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ARSONS

BIOLOGY OF THE REPTILIA

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Edited by

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The Editors would like to dedicate this volume to our friend,

WADE FOX

whose untimely death deprived us of a valued collaborator.

Preface

This, the third volume of the BIOLOGY OF THE REPTILIA deals with aspects of the blood and of the endocrine system. As in the previous morphological volumes, most of the chapters treat only structure, with function relegated to the physiological volumes to follow.

Limited amounts of histophysiology and some general physiology are included for such topics as the parathyroid, the thyroid, and also for the adrenal; they are omitted for the hypophysis because the topic here is much more complicated and clearly requires a separate coverage under the general heading of physiology. It is hoped that the volume projected for that section will also deal with the most important effects of temperature on these systems.

The preface to the first volume of this series emphasized the editors' aim of facilitating future work. The summary statements of the contributing authors document again how much such additional work is needed. The need is threefold; we require a clear statement of interspecific variability, a characterization of the seasonal and physiological changes induced in the organ's histology, and the need to apply new techniques.

Variability is certainly quite inadequately known and the vast majority of the recorded observations have been made on less than one or two dozen species. Unfortunately the pattern of internal structure cannot be assumed to be similar among the orders of the class, among the families of an order, among the genera of a family, or among the species of a genus. The classification of organisms differs from Mendeleyev's periodic table for the chemical elements in that it is *not* predictive; the existing similarity can only be characterized *after* all of the included forms have been examined.

Many aspects here recorded are far more likely to reflect seasonal changes or those due to the animal's behavioral or physiological state than are other aspects of the animal's morphology. Many of the histological observations need to be checked for the influence of such modifying factors. The comparative studies based entirely on studies using light microscopy and histochemistry need to be reviewed with the electron microscope. A comparison of the accounts of H. Saint Girons and Gabe with the phylogenetically far more restrictive comparison of Clark, clearly points to some of the additional work that needs to be done on each of these organs.

It is the hope of the authors and of the editors that this summary of the

present state of our knowledge will serve as a stimulus leading to a more accurate characterization of the similarities and differences between species and larger categories as well as to a more quantitative characterization of the dynamic structural pattern.

I wish to thank the authors, several of whom agreed to repeated and major changes in the nature of their required contributions. Drs. W. E. Adams, W. Andrew, D. Belkin, E. G. Butler, N. B. Clark, E. Cohen, W. B. Elliott, V. E. Engelbert, W. Frair, M. Gabe, J. E. Heath, E. von Herrath, V. H. Hutchison, O. P. Jones, P. Licht, W. G. Lynn, M. Miller, M. Moss, X. J. Musacchia, U de V. Pienaar, H. Rahn, H. I. Rosenberg, H. Saint Girons, M. C. Saint Girons, A. J. L. Strauss, H. Szarski, A. M. Taub, S. R. Telford, Jr., E. E. Williams, and A. Wright reviewed individual manuscripts and Mrs. Gloria Griffin and my wife provided extensive editorial assistance. My co-editor, Dr. Thomas S. Parsons shared all aspects of the work. Drs. James A. Peters and Heinz Wermuth critically read the proofs for usage and accuracy of the Latin names employed. National Science Foundation Grant GN 815 provided for some financial assistance and the Department of Biology of my University paid the considerable bills for postage and copying.

July, 1970 Carl Gans

Contents

CHAPTER 1

Blood Chemistry of Reptiles: Physiological and Evolutionary Aspects

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I. Introduction

Active multicellular organisms require efficient circulatory systems to carry gases, nutrients, and waste materials to and from their tissues. Both volume and composition of the circulating fluids must be maintained within narrow limits (Lockwood, 1961), in spite of the changing availability of water, salts, and metabolites and their exchange with cells and extravascular fluids. In ectotherms, whose cells function or remain viable over broad ranges in temperature, mechanisms regulating blood volume and composition may be different from those characteristic of mammals (Bullock, 1955).

Homeostatic control systems and cellular requirements place far less stringent limits on the composition of the blood of reptiles than on that of endothermal animals. In a single species of turtle an osmotic pressure as low as 150 mOs/liter can occur under one circumstance and one as high as 450 mOs/liter under other conditions. Plasma of crocodilians may become virtually chloride free after feeding, with bicarbonate making up two thirds of total plasma anions. Calcium may exceed 200 mg % during estrus in snakes. Seventy per cent of the total hemoglobin of certain turtles may be nonfunctional in oxygen transport, existing as the methemoglobin derivative. Blood pH's of 6·5 and 8·1 have been observed under physiological conditions. Such values are unheard of in mammalian physiology, and probably are incompatible with endothermal life. "The maxim that life can exist only within a relatively small pH range . . . may only be true for homothermal animals" (Robin, 1962).

There is no such creature as a "typical" reptile. Chemical, physiological, and immunological findings impress upon one the differences between major groups. This chapter gathers information on the composition of the blood of reptiles and attempts to synthesize the resultant data in terms of reptilian

physiology and evolution. Only a bare beginning has been made in these areas. Many intriguing problems await the physiologist interested in reptiles, problems not answered by reference to concepts based upon mammalian physiology. Likewise, the biologist interested in evolution will find in blood a source of much information on speciation and on the interrelationships of living forms.

II. Blood Letting and Handling

Blood usually is obtained from unanesthetized animals, but on occasion, and especially with snakes, animals must be anesthetized. Anesthetics given by inhalation or by injection are useful (Kaplan and Taylor, 1957). As reptiles do not catabolize barbiturates rapidly, the dose of drugs such as nembutal must be chosen carefully (Karlstrom and Cook, 1955). Betz (1962) summarizes the literature on anesthesia of reptiles and describes the use of the tail and tongue reflexes for controlling the surgical plane of anesthesia in snakes. Fluothane appears to be an especially useful inhalation anesthetic (Hackenbrock and Finster, 1963).

Heparin, oxalate, citrate, ethylenediamine tetracetic acid, and ion exchange resins are effective anticoagulants (Kaplan, 1956). For direct decalcification of small samples, collect blood through a small column of resin attached to the syringe (Lund *et al.*, 1957). Heparin is the anticoagulant of choice with turtles such as *Chelydra serpentina* whose red cells hemolyze in solution lacking calcium ion (Lyman, 1945).

Cardiac puncture of crocodilians, lizards and snakes is relatively easy as their hearts are located readily by the pulsations visible on the anteroventral body wall (Tiegel, 1880; Hopping, 1923; Cohen *et al.*, 1964). Cardiac puncture is more complex with turtles (Gandal, 1958). One can approach the heart laterally by directing a long needle through the soft tissues between the plastron and carapace at the level of a front or rear leg. A more common practice is to tap the heart through a hole trephined in the anteromedial corner of the right abdominal plate of the plastron (Rapatz and Musacchia, 1957; Musacchia and Sievers, 1962). Reptiles generally survive a cardiac puncture if it is carefully done. Coulson and Hernandez (1964) have bled individual alligators as often as 10 times in a 24 hour period without causing any apparent injury. Turtles survive numerous blood lettings, living in laboratory tanks for years with the hole in their plastron sealed with a cork, wax or tape.

Other sites for blood letting are often useful in physiological experiments which require multiple sampling of blood. For crocodilians a convenient method is to cut the tip of the tail and "milk" the sample into a tube containing anticoagulant (Coulson and Hernandez, 1964). Major vessels of

large lizards can be cannulated (Tucker, 1966; Moberly, 1968a). Turtle blood may be obtained from a femoral (Robin *et al.*, 1964; Haning and Thompson, 1965) or jugular vein (Lopes, 1955) or a carotid artery (Crenshaw, 1965; Berkson, 1966). Microliter samples can be obtained from the retroorbital space (Riley, 1960; Frair, 1963).

Analyses of blood constituents of reptiles date back to the late nineteenth century. Methods of analyses have undergone great changes in precision over the years. A listing of modern, simple micro-methods applicable to work on reptiles is given in the monograph on the alligator by Coulson and Hernandez (1964).

III. Composition of Blood Plasma

A. General

Plasma, making up some 60 to 80 per cent of blood volume, is a colorless or straw colored fluid in many species but is intensely pigmented in others (Brocq-Rousseu and Roussel, 1934, 1939; Putnam, 1960, 1965). In iguanid lizards and African chameleons its bright orange or yellow color reflects a high content of carotenoid pigments. Plasma of the snakes *Python*, *Bothrops* and *Mastigodryas* is greenish yellow due to a high content of carotenoids and riboflavin (Villela and Prado, 1945; Villela and Thein, 1967).

Plasma of reptiles, like that of all vertebrates, contains a great variety of different substances with most being present in trace quantities. "Representative" levels of major constituents, especially those commonly measured in blood studies, are collected in Tables I and II. When a number of laboratories have contributed data on the same species such results have been averaged. Only analyses on active animals, maintained at room temperature under fasting conditions, have been included. These averaged values represent orders of magnitude rather than fixed levels as the range of variability for most constituents is great even between individuals of a single population sample. Too little is known of reptilian physiology to define strictly basal conditions for any reptilian species.

B. Low Molecular Weight Components

1. *Electrolytes*

a. *Representative levels.* Plasma of each of the several orders of the Reptilia shows certain trends in osmotic pressure, pH, and concentration of sodium, chloride, and bicarbonate ions (Table I; Dittmer, 1961). Total osmolarity, due primarily to electrolytes in all reptiles, is relatively high in snakes, lizards, and sea turtles, but is low in fresh water turtles. Even snakes such as *Natrix*, which live close to or in fresh water, have blood with a high salt content.

TABLE I
Plasma Electrolytes

Species	Osmotic pressure mOs/liter	pH	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-	P_i	$SO_4^=$	Source[a]
				mM/liter							
					TESTUDINES						
Chelydra serpentina	315	7·62	132	3·2	3·8	2·7	76	48	1·3	0·3	12, 29, 40, 47
Kinosternon subrubrum	288		121	4·2	3·5	1·0	98	30	1·7		12, 47
Sternotherus odoratus	282	7·44	126	3·8			84	25	1·8		12
Chrysemys picta		7·77	143	3·2	2·5	4·8	85	47	1·0	0·8	24, 47, 57, 60
Emydoidea blandingii			140	3·8	3·3	2·1	91	39	1·3	1·3	47, 57
Emys orbicularis	249							40	2·1		4, 37, 55
Graptemys geographica			124	2·4	3·4	0·5	87	39	1·2	0·4	47
Pseudemys scripta		7·56	121	4·1	2·8	2·2	81	40	1·1	0·2	12, 7, 24, 26, 45, 47, 50, 51, 52, 57
Terrapene carolina	345	7·68	130	4·7	1·3	3·5	108		2·4	1·2	12, 24, 34
Terrapene ornata	317			4·6	1·7	2·0	104		0·8		12
Caretta caretta	408		157	2·2	3·1	2·9	110	36	3·0		4, 5, 18, 19, 31, 44, 47
Lepidochelys olivacea			163	6·6	5·2	1·4	108	29	3·5	0·3	47
Chelonia mydas		7·45	158	1·5				33			3, 31
Testudo graeca	321			7·8	4·0		100				5
Testudo hermanni	317		127	4·4	2·3		95				25
Trionyx ferox	274		113	6·8	1·7	1·5	90		2·0		12
Trionyx spiniferus			144								22
					SQUAMATA (Sauria)						
Gekko gecko							123				12
Anolis carolinensis		7·26	157	4·6	2·9		127	15	2·6		12, 35
Anolis			171	4·5							42
Ctenosaura acanthura		7·22	159	2·9	2·9	1·1	133	15	2·3		30
Ctenosaura pectinata			171	4·4							49