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

THE EFFECT OF INSULIN ON CARBOHYDRATE METABOLISM IN THE LIVER FLUKE, *FASCIOLA GIGANTICA*

SHLEMON E. S. AL-BARWARI

Institute of Medical Technology, Baghdad

AND

R. F. ADEL - FATTAH

Department of Zoology, University of Kuwait

Abstract. In vitro effect of insulin on carbohydrate metabolism in adult ***Fasciola gigantica*** was studied. The hormone was tested at concentrations ranging from 0.5 to 2 IU/ml; even the lower one is far higher than levels which would be encountered by the parasite in tissue fluids of its vertebrate host. The results obtained show that these flukes metabolize exogenous glucose at a very high rate, and that, on the whole, the tissues of the parasite contain large amounts of reserve glycogen. The results also show that insulin had no significant action on the rate of glucose absorption, or glycogen turnover, in this trematode species.

INTRODUCTION

During the last decade several reports have appeared on the possible action of host hormones and metabolic regulators in the control of metamolism of some endoparasitic helminths. The subject was approached in two different ways. In the first, hormones and metabolic effectors were administered to hosts bearing parasite infections. In the second, the action of these compounds was tested on parasites maintained under *in vitro* conditions.

As to insulin, there exists a remarkable controversy as to whether it induces an effect on the liver fluke, *Fasciola hepatica,* similar to that exhibited in mammalian systems. Pantelouris (1964) found that incubation of *F. hepatica* in Hedon-Fleig's saline, containing insulin, resulted in a drastic depletion of glycogen from its tissues. While in absence of the hormone the loss of glycogen was minimal over an average experimental duration. Later, the same author observed a depressive effect of insulin on the incorporation and retention of methyl carbon of aminoacid methionine in the fluke (Pantelouris 1965b). However, Pantelouris (1965a) found that the net uptake of glucose $-C^{14}$ by *F. hepatica* was apparently unaffected by insulin, although the hormone reduced extremely the counts in wet tissue samples in the case of galactose $-I-C^{14}$. On the other hand, Sekardi (1965) reported that insulin stimulated the rate of glucose $-U-C^{14}$ uptake by *F. hepatica* and he, contrary to Pantelouris (1964), found that glycogen synthesis was enhanced in the insulin-treated flukes by 41%. Sekardi also found that incubation of flukes with insulin, but without glucose, prior to the glucose-uptake experiments, also enhanced the rate of glucose uptake.

Isseroff and Read (1968) re-examined the effect of insulin on *F. hepatica* metabolism. They stated that the hormone had no action on glucose uptake or on glycogen utilization in a 4-hour incubation, nor any effect on uptake of glucose $-U-C^{14}$ during a 1-minute incubation. Hines (1969) agreed with Pantelouris (1964) in that insulin reduced the level of glycogen in *F. hepatica* but, in his experiment, only after the oral suckers of flukes were tied. A similar action of insulin on carbohydrate metabolism, as has been reported by Sekardi (1965) for *F. hepatica,* has been obtained by Esch (1969) in the larval cestode, *Taenia crassiceps.* Buist and Schof eld (1971) reported that insulin, even at a concentration that far exceeded that found *in vivo,* had no effect on the *in vitro* metabolism of glucose by *F. hepatica.*

The present communication reports some data relating to the direct effect of insulin, if any, on glucose uptake and glycogen content in a digenetic trematode, the liver fluke ***Fasciola gigantica.***

Material and Methods

Adult flukes were obtained from the bile ducts of naturally-infected and freshly-slaughtered buffaloes at a local abattoir. They were immediately immersed in Hedon-Fleig's saline, without glucose (Dawes 1954), fortified with penicillin (1 million units/l) and streptomycin (2 g/l), and kept at 37°C in a thermos flask. At the laboratory, flukes with obvious empty guts were selected, and washed repeatedly in saline until they were free of debris and pigments. They were randomly used, except that immature, inactive and senile individuals were discarded.

In order to determine the rate of glucose uptake by normal and by insulin-treated flukes, each sample of flukes, of about equal size, was divided into 4 batches, each consisting of 5 individuals. The first batch was transferred into 10 ml of sterile saline, having the same composition as that reported by Mansour (1959), with glucose concentration of 0.01M. Whereas the other three batches were similarly treated, except that the saline included, in addition, either 0.5, 1 or 2 IU/ml of protamine zinc insulin (Wellcome). All the batches were then incubated at 37°C, under aerobic conditions, for a period of 1 hour. Chemical analysis for glucose in aliquots of media was made before and after the incubation, following the method recommended by Wootton (1964). After each experiment, the flukes were blotted on filter paper to remove any surface moisture, then the wet weight of each batch was subsequently recorded.

In another series of experiments, to show the effect of insulin on glycogen content in normal and in starved flukes, each sample of flukes of about equal size was divided into 7 batches, each consisting of 5 individuals. The glycogen content of flukes from the first batch was immediately determined. The second batch was transferred to 20 ml saline (Mansour 1959), with glucose concentration of 0.01M, and incubated at 37°C under aerobic conditions for a period of 4 hours. The batches 3 to 5 were similarly treated, except that the saline included, in addition, 0.5, 1, and 2 IU/ml of insulin, respectively. The sixth batch was treated as batch No. 2, except that the saline was devoid of glucose. The seventh batch was treated as batch No. 6, except that the saline contained, in addition, 1 IU/ml of insulin. After 4 hours, all flukes were removed and the glycogen content of flukes from various batches was determined on a single-fluke basis. The glycogen was isolated from tissues of flukes following the procedure of Mansour (1959), then determined quantitatively by the method of Odlaug (1955). The wet weight of individual fluke was always recorded.

Results and Discussion

The results obtained in the present study, showing the effects of host mammalian hormone, insulin, on glucose uptake and glycogen content in *F. gigantica*, are summarized in Tables 1 and 2, respectively. In both series of experiments, the hormone was tested at all three comparably identical concentrations, even the lower one (0.5 IU/ml) being far higher than the level which would be encountered by *Fasciola* in the tissue fluids of its vertebrate hosts (Klostermeyer and Humbel 1966, and Isseroff and Read 1968). The data clearly verify the previous findings, in that *F. gigantica* metabolizes the exogenous glucose at a very high rate (Abdel-Fattah and Al-Barwari 1974), and that, on the whole, the tissues of the adult fluke contain a large amount of reserve glycogen, which is utilized during starvation periods (Goil 1961, Al-Barwari and Abdel-Fattah 1973).

It is also interesting to observe that there is an apparent lack of a significant effect of insulin on the rate of exogenous glucose uptake, or on endogenous glycogen turnover, in this parasite. However, it is also clear that the rate of glucose uptake was slightly higher, and the amounts of final glycogen concentrations slightly lower, than the corresponding data obtained in the control experiments. But whether these mild variations can be attributed to the action of insulin alone is not easy to accept, since it has previously been found that the rate of carbohy-

TABLE 1. Effect of Insulin on Glucose Uptake in *F. gigantica*

Flukes from 10 different buffaloes were used. Incubation period 1 hour, under aerobic conditions and at 37°C. Initial glucose concentration 0.01M. The figures for glucose uptake are the means of 10 batches of 5 flukes each.

Medium used	Additions (Insulin)	Glucose Uptake (u moles/g wet wt./h)	Change
With glucose	—	32.5	—
With glucose	0.5 IU/ml	33.4	+0.89
With glucose	1.0 IU/ml	33.1	+0.65
With glucose	2.0 IU/ml	33.8	+1.26

TABLE 2. Effect of Insulin on Glycogen Content in *F. gigantica*

Flukes from 10 different buffaloes were used. Incubation period 4 hours, under aerobic conditions and at 37°C. Initial glucose concentration 0.01M. Initial glycogen determined in flukes immediately obtained from hosts. Glycogen determined on the basis of single fluke, the figures are the means of 10 flukes.

Medium used	Additions (Insulin)	Glycogen Content (g/100 g wet wt.)	Change
Control	—	4.6	—
With glucose	—	4.8	+0.25
With glucose	0.5 IU/ml	4.3	−0.32
With glucose	1.0 IU/ml	4.2	−0.41
With glucose	2.0 IU/ml	4.2	−0.38
Without glucose	—	3.8	—
Without glucose	1.0 IU/ml	3.3	−0.50

drate metabolism in this fluke, when maintained *in vitro,* is affected by a variety of other factors (Al-Barwari 1971). Various host-parasite, and parasite *in vitro* endogenous and exogenous factors may contribute to this phenomenon. In this connection, it is probably noteworthy to mention that such studies could not omit the physiological state of both the parasite and its host, and to a greater degree the nutritional state of the host animals.

So far, there is very little information on hormonal systems regulating metabolism in the invertebrate animals. However, there is some evidence that metabolic hormones of invertebrates are different from mammalian metabolic hormones, by the fact that rarely do mammalian hormones have an effect on invertebrate metabolism (Mansour, 1967). Pantelouris (1965), Isseroff and Read (1968), and Buist and Schofield (1971) reported that insulin had no action on the carbohydrate metabolism in *F. hepatica.* On the other hand, the glycogen-depleting property of insulin and its enhancement of the glucose uptake and glycogen synthesis in *F. hepatica,* as observed by Pantelouris (1964), and Sekardi (1965), have been thoroughly disputed by Isseroff and Read (1968). The latter authors have further implied that it seemed most unusual for a mammalian hormone to produce effects on a flatworm, at least superficially resembling some of its effects on mammals. An unexpected and questionable effect of insulin on the carbohydrate metabolism in *F. hepatica* was reported by Hines (1969). He has found that the hormone remarkably suppressed the level of glycogen content in flukes only when their oral suckers had been tied. Neither insulin nor the tying was alone shown to have any significant effect on glycogen.

As to other platyhelminthes, Esch (1969) reported that he was unable to elicit a significant stimulation of carbohydrate metabolism by insulin in cysticerci of *T. crassiceps* during a short-term (2 hours) incubation. However, under the same conditions, the hormone stimulated the metabolism of larvae following long-term incubations (8, 24, and 48 hours). In analyzing his data, Esch postulated that "while the insulin levels were not physiological with respect to concentration (insulin was used at 1 IU/ml), one might logically expect that, if the very high insulin levels were solely responsible for observed metabolic stimulation, such an effect would occur in both short and long-term experiments".

REFERENCES

Abdel-Fattah, R.F. & Al-Barwari, S.E. 1974. The role of carbohydrates in the biology of the liver fluke, ***Fasciola gigantica***: Utilization of exogenous glucose. Bull. endem. Dis. **15** : 51-62.

Al-Barwari, S.E. 1971. The role of carbohydrates in the biology of the liver fluke, ***Fasciola gigantica***. (Cobbold, 1855). M.Sc. Thesis, University of Baghdad, 99 pp.

Al-Barwari, S.E. & Abdel-Fattah, R.F. 1973. The role of carbohydrates in the biology of the liver fluke, ***Fasciola gigantica*** : Glycogen content, its utilization and resynthesis by starved flukes. Presented at the First Scientific Conference of the Iraqi Biological Society, Baghdad, March 21-23, 1973.

Buist, R.A. & Schofield, P.J. 1961. Some aspects of the glucose metabolism of ***Fasciola hepatica***. Int. J. Biochem. **2** : 377-383.

Dawes, B. 1954. Maintenance in vitro of ***Fasciola hepatica*** . Nature, Lond. **174** : 654-655.

Esch, G.W. 1969. ***Taenia crassiceps***: Insulin and carbohydrate metabolism in larval forms. Expl. Parasit. **25** : 210-216.

Goil, M.M. 1961. Physiological studies on trematodes, ***Fasciola gigantica*** . Carbohydrate metabolism. Parasitology **51** : 335-336.

Hines, W. J. W. 1969. An in vitro effect of insulin on glycogen levels in the common liver fluke, ***Fasciola hepatica*** (Linnaeus, 1758). Comp. Biochem. Physiol. **28** : 1443-1447.

Isseroff, H. & Read, C. P. 1968. Does insulin affect carbohydrate metabolism in ***Fasciola hepatica*** ? Comp. Biochem. Physiol. **24** : 1069 - 1072.

Klostermeyer, H. & Humbel, R.E. 1966. The chemistry and biochemistry of insulin. Angew. Chem. (Internal. Edit.) **5** : 807-822.

Mansour, T.E. 1959. Studies on the carbohydrate metabolism of the liver fluke, ***Fasciola hepatica***. Biochem. biophys. Acta **34** : 456-464.

Mansour, T.E. 1967. Effect of hormones on carbohydrate metabolism of invertebrates. Fedn. Proc. **26** : 1179-1185.

Odlaug, T.O. 1955. The quantitative determination of glycogen in some parasites of Amphibia. J. Parasit. **41** : 258-262.

Pantelouris, E.M. 1964. Localization of glycogen in ***Fasciola hepatica*** L. and an effect of insulin. J. Helminth. **38** : 283-286.

Pantelouris, E.M. 1965a. Effects of host hormones on the internal parasite, ***Fasciola hepatica.*** Res. vet. Sci. **6** : 330-333.

Pantelouris, E.M. 1965b. Utilization of methionine by the liver fluke, ***Fasciola hepatica***. Res. vet. Sci. **6** : 334-336.

Sekardi, L. 1965. Utjecaj inzulina na apsorpciju glukoze i sintezu glikogena u velikom metilju (***Fasciola hepatica.*** L.). Vet. Arh. **35** : 313-320.

Wootton, I.D.P. 1964. Micro-analysis in medical biochemistry. Churchill, London.

(*Received* 8 *January* 1975)

تأثير هرمون الانسولين على ايض الكاربوهيدرات في الدودة الكبدية فاسيولا جايجانتيكا

شليمون البرواري
المعهد الطبي الفني ببغداد

رشدي فتوح عبد الفتاح
قسم علم الحيوان بجامعة الكويت

خلاصـــة

قام الباحثان بدراسة تأثير هورمون الانسولين في عمليات الايض الخاصـــة بالكاربوهيدرات في الدودة الكبدية فاسيولا جايجانتيكا . وقد استعمل الهورمـون بتركيز يتراوح بين نصف ووحدتين دوليتين في المللي لتر الواحد . ويعتبر هذا التركيز أعلى بكثير من تركيز الانسولين في السوائل الجسمية للعائل الذي تتطفل عليه هذه الدودة . وقد أيدت النتائج التي حصل عليها الباحثان نتائج التجارب السابقة التي اجريت على هذا النوع من الديدان ، وهي أن هذه الديدان تستخدم الجلوكوز المعطي لها بدرجة عالية ، وأن انسجة هذا الطفيلي تحتوي على كميات كبيرة من الجلايكوجين المخزون . وقد اظهرت النتائج ايضا أن هورمون الانسولين ليس له أي تأثير في سرعة امتصاص الجلوكوز أو تخزين الجلايكوجين من قبل هذه الدودة .

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

KAMAL S. AL-BADRY AND R. F. ABDEL-FATTAH

Department of Zoology, University of Kuwait

Abstract. Blood glucose, haemoglobin, red cell count, haematocrit and total plasma protein of the lizard *Uromastix microlepis* were determined. The mean values are : 124 mg/100 ml, 5.8 g/100 ml, 0.680 million/mm³, 24.6% and 5.5 g% respectively. The electrophoretic behaviour of serum proteins, plasma proteins and haemoglobin is found to be generally similar within the two representatives of family Agamidae, *Agama persica* and *Uromastix microlepis*, found in Kuwait. The general electrophoretic pattern of serum and plasma proteins consists of five fractions. The first four fractions or globulins are generally lower in concentration, and somewhat less distinctly separated from each other, than the fifth fraction which is the albumin. The electrophoretic patterns of serum and plasma of *Uromastix* look similar to each other.

The protein patterns of *Uromastix* show little variation from those of *Agama*, both in the concentration of the fractions and in the proportion of albumin to globulin. This can be correlated with the phylogenetic relationships of the two genera, as judged by morphological characteristics. No fibrinogen is detected in the plasma pattern of either lizard. Haemoglobin of *Uromastix* moves as a homogeneous single-component fraction, with a relatively higher anodic mobility than in *Agama*.

INTRODUCTION

Morphological, as well as immunological, methods have been used for many years to study the phylogenetic relationships of animals. Recently, electrophoresis seems to offer another useful technique for such studies, since the electrophoretic patterns of blood proteins are characteristic, and distinct differences are found between patterns of closely related forms. Several authors have studied phylogenetic relationships in certain groups of reptiles. Dessauer and Fox (1956, 1958 and 1964), Zweig and Crenshaw (1957), Foreman (1960), Baril *et al.* (1961), Gorman and Dessauer (1965), Maldonado and Ortiz (1966), Hussein *et al.* (1966 and 1968), Dessauer (1970), Guttman (1970) and Gorman and Shochat (1972) have concluded that analysis of blood protein patterns confirms and supplements taxonomic conclusions derived from traditional criteria. Recent work on the application of haemoglobin patterns was conducted by several investigators for obtaining evolutionary information, and in some cases for providing a basis for formulating new taxonomic conclusions (Sydenstricker *et al.* 1956, Ramirez and Dessauer 1957, Rodnan and Ebaugh 1957, Forman 1960, Zarafonetis and Kalas 1960, Dessauer 1970, Horton *et al.* 1972, and Otis 1973).

In a previous paper by the present authors (Abdel Fattah *et al.* 1974), an electrophoretic examination of blood proteins was carried out on the agamid lizard *Agama persica*, along with a determination of some corpuscular constants and of blood glucose. In the present study, the investigation is carried out further on another lizard, *Uromastix microlepis*, which belongs to the same family Agamidae. In addition, the total plasma protein is determined. As previously mentioned, the ultimate aim of this study is to investigate the phylogenetic relationships within different families of suborder Lacertilia living in Kuwait.

MATERIALS AND METHODS

Uromastix microlepis, the lizard chosen for the present study, is one of the commonest diurnal reptiles inhabiting the Arabian Gulf and neighbouring areas. It is large in size, reaching as much as 53 cm in length. It has a strong, heavy tail armed with half-rings of spines. The head is heavy, with a blunt snout, and teeth modified for cutting grass blades. The body is covered by rough skin with wrinkles, particularly on the neck. The colour varies, according to

temperature and light intensity, from blackish to sulphur-yellow, but it darkens on capture. Ventrally, the body is much paler. *Uromastix* partially hibernates during the winter in burrows up to 240 cm long and 120 cm deep. It is usually very wary and shy, not normally aggressive, but can grip with its jaws, and strike with its strong tail. It is almost entirely herbivorous; very rarely beetles are seen among its stomach contents.

Individuals of the lizard *Uromastix microlepis* were collected fresh from the field, and kept in the laboratory for one week before use, with access to water only. They were acclimated at a constant temperature (25°C), and a photoperiod of approximately 12 hour light-dark cycle. In all, 10 specimens of undetermined sex were used. Blood was collected by severing the tail, and allowing the blood to drip into oxalate-coated specimen tubes. For serum determination, no anticoagulant was used. The serum and plasma were prepared by centrifuging the blood sample at 3000 rpm for 30 minutes. For the preparation of haemoglobin, the technique described by Chernoff (1955) was followed. Five ml of oxalated blood were washed once with physiologic saline (0.65% Na_2Cl), centrifuged, and the washings discarded. To one volume of the packed cells 1.5 volumes of distilled water and 0.5 volume of toluene were added. This was shaken vigorously for about 3 minutes, and allowed to stand over night at 4-10°C. The mixture was then centrifuged. The upper and middle layers were pipetted off and discarded, the bottom layer only being used. This was then filtered yielding a clear solution of haemoglobin.

Protein separations were conducted on Elphor-H electrophoresis apparatus following the technique of Block, Durrum and Zweig (1958). In each electrophoretic run, from 0.1 to 0.02 ml of serum, plasma and haemoglobin were applied across one inch strips of Whatman No. 1 filter paper, and fractionated in barbital buffer (pH 8.6, ionic strength 0.05 mv.). The separations were carried out for 18 hours at 20°C and 100 v. After electrophoresis, each strip was dried and stained with bromophenol blue, then washed with 5% acetic acid solution. The stained strips were made translucent with mineral oil, and scanned with Elphor-Integraph. Total protein in plasma was determined by the micro-kjeldahl method of digestion and oxidation (Hawk *et al.* 1954), converting protein nitrogen to ammonia, which is then determined by titrimetric method. From this, total protein was calculated. Packed cell volume (haematocrit), haemoglobin content, red cell count and blood glucose were determined following the same methods previously described by the authors (Abdel-Fattah *et al.* 1974).

Results and Discussion

Corpuscular Constants, Blood Glucose and Total Plasma Protein

Table 1 represents a summary of the results of corpuscular constants, blood glucose and total plasma protein determinations. Each figure represents the mean of 10 individuals in active state, and each character was determined in triplicate. From this table it is clear that the blood glucose is lower in *Uromastix* than in *Agama*. The figures obtained are 124 and 168 mg/100 ml for *Uromastix* and *Agama* respectively. The value obtained for *Uromastix microlepis* is almost the same as that obtained by Zain-Ul-Abedin and Qazi (1965), and Zain-Ul-Abedin and Katorski (1966), for *Uromastix hardwickii*. The mean haemoglobin content is 5.5 g/100 ml, a figure which is also lower than that recorded for *Agama* (7.0 g/100 ml). This figure approaches that presented by Dassauer (1970), but is much lower than that recorded by Goin and Jackson (1965). These two authors also believe that their few data suggest a correlation between haemoglobin value and body size within a group, the larger species having the smaller value. Since *Uromastix* is much larger in size than *Agama*, it may not be surprising to find that it has a smaller value of haemoglobin.

The mean haemotocrit value (packed cell volume) obtained is 24.6%, a value which approaches that for *Agama* (25%), and lies within the range of 20-35% presented by Dessauer (1970) for reptiles. The mean red cell count obtained for *Uromatix* is 0.68 million/mm^3, a value which is somewhat lower than that for *Agama* (0.812 million /mm^3), but agrees somewhat with that obtained by Ryerson (1949), who recorded values varying between 0.506 and 1.204 million/mm^3. The mean total plasma protein obtained is 5.5 g%. This value nearly approaches that reported by Zain-Ul-Abedin and Katorski (1966) and Otis (1973), but is higher than that reported by Dessauer (1952) for the lizard *Anolis carolinensis* (4.19 g%).

TABLE 1. Means of some Corpuscular Constants, Total Plasma Protein, and Blood Glucose of *Uromastix microlepis*.

Red Cell Count (million/mm³)	0.680
Haemoglobin (g/100 ml)	5.8
Packed Cell Volume (P.C.V. %)	24.6
Blood Glucose (mg/ 100 ml)	124
Total Plasma Protein (g %)	5.5

TABLE 2. Relative Proportions of Different Protein Fractions in Serum and Plasma of *Uromastix microlepis*.

Fraction	Serum	Plasma
Albumin	44.6 ± 2.3	46.5 ± 2 1
α_1 - Globulin	8.8 ± 0.9	8.3 ± 0 8
α_2 - Globulin	5.6 ± 0.6	7.4 ± 0.6
β - Globulin	28.9 ± 1.6	27.3 ± 1.2
γ - Globulin	12.1 ± 0.9	10.5 ± 1.0
Albumin/Globulin	0.81 ± 0.06	0.86 ± 0.07

Blood Proteins

Table 2 presents the percentage composition of serum and plasma proteins. Fig. 1 shows the electropherograms of serum proteins, plasma proteins and haemoglobin. Each one of the electropherograms was chosen to represent a certain animal whose protein fractions coincide more or less with the mean values presented in Table 2. Fig. 2 shows the electrophoretic patterns of serum proteins, plasma proteins and haemoglobin. It is clear from Figs. 1 and 2 that:

1. Electrophoretic pattern of serum proteins of *Uromastix microlepis* consists of 5 distinct fractions. The first or albumin fraction is the fastest, the other four fractions are α_1 – globulin, α_2 –globulin, β –globulin and γ –globulin. Comparing the data presented in Table 2 with that presented in the previous paper by the same authors on the lizard *Agama persica* (Abdel-Fattah *et al.* 1974), it is evident that the globulin fractions, in both *Uromastix* and *Agama*, look the same in that they are generally lower in concentration and somewhat less distinctly separated from each other. The albumin fraction in both lizards shows the highest value (44.6% and 34.% in *Uromastix* and *Agama* respectively). The least value recorded for *Uromastix* serum fractions is that of α_2 – globulin (5.6%), in *Agama* the least value was that of γ – globulin.

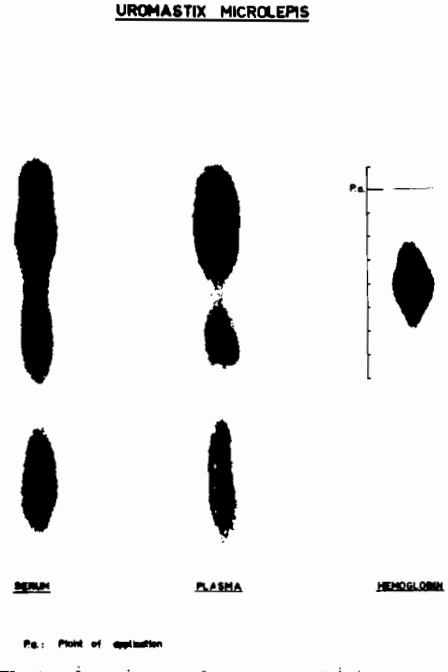

FIG. 1. Electropherograms of serum proteins, plasma proteins and haemoglobin of the lizard *Uromastix microlepis*.

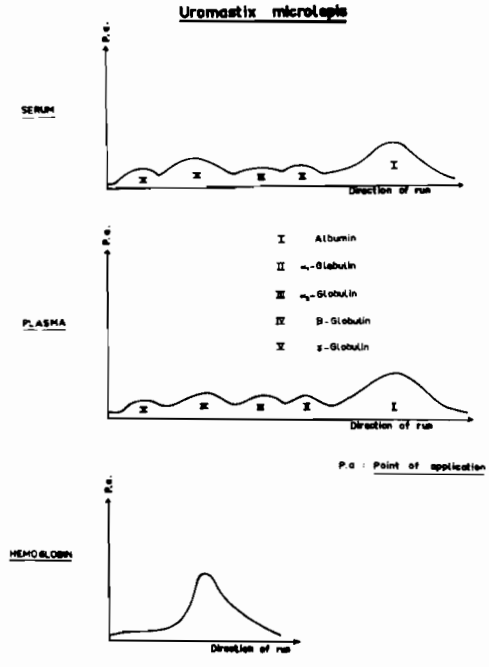

FIG. 2. Electrophoretic pattern for serum proteins, plasma proteins and haemoglobin of the lizard *Uromastix microlepis*.

The albumin: globulin ratio of *Uromastix* serum reaches a value of 0.81, which is higher than that of *Agama*. The patterns obtained for serum proteins of *Uromastix* are similar to those presented by Maldonado and Ortiz (1966) for some West Indian lizards, and also by Hussein *et al.* (1966 and 1968) for some Egyptian lizards.

2. Electrophoretic pattern of plasma proteins of *Uromastix microlepis* is the same as that of serum proteins of the same lizard, except that it shows little variation in some of the fraction values, and in the proportion of albumin to globulin (A/G ratio). The concentration pattern of plasma protein fractions of *Uromastix* differs from that recorded for *Agama*. The highest value in both is that of albumin (46.5% in *Uromastix* and 29.7% in *Agama*). The least value recorded in the plasma of *Uromastix* is that of α_2 – globulin (7.4%); in *Agama* the least value was that of α_1 – globulin. The albumin: globulin ratio in the plasma of *Uromastix* is 0.87, which is again higher than the value recorded for *Agama*. It is to be noted that fibrinogen cannot be detected in the plasma pattern of these lizards. Perhaps this protein migrated with one of the globulin fractions. These findings are in good agreement with the results of Dessauer and Fox (1956 and 1958), and Zain-Ul-Abedin and Katorski (1966).

3. As in *Agama persica,* the haemoglobin of *Uromastix microlepis* moves as a homogeneous compound. It possesses a single-component fraction. This single-component haemoglobin resembles in its direction of mobility the human haemoglobins (Chernoff 1955). It may be interesting to record that the difference between haemoglobins of *Uromastix* and *Agama* lies only in the extent to which the haemoglobin moves towards the anode. In this respect, *Uromastix* haemoglobin moves remarkably faster than that of *Agama*. The rate of haemoglobin movement in *Uromastix* can be compared to the γ – globulin fraction of serum and plasma of the same lizard; haemoglobin moves as rapid as, or slightly slower than, this fraction. These results corroborate those obtained by Sydenstricker *et al.* (1956), Zarafonetis and Kalas (1960), and Guttman (1970).

The results obtained in this study of the agamid lizard *Uromastix microlepis,* together with those previously recorded by the same authors for another agamid lizard *Agama persica* (Abdel-Fattah *et al.* 1974), indicate that all variations recorded between the two lizards are still little if compared with other results on various reptiles, as reported by Dessauer (1970), Guttman (1970) and Otis (1973). This may be correlated with the phylogenetic relationships of the two genera, which belong to the same family Agamidae, as judged by morphological characteristics.

ACKNOWLEDGMENT

The authors are very grateful to Mrs Shahnaz Al-Wakil for her help in the total protein determination.

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.

Baril, E.F., Palmer, J.L. & Bartel, A.H. 1961. Electrophoretic analysis of young alligator serum. Science, N.Y. 133 : 278-296.

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

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

Dessauer, H.C. 1952. Biochemical studies on the lizard ***Anolis carolinensis***. Proc. Soc. exp. Biol. Med. 80 : 742-746.

Dessauer, H.C. 1970. Blood chemistry of reptiles: physiological and evolutionary aspects. In **Gans, C. (Ed.) 1970.** Biology of the Reptiiia, 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.

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

Goin, C.J. & Jackson, C.G. 1965. Hemoglobin values of some amphibians and reptiles from Florida. Herpetologica 21 (2) : 145-146.

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.

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.

Hussein, M.F., Boulos, R. & Al-Badry, Kamal S. 1966. On the serum proteins of the lizards *Chalcides ocellatus* and *Agama stellio* in relation to heat death. Proc. Egypt. Acad. Sci. **20** : 59-64.

Hussein, M.F., Boulos, R. & Al-Badry, Kamal, S. 1968. Studies on serum proteins of the Egyptian snake *Psammophis sibilans* and the Egyptian lizards *Mabuia quinquetaeniata* and *Acanthodactylus boskianus* in relation to heat death. Proc. Egypt. Acad. Sci. **21** : 37-40.

Maldonado, A.A. & Ortiz, E. 1966. Electrophoretic patterns of serum proteins of some West Indian *Anolis* (Sauria : Iguanidae). Copeia **2** : 179-182.

Otis, V.S. 1973. Hemocytological and serum chemistry parameters of the African puff adder *Bitis arietans*. Herpetologica **29** (2) : 110-116.

Ramirez, J. R. & Dessauer, H. C. 1957. Isolation and characterization of two hemoglobins found in the turtle *Pseudemys scripta elegans*. . Proc. Soc. exp. Biol. Med. **96** : 690-694.

Rodnan, G. P. & Ebaugh, F. G. 1957. Paper electrophoresis of animal hemoglobins. Proc. Soc. exp. Biol. Med. **95** : 397-401.

Ryerson, D.L. 1949. A preliminary survey of reptilian blood. J. Ent. Zool. **41** : 49-55.

Sydenstricker, V.P., Oliver, R., Chandler, B.M. & Sydenstricker, O. 1956. Electrophoretic behaviour of some animal hemoglobins. Proc. Soc. exp. Biol. Med. **93** : 396-397.

Zain-Ul-Abedin, M. & Qazi, M.H. 1965. Blood sugar levels of some reptiles found in Pakistan. Can. J. Biochem. Physiol. **43** : 831-833.

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

Zarafonetis, C.J.D. & Kalas, J.P. 1960. Some hematologic and biochemical findings in *Heloderma horridum*, the Mexican beaded lizard. Copeia **3** : 240-241.

Zweig, G. & Crenshaw, J.W. 1957. Differentiation of species by paper electrophoresis of serum proteins of *Pseudemys* turtles. Science, N.Y. **126** : 1065-1066.

(*Received* 12 *June* 1974)

دراسات في دم بعض زواحف الكويت

الجزء الثاني : تعيين بعض ثوابت الكريات ، وجلوكوز الدم ، والمحتوى البروتيني للبلازما، مع فحص كهربي لبروتينات الدم في الضب يوروماستكس ميكروليبس

كمال السيد البدري و رشدي فتوح عبد الفتاح

قسم علم الحيوان بجامعة الكويت

خلاصـــــة

قام الباحثان بقياس بعض ثوابت الكريات الحمراء في الضـــب يوروماستكس ميكروليبس ، وكانت النتائج كالتالي : عدد الكريات الحمراء ... ٦٨٠ كـــرية في المليمتر المكعب ، نسبة الهيما توكريت ٢٤ر٦ ٪ ، كمية الهيموجلوبين ٨ر٥ جم ٪ .كذلك قاما بتعيين كمية الجلوكوز في الدم وكانت ١٢٤ مجم لكل ١٠٠ سم٣ من الـــــدم ، والمحتوى البروتيني للبلازما وكان ٥ر٥ جم لكل ١٠٠ سم٣ من البلازما .

كما أجرى الباحثان دراسة خاصة في بروتينات الدم في المصل والبلازما ، وكذلك في الهيموجلوبين . وقد كان التشابه واضحا بين بروتينات المصل والبلازما ، كمـــا تتشابه بروتينات المصل والبلازما في كل من الضب يوروماستكس ميكروليبس والعظاءة أجاما بيرسيكا التي سبقت دراستها . أما عن الهيموجلوبين فقد ثبت أنه يتحـــرك ككتلة متجانسة نحو القطب الموجب ، ولكنه أسرع قليلا من حالة الاجاما .