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ISOSMOTIC REGULATION IN VARIOUS TISSUES OF THE DIAMONDBACK TERRAPIN *MALACLEMYS CENTRATA CENTRATA* (LATREILLE)*

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INTRODUCTION

Diamondback terrapins are brackish-water turtles that can be caught wild in regions of different or varying salinity (Dunson, 1970). When acclimatized either to fresh water or to sea water they show modifications in the osmotic pressure and composition of their blood (Bentley, Bretz & Schmidt-Nielsen, 1967; Gilles-Baillien, 1970). During summer months terrapins collected in sea water have a higher blood osmotic pressure (460 m-osmoles/l) than animals acclimatized to fresh water for several months (310 m-osmoles/l). This higher osmotic pressure is due to greater Na and Cl concentrations but also and mainly to a higher content of urea (Gilles-Baillien, 1970). As it is usually taken for granted that cells are isosmotic to the blood, our purpose was to investigate which ions or molecules are implicated in the intracellular isosmotic regulation of the tissues. This paper reports the modifications in the inorganic ions and in some nitrogen compounds in various tissues of diamondback terrapins acclimatized either to fresh water or to sea water.

MATERIAL AND METHODS

The tissue sampling was carried out during summer months (from May till the end of September) in diamondback terrapins collected and kept in sea water on the one hand and in others acclimatized for several months to fresh water on the other hand. Several tissues were selected. The muscle tested was the one joining the humerus to the clavicle. Mucosae of bladder, jejunum and colon were excised and by dissection cleared of muscular and connective tissues.

Na, K and Cl were determined, after extraction with HNO_3 0·08 N and adequate dilution, either with a Beckman flame photometer for Na and K or with a Buchler–Cotlove chloridometer for Cl. Amino acids and taurine were estimated with a Beckman amino acid autoanalyser. The urea concentration was measured according to a colorimetric technique involving a digestion by urease (SIGMA Technical Bulletin No. 14).

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Table 1. *Comparison of the inorganic ion content of various tissues in diamondback terrapins acclimatized either to sea water (SW) or to fresh water (FW)*

	Na		K		Cl	
	FW	SW	FW	SW	FW	SW
Muscle	38·2± 5·4	41·3±14·4	81·5±23·4	86·5±12·9	26·0± 4·7	27·3±10·1
	N.S.		N.S.		N.S.	
Bladder	154·2±15·2	134·6±25·8	26·1± 1·9	29·4± 1·2	113·8±13·9	122·5±16·0
	N.S.		H.S.		N.S.	
Colon	103·2± 7·8	120·6±14·3	40·5± 8·0	35·9± 5·1	80·7± 7·3	96·5±15·2
	S.		N.S.		H.S.	
Jejunum	53·8±16·5	79·4±26·7	53·7±18·1	88·2±20·1	50·6± 5·8	69·6±22·8
	N.S.		S.		N.S.	

Results expressed in m.equiv/kg wet weight. Six individuals in each condition.
The probability of differences in the means is reported as follows: N.S., the change is not significant. S., the change is significant (at the 5% level). H.S., the change is highly significant (at the 1% level).

RESULTS

The inorganic ion content of the muscle and of the bladder jejunum and colon mucosae has been estimated in diamondback terrapins collected and kept in sea water and in others acclimatized to fresh water for several months. Table 1 shows the results obtained. In the muscle there is no significant difference in the inorganic ion content. In the bladder mucosa the only significant variation is a higher content in K for the sea-water animals; but in absolute value this variation is far from being important. In the colon mucosa higher concentrations in Na and Cl are found for the terrapins in sea water, but the K concentrations are similar for animals in both conditions. The reverse is the case for the jejunum mucosa: Na and Cl concentrations are comparable for animals in both conditions while the K content is much higher in the sea-water terrapins.

A comparison of the free amino acids has also been carried out in the same tissues between a diamondback in sea water and another in fresh water (Table 2). Considering first the total of the free amino acids estimated, one can say that in the four tissues investigated the free amino acid pool is higher for the animals kept in sea water and the difference is especially important in the muscle and in the jejunum mucosa. In the muscle all amino acids are implicated in the variation except aspartate. In the jejunum mucosa all of them without exception are involved. But while the muscle of sea-water animals shows a higher content not only in NH_3 but also (and mainly) in taurine and urea, in the case of the jejunum NH_3 is lower in sea-water animals, the taurine content is similar in both conditions and the urea concentration is much higher in sea-water animals.

In the colon mucosa and in the bladder mucosa the variation in the amino acid content is less impressive. Nonetheless, in the case of the bladder the total content is more than doubled, the glutamate is tripled; the taurine concentration is also approximately doubled. In the colon mucosa, the glutamate content is also higher in sea-water animals, the taurine content in contrast is lower. In both bladder and colon urea is the compound showing the widest variation.

Table 2. *Comparison of the amino acid, taurine and urea content of various tissues in the diamondback terrapin acclimatized either to fresh water or to sea water*

	Muscle		Bladder		Colon		Jejunum	
	FW	SW	FW	SW	FW	SW	FW	SW
Aspartic acid	0·160	0·108	0·201	0·423	0·196	0·314	0·219	1·696
Threonine	0·080	Tr	0·039	0·133	0·207	0·184	0·100	1·655
Serine	0·163	1·388	0·141	0·377	0·322	0·305	0·343	2·707
Glutamic acid	0·218	3·113	0·563	1·526	1·017	1·451	0·525	5·653
Proline	Tr	0·743	0·145	0·150	0·348	0·387	0·395	2·491
Glycine	1·260	3·819	0·163	0·311	0·291	0·433	0·588	5·414
Alanine	0·410	2·090	0·085	0·297	0·241	0·320	0·661	5·679
Cystine	0·068	0·114	Tr	Tr	Tr	Tr	Tr	Tr
Valine	0·848	1·267	0·046	0·168	0·184	0·212	0·157	2·034
Methionine	Tr	Tr	Tr	Tr	Tr	0·057	Tr	0·583
Isoleucine	0·050	0·206	Tr	0·080	0·072	0·150	0·052	1·326
Leucine	0·073	0·296	Tr	0·140	0·121	0·246	0·075	1·871
Tyrosine	0·068	0·299	Tr	0·094	0·065	0·144	0·051	0·670
Phenylalanine	0·030	0·108	Tr	Tr	0·046	0·040	Tr	0·647
Lysine	0·280	0·894	0·097	0·245	0·133	0·260	0·152	2·320
Histidine	1·180	1·988	Tr	Tr	0·036	Tr	Tr	0·702
Arginine	0·105	0·373	0·107	0·164	0·100	0·173	0·091	1·332
Total A.A.	4·993	16·806	1·587	4·108	3·379	4·676	3·409	36·780
Taurine	15·452	35·233	2·599	4·957	5·652	3·993	13·390	12·711
Ammonia	1·285	3·033	0·219	0·748	0·976	0·973	2·232	1·273
Urea*	Tr	64·1	3·02	47·5	Tr	12·9	Tr	41·1

Results expressed in mmoles/kg wet weight. Tr: traces.

* Urea determinations were obtained by a separate technique (cf. Material and Methods).

DISCUSSION

Our aim in this paper was to search for the compounds involved in the isosmotic regulation of the diamondback terrapin. Indeed when animals in sea water and in fresh water are compared some modifications of the inorganic ion content as well as of the amino acid content in response to the change in the osmolarity of the blood are seen. However, in the muscle no significant change in the inorganic ion content could be detected. This is at variance with results obtained in two euryhaline species of teleosts, *Paralichthys lethostigma* and *Crenimugil labrosus*, where the inorganic ion content varies according to the salinity in their environment (Lasserre & Gilles, 1971). In these same species all the non-essential amino acids especially are subject to changes while in the diamondback terrapin all the amino acids except aspartate are involved. This illustrates once more that the cells of invertebrates are not the only ones where amino acids vary during acclimatization to different salinities (see Schoffeniels & Gilles, 1970). Indeed the variations expressed as percentages are here also of great extent but they refer to values which are much lower than those found in the cells of invertebrates. Therefore when considering the final balance of osmotic pressure that must be achieved by the muscle when the diamondback terrapin goes from fresh water to sea water neither inorganic ions nor amino acids are the important effectors in the osmotic regulation.

But taurine and urea appear to be more important osmo-effectors. For urea one

can assume that it slowly diffuses from the blood. As to taurine it seems to be implicated in the osmotic adjustment of the muscle in all animals investigated.

In the bladder mucosa as far as inorganic ions are concerned only a very small increase in K is recorded in sea-water animals. Amino acids, taurine and NH_3 are also weak osmo-effectors. Only urea appears to play an important part in the intracellular osmotic pressure.

In the colon, Na, Cl and urea are associated with a larger osmotic pressure but other unknown compounds must be involved to a greater extent.

In the jejunum amino acids together with urea are greatly implicated in the osmotic adjustment. Moreover, there is an important increase in K. In a terrestrial tortoise (*Testudo hermanni*) during hibernation there is also an increase in the osmotic pressure of the blood due to Na, Cl and mainly urea (Gilles-Baillien & Schoffeniels, 1965–6). In this species an important increase in K of the jejunum mucosa in response to hibernation has also been recorded (Gilles-Baillien, 1969). In contrast, in other tissues inorganic ions appear to be more involved in osmotic adjustment than they are in the diamondback terrapin.

Among vertebrates it seems therefore that the mechanisms of adjustment of osmotic pressure by amino acids are still present at least in certain teleosts (see for instance Lasserre & Gilles, 1971) in some amphibians (see, for instance, Schoffeniels & Tercafs, 1965) in a chelonian reptile *Malaclemys centrata* (this paper). But their important role in invertebrates is partially supplanted in vertebrates by urea in species that have developed the system of ureogenesis (see Cohen & Brown, 1960; Florkin, 1966) or by devices specialized in achieving salt/water balance, thus ensuring variations of smaller amplitude in osmolarities of the blood and tissues.

SUMMARY

1. Osmotic adjustment is achieved by blood and intracellular fluids in the diamondback terrapin when acclimatized either to fresh water or to sea water.

2. The muscle adjusts its composition to a higher blood osmotic pressure by greater concentrations in ammonia, in taurine and in urea and to a lesser extent in all amino acids (aspartate excepted). The inorganic ion content is not affected.

3. In the bladder mucosa ammonia, taurine and all amino acids are more concentrated in sea-water animals. But essentially urea is responsible for the higher osmotic pressure. Of the inorganic ions only potassium shows a (slight) increase in sea-water animals.

4. In the colon mucosa there is a slight increase in the total amino acid content, in the concentrations of sodium and chloride, and a larger increase in urea.

5. In the jejunum mucosa the concentrations of amino acids, urea and K are much higher in sea-water animals.

6. The results are discussed within the framework of isosmotic regulation of intracellular fluids.

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REFERENCES

BENTLEY, P. J., BRETZ, W. L. & SCHMIDT-NIELSEN, K. (1967). Osmoregulation in the diamondback terrapin, *Malaclemys terrapin centrata*. *J. exp. Biol.* **46**, 161–7.

COHEN, P. P. & BROWN, G. W., Jr. (1960). Ammonia metabolism and urea biosynthesis. In M. Florkin and H. S. Mason, *Comparative Biochemistry*, vol. **2**, pp. 161–244. New York and London: Academic Press.

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.

FLORKIN, M. (1966). *Aspects moléculaires de l'adaptation et de la phylogénie*. Paris: Masson & Cie.

GILLES-BAILLIEN, M. (1969). Seasonal changes in the inorganic ion content of various tissues in the tortoise *Testudo hermanni hermanni* Gmelin. *Life Sci.* **8**, part II, 763–6.

GILLES-BAILLIEN, M. (1970). Urea and osmoregulation in the diamondback terrapin *Malaclemys centrata centrata* (Latreille). *J. exp. Biol.* **52**, 691–7.

GILLES-BAILLIEN, M. & SCHOFFENIELS, E. (1965–6). Variations saisonnières dans la composition du sang de la tortue grecque. *Annls. Soc. r. Zool. Belg.* **95**, 75–9.

LASSERRE, P. & GILLES, R. (1971). Modification of the amino acid pool in the parietal muscle of two euryhaline teleosts during osmotic adjustment. *Experientia* **27**, 1434–5.

SCHOFFENIELS, E. & GILLES, R. (1970). Nitrogenous constituents and nitrogen metabolism in Arthropods. In *Chemical Zoology*, vol. v, part A (ed. by M. Florkin and B. T. Scheer). New York and London: Academic Press.

SCHOFFENIELS, E. & TERCAFS, R. (1965). L'osmorégulation chez les Batraciens. *Annls. Soc. r. zool. Belg.* **96**, 23–39.