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BLOOD CHEMISTRY IN FROGS AND TURTLES

Harold M. Kaplan and Edward H. Timmons
Southern Illinois University
Carbondale, Illinois 62901

ABSTRACT

Blood of frogs and turtles was analyzed quantitatively by clinical instrumentation for chemicals known to be of clinical importance in higher species including man. The determination of such values, particularly for enzymes, is of interest from the standpoint of evolution, biomedical research and laboratory animal science.

INTRODUCTION

Although some blood chemistry values are available for common ectothermic laboratory animals, such as frogs and turtles, the values exist in scattered sources. The knowledge concerning the presence and quantitation of those enzymes known to be of importance in the detection of cellular damage in mammalian disease is especially fragmentary in the ectotherms. Such knowledge has been documented for several mammalian animals (Burns and de Lannoy Jr., 1966; Burns, Timmons and Poiley, 1971; Melby and Altman, 1974; Schalm, Jain and Carroll, 1975) and for man (Bioscience Laboratories Handbook, 1973).

The present data collate and expand the knowledge of blood chemistry in frogs and turtles with special reference to enzyme values.

MATERIALS AND METHODS

The frogs were the northern variety of Rana pipiens (males and females), all about 60 gm. They were fed crickets, then fasted for three days, at 22° C. All frogs used presented a healthy appearance, without muscle flaccidity, postural hypotonia, cutaneous hemorrhage, pigmentation loss, or noticeable abdominal or limb edema. On necropsy evidence of internal parasites was absent or slight.

The turtles used, Pseudemys scripta elegans (males and females), were healthy adults, 5-6 inch shell length. They were fed canned liver dog food wetted with cod-liver-oil, then fasted for three days.

Frogs were rendered unconscious by pithing. After exposing the thorax, blood was drawn from the cardiac ventricle through a 20 gauge needle. For

hemoglobin and the hematocrit, heparinized whole blood was used. For creatine phosphokinase (CPK), heparinized whole blood was centrifuged and the plasma was used. For all other chemicals tested, serum was used.

To obtain serum, untreated whole blood was allowed to clot for 15 minutes. The tube containing clot and supernatant fluid was centrifuged at 4,000 RPM for 5 minutes. If the supernatant serum was colored pink, which denotes red cell hemolysis, the sample was discarded, because hemolysis can interfere with the quantitative determination of enzyme concentrations.

The serum was immediately withdrawn by pipet and microliter samples were utilized in determinations of lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase, uric acid, blood urea nitrogen (B.U.N.), cholesterol, glucose, total protein, and albumin. Determinations were completed within four hours of drawing the blood.

Similar procedures were used for turtle blood. A 1 cm hole was bored in the plastron with an electric drill, and blood was obtained by cardiac puncture using an 18-gauge needle. Euthanasia was by overdose of sodium pentobarbital.

Blood analyses, except for the packed cell volume and the enzyme CPK, were performed with an Accu-Stat System (Clay-Adams Automated Laboratory Equipment, Division of Becton-Dickinson and Co., Parsippany, N.J. 07054). This instrument gives data within a limit of accuracy acceptable for clinical purposes, while permitting the use of very small samples. CPK determinations were obtained by a Sigma procedure (Sigma Chemical Co., St. Louis, MO 63178) involving a Spectronic 70 in which ATP interacts with creatine to produce ADP and phosphocreatine, the reaction being catalyzed by CPK. The phosphocreatine is then hydrolyzed to creatine and inorganic phosphorus. The phosphorus is measured colorimetrically at 520 nm and is proportional to CPK activity. The PCV was determined in a microhematocrit centrifuge (Hematocrit Model No. 34, Chicago Surgical and Electrical Co., Melrose Park, IL 60160).

Sex differences and differences between frog and turtle blood were analyzed by the Student t test for independent means, the level of significance selected at $p = 0.01$.

RESULTS

Data for healthy fasted frogs and turtles are listed in Table 1 along with comparison literature values for the cat and man.

There are no significant sex differences between frogs and turtles for any of the chemicals studied, although such differences exist as tendencies, e.g. PCV is higher in the males of both frogs and turtles.

There are species differences. Alkaline phosphatase is significantly higher in turtles than in frogs, but both values lie approximately within the range of the cat and man.

TABLE 1.

Blood Chemistry baseline values in frog (Rana pipiens) and turtle (Pseudemys scripta elegans)

Chemical constituent	Units used	Mean, standard deviation and range		Cat	Man
		Frog	Turtle		
Alkaline phosphatase	Babson (I.U./L)	24.4 ± 9.1 (6-43) (17 males, 5 females)	42.8 ± 16.2 (10-75) (15 males, 10 females)	8-46 I.U./L	9-35 I.U./L
Glutamic Pyruvic transaminase (GPT)	I.U./L	25.0 ± 6.5 (12-38) (25 males, 4 females)	21.9 ± 10.3 (5-42) (15 males, 10 females)	1-67 I.U./L	10-35 I.U./L
Glutamic oxaloacetic transaminase (GOT)	Karmen/ml	490.2 ± 168.4 (110-785) (16 males, 6 females)	219.8 ± 48.4 (123-317) (15 males, 10 females)	2-43 I.U./L	8-40 Karmen/ml =4-20 I.U./L
Lactic dehydrogenase (LDH)	Wacker/ml	114.7 ± 23.3 (68-161) (12 males, 11 females)	106.2 ± 29.1 (48-164) (15 males, 10 females)	42-420 Wacker/ml	8-20 Wacker/ml =40-60 I.U./L
Creatine phosphokinase (CPK)	Sigma/ml	71.9 ± 25.8 (20-124) (17 males, 14 females)	39.8 ± 18.3 (3-76) (16 males, 10 females)	0.5-1.5 Sigma/ml	0-12 Sigma/ml =0-100 mIU/ml
Packed cell volume (PCV)	ml/100 ml whole blood	35.4 ± 8.15 (25-57) (12 males, 15 females)	28.4 ± 5.4 (18-39) (13 males, 10 females)	37 ml/100 ml	40-50 (male) 37-47 (female)
Hemoglobin	gm/100 ml whole blood	7.1 ± 1.0 (5-9) (16 males, 6 females)	9.2 ± 2.9 (3-15) (13 males, 10 females)	7.9-15.5 gm/100 ml	13-18 (male) 11-16 (female)
Glucose	mg/100 ml	30.8 ± 6.9 (17-45) (15 males, 7 females)	96.0 ± 18.2 (56-132) (15 males, 10 females)	39-130 mg/100 ml	70-110
Cholesterol	mg/100 ml	83.9 ± 43.4 (35-180) (16 males, 6 females)	140.8 ± 45.6 (80-260) (15 males, 10 females)	60-136 mg/100 ml	150-250
Total Protein	gm/100 ml	3.3 ± 0.5 (2-4) (16 males, 6 females)	4.7 ± 0.5 (4-6) (14 males, 9 females)	6.6-8.9 gm/100 ml	6.0-8.2
Albumin	gm/100 ml	1.2 ± 0.2 (1-2) (16 males, 6 females)	1.8 ± 0.9 (0.5-4) (14 males, 9 females)	3.5-4.6 gm/100 ml	3.5-5.5
Blood urea nitrogen (B.U.N.)	mg/100 ml	11.9 ± 4.6 (3-21) (16 males, 6 females)	21.6 ± 8.4 (5-38) (14 males, 9 females)	14-31 mg/100 ml	5-25
Uric acid	mg/100 ml	4.0 ± 2.4 (1-9) (15 males, 6 females)	1.6 ± 0.5 (1-3) (17 males, 15 females)	0.3-3.2 mg/100 ml	2.0-7.8

Frogs (avg. weight 60 gm) and turtles (shell length 5-6 inches), held prior to use at room temperature, were used. Serum was analyzed except where otherwise noted in the Table. The normal values for the enzymes were determined at 37 C. Turtle blood is significantly different from that of frogs ($p<0.01$) in alkaline phosphatase, GOT, CPK, total protein, albumin, hemoglobin, cholesterol, glucose, blood urea nitrogen, uric acid, and packed cell volume. There are no sex differences at $p=0.01$.

GPT and LDH are in the same range for the frog, turtle, cat and man. Differences between frogs and turtles are not significant for either enzyme.

GOT and CPK are highest in the frog and significantly reduced to about half those values in turtles. GOT and CPK are far lower in the cat and man, the two latter species being in the same range.

The PCV is significantly higher in frogs than in turtles, but both these values lie approximately within the lower range of the cat and man.

Hemoglobin is significantly higher in turtles than in frogs. Both values are below the cat and human mean values.

Glucose is significantly higher in turtles than in frogs. Turtle blood glucose lies in the range of the cat and man.

Cholesterol is significantly higher in turtles than in frogs. The turtle is in the high range of the cat and in the low range of man.

The total protein and serum albumin are significantly different between frogs and turtles. They are low in frogs, higher in turtles, and still higher in the cat and man (the latter two being in the same range).

Blood urea nitrogen is significantly higher in turtles than in frogs. The turtle B.U.N. value lies within the range of the cat and man.

Uric acid is significantly higher in frogs than in turtles. The turtle uric acid value lies within the range of the cat and man, and the frog value lies only within the range of man.

DISCUSSION

Baseline values for biochemical blood constituents in common laboratory ectothermal species are necessary in a conveniently collated form.

Because some anticoagulants used in plasma collection interfere with enzyme activity, serum was chosen for determining enzyme concentrations except for CPK.

The effects of anesthesia or pithing on serum enzyme concentration in ectotherms are unknown. Literature values should be listed with known specifications. Our frog data are for pithed specimens.

Our enzyme studies deal with rates, which are temperature dependent. The analyses on drawn blood were performed with blood samples warmed in the machine to 37° C which duplicates conditions in the mammalian system. It allows literature comparison with mammals. For ectotherms *per se*, data obtained at the lower laboratory room temperature at which they were held might more accurately specify the living conditions for enzyme operation, although it might increase the time necessary for the test and it would change the end-point where the values are read.

In accepting the validity of blood chemistry and hematologic values listed for ectotherms, strict attention may have to be paid to seasonal biorhythms. Kaplan (1954) found that in the breeding season of frogs there is a significant decrease in hemoglobin, a tendency for the number of red blood cells to fall, and a loss of plasma, these changes affecting both sexes similarly. Kaplan (1960) also reported on seasonal blood changes in pseudemyd turtles. The present studies are limited to summer and fall.

The enzymes discussed herein have been found in the lowest vertebrates (Eckroat, 1975; Markert et al., 1975). From an evolutionary standpoint, the presence of such enzymes from fish to man indicates that it was not necessary to "invent" new cell enzymes for similar vital processes despite the diversity of the species.

For serum enzyme concentrations (which are ultimately intracellular in origin), frog and turtle blood are usually in the same range as man and some of the enzymes of these ectotherms exceed human values. Intracellular activity in ectotherms is not as sluggish as might be supposed.

One possible application of enzyme analysis in ectotherms may lie in the use of enzymes as tools to diagnose cardiac, liver or bone disorder, as is done in mammals. Also, ectotherms may be an adjunctive resource to mammals for enzyme studies in general.

SUMMARY

Quantitative analysis of important blood chemicals, especially selected enzymes, in frogs and turtles, is reported within a range of accuracy useful (1) in laboratory animal medicine and (2) as baseline values in comparative animal physiology.

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