دراسات فى دم بعض زواحف الكويت
الجزء الرابع : تعيين بعض ثوابت الكريات ، وجلوكوز الدم ، وبروتينات الدم ، مع دراسة الفحص الكهربى للبلازما والمصل والهيموجلوبين فى الورل فار اناس جريسياس والأمفيسبينا دبلوميتوبون زارودنياى

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خلاصـــة

عين الباحثان كمية الهيموجلوبين ، والهياتوكريت ، وعدد كريات الدم الحمراء وحجمها ، وكمية الجلوكوز فى الدم ، والمحتوى البروتينى لكل من البلازما والمصل ، وتركيز الزلال فى الدم . وقد توصلا إلى أن النتائج تعكس اختلافات بين الحيوانين .

وفى دراسة السلوك البروتينى فى المصل والبلازما والهيموجلوبين بطريقة الفصل الكهربى على الورق أو على خلات السليولوز ، تبين أن هناك ما بين أربعة وخمسة مكونات فى المصل والبلازما فى الورل . أما فى الدبلوميتوبون فقد حصل الباحثان على ثلاثة مكونات فقط . وبالنسبة للهيموجلوبين فقد أثبتت النتائج وجود مكون هيموجلوبينى واحد فى الورل ، يتجه معظمه نحو القطب الموجب ، أما هيموجلوبين الدبلوميتوبون فيحوى مكونين مختلفين فى الكمية ، يتجهان نحو القطب الموجب .

ومن خلال النتائج التى أسفر عنها البحث فأنه يمكن القول بصفة عامة بأن للورل معظم صفات دم السحالى ، أما الدبلوميتوبون فأن له صفات مختلفة .

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major one and consists of 88·8% of the total haemoglobin. The second component is the minor one and consists of 11·2% only of the total haemoglobin. Regarding mobility, the first, or the major component, moved at a slightly less rapid rate than human haemoglobin A. The second component, or the minor one, moved at a rapid rate and became well separated from the first component. The major component is comparable to the homogeneous, single component haemoglobins of the lacertid lizards *Acanthodactylus scutellatus* and *Eremias brevirostris* which were obtained when using the same buffer (veronal buffer, pH 8·6) and also the same technique. The second component, as far as our experiments are concerned, is specific to *Diplometopon* and not comparable with any human or squamate haemoglobin.

These observations are only preliminary since different methods of examination of blood proteins have not yet been employed. It is likely that the study of larger numbers of species will discover additional examples of variation in these proteins. The results obtained in this study, although preliminary, reveal that the monitor lizard *Varanus griseus* has the typical lizard haematological characters. The worm-like amphisbaenian *Diplometopon zarudnyi* does not possess all these characters. It has some of the lizard characters, some of the snake characters, and some other specific characters. Undoubtedly, this animal is closely related to snakes and lizards, but has characteristics suggesting that it belongs to a separate group, suborder, among squamates. Reference should be made to Gans (1969) for his similar conclusions which were based on different morphological and anatomical features.

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before. The electrophoretic migration of this haemoglobin differs, but little, from other lacertilian haemoglobins. The electrophoretic pattern shows that only 76·5% of the haemoglobin component migrates to the anode, while 23·5% of this component remains close to the point of origin, but directed to the cathode (Figs. 3–5). In the amphisbaenian *Diplometopon zarudnyi* (Figs. 6–8), haemoglobin shows two widely separated anodic bands. The two-banded pattern shows that the first component is the

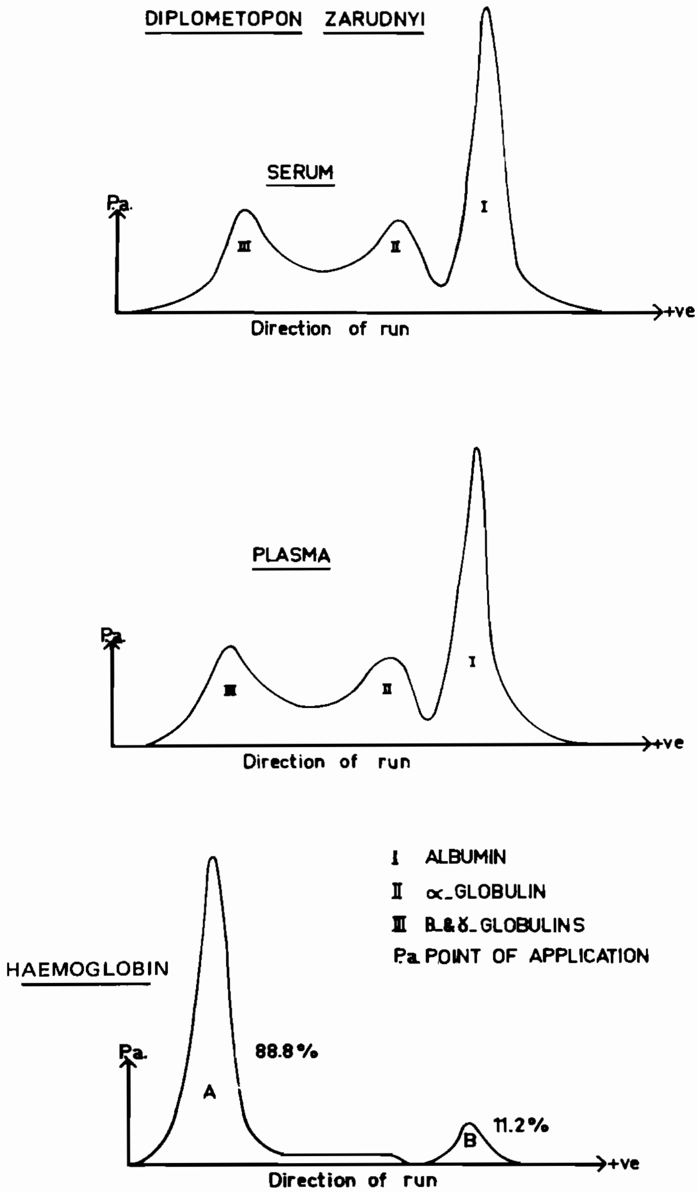

Fig. 8. Electrophoretic patterns of serum proteins, plasma proteins and haemoglobin of *Diplometopon zarudnyi*.

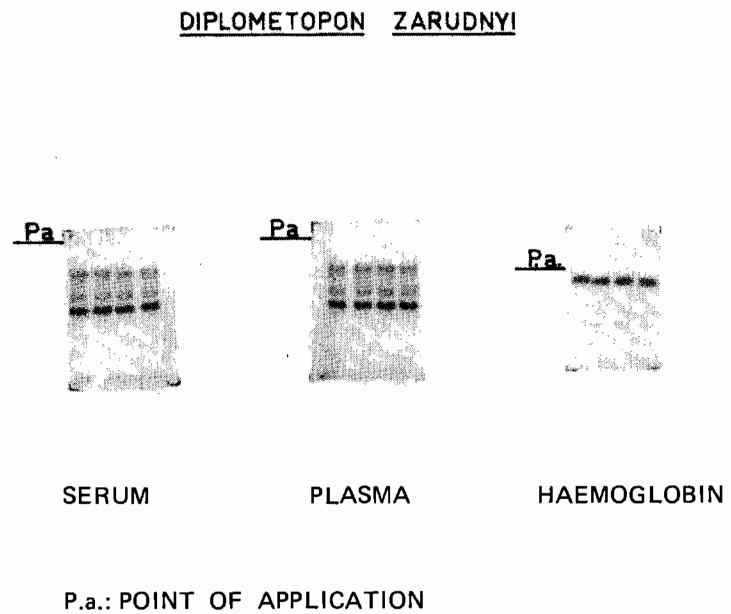

Fig. 7. Cellulose acetate microelectropherograms of serum proteins, plasma proteins and haemoglobin of *Diplometopon zarudnyi.*

among squamates, lizards in general are characterised by higher albumin concentrations. Snakes, on the contrary, are characterised by lower albumin concentrations. The high albumin recorded in the blood of *Diplometopon*, although it may be a reflection of its subterranean life, yet it places this amphisbaenian with lizards in possessing this character.

A common reptilian character also recorded herein for *Varanus* and *Diplometopon* is the absence of a separate fibrinogen fraction in their plasma. As in other reptiles, fibrinogen usually migrates with the alpha or the alpha and beta globulins. This makes the albumin:globulin ratio of plasma always lower than in serum (Tables 3, 4).

As regards haemoglobins, those of many reptiles have been analysed by different electrophoretic methods. The most systematic studies are those of Dessauer *et al.* (1957), Guttman (1970) and Gorman & Schochat (1972). Haemoglobins from various lizards and snakes inhabiting Kuwait were examined, and their electrophoretic behaviour was reported (Abdel-Fattah *et al.* 1974; Al-Badry & Abdel-Fattah 1975; Al-Badry 1975a, b). All the lizards and snakes examined in these investigations showed simple patterns of single-component haemoglobins. Some displayed anodic mobility similar to that of human haemoglobin A (agamid lizards); others possessed lesser anodic mobility (lacertid lizards); and some had slow cathodic mobility (colubrid and viperid snakes).

In the present investigation, Figs. 3–8 show that paper and cellulose acetate techniques revealed similar results. It is evident that *Varanus griseus* possesses a single homogeneous haemoglobin component like that reported for other lizards investigated

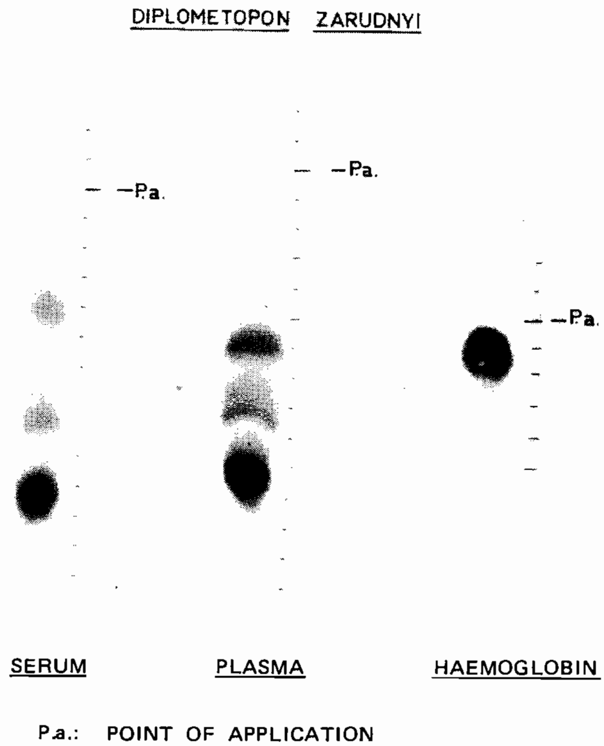

Fig. 6. Paper electropherograms of serum proteins, plasma proteins and haemoglobin of *Diplometopon zarudnyi*.

more or less, within the normal range of lizards (Dessauer & Fox 1964). The albumin percentage is slightly higher than in the agamid lizard *Uromastix microlepis* (Al-Badry & Abdel-Fattah 1975). On the other hand, it is slightly lower than in the lacertid lizards *Acanthodactylus scutellatus* and *Eremias brevirostris* (Al-Badry 1975a). Regarding the electrophoretic mobility of the protein components, Fig. 3 shows that in *Varanus*, as in all reptiles, it is generally low if compared to the rate of migration of the mammalian proteins (Gleason & Freidberg 1953).

For *Diplometopon zarudnyi*, Fig. 8 shows that the electrophoretic patterns obtained seem rare among squamates. Most lacertilian and ophidian blood in our experience resolved into four or five protein fractions. This may be a special electrophoretic behaviour for the amphisbaenian plasma and serum proteins which makes them different from lizards and snakes. On the other hand, the relatively high albumin percentages, and the consequent high albumin: globulin ratios, are similar to those obtained for many lizards. It is still consistently higher than in most snakes. Dawson (1960), Dessauer (1953 & 1970), and Al-Badry (1975b) arrived at the conclusion that with other reptilian levels especially ophidians. Since blood sugar concentration is an indication of animal activity, such low value becomes expected in amphisbaenians due to their special mode of life as burrowing animals.

As far as the available literature is considered, it may be worthy of mention that most of the biochemical data presented here for the amphisbaenian *Diplometopon zarudnyi* may be the first determinations ever presented for such an animal.

(*C*) *Blood proteins*

Evaluations of the plasma and serum proteins in several species of lizards and snakes have been carried out by different authors. Dessauer (1970) presented a table including values of blood proteins of different reptiles as determined by several investigators. Plasma protein concentrations of some reptiles living in Kuwait have been determined by Al-Badry (1974), Abdel-Fattah *et al.* (1974), Al-Badry & Abdel-Fattah (1975), and Al-Badry (1975a, b). The results showed that the lowest value is 4·0 g% recorded for the lizard *Eremias brevirostris*, and the highest 6·5 g, recorded for the snake *Coluber ventromaculatus*. The values reported here (Tables 1, 2) agree with these values. Plasma and serum proteins of the lizard *Varanus griseus* are 5·21 and 5·10 g% respectively. These values are very close to those recorded by Nair (1958) for the same species. For the amphisbaenian *Diplometopon zarudnyi*, plasma and serum protein concentrations are 4·13 and 4·08 g%. These data are in accordance with the values already determined for other reptiles. They may suggest only a limited variation in the plasma and serum proteins within reptiles (Dessauer 1970; Otis 1973).

(D) *Electrophoresis of blood proteins*

The protein patterns obtained for normal human serum on paper and cellulose acetate electrophoresis agree closely with the results of other workers (Moody & Thomas 1975). Higher percentages of albumin fractions were obtained when using cellulose acetate than when using paper techniques (Tables 3–5). It is worthy of mention that the results of albumin percentages given by cellulose acetate electrophoresis coincide with the absolute values of albumin concentrations presented in Tables 1 and 2.

In the monitor lizard *Varanus griseus*, the serum and plasma proteins resolved on paper into four fractions (Fig. 3). These are albumin, alpha, beta and gamma globulins. The alpha fraction was not resolved on paper into its $alpha_1$ and $alpha_2$ components. Applying the cellulose acetate technique, five fractions were obtained, numbered I through V for albumin, $alpha_1$, $alpha_2$, beta and gamma globulins (Figs. 4, 5).

In the amphisbaenian *Diplometopon zarudnyi*, the serum and plasma proteins resolved both on paper and cellulose acetate into three fractions only, numbered I, II and III for the fastest to the slowest in anodic mobility (Figs. 6–8). According to their mobility and percentage composition, these three fractions were comparable to albumin, alpha globulin and beta with gamma globulins. They were not resolved into more fractions under the standard experimental conditions followed in the present and previous investigations.

In order to compare the electrophoretic behaviour of the blood proteins of the present animals with those previously investigated in this series of work, paper electrophoresis results should be considered. For *Varanus griseus*, the percentage composition of the plasma and serum protein fractions, as shown in Table 3, lies,

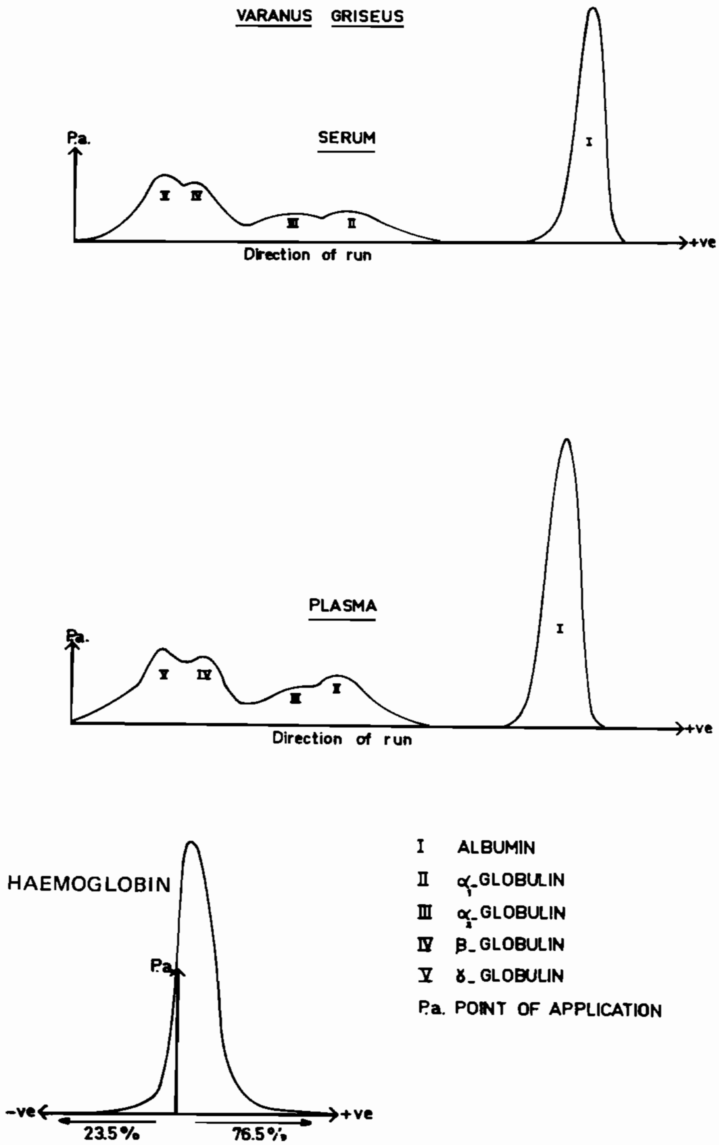

Fig. 5. Electrophoretic patterns of serum proteins, plasma proteins and haemoglobin of *Varanus griseus*.

animal. The data given in Table 1 show that *Varanus*, as a lizard, has a higher glucose level than in other reptiles. Meanwhile, the glucose concentration of *Varanus* blood (109·7 mg%) is still lower than that of most other lizards. The value reported here is very similar to that reported by Dessauer (1970) for the same species (106 mg%). Blood glucose concentration of *Diplometopon*, shown in Table 2 (66·25 mg%), is consistently low when compared with the value recorded for *Varanus* or even for other lizards (Dessauer 1970; Abdel-Fattah *et al.* 1974; Al-Badry & Abdel-Fattah 1975; Al-Badry 1975a). On the other hand, this blood glucose level can compare well

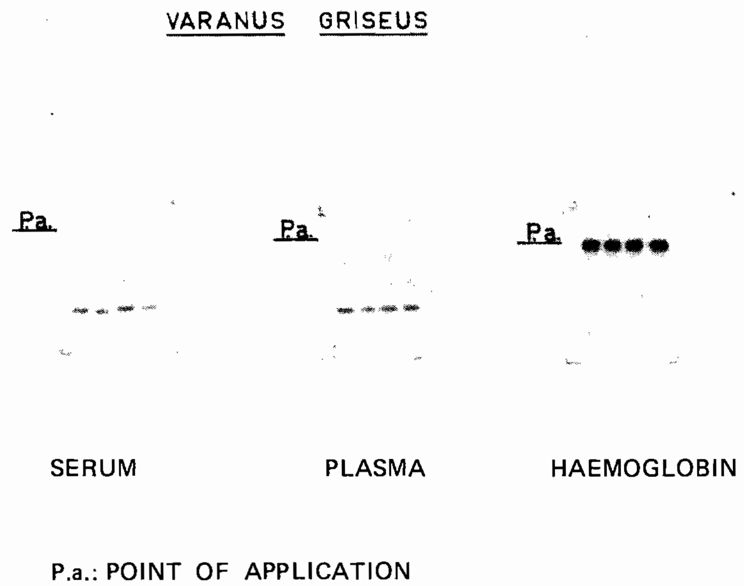

Fig. 4. Cellulose acetate microelectropherograms of serum proteins, plasma proteins and haemoglobin of *Varanus griseus*.

166·76 μ^2. The high figure for red cell count in *Diplometopon*, accompanied by essentially unchanged PCV, can be interpreted on the basis that this animal has small-sized erythrocytes. Kaplan & Rueff (1960) obtained a similar result for some turtles and attributed it to the difference in the plasma volume.

Figs 1 and 2 illustrate the morphology of blood cells in *Varanus* and *Diplometopon*. From these figures it is evident that the red cells (erythrocytes) of both animals are oval and nucleated. Likewise, the nuclei are oval, with dense chromatin strands which are more visible in *Varanus* than in *Diplometopon*. Tables 1 and 2 show that the cell diameter ranges in *Varanus* from 12·25 to 190·3 μ, and in *Diplometopon* from 12·00 to 17·15 μ. St.-Girons (1970) reported lower values for *Varanus griseus* (from 8·9 to 15·6 μ). For the amphisbaenian *Trogonophis wiegmanni*, she reported values ranging from 9·5 to 16·6 μ. St.-Girons & St.-Girons (1969) postulated that within the class Reptilia, the largest erythrocytes occur in *Sphenodon punctatus*, turtles, and crocodilians, which are considered very archaic. They added that the smallest corpuscles are found in the Lacertidae which have retained a wide evolutionary potential. Regarding amphisbaenians, the data presented here in Table 2, and also those presented by St.-Girons (1970), suggest that this group of reptiles, like many lizards and snakes, may not be very archaic among squamates, and perhaps geckos are more archaic.

(*b*) *Blood glucose*

Blood sugar seems to present an important indication of the state of activity of the

Table 5. The percentage of albumin and globulin contents of human serum determined by paper and cellulose acetate electrophoresis

Fraction	Albumin–globulin percentage	
	Paper	Cellulose acetate
Albumin	56·5 ± 4·5	65·8 ± 4·6
α_1-globulin	5·6 ± 0·3	3·3 ± 0·3
α_2-globulin	8·1 ± 0·6	5·9 ± 0·4
β-globulin	11·8 ± 0·9	9·7 ± 0·6
γ-globulin	18·0 ± 1·1	15·3 ± 0·9
Albumin/Globulin ratio	1·30 ± 0·09	1·92 ± 0·15

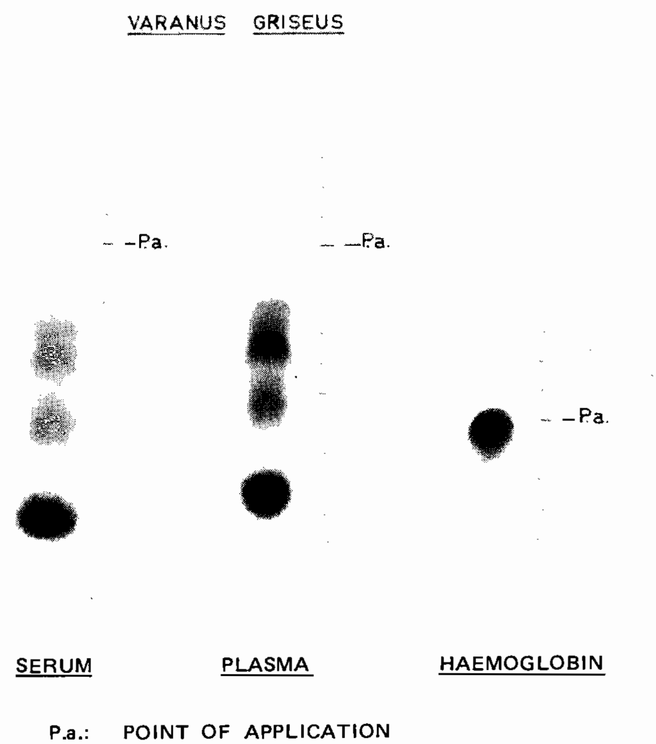

Fig. 3. Paper electropherograms of serum proteins, plasma proteins and haemoglobin of *Varanus griseus*.

Table 3. The percentage of albumin and globulin contents in serum and plasma of *Varanus griseus*

Support	Fraction	Serum	Plasma
Paper	Albumin	49·15 ± 2·95	47·93 ± 2·40
	α_1-globulin } α_2-globulin	19·22 ± 1·06	22·01 ± 1·43
	β-globulin	12·81 ± 0·90	13·25 ± 0·93
	γ-globulin	18·82 ± 1·13	16·81 ± 0·91
	Albumin/globulin ratio	0·97 ± 0·07	0·92 ± 0·06
Cellulose acetate	Albumin	53·01 ± 2·30	51·59 ± 2·00
	α_1-globulin	9·64 ± 1·70	12·10 ± 0·67
	α_2-globulin	7·83 ± 1·49	7·01 ± 1·03
	β-globulin	11·45 ± 1·47	12·74 ± 1·16
	γ-globulin	18·07 ± 1·86	16·56 ± 1·44
	Albumin/globulin ratio	1·13 ± 0·09	1·07 ± 0·09

lizard. This value is lower than that recorded by Haggag *et al.* (1966) for the same species in summer or in winter. For the amphisbaenian *Diplometopon zarudnyi*, the value recorded is 4·15 g% which is low and unexpected if the relation between haemoglobin content and body size was considered. A similar result was achieved by Judd (1974) who preferred not to involve body size as a causative factor in haemoglobin concentration. If such low haemoglobin is obtained for more amphisbaenians, it may reflect a genetic variation between two squamate groups, lizards and amphisbaenians. The underground life and the oxygen-deficient atmosphere around amphisbaenians have to be considered as causative factors of this difference.

Considering haematocrit, red cell count, and cell size, Table 1 shows that the PCV of *Varanus* is 35·61%, the red cell count $1 \cdot 157 \times 10^6/mm^3$, and the cell size 192·02 μ^2. These values lie within the normal range of many lizards as reported by Duguy (1970). The erythrocyte count approaches that recorded by Haggag *et al.* (1966) for the same species. Regarding cell size, St.-Girons (1970) reported a value of 109·2μ^2 for *Varanus griseus*, which is much lower than the value reported here. For *Diplometopon*, Table 2 shows that the PCV is 35·43%, the red cell count $1 \cdot 338 \times 10^6/mm^3$, and the cell size

Table 4. The percentage of albumin and globulin contents in serum and plasma of *Diplometopon zarudnyi*

Support	Fraction	Serum	Plasma
Paper	I (albumin)	49·62 ± 2·79	48·22 ± 2·83
	II (α-globulin)	19·98 ± 1·10	23·92 ± 1·34
	III (β- and γ-globulins)	30·40 ± 2·04	27·86 ± 2·28
	Albumin/globulin ratio	0·98 ± 0·07	0·93 ± 0·07
Cellulose acetate	I (albumin)	50·12 ± 3·21	49·50 ± 3·06
	II (α-globulin)	18·59 ± 1·09	22·93 ± 1·42
	III (β- and γ-globulins)	31·29 ± 2·82	27·57 ± 2·45
	Albumin/globulin ratio	1·00 ± 0·08	0·97 ± 0·07

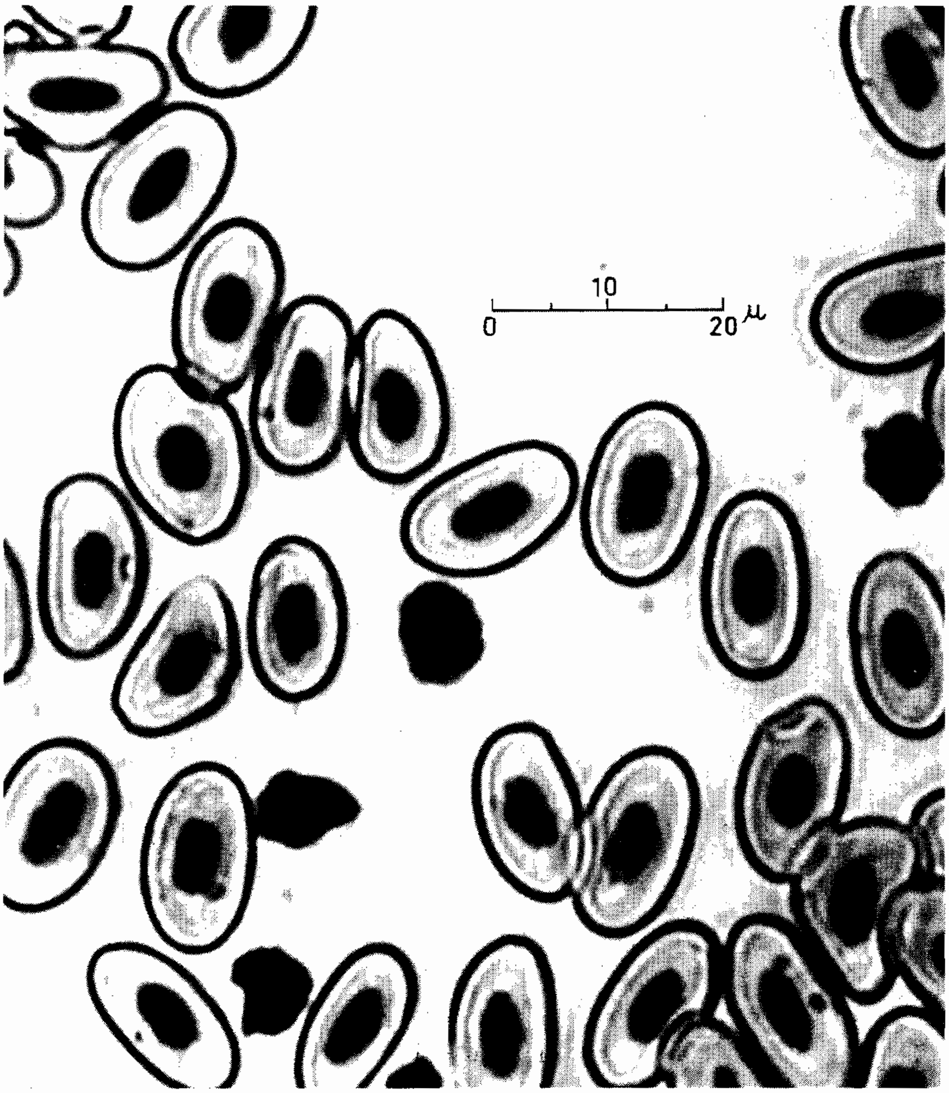

Fig. 2. A photomicrograph showing erythrocytes of *Diplometopon zarudnyi.*

respiratory functioning beyond the limit of tolerance. Thus, the wide range in haemoglobin values reported for reptiles by different authors is not surprising. Goin & Jackson (1965) attributed such variation to different factors: they mentioned that it may be correlated with ontogeny, activity, altitude or temperature. Moreover, they suggested a correlation between the haemoglobin content and body size. In this connection Al-Badry (1975a) and Al-Badry & Abdel-Fattah (1975) recorded haemoglobin values for the small lizard species *Eremias brevirostris* as 9·2 g%, and for the large lizard *Uromastix microlepis* as 5·8 g%.

In the present study, the haemoglobin content recorded for the lizard *Varanus griseus* is 4·4 g%, a value that was expected considering the large size of the monitor

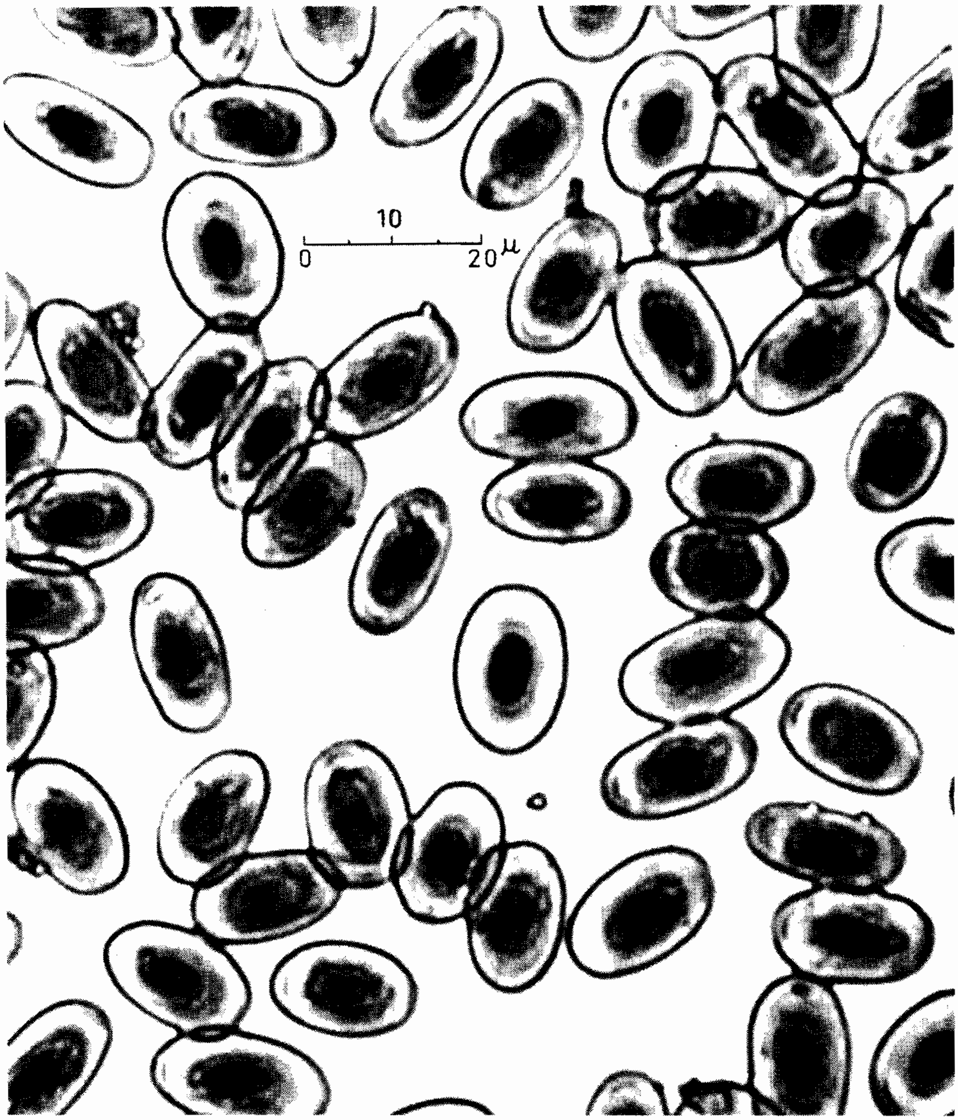

Fig. 1. A photomicrograph showing erythrocytes of *Varanus griseus*.

protein fractions. Photomicrographs of blood cells from *Varanus* and *Diplometopon* are presented in Figs. 1 and 2. Figs. 3 and 4 show paper and cellulose acetate pherograms obtained for serum, plasma and haemoglobin of *Varanus griseus*; their electrophoretic patterns are presented in Fig. 5. For the amphisbaenian *Diplometopon zarudnyi*, the blood protein pherograms and patterns are illustrated in Figs. 6–8.

(*A*) *Corpuscular constants*

In the ectothermal vertebrates, the respiratory function seems to be the product of so many variables that many of these variables can fluctuate widely without carrying

For electrophoretic examination, plasma and serum were prepared by blood centrifugation at 5°C. For the preparation of haemolysate solutions, the technique described by Chernoff (1955) was followed. Electrophoresis of serum, plasma and haemoglobin of the experimental animals was determined under uniform conditions. In each electrophoretic run, a parallel sample of human serum was used as a standard reference. The protein separations were conducted on Elphor-H apparatus for paper electrophoresis following the technique of Block *et al* (1958), and on Beckman apparatus for cellulose acetate electrophoresis following the technique of Moody & Thomas (1975). Scanning of the paper pherograms was conducted on Elphor-H integraph. Cellulose acetate pherograms were scanned on Beckman R-110 microzone densitometer.

RESULTS AND DISCUSSION

The corpuscular constants, blood glucose and blood proteins of *Varanus griseus* and *Diplometopon zarudnyi* are presented in Tables 1 and 2 respectively. Tables 3 and 4 show the percentages of albumin and globulin contents in the serum and plasma of the two animals. Using human serum as a standard reference in both paper and cellulose acetate electrophoresis, Table 5 shows the relative percentages obtained for its different

Table 1. Means of some corpuscular constants, blood glucose and protein of *Varanus griseus*

Haemoglobin (g/100 ml)	4·40 ± 0·59
Packed cell volume (PCV) (%)	35·61 ± 7·10
Red cell count (million/mm^3)	1·157 ± 0·404
Red cell diameter—long axis (μ)	19·03 ± 0·61
Red cell diameter—short axis (μ)	12·25 ± 0·45
Red cell size (μ^2)	192·02 ± 13·44
Blood glucose (mg/100 ml)	109·70 ± 16·09
Total plasma protein (g%)	5·21 ± 1·32
Total serum protein (g%)	5·10 ± 0·87
Albumin concentration (g%)	2·68 ± 0·23

Table 2. Means of some corpuscular constants, blood glucose and protein of *Diplometopon zarudnyi*

Haemoglobin (g/100 ml)	4·15 ± 0·19
Packed cell volume PVC(%)	35·43 ± 1·18
Red cell count (million/mm^3)	1·338 ± 0·110
Red cell diameter—Long Axis (μ)	17·15 ± 0·25
Red cell diameter—Short Axis (μ)	12·00 ± 0·25
Red cell size (μ^2)	166·76 ± 8·34
Blood glucose (mg/100 ml)	66·25 ± 3·77
Total plasma protein (g%)	4·13 ± 0·49
Total serum protein (g%)	4·08 ± 0·55
Albumin concentration (g%)	2·04 ± 0·13

new information to our knowledge of the reptiles inhabiting this part of the world. This work is not intended to answer taxonomic questions, although the data presented may be useful to taxonomists.

MATERIAL AND METHODS

Two reptilian species were chosen for the present study, the monitor lizard *Varanus griseus* (Varanidae, Lacertilia, Squamata), and the amphisbaenian *Diplometopon zarudnyi* (Trogonophidae, Amphisbaenia, Squamata).

Varanus griseus is a diurnal lizard, commonly observed beside burrows formed in hard soils using them as shelters during the hibernation period in the winter. It has a large head, long neck and a heavy body. It is coloured sandy yellow with dark brown spots dorsally. Ventrally, it is more or less sandy, but the dark spots are found only below the head and neck. The tail is extremely long with transverse brown bands along its length. The lizard is carnivorous, usually seen feeding on rodents and small lizards.

Diplometopon zarudnyi is one of the amphisbaenians which were originally regarded as a group of burrowing lizards. Several authors, among them Gans (1969), prefer now to keep them, at least for the moment, in a separate suborder. *Diplometopon* resembles a large stocky earthworm. The head is blunt, with no visible neck. It has a sloping, spade-shaped snout. The eyes are small and sunk beneath smooth, shiny scales. The body is long and limbless, with a groove along the median line of its ventral side. The tail is short and pointed. The scales are set in transverse rows forming annular grooves. The colour is spotted brown above and pinkish white below. This species burrows in damp sandy ground, and rarely appears on the surface. It feeds on earthworms, ants and termites.

Only six adult specimens of *Varanus* have been collected from Kuwait from March to July 1975. The quantity of blood collected from each individual ranged from 15 to 20 ml, which was enough to carry all experiments in triplicate. In the case of *Diplometopon*, 35 adult individuals were collected in the period from March to October 1975. This was a large number considering the scarcity of this animal, and the difficulties faced in obtaining it. Each individual gave only between 1 and 2 ml of blood; this necessitated the use of microtechnipues in determining the blood glucose and total protein concentrations.

This investigation was carried out in the period of March to October 1975, to avoid any variation in the data that could have been induced by cold weather or hibernation of these animals in winter (Acuna 1974). Individuals of undetermined sex were brought to the laboratory immediately after capture, and examined within 24 hr, thereby minimizing physiological changes which could have been induced by capture or prolonged maintenance in captivity. Blood was obtained from living individuals by cardiac puncture.

For corpuscular constants, haemoglobin was determined as cyanomethaemoglobin using Beckman spectrophotometer ACTA C II at 540 nm. Packed cell volume was determined using heparinized microhaematocrit tubes. Erythrocytes were counted using the standard Hawksley haemocytometer. Red cell diameter was measured and cell size calculated using a calibrated ocular micrometer (Dacie & Lewis 1970). Blood glucose and protein concentrations were estimated using Technicon Auto Analyser AAI, and following the methods recommended by Frankel *et al.* (1972).

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Haematological studies on some reptiles from Kuwait

Part IV. Some corpuscular constants, blood glucose, blood proteins, and electrophoretic examination of plasma, serum and haemoglobin of the monitor lizard *Varanus griseus* and the amphisbaenian *Diplometopon zarudnyi.*

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ABSTRACT

Haemoglobin concentration, packed cell volume, red cell count, erythrocyte size, blood glucose, total plasma protein, total serum protein and albumin concentration have been determined for the monitor lizard *Varanus griseus* and the amphisbaenian *Diplometopon zarudnyi*. The mean values reported reflect some variations specific to each animal.

Paper and cellulose acetate electrophoretic separations of serum and plasma proteins have been conducted. Four to five fractions were obtained for the serum and plasma of *Varanus*. Three fractions only were obtained for *Diplometopon*. No qualitative difference between the patterns of serum and plasma was detected. Haemoglobin behaviour on electrophoresis showed a single anodic component for *Varanus* and two widely separated anodic bands for *Diplometopon*. Blood protein examination revealed that the monitor lizard *Varanus griseus* has the typical lizard characters. The amphisbaenian *Diplometopon zarudnyi* has some different characters.

INTRODUCTION

Recent studies on reptilian haematology provided useful results. Some of the data given are concerned with biochemical fields. Most often these characters have been used to serve as a check on the reptilian systematic scheme. However, a survey of the literature shows that little is known about this group of vertebrates, and there is still a need for further haematological work, especially on amphisbaenians. Gans (1967), in his list of recent amphisbaenians, called for further studies on their physiology. Moreover, the same author (Gans 1971), directed attention to the importance of the study of the chemistry of the amphisbaenian blood and plasma to give information on their blood characteristics that may help in the settlement of their taxonomic position. On the other hand, some useful studies were carried out by several authors on some lizards and snakes. Among these studies, those of Dessauer & Fox (1956, 1964), Pearson (1966), Dessauer (1967, 1970), Voris (1967), Guttman (1970), Gorman & Schochat (1972), Horton *et al.* (1972), Otis (1973), Higgins & Rand (1974, 1975), Judd (1974) and MacMahon & Hamer (1975) are probably the broadest.

This paper is the fourth in a series of investigations suggested to study some haematological aspects of the reptiles living in the deserts of Kuwait, aiming to add

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