitrophenylphosphate, with or without  $10 \text{ mmol l}^{-1}$   $\text{MgCl}_2$ ,  $10 \text{ mmol l}^{-1}$   $\text{KCl}$  or  $10^{-3} \text{ mol l}^{-1}$  ouabain. The assay was run at  $22^\circ\text{C}$  for 15 min using 2.5 ml of assay medium and 50  $\mu\text{l}$  of homogenate (50 mg tissue  $\text{ml}^{-1}$  buffer). The incubation was stopped by the addition of 0.5 ml 33% trichloroacetic acid. 3.0 ml of Tris base was added to bring the pH back to 8.9 before measuring the *p*-nitrophenol in the spectrophotometer at 420 nm. All blanks were complete medium including substrate and 50  $\mu\text{l}$  of boiled homogenate.

## Results

*Malaclemys* kept in 100% sea water gradually lose weight. To test at which salinity this weight loss stopped, *Malaclemys* were acclimated to slowly decreasing salinities as shown in Fig. 1. In the first experiment the turtles continued to lose weight until the salinity was 55%. In the second experiment weight loss stopped at 66%. After 16 days in 66% sea water body weight increased 28%. Abrupt transfers to 33% sea water or to fresh water (Fig. 1) caused immediate increases in body weight (16% and 14% respectively). These differences are highly significant when analyzed as paired observations.

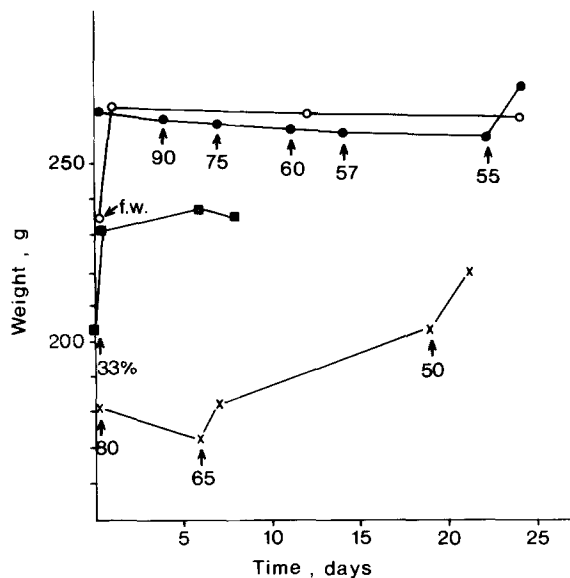


Fig. 1. The effect on body weight of abrupt or gradual transfer of *Malaclemys* from higher to lower salinity. Numbers on graph give salinity. In this and all other figures the data was analyzed as paired samples over time therefore S.E.M. bars are not indicated. Ten animals were used for each of the four experiments in this figure

In the next series of experiments, animals were taken from 100% sea water and salt loaded. Half of the loaded animals were returned to 100% sea water and half were placed in 55% sea water. A third group was taken from fresh water, salt loaded, and returned to fresh water. In the first experiment the animals were killed 24 h post-loading (Fig. 2, top), in the second they were killed 6 days after salt loading (Fig. 2, bottom). The animals salt loaded and placed in either 100% or 55% sea water continued to lose weight and both groups sustained elevated plasma sodium concentrations. The salt-loaded animals placed in fresh water gained weight immediately. Over the longer 6 day trial the animals salt-loaded and placed in 100% sea water continued to lose weight, while the animals placed in 55% sea water or fresh water gained weight over the six day period. Despite the differences in weight changes, all groups appeared to be able to ionregulate equally well, as judged by the return of plasma sodium concentrations to control values.

A salt load of  $2 \text{ mmol } 100 \text{ (g body weight)}^{-1}$  should raise plasma sodium concentration approximately  $100 \text{ meq l}^{-1}$ . At 24 h post-loading the increase in plasma sodium concentration was only  $30 \text{ meq l}^{-1}$ . To see if the plasma sodium concentration had reached a maximum and had then declined before 24 h, 36 *Malaclemys* were given the same salt loading placed in 100% sea water and samples of 6 were killed at intervals within the 24 h period (Table 1).

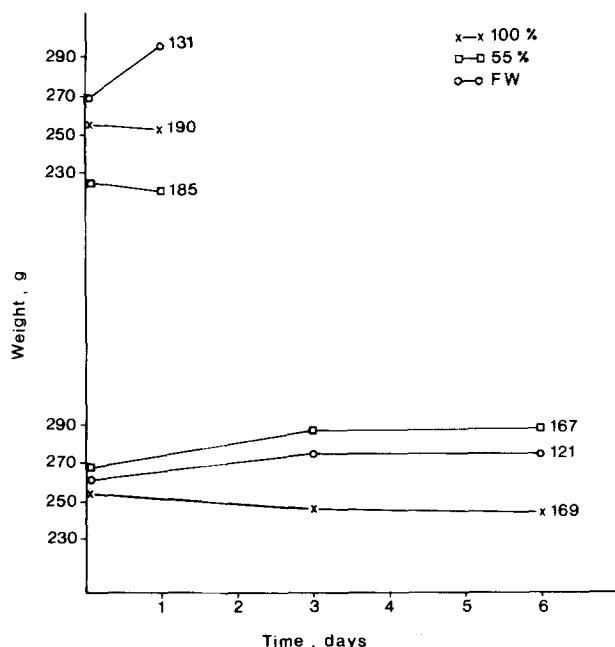


Fig. 2. The effect of salt loading on body weight of *Malaclemys* acclimated to fresh water, 55% or 100% sea water. Numbers on graph give the plasma sodium concentration at the termination of each experiment. Two series of experiments ( $n=10$ )

Table 1. Effect of salt loading on blood osmotic pressure and ion concentrations in *Malaclemys* acclimated to 100% sea water

Time (h)	Load (mmol 100 g <sup>-1</sup> )	Osmotic pressure	Na (meq l <sup>-1</sup> )	K (meq l <sup>-1</sup> )
0	0	337	155	3.5
1.5	2	394	180	3.1
3.0	2	384	193	2.6
6.0	2	360	192	3.2
24	2	355	190	3.1
168	2	363	167	3.7

This data showed that the maximal sodium concentration was 193 meq l<sup>-1</sup> (compared to the expected value of 260 meq l<sup>-1</sup>).

Figure 2 indicates that salt loaded *Malaclemys* can ionregulate when in 100% or 55% sea water. The role of the salt gland in this ionregulation was studied first by the head rinse technique. The results show (Table 2) that the secretion rates for sodium and for potassium from the cranial region are very low in the unloaded animals irrespective of the salinity of acclimation. Salt loading with 3.2 mmol 100 g<sup>-1</sup> body weight increased sodium secretion significantly ( $P < 0.05$ ) in all cases except in the animals acclimated to 100%. This was also found if the load was 2.1 mmol sodium chloride plus 1.1 mmol potassium chloride. In both types of experiment there was no increase in potassium secretion.

Table 2. Sodium and potassium secretion rates, determined by head rinse technique, in preloaded and salt loaded *Malaclemys* acclimated to various salinities (means  $\pm$  S.E.)

Ac-clima-tion salinity	Preload		Load		Postload				
	1 h		2 h		2 h		4 h		
	Na <sup>a</sup>	K	Na	K	mM/100 g		Na	K	
30%	1.16	0.46	5.2	1.7	NaCl 3.2	9.2	1.2	10.6	1.3
S.E.	0.06	0.56	4.7	1.6		5.1	1.6	6.3	1.6
55%	2.17	1.1	2.7	0.6	NaCl 4.8	10.7	0.9	20.3	1.0
S.E.	1.56	1.2	3.4	0.7		7.3	1.4	7.2	1.3
55%	0.65	0.9	2.3	0.8	NaCl 3.2	12.6	0.2	12.1	0.2
S.E.	0.3	0.3	0.5	0.3		3.0	0.2	5.9	0.1
55%	1.95	0.1	0.7	0.1	NaCl 2.2	4.9	0.2	7.8	0.4
S.E.	0.4	0.1	0.2	0.1	KCl 1.1	0.7	0.2	1.9	0.1
100%	0.52	0.1	0.7	0.1	NaCl 3.2	2.4	0.2	4.3	0.2
S.E.	0.4	0.2	0.2	0.1		3.8	0.1	6.1	0.1

<sup>a</sup> All values are  $\mu\text{mol } 100 \text{ g}^{-1} \text{ h}^{-1}$

To overcome weaknesses in earlier techniques, the eye cup method was devised. Various tests were made to ensure its validity. When the eye cup was applied first in early trials there was spontaneous orbital secretion, due perhaps to irritation of the eye. The tears obtained when this happened had a high potassium concentration (60 meq l<sup>-1</sup>) but very low sodium. With practise this irritation and spontaneous flow were avoided. To test for the addition of ions from the glue or eye cup itself trials were done with the eye cup placed in dummy sites. Quantitative additions, recoveries and analysis of ions in the recovered fluid show no addition of ions or loss of fluid from the eye cup.

The following protocol was adopted. The head was washed thoroughly, dried with cheesecloth and the eye cup was applied. If spontaneous tearing, evidence of leakage or exogenous addition of ions occurred the animal was removed from the experiment. It should be noted that the eye cups were so placed that added or secreted fluid did not bathe the eye; it collected in the cup beneath the eye.

Before salt loading animals acclimated to fresh water there was very little accumulation of secretion in the eye cup (Fig. 3). The sodium concentration was equal to that in plasma and the potassium concentration was high (43 meq l<sup>-1</sup>) in this basal secretion. Salt loading caused significant increases in secretory rate and sodium concentration in the tears, and a reduction in potassium concentration. The peak fluid secretory rate was 60  $\mu\text{l } 100 \text{ g}^{-1} \text{ h}^{-1}$  which means the sodium secretion was 33.6  $\mu\text{mol } 100 \text{ g}^{-1} \text{ h}^{-1}$ .

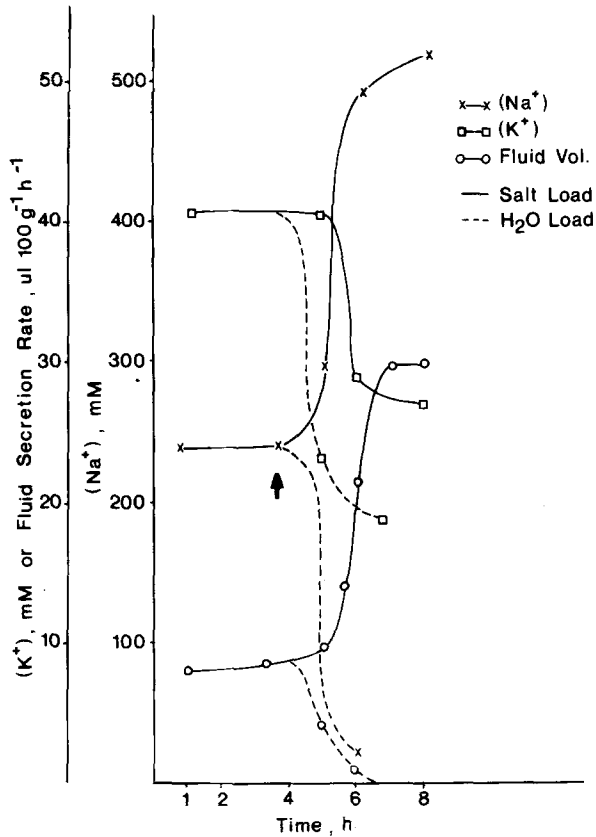


Fig. 3. Effect of salt or water loading on the rate, sodium concentration and potassium concentration of orbital gland fluid in *Malaclemys* acclimated to fresh water for two months. Arrow indicates time of loading. ( $n=6$ )

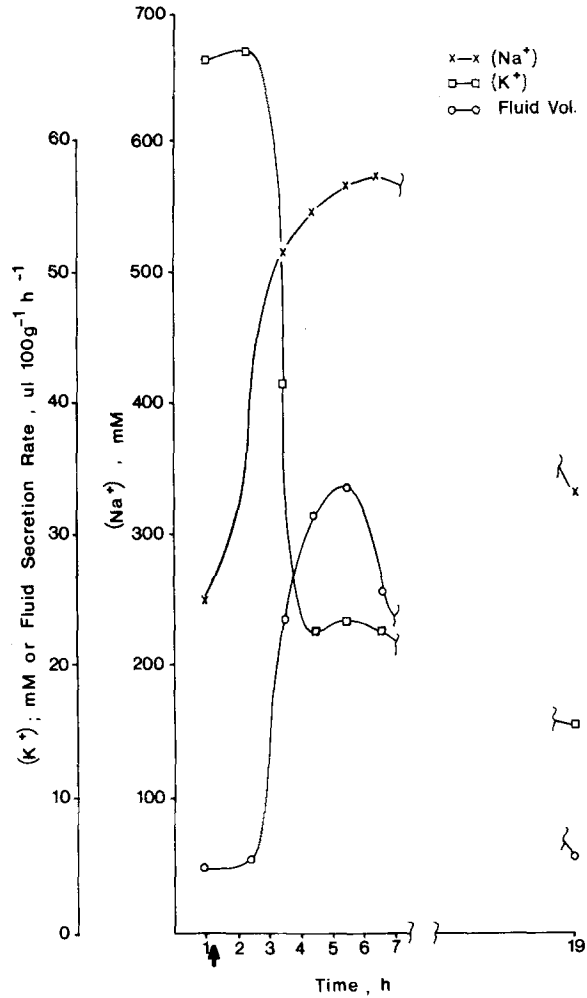


Fig. 5. Effect of salt loading on rate, sodium concentration and potassium concentration of orbital gland fluid in *Malaclemys* acclimated to 55% sea water for two months. In this case the experiment continued for 19 h. Arrow indicates 1 h post-loading. ( $n=6$ )

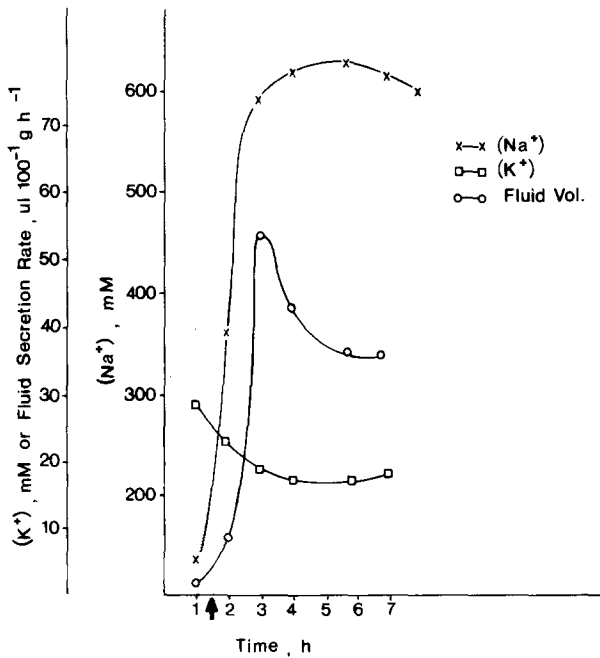


Fig. 4. Effect of salt loading on the rate, sodium concentration and potassium concentration of orbital gland fluid in *Malaclemys* acclimated to 100% sea water for two months. Arrow indicates time of loading. ( $n=6$ )

Essentially similar results were found when animals acclimated to 100% sea water were salt loaded (Fig. 4). The response to salt loading was secretion of a fluid with sodium concentration greater than sea water. The sodium concentration in the tears was not greater than found after salt loading animals acclimated to fresh water, but the secretory rate was significantly greater ( $64 \mu\text{mol } 100 \text{ g}^{-1} \text{ h}^{-1}$ ).

The animals in the previous two experiments were killed five hours after loading so that *p*-nitrophenylphosphatases could be assayed at the time when maximum secretion by the lachrymal gland was occurring. If the experiment is allowed to continue the results in the first five hours are essentially similar to the previous experiments, with a response of copious secretion with high sodium and low potassium concentrations (Fig. 5). However this protocol showed that the response had diminished within 24 h before a maximum of 20% of the injected load had been secret-

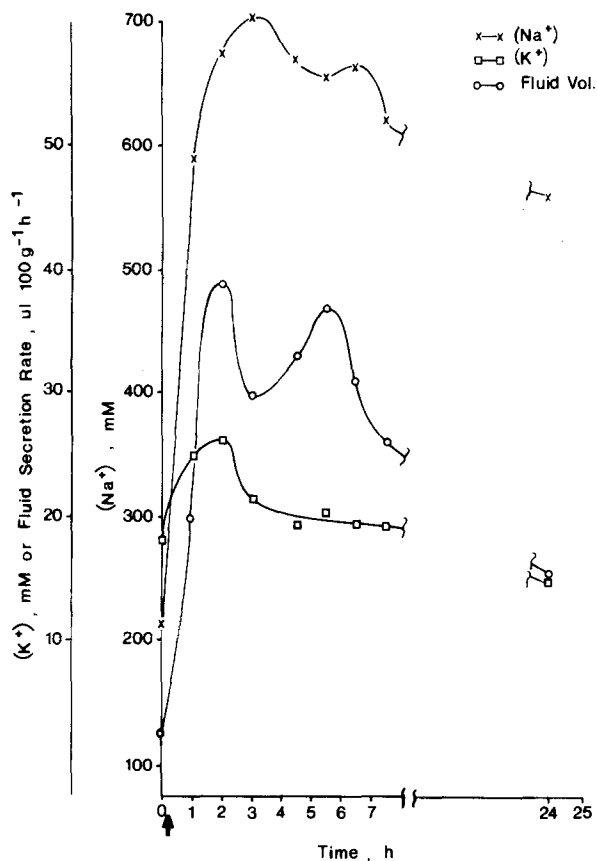


Fig. 6. Effect of a second salt load plus 1.8 mg 100 g<sup>-1</sup> theophylline on the rate, sodium concentration and potassium concentration of orbital gland secretion in *Malaclemys*, acclimated to 55% sea water for 2 months. This is a continuation of the experiment shown in Fig. 5. The second load was three days after the first

ed. This return to basal levels of lachrymal gland secretion occurred even though plasma sodium values are still high.

Figure 6 shows a continuation of the experiment in Fig. 5. Three days after the initial load a second load was given using 3.2 mmole sodium chloride plus 1.8 mg theophylline 100 g<sup>-1</sup> body weight. The maximum sodium concentration in the tears (706 meq l<sup>-1</sup>) and the rate of fluid secretion were not significantly greater than after the first load. However in this case the hypertonic secretion was sustained for at least 24 h. This effect could be a result of the cumulative effects of two loads or to the addition of theophylline with the second load. This was tested in another experiment, not shown, in which the animals received a single load of sodium chloride and theophylline. The maximum sodium concentration in the fluid collected six hours after loading was 612 meq l<sup>-1</sup> and this concentration was maintained for at least 24 h. Thus theophylline appeared to prolong the response of the lachrymal gland to salt loading, but does not effect the other parameters.

Table 3. Nitrophenylphosphatases<sup>a</sup> in the lachrymal salt gland of *Malaclemys* acclimated to fresh water or to sea water, and water loaded or salt loaded

Activator or inhibitor	Acclimated to fresh water		Acclimated to sea water	
	Salt load	Water load	Salt load	Water load
No ions	0.058 ±0.026	0.053 ±0.09	0.09 ±0.022	0.088 ±0.008
Mg <sup>2+</sup> stimulated	0.035 ±0.007	0.019 ±0.014	0.036 ±0.049	0.046 ±0.027
Mg <sup>2+</sup> +K <sup>+</sup> stimulated	0.138 ±0.028	0.147 ±0.089	0.165 ±0.08	0.130 ±0.08
Ouabain inhibited	0.143	0.132	0.157	0.125
L-Tetramisole			0.091	0.074

<sup>a</sup> Activities expressed as μmol nitrophenyl liberated mg<sup>-1</sup> tissue h<sup>-1</sup> (Means ± S.E.)

At the conclusion of each experiment the lachrymal glands were removed and assayed for *p*-nitrophenylphosphatases. An example of this data shown in Table 3 includes the same animals depicted in Fig. 3 and 4. The data shows no significant differences for K<sup>+</sup>-stimulated *p*-nitrophenylphosphatase for any possible pairing of groups. Salt loading of animals acclimated to fresh water or 100% sea water, which results in stimulation of hypertonic lachrymal gland secretion in both groups does not result in an increase in transport enzyme compared to the unloaded controls. Comparing unloaded animals acclimated to fresh water to those acclimated to sea water also shows no statistical difference in the levels of K<sup>+</sup>-stimulated *p*-nitrophenylphosphatase (*y*) versus plasma sodium concentration (*x*) for *n*=30 yielded the equation  $y=0.076+0.004x$ , with  $r=0.184$ . The slope of this line is not statistically different from zero.

There was a statistically significant difference in the activity of one of the other enzymes. Mg<sup>2+</sup>-stimulated *p*-nitrophenylphosphatase was increased significantly ( $P<0.05$ ) in fresh water acclimated animals which were salt loaded as compared to the unloaded controls. This enzyme is described in detail elsewhere (Cowan, in press).

## Discussion

The lachrymal salt gland of *Malaclemys* is potentially a good test system for the study of salt secretion. The estuarine habitat means that the gland should secrete at various levels of activity under relatively physiological conditions. In addition the salt gland in *Malaclemys* is a lachrymal gland (Cowan 1969)

and not a nasal gland as it is in some other reptiles and birds. Thus study of the lachrymal gland might reveal some features of the general phenomenon of salt secretion not readily apparent in other systems.

There are however some drawbacks. The gland opens through numerous and very small pores on the nictitating membrane. This is not true in the sea turtle which also has a lachrymal salt gland, but these animals are difficult to obtain at a size useful in the laboratory. The lachrymal gland of *Malaclemys* is too small to use for perfusion as has been done with the rectal gland of elasmobranch (Silva et al. 1977), and attempts at isolating lachrymal gland tubules for in vitro perfusion have only been partially successful. Thus a potentially good study system has not as yet been too useful, to the point that Peaker and Linzell (1975) conclude in their review on salt glands that next to nothing is known about the physiology of salt secretion in reptilian salt gland systems. This is especially true about the lachrymal gland in *Malaclemys*.

Schmidt-Nielsen and Fänge (1958) first suggested that an orbital gland was a salt secreting organ in *Malaclemys*. Cowan (1969, 1970) showed that of the several orbital glands present, the lachrymal gland was the most likely source of the secretion collected in that region. Cowan (1974b) also showed that the lachrymal gland has high activities of  $K^+$ -stimulated *p*-nitrophenylphosphatase. He did not find an increase in the activity of the enzyme in *Malaclemys* acclimated to sea water as compared to those acclimated to fresh water. Dunson and Dunson (1975) disagreed with this observation. Both Cowan (1970) and Dunson (1970) found a significant secretion of sodium from some cranial source using the head rinse technique. Dunson (1970) also collected secretion from the orbit using a micropipette. He acknowledged that evaporation of water before collection might artificially raise the sodium concentration in the collected tears.

Thus very little is known about salt secretion in *Malaclemys*, in fact it has not been fully established that *Malaclemys* can successfully ionregulate and osmoregulate when kept in sea water for long periods. Bentley et al. (1967) noticed that *Malaclemys* in sea water gradually lose weight, and this was confirmed by Cowan (1974b) for much larger samples. To learn more about this the experiments in Fig. 1 and 2 were done. These indicate, as before, that *Malaclemys* lose weight in any salinity greater than 60%. Transferred to 55% sea water they gain weight within 30 min, presumably due to drinking, indicating they have some receptor for salinity. Animals kept in fresh water or 55% sea water and salt loaded also gain weight

when returned to these two salinities, but not if placed in 100% sea water. Thus the conclusion seems to be that animals in 60% sea water or less do drink to aid in ion regulation after sodium loading. However it should be noted that although they do not drink 100% sea water and that they continue to lose weight, salt loaded animals in 100% sea water also appear to ionregulate successfully. This suggests that *Malaclemys* have a mechanism for excreting sodium hypertonic to sea water. It is possible that the rate of generating free water by this mechanism is slower than hypotonic loss of water by other routes.

Table 1 indicates another interesting aspect of osmoregulation in *Malaclemys*. When a load is administered which should cause a rise in plasma osmolality of 100 mosmols, the maximal observed rise is only 30 mosmol indicating that the load is equilibrated across a space larger than the normal inulin extracellular space. Preliminary data (personal observations) showed that some of the sodium enters the cells. There is another pool which may act as a sink for an injected sodium load. The amount of urine stored in the bladder is very variable, even in animals kept in 100% sea water for many months. In *Malaclemys* kept in fresh water the bladder urine is very hypotonic, thus when an animal is transferred from fresh water to sea water it carries with it a store of virtually free water to be used at a variable rate depending on the conditions met. Experimentally this variable pool of free water possibly accounts for the wide variation in plasma ionic concentrations.

To study the lachrymal gland's contribution to osmoregulation the head rinse technique was used first. The results agreed well with earlier observations (Dunson 1970; Cowan 1970). Animals acclimated to 30%, 50% and 100% sea water all had very low rates of orbital secretion if not salt loaded. The sodium: potassium ratio in this basal secretion from the head region was approximately 10:1. After salt loading animals from all salinities responded in a similar way: increased sodium secretion, decreased potassium secretion and an increase in the sodium: potassium ratio to 60:1. Loading with a 2:1 mixture of sodium chloride-potassium chloride resulted in increased sodium secretion, but not in potassium secretion. This distinguishes the lachrymal gland of *Malaclemys* from the salt glands of other reptiles (see Dunson 1976, for a review).

As the head rinse technique has many weaknesses the eye cup method was devised. Tests with this system showed it was quite reliable in catching all the secretion, in localizing the source to the orbit alone, and in reducing evaporative loss. It was found that orbital secretions in unloaded animals were very

small. The amounts of ions secreted into the eye cup are in fact less than those collected by the head rinse method, showing that the latter method also collects fluid from some other source. As was found with the head rinse technique the basal secretion is the same in specimens acclimated to sea water or fresh water even though plasma sodium concentration is 50 meq l<sup>-1</sup> greater in the former.

The surprising result using the eye cup was found after salt loading. Salt loading animals in fresh water results in a secretion of a fluid with a hypertonic sodium concentration similar to that found in tears of salt loaded animals acclimated to sea water for long periods. It has to be acknowledged that during the collection period the animals acclimated to fresh water do not have access to water. Even so the experiments do show that the lachrymal glands from these animals that are well hydrated have the immediate capability of forming a lachrymal gland fluid similar in sodium concentration to that collected from animals acclimated to sea water. In other words the salt gland is kept in a state of readiness when in fresh water. This could explain why I found no change in levels of transport enzyme in glands from animals acclimated to either salinity. The overall conclusion is that animals acclimated to fresh water, 55% or 100% sea water all respond to a salt load in a similar way: the immediate secretion of a fluid highly concentrated in sodium but with low potassium concentrations.

The present experiments show that theophylline appears to stimulate sodium secretion when given with a salt load. The only effect is to prolong the response. These results are similar to those found in the shark rectal gland (Silva et al. 1977), and perhaps depend on an interaction with the cyclic AMP system. Theophylline's complex interaction with alkaline phosphatase is another possibility (McComb et al. 1979).

In some longer term experiments it was found that the levels of secretion declined to the control preloaded levels well before the total injected load was secreted. They also showed that the rate and concentration of secretion returned to control levels before plasma sodium concentration returned to pre-load levels. The peak secretion in terms of rate and sodium concentration occurred in sea water acclimated animals when plasma sodium was about 200 meq l<sup>-1</sup>. A similar peak in salt loaded but fresh water acclimated animals occurred when the plasma sodium was 160 meq l<sup>-1</sup>. Note that this latter figure equals plasma sodium concentrations of unloaded sea water acclimated animals in which there is no stimulation of the lachrymal gland. The conclusion from this is

that plasma sodium concentration per se is not the primary stimulus for secretion. The linear regression equation of sodium secretion rate versus plasma sodium concentration ( $n=40$ ) gives  $y=14+0.04x$  with  $r=0.129$  which also indicates that plasma sodium concentration is not a very good indicator of salt secreting activity of the gland. Perhaps one could postulate that it is the perturbation of the sodium ion gradient across cell membranes which is the primary stimulus for secretion.

There is an interesting correlation between sodium concentration in the tear fluid and flow rate of the secretion. The equation is  $y=405+4.94x$  ( $r=0.72$ ). This if anything is a positive correlation and as such would argue against a model suggesting concentration of tear fluid by ductal reabsorption of water. That model applied to the avian salt gland (Ellis and Goertemiller 1977) envisions reabsorption of water into a highly hypertonic intercellular space along the duct. If such a model were applicable to the lachrymal gland of *Malaclemys* a negative correlation should be found between flow rate and sodium concentration.

The data with respect to K<sup>+</sup>-NPPase confirms the earlier data of Cowan (1974b) (compare to Dunson and Dunson 1975) that there is no increase in activity of the enzyme with acclimation to sea water. There is no correlation between enzyme activity with sodium excretion rate, or with sodium concentrations in the plasma. The linear regression of enzyme activity versus plasma sodium yields  $y=0.76+0.004x$  with  $r=0.184$ . The regression line of enzyme activity vs sodium concentration in the tear fluid is  $y=415+1161x$  ( $r=0.37$ ). This data indicates that plasma sodium can not be used to predict enzyme activity and also disagrees with Dunson and Dunson (1975). Rough analysis of their published data (Fig. 1, Dunson and Dunson 1975) indicates a correlation coefficient of approximately 0.55. Those authors do suggest a reason for this difference; perhaps maximum dehydration is needed before the correlation is found. In many of their specimens the plasma sodium concentration had been raised above 200 meq l<sup>-1</sup>, higher than used in this paper. Nevertheless the present paper does show in conditions which can bring about high sodium secretion rates there is no increase in K<sup>+</sup>-NPPase. This also could be explained, as stated above, by assuming the lachrymal gland in *Malaclemys* is able to respond to salt loading immediately and without lengthy periods of induction. This may be relatively unique to the estuarine habitat occupied by *Malaclemys*.

## References

- Bentley PJ, Braetz WL, Schmidt-Nielsen K (1967) Osmoregulation in the diamondback terrapin, *Malaclemys terrapin centrata*. *J Exp Biol* 46:161–167
- Cowan FBM (1969) Gross and microscopic anatomy of the orbital glands of *Malaclemys* and other emydine turtles. *Can J Zool* 47:723–729
- Cowan FBM (1970) Comparative studies on the cranial glands of turtles, with special reference to salt secretion. Ph D thesis, University of Toronto, Toronto, Canada
- Cowan FBM (1974a) Observations on extra-renal excretion by the orbital glands, and osmoregulation in *Malaclemys terrapin*. *Comp Biochem Physiol [A]* 48:489–500
- Cowan FBM (1974b) Observations on K<sup>+</sup>-stimulated *p*-nitrophenylphosphatase in the lachrymal gland of *Malaclemys terrapin* during adaptation to salt and fresh water. *Comp Biochem Physiol [A]* 49:775–785
- Dunson MK, Dunson WA (1975) The relationship between plasma Na concentration and salt gland Na-K ATPase content in the diamondback terrapin and the yellow-bellied sea snake. *J Comp Physiol* 101:89–97
- Dunson WA (1970) Some aspects of electrolyte and water balance in three estuarine reptiles, the diamondback terrapin, 'American' and salt water crocodiles. *Comp Biochem Physiol* 32:161–174
- Dunson WA (1976) Salt glands in reptiles. In: Gans C, Dawson WR (eds) *Biology of the reptilia*, vol 5. Academic Press, New York
- Ellis, RA, Goertemiller CC Jr, Stetson DL (1977) Significance of extensive leaky junctions in the avian salt gland. *Nature* 268:555–556
- McComb RB, Bowers GN Jr, Posen S (1979) *Alkaline phosphatase*. Plenum Press, New York
- Peaker M, Linzell J (1975) *Salt glands in birds and reptiles*. Cambridge University Press, London
- Schmidt-Nielsen K, Fänge R (1958) Salt glands in marine reptiles. *Nature* 182:782–785
- Silva P, Stoff J, Field M, Fine L, Forrest JN, Epstein FH (1977) Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. *Am J Physiol* 233:F298–F306