Biochemical Studies on the Lizard, *Anolis carolinensis.* (19748)

HERBERT C. DESSAUER. (Introduced by Roland A. Coulson.)

From the Department of Biochemistry, Louisiana State University Medical School, New Orleans, La.

Although those mammals of interest to biochemistry have been studied in detail for some 50 years, comparative biochemistry as a field of study is little more advanced than was the field of comparative anatomy a hundred years ago. It is curious to note that a century old knowledge of the anatomy of most of the common reptiles has not stimulated research in the chemical composition and biochemistry of this important class of animals.

Among reptiles the "American chameleon," *Anolis carolinensis,* is plentiful, inexpensive and has many other advantages in research requiring the sacrifice of large numbers of animals. Before this animal could be used for biochemical research, a knowledge of the composition of the blood, urine, and ash was thought to be necessary. Since *Anolis carolinensis* when fully grown weighs only 5 to 7 g, micro and ultramicro analytical technics were required in this survey study.

All analyses were made on recently captured animals which were fasted 4 days before use. Blood for glucose determinations was obtained by decapitation; blood for all other analyses was obtained by cardiac puncture in the following manner: About 0.001 ml of a 0.004% solution of heparin was added to the tip of a tuberculin syringe fitted with a No. 26 needle. While the animal was held ventral surface up on a table by an assistant, the needle of the syringe was placed just anterior to the pectoral girdle in the midline, parallel to the body of the animal and pointed toward its tail. The needle was then pushed carefully through the skin into the region just beneath the pectoral girdle. With careful probing the heart was penetrated, and following slight suction on the syringe, blood was obtained. From a single animal one can obtain a maximum of 0.05 to 0.20 ml of blood. If less than one-half of the total estimated blood volume is drawn, 7 out of 10 animals will survive such a blood letting.

If blood pH was to be determined or if whole blood was required, the capillary tube used in pH determinations, and the blood pipettes were filled directly from the blood in the syringe. If plasma was required, a metal clamp was fitted to the syringe between the head of the plunger and the body of the syringe and the blood was then centrifuged in the syringe. If acid base studies were to be done, a drop of mineral oil was layered above the blood. After centrifuging the syringe was held vertically and the plunger carefully twisted upward. By this technic practically all of the 0.03 to 0.15 ml of plasma was measured directly into blood pipettes for analyses without disturbing the packed cell layer in the syringe. Using such methods and ultramicroanalysis pH, Na, K, Cl and CO_2 could be done on the plasma of one animal.

Urine was obtained by exerting slight pressure on the abdomen just anterior to the anus and collecting the drops of urine in measuring pipettes. Using this method 0.01 to 0.20 ml of urine was obtained per animal.

Ash studies were carried out in November, on animals recently caught. After a 4-day fast they were weighed, decapitated, dried at 75°C to constant weight, and ashed in a muffle furnace at 450-500°C. The resulting ash was weighed, ground to a very fine powder, placed in a 10 ml volumetric flask, diluted to mark with distilled water, stirred thoroughly and allowed to settle. The supernatant fluid was analyzed then for Cl, Na and K. Following the analyses of these water-soluble constituents, enough HCl was added to the flask to dissolve the water-insoluble Ca and Mg salts. Water was added to mark and samples of the acid solution were analyzed for Ca and Mg.

Analytical methods. Packed cell volume determined by the method of van Allen(1). Na and K were analyzed with the Beckman flame spectrophotometer. Chlorides were determined in plasma, urine and protein-free filtrates of blood by the method of Schales and Schales(2) adapted to small volumes of sample through the use of the ultramicro

TABLE I. Blood Constituents.

		Avg	Analyses	Range
Packed cell vol (%)		28	26	20 – 34
Red blood cells (10^6/mm^3)		.96	26	.61– 1.21
Oxygen capacity (vol %)		9.3	15	7 – 12.5
Plasma	pH	7.26	25	6.93– 7.63
	Na (mM/L)	157	41	139 –186
	K "	4.59	15	2.80– 5.93
	Cl "	127	30	113 –133
	CO_2 "	15.4	25	9.6 – 22.5
	Ca "	2.9	20	2 – 4.2
	P "	2.6	19	1.7 – 3.2
	Protein (%)	4.1	12	3 – 5.7
Blood	Na (mM/L)	130	15	118 –135
	K "	31.8	15	20.9 – 38.4
	Cl "	105	18	78 –116
	CO_2 "	14	15	9.3 – 17.1
	Protein (%)	11.5	16	9.1 – 13.6
	Uric acid (mg %)	7.9	20	4.1 – 10.8
	Urea "	7.2	15	5.5 – 9.8
	NH_3 "	Tr	15	0 – .9
	Glucose "	172	715	58 –305

TABLE II. Urine Constituents.

	Avg	Analyses	Range
pH	5.54	19	4.62– 6.50
Na (mM/L)	20.2	18	5.7 – 33.7
K "	17.1	18	5.1 – 36.9
Cl "	23.4	22	4 – 49
CO_2 "	Tr	15	0 – 1
Uric acid N (mg %)	45.6	19	5.3 –166.5
Urea N "	8.8	20	4.9 – 18.5
Ammonia N "	8.2	20	4.9 – 22.8
Creatinine N "	1.3	18	.6 – 1.9

burette of Gilmont(3) and technics of microanalysis suggested by Kirk(4). CO_2 and O_2 capacity were measured in the Scholander syringe analyzer(5,6). The Beckman model G pH meter fitted with the Beckman No. 290-83 capillary glass electrode was used to determine the pH of blood and urine. Plasma Ca was determined by the method of Sobel and Sobel(7), plasma P by the method of Kuttner and Cohen(8). Ash Ca was determined by permanganate titration(9), Mg by the method of Denis(10). Blood and plasma were digested and fractions of the digest were added to Conway diffusion cells and the protein determined as ammonia(11). Ammonia, urea and ash Cl also were analyzed in Conway cells(11). Blood uric acid was determined by the method of Brown(12), urine uric acid by the method of Folin(13). Creatinine in the urine was measured by the method of Folin and Wu(14). A micro adaptation of the method of Folin and Wu(15) was used to determine blood glucose.

The results of these analyses appear in the accompanying tables. Compared to the alligator(16) and turtles(17), *Anolis* has a higher blood Cl and lower pH. Inorganic P is high as compared to the alligator and human. The distribution of body water may be approximated from the average values of Cl in the ash and plasma on the assumption that all Cl is extracellular. In the average animal in November intracellular fluid is 44.7% and extracellular fluid 22.7% body weight.

Blood glucose averages 172 mg % over the year but there is a seasonal variation, an average minimum of 110 mg % in August, average maximum of 197 mg % in February. There are no sex differences. In order to check the high values of blood sugar by a more specific method, a series of fermentation studies were carried out using a yeast microdiffusion method in a special Conway cell(18). Fermentable sugar averaged 159 mg % as compared with the value of 186 mg % obtained on a control series using the Folin and Wu method.

Summary. The following determinations have been done on freshly caught fasting specimens of *Anolis carolinensis*: red cell count, hematocrit, O_2 capacity, blood glucose, blood

TABLE III. Composition of Anolis Ash.

Sex	Wet wt	Dry wt	Ash wt	Na	K	Ca	Mg	Cl
	g			mg				
♂	3.50	1.32	.175	3.35	5.87	58.3	1.34	3.85
♂	3.26	.99	.135	2.86	5.23	43.2	1.10	2.73
♂	3.02	.90	.132	2.23	5.03	45.6	1.19	3.30
♂	3.71	1.26	.173	2.39	6.23	57.9	1.30	4.20
♀	3.22	1.00	.153	2.80	5.65	51.6	1.39	3.12

and urine urea, ammonia, uric acid, Cl, Na, K, pH, CO_2; blood and plasma proteins; urine creatinine, and Na, K, Ca, Mg and Cl of the ashed animal.

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Received July 10, 1952. P.S.E.B.M., 1952, v80.

Effects of Liver Injury and Nephrectomy on the Anticonvulsant Activity of Phenacemide.*† (19749)

LAWRENCE C. WEAVER, EWART A. SWINYARD, AND LOUIS S. GOODMAN.

From the Departments of Pharmacology, University of Utah College of Pharmacy and College of Medicine, Salt Lake City, Utah.

Although phenacemide (phenacetylurea; Phenurone) is one of the most useful agents for the treatment of psychomotor epilepsy, comparatively little is known concerning the fate and the excretion of this agent in animals or man. In a brief report on the pharmacology of Phenurone, Everett(1) mentioned experiments in which the duration of anticonvulsant action remained unchanged in bilaterally nephrectomized mice, but was significantly prolonged by carbon tetrachloride-induced liver damage and hepatectomy. We are aware of no published information concerning the fate and excretion of Phenurone in man.

We have reported(2,3) the results of experiments in which biological methods were used to measure the effect of liver injury and nephrectomy on the potency and the duration of anticonvulsant action of clinically employed hydantoins and oxazolidine-2,4-diones. We have now applied the same biological methods in the study of Phenurone. It was anticipated that the results would contribute information

* This investigation was supported by research grants from the National Institutes of Health, Public Health Service, and the University of Utah Research Fund.

† Phenacemide was kindly supplied by Dr. R. K. Richards, Abbott Laboratories.