J. Univ. Kuwait (Sci.) 2, 1975

HAEMATOLOGICAL STUDIES ON SOME REPTILES FROM KUWAIT

PART III. SOME CORPUSCULAR CONSTANTS, BLOOD GLUCOSE, TOTAL PLASMA PROTEIN AND ELECTROPHORETIC EXAMINATION OF BLOOD PROTEINS OF THE LIZARDS *ACANTHODACTYLUS SCUTELLATUS* AND *EREMIAS BREVIROSTRIS*

KAMAL S. AL-BADRY

Department of Zoology, University of Kuwait

Abstract. Corpuscular Constants : hemoglobin, hematocrit (packed cell volume), and red cell count; also blood glucose and total plasma protein of the lizards ***Acanthodactylus scutellatus*** and ***Eremias brevirostris*** were determined. For ***Acanthodactylus scutellatus*** the mean values are : 8.5 g/100 ml, 30.0%, 1.135 $\times$ 10^6/mm^3, 224.6 mg/100 ml, and 4.7 g %. For ***Eremias brevirostris*** the mean values are : 9.2 g/100 ml, 30.8%, 1.424 $\times$ 10^6/mm^3, 217 mg/100 ml, and 4.0 g % respectively.

Electrophoretic mobilities of the three protein systems, serum, plasma and hemoglobin are similar in the two lacertid lizards, but different from those previously obtained by the same author for two agamid lizards. Electrophoretic patterns of serum and plasma proteins consist in both lizards of four fractions, albumin, α—globulin, β—globulin and γ—globulin. The α—globulin fraction was not resolved into α_1 and α_2 as obtained in the agamid lizards. Regarding the relative proportions of the different protein fractions and the albumin : globulin ratio, both lizards showed but little variation from each other. In all individuals of the two species examined, no fibrinogen fraction was detected in their plasma patterns. Hemoglobin of both lizards behaved as a homogeneous single fraction slightly moving towards the anode. The rate of movement was nearly the same in both lizards, but it differed from that recorded for the agamid lizards.

INTRODUCTION

Blood chemistry and serology have been successfully employed in systematic studies. Most of these studies have been used to serve as a check on an established classification. Phylogenetically, the characteristics of blood proteins emphasize the evolutionary divergence of major groups of animals. Dessauer (1970) reported that in reptiles, major structural differences exist between the hemoglobin, plasma and serum proteins and other blood constituents of turtles, crocodiles and squamates. He added that homologous blood proteins exhibit hierarchies of variation that parallel degrees of divergence of taxa of close and distant relationships. Comparative evidence on proteins could clarify difficult problems of relationship. Structural differences between blood proteins of different reptiles have been related to their evolutionary, physiological and ecological factors (Dessauer 1970). Some studies on different aspects of reptilian blood have been carried out by several authors. Dessauer and Fox (1956, 1958 and 1964), Gorman and Dessauer (1966), Dessauer (1970), Guttman (1970), Gorman and Shochat (1972), Horton *et al.* (1972), Otis (1973), Abdel-Fattah *et al.* (1974), Burbidge *et al.* (1974) and Judd (1974) have concluded that blood properties of reptiles not only substantiate the placement of different species of this group, but also alter and amplify our understanding of relationships among them.

In the present investigation, a trial was made to add further information to our knowledge about the group of lizards living in Kuwait. The final aim of this series of papers is to establish some phylogenetic relationships between the different families of suborder Lacertilia living in this part of the world. For this reason a determination of some corpuscular constants, blood glucose and total plasma protein, along with an electrophoretic examination of blood proteins are carried out on different representatives of the lizard families. The work in this paper was confined to the two lacertid lizards *Acanthodactylus scutellatus* and *Eremias brevirostris*.

MATERIALS AND METHODS

Acanthodactylus scutellatus (family Lacertidae), one of the lizards used in the present investigation, is a common lizard in the desert of Kuwait. It is characterised by having a short and slender body with a relatively long tail. The

body is covered by small and granular scales of which four rows are found around the fingers. Toes, especially the fourth one, have well-developed lateral fringes. The lizard is usually seen running very fast over loose surfaces even over wind-blown sand dunes. It keeps to a small area, running, hiding or burrowing under bushes but not climbing them. It is diurnal and active all over the year. It is almost entirely insectivorous.

Eremias brevirostris (family Lacertidae), the other lizard in the present study, is found usually living with *Acanthodactylus* in the same localities but in separate populations. It is characterised by having a smaller body with a thin and long tail. It has a short snout with raised nostrils. Digits are long, slender and unfringed. This lizard is very active through day-time most of the year. It may be observed hiding under any object or in a small burrow. It is insectivorous, feeding on flies, sand-hoppers, etc.

Individuals of the two lizard species *Acanthodactylus scutellatus* and *Eremias brevirostris* were collected from the field several times through January to April, 1975. Each time the individuals were kept in captivity for one week before use, with access to water only, and under a constant temperature (25°C) and a photoperiod (12 hour light-dark cycle). Sixty specimens of undetermined sex from each species were used in this investigation. Blood samples were obtained through heart punctures. For the determination of corpuscular constants and for the preparation of plasma and hemolysate solutions, blood was allowed to drip into oxalate-coated specimen tubes. When the serum was needed, no anticoagulant was used. Hemoglobin content was determined using Sahli's hemoglobinometer. Packed cell volume (hematocrit) was determined by microhematocrit tubes. Red cell counts were made in a standard hemocytometer. Blood glucose was determined by using the titrimetric method described by Wootton (1964). Total plasma protein was estimated by the method of digestion and oxidation described by Hawk *et al.* (1954). Serum and plasma were prepared by centrifuging the blood samples at 3000 rpm for 30 minutes under constant temperature (5°C). For the preparation of hemolysate solutions, the technique recommended by Chernoff (1955) was followed. Protein separations were carried out on Elphor-H paper electrophoresis apparatus following the technique of Block, Durrum and Zweig (1958), using barbital buffer, pH 8.6 and ionic strength 0.05 mv. Scanning was conducted on Elphor integraph.

Results and Discussion

Corpuscular Constants, Blood Glucose, and Total Plasma Protein

The results of hemoglobin, hematocrit, red cell count, blood glucose and total plasma protein of *Acanthodactylus scutellatus* are given in Table 1. Those of *Eremias brevirostris* are given in Table 2. Each figure represents the mean of 20 individuals.

From these tables it is evident that hemoglobin is slightly higher in *Eremias* than in *Acanthodactylus* (9.2 and 8.5 g/100 ml respectively). Both values are still higher than those recorded by Al-Badry and Abdel-Fattah (1975) for the lizards *Agama persica* (7 g/100 ml) and *Uromastix microlepis* (5.8 g/100 ml). Dawson and Poulson (1962) recorded hemoglobin values of 8.2 g/100 ml for the lizard *Sceloporus graciosus*, and 9.1 g/100 ml for the lizard *Phrynosoma modestum*. The relatively higher values of hemoglobin content obtained for the two lizards in the present study may be attributed to their considerably small size. This agrees with the findings of Goin and Jackson (1965). The means of the hematocrit values were 30.0% and 30.8% for *Acanthodactylus* and *Eremias*, which are also higher than those recorded for *Agama* (25%) and for *Uromastix* (24.6%). Hernandez and

TABLE 1. Means of Some Corpuscular Constants, Total Plasma and Serum Proteins and Blood Glucose of *Acanthodactylus scutellatus*

Hemoglobin (g/100ml)	8.5 ± 0.6
Packed Cell Volume P.C.V. (%)	30.0 ± 2.8
Red Cell Count (million/mm³)	1.135 ± 0.417
Blood Glucose (mg/100 ml)	224.6 ± 37.1
Total Plasma Protein (g%)	4.7 ± 0.4
Total Serum Protein (g%)	4.6 ± 0.6

TABLE 2. Means of Some Corpuscular Constants, Total Plasma and Serum Proteins and Blood Glucose of *Eremias brevirostris*

Hemoglobin (g/100 ml)	9.2 ± 0.9
Packed Cell Volume P.C.V. (%)	30.8 ± 2.3
Red Cell Count (million/mm³)	1.424 ± 0.402
Blood Glucose (mg/100 ml)	217.0 ± 29.8
Total Plasma Protein (g%)	4.0 ± 0.6
Total Serum Protein (g%)	3.8 ± 0.7

Coulson (1951) and also Thorson (1968) found almost the same value (30%) for the lizard *Iguana iguana*. The mean red cell count was 1.135 × 10^6/mm³ in *Acanthodactylus* which is lower than that of *Eremias* (1.424 × 10^6/mm³). Both figures are higher than those found in *Agama* (0.812 × 10^6/mm³) and in *Uromastix* (0.680 × 10^6/mm³), but they approach the figures reported by Duguy (1970) for the lizard *Lacerta agilis* (1.420 × 10^6/mm³).

Blood glucose values are consistently higher in both of the lacertid lizards than in the agamid lizards. The values are 224.6 mg/100 ml in *Acanthodactylus*, 217.0 mg/100 ml. in *Eremias*, 168.0 mg/100 ml in *Agama* and 124.0 mg/100 ml in *Uromastix*. The highest figures in the available literature are 191.0 mg/100 ml for the lizard *Phrynosoma cornutum* and 192.0 mg/100 ml for the lizard *Ctenosaura acanthura* as reported by Dessauer (1970). All these results were obtained for individuals that have been fasted for 7 to 10 days after capture. The hyperglycemia found in these lizards supports Dessauer's contention that there is a considerably high concentration and less rigid regulation of blood glucose levels in lizards than in other reptiles. Miller and Wurster (1956) added that reptiles, in general, have much less rigid regulation of blood glucose level than mammals. The mean total plasma protein of the lizard *Acanthodactylus scutellatus* was 4.7 g% which is a higher value than that obtained for the lizards *Eremias brevirostris* (4.0 g%), but lower than that recorded for the lizard *Uromastix microlepis* (5.5 g%). Dessauer (1970) reported the total plasma protein of the lizards *Anolis carolinensis* as 4.1 g%, *Iguana iguana* as 4.5 g%, *Phrynosoma cornutum* as 4.4 g% and *Eumeces fasciatus* as 3.0 g%.

Blood Proteins

The relative proportions of different protein fractions in the serum and plasma of the lizards *Acanthodactylus scutellatus* and *Eremias brevirostris* are presented in Tables 3 and 4. Figs. 1 and 2 represent the electropherograms of serum proteins, plasma proteins and hemoglobin of both lizards. Their electrophoretic patterns are given in Figs. 3 and 4. Each figure is chosen to represent values that more or less approach those presented in the tables.

Serum protein patterns of the two lizards are generally similar to each other. Both sera have resolved into four fractions; the fastest was albumin which is slightly higher in concentration

TABLE 3. Absolute Values (g%) and Relative Proportions (%) of Different Protein Fractions in Serum and Plasma of *Acanthodactylus scutellatus*

Fraction	Serum	Plasma
Albumin	2.54 ± 0.13 55.2 ± 5.1	2.51 ± 0.12(g%) 53.3 ± 3.3 (%)
α — Globulin	0.55 ± 0.03 11.9 ± 1.0	0.37 ± 0.02 7.8 ± 0.9
β — Globulin	0.43 ± 0.02 9.3 ± 1.5	0.70 ± 0.04 14.9 ± 1.8
γ — Globulin	1.08 ± 0.05 23.6 ± 2.2	1.13 ± 0.06 24.0 ± 2.7
Albumin/Globulin Ratio	1.23 ± 0.11	1.14 ± 0.09

in *Acanthodactylus* than in *Eremias*. This is reflected on the albumin: globulin ratio which is again slightly higher in *Acanthodactylus* than in *Eremias*. In the two lizards the other three fractions are α –, β – and γ – globulins, of which the α – globulin was not refractionated into its α_1 and α_2– globulins. In the previously studied agamid lizards, although almost using the same conditions, five clear fractions were obtained. This may indicate that α_1 – and α_2 – globulins in the lacertid lizards have more or less similar particular size that makes it difficult to separate them by the ordinarily used technique. Dessauer and Fox (1964), using human plasma as reference for comparisons, have obtained similar results for some lizard and turtle plasma. Also the present author (Al-Badry 1974) in his work on the blood of the turtle *Testudo kleinmanni* obtained only four serum protein fractions.

One of the characteristic features of the

TABLE 4. Absolute Values (g%) and Relative Proportions (%) of Different Protein Fractions in Serum and Plasma of *Eremias brevirostris*

Fraction	Serum	Plasma
Albumin	1.91 ± 0.09 50.3 ± 4.0	1.93 ± 0.1 (g%) 48.2 ± 2.9 (%)
α — Globulin	0.58 ± 0.03 15.3 ± 3.1	0.28 ± 0.01 6.0 ± 1.1
β — Globulin	0.38 ± 0.02 10.1 ± 2.5	0.09 ± 0.05 22.5 ± 3.4
γ — Globulin	0.92 ± 0.05 24.3 ± 2.7	0.89 ± 0.04 22.2 ± 3.2
Albumin/Globulin Ratio	1.01 ± 0.09	0.93 ± 0.12

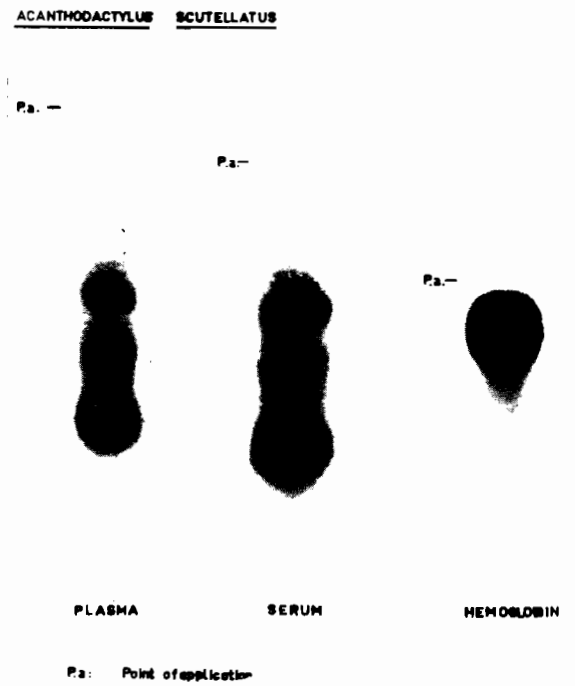

FIG. 1. Electropherograms of serum proteins, plasma proteins and hemoglobin of the lizard *Acanthodactylus scutellatus*

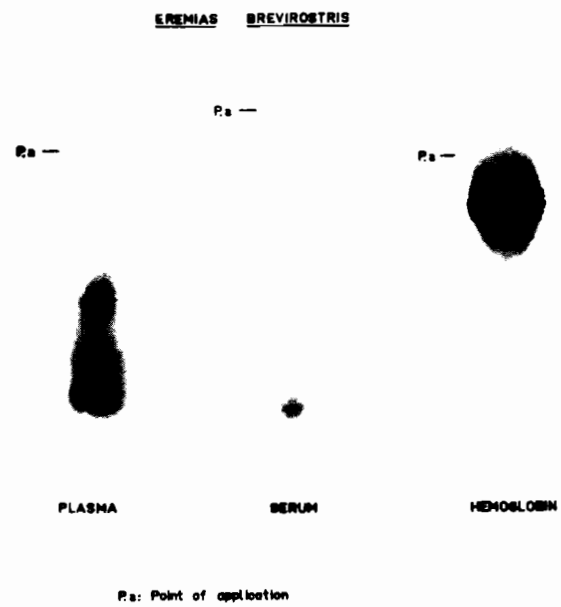

FIG. 2. Electropherograms of serum proteins, plasma proteins and hemoglobin of the lizard *Eremias brevirostris*

lacertid serum proteins studied is their slow electrophoretic mobility. The total mirgration was less than 7 cm. In the agamid serum proteins studied under the same experimental conditions the total migration was more than 10 cm. This could be attributed to the different nature of blood proteins in the two lizard families. Dessauer and Fox (1956) have succeeded in finding some taxonomic characteristics for some amphibians and reptiles based on their plasma protein pattern and mobility. Also, Burbidge *et al.* (1974) studied the relation between some reptilian species on serological grounds.

Plasma electrophoresis which was conducted simultaneously in the same run together with serum and hemoglobin revealed that the plasma pattern is not basically different from the serum pattern. These patterns showed only little variation in some of the fraction values. In *Acanthodactylus,* the percentage composition of the α – globulin decreased from 11.9 in serum to 7.8 in plasma, the β – globulin, on the other hand, increased from 9.3 in serum to 14.9 in plasma. In *Eremias,* the α – globulin decreased from 15.3 to 7.0 and the β – globulin increased from 10.1 to 22.5. This may be due to the migration of fibrinogen and β – globulin together. Consequently, the fibrinogen could not be detected as a separate fraction in the plasma patterns of these lizards. This is also the case with the agamid lizards examined before. It also agrees with the results of Dessauer and Fox (1956 and 1958) and Zain-Ul-Abedin and Katorski (1966).

The migration of hemoglobins in electrophoresis depends upon the size and shape of their molecules and also on their electric charge that is determined by their isoelectric point and the buffer used. The electrophoretic mobility of the hemoglobin molecule is, therefore, an important characteristic of that molecule. It was postulated by Foreman (1960) that differences in the electrophoretic properties of hemoglobins could contribute significantly to the differentiation of species which are morphologically similar. Guttman (1970) studied the hemoglobin patterns of several sand lizards and found that two genera were closely related even more than their current taxonomic status indicated. In agamid lizards living in Kuwait, the present author reported homogeneous single fractioned hemoglobins for *Agama persica* and *Uromastix microlepis* (Al-Badry and Abdel-Fattah 1975).

Figs. 1 to 4 show that the lacertid lizards, *Acanthodactylus scutellatus* and *Eremias brevirostris* have also hemoglobins that move as anodic homogeneous components and are not re-

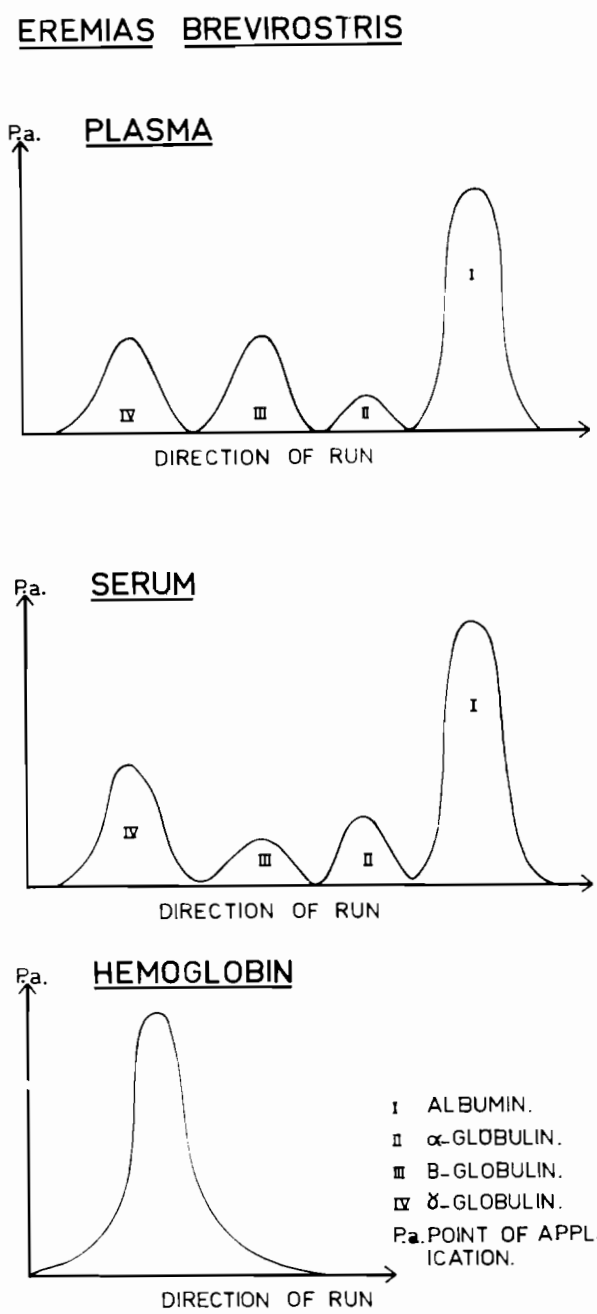

FIG. 3. Electrophoretic patterns for serum proteins, plasma proteins and hemoglobin of the lizard *Acanthodactylus scutellatus*

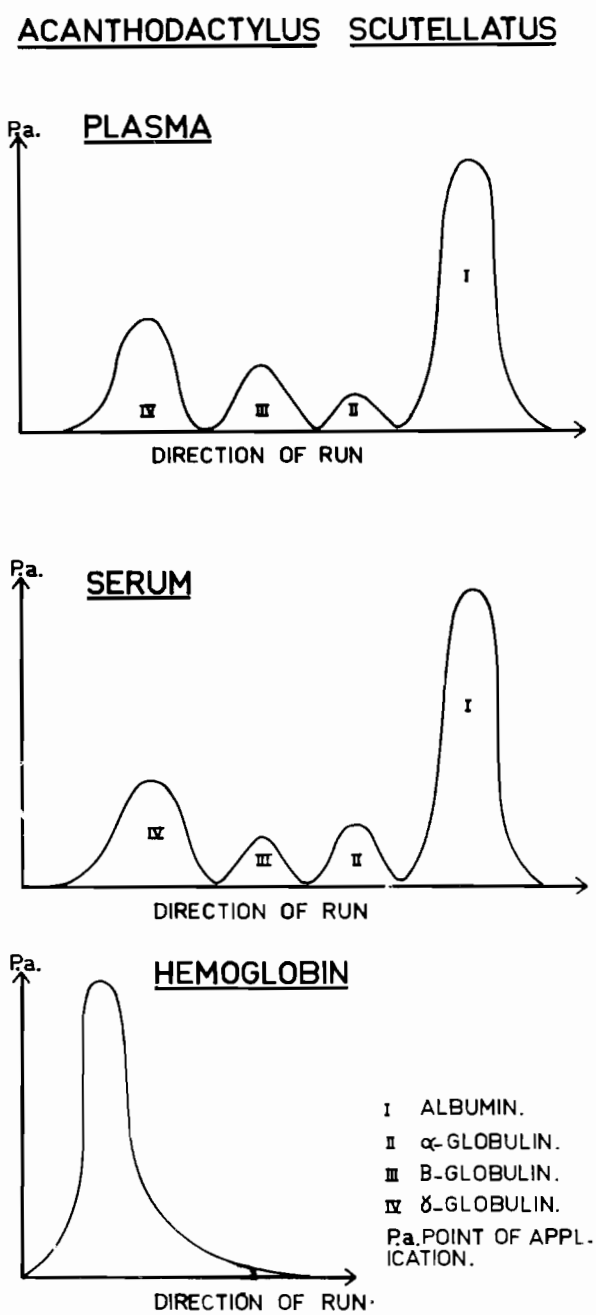

FIG. 4. Electrophoretic patterns for serum proteins, plasma proteins and hemoglobin of the lizard *Eremias brevirostris*

solved into fractions. The electrophoretic mobility of the lacertid hemoglobins studied is much slower than that recorded for the agamid lizards and especially for *Uromastix*. For the present lizards, the hemoglobin electropherograms and electrophoretic patterns presented in the figures indicate that in *Acanthodactylus* the migration is slightly faster than in *Eremias*. This can be

regarded as evidence of the close relationship between the two lacertid lizards. Gorman and Shochat (1972) arrived at similar conclusions when they studied the electrophoretic patterns of the hemoglobins of some agamid lizards. They found two species having single major component hemoglobins that migrate in similar mobilities toward the anode.

The results obtained in this part of study indicate that there is a considerable similarity between the two lacertid lizards in most of the aspects studied, especially the electrophoretic analysis of blood proteins. These two genera of family Lacertidae could be considered as being more closely related than the two agamid lizards previously studied, where there are clear intergeneric variations existing between them. The principal conclusion is that the present results support the previous opinions concerning the close ecological and evolutionary relationships of these two lacertid lizards.

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to the Head and Staff of the Department of Zoology, Faculty of Science, University of Kuwait, for providing facilities and for their continuous help and encouragement.

REFERENCES

Abdel-Fattah, R.F., Al-Badry, K.S. & Al-Balool, F. 1974. Haematological studies on some reptiles from Kuwait. Part I. Some corpuscular constants, blood glucose, and electrophoretic examination of blood proteins of the lizard *Agama persica*. J. Univ. Kuwait (Sci.) 1 : 129-135.

Al-Badry, K.S. 1974. Some aspects of the haematology of the tortoise *Testudo kleinmanni*. Bull. Fac. Sci. Cairo Univ. (In press).

Al-Badry, K.S. & Abdel-Fattah, R.F. 1975. Haematological studies on some reptiles from Kuwait. Part II. Some corpuscular constants, blood glucose, total plasma protein and electrophoretic examination of blood proteins of the lizard *Uromastix microlepis*. J. Univ. Kuwait (Sci.) 2 : 153 - 158.

Block, R.J., Durrum, E.L. & Zweig, G. 1958. A manual of paper chromatography and paper electrophoresis. Academic Press.

Burbidge, A.A., Kirsch, A.R. & Main, A.R. 1974. Relationships within the chelidae (Testudines : *Pleurodira*) of Australia and New Guinea. Copeia, 2 : 392-409.

Chernoff, A.I. 1955. The human hemoglobins in health and disease. New Engl. J. Med. 253 : 322-331.

Dawson, W.R. & Poulson, T.L. 1962. Oxygen capacity of lizard blood. Am. Midl. Nat. 68 : 154-164.

Dessauer, H.C. 1970. Blood chemistry of reptiles : physiological and evolutionary aspects. In **Gans C. (Ed.) 1970.** Biology of Reptilia, Vol. 3. Academic Press.

Dessauer, H.C. & Fox, W. 1956. Characteristic electrophoretic patterns of plasma proteins of orders of Amphibia and Reptilia. Science, N.Y. 124 : 225-226.

Dessauer, H.C. & Fox, W. 1958. Geographic variation in plasma protein patterns of snakes. Proc. Soc. exp. Biol. Med. 98 : 101-105.

Dessauer, H.C. & Fox, W. 1964. Electrophoresis in taxonomic studies illustrated by analyses of blood proteins. In **Leone C.A. (Ed.) 1964.** Taxonomic Biochemistry and Serology. Ronald Press.

Duguy, R. 1970. Number of blood cells and their variation. In **Gans, C. (Ed.) 1970.** Biology of Reptilia, Vol. 3. Academic Press.

Foreman, C.W. 1960. Electromigration properties of mammalian hemoglobins as taxonomic criteria. Am. Midl. Nat. 64 : 177-186.

Goin, C.T. & Jackson, C.G. 1965. Hemoglobin values of some amphibians and reptiles from Florida. Herpetologica 21 : 145-146.

Gorman, G.C. & Dessauer, H.C. 1966. The relationships of *Anolis* of the *roquet* species group (Sauria : Iguanidae), I. Electrophoretic comparison of blood proteins. Comp. Biochem. Physiol. 19 : 845-853.

Gorman, G.C. & Shochat, D. 1972. A taxonomic interpretation of chromosomal and electrophoretic data on the agamid lizards of Israel with notes on some East African species. Herpetologica 28(2) : 106-112.

Guttman, S.I. 1970. Hemoglobin electrophoresis and relationships within the lizard genus *Sceloporus* (Sauria : Iguanidae) Comp. Biochem. Physiol. 34 : 563-568.

Hawk, P.B., Oser, B.L. & Summerson, W.H. 1954. Practical physiological chemistry. McGraw-Hill.

Hernandez, T. & Coulson, R.A. 1951. Biochemical studies on the Iguana. Proc. Soc. exp. Biol. Med. 76 : 175-177.

Horton, B., Fraser, R., Dupourque D. & Chernoff, A. 1972. An analysis of the hemoglobins from some common turtles. J. Exp. Zool. 180(3) : 373-384.

Judd, F.W. 1974. Intraspecific variation in blood properties of the keeled earless lizards, *Holbrookia propinqua*. Herpetologica 30(1) : 99-102.

Miller, M.R. & Wurster, D.H. 1956. Studies on the blood glucose and pancreatic islets of lizards. Endocrinology 58 : 114-120.

Otis, V.S. 1973. Hemocytological and serum chemistry parameters of the African puff adder *Bitis arietans*. Herpetologica 29 : 110-116.

Thorson, T.B. 1968. Body fluid partitioning in Reptilia. Copeia 4 : 592-601.

Wootton, I.D.P. 1964. Micro-analysis in Medical Biochemistry. Churchill.

Zain-Ul-Abedin, M. and Katorski, B. 1966. Increased blood clotting time in a hibernating lizard. Can. J. Physiol. Pharm. 44 : 505-507.

(*Received* : 1975)

دراسات في دم بعض زواحف الكويت

الجزء الثالث : تعيين بعض ثوابت الكريات ، وجلوكوز الدم ، والمحتوى البروتيني للبلازما ، مع فحص كهربي لبروتينات الدم في العظائتين اكانثود كتليس سكيوتلاتس واريمياس بريفيروسترس

كمال السيد البدري

قسم علم الحيوان بجامعة الكويت

خلاصـــــة

في دراسة الثوابت الكروية توصل الباحث الى ان كميـــة الهيمـــــوجلوبين ، والهيما توكريت ، وعدد كريات الدم الحمراء هي على التوالي : ٥ر٨ جم ، ٣٠٪ ، ٠٠٠ ١٣٥ ١/مم٣ في اكانثود كتيلس ، وكانت ٢ر٩ جم٪ ، ٨ر٣٠٪ ، ٠٠٠ ٤٢٤ ١/مم٣ في اريمياس . أما جلوكوز الدم والمحتوى البروتيني فقـــد كان ٦ر٢٤٤ مجم٪ ، ٧ر٤ جم٪ في اكانثودكتليس ، و ٢١٧ مجم٪ ، ٠ر٤ جم٪ في اريمياس .

وبدراسة الفصل الكهربي لبروتينات الدم في كلا العظائتين توصل الباحث الى ان بروتينات المصل والبلازما متشابهة في الحالتين ، وتتكون فقط من أربعة اجزاء هي : البيومين ، ألفاجلوبيولين ، بيتا جلوبيولين ، جاما جلوبيولين . والعظائتين تختلفان في ذلك عن الاجاما والضب اللذين سبقت دراستهما . وفي دراســة الهيموجلوبين كهربيا ثبت انه جزء واحد متجانس ، ويتحرك في العظائتين بسرعة متشابهة نحــو القطب الموجب ، ولكن تختلف هذه السرعة عنها في حالة الاجاما والضب .