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Seasonal Variation in blood plasma sodium and potassium concentrations in the lizard *Agama nupta* (Agamidae)

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ABSTRACT. Seasonal changes in the concentrations of Na and K ions in the blood of *Agama nupta* in summer are reported from a population in Iran. These lizards do not have access to free water and acquire all their required water from their diet of insects. The hypernatraemia and hyperkalaemia reported here may be the result of chronic dehydration but could also result from the accumulation of ions in the extracellular fluid, as has been shown in other species of lizards. In contrast to herbivorous species of lizards, *A. nupta*, which is insectivorous, does not appear to have an active nasal salt gland for excreting sodium and potassium as salt-loading in the laboratory failed to evince any secretions.

KEY WORDS : *Agama nupta,* sodium, potassium, blood.

INTRODUCTION

There have been a number of published studies with lizards showing that many species, but not all, experience increased blood sodium (hypernatraemia) and potassium (hyperkalaemia) concentrations in summer when supplies of water are at a premium (BRADSHAW, 1986; BRADSHAW, 1997). Hypernatraemia has been shown to result from excessive dietary intake of sodium ions (BRADSHAW & SHOEMAKER, 1967). This study is the first to examine water and electrolyte balance in the Iranian agamid lizard *Agama nupta*, and to examine the maintenance of electrolyte homeostasis during the hot dry season near Khoramabad. Another desert agamid lizard, *Uromastix acanthinurus* has been reported as being able to maintain electrolyte constancy in its blood throughout the summer (BENTLEY, 1976; GRENOT, 1976), but subsequent careful analysis of changes in plasma sodium levels from separate populations near Beni-Abbes in Algeria showed that this species also experiences hypernatraemia in summer (LEMIRE et al., 1979; LEMIRE et al., 1982; BRADSHAW, 1986).

The study of changes of the amount of electrolytes in the blood of different lizard species and during various seasons has attracted a considerable deal of attention. BENTLEY (1959), working with the skink, *Tiliqua rugosa*, reported the existence of high concentrations of Na^+ in plasma during mid-summer. In addition, he found that sodium injections in the laboratory also resulted in hypernatraemia. BRADSHAW & SHOEMAKER (1967), carried out field studies on water and electrolytes balance in the agamid lizard *Amphibolurus* (now *Ctenophorus*) *ornatus* in Western Australia and found that high concentration of Na^+ in the plasma of this lizard during summer, were associated with a significant isosmotic expansion of the extracellular fluid. Plasma sodium levels were found to increase to as high as 300mmol.L^{-1} in extended droughts and were associated with significant mortality.

In lizards, an active nasal gland for discharging salt has attracted zoologists' attention, and in some species the role of this active nasal gland in excreting ions has been investigated. In some lizards species however, no research has been performed on nasal glands and their function remains unknown.

Since the discovery of nasal salt glands in many species of reptiles some zoologists have shown that in natural and laboratory conditions, a group of terrestrial reptiles can exude the salt in a highly-concentrated solution from the nasal gland (SCHMIDT-NIELSEN et al., 1963; DUNSON, 1974; 1976).

This subject has been reviewed by CLOUDSLEY-THOMPSON (1971), and LEMIRE & VERNET (1982), reported that some species of terrestrial reptiles usually exude relatively smaller amounts of salt relative to marine and estuarine species. A nasal gland in the form of a special gland specialized for salt secretion is common in herbivorous desert lizards, in particular those where their only water sources are the plants that they eat (GABE & SAINT GIRONS, 1971; 1976). These animals ingest significant amounts of electrolytes particularly potassium and they must adapt to quantitative changes in ion concentrations in their food. The importance of nasal gland secretions in regulating plasma electrolyte concentrations in the family Iguanidae, residing in deserts of Northern America, has been demonstrated in both *Sauromalus obesus* (NORRIS & DAWSON, 1964; TEMPLETON, 1964; SHUTTLEWORTH et al., 1987); and *Dipsosaurus dorsalis* (TEMPLETON, 1966; SHOEMAKER et al., 1972; HAZARD, 2001). GRENOT (1976) showed that these lizards are similar in their ecology to the north-Africa agamid lizard, *Uromastix. acanthinurus*. However despite salt being gathered on the nasal cavities in normal conditions (GRENOT, 1968), and having a salt gland in the nose which has specialized morphologically (LEMIRE et al., 1970; 1972; LEMIRE, 1975), the manner of adaptation of the nasal gland in Agamidae against increasing salt ions in comparison with Iguanidae of Northern America has remained unknown. Nasal gland structure in *U.acanthinurus* has been studied by LEMIRE & GRENOT (1974), LEMIRE et al. (1970), and in *Agama* *mutabilis* by LEMIRE & GRENOT (1973), and its function has been investigated by GRENOT (1968), LEMIRE & VERNET (1981; 1982; 1983) and by BRADSHAW et al. (1984).

SAINT GIRONS & BRADSHAW (1987) have studied the histology and cytology of nasal glands among Australian lizards such as Varanidae, Scincidae, Gekkonidae, Agamidae and Pygopodidae families. They reported that nasal salt secreting of glands are most highly developed in the Varanidae and to a lesser extent in Scincidae. Salt- secreting elements have been reported only and then in Gekkonidae and Agamidae, and never to date in the Pygopodidae.

Agama nupta is an insectivorous lizard that never drinks water and obtains its required water for body activity through its food. Adaptations of *A. nupta* to its environment and the manner of its reaction against effective factors in critical conditions have not been studied previously. The aim of this study was to investigate whether *A. nupta* lizards around Khoramabad are able to regulate concentrations of Na^+ and K^+ in the blood during summer when free water is scarce and whether they possess an active salt-secreting gland that assists in this process.

MATERIALS AND METHODS

Lizards weighting between 103 to 170g were collected weekly or biweekly (some times more) over a two-year period from May to September. 90 *Agama nupta* were collected during the investigation as follow:

5 individuals in May, 17 in June, 17 in July, 29 in August and 22 individuals in September in Khoramabad (50° 3'E, 34º 22'N). The animals were captured by hand (FERGUSSEN & BRADSHAW, 1992) usually in the morning to about noon. The mean size/weight ratio of animals was 5.6. Animals were collected and bled several times per month, 2 times in May, 4 times in June, 5 times in July, 6 times in August and 7 times in September. Habitat, temperature, and humidity were all recorded. Animals were transferred to laboratory, and blood samples in triplicate were collected into heparinised microhaematocrit tubes, from the infra orbital sinus of each lizard (HALPERN & PACAUD, 1951). Blood was obtained 2-4 hours after animals captured, and then the animals were released. Blood was centrifuged at 1500g and plasma was separated and then stored frozen at –20°C until analyzed (SAINT GIRONS et al., 1992).

Subsequently Na^+ and K^+ concentrations were measured with a flame photometer (Cornic 405). In addition to the above 90 mentioned lizards, and in order to stimulate nasal gland functions of *A. nupta*, for becoming active and secreting salt, 15 adults weighing between 103 to 170g, were trapped and transferred to the laboratory of zoology, they were divided into two groups, each group housed in a special cage, then they placed in climatic chamber for experimental conditions (see LEMIRE et al., 1980). The lizards were not fed during the experiments. One group was given daily subcutaneous injections of 2mL of sodium chloride (2mmol NaCl per 100g body weight) and the other group received 2mL potassium chloride (2mmol KCl per 100g body weight) for 5 days at 24 hours (LEMIRE & VERNET, 1983). The encrustations around the nostrile were checked every day to find the nasal secretions (because in lizards possessing active nasal gland, salt secretions are crystallized spontaneously on those areas). But no secretions of Na or K ions were seen through the nasal gland, after injections, to measure the nasal salt secretions. ANOVA was applied to determine significant differences between mean electrolyte concentrations. The confidence limit in all analyses was $P<0.05$. To detect differences between the different months Sceffe test was used.

RESULTS

Results obtained from tests on *A. nupta* collected around Khoramabad, indicate that the concentration of sodium ions in the plasma rise during the warm season (differ significantly from May to September, $P<0.05$) and is associated with small changes in the concentration of potassium ions (see Fig. 1). Temperature rises from a typical 30°C in May to 37°C in June, then to 42ºC in July, finally reaching 46ºC in August. Plasma sodium concentrations show a progressive rise through this period, reaching a maximum of approximately 175mmol.L^{-1} in late August, before falling in September. Changes in potassium concentrations mirror sodium reaching a maximum of 7mmol.L^{-1} in late summer (see also Fig. 1). This increase continues until early September. As the temperature of the environmental decreases, ions concentrations reach their normal level, means 140-150mmol.L^{-1} for sodium and 5mmol.L^{-1} for potassium. There was significant difference for sodium among August and June, July, May, September, and also among May and June, July and September ($P<0.05$). Significant change was observed between June and July ($P<0.05$). No significant difference for potassium concentrations was observed in the blood plasma among May and June, September and also between June and September ($P>0.05$). There was no mean difference for potassium between July and August ($P>0.05$). But significant difference for potassium concentrations was observed in the blood plasma among May and July, August and also among June and July, August ($P<0.05$). There was significant difference for potassium between July and September and also between August and September ($P<0.05$).

The 15 *A. nupta* collected to check whether or not they have an active salt secreting gland were exposed to sodium chloride or potassium chloride. During their keeping period in laboratory, no food was given to them. 2mL sodium chloride or 2mL potassium chloride (see Materials and Methods) were injected over 5 days through subcutaneous injections. Before injecting the salt solutions, no a signs of secreted salt were observed around the nostrils. Also, after injecting the salt solutions in a number of days to the lizards, no secretions of Na^+ or K^+ from the nasal gland were identified in *A. nupta*. This means that, in contrast of other species of herbivorous lizards, such as *U. acanthinurus* and members of the Iguanidae, *A. nupta* do not appear to possess an active nasal salt gland.

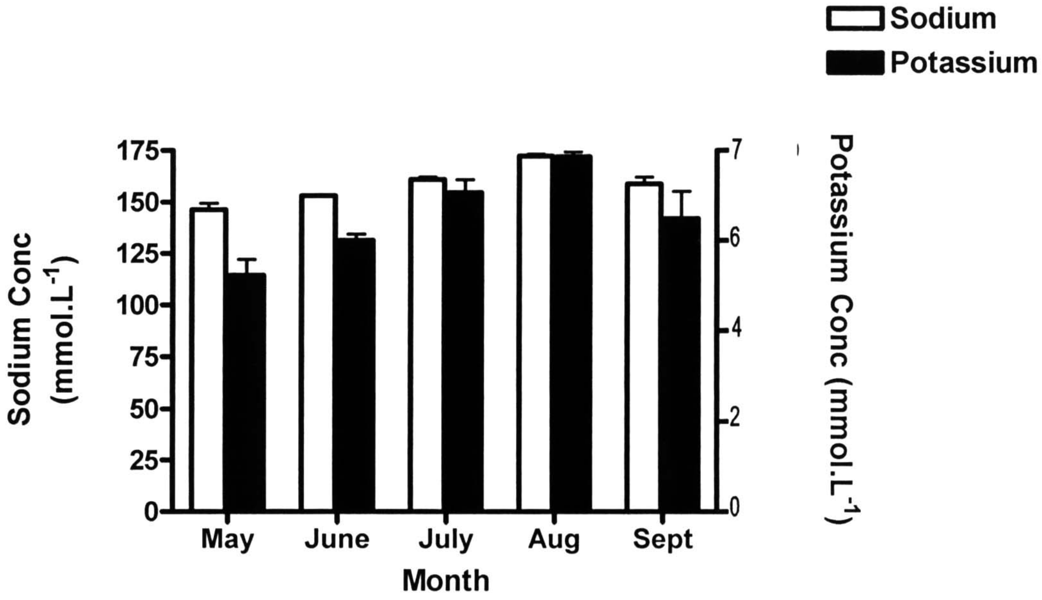

Fig. 1. – Bar chart of seasonal variation in Na^+ and K^+ concentration in the blood plasma of *Agama nupta* in Khoramabad.

DISCUSSION

Our investigation which carried out with *A. nupta* around Khoramabad has indicated that lizards fall into hibernation during the cold season, emerging to become active at the beginning of spring. The lizards are typical heliotherms, during the warm season. They use sunlight to regulate their body temperature. Typical thermoregulatory behaviour is indicated by *A. nupta* including the changes of dermal colour, which may assist in thermoregulation (Rice & Bradshaw, 1980). *A. nupta* do not drink water and their required water is supplied solely through their insect diet.

The results obtained from the present investigation on *A. nupta* in Khoramabad indicate that plasma electrolyte concentrations increase significantly during the summer months with clear evidence of both hypernatraemia and hyperkalaemia. As in desert populations of lizards studied by Lemire & Vernet (1981) the plasma salt level changed according to season, increasing markedly during the warm season. In spring when environmental heat is lower than in summer, the concentration of sodium and potassium is lower in these lizards, and in summer through increasing environment temperature, the concentration of the ions particularly sodium goes up, and reaches a relatively high level in August and early September. Then, as September progresses, and temperatures fall, sodium levels decrease to reach essentially normal values. It should be considered that the percentage of humidity in spring is higher than in summer, and this factor may increase the amount of water present in the lizards' food. In summer when the humidity is low, the rate of evaporative water loss from the lizards' body is high and there is a potential for plasma Na^+ and K^+ concentration to increase through dehydration. Bentley (1959) reported high levels of sodium in the Australian skink *Tiliqua rugosa* in the middle of summer. Bradshaw & Shoemaker (1967) studied *Amphibilurus ornatus* in Western Australia and reported a similar case of hypernatraemia, but one induced by sodium loading from the diet, rather than from dehydration. Increasing sodium concentration in some of the species depends on environment conditions and type of feeding, and may reach to 300mmol/L^{-1} and for potassium 12mmol.L^{-1} (Bradshaw, 1997).

The secretory capacity of nasal salt gland has been examined in some species of lizards (Minnich, 1981; Lemire & Vernet, 1982) with experimental loads, but due to differences in morphology and in the doses used, it is not always easy to obtain a clear idea of the potentialities of the nasal gland.

The first investigation on salt secretion on reptiles indicated that many of terrestrial lizards have a special gland in their nose, which has the property of producing a hyperosmotic secretion (Schmidt-Nielsen & Fange, 1958; Schmidt-Nielsen, 1964). In the desert lizard *Uromastix acanthinurus*, which feeds on plants, electrolytes excreted from the nasal gland assist in maintaining the water and electrolyte balance in the animal (Lemire, 1976). There is a nasal gland with high electrolyte excretion to adapt to environment in desert reptiles. The importance of the function of nasal gland in excreting salt and regulating electrolytes concentration in some species of North America Iguanidae has been highlighted by Norris & Dawson (1964), Tempelton (1964; 1966), Shoemaker et al. (1972).

Other investigations indicated that in intertidal lizard, *Uta tumidarostra*, as in other lizards, the nasal glands are able to eliminate of ions (Hazard et al., 1998), and in desert Iguana, *Dipsosaurus dorsalis*, (Hazard, 2001) nasal glands are capable to secret ions sodium, potassium, chloride and bicarbonate in different rates. The rate of secretions may be related to diet of lizards.

The structure of nasal gland has been studied in some groups of lizards with regard to the function of the secretory cells. In a group of lizards, like herbivorous species, such as *Dipsosaurus dorsalis*, *Sauromalus obesus* and *U. acanthinurus*, the nasal gland is obvious and cells with electrolytes-active transport features (the so called principle cells or cellules strièes) can be recognized easily in histology sections (Lemire, 1976). In another group, consisting of desert's insectivorous Agamids like *Agama mutabilis,* the nasal gland is small and has no special cells for active transport of ions (Lemire, 1976). Therefore, the nasal gland is unable to act as a salt-secreting gland (Saint Girons et al., 1977) to produce hyperosmotic solution. Interesting, the skink *Tiliqua rugosa* has a mixed cell population in its nasal gland but is capable of producing a mildly hyperosmotic solution (Bradshaw, Tom & Bunn, 1984; Saint Girons et al., 1977).The present study suggests that *A. nupta*, possesses a nasal gland with a structure similar to other insectivorous lizards such as *A. ornatus* and *A. mutabilis* (Lemire & Grenot, 1974) that can not excrete sodium and potassium ions in an hyperosmotic solution, but this needs histological confirmation.

During the period of experiment, when *A. nupta* were exposed to sodium chloride or potassium chloride, no secretions from the nasal gland were seen. This data suggests strongly that *A. nupta* does not possess an active salt-secreting gland. Plasma sodium levels were not measured in these salt injected lizards, however, and confirmation would be needed that these were elevated by the treatment before confirming this conclusion. Histological examination of the gland is also needed to finally confirm that the gland lacks any salt-secreting ability. At this stage, it is not clear whether the hypernatraemia and hyperkalaemia evidenced by *Agama nupta* is the result of dehydration or of salt loading from the diet. To determine this, a body-mass length regression would be needed to examine whether field-caught lizards in summer were dehydrated (Bradshaw & Death, 1991). If the changes on the other hand are diet induced, one would need to analyses the sodium content of the insects eaten by *Agama nupta* to ascertain whether this is hyperosmotic with respect to lizard plasma (Bradshaw, 1986).

What is clear from the data is that *A. nupta* survives the very hot summer period in the region of Khoramabad with relatively modest increases in blood sodium and potassium concentrations. Plasma sodium concentrations of 175mmol.L^{-1} are quite low compared with other reported in literature and suggests that this species is well-adapted to its desert environment. Future research should focus on measuring a number of aspects of the physiology of this species, including its rate of evaporative water loss, the nature of its insect diet and also kidney function as all these factors interact to facilitate survival.

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