

UREA AND OSMOREGULATION
IN THE DIAMONDBACK TERRAPIN *MALACLEMYS*
CENTRATA CENTRATA (LATREILLE)*

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INTRODUCTION

The diamondback terrapin, *Malaclemys centrata centrata* (Latreille), is a thoroughly aquatic turtle living in salt and brackish marshes and estuaries. From its habitat, it is obvious that this animal has to contend with various osmotic conditions. Bentley, Bretz & Schmidt-Nielsen (1967) and just recently Dunson (1969) have already investigated the problem of the salt and water balance in that species. Our preliminary experiments have shown that inorganic ions are not the only effectors of the osmoregulation in the blood. This paper reports a comparison of the osmotic pressure of the blood and of the substances contributing to it in diamondback terrapins adapted to various salinities. The part played by the bladder has also been investigated in terms of modifications in the osmotic pressure and in the composition of the urine.

MATERIAL AND METHODS

Diamondback terrapins were collected in the marshes of Beaufort Harbour and kept in tanks of sea water directly supplied with the water of the estuary. A batch of terrapins has also been obtained from an animal dealer who was keeping them in fresh-water ponds. The sampling of blood was taken directly at the level of the aorta after the animal had been pithed and the shell sawn open. After defibrination and low-speed centrifugation, the serum was adequately diluted with de-ionized distilled water for subsequent analysis. Urine samples were collected by direct puncture of the bladder (after the shell had been sawn open) and centrifuged in order to get rid of the precipitate of urates.

Sodium and potassium concentrations were determined partly with an atomic absorption spectrophotometer and partly with a flame photometer.

Chloride estimations were performed on a Buchler Cotlove chloridometer.

Measurements of the osmotic pressure were made with a cryoscopic osmometer.

Urea and ammonia were determined by the microdiffusion technique of Conway.

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RESULTS

Blood

Na, K, Cl, urea and osmotic pressure were determined in the serum of animals originating from three batches. 1. Turtles having lived in fresh water for several months. 2. Turtles from group 1 adapted to 50% sea water for at least 15 days.* 3. Turtles collected in sea water.

Table 1 shows the results obtained and suggests the following conclusions:

(a) The osmotic pressure of the serum increases when the animal originating from fresh water is adapted to 50% sea water ($0.01 < P < 0.05$). A further increase is measured in animals living in sea water ($P < 0.01$).

(b) The Na and Cl concentrations increase significantly ($P < 0.01$ for Na and $0.01 < P < 0.05$ for Cl) when the animal originating from fresh water is adapted to 50% sea water. In this case, K and urea concentrations are not significantly affected.

(c) When compared with terrapins adapted to 50% sea water those adapted to 100% sea water do not show any significant variation in the Na and Cl concentrations. But in contrast, the urea concentration is much greater ($P < 0.01$).

It may be remarked that in all cases the 'calculated' osmotic pressure (cf. * and †, Table 1) represents 85–90% of the osmotic pressure measured experimentally.

In conclusion in the first stage of the adaptation (fresh water → 50% sea water), the Na and Cl concentrations increase proportionately with the osmotic pressure. Between 50% sea-water animals and sea-water animals the Na and Cl concentrations do not appear to be significantly different; the percentage of osmotic pressure represented by the Na concentration varies approximately from 42 to 35%; in contrast, the variation in the urea concentration, which was not significant in the first stage of adaptation, is tremendously increased in sea-water animals, the percentage of osmotic pressure represented by urea going from 8 to 25%.

Shorter periods of adaptation have also been studied:

(1) Terrapins from fresh water and immersed for 3 days in 50% sea water already show the typical pattern of blood composition as reported in Table 1 for terrapins adapted to 50% sea water for 15 days and more; and if they are then further adapted to sea water for another 3 days the pattern obtained for the osmotic pressure and osmotically active substances is again similar to the one obtained for animals directly collected in sea water.

(2) Starting with animals living in sea water, the reverse adaptation in contrast appears as a much slower phenomenon; after 3 days in 50% sea water the animal has still high levels of Na, Cl and urea. Three more days in fresh water allow the animal to get rid of most of its urea but Na and Cl concentrations remain high; 11 days later, however, the pattern obtained is similar to the one obtained for animals which have lived in fresh water for several months.

* Water loss occurs in the first 3 days after transfer from 50% sea water to fresh water, and the animal suffers general oedema after transfer in the reverse direction. The same changes are also seen in the water content of the muscle joining humerus to clavicle; however, after 11 days of adaptation the water content of that muscle is restored to its usual value of 80%.

Table 1. Na, K, Cl, urea and osmotic pressure in the serum of the diamondback terrapin adapted to different salinities

	Na (m-equiv./l.)	Cl (m-equiv./l.)	K (m-equiv./l.)	Urea (mm/l.)	Osmotic pressure (m-osmoles/l.)	
					Measured*	Calculated†
1 Fresh water	(9) 129.0 ± 11.7	(8) 88.0 ± 17.4	(6) 3.1 ± 0.8	(5) 21.5 ± 8.8	(7) 308.8 ± 20.8	(3) 271.8 ± 22.1
2 50% fresh water	(4) 155.8 ± 10.8	(4) 112.7 ± 12.8	(4) 4.1 ± 0.5	(4) 30.1 ± 15.4	(4) 355.5 ± 50.8	(4) 325.6 ± 51.7
3 Sea water	(10) 163.4 ± 20.8	(9) 136.6 ± 20.7	(10) 3.8 ± 1.4	(5) 115.2 ± 11.8	(7) 458.8 ± 30.4	(3) 430.7 ± 14.2
Significance of differences						
1-2	$P < 0.01$ H.S.	$0.01 < P < 0.05$ S.	$P > 0.05$ N.S.	$P > 0.05$ N.S.	$0.01 < P < 0.05$ S.	
2-3	$P > 0.05$ N.S.	$P > 0.05$ N.S.	$P > 0.05$ N.S.	$P < 0.01$ H.S.	$P < 0.01$ H.S.	
1-3	$P < 0.01$ H.S.	$P < 0.01$ H.S.	$P > 0.05$ N.S.	$P < 0.01$ H.S.	$P < 0.01$ H.S.	

Mean values ± standard deviation. Number of determinations between parentheses.

† Tests were applied between groups 1 and 2, 2 and 3, and 1 and 3.

Significance of differences is based on the following criteria:

$P > 0.05$: the difference is not significant (N.S.). $0.01 < P < 0.05$: the difference is significant at 5% level (S.). $P < 0.01$: the difference is highly significant (H.S.)

* The osmometer being standardized with NaCl solutions, the osmotic pressure 'measured' gives in fact arbitrary values assuming that the osmotic pressure is due only to NaCl.

† The osmotic pressure 'calculated' is obtained by adding the ionic concentrations for Na, K and Cl to the molarity obtained for urea. This assumes that Na and K salts present in the serum are wholly dissociated while urea is not at all dissociated. This approximation is quite satisfactory for our purpose.

Urine

In the same three groups of terrapins urine samples obtained by puncture of the bladder have been analysed. Table 2 gives the results obtained for Na, K, urea and osmotic pressure. In spite of very large standard deviations, the variations in osmotic pressure and in urea concentration between fresh-water terrapins and sea-water terrapins appear to be highly significant ($P < 0.01$). The higher K concentration found in the urine of sea-water terrapins is also significant at 5% level when compared to the K concentration in the urine of fresh-water terrapins. The Na concentration, though showing very large standard deviations, remains very low in the three groups

Table 2. *Na, K, urea and osmotic pressure in the urine of the diamondback terrapin adapted to different salinities*

		Na (m-equiv./l.)	K (m-equiv./l.)	Urea (mm/l.)	Osmotic pressure (m-osmoles/l.)
1	Fresh water	(6) 4.4 ± 4.9	(6) 16.8 ± 18.7	(4) 22.0 ± 10.6	(5) 107.0 ± 44.7
2	50% sea water	(3) 25.8 ± 18.7	(3) 30.0 ± 8.1	(5) 67.9 ± 55.3	(5) 325.4 ± 103.9
3	Sea water	(7) 7.9 ± 6.1	(5) 59.7 ± 34.3	(5) 107.4 ± 30.2	(9) 372.2 ± 94.4
Significance of differences					
1-3		$P > 0.05$ N.S.	$0.01 < P < 0.005$ S.	$P < 0.01$ H.S.	$P < 0.01$ H.S.

Mean values \pm standard deviation. Number of determinations between parentheses.

t-Tests were applied between groups 1 and 3.

The significance of differences is based on the following criteria: $P > 0.05$: the difference is not significant (N.S.). $0.01 < P < 0.05$: the difference is significant at 5% level (S). $P < 0.01$: the difference is highly significant (H.S.).

It has not been possible technically to measure the Cl concentration in all the samples of urine because of abnormal potentiometric titration curves obtained by the Cotlove technique, and it is probable that some ion or a molecule is interfering. However, some samples showing a more reliable curve indicate that the Cl concentration is probably very low in the urine of all terrapins. It should also be mentioned that a high ammonia concentration can be detected in the urine of sea-water terrapins (94.3 ± 53.3 m-equiv./l.; five determinations) while in terrapins from fresh water and from 50% sea water it is always very low or undetectable by the Conway microdiffusion technique. The osmotic pressure obtained by adding up the osmolarities due to Na, K, urea and even NH_3 in the case of sea-water terrapins is always much lower than the osmotic pressure measured experimentally. This osmotic deficit is probably due mainly to the end products of the nitrogen metabolism which have not been determined.

Comparing urine and blood composition of each terrapin individually additional conclusions can be drawn:

(a) The urine of fresh-water terrapins is always hypo-osmotic to the blood while in 50% sea-water terrapins and in sea-water terrapins the urine is most of the time isosmotic to the blood.

(b) The urea concentration is always higher in the urine than in the corresponding serum whatever the salinity the animals are living in, and independently of the fact that the urine is hypo-osmotic or isosmotic to the blood.

DISCUSSION

The diamondback terrapin, *Malaclemys centrata centrata* (Latreille), is able to bear considerable changes in the salinity of its environmental medium. Its ecological distribution, however, seems to indicate that this species has advanced in evolution to a typical salt-water and brackish-water habitat (Dunson, 1969). Our results allow us to compare the osmotic pressure and the composition of the blood serum as well as of the urine of diamondback terrapins adapted to three different salinities: fresh water, 50% sea water and sea water. When the terrapin originating from fresh water is adapted to 50% sea water a significant increase in the blood osmotic pressure is observed and appears to be due exclusively to an increase in Na and Cl concentrations. This suggests that during this first step of adaptation a progressive entry of NaCl takes place. However, one can reasonably assume that excretion of NaCl occurs rapidly, sufficient after three days to balance the entry of NaCl, thus maintaining the osmotic pressure of the blood at a lower level than that of the surrounding medium (355.5 m-osmoles/l. in the blood, 450 m-osmoles/l. in 50% sea water). The urine of these animals, which is generally hypo-osmotic in fresh-water animals, becomes generally isosmotic to the blood. This phenomenon appears to solve, at least partially, the problem of water economy; however, according to our results Na, Cl, K and urea do not contribute to this increase in the osmotic pressure. The entry of NaCl must therefore be balanced through an extrarenal route, probably the orbital salt gland (Schmidt-Nielsen & Fänge, 1958; Bentley *et al.* 1967; Dunson, 1969).

Now, if we compare the sea-water terrapins to the 50% sea-water terrapins, the osmotic pressure of the serum appears once more considerably higher: but in this second step of adaptation Na and Cl concentrations are no more involved; urea alone appears as responsible for this increase. Indeed, the sum of the osmolarities due to Na, Cl, K and urea accounts for 85 to 90% of the osmotic pressure measured experimentally in any salinity. The urine of the sea-water terrapins is generally isosmotic to the blood and its osmotic pressure in this second step of adaptation seems to be increased mainly by urea but also by high amounts of ammonia. Since the urea concentration is always higher in the urine than in the corresponding serum when each animal is individually considered, one could reasonably assume that the increase in the urea concentration of the blood is due to a reabsorption from urine probably at the level of the bladder where urea accumulates; this accumulation of urea in the bladder does not necessarily implicate a modification in the catabolism of nitrogen compounds; for one knows that the urine volume voided by sea-water animals is about one fifth of the urine volume voided by turtles adapted to tap water (Bentley *et al.* 1967); indeed, a longer stay of the urine in the bladder not only allows the animal to recuperate a greater amount of water through the vesicular epithelium but also enables it to use one of the end-products of nitrogen metabolism as an osmoregulatory effector. The high amount of ammonia detected in the urine of sea-water animals could also simply result from an accumulation in the bladder, this accumulation being still more acute than for urea since ammonia does not appear in the serum except at very low concentrations and therefore is probably not reabsorbed at the level of the vesicular epithelium. However, additional experimental support is required before it is known whether nitrogen catabolism of the diamondback terrapin, which is at one and the

same time uricotelic and ureotelic, is affected by the salinity of the external medium. For that purpose the percentages contributed by urea, uric acid and ammonia should be established taking account of the possible transfer of urea into the blood and accumulation of uric acid and ammonia in the bladder, and also of the rate of urine production in various conditions of salinity.

In summary, the terrapin going from fresh water to 50% sea water shows an increase in Na and Cl concentrations of its blood. This entry of NaCl is rapidly balanced, probably through the intermediary of the orbital salt gland, since a steady-state of concentration is obtained in the blood after only 3 days. Going from 50% sea water to sea water the animal calls upon another mechanism to avoid an excess of NaCl together with a too important loss of water using one of the end-products of the nitrogen metabolism, urea. The resulting osmotic pressure of the blood remains, however, half that of the sea water.

According to this view it is most interesting to remember the experiments of Schoffeniels & Tercafs (1965-6*a*), who have adapted a marine turtle *Caretta caretta* L. to fresh water, and a fresh-water turtle *Clemmys leprosa* L. to sea water. These authors came to the conclusion that in both cases the osmoregulatory mechanisms are overwhelmed, inducing in both cases a tremendous variation of the blood osmotic pressure. This could be explained in the case of *Clemmys* by the absence of salt glands and inability to use urea as an osmoregulatory effector. As for *Caretta* when living in sea water, its osmotic pressure and its Na and Cl concentrations are rather similar to those measured in the diamondback terrapin adapted to sea water (present paper) but its urea concentration is much lower. This implies that *Caretta* is able to maintain its osmotic pressure considerably lower than that of sea water without involving urea. This could be achieved if the salt gland had a higher capacity in secreting NaCl, which is probably the case. Indeed, Dunson (1969) reports in the diamondback terrapin a Na secretion rate of 26.6 μ -moles/100 g. hr., while Holmes & McBean (1964) in the sea turtle, *Chelonia mydas*, report a Na secretion rate of 134 μ -moles/100 g. hr.

Therefore it seems that animals like the diamondback terrapin represent the intermediary stage in evolution from fresh water to sea water.

Osmoregulatory mechanisms involving both salts and urea also operate in amphibians when adapted to sea water (Gordon, Schmidt-Nielsen & Kelly, 1961; Tercafs & Schoffeniels, 1962; Schoffeniels & Tercafs, 1965-6*b*). However, the part played by the salt gland and the bladder in chelonians would be played in amphibians mainly by the skin. Moreover it should be noted that, while in amphibians the osmotic pressure of the blood approximately equals that of the external medium when adapted to sea water, in chelonians in contrast the osmotic pressure remains much lower.

According to Florkin (1966) ureogenesis, an invention of primitive fishes for their transfer from fresh water to sea water, would be at work in various ecological aspects in the life of amphibians: euryhalinity of certain aquatic species, resistance to evaporation in some terrestrial forms. A parallel utilization of ureogenesis appears to exist in chelonians. Indeed, ureogenesis allows the transfer from fresh water to sea water in the diamondback terrapin; moreover, in the dehydrated desert tortoise, *Gopherus agassizii*, the increase in the osmolality of the blood is principally due to an increase in urea concentration (Dantzler & Schmidt-Nielsen, 1966). It is also most interesting to note that urea also plays a part in the terrestrial tortoise, *Testudo hermanni hermanni*

Gmelin, in the course of hibernation (Gilles-Baillien & Schoffeniels, 1965-6; Gilles-Baillien, 1966, 1969).

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SUMMARY

1. The blood of the diamondback terrapin going from fresh water to 50% sea water shows an increase in its osmotic pressure which is mainly due to an increase in Na and Cl concentrations.

2. The blood of terrapins living in sea water compared with the blood of terrapins living in 50% sea water shows a higher osmotic pressure which is the result solely of a higher urea concentration; Na and Cl concentrations are no longer affected in this second stage of adaptation.

3. Urine of 50% sea-water terrapins and of sea-water terrapins is generally isosmotic to the blood while the urine of fresh-water terrapins is usually hypo-osmotic.

4. The bladder appears to play an essential part in reducing water loss in the sea-water terrapins but is not implicated in the salt balance.

5. When each animal is considered individually, the urea concentration in the urine is always higher than in the serum, suggesting that the high urea concentration in the blood of terrapins adapted to sea water is due to an urea accumulation in the bladder.

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