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HAEMATOLOGICAL VALUES OF THE RAINBOW LIZARD *AGAMA AGAMA* L.

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Haematological values of blood of 82 rainbow lizards, *Agama agama* L. collected during the rainy season in Ago-Iwoye, Nigeria were determined and the influence of age, sex and reproductive condition were investigated. Red blood cells were oval and nucleated and had a mean size of 18.6 by 13.0μ. Means of parameters commonly used in patho-physiological investigations were: red blood cell (RBC) - 0.78 x 10^{12}/l, white blood cell (WBC) – 0.46 x 10^{11}/l, packed cell volume (PCV) – 28.9% and haemoglobin (Hb) - 6.1g/dl. There were differences in blood parameters between males and females (thrombocyte, WBC, Hb and red cell indices), adult and subadult males (Hb and PVC) and non-breeding, vitellogenic and ovigerous females (WBC, RBC and PCV).

INTRODUCTION

Studies on the blood of reptiles have been reviewed by Pienaar (1962) Dessauer (1970), Saint Girons (1970) and Duguy (1970). Data from these studies show that reptilian blood plasma makes up 60 to 80% of the blood volume and contains inorganic and organic substances such as sodium, haemoglobin and urea, most of which occur in trace quantities. The pH ranges between 7.2 and 7.8, the haematocrit or packed cell volume between 20 and 35% and the blood contains 6 to 12g/dl haemoglobin. Erythrocytes or red blood cells are oval, 14-23 μ by 7-14 μ in size, each with an oval nucleus which is centrally located such that its long axes lie parallel to those of the red blood cells. The number of erythrocytes in the reptilian peripheral blood ranges between 0.15 and 2.05 x 10^{10}/l. White blood cells are highly variable in number.

Pienaar (1962) and Duguy (1970) identified age, sex, reproductive state, disease, nutrition, season and environment as factors which influence haematological values in reptiles and Newlin & Ballinger (1976) reported differences in haemoglobin content of lizards living at different altitudes.

In the studies on reptilian blood, only few species were investigated (Saint Girons, 1970) and detailed studies on African reptiles were few (Pienaar, 1962; Otis, 1973; Simbotwe, Ghosh & Grimms, 1983) despite the need to establish normal haematological baseline values for healthy animals relating to pathological and physiological condition (Duguy, 1970; Kocan & Pitts, 1976; Brown, 1980; Gee, Carpenter & Hensler, 1981; Needham, 1981; Ugochukwu, 1982).

In this paper, we present haematological values for apparently healthy rainbow lizards, *Agama agama* L. a species which is widespread in Africa (Harris, 1964) and which is used in epidemilogical studies of malaria infections (Dipeolu & Nutinga, 1989). We have also examined the influence of age, sex and reproductive state on haematological values of rainbow lizards. For comparison, haematological data for other vertebrate classes are presented.

MATERIALS AND METHODS

Lizards were obtained from Ago-Iwoye, Nigeria 6°58'N, 3°54'E) during rainy season months (May and June 1991) when the lizards enjoy their best health status (Harris, 1964; Ekundayo & Otusanya, 1969). Subsequently, each lizard was anaesthetized with chloroform for 10 to 25 minutes depending on the size and was bled thereafter from the severed jugular vein directly into small EDTA-coated bottles.

Lizard body weight (nearest g) before blood was taken, and snout-vent length (SVL) (nearest mm) were recorded. Reproductive condition of females whether breeding or non-breeding based on the criteria reported by Sodeinde & Kuku (1989), and relative age of males were determined. Breeding females were either vitellogenic having yolked ovarian follicles, or they were ovigerous with shelled oviductal eggs. Non-breeding females had undeveloped, milky-white ovarian follicles and no oviductal eggs. Subadult males were non-rainbow coloured and had SVLs of 75-125 mm while adults were rainbow-coloured and longer than 125 mm SVL (Harris, 1964).

Blood values were determined using methods described in Brown (1980) and which have been used in the study of reptilian blood (see Pienaar, 1962; Engbretson & Hutzlison, 1976; Needham, 1981; Simbotwe *et al.*, 1983). The packed cell volume was measured by a microhaematocrit technique. Whole blood drawn to marked levels in two haematocrit tubes was centrifuged for 5 min. in a microcapillary centrifuge and the space occupied by the packed red cells, the haematocrit reading, was taken. White blood cells, red blood cells and thrombocytes were counted in the Improved Neubauer haemocytometer after 1:200 dilution of uncentrifuged whole blood with the appropriate diluents (0.85% sodium chloride - RBC, Turk's solution - WBC, platelet diluting fluid - thrombocytes). The number of cells of each type in undiluted whole blood (N) was calculated using the formula, N = n.DF.VCF, where n is the number of cells in diluted blood, DF is the dilution factor (200 for the 3 cells types) and VCF,

the volume correction factor (50 for RBC, 2.5 for WBC and thrombocytes).

Haemoglobin content was determined colorimetrically by the cyanomethaemoglobin method. For each parameter duplicate samples were run for each lizard and the values were averaged. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin content (MCHM) were calculated from RBC, PCV and Hb values using standard formulae (Thompson, 1969).

Blood films were made from blood collected directly from each lizard and were stained with Giemsa stain. On each film, length and breadth measurements of five non-parasitized red blood cells and their nuclei were recorded using an ocular micrometer. A measure of the shape of red blood cells was obtained based on the ratio: RBC length/RBC breadth (Simbotwe *et al.*, 1983). Values greater than 1.0 indicated oval shapes where 1.0 represents round cells.

Means were expressed ± SD, pair-wise and multiple comparisons were made using the *t*-test and one-way analysis of variance (ANOVA) and multiple means were separated using the New Duncan's Multiple Range Test.

Sex	RBC Measurement	Mean	SD	Range
Male (5)	length	18.9	3.5	12.6-25.2
	breadth	13.1	2.5	8.4-16.8
	L/B ratio	1.48	0.16	1.25-1.75
	nucleus length	9.2	1.3	8.4 -12.6
	nucleus breadth	5.0	1.3	4.2-8.4
Female (5)	length	18.3	2.0	16.8-21.0
	breadth	12.8	2.3	8.4-16.8
	L/B ratio	1.46	0.21	1.25-2.0
	nucleus length	9.3	1.7	8.4-12.7
	nucleus breadth	5.4	2.0	3.8-10.5
Male & female pooled (10)	length	18.6(NS)	2.8	12.6-25.2
	breadth	13.0(NS)	2.4	8.4-16.8
	L/B ratio	1.47(NS)	0.19	1.25-2.0
	nucleus length	9.3(NS)	1.5	8.4-12.7
	nucleus breadth	5.2(NS)	1.7	3.8-10.5

TABLE 1. Means, SDs and ranges of red blood cell sizes (in μ) of male and female rainbow lizards. Number of lizards examined in each category is shown in parentheses. NS, male and female lizards means not significantly different ($P > 0.10$).

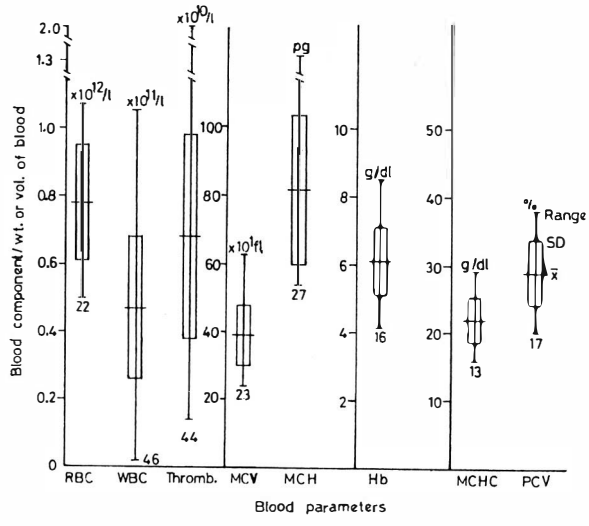

FIG. 1. Ranges, means, standard deviations and coefficients of variation of blood values of rainbow lizards ($n = 82$). The unit of measure for each blood parameter is given on top of the bar drawing and coefficients of variation are given at the base.

RESULTS

Morphometry of Red Cells

Dimensions of the RBC of rainbow lizards are presented in Table 1. Red blood cell length ($t = 1.20$, df = 24, $P > 0.30$) and RBC breadth ($t = 0.62$, df = 24, $P > 0.50$) were not significantly different in male and female lizards. The oval appearance of the cells each of which had a centrally-placed nucleus was confirmed by the RBC length/RBC breadth ratios which were greater than 1.25.

Haematological Values

Values of the blood parameters (Fig. 1) showed that thrombocytes and WBC had the greatest variances around the mean. They had coefficients of variation of 44% and 66%, respectively. The MCHC had the lowest coefficient of variation.

Male lizards had higher body weights and SVL (both non-haematological attributes), WBC, Hb, MCHC and MCH than females (*t*-test, df 80, $P < 0.05$) while thrombocytes were more numerous in females (Table 2). Differences between sexes in other blood values were not significant ($P > 0.10$).

Analysis of blood values of males by relative age showed that adults that were bigger and heavier, had higher Hb and PCV values than did subadults (*t*-test, df 39, $P < 0.05$) but did not differ from subadults in other values (Table 2).

There were no size and weight differences between non-breeding (non-gravid), vitellogenic and ovigerous female lizards (*F*-tests; df = 2,38; $P > 0.10$) (Table 2). Lizards in these groups differed in WBC, RBC and PCV only. Ovigerous and non-gravid females had the highest RBC and PCV values.

Characteristic	A		B		C		
	Male (41)	Female (41)	Sub-adult (21)	Adult (20)	Non-gravid (6)	Vitellogenic (31)	Ovigerous (4)
Body wt (g)	52.1±14.7*	34.8±8.3	39.7±13.4*	63.0±7.7	35.5±8.2	34.5±8.8	36.1±4.9NS
SVL (mm)	118±12.0*	104±7.0	109±10.0*	128±4.0	105±8.0	104±8.0	100±3.0NS
WBC ($x10^{11}$/l)	0.54±0.26**	0.40±0.16	0.48±0.20NS	0.58±0.31	$0.35±0.02^{b}$	$0.38±0.16^{b}$	$0.60±0.09^{a}$
RBC ($x10^{12}$/l)	0.80±0.19NS	0.76±0.15	0.77±0.22NS	0.83±0.17	$0.83±0.10^{ab}$	$0.73±0.16^{b}$	$0.90±0.03^{a}$
Thrombocytes ($x10^{10}$/l)	0.56±0.30**	0.79±0.29	0.51±0.14NS	0.62±0.40	0.88±0.53	0.77±0.24	0.83±0.08
PCV (%)	29.3±4.6NS	28.5±4.9	26.7±3.7**	32.0±3.8	$32.3±5.7^{a}$	$27.2±4.5^{b}$	$32.1±1.7^{a}$
Hb(g/dl)	6.6±1.2**	5.7±0.7	6.2±1.1*	7.0±1.2	5.6±0.6	5.6±0.7	6.2±0.6
MCHC (g/dl)	22.9±3.1**	20.8±3.6	23.3±3.1NS	21.8±2.4	17.6±1.9	20.8±3.1	19.4±0.8
MCH (pg)	88.1±27.7*	76.7±16.8	86.3±31.2NS	87.5±22.5	68.2±8.5	79.4±18.3	69.0±4.4NS
MCV (fl)	388.8±103.9NS	381.6±66.9	378.8±121.8NS	399.1±82.9	392.3±83.0	382.7±68.6	358.0±9.9NS

TABLE 2. Mean body weight, SVL and haematological values (±SD) of rainbow lizards by sex (A), male relative age (B) and female reproductive condition (C). Number of lizards in each category is shown in parentheses. NS, non-significant; * $P < 0.05$; ** $P < 0.01$; multiple means along rows with the same superscripted letter are not different ($P > 0.05$).

DISCUSSION

The red blood cells of rainbow lizards and those of other reptiles are oval and have centrally-placed nuclei (Pienaar, 1962; Saint Girons, 1970; Simbotwe *et al.*, 1983). The mean RBC size of rainbow lizards falls within the range for reptiles (Saint Girons, 1970) but is less than the 23.8 by 16.2 μ given for the frog *Rana pipiens* (Rouf, 1969), and greater than the 13.1 by 6.6 μ described for the duck, *Aythya valisineria* (Kocan, 1972) and the RBC diameter of 7.2 μ for man (Brown, 1980).

The PCV for rainbow lizards falls within the 20% to 35% range reported for reptiles (Table 3, Pienaar, 1962; Duguy, 1970). The PCV can be an index of health status; a reduced value for a species can indicate anaemia while a raised value can indicate decreased plasma volume or dehydration (Pienaar, 1962; Dessauer, 1970; Thompson, 1969). Comparison with other vertebrates shows that PCV, RBC and Hb are lower in ectotherms than endotherms (Table 3). Emery (1986) ascribed this to different oxygen requirements by the animal groups. Sheeler & Barber (1965) obtained higher values of these parameters in the rabbit and rat than in the turtle.

Although values of MCHC, MCV and MCH were given in some reports on reptiles (Wintrobe, 1933; Pienaar, 1962) their significance was not discussed. These red cell indices are measures of blood iron content and are usually low in iron-deficiency anaemia (Thompson, 1969). For ectothermic vertebrates such as rainbow lizards which have larger-sized but fewer red blood cells compared with endotherms, MCV and MCH values are higher (Table 3). This is because the RBC is a denominator term in the formulae for calculating the parameters; MCV = (PCV/RBC) x 10 and MCH = (Hb/RBC) x 10 (units of expression as in Table 2). The relatively low RBC count in rainbow lizards is, therefore, responsible for the high MCV and MCH. The MCHC is not affected by RBC size. According to Thompson (1969) MCHC is the most accurate of the three red cell indices because it is estimated from the most accurately determined parameters, the PCV and Hb. These parameters also had the lowest coefficients of variation in this study.

The large individual variation in WBC and thrombocytes observed here has been reported for other ectothermic species and is considered normal (Pienaar, 1962; Rouf, 1969; Duguy, 1970).

Whereas male and female rainbow lizards differed in many haematological vlaues (Table 2), males and females of the lizard *Cordylus vittifer* (Pienaar, 1962) and

Species & Class		PCV	Hb	RBC	MCHC	MCH	MVC	Source
PISCES								
Sandbar shark *Carcharhinus plumbeus*		14.9	5.1	+NG	35.0	+NG	+NG	Emery (1986)
AMPHIBIA								
Leopard frog *Rana pipens*		24.7	6.8	0.31	27.4	211	772	Rouf (1969)
REPTILIA								
Boomslang *Dispholidus typus*		27.8	7.4	1.42	26.5	52	196	Simbotwe *et al.* (1983)
Lizard *Agama atra*		32.5	7.8	1.25	24.5	64	260	Pienaar (1962)
Lizard *Cordylus vittifer*		32.0	8.4	0.83	23.5	95	415	Pienaar (1962)
Chameleon *Chamaeleo dilepsis*		36.5	10.8	1.18	29.5	94	300	Pienaar (1962)
Rainbow lizard *Agama agama*		28.9	6.1	0.78	21.9	82	385	This study
AVES								
Canvasback duck *Aythya valisineria*		56.5	16.6	2.75	29.0	60	206	Kocan & Pitts (1976)
MAMMALIA								
Vampire bat *Desmodus rotundus murinus*		54.2	19.4	10.72	35.8	18	51	Krutzsch & Wimsatt (1963)
Man	Male	40 - 54	13.5 - 18.0	4.5 - 6.5	++32.0 - 36.0	++27 - 32	++76 - 79	Thompson (1969)
	Female	36 - 47	11.5 - 16.4	3.9 - 5.6				

TABLE 3. Comparative red cell values for ectothermic and endothermic species belonging to the same or other vertebrate classes as the rainbow lizard. Except man all values are means. Units of measurement are the same as in Table 2. $^{+NG}$, values not given; $^{++}$, pooled male and female values.

the snakes *Vipera aspis* and *Natrix maura* (Duguy, 1970) differed only in RBC and WBC. Engbretson & Hutchinson (1976) found no difference in PCV, Hb and RBC between males and females of the lizard, *Liolaemus multiformis*. The implication of observed gender-related differences in blood values where such exist, is that the values for each sex should be used for patho-physiological comparison between the same sex only as is done for humans (Thompson, 1969; Brown, 1980). Such differences in values due to gender have been attributed to hormonal influence (Duguy, 1970; Nirmalan & Robinson, 1971; Gee *et al.*, 1981).

Higher PCV values are frequently reported in adults than subadult and juvenile ectothermic vertebrates (Duguy, 1970; Nirmalan & Robinson, 1971; Kocan & Pitts, 1976). Differences in other values are not as frequent. Nirmalan & Robinson (1971) reported that young quail differed from adults in RBC, Hb, MCHC and thrombocytes. We also observed a difference in Hb between adult and subadult male rainbow lizards.

In birds, egg production (vitellogenesis) decreases PCV, Hb (Nirmalan & Robinson, 1971) and RBC (Ronald, Foster & Dyer, 1968) and it probably does likewise in female rainbow lizards. Among female rain-

bow lizards, only vitellogenic ones commonly have developing yolked ovarian follicles (Sodeinde & Kuku, 1989). These had the lowest RBC and PCV values. Duguy (1970) also reported a decrease in the number of RBCs in pregnant females of the snake *V. aspis*.

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