Science and Technology, 8 (2003) 1-10

Effect of Cold and Hot Temperature on Behavioral and Selected Physiological Measures of *Uromastyx aegyptius* (Agamidae)

A.A. El-Banna and A.M. Al- Johany

Department of Zoology, College of Science, King Saud University, P.O.Box 2455, Riyadh 11451, Saudi Arabia, Email: ajohany@ksu.edu.sa.

أثر درجة الحرارة الباردة والعالية على السلوك وبعض القياسات الفسيولوجية للضب المصري

أمين عبدالحميد البنا وعوض الجهني

خلاصـة : تمـت دراسة الاستجابات السلوكية والفسيولوجية والكيميائحيوية لدرجات الحرارة الباردة والعالية للضب المصري . Uromastyx aegyptius. وقـم تـم تقسيم اثني عشر ضباً إلى مجموعتين اختباريتين. بالإضافة إلى مجموعة ثالثة ضابطة حفظـت تحت درجة حرارة 22 °م . وقد أعطيت الثلاث مجموعات فترة 24 ساعة للتأقلم عند درجة حرارة 22 °م . جمعت عينات الدم من كل حيوان عندما تصل درجة حرارة الجسم الدرجة المحددة ، وقد أظهرت النتائج فروقاً معنوية ($P<0.05$) في انخفاض جلوكوز الدم ومستوى الكولسترول خلال فترة تعرض الحيوانات للبرودة (2°م) هذا الانخفاض في مواد التمثيل خارج الخلايا يعكس زيادة في امتصاص الخلايا لهذه المواد. أن رفع درجة حرارة الحيوان إلى (45°م) أدى إلى زيادة معنوية ($P<0.05$) فـي كمـية البروتين الكلي واليوريا وحمض اليوريك. هذه التغيرات يمكن أن تعزي إلى زيادة فقد الماء بالتبخير وبخاصة بسبب زيادة اللهث وما يتبعه من نقص في حجم البلازما. لم تظهر أي تغيرات معنوية ($P<0.05$) في *T3*، *T4* خلال فترة التبريد أو التسخين. وقد أشارت نتائج هذه الدراسة إلى زيادة في حجم الأيض اللاهوائي في الضب خلال فترة التبريد بديل نقص مستوى الجلوكوز من خارج الخلايا إلى داخل الخلايا مما يشير إلى استعداد الحيوان لتفادي حدوث التجمد الكامن لكي تتم حماية الحيوان من تجمد السوائل داخل الخلايا.

ABSTRACT: The behavioral, physiological and biochemical response to low (2°C) and high (45°C) temperatures was studied in *Uromastyx aegyptius*. Twelve animals were divided into two experimental groups. A third control group was kept at 22 °C. All animals in the cooling, warming, and control groups were allowed a period of 24 hours for adjustments at 22 °C. Blood samples were collected from each animal when body temperature reached the corresponding levels. The results showed a significant ($P<0.05$) decrease in blood glucose and cholesterol levels during cooling (2°C). This reduction in extracellular fluid substrates reflects an increase in cellular uptake of these substrates. Warming (45°C) resulted in a significant ($P< 0.05$) increase in total proteins, urea, and uric acid. These later changes could be attributed to an increase in the evaporative water loss, particularly due to the increased observed panting, and the associated reduction in plasma volume. There were no significant ($P>0.05$) changes in T3 and T4, during cooling nor during warming. The results of this study suggest augmentation of anaerobic metabolism of the *U.aegyptius* during cooling as evident by reduction in blood glucose levels. Furthermore, shift of glucose from the extracellular to the intracellular fluids demonstrates anticipation against potential freezing in order to protect the animal from intracellular freezing.

KEYWORDS: Metabolism, Temperature; Behaviour; Blood Constituents; Plasma; *Uromastyx*; Lizard.

1. Introduction

The spiny tailed lizard *Uromastyx aegyptius* is the largest agamid lizard and is widespread in Arabia (Arnold, 1986). It is a diurnal animal and becomes active during the warm part of the day. Many terrestrial ectotherms, like the *U. aegyptius*, have a considerable capacity for behavioral and physiological thermoregulation (Bartholmew, 1981). Although many ectotherms are

physiologically adapted to survive at low ambient temperatures, terrestrial ectotherms are particularly susceptible to freezing, and there are a variety of strategies that enable ectothemic animals to survive freezing conditions (Hew *et al.* 1986). Ectotherms exposed to high temperature can either attempt to regulate their body temperature below the air temperature or rely on biochemical adaptation for tolerance of high body temperature (Cossins and Bowler, 1987).

Additionally, high temperature causes proteins to unfold and hence enzymes protein can no longer function. However, heat shock protein (HSP) response by programming cellular activities that bind and help mutated protein to restore their shape. Ulmasov, *et al.*, (1992) and Zatespina, *et al.*, (2000) found that HSP plays a major role in the cellular response during heat stress. Their findings also included a positive relationship between HSP and the magnitude of thermotolerance among lizard species. We thought that HSP dynamic relates to total plasma protein, and related measures such as albumin, and globulin. Thyroid hormones are involved indirectly in thermoregulation by increasing metabolic rate and subsequently heat production. Despite this, controversy exist regarding the initial calorigenic effects of thyroxin. Sinha and Choubey (1981) reported a positive relationship between thyroid activity and environmental temperature in *U. hardwickii* lizards. As thyroxin enhances cellular absorption of glucose and also influence plasma cholesterol, we included these measures in our study.

Thermoregulatory adjustment of the lizards are behavioral or physiological changes to deflect the body temperature from environmental heat loads (Firth and Belan, 1998). Measurements of blood composition are commonly used to assess the way in which the environment affects the physiology of animals. Therefore the objective of the present study was to investigate some aspects of physiological and biochemical responses of the *U.aegyptius* when exposed to low and to high temperatures.

2. Materials and methods

Eighteen *U. aegyptius* (mean wt. 760 gms) were collected from the field (Rumah and Haradh townships around Riyadh city, Saudi Arabia). The animals were housed in an open vivarium (2m x 1/4 m) with overhead heat and light lamps with timer control set at 12:12 for light and dark periods and kept at a constant air temperature of 22°C (room temperature). The animals were then divided into three groups (6 animals in each) of matched body weight.

Blood samples were collected from animals in the control group at 22°C. Animals in the two experimental groups were exposed to the low and high temperatures, as described below. Body temperature was maintained at 45°C for the warm group and 2°C for the cold group for 24 hrs and 48 hrs, respectively. Blood samples were collected at the end of exposure time. All blood samples were collected by cardiac puncture method using vacutainers. The vials were kept at room temperature for one to two hours, then, centrifuged at 3000 rpm for 10 minutes to separate the plasma which was stored at - 20°C for later analysis.

2.1. Cooling procedure: exposure to low temperature

All animals of the second group were placed in Plexiglas chamber. A YSI model 423 thermocouple temperature probe was inserted 2 cm into the cloaca of the animal and held tight with a tape on the tail of the animal. The probe was attached to an YSI telethermometer (Model 44) in conjunction with a Omni scribe chart recorder to monitor the internal body temperature of the animal. Each animal was placed on a multiple benched mini-cold lab model 2203 (LKB-Produkter AB), and the temperature was set to 2ºC. Animals were left in this environment for 48 hours before taking the blood sample.

2.2. Warming procedure: exposure to the high temperature

The same probe and recording instruments were tied to the animals while exposing them to the high temperature. A Plexiglas chamber animal was fixed in a water-bath (Gallenkemp Gmbh) with temperature control thermostat set at 45ºC. Each animal was introduced into the chamber and then

the temperature was gradually raised till the water-bath temperature and the animal body temperature reached 45 ±1ºC. After constant exposure to this temperature for 24 hr, blood samples were collected.

2.3. Analysis of plasma

The following components were determined quantitatively in the plasma collected from the three groups of *U.aegyptius*, using the BioMerieax Vitek (Missouri, USA) kits. Total protein following the principal of Biuret reaction according to Peters (1968); albumin according to Drupt *et al*. (1974); triglyceride according to Fossati and Prencipe (1982); cholesterol according to Anonymous (1989); glucose according to Tender (1969); urea according to the modified Berthelot reaction after Patton and Crouch (1977); uric acid as described by Artiss and Entwistle (1981). T_3 and T_4 were determined using enzyme immuno assay (EIA). Plasma globulin concentration was calculated by subtraction. Fractionation of total protein was carried-out using cellulose acetate electrophoresis (Helena Lab. Instruments Ltd. U.K), and the electropherograms were then scanned using Personal densitometer SI (Amersham Pharmacia Biotech), and the percentages of the different protein fractions were determined.

2.4. Statistical analysis

Analysis of Variance (ANOVA) was utilized for testing the overall effects of cooling and warming on selected blood measures. Post-hoc comparisons were conducted between pairs of means, via LSD procedure, on total protein, albumin, cholesterol, globulin, urea, uric acid, T3, and T4.

3. Results

3.1 Behaviour

U.aegyptius are known to tolerate extreme low and high temperatures (Bartholmew, 1981). The results of this study showed that when the animals were exposed to low temperature (2°C), they became lethargic and immobile. No change of color was noticed during this period. At 2°C blood circulation was very slow and the animal needed to be rubbed on the neck and back to facilitate the flow, when drawing blood. At high temperature *U.aegyptius* were noticed to fight for release when body temperature reached 40°C. Defecation was observed after two hours of high temperature exposure. They started to pant when temperature crossed the 40°C limit. Body colouration changed from a dark tan to pale yellow at 42°C.

3.2. Blood components

Table 1 shows the concentrations of plasma proteins in *U.aegyptius* exposed to the low and high temperatures compared to that in *U.aegyptius* exposed to the room temperature (22°C). No significant difference was observed in plasma total proteins between animals exposed to the 2°C and the 22°C, but there was a significant ($P<0.05$) increase in plasma total proteins when animals were exposed to the 45°C. The main increase in plasma total proteins at the 45°C was due to an increase in albumin and α 1-globulin fractions. Plasma albumin and the albumin/globulin ratio were significantly ($P<0.05$) lower in animals exposed to the 2°C (Table 2).

The electrophoretic separation of plasma proteins indicates that there was a significant decrease ($P<0.05$) in the α 2-globulin fraction when animals were exposed to either the 2°C or the 45°C. No significant change was observed in the percentages of the β and γ-globulin fractions when animals were exposed to the three different temperatures (Table 2).

The mean concentrations of some plasma constituents in *U.aegyptius* exposed to the three different temperatures are presented in Table 1. There was no significant ($P>0.05$) difference in the concentrations of plasma triglycerides in *U.aegyptius* exposed to the different temperatures (Table2). Plasma cholesterol concentrations in animals exposed to the 22°C and the 45°C were

about the same, but a significant (P<0.05) decrease in cholesterol concentration was found in animals exposed to the 2°C (Table 3). The mean plasma glucose concentration in *U.aegyptius* at room temperature (22°C) was significantly (P< 0.05) decreased in animals exposed to the 2°C, and was significantly (P<0.05) increased in animals exposed to the 45°C (Table 3).

Table 1: Mean concentration of plasma proteins in *U.aegyptiuss* exposed to low, Room and high temperature.

Measure	Temperature		
	Cold (2°C)	Room (22°C)	Warm (45°C)
Total Protein (g/dl)	2.53 ± 0.27	2.90 ± 0.15	3.65 ± 0.17
Albumin (g/dl)	0.98 ± 0.09	1.27 ± 0.10	1.67 ± 0.09
Globulin (g/dl)	1.98 ± 0.30	1.74 ± 0.19	2.30 ± 1.10
Albumin/Globulin ratio	0.49 ± 0.06	0.72 ± 0.05	0.73 ± 0.05
Triglyceride (mg/dl)	19.2 ± 3.5	18.3 ± 4.0	22.5 ± 1.8
Cholesterol (mg/dl)	104.7 ± 15.9	234.3 ± 28.3	226.4 ± 28.4
Glucose (mg/dl)	206 ± 8.8	281.9 ± 16.8	358.0 ± 12.8
Urea (mg/dl)	12.3 ± 1.3	8.8 ± 0.5	12.0 ± 8.0
Uric Acid (mg/dl)	2.8 ± 0.3	3.1 ± 0.4	5.8 ± 0.7
T3 (ng/dl)	7.0 ± 0.5	7.6 ± 1.0	7.6 ± 0.8
T4 (µg/dl)	3.0 ± 0.2	4.1 ± 0.4	5.4 ± 0.3

Table 2: Summary table of ANOVA conducted on Dependent Variables (measures).

Measure	Source of Variation	SS	df	MS	$F_{(value)}$	$F_{(probability)}$
Total Protein	Between Group	4.32	2	2.15	17.21*	3.63
	Within Group	3.85	16	0.24		
Albumin	Between Group	1.42	2	0.71	12.29*	3.63
	Within Group	0.87	16	0.05		
Globulin	Between Group	1.03	2	0.51	1.54	3.63
	Within Group	5.32	16	0.33		
Albumin / Globulin Ratio	Between Group	0.36	2	0.18	2.25	3.63
	Within Group	1.28	16	0.08		
Glucose	Between Group	74174.67	2	37087.34	33.97*	3.63
	Within Group	17467.53	16	1091.72		
Triglycerides	Between Group	51.69	2	25.84	.41	3.63
	Within Group	989.49	16	61.84		
Cholesterol	Between Group	64793.73	2	32396.86	7.97*	3.63
	Within Group	65184.06	16	4074.00		
Urea	Between Group	45.62	2	22.81	4.44*	3.63
	Within Group	82.16	16	5.14		
Uric Acid	Between Group	35.68	2	17.84	10.06*	3.63
	Within Group	28.36	16	1.77		
T3	Between Group	1.25	2	0.62	0.16	3.63
	Within Group	60.09	16	3.75		
T4	Between Group	18.84	2	9.42	1.92	3.63
	Within Group	78.26	16	4.89		

* P<0.05

EFFECT OF COLD AND HOT TEMPERATURES

There was a significant ($P<0.05$) increase in the mean plasma urea concentration when *U.aegyptius* were exposed to either the 2°C or to the 45°C (Table 3). Also, there was a significant ($P<0.05$) increase in the mean plasma uric acid concentration when *U.aegyptius* were exposed to the 45°C, but no change in plasma uric acid was observed in animals exposed to the 2°C and 22°C (Table 3).

Table 3: Multiple Comparisons of Differential Mean Effect Using LSD Method for only Those Variable which Were Significantly Different.

Total Protein			**Albumin**			**Glucose**		
Group	Mean Diff.	Sig.	Group	Mean Diff.	Sig.	Group	Mean Diff.	Sig.
LT RT	-0.5333*	0.034	LT RT	-0.4333*	0.033	LT RT	-74.700*	0.001
HT	-1.2905*	0.000	HT	-0.7988*	0.000	HT	-151.328*	0.000
RT LT	0.5333*	0.034	RT LT	-0.4333*	0.033	RT LT	74.700*	0.001
HT	-0.7571*	0.004	HT	-0.3655	0.058	HT	-76.628*	0.001
HT LT	1.2905*	0.000	HT LT	0.7988*	0.000	HT LT	151.328*	0.000
RT	0.7571*	0.004	RT	0.3655	0.058	RT	76.628*	0.001

Cholesterol			**Uric Acid**		
Group	Mean Diff.	Sig.	Group	Mean Diff.	Sig.
LT RT	-129.746*	0.003	LT RT	-0.2667	0.733
HT	-121.728*	0.003	HT	-2.9657*	0.001
RT LT	129.746*	0.003	RT LT	0.2667	0.733
HT	8.018	0.824	HT	-2.6990*	0.002
HT LT	121.728*	0.003	HT LT	2.9657*	0.001
RT	-8.018	0.824	RT	2.6990	0.002

*The mean difference is significant at the 0.05 level.

The mean plasma T_3 and T_4 concentrations were not significantly different among animals exposed to the three different temperatures, although T_4 concentrations were somewhat higher at the 22°C and the 45°C (Table 2).

4. Discussion

In their natural habitat, *U.aegyptius* are exposed to low or high temperatures. In many instances they continue activity while air temperature is around 40°C and above (Louw and Gideon, 1993). They remain in their burrows when surface temperature falls below 10°C. They are not true hibernators; they may come out during winter when it is warm enough like many other lizards. They usually become active when temperature reaches 30°C, and when temperatures reache above 40°C they seek shelter in their deep underground burrows.

In our study, a significant decrease in blood glucose and cholesterol levels were found in *U.aegyptius* exposed to 2°C for about two days. This was also associated with a significant decrease in albumin fraction of the plasma proteins, and with a significant increase in the levels of plasma urea. On the other hand, a significant increase in blood glucose, as well as a significant increase in plasma total proteins, urea and uric acid were found in *U.aegyptius* exposed to 45°C for 24 hours. To adjust for changes in environmental temperature many ectothermic vertebrates

lower the selected body temperature during winter (Hazel and Prosser, 1974; Case, 1976). Ectotherms exposed to higher temperatures attempt to regulate their body temperature below the air temperature (Huey, 1982). These alterations in the selected body temperature lead to blood biochemical changes. Such modification seem to be achieved by metabolic changes which lead to changes in the composition of blood and tissues (White and Somero, 1982; Geiser *et al.*, 1992). Therefore, measurements of blood composition are commonly used to assess the way in which the environment affects the physiology of animals.

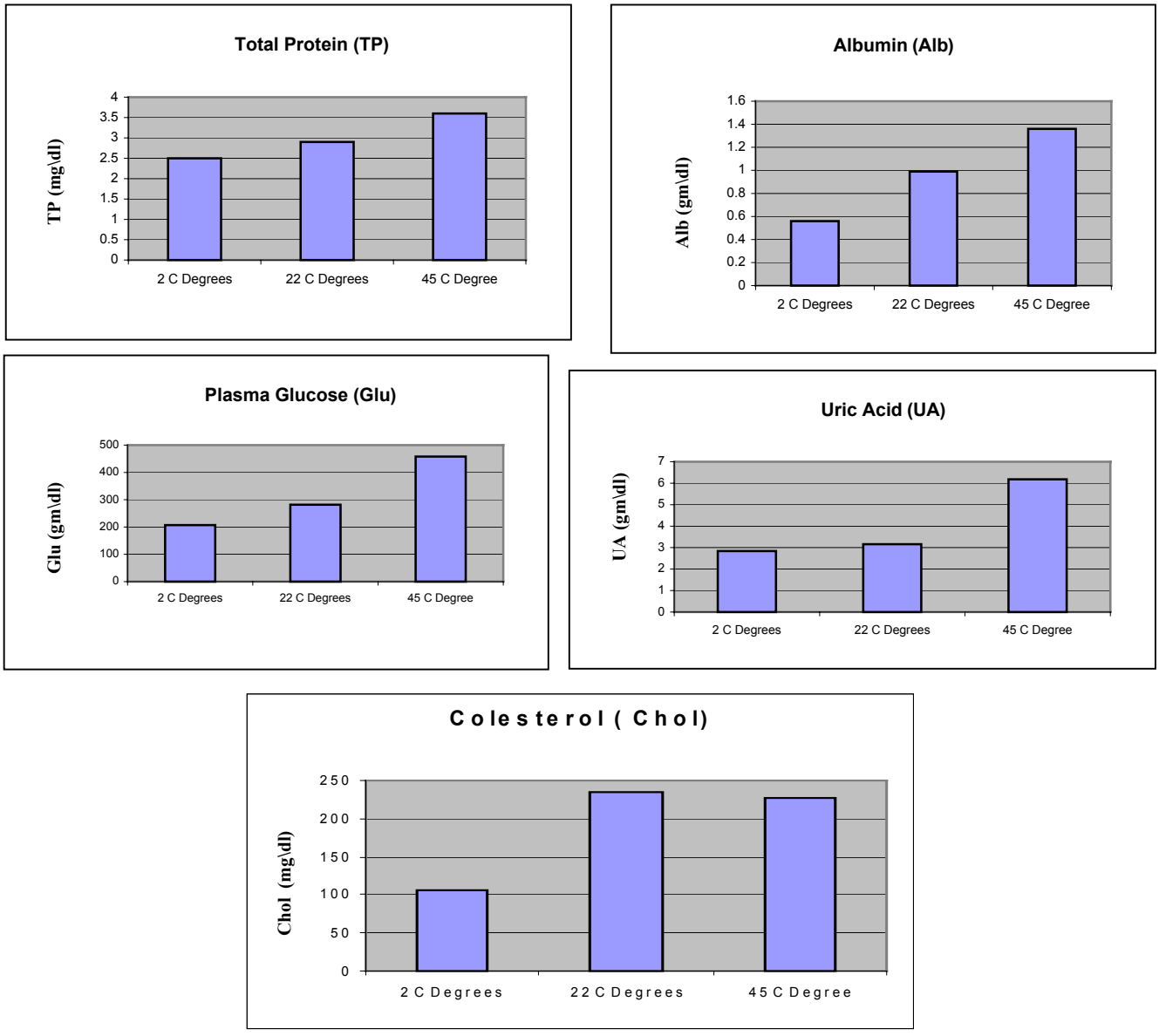

Figure 1. Plot of the changes in total protein (TP), Albumin (Alb), Glucose (Glu), uric acid (UA), and cholesterol (Chol) among variation in environmental temperature.

When lizard species normaly encounter temperatures close to or below 0°C, they have exceptionally high survival rates (Geiser and Firth, 1992). These lizards survive exposure to subfreezing temperatures by supercooling, i.e., by remaining unfrozen at temperatures below the equilibrium crystallization temperature of body fluids (Grenot and Heulin, 1990). Also, some lizards are freeze-tolerant (Storey and Storey, 1992; Costanzo *et al.*, 1995). Ectotherms can counteract a potentially freezing ambient temperature by increasing the osmotic concentration of

their body fluids so that its freezing point is depressed below the ambient temperature (Withers, 1992). Some ectotherms accumulate high concentration of specific solutes to depress their freezing point; these solutes are typically sugar, such as glucose or sugar alcohols. Their low molecular weight maximizes the freezing point depression per mass of solute (Withers, 1992; Lee and Costanzo, 1993). These sugars apparently have cryoprotectant effect, and they protect membranes and enzymes against cold denaturation and cold shock injury. It is important in these animals to avoid the freezing of their intracellular fluids, since intracellular ice crystals apparently destroy the integrity of intracullar membranes and organelles. In contrast, extracellular freezing does not disrupt essential cell structures and is not lethal, at least to the freeze-tolerant species (Withers, 1992; Lemos-Espinal and Ballinger, 1992). The decrease in blood could reflect a shift of glucose from the blood (extracellular fluid) to the intracellular fluids, probably through increasing cellular uptake of glucose, in anticipation of potential freezing ambient temperature. In doing that, the animals guard against intracellular freezing by depressing intracellular freezing point. The accumulation of the putative cryoprotectant glucose in tissues of lizards subjected to freeze tolerance trials had been reported (Costanzo *et al*. 1995).

The mean blood cholesterol level in *U.aegyptius* at room temperature (234.3 ± 28.3 mg/dl) is relatively higher than that in many mammalian species (Al-Tuwaijri, 1987). Also, cholesterol content in *U.aegyptius's* tissues is more than twice the cholesterol content of camel, lamb and cattle tissues (Abu-Tarboush *et al*. 1996). Cholesterol could also have cryoprotectant effect in lizards exposed to low temperatures, and the changes in blood cholesterol level observed in the present study may indicate a role for cholesterol in thermoregulation. Changes in the lipid composition of tissues and cell membranes are apparently required in order to maintain normal function at the lower body temperatures (Bauwens, 1981).

Lizards exposed to high temperature try to reduce their body temperature below the air temperature, and this strategy of thermoregulation can only be accomplished by evaporative cooling. Evaporation of water can dissipate a considerable amount of heat and lower the temperature (Withers, 1992). Relatively waterproof terrestrial animals are generally able to enhance evaporative cooling by markedly increasing their cutaneous or respiratory water loss. The lizard *Dipsosaurus* totally dissipates its metabolic heat production by panting at air temperature more than 40°C, and the large agamid lizard *Amphibolurus* can reduce its body temperature about 3.2°C below air temperature of 43°C, and the chuckwalla lizard *Sauromalus obseus* can considerably lower its body and brain temperatures by evaporative cooling by panting (Withers, 1992). In this study evoparative water loss (EWL) was not measured. However, we observed, particularly because of the increased panting, that the reduction in plasma volume could be the reason for the increased concentrations of plasma glucose, total proteins, urea and uric acid observed in *U.aegyptius* exposed to the 45°C. During dehydration, animals show elevated concentrations of osmolytes and proteins in their blood (Dunlap, 1995). The increased plasma glucose concentration in *U.aegyptius* exposed to the higher temperature could also reflect a decrease in glucose uptake by tissues in order to avoid excess intracellular glucose breakdown and energy production, and excess heat load.

Lizards exposed to high temperature can also rely on biochemical adaptations for tolerance of high body temperature. Heat stress has been shown to induce the synthesis of a family of proteins, the so-called heat shock proteins (HSPs) in cells of a wide spectrum of ecologically different lizard species (Ulmasov *et al.* 1992; Feder and Hofmann, 1998; Zatsepina *et al*. 2000). The level of HSPs were found to be higher in desert species than in the non-desert species (Zatsepina *et al*. 2000), and the induction and active synthesis of HSPs occurs within a temperature interval normal to the species (Ulmasov *et al.* 1992). HSPs play a key role in the cellular response to heat shock by maintaining the native state and proper folding of cellular proteins during physiological stress and by facilitating the restoration of cellular functions (Morimoto, 1993; Morimoto, *et al.*, 1994). The expression of the HSP gene is tightly regulated to ensure that the response is proportional to the level of heat stress, and then repressed and terminated when normal physiological conditions recur (Lindquist, 1986). We did not measure the synthesis of HSPs in the present study, but these

proteins can be synthesized, particularly by liver cells, within 1 to 2 hrs of exposure to the high temperature, and in desert lizards, the temperature range of induction and continuing synthesis of HSPs was reported to be 36-50°C (Ulmasov, *et al.*, 1992). The changes in plasma protein and protein fractions observed in *U.aegyptius* exposed to the 45°C in the present study could reflect induction and synthesis of HSPs.

U.aegyptius exposed to different temperatures in the present study showed no changes in plasma levels of the thyroid hormones T_3 and T_4. It is not certain whether the thyroid hormones have a direct and immediate role in thermoregulation in lizards. Sinha and Choubey (1981) reported that the thyroid gland of the Indian spiny tailed, *U. harrdwickii,* lizard appeared most active when the environmental temperature was high. When the temperature was low, the activity of thyroid gland was decreased. These authors also noted a reduction in thyroid activity in September. They did not reach a final conclusion because they found a parallel relation to the gonadal cycle.

Based on the results of the present study, it can be concluded that behavioral, physiological, and biochemical adjustments are altered in response to environmental temperature changes. However, the exact biological mechanisms are species specific in lizard populations. The physiological and biochemical control of thermoregulation in reptiles, and in *U.aegyptius* in particular, need more investigations, and as indicated by Grigg and Seebacher (1999) that the control of thermoregulation in reptiles is more complex than has been previously recognized.

5. Acknowledgements

We wish to thank Dr. S. Haffor for reviewing the manuscript. Mr. Awni El-Gazawi and Mr. M.Y. AbdulRawoof are also thanked for their technical help in the laboratory.

References

ARNOLD, E.N. 1986. A key and annotated checklist to the lizards and amphisbaenians of Arabia. *Fauna of Saudi Arabia* **8:** 335-435.

ARTISS, J.D. and ENTWISTLE, W.M. 1981.The application of a sensitive uricase-peroxidase couple reaction to a centrifugal fast analyser for the determination of uric acid. *Clin Chim Acta*.; **116(3):**301-309.

ABU-TARBOUSH, H. M., AL-JOHANY, A.M. and AL-SADOON, M.K. 1996. Proximate composition and fatty acids and cholestrol content of U.aegyptius's meat (*Uromastyx aegyptius* Blanford 1874) at the end of winter and during spring. *Journal of College of Agriculture of King Saud University*. **8 :** 79-92.

AL-TUWAIJRI, A.S. 1987. *Standard Biological Data. Human and Experimental animals*. King Saud University Press, Riyadh, Saudi Arabia.

ANONYMOUS 1989. The French consensus on cholesterol and dyslipoproteinemias. Administration Council of ARCOL. *Rev. Prat.*; **39(11):** 972-974. Review. French.

BARTHOLMEW, G.A. 1981. Physiological thermoregulation. In *Biology of the reptilia. Vol. 12, Physiological ecology* eds C. Gans and F. H. Pough). Academic press, New York. pp. 167-212

BAUWENS, D. 1981. Survivorship during hibernation in the European common lizard, *Lacerta vivipara*. *Copeia* **1981:** 741-744.

CASE, T. J. 1976. Seasonal aspect of thermoregulatory behavior in the chuckawalla, *Sauromalus obesus* (Reptilia, Lacertilia, Iguanidae). *Journal of Herpetology* **10:** 85-95.

COSSINS, A. R., and BOWLER, K. 1987. *Temperature biology of animals*. Chapman & Hall, London.

COSTANZO, J.P., GRENOT, C. and LEE, R.E.Jr. 1995. Supercooling ice inoculation and freeze tolerance in the European common lizard, *Lacerta vivipara*. *Journal of Comparative Physiology* B, **165:** 238-244.

DRUPT, F., PARIS, M., FRYDMAN, A., LECLERC, M. 1974. Plasma albumin assay by bromocresol green method: application to different automatic apparatus. *Ann Pharm Fr.* **32(5):**249-256. French.

DUNLAP, K.D. 1995. External and internal influences on indices of physiological stress: II. Seasonal and size related variations in blood composition in free-living lizards, *Sceloporus occidentalis. Journal of Experimental Zoology* **272:** 85-94.

FEDER, M.E. and HOFMANN, G.E. (1998). Evolutionary and ecological physiology of heat-shock proteins and the heat-shock response: comparative bibliography. *Annual Review of Physiology* **61:** 243-282.

FIRTH, B. T. and BELAN, I. 1998. Daily and seasonal rhythms in selected body temperature in the Australian lizard *Tiliqua rugosa* (Scincidae): Field and laboratory observations . *Physiological Zoology* **71:** 303-311.

FOSSATI, P. and PRENCIPE, L. 1982 Plasma triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry* Oct; **28(10):**2077-80.

GEISER, F., FIRTH, B.T., and SIGMOUR, R. S. 1992. Poly-unsaturated dietary lipids lower the selected body temperature of a lizard. *Journal of Comparative Physiology* B, **162:** 1-4.

GRENOT, C., and HEULIN, B. 1990. Sur la palasticite ecophysiologique du lezard vivipare, *Lacerta vivipara* (Reptilia, Lacertidae). *Bulletin of Society for Herpetology*, France. **54:** 1-22.

GRIGG, G.C. and SEEBACHER, F. 1999. Field test of a paradigm: hysteresis of heart rate in thermoregulation by a free-ranging lizard (*Pogona barbata). Proceeding of Royal Society London. B. Biological Science* **266:** 1291-1297.

HAZEL, J. R. and PROSSER, C.L. 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiology Review* **54:** 620-677.

HEW, C.L., SCOTT, G. K. and DAVIES, P.L. 1986. Molecular biology of antifreeze. In *Living in the cold: Physiological and biochemical adaptations* eds H. C. Heller., X.J. Musacchia and L.C.H. Wang Elsevier, New York. pp. 117-123.

HUEY, R.B. 1982. Temperature, physiology and the ecology of reptiles. In *Biology of the reptilia Vol. 12, Physiological ecology* ed. F.H. Pough. Academic Press, London. pp. 26-90.

LEE, R.E., and COSTANZO, J.P. 1993. Integrated physiological responses promoting anuran freeze tolerance. In *Life in the cold III: ecological, physiological and molecular mechanisms* eds C. Carey *et. al*. Westview Press, Boulder, Colarado. pp. 501-510.

LEMOS-ESPINAL, J. A., and BALLINGER, R.E. 1992. Observations on the tolerance to freezing by the lizard, *Sceloporus grammicus*, from Iztaccihuattl volcano, Mexico. *Herpetological Review* **23:** 8-9.

LINDQUIST, S. 1986. The heat shock response. *Annual Review of Biochemistry*. **55:**1151-1191.

LOUW, G.N. and GIDEON, N. 1993. *Physiological animal ecology*. John Wiley & Sons Inc. New York.

MORIMOTO, R.I. 1993. Cells in stress: transcriptional activation of heat shock genes. *Science* **259:** 1409-1410.

MORIMOTO, R.I., TISSIERES, A. and GEORGOPOLOUS, C. 1994. The biology of heat shock proteins and molecular chaperones. Cold Spring Harbor Laboratory Press, Plainview, NY.

PATTON, C. J. and CROUCH, S.R. 1977. Spectrophotometric and kinetic investigation of Berthelot reaction for the determination of ammonia. *Anal. Chem*.; **49 (1-4):** 464-469.

PETERS, T. Jr. 1968. Proposals for standardization of total protein assays. *Clinical Chemistry* Dec; **14(12):**1147-59.

SINHA, A.K. and CHOUBEY, B.J. 1981. Seasonal changes in the thyroid gland of Indian spiny tailed, sand lizard, *Uromastix hardwickii* (Gray) male. *Z. Mikrosk. Anat. Forsch.* **95:**72-80.

STOREY, K.B. and STOREY, J. M. 1992. Natural freeze tolerance in ectothermic vertebrates. *Annual Review of Physiology* **54:** 619-637.

TRINDER, P. 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. *Journal of Clinical Patholology*.; Mar; **22(2):**246.

ULMASOV, K.A., SHAMMAKOV, S., KARAEV, K. and EVEGENEV, M.B. 1992. Heat shock proteins and thermoresistance in lizards. *Proceeding of National Academy of Science*. **89:** 1666-1670.

WHITE, F.N., and SOMERO, G. 1982. Acid-base regulation and phospholipid adaptation to temperature: Time course and physiological significance of modifying the milieu for protein function. *Physiology Review* **62:** 40-90.

WITHERS, P. C. 1992. *Comparative Animal Physiology*. Saunders College Publishing, New York.

ZATESPINA, O.G., ULMASOV, K.A., BERESTEN, S.F., MOLODTSOV, V.B., RYBTSOV, S.A. and EVEGENEV, M.B. 2000. Thermotolerant desert lizards characteristically differ in terms of heat-shock system regulation. *Journal of Experimental Biology,* **203:** 1017-1025.

Received 18 May 2002
Accepted 19 April 2003