

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

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INTRODUCTION

How the salt water balance is achieved in the chelonian reptile *Malaclemys centrata* when acclimatized to various salinities is a problem which has aroused some interest.

Indeed it is well established that the blood of this terrapin living in sea water has a higher osmotic pressure than when acclimatized to fresh water, and that this higher osmotic pressure is due to higher sodium and chloride concentrations and also and mainly to a greater content in urea (see Bentley, Bretz & Schmidt-Nielsen, 1967; Dunson, 1970; Gilles-Baillien, 1970*a*). However, this higher osmotic pressure is maintained at a value which is still less than half that of sea water. This indicates that the animal is able to limit salt gain and water loss. The activity of the salt gland (Schmidt-Nielsen & Fänge, 1958; Dunson, 1970) together with the increase in urea concentration are apparently the two main devices at work; the latter would be operated not by an enhanced production of urea but rather by a longer retention of the urine in the bladder from where urea could pass back to the blood (Gilles-Baillien, 1969*a*, 1970*a*). This assumption is supported by the fact that the urine flow is lower in sea-water animals (Bentley *et al.* 1967). Furthermore, while the urine of the animals in sea water is isosmotic to the blood, the urine of the freshwater animals is always hypo-osmotic which helps the animal to maintain its salt balance when in fresh water (Gilles-Baillien, 1970*a*).

Among reptiles that have colonized the northern hemisphere many species undergo hibernation during the cold season. The diamondback terrapin is one of these. It is said to bury itself in the mud lining marshes close to estuaries, or even in the mud covering the bottom of brackish-water marshes; but there is no official confirmation of this. Nonetheless, it is known that two turtles (*Graptemys geographica* and *Chelydra serpentina*) do hibernate on the bottom of Lake Ontario which is covered with ice for most of the winter (Semple, Sigsworth & Stitt, 1969). Indeed as far as seasonal variations in reptiles are concerned currently available information is contradictory; but it is agreed that during hibernation certain active mechanisms are in abeyance. It is known, for instance, that active transport of sugars (Latif, Zain & Zain-ul-Abedin, 1967), amino acids (Gilles-Baillien, 1970*b*; Quadri, Zain & Zain-ul-Abedin, 1970)

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and sodium (Gilles-Baillien, 1970*b*) is strongly inhibited in the intestine during that time. Furthermore, it has been shown that the water permeability of the bladder mucosa is altered during hibernation (Gilles-Baillien, 1969*b*). These observations being made essentially on terrestrial species of reptiles, it was of major interest to start an investigation on an aquatic species which, furthermore, has the ability to survive either in fresh water or in sea water. We have therefore undertaken a study on the seasonal variations occurring in the composition of the blood when these variations are superimposed on conditions of hyper-osmotic or hypo-osmotic stress.

MATERIAL AND METHODS

Diamondback terrapins were collected in the brackish-water marshes in the area of Beaufort N.C. Two batches of turtles were kept for at least a whole year, one in sea water and the other in fresh water. The technique used for blood sampling was carried out as previously described (Gilles-Baillien, 1970*a*). But while in the earlier publication the blood sera were analysed after a more or less long stay in a deep-freeze, in the present study the plasma obtained after heparinization and further centrifugation was immediately diluted with 0.08 N nitric acid for determinations of Na, K and Cl. For animals in similar conditions, Na values were always higher than were found using the earlier technique.

Equipment used for determinations of Na, K and Cl and for the measurement of the osmotic pressure were the same as those reported earlier. The urea concentration was determined according to a colorimetric method prescribed by SIGMA (Technical Bulletin No. 14).

RESULTS

Table 1 shows the changes in the osmotic pressure and in the composition of the blood plasma over a year when diamondback terrapins were maintained either in fresh water or in sea water. In both fresh-water and sea-water terrapins, the lowest values of osmotic pressure were recorded in July. But in September, before entering hibernation (which occurred that year at the end of October), an increase of osmotic pressure was observed in the fresh-water animals which declined progressively during hibernation. In the sea-water animals the blood osmotic pressure started to increase in September but kept on increasing during hibernation to reach maximal values at the period of arousal (middle of April). A rather abrupt decrease occurred only after May when the animals were again feeding regularly.

The urea concentration followed approximately the same pattern. For the terrapins kept in fresh water a peak value was recorded in September before the animals entered hibernation. In the terrapins maintained in sea water the urea concentration began to increase in the period September to November and stayed high until the end of the hibernating period.

As far as inorganic ions are concerned, Na and Cl concentrations were maximal for fresh-water animals from May to September. Then the Na, and also the Cl, concentrations were progressively lowered during hibernation but increased again just before arousal. In the sea-water animals the variations in Na and Cl were rather similar. A slow decrease was observed during hibernation and just before arousal a renewed increase was initiated. But a striking phenomenon was the invasion by Na

Table 1. Seasonal variations in the sodium, potassium, chloride and urea concentrations and in the osmotic pressure in the blood plasma of *Malaclemys centrata* when kept in sea water (SW) or in fresh water (FW)

Month	Na		K		Cl		Urea		Osmotic pressure	
	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW
February	118.9 ± 13.2 (3)	152.4 ± 13.5 (3)	2.5 ± 0.9 (3)	4.2 ± 1.1 (3)	51.4 ± 7.4 (3)	138.5 ± 3.5 (3)	12.9 ± 6.0 (3)	80.7 ± 11.2 (3)	277.7 ± 10.3 (3)	440.4 ± 28.6 (3)
April beginning	129.7 ± 7.4 (6)	199.6 ± 14.3 (5)	1.8 ± 0.5 (4)	3.0 ± 0.6 (4)	73.1 ± 8.2 (8)	150.5 ± 13.6 (7)	12.1 ± 5.1 (9)	95.5 ± 23.1 (8)	281.1 ± 11.8 (8)	512.3 ± 43.7 (7)
April end	133.8 ± 1.6 (3)	198.6 ± 11.1 (3)	3.5 ± 0.9 (3)	4.3 ± 0.4 (3)	97.0 ± 12.0 (3)	205.0 ± 12.0 (3)	10.4 ± 5.4 (3)	83.7 ± 11.3 (3)	288.3 ± 9.8 (4)	532.7 ± 33.6 (3)
May	157.1 ± 9.1 (3)	227.8 ± 26.0 (3)	4.8 ± 1.5 (3)	5.5 ± 0.7 (3)	111.6 ± 8.9 (3)	316.4 ± 35.3 (3)	12.4 ± 2.5 (3)	67.4 ± 4.8 (3)	277.8 ± 9.0 (5)	492.3 ± 43.2 (5)
July	152.3 ± 2.8 (3)	176.7 ± 16.6 (3)	6.1 ± 1.1 (3)	5.7 ± 0.5 (3)	95.0 ± 7.8 (3)	119.8 ± 11.8 (3)	8.5 ± 3.6 (3)	14.6 ± 3.9 (3)	255.7 ± 35.6 (5)	333.5 ± 13.1 (3)
September	150.5 ± 5.4 (3)	181.0 ± 2.8 (3)	5.5 ± 0.5 (3)	5.7 ± 0.3 (3)	87.4 ± 4.6 (3)	126.2 ± 2.6 (3)	27.0 ± 3.9 (3)	43.8 ± 20.3 (3)	341.7 ± 13.2 (3)	406.3 ± 10.6 (3)
November	126.9 ± 18.6 (4)	179.3 ± 19.9 (4)	2.3 ± 0.3 (3)	2.9 ± 0.6 (4)	71.2 ± 5.7 (7)	134.0 ± 27.3 (6)	40.5 ± 6.8 (3)	85.8 ± 24.4 (6)	324.6 ± 12.0 (3)	447.6 ± 55.2 (5)
December	118.8 ± 14.2 (5)	162.7 ± 17.2 (3)	1.8 ± 0.1 (4)	2.4 ± 0.3 (4)	72.9 ± 11.7 (5)	149.1 ± 16.6 (4)	25.4 ± 10.6 (3)	83.6 ± 29.7 (3)	320.0 ± 19.1 (5)	479.3 ± 27.1 (4)

Concentrations in m equiv/l or mm/l. Osmotic pressure in m osmoles/l. Mean values ± s.d.; Numbers in between brackets = number of individuals tested.

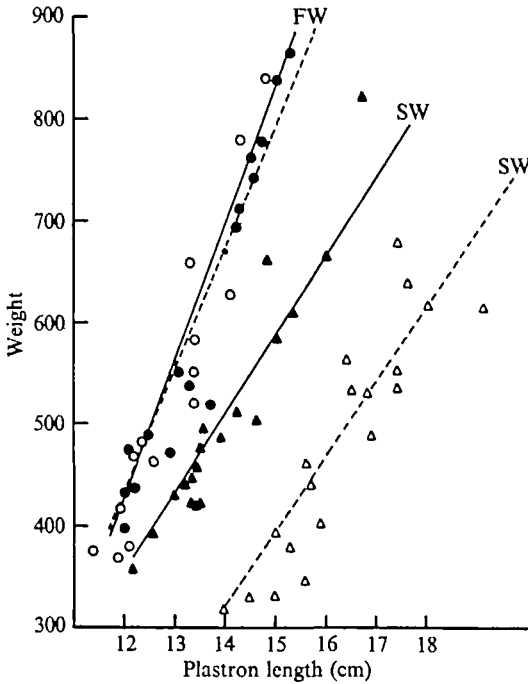


Fig. 1. Mean regression curve of the weight of *Malaclemys centrata* as a function of the plastron length, for animals maintained either in fresh water or in sea water. ●, Fresh water, 5 April; ○, fresh water, 27 July; ▲, sea water, 5 April; △, sea water, 27 July.

and mainly by Cl which occurs at arousal. The Cl concentration recorded in May is exceptionally high (316 m-equiv/l), while in July it has resumed a very low value (120 m-equiv/l).

As to K, the highest values are measured in summer and the lowest during hibernation, this being true for both groups of terrapins, but this ion tends always to be higher in the sea-water animals.

What appears to us as the most surprising event in these seasonal variations occurring in the blood plasma composition is the drastic invasion by Na and above all by Cl that happens at the arousal of the terrapins maintained in sea water. This salt gain is probably linked to a passive entry of sea water into the animal at the moment of arousal. Indeed when comparing the mean regression curve obtained by plotting the weight of the animal versus the length of the plastron, it is shown that the sea-water animals have a higher weight at the period of arousal than three months later in July (Fig. 1). In contrast no significant change is observed between these periods in the fresh-water animals. The weight gain observed in the sea-water animals at the moment of arousal and the concomitant increase of the salt concentration in the blood can therefore only be supplied by the environmental sea water, since the animals are not fed at that period. It is also very important to note the tremendous difference of weight in July between the sea-water animals and the fresh-water animals.

DISCUSSION

The results presented above demonstrate that the blood of terrapins acclimatized to sea water has always a higher osmotic pressure than in those acclimatized to fresh water, this being true in every month of the year. However, it is in July that the difference is the least marked. It is therefore in midsummer that the terrapins are the more able to contend with the osmotic gradient of their environment, but this is achieved to the detriment of the water balance as inferred by the difference in the weight of the animals in the two conditions of salinity (Fig. 1). It is at that period too that the terrapins in both conditions have the lowest osmotic pressure. From there on the sea-water animals show a progressive increase in their osmotic pressure until December, followed by a slight depression. But at the period of arousal a sudden important increase again occurs which is reduced only after May. Whereas urea is mainly involved in the first increase, in the second increase, occurring at arousal, it is mostly Na and Cl which are implicated. It is rather remarkable that during the whole period of hibernation the animal in sea water is able to prevent any salt gain, and even seems to lose some NaCl from December to February, and in this situation urea plays an important part as osmo-effector. The accumulation of urea in the blood could reflect a partial or total anuria as is the case in a terrestrial chelonian *Testudo hermanni* during hibernation (Gilles-Baillien & Schoffeniels, 1965-6; Gilles-Baillien, 1969*a*). As to the invasion by Na and Cl occurring at arousal, it could result from a suddenly increased permeability of the integument but also and more probably of the digestive tract. The permeability characteristics of the intestinal epithelium of *T. hermanni* have been shown to be subject to seasonal variations; and more specially the active transport of Na appears to be strongly inhibited at this level during hibernation (Gilles-Baillien, 1970*b*). If in the diamondback terrapin the salt gain at arousal results from the re-establishment of active Na uptake from the intestine this would imply that the animal drinks sea water; indeed the animals were not fed before the end of May but on the other hand the fact that the diamondback terrapin drinks sea water has been denied by Bentley *et al.* (1969).

In *T. hermanni* it has also been shown that the water permeability of the bladder mucosa is higher in active animals than in torpid ones, and moreover that this change of permeability appears rather abruptly at arousal (Gilles-Baillien, 1969*b*). If such modifications are also at play in the diamondback terrapin in sea water at the moment of arousal, it could explain at least partially the salt gain in the blood as well as the weight gain of the animal at arousal. Then the observed increased tonicity of the blood could act on the secretory activity of the salt gland in the same way as that proposed for the avian salt gland (see Peaker, 1971) re-setting the animal's metabolism to active life and feeding.

Let us now consider what happens to the animal which is undergoing hibernation in fresh water. An important fact is the increase in osmotic pressure of the blood in September. This increase, attributable in small part to an increase in urea concentration, must be explained by the appearance in the blood of another compound than those tested. For although in July the sum of Na, K, Cl and urea accounts rather well for the total osmotic pressure measured, this is far from being the case in September or during the whole hibernating period. The Na and Cl concentrations

unaffected in September, start to decrease along the hibernating period, together with the urea concentration which was maximal in September. This last fact would mean that the animal still urinates when kept in fresh water, thus helping the animal to contend with the dilution of its fluids. No significant change in the weight of the animal occurs between arousal and full summer, which probably indicates that the animal controls its fluid volumes more adequately than its ionic composition during hibernation. This is also the case for the common snapping turtle (*Chelydra serpentina*) when hibernating on the bottom of the Lake Ontario (Semple *et al.* 1969). Higher values of Na and Cl in the blood during summer could also indicate, along with the results obtained with the terrestrial tortoise (Gilles-Baillien, 1970b) that active transport of Na is more effective during summer at both levels of the intestine, small intestine and colon. A better ability to excrete a hypotonic urine could also contribute to it by a better resorption of Na in the kidney or in the bladder. The part played by the integument in salt/water balance has long been assumed to be restricted. However, it is now well established that the skin of two aquatic turtles *Tryonix spinifer* and *Pseudemys scripta* is not totally impermeable to water (Bentley & Schmidt-Nielsen, 1970).

The general decrease in the K concentration of the blood during hibernation for both groups of terrapins could be explained either by a redistribution of K between extracellular and intracellular fluids or by a storage in the urine as is the case for *Testudo hermanni* (Gilles-Baillien, 1970b).

In conclusion the results presented here indicate that diamondback terrapins are able to support wide variations in the composition and osmotic pressure of their blood. They represent in chelonians an intermediate step in the evolution of fresh-water species to sea-water species since they still have the capacity to survive in fresh water and they have already acquired the indispensable devices needed for the survival of chelonian species in sea water, namely the salt-gland. Their ability to retain urea is profitably used to help the salt gland in maintaining the salt/water balance. The secretory capacity (rate of secretion) of the salt gland has indeed not yet reached in this species (Dunson, 1970) the level acquired in pelagic turtles such as *Chelonia mydas* (Holmes & McBean, 1964). Another pelagic turtle *Caretta caretta* equipped with a salt gland which excretes Na at a very high concentration (Schmidt-Nielsen & Fänge, 1958) has on the other hand lost the ability to use urea as an osmo-effector (Schoffeniels & Tercafs, 1965-6).

Our results demonstrate that diamondback terrapins are able to withstand hibernation and the seasonal modifications implicated in the process in sea water as well as in fresh water. Hibernation in reptiles appears to be characterized by important modifications of the permeability characteristics of the cellular membranes and more specially of those of the limiting epithelia probably through hormonal influence (Gilles-Baillien, 1972). It is probably at these levels that one must look for the origin of the seasonal variations of the blood composition in the diamondback terrapin. However, the aptitude of the species to face these variations in sea water or in fresh water and to survive, probably explains its large distribution in regions where it has to contend with large and sometimes abrupt changes in the salinity of the environment (see Dunson, 1970).

SUMMARY

1. Two batches of diamondback terrapins have been kept for a whole year, one in sea water the other in tap water, and seasonal variations have been recorded in the composition and osmotic pressure of the blood plasma.
2. All year round the sea-water animals have a higher osmotic pressure and higher concentrations of Na, K, Cl and urea than fresh-water animals. It is in July, however, that these differences are the least marked.
3. The seasonal variations recorded are linked in particular to the conditions of osmotic stress imposed by the environment.
4. The results are discussed within the framework of hibernation and of the evolution among chelonians from fresh water to sea water.

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REFERENCES

- BENTLEY, P. J. & SCHMIDT-NIELSEN, K. (1970). Comparison of water exchange in two aquatic turtles, *Trionyx spinifer* and *Pseudemys scripta*. *Comp. Biochem. Physiol.* **32**, 363-5.
- BENTLEY, P. J., BRETZ, W. L. & SCHMIDT-NIELSEN (1967). Osmoregulation in the diamondback terrapin, *Malaclemys terrapin centrata*. *J. exp. Biol.* **46**, 161-7.
- DUNSON, W. A. (1970). Some aspects of electrolyte and water balance in three estuarine reptiles, the diamondback terrapin, American and 'salt water' crocodiles. *Comp. Biochem. Physiol.* **32**, 161-74.
- GILLES-BAILLIEN, M. (1969a). Seasonal variations in blood and urine constituents of the tortoise *Testudo hermanni hermanni* Gmelin. *Archs. int. Physiol. Biochim.* **77**, 427-40.
- GILLES-BAILLIEN, M. (1969b). Seasonal changes in the permeability of the isolated vesical epithelium of *Testudo hermanni hermanni* Gmelin. *Biochim. biophys. Acta* **193**, 129-36.
- GILLES-BAILLIEN, M. (1970a). Urea and osmoregulation in the diamondback terrapins *Malaclemys centrata centrata* (Latreille). *J. exp. Biol.* **52**, 691-7.
- GILLES-BAILLIEN, M. (1970b). Permeability characteristics of the intestinal epithelium and hibernation in *Testudo hermanni hermanni* Gmelin. *Arch. int. Physiol. Biochem.* **78**, 327-8.
- GILLES-BAILLIEN, M. (1973). Seasonal variations. In *Chemical Zoology*, vol. IX, Section IV. Edited by M. Florkin and B. T. Scheer. New York and London: Academic Press. (In the Press.)
- GILLES-BAILLIEN, M. & SCHOFFENIELS, E. (1965-6). Variations saisonnières dans la composition du sang de la tortue grecque *Testudo hermanni* J. F. Gmelin. *Annls. Soc. r. zool. Belg.* **95**, 75-9.
- HOLMES, W. N. & MCBEAN, R. L. (1964). Some aspects of electrolyte excretion in the green turtle, *Chelonia mydas mydas*. *J. exp. Biol.* **41**, 81-90.
- LATIF, S. A., ZAIN, B. K. & ZAIN-UL-ABEDIN, M. (1967). Intestinal transport of sugars in a lizard during hibernation and activity. *Comp. Biochem. Physiol.* **23**, 121-8.
- PEAKER, M. (1971). Avian salt glands. *Phil. Trans. R. Soc. B* **262**, 289-300.
- QUADRI, M., ZAIN, B. K. & ZAIN-UL-ABEDIN, M. (1970). Intestinal transport of amino-acids in a lizard during hibernation and activity. *Comp. Biochem. Physiol.* **36**, 569-77.
- SCHMIDT-NIELSEN, K. & FANGE, R. (1958). Salt glands in marine reptiles. *Nature, Lond.* **182**, 783-5.
- SEMPLE, R. E., SIGSWORTH, D. & STITT, J. T. (1969). Composition and volume of fluids in winter and summer of turtles native to Ontario, Canada. *J. Physiol.* **204**, 39-40P.

