

Does the lachrymal salt gland of *Malaclemys terrapin* have a significant role in osmoregulation?

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Several reptiles possess glands capable of secreting fluids with sodium chloride as the major solute. Almost all studies have measured total sodium chloride secreted by these glands, not the concentration of ions. Thus, it has been difficult to evaluate the role of these glands in osmoregulation. The results reported herein on a large sample of *Malaclemys terrapin* indicate the lachrymal gland is capable of secreting tears with concentrations of sodium chloride exceeding 500 mM. However, the absolute amount of sodium chloride secreted and the generation of "free water" is relatively low, and the response to salt loading is somewhat inconsistent. Furthermore, the lachrymal gland responds consistently to the presence of irritating fumes not apparently related to osmo- or iono-regulation. The ionic and organic contents of the tears resulting from ionic and nonionic stimuli are similar, suggesting that the lachrymal gland is not a dedicated salt gland, but a gland differentiated to serve some other role, as in many other animals. The gland may be adapted to carry out that role with minimal water loss, as expected in euryhaline animal. The evidence indicates that the control of the lachrymal gland in *M. terrapin* is considerably more complex than that of other known salt glands.

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Plusieurs reptiles possèdent des glandes capables de sécréter des fluides dont le principal soluté est le chlorure de sodium. Dans presque toutes les études, on trouve des mesures du chlorure de sodium total sécrété par ces glandes et non les concentrations ioniques. Il est donc toujours resté difficile d'évaluer le rôle de ces glandes dans l'osmorégulation. Les résultats présentés ici sur un échantillon important de *Malaclemys terrapin* indiquent que la glande lacrymale est capable de sécréter des larmes dont les concentrations de chlorure de sodium dépassent 500 mM. Cependant, la quantité absolue de chlorure de sodium sécrétée et la production « d'eau libre » est relativement faible et la réaction à la concentration de sel est plutôt imprévisible. De plus, la glande lacrymale réagit ordinairement à la présence de vapeurs irritantes sans lien apparent avec l'osmorégulation ou l'ionorégulation. Les contenus ionique et organique des larmes à la suite de stimulus ioniques ou non ioniques sont semblables. Il semble donc que la glande lacrymale ne soit pas une glande spécifiquement consacrée à la sécrétion de sel, mais une glande à fonction toute autre, comme c'est le cas chez plusieurs autres animaux. La glande peut servir de glande à sel avec une perte minimale d'eau, comme on pourrait s'y attendre chez un animal euryhalin. Les résultats démontrent que le contrôle de la glande lacrymale de *M. terrapin* est beaucoup plus complexe que celui de toute autre glande à sel.

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Introduction

Schmidt-Nielsen and Fänge (1958) first suggested that an orbital gland in the euryhaline turtle, *Malaclemys terrapin*, functions in osmoregulation. Later work indicated that the lachrymal gland might be the source of the secretion that they obtained (Cowan 1970). The measurements of Schmidt-Nielsen and Fänge (1958) and of most subsequent authors were of the total amounts of ions secreted, not of concentrations and flow rates. In many cases this has been due to the difficulty in making quantitative collection of fluids from the various putative salt glands. Dunson (1976) has carefully defined a salt gland as a gland which "secretes a solution of electrolytes that is hyperosmotic to plasma. In the event that this physiological test is difficult to apply, a morphological examination of the tissue with the electron microscope may be fruitful." Most reptilian salt glands have not been shown to fulfill the physiological criterion. Thus, little can be said concerning the role of salt glands in many reptiles (Peaker and Linzell 1975). The study by Cowan (1981) allowed the quantitative collection of orbital gland fluid in *Malaclemys* with the use of an eyecup. The fluid had sodium concentrations which were greater than seawater, and thus suggested that *Malaclemys* could obtain "free water" by ingesting seawater. The amount of free water so obtained appeared to be less than in other species (Cowan 1974; Peaker and Linzell 1975; Robinson and Dunson 1976).

The results showing hyperosmotic secretion in *Malaclemys* were obtained on a relatively small sample (Cowan 1981).

Subsequent attempts (F. B. M. Cowan, personal observations) to more fully describe secretion parameters showed there was great variability. Not all animals responded to salt loading, and some animals secreted large tear volumes in the absence of ionic loading. Furthermore, animals kept in fresh water and given a salt load usually secreted tears with high sodium concentrations. Animals kept in seawater, but not loaded, usually did not secrete even though their plasma sodium concentration was greater than that of animals kept in fresh water and salt loaded. These experiments indicate that plasma sodium concentration therefore is not the sole, or even an important, stimulus for secretion. This paper reports on experiments on lachrymal gland function in *M. terrapin* and explores the possibility that its function is not solely, or even primarily, related to osmoregulation. The experiments described indicate responses of the gland to stimuli which are neither osmotic nor ionic, giving evidence for a nonosmoregulatory function. The results show that exposing the eye to irritating fumes yields the same response as salt loading.

Materials and methods

Malaclemys terrapin centrata were caught off Chincoteague, VA. The mean (\pm SD) body mass of all animals reported on in this study was 306 ± 10 g, with a range of 176–1250 g. The animals were flown to the laboratory in Fredericton and upon arrival, were housed in half-strength (50%) Instant Ocean. The tanks were 4.6×0.9 m, with an elevated and sloped basking area between two pools of recirculated water. After 3 weeks acclimation to 50% seawater, the animals were

randomly assorted into three groups and kept in fresh spring water, 50% artificial seawater, or 100% artificial seawater (full-strength Instant Ocean). The animals were fed shrimp and lettuce. A natural light cycle was followed and room temperature varied between 20 and 26°C.

Most experiments were done with animals acclimated to either 50 or 100% seawater, but a few were done on animals acclimated to fresh water to see if acclimation to salinity effected the response of the gland. To measure the response of the gland, an eyecup was affixed in the orbital region, as described earlier (Cowan 1981). On the following day, experiments usually began at 09:00 by clearing any accumulated fluid from the eyecup. After 1 h, the eyecup was again cleared to test for the presence of spontaneous secretion. After the control collection period, the animals were injected with physiological saline or salt loads in an equivalent amount of saline. The usual salt load was 3.2 mmol NaCl/100 g body weight. In some experiments, the animals were loaded with KCl (0.03–0.8 mmol/100 g body weight, with or without NaCl). In other experiments, sucrose or glucose was injected in attempts to raise plasma osmolality without increasing ion concentration. The sugars were injected to give a calculated increase in plasma osmolality of 100 mosmol. The actual increase was checked by measurement of freezing point depression (Advanced Instruments osmometer). The volume of each injection was 0.7 mL/100 g body weight. After loading, fluid in the eyecup was collected at hourly or 20-min intervals, for a 4-h period. The rate of volume secretion was expressed in $\mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. Sodium and potassium concentrations were measured with an IL143 flame photometer (Instrumentation Laboratories Inc.). Sodium and potassium secretion rates were derived by multiplying the rate of secretion times the ion concentration ($\mu\text{E} \mu\text{L}^{-1}$). The results in this paper are expressed on a per gland basis unless stated otherwise as only one eyecup was used on each animal. In discussing the significance of ion excretion, it is often useful to write of total ions secreted. Collections from the other eye were often made using different techniques (rinse or cotton swabs) and confirmed that secretion from the side with the cup was not due to the presence of the eyecup and that both eyes secreted approximately equally (Cowan 1981).

The fumes from the cyanoacrylate glue used to affix the eyecup cause tearing in humans, and it does so in turtles. When the eyecup is first applied there is considerable tear flow, which is of short duration (this is the reason eyecups were installed the day before salt loading). In some experiments, drops of glue were placed on the outer rim of the eyecup at regular intervals. No glue touched the eye or surrounding area, but the fumes created were sufficient to cause significant secretion.

In addition to analysis of tear volume and ion concentrations, organic constituents were also measured. Protein was measured with the BioRad technique, sialic acid, with the resorcinol technique, and glucose concentration, with a commercial kit (Sigma). SDS-PAGE electrophoresis, followed by Coomassie blue and silver staining, was also carried out with many tear samples from freshwater- and seawater-acclimated animals and with samples from animals that were salt loaded or stimulated by the fumes from the glue.

Preinjection (control) and postinjection values were compared by paired *t*-test. Comparisons between animals subjected to different acclimations, different stimulations, or other factors, including weight, length of stay in captivity, season, or year, were done using unpaired *t*-tests for single factors or analysis of variance (ANOVA) for multiple tests. The secretion rate from control animals (at rest with no known stimulation) were not normally distributed and therefore, square root transformations were used for statistical analysis. Graphical tests for goodness of fit including probability plots are described by d'Agostino and Stephens (1986).

Results

Lachrymal gland secretion was measured in 213 animals in which there was no stimulation or ion loading. Results are given as the mean \pm SE. This is termed basal or resting secretion and is considered spontaneous (Fig. 1). The overall average for

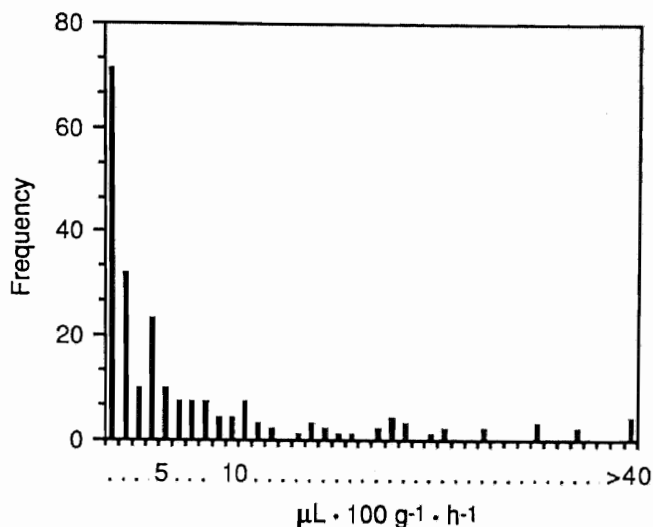


FIG. 1. Frequency distribution curve of spontaneous or resting secretion rates of the lachrymal gland of *M. terrapin*.

these 44 samples of five animals each, using raw data, was $5.8 \pm 0.5 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. With square root transformation, the corresponding value was $5.62 \pm 0.3 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. The empirical cumulative frequency distribution indicated the secretory rates were not normally distributed, and the frequency curve resembled more closely that expected for an exponential distribution, but with the right side of the curve showing considerable deviations. Probability plotting (d'Agostino and Stephens 1986) showed that values over $7 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$ appeared to be normally distributed, which suggests these data include two subpopulations. Thus, there seemed to be sufficient evidence to warrant the subsequent use of square root transformation.

The above observations applied to animals at the end of the 1st h of the control period, that is, 1 h after initial handling for the day. If the animals were left for an additional hour prior to further treatment, the baseline secretion dropped to a mean of $3.1 \pm 2.6 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. The characteristics of the distribution were the same as at the end of the 1st h, with the indication that the data represented a contaminated exponential distribution.

Of the 220 control animals just described, there was sufficient secretion in only 60 animals to allow accurate analysis of sodium and potassium concentration. The mean sodium concentration in the tears of these animals was $394 \pm 50 \text{ mmol/L}$. The data were symmetrical and approximated a normal distribution, which was platykurtotic. The secretory rates in this subset of control animals represented the 60 highest values for spontaneous secretion, with an average of $11 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. In these animals, the total rate of sodium secretion by both orbital glands was $8.7 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$, assuming that both glands were equally active. If the sodium concentration was the same in the animals with volumes of secretion too small to measure ion concentration (overall average = $3.1 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$), the spontaneous loss of sodium overall in control animals would have been $2.4 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. This is probably an overestimate as there is a positive correlation between flow rate and sodium concentration with flow rates between 3 and $9 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$ ($r^2 = 0.53$). The mean potassium concentration among spontaneous secretors was $37 \pm 4 \text{ mmol/L}$. For these animals, the rate of potassium secretion would have been $0.4 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. The distribution for potassium

concentrations was positively skewed, with potassium concentration never falling below 15 $\mu\text{mol/L}$. There were no differences in any of the spontaneous secretion parameters for animals kept in freshwater or salt water, nor was there any statistically significant evidence of effects of year, season, length of stay in captivity, or weight on secretory rate.

As it was noted earlier that the glue itself irritated the eye and caused lachrymal gland secretion, I studied the use of glue as a primary stimulus to lachrymal gland secretion. A total of 47 animals were stimulated by exposure to cyanoacrylate fumes at a tested level which is disconcerting, but not uncomfortable, to humans. This exposure to fumes caused copious, but short-lived, secretion. The secretion rate over the 1st h after stimulation was $16.7 \pm 2.9 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. This is significantly greater than spontaneous secretion rates ($p < 0.05$). The secretion returned to levels not significantly different from spontaneous levels by the end of the 2nd h. A second application of glue at this time caused a renewed burst of secretion. Sodium and potassium concentrations in the secretions after glue stimulation were 492 ± 27.1 and $45.1 \pm 1.6 \text{ mmol/L}$, respectively. The sodium concentration was significantly greater than the concentration found in spontaneous secretion ($p < 0.05$). The calculated amount of sodium and potassium lost during the response to glue stimulation would have been 15.5 and $1.45 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$, respectively, if the right and left glands secreted equally.

Twenty-two NaCl loading experiments were completed; n for each experiment varied from 4 to 6. Ten samples were lost from the experiments as a result of eyecup leakage. Flow rates per gland were available for 100 animals and Fig. 2 shows a significant increase in secretion 1, 2, and 3 h after salt loading ($p < 0.5$), compared with the rate of spontaneous secretion in the same animals at time zero. Three hours was the usual duration of each experiment. Several experiments were carried out for longer periods and showed secretion rates of 32.6 ± 2.3 , 27.7 ± 1.7 , and $14.0 \pm 1.4 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$ at 4, 5, and 6 h after loading, respectively. The last figure describes data from only 10 animals. The distribution of secretion rates 1 h after salt loading was definitely not normal, while the distribution at 2 and 3 h after salt loading was normal. There were 15 experiments (in each $n = 5$) which could be subjected to ANOVA. These experiments differed as to time of year, salinity of acclimation, length in captivity, weight, year, and left or right gland. Two-way ANOVA using raw data showed that in 14 of the 15 experiments there was a significant increase in secretory response for the 3 h after salt loading compared with the 2-h period prior to salt loading. In one experiment, the secretory rate did not increase significantly, although no reason for the weak response was apparent. The 15 experiments could not identify any other factor which by itself caused a significant alteration in secretory response.

Sodium concentrations increased at 1 h after salt loading and remained elevated for the remaining 2 h of the experiment (Fig. 2). Because of the large number of control animals with resting secretion rates below the limit of accurate measurement, comparisons of ion concentrations before and after loading were available for only 48 animals. A paired t -test for all 48 animals at time zero and 1 h after salt loading indicates the increase in sodium concentration is significant ($p < 0.01$). One-way ANOVA of each experiment ($n = 5$) showed that the concentration of sodium in the secretion was significantly greater for all 3 h following salt loading ($p < 0.05$). The calculated sodium excretion rate was $31 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. This is only about two

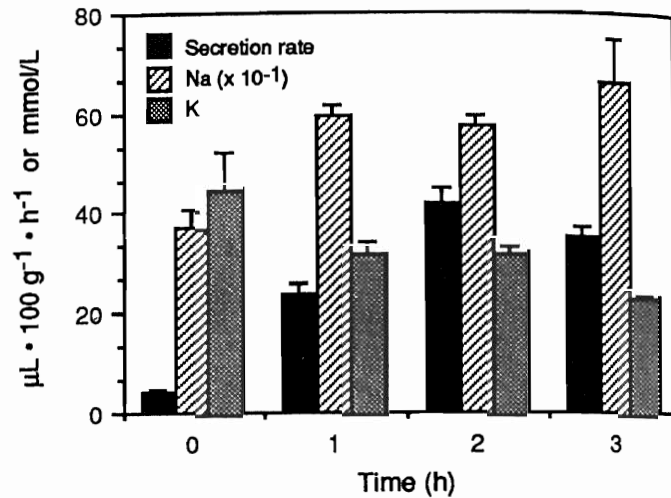


FIG. 2. Rate of fluid secretion by the lachrymal gland ($\mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$) and the sodium and potassium concentrations (mmol/L) in the tears following NaCl loading (3.2 mmol/100 g) in *M. terrapin* (mean \pm SE, $n = 60$).

times the sodium loss following glue stimulation and represents a small fraction of the injected load. This rate remained elevated over the next 2 h and then began to decline slowly, owing to a decline in volume secreted rather than concentration of sodium. Correlating sodium concentration with flow rate indicates the best fit is obtained with a logarithmic curve ($r^2 = 0.4624$, $p < 0.05$), which indicated that sodium concentration increases with flow rate up to $10 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. At higher flow rates, sodium concentration remains constantly above the concentration of sodium in seawater. Figure 2 also shows potassium concentration in the tears was less after salt loading than in spontaneous secretions ($p < 0.05$). However, the amount secreted rose substantially (to $1.6 \mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$) for both glands, which is greater than the potassium loss in the spontaneous secretion, but not greater than the loss after glue stimulation.

Attempts to stimulate tear flow with an osmotic load were unsuccessful. Sucrose ($n = 10$) and glucose ($n = 10$) injections that gave increases in plasma osmolality of up to 100 mosmol did not increase secretion above baseline levels.

Seven experiments used KCl for ion loading, to ascertain if potassium loading would lead to secretion as it does in some lizards (Dawson 1976). KCl in amounts ranging from 0.3 to 0.7 mmol/100 g caused significant increase in secretion above resting levels (mean difference in secretory rate = $12.3 \pm 2 \mu\text{L}/100 \text{ g}$; $p < 0.01$) for at least 1 h after loading as judged by a paired t -test on all 35 animals. Analysis of the seven individual experiments using raw or transformed data ($n = 5$ in each) showed that in three of the experiments potassium loading did not significantly increase secretion. The distribution of secretory rates 1 h after loading was symmetrical about the mean, but very platykurtotic. The results seemed to indicate a marginal secretion in response to potassium loading. There was no correlation between the secretory response and the size of the potassium load within the range tested. One experiment compared the response to loading (0.3 mmol/100 g) with either KCl or NaCl. The secretory response was equal for the two ions. Electrocardiography showed that a potassium load of 0.8 mmol/100 g caused arrhythmias in all five animals in the one experiment it was used. The potassium concentration in the tears after KCl loading was significantly greater than in tears resulting from

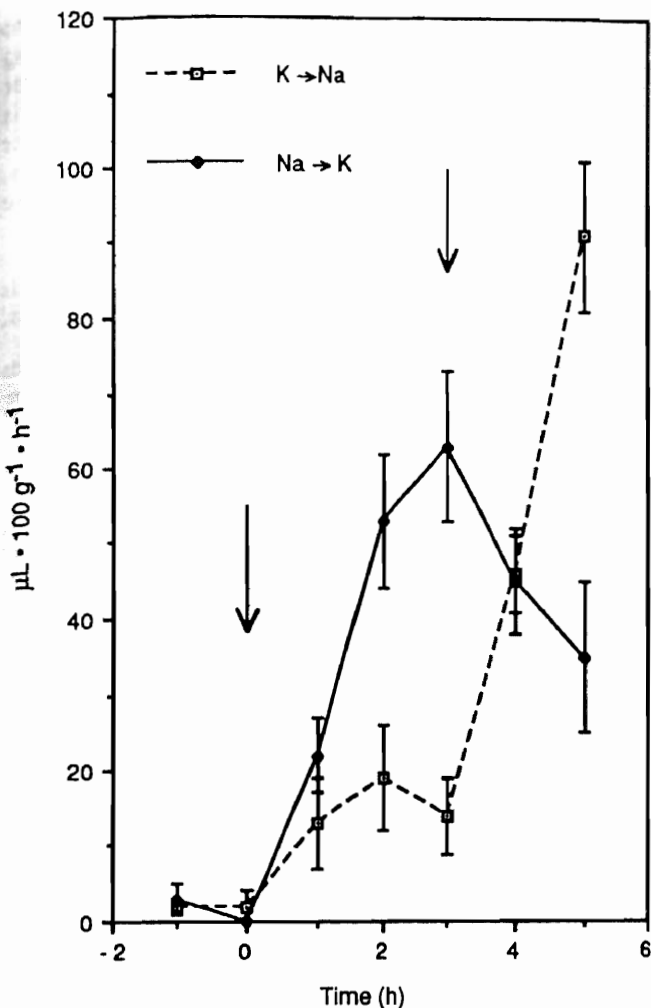


FIG. 3. Rate of fluid secretion by the lachrymal gland of *M. terrapin* following KCl loading (0.6 mmol/100 g) at time zero and NaCl loading (3.2 mmol/100 g), 3 h later, or loading in the reverse sequence (mean \pm SE, $n = 8$).

NaCl loading (60 ± 5 vs. 27 ± 4 mmol/L; $p < 0.05$). The sodium concentration in orbital fluid was equal after sodium or potassium loading. Thus, potassium loading caused a drop in the Na/K ratio of the secretion from 22:1 to 7:1. However, the relatively low secretory flow rates after KCl loading meant that potassium excretion was in fact lower than it was during the response to sodium loading.

One experiment tested for any interaction between KCl and NaCl loading (Fig. 3). Eight animals were loaded at time zero with KCl (0.5 mmol/100 g), followed by the standard NaCl load (3.2 mmol/100 g) 3 h later. This protocol was reversed in eight other animals. The figure shows a small, but significant, response to primary potassium loading of the type described above. With secondary NaCl loading, the secretory response in these animals rose to the highest levels seen, which were significantly greater ($p < 0.05$) than those found in a control group which was only sodium loaded. Conversely, the animals loaded with NaCl at time zero showed the usual primary response to NaCl loading, but the additional imposition of a KCl load 3 h later caused the secretory rate to fall to levels found with KCl loads alone. Sodium concentrations in the secretions were constant throughout the experiment for both groups, whereas potassium concentrations were higher ($p < 0.05$) immediately

after the administration of KCl than they were after the administration of NaCl loads. Thus, in the same animals over a 3-h period, the Na/K ratio varied between 25:1 and 6:1.

Attempts to study secretion with more invasive procedures under anesthesia were frustrated. Sodium pentobarbital (12–20 mg/kg) and ketamine hydrochloride both prevent tear secretion in response to a subsequent NaCl load. Both drugs also stop lachrymal gland secretion established by a prior salt load. Sodium pentobarbital (12 mg/kg) at the minimum acceptable dose had prolonged effects, such that the secretory rates to NaCl loads given 24 h later were only 26% of the normal response.

As the lachrymal glands of *M. terrapin* responded to both osmotic and nonosmotic stimuli in a similar way with respect to both flow rate and ionic concentrations, I attempted to establish if differences in the organic constituents might exist. Protein concentrations in the secretions following either stimuli were similar (0.21 ± 0.2 and 0.25 ± 0.03 mg/mL, respectively). There also were no differences in the concentrations of glucose or sialic acid in the secretions resulting from glue or ionic stimulation. Preliminary results with SDS-PAGE electrophoresis have shown no detectable and consistent differences in the band patterns following the two types of stimuli.

Discussion

The experiments reported here show that sodium loss from the orbital gland under basal or resting conditions is in the range of 2.4 – 8.0 $\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$. This would substantially offset the influx of sodium (6 – 10 $\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$) reported by Robinson and Dunson (1976). The rate of basal secretion is highly variable and not normally distributed. A significant proportion of the animals acclimated to both freshwater and seawater have no basal measurable secretion, and the level of basal secretion does not correlate with plasma sodium concentration. The frequency distribution curve of basal secretory rates in *M. terrapin* gives some indication that it is a composite curve. It closely resembles the frequency distribution curve for spontaneous secretory rates in the human lachrymal gland (Holley *et al.* 1984). These workers were unable to explain the shape of the curve they observed, but it also was indicative of multiple functional populations.

The frequency distribution and dispersion of the baseline data indicate caution has to be exercised in treating the data statistically; paired *t*-tests with transformed data or nonparametric tests should be used. As an example of the need for caution, the large samples used here to establish the level of spontaneous secretion indicate that one previous report (Dunson 1976) on the control of lachrymal gland secretion in *M. terrapin* needs to be corroborated. The rates of secretion after the injection of cholinergic agents in that study (Dunson 1976) were no greater than the baseline rates found in my experiments. Thus, little is known about the control of salt secretion in lachrymal glands of turtles beyond the possibility that cAMP is involved (Cowan 1981; Shuttleworth and Thompson 1987). Various factors were tested for their effect on spontaneous secretion using two-way ANOVA, but the variability precluded any firm conclusions.

The second significant finding concerning the lachrymal gland in *M. terrapin* is that it responded both to salt loading and to irritating fumes. Furthermore, it responds in a similar fashion, with sodium and chloride concentrations greater than seawater. After salt loading, less than one tenth of the administered load is excreted. This is much lower than sodium excretion rates seen in other salt glands (Peaker and Linzell 1975). Some free water can be obtained from seawater;

however, an upper estimate would be approximately $250 \mu\text{L} \cdot 100 \text{ g}^{-1} \cdot \text{day}^{-1}$. Potassium concentrations in the secretions are above plasma levels and the Na/K ratio variable. Studies of the proteins and other organic constituents in the secretions are similar both qualitatively and quantitatively after either osmotic or noxious stimuli. Further studies with tritiated *N*-acetylmannosamine and other carbohydrate precursors indicate similar rates of incorporation and release in secretory products after the gland is stimulated by salt loading or by the introduction of irritating fumes (F. B. M. Cowan, personal observations).

All of these observations might be taken to suggest that the lachrymal gland of *M. terrapin* may not be a dedicated "salt" gland solely, or even primarily, involved in ion and water balance. Postulating multiple functions for the gland might partially account for the frequency distribution curve of resting secretory rates, as well as some of the well known differences between the salt gland of *M. terrapin* and other known salt glands, such as the avian nasal gland. As reviewed by Peaker and Linzell (1975), the lachrymal gland of *M. terrapin* has been shown to excrete much less NaCl in response to a salt load than does the avian nasal gland. The lachrymal gland of *M. terrapin* also does not show functional hypertrophy in response to salt acclimation (F. B. M. Cowan, personal observation), nor are there morphometrically detectable changes in the ultrastructure of the gland in response to repeated salt loading or acclimation to seawater. One might also hypothesize that the neurohumoral control of the lachrymal gland function in *M. terrapin* is different or perhaps more complex than in other better studied salt glands. Multiple types of stimuli controlling lachrymal gland function in *M. terrapin* might also easily confound experimental study of the gland. Thus, Dunson and Dunson (1976) found in *M. terrapin* a correlation between plasma sodium and lachrymal gland secretory rate when plasma sodium was above 200 mmol/L. Cowan (1981, this study), on the other hand, found that animals acclimated to fresh water responded to a salt load which raised their plasma sodium concentration to only 165 mmol/L, whereas *M. terrapin* acclimated to seawater and with plasma sodium concentrations greater than 180 mmol/L did not respond unless salt loading pushed their plasma sodium concentrations even higher. Thus, there is no simple correlation between plasma sodium concentration and lachrymal gland function.

It might be that the lachrymal gland of *M. terrapin* evolved to serve some exocrine function related to the orbit but in doing so also developed mechanisms to conserve water and excrete excess ions, both of which might be useful strategies for this brackish water animal. The absolute necessity of the gland for the maintenance of ion and water balance remains to be proven, and the multiple functions and variability of the data might make it difficult to prove.

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