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OSMOREGULATION IN THE DIAMONDBACK TERRAPIN, *MALACLEMYS TERRAPIN CENTRATA*

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INTRODUCTION

Survival of vertebrates in hypertonic environments such as the sea is opposed by diffusional loss of water and gain of sodium, and is aided by physical barriers to such diffusion along with the ability to transport ions actively. The energy expenditure for active transport obviously must be related to the efficiency of the physical barriers that impede diffusion. The description of these processes in the primarily aquatic fish has received much more attention than in secondarily aquatic tetrapods.

In marine fish homeostasis is achieved by active extrusion of sodium through extrarenal channels, and Schmidt-Nielsen & Fänge (1958) have shown that in some marine turtles an orbital gland ('salt gland') can fulfil this function by excreting solutions hypertonic to sea water. Circumstantial evidence indicates that electrolyte excretion through this gland in the marine turtle *Chelonia mydas* is far greater than from the kidney (Holmes & McBean, 1964). However, the precise roles of the outer integument, the gut and the kidneys in osmotic regulation of reptiles living in a marine environment have not been reported. The present work describes the water, salt, and energy metabolism of an estuarine turtle both in fresh water and in marine situations.

The diamondback terrapin, *Malaclemys centrata*, lives in estuaries but has not been found in areas beyond tidal influence. Thus it must contend with a variety of osmotic conditions, from sea water to almost completely fresh water, a situation which contrasts with that of most other aquatic turtles, which are either exclusively marine or fresh water in habitat.

METHODS

Animals. Turtles (*Malaclemys terrapin centrata*) weighing about 400–900 g. (mean 670 g.) were obtained from an animal dealer and kept, without feeding, in tanks of fresh water at 21° C. They were allowed 2 weeks to adjust to these conditions before the experiments were commenced.

Collection of body fluids

Urine. The urinary bladder was emptied by catheterizing with a piece of plastic tubing (diameter 3 mm.). Twenty-four-hour urine samples were collected by suturing into the turtle's cloaca a polyethylene cannula to which a small rubber bag was attached.

Blood was collected either after the animals had been decapitated (in terminal

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experiments) or by puncturing a limb with a sharp scalpel. The whole blood was immediately diluted in preparation for sodium and potassium determinations.

Measurement of drinking. Phenol red (25 mg./l.) was placed in the bathing media, and recovery, if any, from gut and urine was measured according to Smith (1930).

Sodium and potassium concentrations in bathing media, urine and blood were determined by flame photometry. The osmotic concentration of the urine was measured with an osmometer (Fiske model H).

Oxygen consumption was determined by placing the animals in chambers through which a measured flow of air was passed. The partial pressure of oxygen in the effluent air was measured with a Beckman paramagnetic oxygen analyser. The animals were allowed 2 hr. to equilibrate with conditions in the chambers; the oxygen consumption was then recorded at 15 min. intervals for the next hour and the mean value for this period calculated.

Evaporative water loss. This was measured gravimetrically by weighing the animals before and after 24 hr. in a chamber through which dry air (at 23° C.) was passed at about 1·2 l./min. Since the animals were cannulated, urinary and faecal water losses were excluded. Catabolic carbon loss as calculated from rates of oxygen consumption make up less than 5 % of the observed weight losses and were ignored.

RESULTS

Survival of Malaclemys *in hypertonic saline*

Malaclemys have been reported to survive months in sea water (Coker, 1951). In the present experiments it was found that turtles remained in good condition after 14 days in 3·3 % NaCl (equivalent in concentration to sea water) followed by another 10 days in 6·6 % NaCl. Three box turtles (*Terrapene carolina*), on the other hand, died within 4 days when placed in 3·3 % sodium chloride solution.

The turtles in different solutions were weighed daily; five *Malaclemys* in tap water did not change significantly in weight; they gained on the average 0·1 ± 0·2 (S.E.) % of their body weight per day. Another five animals in 3·3 % NaCl lost 0·56 ± 0·07 % daily, while a similar group in 6·6 % NaCl lost 1·21 ± 0·23 % per day.

Water and sodium balance in Malaclemys *replaced in fresh water after prolonged immersion in saline*

Water. Five turtles placed in fresh water after 14 days in 3·3 % saline had gained 10·1 ± 2·7 % of their body weight when they were weighed 3 days later. Another five animals placed in 6·6 % NaCl for 10 days had gained 7·2 ± 0·5 % of their body weight when they were weighed 24 hr. after being replaced in fresh water.

Sodium. Two groups each of five turtles, one of which had been in 3·3 % NaCl for 14 days and the other in tap water, were placed in fresh distilled water after a brief thorough rinsing in distilled water for 30 min. The total net sodium loss (urine, skin and orbital gland) was measured after 6 hr. and again at 72 hr. During the first 6 hr. the animals which had been in saline lost sodium 6 times as rapidly as those that had been in tap water, but after 6 hr. the rates of sodium loss were almost identical (Table 1). The total excess sodium loss of the animals in saline compared to those in tap water was 480 μ-equiv./kg. in the 6 hr.

The experiments suggest that *Malaclemys* experiences a water deficit and an excess of sodium when it is in hypertonic sodium chloride solutions, and that this is corrected on return to fresh water.

Renal and extrarenal water loss in 3·3 % NaCl or in sea water

Total intake and output of water was measured in seven *Malaclemys* (4 in 3·3 % NaCl and 3 in sea water). There was no apparent difference between the animals in sea water and those in 3·3 % NaCl, and the results have been combined. During the 24 hr. period of measurement, drinking (as detected by the presence of phenol red in the gut or urine) could be detected only in one animal, and the amount was quite small, 0·2 ml.

Table 1. *Total sodium loss of* Malaclemys *in distilled water after prior maintenance for 14 days in 3·3 % NaCl or in tap water. Mean* ± S.E.

	Initial 6 hr. (μ-equiv./kg. hr.)	Subsequent 66 hr. (μ-equiv./kg. hr.)
Tap water (5)	15 ± 1·0	13 ± 1·6
3·3 % NaCl (5)	95 ± 8·5	13 ± 2·0

Table 2. *Urine volume and solute composition of* Malaclemys *in fresh water and in sea water. Mean* ± S.E.

	Volume (ml./100 g. day)	Sodium (m-equiv./l.)	Potassium (m-equiv./l.)	Total conc. (m-osm./l.)
Tap water (7)	2·4 ± 0·46	0·7 ± 0·19	4·9 ± 0·96	57 ± 20
Sea water (10)	0·44 ± 0·18	28·0 ± 13·7	31·0 ± 6·0	207 ± 42

Extrarenal water loss was small, 0·4 ± 0·08 (7 %) of the body weight in 24 hr., and the loss of urine was similar (Table 2). The combined total water loss (0·84 ± 0·18 (7 %) was not significantly different from the daily weight losses of animals kept for prolonged periods in 3·3 % saline (0·56 ± 0·07 %), again indicating that such animals drink little or none of the bathing salt solutions in order to gain water.

Urine composition in tap water and in 3·3 % NaCl

Turtles kept in tap water took up variable amounts of water, 0·8–4·3 % of the body weight in a day. The differences may reflect drinking rather than variability in osmotic uptake through the skin. The water is excreted as a dilute urine containing very little sodium (Table 2).

In 3·3 % sodium chloride solution the urine volume was only about one-fifth as great as in animals in fresh water, and the urine concentration was higher (Table 2). The potassium concentration increased proportionately to the drop in urine volume while the sodium concentration increased about 40 times but was very variable. However, considering the small volumes of urine formed, only small total quantities of sodium would be excreted in this manner. Thus for a urine volume of 0·5 % of the body weight a day and a sodium concentration of 28 m-equiv./l. only 140 μ-equiv. Na per kg. body weight would be excreted through this channel.

Blood electrolyte (Na + K) concentration in tap water and in 3·3 % NaCl

Turtles in hypertonic sodium solutions lose water (and, in addition, may gain sodium) by diffusion through the skin or gut. Measurement of blood electrolyte (Na + K) concentrations may provide information relevant to both these processes, as well as indicate whether physiological regulation occurs.

The blood electrolyte concentration increased by 10 % after the animals had been 2 days in 3·3 % NaCl (Fig. 1). During this period the water loss was only about 1 % of the body weight, and the observed increase must therefore be due to sodium gain

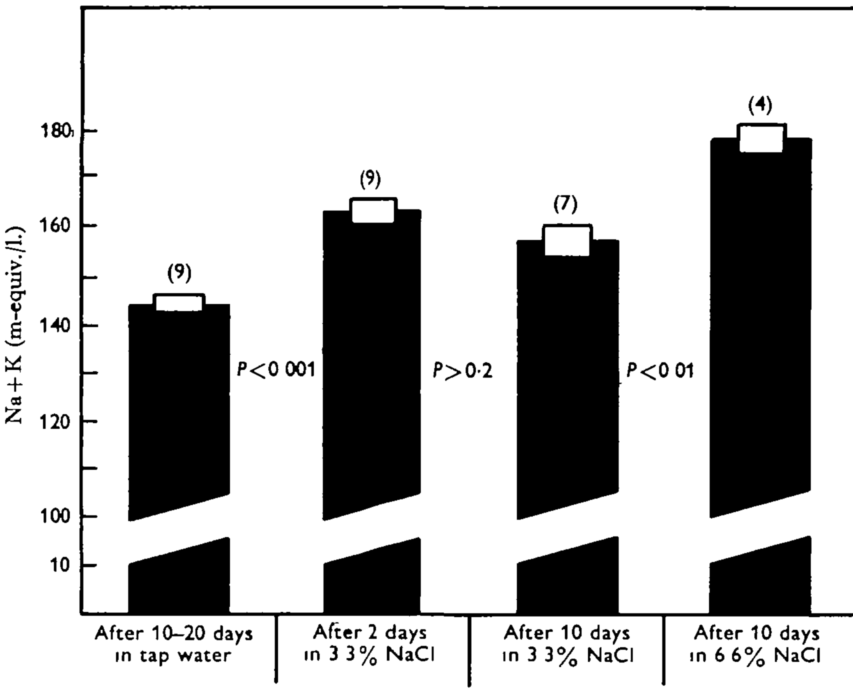

Fig. 1. Electrolyte (Na + K) concentration of blood from *Malaclemys* kept in different media. Results are given as mean values. Boxes inset are ± *s.e.* In parentheses, number of animals.

through the skin or gut or both. In seven parallel experiments on fasting turtles we found evidence of drinking only in one animal (0·2 ml.), but the possibility that drinking may occur irregularly cannot be ruled out.

The blood electrolyte concentration was the same after 10 days in 3·3 % NaCl as it had been after 2 days, suggesting that regulation of electrolyte concentration was taking place. As the kidney has been shown to excrete little sodium this probably signifies extrarenal excretion through the orbital gland.

Evaporative water loss

The rate of evaporative water loss from an animal may reflect the permeability of its integument to water. *Malaclemys* that previously had been kept in tap water lost water in dry air at 23° C. at a rate of 0·55 ± 0·14 mg./hr. cm.^{2} body surface (7). The rate of oxygen comsumption of these animals was 0·028 ml./g. hr. The rate of water loss in these estuarine turtles is similar to that in the freshwater species *Pseudemys scripta* under the same conditions, 0·66 mg./hr. cm.^{2} for an oxygen consumption of 0·038 ml./g. hr. (Bentley & Schmidt-Nielsen, 1966).

Oxygen consumption of Malaclemys *in tap water and in* 3·3 % *NaCl*

Animals in media which impose differing osmotic stresses may have different rates of oxygen consumption reflecting changes in energy required to maintain homeostasis. The oxygen consumption of *Malaclemys* in 3·3% NaCl was twice that of animals kept in tap water; when turtles that had been kept in hypertonic saline were returned to tap water their oxygen consumption decreased accordingly (Fig. 2).

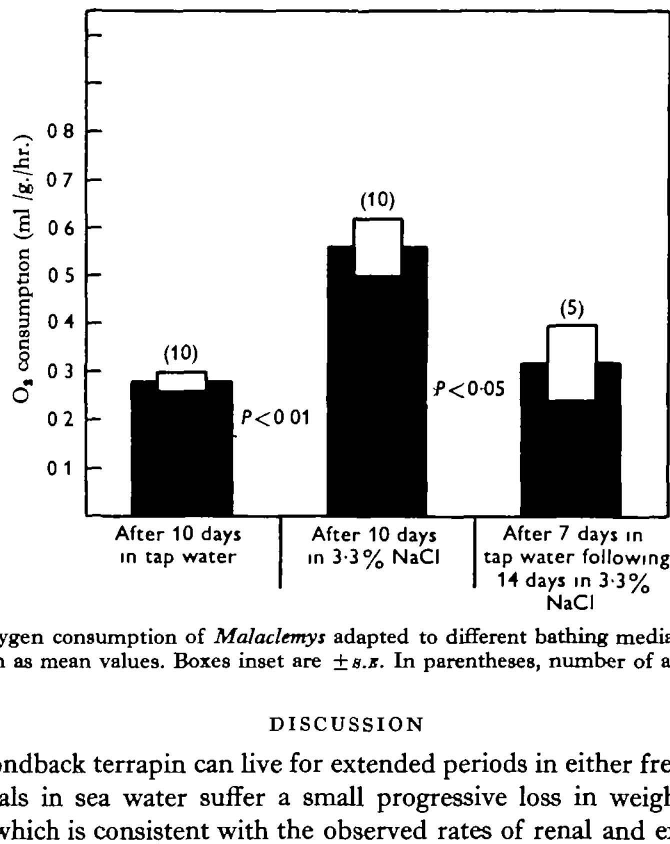

Fig 2. Oxygen consumption of *Malaclemys* adapted to different bathing media. Results are given as mean values. Boxes inset are ±S.E. In parentheses, number of animals.

DISCUSSION

The diamondback terrapin can live for extended periods in either fresh water or sea water. Animals in sea water suffer a small progressive loss in weight, not seen in fresh water, which is consistent with the observed rates of renal and extrarenal water loss. This weight loss takes place more rapidly the more hypertonic the bathing media, suggesting that it is at least partly due to osmotic problems. When turtles kept in sea water are moved to fresh water they rehydrate and rapidly excrete excess salt. While the turtles drink fresh water there is no evidence that they normally imbibe saline media in order to regain lost water. Whether such uptake takes place in connexion with feeding has not been studied.

The diamondback terrapin regulates the composition of its body fluids both in fresh water and in salt water. While there is no indication that animals in hypertonic saline gain appreciable amounts of sodium through the gut, circumstantial evidence from changes in blood concentration and total excretion of sodium indicate that a small gain continually takes place through the integument. Conversely, in fresh water total sodium excretion exceeds that from the kidney, suggesting that small amounts of sodium are being lost extrarenally. At the same time water is being lost from animals in saline and gained by those in fresh water. In fresh water this water is excreted by the kidney as a dilute urine with a very low sodium concentration. In sea water the urine volume decreases about fivefold while urine osmotic concentration increases proportionately. Potassium continues to be excreted at the same rate, and there is a relatively minor increase in total sodium excretion by the kidney. Calculations based on total osmolarity indicate that the urine from animals in either fresh water or salt water contains considerable solute apart from that attributable to sodium and potassium and their accompanying anions, so that even when the turtles are in saline solutions the kidney is probably an important channel for the excretion of catabolic solutes. This probability is supported by the regular appearance of a thick paste of uric acid in the cloaca.

Marine teleost fish, like marine reptiles, have body fluids hypotonic to sea water but lose water at the rate of about 5 % of their body weight per day through their skin, gills, and kidneys. Teleosts regain this water by drinking an equivalent amount of sea water and excreting the excess sodium through the gills (Smith, 1930). *Malaclemys*, on the other hand, loses water at only about one-tenth this rate in 3·3 % saline, and we could find no evidence to suggest that fasting animals replace the water loss by drinking the saline and excreting the salt. The basic difference between the behaviour of marine teleosts and *Malaclemys* is probably related to the absence of gills and to the relative impermeability of the integument of the reptile. The slow dehydration is at least partly compensated by drinking when fresh water becomes available, a chatacteristic of most terrestrial vertebrates. It has previously been noted that *Malaclemys* in captivity require a supply of fresh water (Coker, 1951). It is possible that estuarine animals like *Malaclemys* can tolerate long periods in hypertonic environments by undergoing slow dehydration, and that the water deficit is corrected at intervals when fresh water becomes available.

The energetics of osmoregulation in fresh water and sea water have been the subject of much speculation but the precise contribution of osmoregulation to total energy expenditure is not understood. Large differences in the oxygen consumption of animals living in sea water as compared to fresh water have been observed (Potts & Parry, 1964). Osmoregulatory excretion of sodium by the orbital gland probably results in increased consumption of oxygen (Borut & Schmidt-Nielsen, 1963), but it is inconceivable that it could result in a doubling of the oxygen consumption of the animal. It has been established that the rate of tissue metabolism is markedly influenced by the sodium concentration in the extracellular fluid (see Whittam, 1963), and such a general increase in tissue metabolism could be occurring in *Malaclemys*. However, the possibility that other factors, such as endocrines, are involved cannot be ruled out. The overall economics of osmoregulation and energy metabolism of turtles in fresh water as compared with salt water is worthy of further consideration, especially in relation to the ecological distribution of the Chelonia.

SUMMARY

1. While in hypertonic environment diamondback terrapins (*Malaclemys centrata*) slowly lose water by osmosis through the integument and as urine through the kidney.

2. Small amounts of sodium are gained, probably largely as a result of diffusion through the integument rather than by drinking, and this sodium is principally excreted extrarenally. Nevertheless, the solute concentration in the blood of such turtles increases.

3. When returned to fresh water the animals rehydrate and excrete accumulated excess sodium.

4. In these ways they could undoubtedly survive for extended periods in the absence of fresh water, but it is not clear whether they could do so indefinitely.

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