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-	HCO3-	Pi	SO4-	Sourcea
				mM/liter							
					TESTUDINES						
Chelydrasetpentina	315	7 62	132	32	38	27	76	48	13	03	12, 29,40,47
Kinostenonsubrubrum	288		121	42	35	10	98	30	17		12,47
Sternotherusodoratus	282	7 44	126	38			84	25	18		12
Chrysemyspicta		7 77	143	32	25	48	85	47	10	08	24,47,57,60
Emydoideablandingii			140	38	33	21	91	39	13	13	47,57
Emysorbicularis	249							40	21		4,37,55
Graptemysgeographica			124	24	34	05	87	39	12	04	47
Pseudemysscripta		7 56	121	41	28	22	81	40	11	02	12,7,24,26, 45,47,50,51, 52,57
Terrapenecarolina	345	7 68	130	47	13	35	108		24	12	12,24,34
Terrapeneornata	317			46	17	20	104		08		12
Caretta caretta	408		157	22	31	29	110	36	30		4, 5,18,19, 31,44,47
Lepidochelysolivacea			163	66	52	14	108	29	35	03	47
Cheloniamydas		7 45	158	15				33			3,31
Testudograeca	321			78	40		100				5
Testudohetmannt	317		127	44	23		95				25
Trionyx ferox	274		113	68	17	15	90		20		12
Trionyxspiniferus			144								22
					SQUAMATA (Sauria)						
Gekkogecko							123				12
Anoliscarolinensis		7 26	157	46	29		127	15	26		12,35
Anolis			171	45							42
Ctenosauraacanthura		7.22	159	29	29	11	133	15	23		30
Ctenosaura pectinata			171	44							49

Iguana iguana	7.48		157	3.5	2.7	0.9	118	24	2.0		30,56,58
Sauromalus obesus	7.50	16		9	4.9		127				16,49
Sceloporus occidentalis					2·5						41
Amphibolurus ornatus			150	5.0							61
Agama agama		179		5.2							53
Agama impalearis		349	152	7.1	2.9						59
Uromastyx acanthinurus		307	150		2.5						59
Chamaeleo chamaeleon					114						12
Eumeces fasciatus							130				12
Trachydosaurus rugosus			151	4.7							1,46
Cnemidophorus sexlineatus							128				12
Tupinambis nigropunctatus			136		3.5		110				12
Ophisaurus ventralis	388	7.16									12
Gerrhonotus multicarinatus									353		12
Heloderma suspectum				7.20		130			27		23
Heloderma horridum			158		4.1		114				54
Varanus griseus			181	3.5	3.1	2.3	148	31	2.5		27
SQUAMATA (Ophidia)											
Lichanura roseofusca							106				12
Coluber constrictor	375	7.63	151	4.1	3.2	1.5	101	14			12,32
Elaphe obsoleta	384		162	4.9	3.6	2.5	131		2.5		12
Farancia abacura	313		147	5.4	3.3		115				12
Heterodon platyrhinos		7.53	155		4.4		126	12			12
Lampropeltis getulus	345	7.46	148	4.5	2.9	1.9	121	10	2.4		12,32
Masticophis flagellum	341		156	3.9	3.4	2.1	120		1.1		12
Pituophis catenifer		381	176	5.9	3.6		136				12
Rhinocheilus lecontei		164			4.1			122			12
Natrix erythrogaster	7.22		192		5.0		146	10			12
Natrix natrix			159	6.4							42
Natrix rhombifera	359	7.32	155	4.0	3.9		139	7			12
Natrix sipedon	318	7.29	159	4.6	3.8	1.3	127	11	2.3		10,12,32,38
Regina grahamiae		354	156	3.5	3.8		120				12
Thamnophis elegans	347	7.20	161	4.3	3.4	0.8	134	14	1.9		12
Thamnophis ordinoides	349	7.27	159		5.4		126	10			12
Thamnophis sauritus	324	7.19	159	5.4	2.7	2.0	125	14	1.6		12,13

TABLE I—*continued*

Species	Osmotic pressure mOs/liter	PH	Na+	K+	Ca++	Mg++	CL-	HCO-3	Pi	SO4	Source a
				mM/liter							
SQUAMATA (Ophidia)—*continued*											
Thamnophissirtalis	329	7.30	152	5.9	3.0	1.5	130		***0.7***		12,14
Homalopsisbuccata			162	4.8	4.2						2
Micrurus fulvius							134				12
Agkistrodoncontortrix	361	7.32	154	5.1	3.5	2.3	138	12			12
Agkistrodonpiscivorus	401		151	5.0	3.4	1.7	116	10	1.8		12,32
Crotalusatrox	345		154	3.7	3.7	1.8	131				12,39
Crotalushorridus							112				6
Crotalusviridis	325		146	3.6			123				12
Viperaaspis			170	6.5	3.5		130		3.5		36
Laticaudasemifasciata	320		159								21
CROCODILIA											
Alligatormississippiensis	284	7.48	141	3.8	2.6	1.5	112	20			8,9
Crocodylusacutus			149	7.9	3.4	1.9	117	11			15

a - Numbers designate reference source given in key below:

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49. Templeton, 1964
50. Urist and Schjeide, 1960/61
51. Williams, Unpublished
52. Wilson, 1939
53. Wright and Jones, 1957
54. Zarafonetis and Kalas, 1960
55. Verbiinskaya, 1944
56. Tucker, 1966
57. Stenroos and Bowman, 1968
58. Moberly, 1968a, b
59. Tercafs and Vassas, 1967
60. Clark, 1967
61. Bradshaw and Shoemaker, 1967

TABLE II

Packed Cell Volume and Certain Organic Constituents of Blood

Species	Packed Cell Volume %	Hemoglobin g%	Total Plasma Protein g%	Glucose mg%	Urea mg%	Uric Acid mg%	Source-a
			TESTUDINES				
Chelydra serpentina	25	5.9*	4.7	33	96	2	6,13,20,23,35,37, 42, 66, 76, 79, 84, 86,102
Kinosternon subrubrum	23		5.6				20,35
Sternotherus odoratus	33	**11.2**	4.5				20,35,39
Sternotherus minor	35	9.9	4.0				39,79
Deirochelys reticularia	20	8.3	4.2				35,39,79
Chrysemys picta	23	**11.2**	4.4	76	37	2	35,37,57,70,75, 79,92,98
Clemmys guttata	4.5*		6.1				20,71
Emys orbicularis	24	6·6*		50			2,35,52,54,74, 87,95
Malaclemys terrapin		9.2					39
Kachuga smithii				78			97
Pseudemys dorbigni				*91*			15,34,63,89,99
Pseudemys floridana		8·1					82
Pseudemys scripta	26	8.0	3.6	70	22	1	20,35,36,39,44,45, 48,50,55,56,91,92, 98
Terrapene carolina	28	5·9*	4.5	36	30	2	3,4,20,35,37,50
Testudo kleinmanni	27		6·6				60
Testudo hermanni					78		38
Gopherus polyphemus	30		3.5				35,79,102
Caretta Caretta	32	4.7		60	45		12,32,35,62,85,102
Chelonia mydas	30	6.6*	2.9		52	8	8, 35, 58, 62, 79, 102
Dermochelys coriacea			3·8		70	4	12,50
Trionyx ferox			5·3				20,35
Lissemys punctata				46			97
Pelusios			4.3				35
Chelodina longicollis			3.3				35
Phrynops geoffroanus				99			34
			SQUAMATA (Sauria)				
Gekko gecko				93	4		20
Coleonyx variegatus				94	4		39,83
Hemidactylus	-	10.8		54			39,97
ANOLIS *carolinensis*	28	7.0*	4.1	172	7	8	20,51,67
Crotaphytus collaris		7.8*					19
Ctenosaura acanthura	35	6.0*	6.8	**192**	2	4	43
Iguana iguana	30	7.1*	4.5	155	1	5	43,79,102
Phrynosoma cornutum		7.0*	4.4	**191**	2		19,93
Phrynosoma douglassii		7.7*					19
Phrynosoma modestum		9.1*					19
Sauromalus obesus	31	8.4*	4.9				26
Sceloporus clarkii		6.2*					19
Sceloporus graciosus		8.2*					19
Sceloporus jarrovii		6.1*					19
Sceloporus occidentalis		7.1*					19
Sceloporus poinsettii		7.5*					19
Sceloporus undulatus	7.2*		3.0	**141**			19,20
Uta *stansburiana*	35	6.5*					19,101
Cordylus cataphractus						1	20
Uromastyx	4.6			**120**			97,104
Lacerta muralis		9.0					2,74
Lacerta viridis			4.6				65
Chamaeleo Chamaeleo				*173*	3		20
Eumeces fasciatus		*3.0*		**107**			20
Eumeces obsoletus		9.4*		**112**			18,67,68
Anguis fragilis		**11.3**					2,29,74
Cnemidophorus sexlineatus				93			20
Cnemidophorus tigris		7.2*					19
Cnemidophorus sackii		8·7*					19

TABLE II—*continued*

Packed Cell Volume and Certain Organic Constituents of Blood

Species	Packed Cell Volume %	Hemoglobin g%	Total Plasma Protein g%	Glucose mg%	Urea mg%	Uric mg%	Source-a
			SQUAMATA (Sauria)—*continued*				
Tupinambis nigropunctatus			7.8		2		20,79
Tupinambis teguixin				104			72
Gerrhonotus multicarinatus		7.2 *	4.5				19,20
Ophisaurus ventralis	31	6.9	5.4				20
Heloderma horridum	30	8.0		45	3		94
Heloderma suspectum	26	8.1*	6.3	109	1		20,31,76
Varanus	27		6.9	106	2		20,40,41,60,97,103
			SQUAMATA (Ophidia)				
Boa constrictor	29		6.5	70			9,102
Lichanura roseofusca				73	6	1	20,79
Epicrates cenchria				93			9
Eryx johnii				25			97
Coluber constrictor	26		5'0	75	4	6	11,12,13,20,48
Coluber florulentus	26		6.2				60
Heterodon platyrhinos			4.5	52	5		20
Lampropeltis getulus	23		5.8	59	2	6	12,48,76,77,79
Rhinocheilus lecontei				88	2		20
Coluber viridiflavus				65			78
Xenodon merremii				55			47
Natrix cyclopion		6.5		65	5		20,39
Natrix natrix	37		4.3	57			69,80
Natrix tessellatus	33		6.2				59,60
Natrix rhombifera			5.5	30	5		20
Natrix sipedon	23		5.7	49	3	6	12,13,17,20,22,23, 4S
Philodryas				63			73
Thamnophis sirtalis	33	8.1	4.5		5		11,12,20,22,79
Thamnophis elegans	25		4.0				20,22
Thamnophis sauritus	30		4.4				20,22
Farancia abacura		7.5	4.9				20,39,79
Storeria dekayi		10.8	3.5				20,39
Micrurus nigrocinctus				107			9
Naja naja			4.4	29			12,13,79,97
Agkistrodon contortrix	28		5.2				20
Agkistrodon piscivorus	19		4.6	52	5	6	12,13,20,23,48,76, 79
Bothrops atrox				60			9;73
Crotalus atrox			5.2	60	1	2	20,23,64,79
Crotalus horridus	45	8.6	3.5	60	11	3	12,23,79
Crotalus ruber			5.2	70			9,12,13,20
Crotalus viridis			2.9	48	0	2	12,13,20
Vipera aspis		10.5	5.5	40	10	4	1,28,33
Vipera				34			88,97
Typhlops				84	1		20
			CROCODILIA				
Alligator mississippiensis	20	7.1*	5.1	74	0	3	5, 7, 16, 25, 46, 102
Caiman	26	8.6*	5.9				20,24,79,102
Crocodylus niloticus	35		6.5				60
Crocodylus acutus	26	9·0*		101			9,24,102

*Calculated from oxygen capacity measurement: values based on oxygen capacity were given preference in tabulation.

[a] Numbers designate reference source given in key below:

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TABLE II—*continued*

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103. Menon, 1952
104. Khalil and Yanni, 1959

Osmolarity of blood of crocodiles and fresh water turtles most commonly is equal to or slightly less than that of human blood, about 290 mOs/liter. Sodium, chloride, and bicarbonate ions account for over 85 per cent of the osmotically active components in plasma of all reptiles. Sodium, contributing about 90 per cent of the cations, is high in lizards, snakes, and marine turtles. Chloride plus bicarbonate contribute 80 to 90 per cent of the anions. Chloride levels above 115 mEq/liter typify lizards and snakes; other reptiles have lower levels. Bicarbonate is generally high in turtles, making up about a third of the total anions; it contributes only 10 to 15 per cent of the anions in other reptiles. Carbonic anhydrase is involved in renal aspects of the control of anion levels in the alligator (Hernandez and Coulson, 1954; Coulson and Hernandez, 1957). Curiously, the alligator produces an alkaline urine, conserving both sodium and chloride of plasma by excreting high concentrations of ammonium bicarbonate (Coulson and Hernandez, 1955). Blood pH of turtles is relatively alkaline, paralleling the elevated bicarbonate. A pH of 7.8 is common in control animals; blood of other reptiles is more acidic, often with a pH below 7.4. Marked changes in rate and depth of breathing are common features of reptilian behavior. These affect carbon dioxide tension and may lead to sudden changes in blood pH (Stullken *et al.*, 1942; Andersen, 1961; Schmidt-Nielsen *et al.*, 1966). Ionic gradients between cells and plasma are also affected (Collip, 1921b).

Plasma potassium concentration appears to be under relatively stringent control. A level of 3 to 6 mEq/liter characterizes all reptiles; higher values usually indicate analyses on hemolyzed samples. The significance of the low potassium values for the turtles *Chelonia* and *Caretta* is not known. Potassium levels undergo only minor alterations during feeding, temperature changes, osmotic stress, and other episodes in the life cycle of reptiles which drastically alter electrolyte balance. The reptilian kidney is very efficient in clearing potassium from the blood (Smith, 1951; Coulson and Hernandez, 1964; Shoemaker *et al.*, 1966). Nasal glands play an effective role in potassium excretion in the lizards *Ctenosaura pectinata* and *Sauromalus obesus* (Templeton, 1964). The adrenal gland is involved in the control of potassium and sodium levels in the lizards *Trachydosaurus rugosus* and *Agama agama* and a snake *Natrix natrix* (Wright and Jones, 1957; Bentley, 1959).

Magnesium increases during winter torpor in the turtles *Pseudemys* and *Terrapene* (Hutton and Goodnight, 1957) and in the lizard *Varanus griseus (Haggag et al.*, 1965). Total plasma calcium and magnesium attain remarkably high levels during estrus (see Sect. III C, Part 6), but levels of ionic calcium and magnesium may not undergo great change (Grollman, 1927) as most of the alkaline earths appear to be protein bound (Dessauer and Fox, 1959). Large fluctuations in ionic calcium or potassium seriously affect cell permeability (Maizels, 1956; Lockwood, 1961) and cardiac rhythms (Mullen,

1962). However, the lizard *Ctenosaura pectinata* survived an injection of potassium which tripled its usual plasma level (Templeton, 1964).

b. *Temperature and electrolytes.* Body temperature has a major role in the control of fluid and electrolyte balance in reptiles; the physical properties of electrolytes in solution and also the metabolic rate of the organisms are altered by temperature change. Carbon dioxide production and its hydration, solubility, and dissociation are all affected (Edsall and Wyman, 1958). Temperature changes can drastically affect acid base balance. In both a living turtle *Pseudemys scripta* and in an *in vitro* sample of its plasma, a rise in temperature results in an increase in carbon dioxide tension and a decrease in pH; bicarbonate remains constant in the *in vitro* system but rises in the intact animal (Robin, 1962; see also Dontcheff and Kayser, 1937; Gordon and Frankel, 1963; Frankel *et al,* 1966). Similar responses have been observed in crocodiles (Austin *et al.,* 1927) and lizards (Edwards and Dill, 1935; Tucker, 1966). Blood pH dropped as low as 6.75 in a chuckwalla, *Sauromalus obesus,* maintained at 38°C (Dill *et al.,* 1935). Acid base changes noted in reptiles during winter torpor may result from low temperature (Grundhauser, 1960; Haggag *et al.,* 1965).

Inhibition of ion-transport at low temperatures has the overall effect of lowering plasma sodium. The emydine turtles *Chrysemys picta* and *Pseudemys scripta* often incur hemodilution during cold torpor (Musacchia and Sievers, 1956; Musacchia and Grundhauser, 1958; Hutton, 1960). The softshell turtle *Trionyx spiniferus* may lose over half of its plasma sodium during winter because active sodium transport by pharyngeal villi is suppressed by cold (Dunson and Weymouth, 1965). Short term cold exposure of the snake *Natrix* and the lizard *Anolis* leads to a decrease in both sodium and potassium (Munday and Blane, 1961). Contributing to such changes is the shift of sodium into intracellular fluids (Maizels, 1956) and probably losses of sodium through the kidney whose tubules lose their capacity for reabsorbing sodium when cooled (Hernandez and Coulson, 1957; Coulson and Hernandez, 1964). In the Greek tortoise *Testudo hermanni,* however, osmotic pressure and plasma sodium and chloride attain maximum values during the winter (Gilles-Baillien and Schoffeniels, 1965).

c. *Water and salt availability.* Reptiles tolerate marked extremes of hydration. Regulation of plasma Osmolarity seems less critical than in many other vertebrates. The range of variation among 7 specimens of a tortoise (species not given), 278 to 400 mOs/liter, was three times greater than ranges observed in a frog and a number of birds and mammals (Aldred, 1940). Sea turtles *Chelonia mydas* (Holmes and McBean, 1964) and *Caretta Caretta* are also able to live in fresh water. The osmotic pressure of *Caretta Caretta,* accidently maintained in fresh water for three years, dropped from above 350 mOs/liter to 211 mOs/liter. The change chiefly involved a marked

decrease in sodium and chloride (Tercafs *et al.,* 1963). A turtle of estuarian ecology, *Malaclemys terrapin centrata,* survived an experiment in which it was maintained for 14 days in 3.3% saline followed by 10 days in 6.6% saline - a solution of twice the Osmolarity of sea water. Plasma sodium rose to about 170 mEq/liter in the hypertonie environment. The land turtle *Terrapene carolina* survived only 4 days in the 3.3% saline (Bentley *et al.,* 1967). Some regulation of salt content occurs, however, since blood Osmolarity of species that survive such treatment remains very different from that of either sea or fresh water.

Some lizards and probably also snakes have high tolerance to hypernatremia. In desert dwelling agamid lizards blood osmotic pressure during dehydration may exceed 400 mOs/liter (Tercafs and Vassas, 1967). *Trachydosaurus rugosus,* a lizard of the Australian desert, usually has 150 mEq/liter of plasma sodium. During the summer water loss through the kidney is nil, but sodium rises to an average 195 mEq/liter as a result of evaporation. In their arid habitat *Trachydosaurus* apparently "conserves body water at the expense of abandoning maintenance of body fluid composition" (Bentley, 1959). Similar changes occur in *Amphibolurus ornatus* (Bradshaw and Shoemaker, 1967). During periods of water excess the lizard *Varanus griseus* stores water in its tissues (Khalil and Abdel-Messeih, 1954, 1959a and b, 1961). Subspecies of the water snake *Natrix sipedon* adapted to brackish water habitat avoid drinking salty water (Pettus, 1958). Widely different values for plasma sodium are often reported for the same species of lizard or snake, e.g. for *Lampropeltis getulus* of 124 mEq/liter (Hutton and Goodnight, 1957) and 165 mEq/liter (Dittmer, 1961).

In maintaining the osmotic pressure of the blood, the kidney is of prime importance (Smith, 1932, 1951), but specialized accessory organs contribute to the control. Fresh water turtles lack sodium ion in their environment. Their kidneys conserve sodium by excreting a dilute urine low in sodium. Specialized cells in the pharynx of *Trionyx spiniferus* contribute to salt stores by absorbing sodium from "fresh water" which contains as little as 5 mM/liter (Dunson and Weymouth, 1965). The kidney of reptiles cannot produce a urine of higher Osmolarity than blood. Turtles living in marine habitats, *Caretta Caretta* and *Malaclemys terrapin,* maintain the osmotic pressure of their blood lower than that of sea water by excreting concentrated salt solutions through orbital salt glands. In the marine iguana *Amblyrhynchus cristatus* a nasal salt gland performs this function (Schmidt-Nielsen and Fänge, 1958; Schmidt-Nielsen, 1962-1963). The sea snake *Laticauda semifasciata* maintains an osmotic pressure of between 290 and 350 mOs/liter by extrarenal salt excretion through a gland lying in the roof of its mouth (Dunson and Taub, 1967).

d. *Excitement and feeding.* No other group of animals is known to undergo

the drastic shifts in blood pH and anion distribution that occur in reptiles during daily physiological events. Moderate excitement can lead to falls in pH which could be considered severe acidosis in a mammal. A drop in pH follows a rise in lactic acid; the lactate produced in mild excitement displaces, equivalent for equivalent, bicarbonate ions in the extracellular fluids. Periods of apnea may contribute to the fall in pH (Schmidt-Nielsen *et al.,* 1966). Handling, marked sudden changes in temperature (Austin *et al,* 1927), and anesthesia will induce such responses. Bicarbonate levels of snakes anesthetized with ether may drop to 4 or 5 mEq/liter and blood pH may fall to 6.7 (Dessauer, unpublished). In alligators injected with epinephrine to magnify the response, lactic acid rose to 30 mEq/liter and the pH dropped as low as 6.54. Early in the experiment the rise in lactate was matched by an equivalent fall in bicarbonate; later, lactate production was buffered by the release of sodium from intracellular spaces (Hernandez and Coulson, 1956, 1958; Coulson and Hernandez, 1964). Anaerobic glycolysis, which often provides most of the energy during activity, contributes high concentrations of lactate to the blood. The return of lactate to the resting level is most rapid in lizards near their preferred body temperature (Moberly, 1968a).

All reptiles become acidemic when excited, but with alligators "the simple act of eating forces them into an equally pronounced alkalosis" (Coulson and Hernandez, 1964). Soon after a meal a shift in the anion distribution begins. Plasma chloride is slowly replaced, equivalent for equivalent, by bicarbonate. Total osmotic pressure and sodium and potassium concentration remains almost constant (Fig. 1). In one alligator plasma chloride dropped to 7 mEq/liter and bicarbonate rose to 105 mEq/liter. The maximum blood pH observed

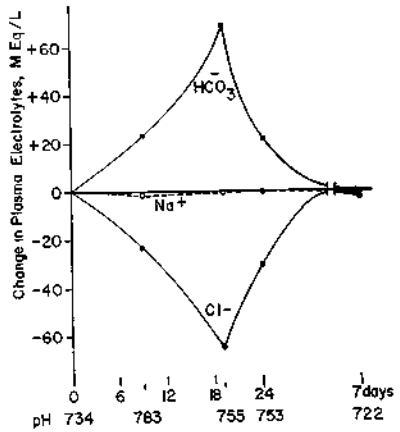


FIG. 1. Electrolyte changes in blood plasma of an alligator *(A. mississippiensis)* following feeding. Graph illustrates the reciprocal nature of the exchange of chloride for bicarbonate and the reverse exchange after the 18th hour of the experiment (from Coulson and Hernandez, 1964).

following feeding was 8.09. Bicarbonate rises for about 18 hours after which the reverse exchange commences. Ingestion of food apparently stimulates the cells of the stomach to secrete gastric juice containing copious quantities of hydrochloric acid. Chloride is transferred from the blood with hydrogen ions produced by the metabolic activities of stomach cells. Bicarbonate, another product of the cells, replaces the chloride in the blood (Coulson *et al.,* 1950b). The lizards *Iguana iguana* and *Ctenosaura acanthura* do not exhibit such a marked "alkaline tide" (Hernandez and Coulson, 1951). Although feeding leads to the secretion of hydrochloric acid into the stomach of *Chrysemys picta* (Fox and Musacchia, 1959), the effect of feeding on blood electrolytes of turtles is not known.

e. *Diving and burrowing.* Diving and burrowing reptiles are often exposed to oxygen lack for prolonged periods (Andersen, 1966). Crocodiles, lizards, and snakes are usually able to survive without oxygen about 45 minutes at 22°C. The tolerance of turtles to anoxia is remarkable, exceeding that of all other tetrapod vertebrates. Turtles of all families except the Cheloniidae (sea turtles) commonly survive without oxygen at 22°C for about 12 hours; sea turtles are less tolerant, having an average survival time of 2 hours (Belkin, 1963, 1965). In the laboratory some *Chrysemys picta* have lived for 3 to 4 months submerged at 1.5 to 3.5°C. Many turtles probably spend the entire winter under water (Musacchia, 1959).

Blood composition changes drastically during a dive, but the patterns of changes are different in land and fresh water turtles than in other reptiles. In 1933 Johlin and Moreland found that a fresh water turtle (species not given) would survive over 24 hours in an atmosphere of pure nitrogen or carbon monoxide even though its blood pH fell to 6.8 and lactate rose to a reported 110 mEq/liter. Bellamy and Petersen (1968) have noticed similar responses in *Testudo* and *Podocnemis.* Robin and coworkers (1964) have followed the course of blood changes in such turtles during an extended submergence (Fig. 2). Soon after *Pseudemys scripta* submerge, blood oxygen tension drops to near zero where it remains throughout the dive; carbon dioxide tension increases to about 100 to 150 mm of mercury, reaching a plateau after 24 hours. Blood pH falls steadily to about 6.8 and then decreases more slowly. Lactate rises throughout submersion; increases of 50 mEq/liter occur in 24 hours. Nothing is known of the fate of other electrolytes during the dive or of the pattern of blood changes which occur after the turtle emerges from the water.

A number of adaptations make possible the remarkable diving capability of turtles. Bucco-pharyngeal and cloacal absorption contribute minor quantities of oxygen (Root, 1949; Girgis, 1961; Musacchia and Chládek 1961; Belkin, 1962; Robin *et al.,* 1964). The heart beats more slowly (Belkin, 1964) and a functional ventricular shunt allows blood to bypass the lungs (Millen *et al,*

1964). Their energy requirement under water may be less than in air, allowing glycolysis and other anaerobic pathways to meet their needs (Belkin, 1963a, 1968; Robin *et al,* 1964). Stores of glycogen in tissues of turtles

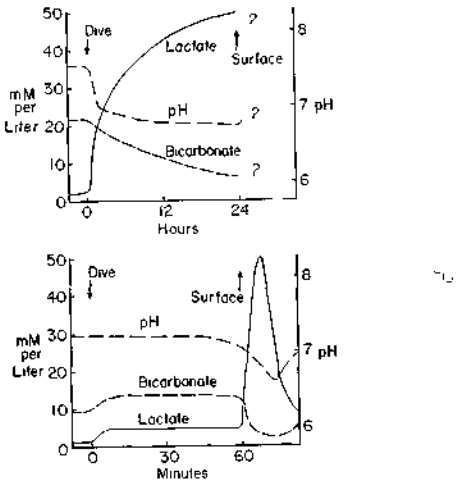


FIG. 2. Electrolyte changes in blood plasma of reptiles following a dive. Upper curves: time sequence of changes in blood of the turtle *Pseudemys scripta* (plotted from data of Robin *et al.,* 1964). Lower curves: time sequence of blood changes in *Alligator miisissippienus;* note difference in time scale from upper graph (plotted from data of Andersen, 1961).

(Issekutz and Végh, 1928; Daw *et al,* 1967) exceed those of other reptiles (Dessauer, 1953; Agid *et al,* 1961b; Coulson and Hernandez, 1964). Although pyruvate is a receptor for the hydrogen produced by glycolysis, other acceptors probably are involved which yield products causing less disturbance of tissue pH than lactic acid (Belkin, 1963a). The disulfide-sulfhydryl interchange involved in polymerization of turtle hemoglobins may represent such an acceptor system (see Section IV B below). Hydrogen ions generated during the dive are partially buffered by bicarbonate of plasma and probably by pools of coelomic fluids. Fresh water and land turtles store large volumes of such extravascular, extracellular fluids. These contain high concentrations of sodium bicarbonate (Smith, 1929; Thorson, 1963; Robin *et al,* 1964).

Adaptations to diving in alligators, lizards, snakes, and, to some extent, sea turtles are similar to those of diving birds and mammals: (1) marked bradycardia with decreased cardiac output, and (2) vasoconstriction, preventing blood from circulating through muscle beds during the dive (Andersen, 1961, 1966; Murdaugh and Jackson, 1962; Murphy *et al,* 1964; Berkson, 1966). Andersen (1961) has analyzed certain blood constituents of alligators during and after emergence from a dive (Fig. 2). In contrast to

Pseudemys scripta, blood changes are relatively slight in the alligator during submergence. The pH of the blood and levels of bicarbonate and lactate do not change as excessively. The animals apparently remain submerged only as long as some oxygen is present in circulating blood. When the animal surfaces, the vascular beds of the muscles dilate and the lactate produced during submersion diffuses into the blood. The profuse influx of lactate causes a sharp drop in pH and a marked decrease in bicarbonate. Within an hour blood levels adjust to predive values. The lizard *Iguana iguana* (Moberly, 1968b) and the water snakes, *Natrix cyclopion* and *N. sipedon,* undergo similar changes during a dive (Murdaugh and Jackson, 1962). Sea turtles *Chelonia mydas* do not exhibit large volumes of interstitial fluids (Smith, 1929; Thorson, 1963) and seem to respond to oxygen lack more like the alligator than like other turtles (Berkson, 1966). Most such experiments involve animals forced to dive. The demonstration that changes in physiological parameters may involve both submergence and withdrawal components (Gaunt and Gans, 1969) suggests the merit of partitioning as well as the observed changes in the blood.

2. *Organic Components*

Blood plasma of reptiles contains a large variety of organic compounds of low molecular weight. Some, such as glucose, are present in moderate concentration but many occur only in trace amounts. Daily events in the life of a reptile as well as seasonal metabolic cycles influence the blood levels of a number of these substances.

a. *Glucose.* Representative levels of one major metabolite, glucose, appear in Table II. The majority of these values represent total reducing substance and give somewhat high estimates of glucose. The data of Coulson and Hernandez (1964) on *Alligator mississippiensis* and that of Zain-ul-Abedin and Qazi (1965) on a variety of turtles, snakes, and lizards were obtained by means of the glucose oxidase method, a technique of high specificity. Glucose levels in lizards are consistently higher than in other reptiles. Values exceeding 150 mg% are commonly observed, especially among the Iguanidae. Among other vertebrates only birds have such elevated blood sugars (Miller, 1961). Most of the reducing substance of lizards is probably glucose; only 27 mg% of reducing substance in *Anolis carolinensis* was not fermentable (Dessauer, 1953). Analyses for glucose are commonly carried out on blood rather than plasma, but glucose is largely absent from red blood cells. Cells of the snapping turtle (McCay, 1931; Andreen-Svedberg, 1933) and the American alligator (Coulson and Hernandez, 1964) do not contain glucose.

Physiological events are accompanied by appreciable fluctuations in blood sugar in all major groups of reptiles. A rise in temperature leads to an increase in glucose in alligators (Austin *et al,* 1927) and in the snake *Vipera aspis*

(Agid *et al,* 1961b), but a fall in glucose in the turtle *Emys orbicularis* (Vlädescu, 1964). Glucose rises sharply in slider turtles (*Pseudemys)* subjected to sudden drops in temperature (Hutton, 1964). Crocodiles and snakes (Britton and Kline, 1939) exhibit an emotional hyperglycemia of between 10 and 25 per cent. During extended dives the fresh water turtles *Pseudemys scripta* and *Chrysemys picta* utilize anaerobic glycolysis as a source of energy and their blood glucose increases markedly (Robin *et al,* 1964; Daw *et al.,* 1967). Similarly, after 24 hours in an atmosphere of nitrogen or carbon monoxide, glucose rises to as high as 1200 mg% (Johlin and Moreland, 1933). Neither force-feeding meat to the snake *Bothrops jararaca* (Prado, 1946b) nor fasting the adder *Vipera aspis* (Agid *et al.,* 1961a, b; Duguy, 1962) or the house snake *Natrix natrix* (Vlädescu and Baltac, 1967) will cause an appreciable change in blood sugar. Fasting the turtle *Chrysemys picta* (Rapatz and Musacchia, 1957; see also Vlädescu, 1965d) for six to eight weeks results in a drop in glucose from 79 mg% to 49 mg%.

Glucose given by mouth is rapidly absorbed, producing hyperglycemia in turtles (Corréa *et al.,* 1960), snakes (Vlädescu, 1964), and other reptiles. Glucose apparently distributes throughout the extracellular fluid, so that the magnitude of the rise depends upon the volume of extracellular fluid as well as upon the quantity of glucose fed (Coulson and Hernandez, 1953). The rate at which glucose returns to control levels depends upon metabolic rate and thus upon temperature. Such observations, analogous to glucose tolerance studies in mammals, have been made on turtles (Lopes *et al.,* 1954; Vlädescu, 1964, 1965d), alligators (Coulson and Hernandez, 1953), lizards (DiMaggio and Dessauer, 1963; Vlädescu, 1965c; Vlädescu *et al.,* 1967),

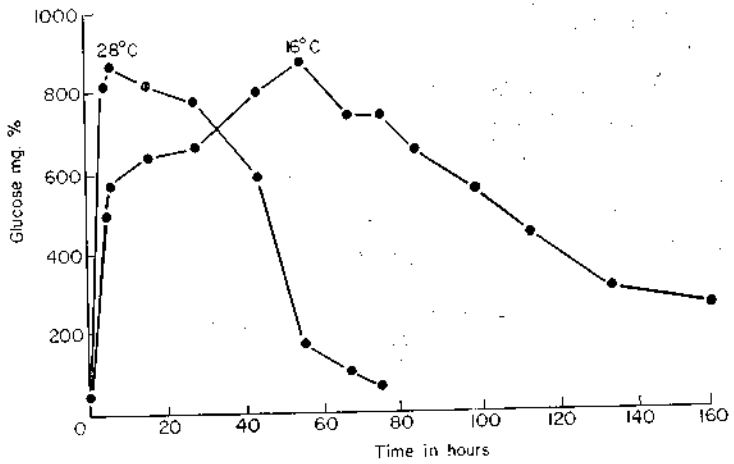


FIG. 3. Effect of temperature on blood glucose utilization in *Alligator mississippiensis* (from Coulson **and** Hernandez, 1964).

and snakes (Prado, 1946b; Vlädescu and Baltac, 1967). In *Alligator mississippiensis* glucose decreased three times faster at 20°C than at 16°C (Fig. 3; Coulson and Hernandez, 1953, 1964). A turtle *Emys orbicularis* utilized glucose more rapidly at its optimal temperature of 20°C than at either 10° or 32°C (Vlädescu, 1964).

The concentration of glucose in the blood varies with the season in a number of reptiles (Fig. 4). Of the species examined only the turtles *Emys*

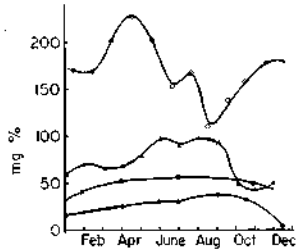


FIG. 4. Seasonal variation in blood glucose levels of fasted reptiles. Open circles = the lizard *Anolis carolinensis* (from Dessauer, 1953); closed circles = the snake *Vipera aspis* (from Agid *et al.,* 1961a); triangles = *Alligator mississippiensis* (from Coulson and Hernandez, 1964); squares = the turtle *Emys orbicularis* (from Vlädescu, 1964).

orbicularis (Vlädescu, 1964) and *Pseudemys scripta* (Hutton, 1960) failed to exhibit a seasonal cycle in blood sugar. The most rapid fall in glucose level in the lizard *Anolis carolinensis* occurs in later summer when the species begins to store fat and glycogen in its tissues (Dessauer, 1953, 1955). In the lizard *Varanus griseus* glucose averages 95 mg% during the summer when the animals are active and 36 mg% during winter torpor (Haggag *et al.,* 1966). The lowest blood glucose levels in alligators (Hopping, 1923; Coulson *et al.,* 1950a; Hernandez and Coulson, 1952; Coulson and Hernandez, 1964), and in snakes (Agid *et al.,* 1961b; Duguy, 1962) occur in autumn or early winter. Seasonal cycles in metabolic controls, rhythms inherent or related to photoperiod, are responsible in part for such differences (Bartholomew, 1959). Even when maintained at constant summer temperature, *Anolis carolinensis* (Fig. 5; DiMaggio and Dessauer, 1963) and *Alligator mississippiensis* (Coulson and Hernandez, 1964) remove glucose from the blood more rapidly in spring and summer than in winter (see Barwick and Bryant, 1966).

The control of blood sugar levels of reptiles is only poorly understood (Vlädescu, 1967), but it appears to be somewhat different in Squamata than in Testudines and Crocodilia. Small quantities of bovine insulin lead to marked decreases in glucose in alligators (Coulson and Hernandez, 1953, 1964; Stevenson *et al,* 1957) and turtles (Issekutz and Végh, 1928; Lopes et *al,* 1954). Insulin is present in the plasma *of Chrysemys* (Marques, 1967) and reaches its highest level in the turtle pancreas in winter when blood

glucose is minimum (Marques and Kraemer, 1968). Bovine insulin, even in large doses, produces only small drops in blood sugar in lizards (Miller and Wurster, 1956, 1958, 1959; Miller, 1961; DiMaggio, 1961, 1961/1962; see

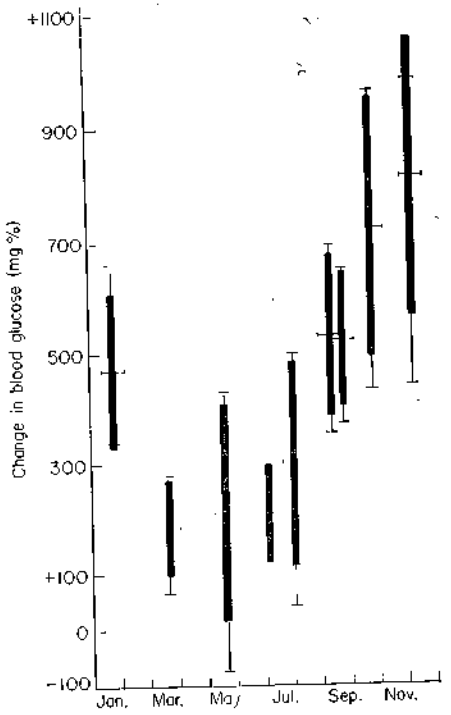


FIG. 5. Effect of season on blood glucose utilization in the lizard *Anolis carolinensis,* illustrating the presence of an annual metabolic rhythm. Each bar graph shows differences in blood glucose levels of lizards one day after receiving a standard amount of glucose. Horizontal I = mean; vertical I = range; vertical bar indicates standard deviation (from DiMaggio and Dessauer, 1963).

also Vlădescu *et al,* 1967, and Vlădescu and Motelica, 1965) and snakes (Prado, 1947; Saviano, 1947a,b; Saviano and Francisis, 1948). Beta cells are sparse in pancreatic islets of lizards such as *Eumeces* (Miller, 1960).

Marked hyperglycemia is produced in reptiles just as in mammals by injections of glucagon (Coulson and Hernandez, 1953; Stevenson *et al,* 1957; Miller and Wurster, 1959; Miller, 1961; DiMaggio, 1961, 1961/1962; Marques, 1967). The initial rise in glucose level commonly observed following injections of many insulin preparations into reptiles seems to be largely, but not entirely, due to glucagon contamination (Coulson and Hernandez, 1953, 1964). Epinephrinc (Prado, 1947; Lopes *et al.,* 1954; Stevenson *et al,* 1957; DiMaggio, 1961, 1961/1962), bovine somatotropin (Marques, 1955b; Stevenson *et al,* 1957; DiMaggio, 1961,1961/1962; Coulson and Hernandez,

1964), and hydrocortisone (Coulson and Hernandez, 1953, 1964; DiMaggio, 1961, 1961/1962; Vlădescu *et al,* 1967) also produce hyperglycemia. Xanthine derivatives such as caffeine cause a rise in glucose and have pronounced sympathomimetic effects in alligators (Hernandez and Coulson, 1956).

Intense hyperglycemia followed pancreatectomy in the turtle *Phrynops geoffroanus hilarii* (Foglia *et al,* 1955; Marques, 1955a) and alloxan injections in the turtle *Pseudemys d'orbigni* (Lopes, 1955). Pancreatectomy or alloxan injections caused only mild hypoglycemia in the lizard *Eumeces obsoletus* (Miller and Wurster, 1959). Hypophysectomy caused a marked decrease in the blood sugar of turtles (Houssay and Biasotti, 1933; Lopes *et al,* 1954; Wagner, 1955; Foglia *et al,* 1955), but led to only a mild hypoglycemia in *Eumeces obsoletus* (Miller and Wurster, 1959).

b. *Amino acids.* Plasma of reptiles contains a complex of small molecular weight, ninhydrin-positive compounds. These separate into about 35 fractions upon ion-exchange chromatography. These fractions contain the nineteen amino acids that are common constituents of proteins, ornithine, citruline, alpha-amino butyric acid and ten to fifteen unidentified compounds. The latter include cardioactive peptides in the turtles *Chelodina longicollis* and *Pseudemys scripta,* the lizard *Tiliqua scincoides,* and the crocodilian *Alligator mississippiensis.* They reach a bradykinin equivalent of 0.2 mg% in

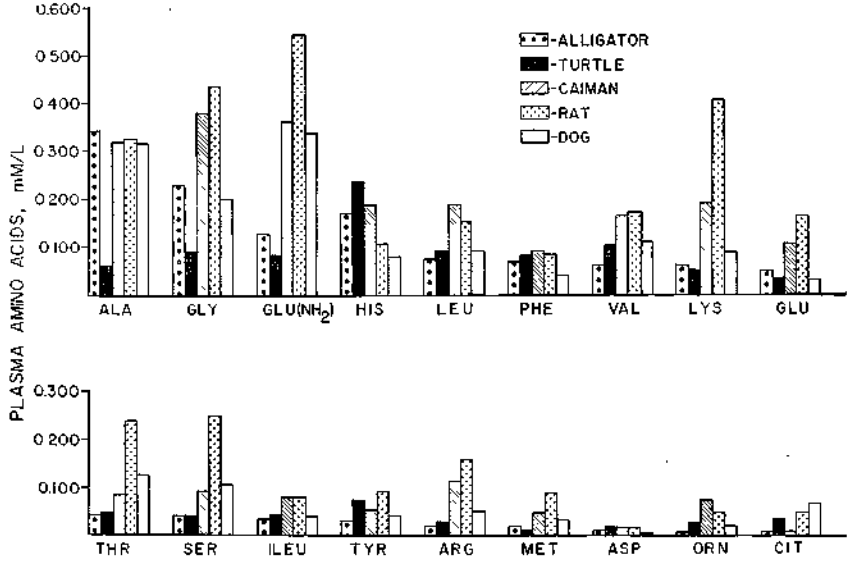


FIG. 6. Amino acid composition of plasma of fasted reptiles and mammals (from Coulson and Hernandez, 1965).

Pseudemys and 0.14 mg% in *Alligator* (Nayler *et al.,* 1965; Erdos *et al.,* 1967).

Amino acid nitrogen, a composite measurement of this complex mixture of substances, ranges from 1 to 10 mM/liter in fasted reptiles (Wiley and Lewis, 1927; Nera, 1925; Carmichael and Petcher, 1945; Khalil, 1947; Nair, 1955a; Hutton, 1958, 1960; Menon and Sathe, 1959; Coulson and Hernandez, 1959, 1961, 1962, 1964, 1965, 1966, 1968; Nair, 1960; Izard *et al,* 1961; Hernandez and Coulson, 1961; Herbert *et al,* 1966). Relative concentrations of specific amino acids vary over 100 fold. Some such as aspartic acid are often barely detectable, but together glycine, alanine, and glutamine make up half of total amino nitrogen. Distributions of amino acids in blood plasma of vertebrate animals show remarkable similarities (Fig. 6).

After feeding, amino nitrogen rises higher and falls more slowly in reptiles

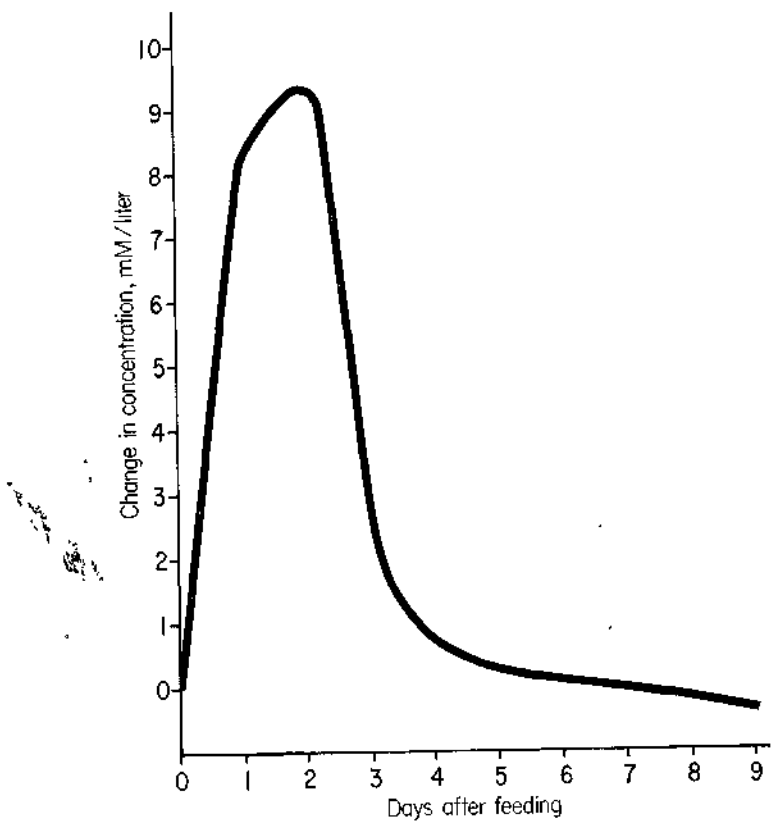


FIG. 7. Effect of feeding on total amino acid content of plasma of individual alligators. Heavy curve = average of 24 alligators (from Coulson and Hernandez, 1965).

than in endothermal animals. Coulson and Hernandez (1964, 1965) have followed the fate of absorbed amino acids in the Crocodilia and to a lesser extent in other reptiles. One to 3 days after alligators consumed 7 per cent of their body weight in fish, total amino acids reached a peak of between 8 and 15 mM/liter in the plasma and did not return to the control level for 5 or 6 days (Fig. 7). Glycine, alanine, and glutamine increased out of proportion to their content in the diet, suggesting their synthesis from other amino acids. Histidine, glutamic acid, and aspartic acid were concentrated in the extracellular fluid, but all other amino acids were distributed throughout body water (Hernandez and Coulson, 1967).

The rate at which each amino acid returns from an elevated to its fasting level has been followed in *Alligator* and in the turtle *Pseudemys scripta.* The concentrations of the majority of those amino acids known to be "essential" nutrients for mammals decrease slowly in the reptilian as in mammalian forms. Only lysine and histidine in the alligator and histidine in the turtle fall more rapidly than might be expected. Glycine, alanine, and glutamine are catabolized most rapidly. They appear to be the transport form of amino acid nitrogen fated for excretion, the major precursors of urinary ammonia, urea and uric acid. The plasma levels of amino acids are affected by insulin and a number of the other hormones that are involved in controlling blood glucose (Coulson and Hernandez, 1964, 1965, 1966, 1968; Hernandez and Coulson, 1961, 1968; Herbert *et al,* 1966).

c. *Ammonia, uric acid and urea.* Representative levels of uric acid and urea, which with ammonia form the major products of protein catabolism in reptiles, appear in Table II. Ammonia is probably present in only trace amounts in the blood (Dessauer, 1952; Hutton and Goodnight, 1957; Coulson and Hernandez, 1964). The larger amounts occasionally reported in the blood (Hopping, 1923; Khalil, 1947; Coulson *et al,* 1950a) may have originated from the decomposition of amide residues of blood proteins.

Uric acid does not normally reach a much higher blood level in reptiles than in mammals in which uric acid is a less important catabolite (Table II). Kidney tubules of reptiles are highly efficient in clearing uric acid from blood (Smith, 1951; Coulson and Hernandez, 1964; Dantzler, 1967). The decreased tubular function at low temperatures, observed in *Alligator mississippiensis* and *Pseudemys scripta* (Hernandez and Coulson, 1957), may explain the high uric acid level observed during winter torpor in a lizard *Varanus griseus* (Haggag *et al,* 1966) and in the turtles *Terrapene carolina* and *Pseudemys scripta* (Hutton and Goodnight, 1957) and *Testudo hermanni* (Gilles-Baillien and Schoffeniels, 1965). Dehydration does not greatly impair uric acid excretion in alligators. The feeding of D-serine, however, causes severe renal damage leading to uric acid concentrations as high as 70 mg%. Uric acid deposits in the tissues, produce a condition resembling gout in

these alligators (Coulson and Hernandez, 1961, 1964). Gout has also been described in turtles and lizards (Appleby and Siller, 1960).

Urea is present in relatively high concentrations in turtles, and low concentrations in lizards and snakes; has not been detected in the blood of crocodilians (Table II). The quantity of urea in the blood of lizards and snakes is small, generally less than 5 mg%, but this value is significant as analyses were by the highly specific urease method. Livers of Squamata lack the full complement of urea-cycle enzymes (Cohen and Brown, 1960), yet they do synthesize some urea. During pregnancy in the viviparous snake *Thamnophis sirtalis* blood urea rises to about 10 mg% due to urea produced by the embryo. Sixty per cent of the nitrogenous waste of the developing snake is urea (Clark, 1953, 1955). If carefully collected from hydrated animals, the urine of adult Squamata also contains urea (Hernandez and Coulson, 1951; Dessauer, 1952). Urea reaches an extremely high level in the spring in the turtle *Testudo hermanni* when the animal is at the end of a season of inactivity (Gilles-Baillien and Schoffeniels, 1965).

d. *Lipids.* Total lipid levels of between 300 and 1670 mg% are reported for reptilian blood (Nera, 1925; Chaikoff and Entenman, 1946; Menon, 1954; Izard *et al.*, 1961). Carotenoid pigments are present in relatively high concentrations in many squamates (see Section III C, 3 d below). Neutral fat accounts for the major share of the total. Fat levels change slowly during starvation and cold torpor in the turtle *Chrysemys picta.* Total fatty acids decreased only 89 mg% in turtles fasted 6 to 8 weeks (Rapatz and Musacchia, 1957). Total cholesterol averages 50 mg% in *Alligator mississippiensis* (Coulson and Hernandez, 1964), 100 to 172 mg% in the blood of rattlesnakes (Luck and Keeler, 1929; Carmichael and Petcher, 1945), and over 220 mg% in the snake *Vipera aspis* (Izard *et al.*, 1961). Averages as low as 69 mg% and as high as 480 mg% are reported in different species of emydine turtles (Chaikoff and Entenman, 1946; Jackson and Legendre, 1967; Stenroos and Bowman, 1968). Approximately half of the cholesterol is esterified in *Chrysemys* and *Vipera.* Total fat, cholesterol, cholesterol esters, and phospholipids are elevated in *Chrysemys* and probably in other reptiles during estrus (See Section III C, 6 below). Phospholipids are elevated in both male and female *Pseudemys* during the spring (Hutton, 1960). Atherosclerosis has been observed in reptiles (Finlayson, 1964).

C. HIGH MOLECULAR WEIGHT COMPONENTS

1. *General*

Three to 7 per cent of blood plasma is comprised of a complex mixture of proteins (Table II). This mixture separates into three or four molecular weight fractions with sedimentation constants in three ranges: (1) 3.3 to 4.8,

(2) 5.8 to 9.6, and (3) 12.2 to 16.2 Svedbergs. A small percentage of heavier components is occasionally present (Svedberg and Andersson, 1938; Baril *et al.*, 1961; Roberts and Seal, 1965). Molecular weights as estimated with molecular sieves (Fig. 8) include fractions (1) between 40,000 and 120,000, (2)

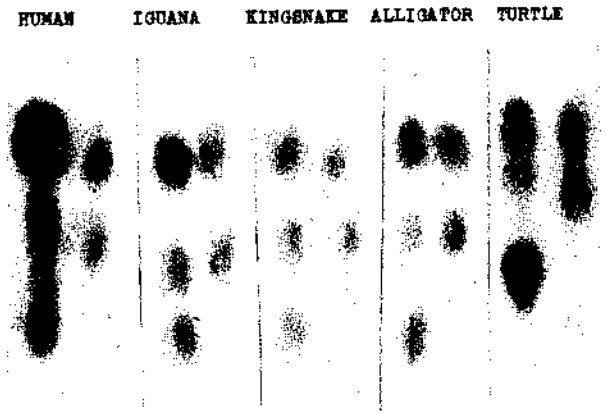
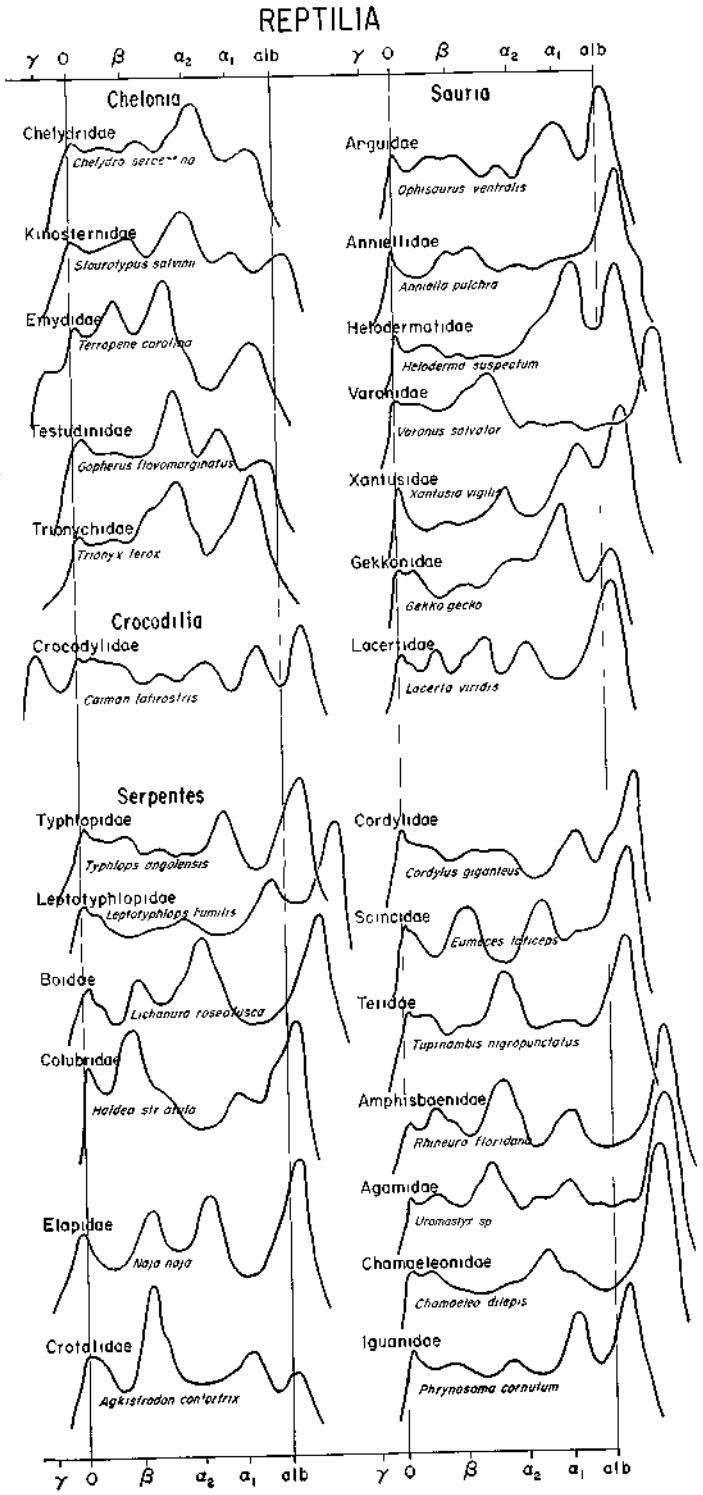


FIG. 8. Molecular weight fractions of plasma proteins of reptiles. For each species, patterns on the left are of unfractionated plasma; patterns on the right are of rivanol soluble proteins. Separation was carried out on thin layers of Sephadex G-200 (Pharmacia Co.). Uppermost spots on plasma patterns are due to proteins of lowest molecular weight, primarily albumin and transferrin; lowermost spots are due to proteins of highest molecular weight, e.g. fibrinogens; intermediate spot on human pattern contains the 7 S gamma-globulins. Uppermost spots of the rivanol soluble proteins represent transferrins; the fraction of intermediate size in the human sample is gamma-globulin; the identity of this protein in reptiles is not known.

between 120,000 and 190,000, and (3) greater than 180,000 (Masat and Dessauer, 1968).

Plasma proteins resolve into 5 to 7 major fractions of different charge density upon electrophoresis in alkaline buffers (Fig. 9). Although mobilities fall within ranges comparable to the albumin, alpha, beta, and gamma fractions of human plasma protein patterns (Seniow, 1963), one should use such designations only as reference standards of mobilities. Proteins migrating in fractions of similar mobility may often have very different structures (Dessauer and Fox, 1964). The extreme heterogeneity of plasma proteins is shown by high resolution techniques such as starch gel electrophoresis (Smithies, 1959) and immunoelectrophoresis (Lewis, 1964; Neužil and Masseyeff, 1958). Proteins of individual reptiles resolve into as many as 30 components. Stage of development (Kaplan, 1960a), sex, season, and physiological state affect the protein complement. Individuals of a population may exhibit inherited differences in specific proteins (Dessauer and Fox, 1964; Masat and Musacchia, 1965; Dessauer, 1966). Hypophysectomy



of the turtle *Clemmys caspica leprosa* leads to an increase in plasma protein and to a relative decrease in albumin (Aron *et al.,* 1959).

Some plasma proteins of reptiles have specialized transport functions; others are involved in the complex immune and coagulation functions of blood. Most are conjugated with either lipid or carbohydrate. Two or three electrophoretic fractions contain lipoproteins (Uriel *et al,* 1957; Crenshaw, 1962; Coulson and Hernandez, 1964). Plasma proteins are conjugated to considerable carbohydrate. Lustig and Ernst (1936) found that an average of 3.0% of the protein mass was carbohydrate in *Testudo,* 3.4% in *Lacerta,* 6.4% in *Anguis,* 6.9% in *Natrix,* 4.5% in *Vipera* and 6.3% in *Elaphe.* The albumin-like component, based upon experiments with concanavalin A, appears to be the only quantitatively important plasma protein that lacks carbohydrate in its structure (Nakamura *et al.,* 1965; Masat and Dessauer, 1968). Plasma proteins of all groups of reptiles contain sialic acid as part of their structure, but those of snakes contain unusual amounts of this carbohydrate (Seal, 1964). A number of varieties of enzymes have been observed in the plasma of reptiles. Very active esterases of a number of varieties are present (Augustinson, 1959, 1961; Dessauer *et al.,* 1962b; Dessauer, 1967; Holmes *et al,* 1968). Leucine aminopeptidase (Ehrensing, 1964; Dessauer, 1967, see also Section V, C 3 below and Fig. 14), arginase (Goodcase *et al,* 1964), phenylenediamine oxidases (See Section 3 c below) trehalase, maltase, (Van Handel, 1968) and proteins such as haptoglobin with peroxidase activities (Liang, 1957) are present in the plasma of some reptiles.

2. *Albumin and Plasma Volume*

The volume of fluid within the circulatory system must be maintained even while water, metabolites, and electrolytes exchange with intracellular material. The delicate balance between hydrostatic and colloid osmotic pressures across capillary walls controls plasma volume (Macfarland and Robb-Smith, 1961). Colloid osmotic pressure in mammals is exerted largely by proteins of low molecular weigh*; and high charge such as albumin which is responsible for 75 per cent of osmotic pressure of human plasma.

The identity of the principal "volume expander" of reptilian plasma has been in question (Cohen and Stickler, 1958). Plasma of reptiles of all major groups, however, contains a protein with properties analogous to human albumin: a low molecular weight, hydrophilic protein of relatively high charge. One fraction of their plasma proteins falls in the molecular weight range of albumins, 50,000 to 100,000 (Svedberg and Andersson, 1938; Roberts

FIG. 9. Electrophotetic patterns of plasma proteins of representative species from different families of Reptilia. Patterns were obtained under comparable conditions. Migrations of human plasma fractions form a frame of reference for comparisons (from Dessauer and Fox, 1964).

and Seal, 1965; Baril *et al,* 1961; Masat and Dessauer, 1968). The low molecular weight fraction of *Pseudemys, Alligator, Natrix,* and *Iguana* resolves into two major subfractions upon electrophoresis, with most of the protein migrating with the subfraction of highest charge density. Protein of this subfraction is very hydrophilic, being soluble in water, 2 M ammonium sulfate, and alcoholic trichloracetic acid. Also like the mammalian albumins it is insoluble in rivanol (Masat and Dessauer, 1966, 1968). Proteins of the most highly charged electrophoretic fractions of plasma of a great variety of turtles are soluble in 2 M ammonium sulfate (Leone and Wilson, 1961; Frair, 1962a and b; Crenshaw, 1962). That of the turtle *Clemmys japonica* has the same isoelectric point as human albumin (Michaelis and Nakashima, 1923).

The contribution of the albumin to plasma volume appears to vary greatly among different reptiles. In *Alligator, Iguana,* and *Lampropeltis* albumin appears to be responsible for at least 50 per cent of the colloid osmotic pressure, whereas its contribution in emydine turtles such as *Pseudemys* is often less than 20 per cent (Masat and Dessauer, 1966). High concentrations of albumins with high charge densities are found in active species with high metabolic rates and in those living in dry, hot environments, e.g. in those lizards which generally have the highest metabolic rates among reptiles (Benedict, 1932; Dessauer, 1953; Dawson, 1960) and high preferred body temperatures (Dawson and Poulson, 1962). Total blood water of desert species seems to relate directly to the amount of albumin in their plasma (Khalil and Abdel-Messeih, 1963). At the other extreme are the fresh water turtles which generally have low concentrations of albumins (Fig. 8) of low charge (Fig. 9; Section V, C 2 below). Probably the "functional ascites" noted in such turtles (Smith, 1929; Thorson, 1963) directly correlate with the low colloid osmotic pressure of their plasma. In *Pseudemys scripta* this was 3 to 8 mm of mercury, only 1 or 2 mm of mercury higher than the pressure of perivisceral fluids (Campbell and Turner, 1937). Pressures were higher for other reptiles equalling 26 mm of mercury for the sea turtle *Caretta,* 23 mm for *Caiman,* 22 mm for *Iguana,* and 24 mm for *Boa constrictor* (Scholander et al., 1968).

3. *Transport Proteins*

a. *Albumin and anion transport.* Mammalian albumins transport fatty acids and buffer a great variety of potentially toxic anions. Practically nothing is known of mechanisms of such transport in reptiles, although they exhibit marked shifts in lipid distribution during estrus (Fig. 11; Section III C, 6 below) and seasonal metabolic cycles (Dessauer, 1955; Hutton and Goodnight, 1957; Rapatz and Musacchia, 1957;Duguy, 1962;Barwick and Bryant, 1966). Albumin may be involved in fatty acid transport in the lizard *Uta*

stansburiana (Hahn, 1965, 1967). Albumins of all major groups of reptiles bind anions, but their binding affinities for dyes such as bromphenol blue are far weaker than those of the albumins of mammals (Masat and Dessauer, 1968). Alligator albumin binds indole-3- propionate and certain other indole derivatives (McMenamy and Watson, 1968).

b. *Transferrins and iron transport.* Transferrins are major constituents of blood plasma in all vertebrates. Together with albumin they make up 95 per cent of the mass of small molecular weight proteins of reptilian plasma. Their molecular weights lie between 70,000 and 90,000 and are similar in reptiles of all major groups (Fig. 8; Masat and Dessauer, 1968). In contrast, their surface charges as indicated by electrophoresis exhibit marked variability (Figs 10 and 14). Electrophoretic mobilities of turtle transferrins are

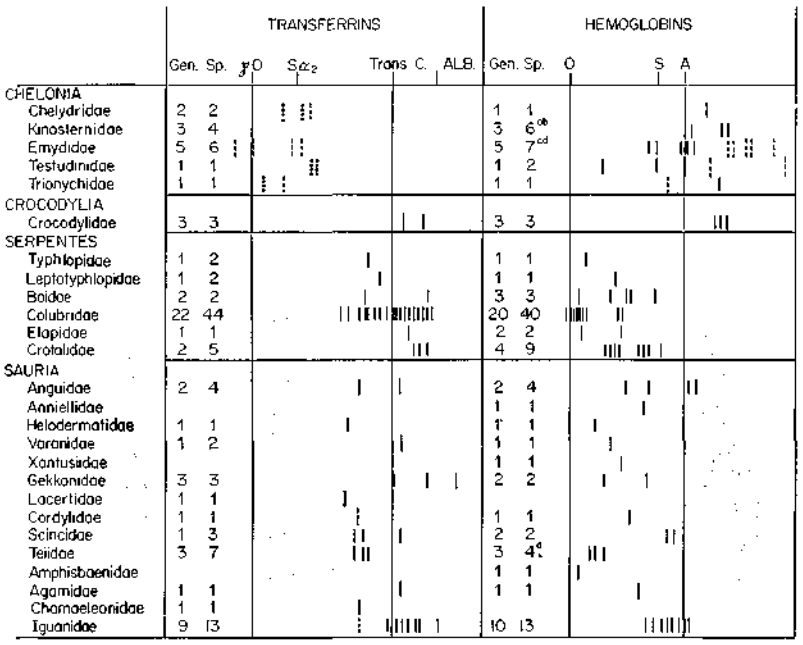


FIG. 10. Relative electrophoretic migrations of transferrins and hemoglobins of species of reptiles. Migration rates of human blood proteins form the frame of reference for comparisons (from Dessauer and Fox, 1964).

as slow as human gamma-globulin, whereas those of some gekonid lizards are as fast as human albumin. Usually one but often two iron-binding proteins are present in the plasma of a single reptilian species. Marked individual variation, both within geographically limited populations and

between populations from different geographic areas, occurs in a number of species (Dessauer *et al.,* 1962a).

By complexing metal ions specialized transport proteins such as transferrin effectively remove the free ions from the blood. Heavy metal ions are generally toxic to living cells. Transferrins of reptilian plasma have considerable iron-binding capacity. Most often less than half of the transferrin molecules are saturated with iron (Dessauer *et al.,* 1962a; Barber and Sheeler, 1961; Sheeler and Barber, 1964). Plasma iron was reduced in starved turtles (*Pseudemys scripta)* to approximately one-third the normal level (Hirschfeld and Gordon, 1965). The two transferrins of *Pseudemys scripta* have different unloading pH's and heat stabilities (Barber and Sheeler, 1963). Transferrins of reptiles are soluble in water, 2 M ammonium sulfate, and rivanol (Fig. 8; Masat and Dessauer, 1966, 1968). They retain their iron-binding capability and electrophoretic mobilities after being stored at deep freeze temperatures for as long as ten years (Dessauer, personal observation).

Transferrins of *Pseudemys scripta* release iron to reticulocytes, demonstrating that they can serve as a source of iron for hemoglobin synthesis. Rat and human transferrin give up their iron readily when exposed to a pH below 6, but some iron remains in equilibrium with transferrins of turtles even at pH 5 (Barber and Sheeler, 1963; Sheeler and Barber, 1965). By binding traces of other metal ions, transferrins may also perform an antioxidant function, e.g. inhibition of lipid peroxide formation (Barber and Sheeler, 1961).

c. *Ceruloplasmin and plasma copper.* The copper binding protein of blood plasma, ceruloplasmin, has been implicated in copper transport, mechanism of iron release to the tissues, and plasma oxidase reactions (Putnam, 1965). Plasma of the Mexican beaded lizard, *Heloderma horridum,* contains 196 mcg% of copper; all but 8 mcg% of this is bound to protein (Zarafonetis and Kalas, 1960; see Beck, 1956). Ceruloplasmin has been assayed in reptiles of all major groups on the basis of its p-phenylene diamine oxidase activity (Seal, 1964). Activity is completely absent from plasma of some species and relatively high in others. The species distribution of activity is almost random. Protein with oxidase activity is present in the fraction with molecular weights between 120,000 and 190,000 (Masat and Dessauer, 1968). Its electrophoretic migration rate is similar in widely divergent species (Dessauer and Fox, 1964).

d. *Hormone and vitamin transport.* A number of substances which are known vitamins or hormones for mammals and birds are transported in combination with specific plasma proteins. Thyroxine binding proteins have been demonstrated in plasma of major groups of reptiles, although their capacity appears to be below that of mammalian forms (Farer *et al.,* 1962). Protein bound iodine of alligators averages less than 0.1 mcg/100 ml of plasma (Coulson and Hernandez, 1964). Transcortin, a corticosterone binding protein, varies

in binding capacity with temperature. Its capacity in the alligator rises from 5 mcg/110 ml of plasma at 6°C to 43 mcg% at 14°C and drops to 8 mcg% at 37°C. Transcortin of the garter snake has been isolated and contains hexose and sialic acid (Seal and Doe, 1963).

A lipoprotein which contains carotenoid pigments is present in plasma of iguanid lizards such as *Iguana iguana* and *Anolis carolinensis.* The protein migrates in the alpha-2 region in electrophoresis (Dessauer, personal observation). Plasma of the lizard *Varanus komodoensis* (Jensen and With, 1939) and *Uromastyx hardwickii* (Zain and Zain-ul-Abedin, 1967) and the snakes *Bothrops jararaca* and *Mastigodryas bifossatus* (Villela and Prado, 1945) contains considerable carotenoid pigment. Riboflavin, both free and protein bound, is present in the remarkably high concentrations of between 165 and 333 mcg% in plasma of these snakes (Villela and Prado, 1944, 1945; Villela, 1945) and *Python molurus* (Villela and Thein, 1967). Proteins to which riboflavin is bound have L-amino acid oxidase activity and migrate as fluorescent bands in the alpha and beta regions during electrophoresis (Ribeiro *et al.,* 1955; Villela *et al.,* 1955). Vitamin B_{12} content and binding capacity of plasma varies widely among animal species. Most of the B_{12} present in blood is part of red cell structure. In 100 ml of blood *of Alligator mississippiensis* 548 millimicrograms are present in the cells but only 4mmcg in the plasma; a nearly equal amount is present in plasma of a turtle. Plasma of alligators can bind an additional 94 mmcg% of B_{12}. The unsaturated B_{12} binding capacity of the turtle *Pseudemys scripta* is much higher, averaging 1·8 mcg% (Couch *et al.,* 1950; Rosenthal and Brown, 1954; Rosenthal and Austin, 1962).

4. *Blood Clotting*

Injury to the vascular system of reptiles is followed by a series of phenomena including vasoconstriction and blood coagulation (Brocq-Rousseu and Roussel, 1939; Engle and Woods, 1960; Macfarland and Robb-Smith, 1961; Gregoire and Taynon, 1962). Proteins involved in blood coagulation appear to be similar to those of mammals, although their concentrations may be very different. Cellular factors seem to have great importance. Plasma, if carefully collected, usually exhibits slow clotting times (Dorst and Mills, 1923; Fantl, 1961; Hackett and Hann, 1967). The addition of traces of tissue fluids, however, greatly accelerates clot formation. Clotting times of blood are often high (Hutton and Goodnight, 1957; Rabalais, 1938). Calcium is required for clot formation. Ion exchange resins (Lund *et al.,* 1957) and agents which precipitate or chelate calcium stop the clotting process (Kaplan, 1956). Heparin is also an effective anticoagulant. A related polysaccharide is present in low concentration in plasma of active *Pseudemys scripta* but rises to 4 to 6 mg% during winter torpor (Jacques and Musacchia, 1961; Jacques, 1963).

Serum expressed from blood clots of mammals and the lizard *Tupinambis nigropunctatus* retains clot promoting activity. Serum of a number of colubrid and viperid snakes, however, exhibits marked anticoagulant activity (Brazil and Vellard, 1928).

Fibrinogen is present in plasma of all major groups and will form a fibrin clot if treated with bovine thrombin. The rate of fibrin formation is slower than for mammalian plasma, presumably because of some species specificity. Fibrinogens of *Chelydra* and *Pseudemys* are less soluble than are those of amphibians but more soluble than those of mammals in plasma-ethanol mixtures (Morrison *et al.,* 1951). Fibrinogen content of plasma has been estimated in a number of forms: *Testudo graeca* = 970 mg%; *Dermochelys coriacea* = 120 mg%; *Alligator mississippiensis* = 380 mg%; *Iguana iguana* = 1200 mg% *Trachydosaurus rugosus* = 140 mg%; *Coluber constrictor* = 115 mg%; *Notechis scutatus* =140 mg%; *Vipera aspis* = 310 mg% (Nera, 1925; Dunlap, 1955; Nair, 1958; Izard *et al,* 1961; Fantl, 1961; Dessauer, personal observations).

Less is known of other clotting factors. Prothrombin level in the turtle *Chrysemys picta* is only 42 per cent of that of dogs (Warner *et al.,* 1939), but its synthesis by the turtle also seems to require vitamin K (Brambel, 1941). Prothrombin concentration is very low in plasma of the tiger snake *Notechis scutatus.* One can isolate prothombin by adsorption on barium sulfate (Hackett and Hann, 1967). The Hageman or surface factor is lacking in the tiger snake, but is present in the lizard *Trachydosaurus rugosus,* the turtles *Chelodina longicollis* (Fantl, 1961), *Pseudemys scripta* and *Chelydra serpentina* and in *Alligator mississippiensis* (Erdos *et al.,* 1967). Proccelerin activity (Ac-globulin) is low in plasma of turtles and chickens but high in mammals (Murphy and Seegers, 1948).

5. *Antibodies, Complements, and Cellular Antigens*

Plasma of reptiles contains proteins that are intimately involved in natural immunity, anaphylaxis, and tissue tolerance (Brocq-Rousseu and Roussel, 1939; Favour, 1958; Putnam, 1960; Hildemann, 1962; Smith et al., 1966). Isoagglutinins are present in numerous turtles (Bond, 1940a; Frair, 1962a, 1962b, 1963), but have not been found in the American alligator (Bond, 1940b) nor in a number of colubrid and crotalid snakes (Bond, 1939). Heteroagglutinins have been discovered in all species examined, including turtles (Bond, 1940a; Frair, 1962a, 1962b, 1963), crocodilians (Bond, 1940b), and snakes (Bond, 1939; Dujarric de la Riviere *et al.,* 1954; Timourian and Dobson, 1962). Autoantibodies in plasma of the Gila monster, *Heloderma suspectum,* can neutralize its venom (Tyler, 1946). Similarly, a plasma anti-phosphatidase of *Vipera aspis* can neutralize the toxicity of its venom (Izard *et al,* 1961). Plasma of the kingsnake *Lampropeltis getulus* protects mice

against a dose of 7 LD_{50} of venom of the water moccasin *Agkistrodon piscivorus* (Philpot and Smith, 1950). Immune hemolytic systems involving complement occur in turtles (Frair, 1963) and are common in snakes. Plasma of numerous snakes will hemolyze mammalian red blood cells (Bond and Sherwood, 1939; Timourian and Dobson, 1962).

Reptiles of all groups respond on initial exposure to a variety of antigens with the production of antibodies (Evans *et al,* 1965); in the light of present knowledge, earlier negative responses can be traced to technical shortcomings (Hildemann, 1962). Alligators (Lerch *et al.,* 1967) have an immunologic memory, that is they will respond to a second exposure to an antigen with a rapid increase in circulating antibody. Circulating antibodies in the carpet snake, *Moreha argus,* react with body fluid antigens of infecting nematodes. The presence of a specific antibody in the plasma correlates with a particular nematode infection (Timourian *et al.,* 1961). The immune response, like many other metabolic activities of reptiles, depends upon temperature (Evans and Cowles, 1959; Hildemann, 1962). The lizards *Dipsosaurus dorsalis* and *Sauromalus obesus* synthesize antibodies against *Salmonella typhosa,* bovine serum albumin, and rabbit gamma-globulin most effectively when the animals are maintained at 35°C. Synthesis is poor in lizards kept at 25°C and at 40°C. If immunized at 25°C and later transferred to 35°C, the lizards form antibodies without further immunization (Evans, 1963a, 1963b). High titers of antibodies resulted in rat snakes and slider turtles maintained at temperatures near the upper end of their temperature range (Frair, 1963). The turtles *Chrysemys* and *Chelydra* maintained at 23 to 28°C developed antibodies against bacteria (Gee, 1941). Schedules of injections must be controlled to obtain effective antibody production and to avoid anaphylaxis (Downs, 1928; Placidi and Placidi, 1960; Hildemann, 1962). Slider turtles appear to vary in their resistance to infection during different periods of the year (Kaplan and Rueff, 1960).

Proteins involved in the immune responses of reptiles have been poorly characterized. Only crocodilians and turtles have electrophoretic fractions which migrate as slowly as mammalian gamma-globulins (Dessauer and Fox, 1964; see Fig. 9). Fractions of slowest mobility in the lizards *Dipsosaurus dorsalis* and *Sauromalus obesus* (Evans, 1963a, 1963b) contain antibodies. Immunoelectrophoretic patterns of antisera from these lizards (Evans, 1963b), alligator, and loggerhead turtle (Lewis, 1964) exhibit precipitin arcs comparable in position and appearance to human gamma-globulin patterns. *Alligator* exhibits at least two immunoglobulins that differ in electrophoretic mobility and perhaps molecular weight (Lerch *et al.,* 1967). Plasma of reptiles of all major groups contains a fraction which, like human gamma-globulin, is soluble in rivanol and is of intermediate molecular weight (Fig. 8). Antibodies of the carpet snake *Morelia argus* (Timourian and Dobson, 1962)

and autoantibodies of the Gila monster (Tyler, 1946) are insoluble in half saturated ammonium sulfate. Complement of turtles (Bond, 1940a; Frair, 1963) and of snakes (Bond and Sherwood, 1939; Timourian and Dobson, 1962) are thermolabile, being destroyed if incubated at 56° C for 5 or 6 minutes. Complement of the carpet snakes is somewhat soluble in half saturated ammonium sulfate (Timourian and Dobson, 1962).

6. *Plasma Vitellin and Estrus*

Striking increases in levels of alkaline earths, phosphorus and lipid fractions, and protein accompany estrus in reptiles, having been noted in turtles (Laskowski, 1936; Chaikoff and Entenman, 1946; Clark, 1967), the lizard *Uta stansburiana* (Hahn, 1965), and numerous snakes (Dessauer *et al.*, 1956; Dessauer and Fox, 1958, 1959, 1964; Izard *et al,* 1961; Jenkins and Simkiss, 1968). Stimulation with estrogens, presumably from the developing ovary, induces the liver to enlarge and to synthesize a calcium-binding, lipophosphoprotein complex known as plasma vitellin. The latter moves via the blood stream to the ovary where it is involved in the synthesis of yolk (Simkiss, 1961, 1967). The rise and fall in vitellin in the plasma correlates with successive changes in follicle composition and with weight cycles of the liver and fat bodies in the ribbon snake, *Thamnophis sauritus* (Fig. 11). Vitellin appears in the blood shortly after the follicles become hydrated but before they begin to accumulate yolk. On the average each 100 ml of plasma contains about a gram of phosphoprotein and an additional 20 mg of calcium throughout the period of yolk production. About the time of ovulation extreme plasma levels occur, e.g. a total protein as high as 8.8 g% and calcium of 360 mg%. By the time eggs attain early cleavage stages, protein, calcium, etc. return to anestrous levels (Dessauer and Fox, 1959).

Such plasma changes can be induced in turtles (Schjeide and Urist, 1960; Urist and Schjeide, 1960/61; Seal, 1964; Clark, 1967; Rao, 1968), lizards (Hahn, 1965, 1967; Suzuki and Prosser, 1968), snakes (Dessauer and Fox, 1959), and crocodiles (Prosser and Suzuki, 1968) by injections of estrogens. Natural and artificial androgens cause, at best, only slight increases in plasma protein (Rao and David, 1967). Five days after treating turtles with estrone total lipid increased 3 fold, calcium 4 fold, and protein 1.5 times. There was an 8 fold rise in protein bound phosphorus. Lipid contained 38% triglyceride, 35% phospholipid, 15% sterol and 12% sterol esters (Urist and Schjeide, 1960/61). Seal (1964) induced a protein rise to 12 g% in a turtle injected daily with 100/mcg of 17-beta-estradiol for six weeks. Clark (1967) needed only 20 mcg of estradiol to induce a significant response in *Chrysemys.* Injections of one mcg/g body weight of estradiol into a lizard were followed within twenty-four hours by the appearance of plasma vitellin; after injections for 4 to 6 days vitellin made up over 60% of the plasma protein (Hahn, 1965 1967)· Rates of increase of calcium and phosphoprotein were equal and almost linear in male ribbon snakes *{Thamnophis sauritus)* injected with estradiol. Plasma and associated tissue changes occurred even though the snakes received no food for at least two weeks before or during the experiment (Dessauer and Fox, 1959). Reptiles may be under some stress to obtain calcium during reproduction (Simkiss, 1961, 1962). For anoles and geckos one source of calcium may be the calcareous material that fills the *saccus endolymphaticus* (Whiteside, 1922; Jenkins and Simkiss, 1968).

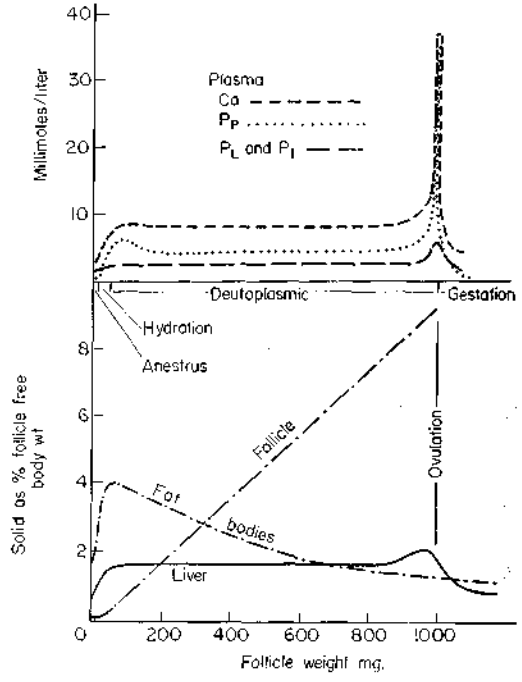


FIG. 11. Correlation of plasma vitellin levels with organ changes during the estrous cycle of the ribbon snake *Thamnophis sauritus.* P_p = protein bound phosphorus, a measure of plasma vitellin concentration; P_T + Pi = lipid plus inorganic phosphorus; Ca = plasma calcium (from Dessauer and Fox, 1959).

Plasma vitellin appears to be a complex colloidal micelle of alkaline earths and lipid stabilized by phosphoprotein. Mere ten fold dilution of plasma with water causes it to precipitate (Laskowski, 1936). Its gross composition is similar to that of yolk. Plasma vitellin of *Thamnophis elegans* contains 43% lipid and 57% protein. The protein fraction contains 1.7% phosphorus; calcium associates with the protein in equimolar quantities to its phosphorus content (Dessauer and Fox, 1959). Vitellin of *Pseudemys scripta* is of a similar gross composition; it sediments in the ultracentrifuge as a single peak with a

sedimentation constant of approximately 17 S (Urist and Schjeide, 1960/61). Calcium sediments with vitellin and not as colloidal calcium phosphate (Schjeide and Urist, 1960). Electrophoretic patterns of plasma proteins of estrous females and those of male or anestrous females treated with estrogens exhibit a vitellin fraction in the alpha-2 or beta-globulin regions (Dessauer and Fox, 1958, 1959,1964; Urist and Schjeide, 1960/61; Hahn, 1965; Suzuki and Prosser, 1968).

IV. Composition of the Red Blood Cells

A. NUMBER, SYNTHESIS AND LONGEVITY

The packed cell volume or hematocrit of reptiles generally falls between 20 and 35 per cent (Table II), although the value may vary with season (Kaplan and Rueff, 1960) and temperature (Musacchia and Sievers, 1956). Fundamental factors which control the number of circulating red cells and erythropoesis are unknown (Pienaar, 1962). Hypoxia and cobalt salts, which are effective stimulants of mammalian erythropoesis, do not induce red cell formation in the box turtle *Terrapene carolina.* Turtles kept for long periods at simulated altitudes over 45,000 feet retain the same hematocrit and hemoglobin levels (Altland and Parker, 1955; Altland and Thompson, 1958). Lizards which live at high elevations have hematocrits and hemoglobin levels similar to those of species of the same genus which live in the lowlands (note data on *Sceloporus* in Table II; Dawson and Poulson, 1962). Likewise hematocrits do not correlate with temperature tolerances of lizards (Dawson and Poulson, 1962). Anemia, whether resulting from phenylhydrazine injection (Tipton, 1934; Sheeler and Barber, 1965) or blood letting (Altland and Thompson, 1958; Hirschfeld and Gordon, 1961, 1965), is the only known stimulant of erythropoesis in turtles and alligators. Maximum reticulocytosis occurs about 3 to 5 weeks following onset of anemia. Folic acid is probably necessary for blood formation in turtles as aminopterin, a folic acid antagonist, inhibits reticulocytosis in *Terrapene carolina* (Altland and Thompson, 1958). Hirschfeld and Gordon (1964) did not find an erythropoesis stimulating factor in the blood of *Pseudemys scripta.* Hypophysectomy of a turtle, *Clemmys caspica leprosa,* did not affect its hematocrit (Aron *et al.,* 1959). Starving of this turtle caused no changes in its hematocrit or hemoglobin levels, but such starved animals appeared incapable of erythropoesis (Hirschfeld and Gordon, 1965).

Red cells of turtles and alligators have exceptionally long life spans. The mean life span of red cells of *Terrapene carolina* is 600 to 800 days (Brace and Altland, 1955; Altland and Brace, 1962). Rate of erythrocyte turnover relates directly to the low metabolic rate of the animal (Altland and Brace, 1962; Rodnan *et al.,* 1957). Mean life span of red cells of *Alligator mississippiensis* is shorter if the animal is maintained at warm rather than at cool temperatures, equaling 300 days at 31°C and exceeding 1320 days at 16 to 17°C (Cline and Waldmann, 1962). Alligator erythrocytes are also very resistant to osmotic and mechanical stress (Cohen *et al.,* 1961; 1964). Hoffert and Fromm (1964) describe the tagging of erythrocytes of *Chrysemys picta* with hexavalent chromium.

B. HEMOGLOBIN AND OXYGEN TRANSPORT

Hemoglobin makes up the greater share of the solid content of the red blood cell. Total solids of red cells of the turtle *Testudo graeca* equal 37 per cent (Nera, 1925). The concentration of hemoglobin in erythrocytes is similar in all vertebrates (Wintrobe, 1934). Most estimates fall between 25 and 32 per cent of net weight in reptiles. Blood contains between 6 and 12 grams % of hemoglobin (Table II). The concentration is directly proportional to packed cell volume, unless large numbers of immature cells are present (Sheeler and Barber, 1965). Methemoglobin is often present in significant quantity. Prado (1946c) found 6 to 28 per cent inactive hemoglobin in the snake *Bothrops jararaca.* Methemoglobin is commonly found in turtles, ranging from 5 to 60 per cent of total heme protein in newly captured *Pseudemys scripta* (Sullivan, 1966; Sullivan and Riggs, 1964). Thus hemoglobin estimates based on measurements of total iron (Menon, 1955; Nair, 1955b) or total heme are often considerably higher than estimates based on oxygen capacity measurements (see Dawson and Poulson, 1962).

Blood of individual reptiles often contains 2 or more hemoglobins, distinguishable by molecular weight, surface charge (Fig. 10), and chemical properties. Hemoglobins with molecular weights approximately twice 68,000 are present along with those of molecular weight 68,000. Svedberg and Hedenius (1934) first discovered these "double hemoglobins" in the turtle *Chrysemys picta,* the lizard *Lacerta vivipara,* and the snake *Coluber constrictor.* Such polymerization is of common occurrence among turtles; apparently it results from sulfhydryl-disulfide exchange reactions (Riggs *et al.,* 1964; Sullivan and Riggs, 1964, 1967a, d; Sullivan, 1966). Two or more hemoglobins of different electrophoretic mobilities are present in most turtles and in many snakes and lizards (Sydenstricker *et al.,* 1956; Dessauer *et al,* 1957; Dessauer and Fox, 1964; Rodnan and Ebaugh, 1957; Nakamura, I960; Crenshaw, 1962; Gorman and Dessauer, 1965; Sullivan and Riggs, 1967b). Both alkali stable and alkali unstable hemoglobins are present in turtles, alligators, and snakes (Ramsey, 1941) and the lizard *Heloderma horridum* (Zarafonetis and Kalas, 1960). The alkali stable and alkali unstable hemoglobins of the turtle *Pseudemys scripta* have been isolated and characterized (Ramirez and Dessauer, 1957; Manwell and Schlesinger, 1966; Sullivan and Riggs, 1967b).

The source of hemoglobin variability resides in the structure of the globin;

the iron porphyrin (heme) probably has the same structure in reptiles as in other vertebrates (Anson *et al,* 1924; Korzhuev and Kruglova, 1957; Ramirez and Dessauer, 1957). Heme is easily separated from globin (Dozy *et al,* 1964; Sullivan, 1966; Sullivan and Riggs, 1967b). In acid buffers globin dissociates into polypeptides which can be separated by electrophoresis. Commonly 2 or 3 but occasionally as many as 6 bands characterize globin patterns of reptiles (Dozy *et al.,* 1964; Sullivan and Riggs, 1967b; Dessauer, 1967).

In light of the remarkable degree of physiochemical variability noted among reptilian hemoglobins, it is surprising how little is known of the structural basis of these differences. Amino acid composition of acid digests of unfractionated hemoglobins appear in Table III. As compared to hemoglobins of fowl, lamprey, and man (Gratzer and Allison, 1960), those of these reptiles have a high content of glycine and glutamic acid and very little proline. Threonine concentration is lower in hemoglobins of *Alligator* and higher in those of *Iguana* and *Clemmys* than in hemoglobins of the other vertebrates. The presence of tryptophane is indicated by tryptophane fine structure band at 290.0 mmcm, of absorption spectra of hemoglobin of the garter

TABLE III

Amino Acid Composition of Reptilian Hemoglobins (Mole %)

Amino Acid Residue	Snapping turtle [a]	Emydid turtle [b]	American alligator [a]	Common iguana [a]	Water snake [a]
Glycine	10.0	5.5	6.3	9.3	10.8
Alanine	5.9	10.0	11.4	10.7	8.1
Serine	5.0	7,4	7,2	2,3	5,7
Threonine		6,2	0,8	6.1	3.6
Proline		3.0	0.6	0.7	0.2
Valine	8.9	9.5	9.2	9·7	8.1
Isoleucine	1.4	3.7	2.2	0.6	1.6
Leucine	**130**	12.6	10,1	12.1	13.0
Phenylalanine	6.2	5.3	7.7	6.2	5.9
Tyrosine	2.2	2.5	2.6	2.6	2.5
Methionine	0.8	0.4	1.8	1.0	0.5
Aspartic acid		7.9	7.7	11.8	9.6
Glutamic acid	12.5	9.0	9.6	7.3	8.9
Arginine	2.6	2.6	4.3	5.0	3.7
Histidine	11.6	7.4	10.8	6.6	9.8
Lysine	9.5	7.2	8.3	7.3	7.4

a Macroclemys temminckii, Alligator mississippiensis, Iguana iguana and *Natrix rhombifera.* Acid digests analyzed by ion-exchange chromatography (courtesy R. A. Coulson).

b Clemmys caspica (= *Emys caspica).* Acid digest analyzed by ion-exchange chromatography (Christomanos and Pavlopulu, 1968; see also Georgatsos, 1960).

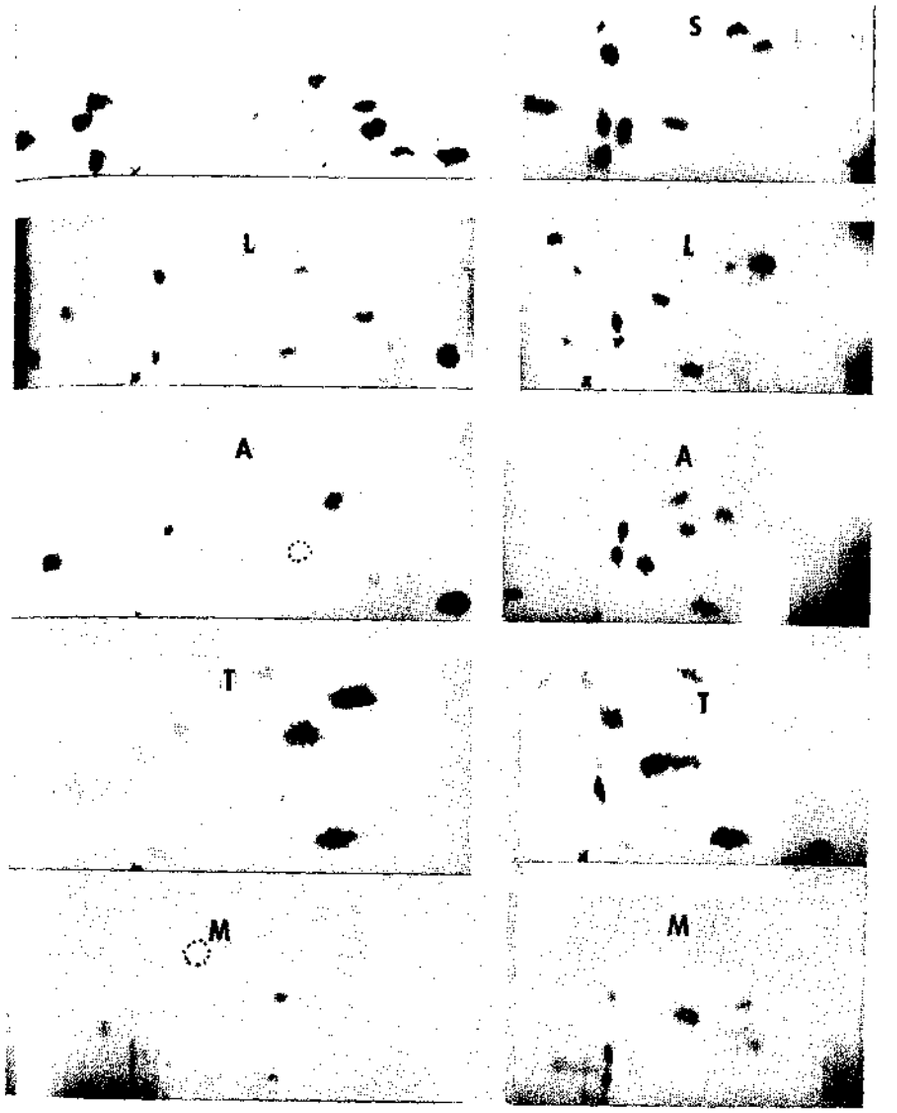


FIG.12. Fingerprints of arginine and histidine peptides of tryptic digests of hemoglobins of representative reptiles. Peptide mixtures were applied at spots marked x, subjected to electrophoresis in the horizontal direction (pyridine-acetic acid buffer, pH 6.4), chromatography in the vertical direction (pyridine: isoamyl alcohol: water, 30:30:35), and then stained to localize peptides. Arginine fingerprints shown on the left were developed with the Sakaguchi reaction; histidine fingerprints on the right were developed with the Pauly reaction. S = the snake *Natrix taxispilota; L* = the lizard *Iguana iguana;* A = *Alligator mississippiensis;* T = the turtle *Macroclemys tern mmckii;* M = *Homo sapiens* (hemoglobin A) (from Sutton and Dessauer, unpublished).

snake, *Thamnophis sirtalis* (Gratzer and Allison, 1960). "Fingerprints" of tryptic digests of hemoglobins of reptiles (Fig. 12) exhibit from one to seven tryptophane peptides, 12 to 17 histidine peptides, and 8 to 16 arginine peptides. A large percentage of insoluble "core" remains after treatment of reptilian hemoglobin with trypsin (Dessauer and Fox, 1963; Dessauer and Sutton, 1964). If the sulfhydryl groups are inactivated prior to digestion, the quantity of "core" is diminished greatly (Christomanos and Pavlopulu, 1968). Each of the two hemoglobins of *Pseudemys scripta* when isolated and digested with trypsin yields 30 peptides. The "slow" hemoglobin differs from the "fast" hemoglobin by 10 peptides (Manwell *et al,* 1963; Manwell and Schlesinger, 1966). The N-terminal amino group of the hemoglobin of *Clemmys caspica* is threonine (Christomanos and Pavlopulu, 1968). The Val. Gly- and Val. Glu N-terminal sequences of snake hemoglobin (Ozawa and Satake, 1955) also characterize hemoglobin of a number of mammals (Gratzer and Allison, 1960).

Oxygen transport, the primary function of hemoglobins, depends upon hemoglobin's capability of combining reversibly with oxygen. Oxygen equilibrium curves describing this property are available for reptiles of all major orders (Figs 13a and b). These display the familiar S-shape typical of equilibrium curves of mammalian hemoglobins (Redfield, 1933; Prosser *et al.,* 1950; Manwell, 1960; Riggs, 1965). The sigmoid shape is caused by interactions between oxygen combining sites. Sigmoid coefficients, which measure the extent of this interaction, lie between 1 and 3 for reptilian hemoglobins (Sullivan and Riggs, 1967c; Sullivan, 1968; Dawson, 1960). Interactions between the iron porphyrin groups of turtle hemoglobins are generally lower than for human hemoglobin. The lowest yet found are for *Terrapene carolina* (Fig. 13b) and *Clemmys guttata* which have nearly hyperbolic oxygen equilibrium curves. The hemoglobins of *Clemmys, Phrynops, Malayemys,* and *Kinosternon* show little change in interaction constants with pH, but those of *Chelydra, Podocnemis, Terrapene, Trionyx, Gopherus,* and *Pseudemys* change with pH. Maximum interaction occurs at pH 7.0 in *Chelydra, Gopherus,* and *Trionyx*; in the others interactions are highest at pH 8 (Sullivan, 1966; Sullivan and Riggs, 1967c).

A rise in temperature (Fig. 13b) or a fall in pH (Bohr effect; Figs 13c and d) within the physiological range leads to a decrease in oxygen affinity. Considering the broad ranges of temperature and pH which the blood of reptiles experiences under physiological conditions, these influences on oxygen affinity of hemoglobin must be highly significant. Usually minimum oxygen affinity occurs near the lower limit and maximum affinity near the upper limit of the physiological pH range (Wilson, 1939; Manwell, 1960; Sullivan, 1966; Sullivan and Riggs, 1967c). Oxygen equilibrium curves vary from one reptilian species to another (Figs 13b, c, and d). The alkaline Bohr

effect of hemoglobin in intact red cells (Fig. 13c) is slightly greater for *Pseudemys* than *Alligator* (Wilson, 1939); similarly the Bohr effects for *Alligator* and *Crocodylus* (Dill and Edwards, 1931a, 1931b, 1935) are greater than for the lizards *Sauromalus* (Dill *et al.,* 1935) and *Heloderma* (Dill and Edwards, 1935). Oxygen affinities of the red cells of *Uma, Sceloporous, Dipsosaurus,* and *Gerrhonotus,* though very different when tested at the same temperature, are remarkably similar when measured at the activity temperature of each species (Pough, 1969).

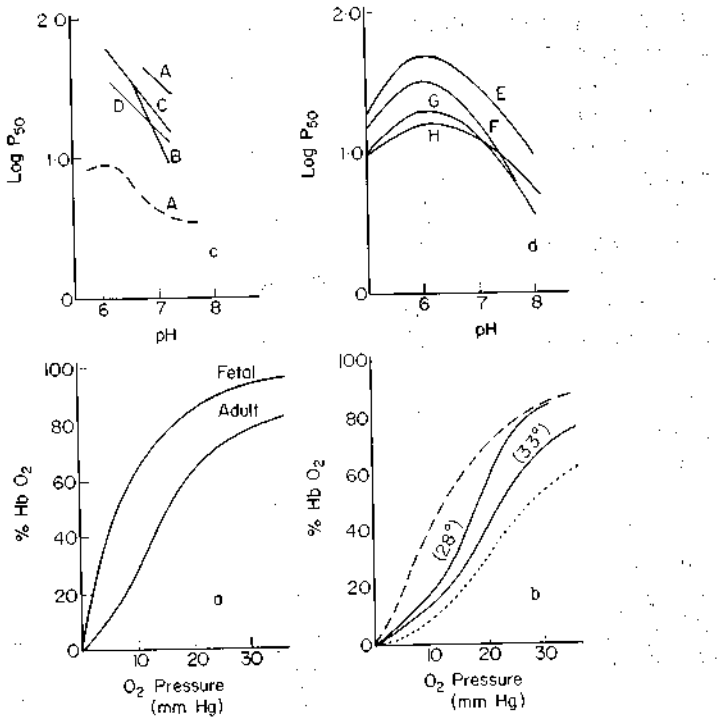


FIG. 13. Oxygen affinities of reptilian hemoglobins.

a. Affinities of fetal and adult hemoglobins of the diamondback terrapcne compared at the same conditions of pH and temperature (from McCutcheon, 1947).

b. Extreme species differences and dependence on temperature.———the turtle *Terrapene carolina,* and. . . . the turtle *Caretta Caretta* (25.5°C, pH 7.4; from McCutcheon, 1947)-_____; the lizard *Eumeces obsohtus* at two different temperatures (pH = 7.2; from Dawson, 1960).

c. Affinity of intact red blood cells under comparable conditions of temperature. For comparison, the affinity of a dilute solution of alligator hemoglobin is plotted as the dashed line. P_{50} = oxygen Pressure at which 50% of active hemoglobin is oxyhemoglobin and 50% reduced hemoglobin. A = the turtle *Pseudemys scripta;* B = *Alligator mississippiensis* (from Wilson, 1939); C = *Heloderma suspectum;* D = *Sauromalus obesus* (from Edwards and Dill, 1935; Dill *et al.,* 1935).

d. Oxygen affinities of dilute solutions of turtle hemoglobins at the same temperature. E = *Terrapene carolina;* F = *Chelydra serpentina;* G = *Gopheruspolyphemus; H* = human hemoglobin A (from Sullivan, 1966).

More extensive data are available for hemoglobins in dilute solutions (Fig. 13d). Hemoglobin solutions of the alligator (See Fig. 13c), the turtle *Pseudemys* (Wilson, 1939), and garter snakes *Thamnophis* (Manwell, 1960) have stronger affinities for oxygen than intact red blood cells of the same species. Hemoglobin of the *Alligator* has one of the highest oxygen affinites (Fig. 13c; Wilson, 1939) and that of the loggerhead sea turtle *Caretta Caretta* (Fig. 13c) the lowest oxygen affinity at pH 7.4 of any reptile yet studied (McCutcheon, 1947; Sullivan and Riggs, 1967c). Hemoglobins of *Natrix taxispilota* and probably other snakes have very high affinities at pH 7 and are unusual in exhibiting larger acid than alkaline Bohr effects (Sullivan, 1968). Hemoglobins of numerous turtles (Figs 13b and d) have been compared (Southworth and Redfield, 1925/26; Wilson, 1939; McCutcheon, 1947; Gaumer and Goodnight, 1957; Sullivan and Riggs, 1964, 1967c; Sullivan, 1966). At the upper limit of the physiological pH range, hemoglobins of active underwater foragers and swimmers (Chelydridae, Kinosternidae, and Trionychidae) tend to bind oxygen stronger than hemoglobins of sluggish, terrestrial species. The latter have higher affinities between pH 6.5 and 6.7, as their Bohr effects are lower (Sullivan, 1966). Marine turtles, which are very active animals, have the lowest oxygen affinities (McCutcheon, 1947; Sullivan, 1966; Sullivan and Riggs, 1967c).

During development the structure of reptilian hemoglobins changes as does that of mammals. Fetal hemoglobin of man is much more resistant to solutions of high pH than adult hemoglobin. Red cells of alligators less than 2 years old contain a higher percentage of an alkali resistant hemoglobin than do those of older animals (Ramsey, 1941). Hemoglobin of embryonic diamond back terrapins has a higher oxygen affinity than hemoglobin of adult animals (Fig. 13a; McCutcheon, 1947). The fetal-maternal difference in oxygen affinity observed in viviparous garter snakes *Thamnophis sirtalis* and *Thamnophis elegans vagrans* may result from differences in the intracellular environment of the hemoglobin (Manwell, 1958, 1960).

C. NON-HEMOGLOBIN COMPONENTS

Red cells of reptiles probably have broader and more typical metabolic potentials than the highly specialized erythrocytes of mammals (Bishop, 1964). Nuclei and thus deoxyribonucleic acids (DNA) are present; from 2.8 to 3.7 per cent of cell mass in snakes, alligator, and turtle is DNA. Each red cell of the turtles *Chelonia, Clemmys,* and *Chelydra,* the alligator and the snake *Natrix* contains about 5 x 10^{-9} mg of DNA, slightly less than is present in mammalian somatic cells. The DNA content per cell of other snakes *(Elaphe* = 4.28 x 10^{-9} mg, *Coluber* = 2.85 x 10^{-9} mg) approaches levels characteristic of birds (Villela, 1947, 1949; Mirsky and Ris, 1951; Gerzeli *et al,* 1956; Vendrely, 1958).

Presumably nucleic acid metabolism and protein synthesis occur throughout the long life span of the reptilian erythrocyte. Nucleotides occur in high concentration (Rapoport and Guest, 1941). Nucleic acid phosphatases are very active in cells of the snakes *Natrix* and *Agkistrodon,* but are of weak activity in a turtle (Rapoport *et al.,* 1942). Carbons of labeled glucose and succinate are incorporated into alanine, glutamic acid, and aspartic acid by the blood of the lizard *Egernia cunninghami* (Barwick and Bryant, 1966). All amino acids that are common constituents of proteins are present within the cell. Glycine, alanine, and glutamic acid occur in higher concentration in the red cells of *Caiman* than in its plasma. The level of 3-methyl-histidine, an amino acid of unknown function, exceeds the sum of all other amino acids in red cells of Crocodilia but is absent from erythrocytes of the turtle *Pseudemys scrïpta* (Herbert *et al,* 1966).

Electrolyte gradients also exist between plasma and red cell interiors. In an African tortoise (species not given) the red cell contains only 3.5 to 4 mEq/liter of sodium; plasma sodium exceeds 100 mEq/liter. Potassium makes up the osmotic deficit of cations within the cell (Maizels, 1956). Similiar electrolyte gradients between red cells and plasma characterize blood of lizards (Dessauer, 1952), alligators (Coulson *et al,* 1950a), and snakes (Hutton, 1958). Carbonic anhydrase, which is important in facilitating shifts of anions between plasma and the red cell, is present in erythrocytes of all major groups. That of a sea turtle *Caretta Caretta* (?) has been isolated and purified 300 fold. It contains 0.2 per cent zinc and has a minimum molecular weight of 32,500. Activity is proportional to its zinc content (Leiner *et al,* 1962). The level of carbonic anhydrase in the red cells of the alligator is less than 10 per cent of that of the dog. It is apparently inhibited by acetazolamide (Wistrand and Whitis, 1959; Coulson *et al,* 1957). In one comparison activity was highest in red cells of *Iguana* and decreased in the order: man, kingsnake, slider turtle, and alligator (Magee and Dessauer, unpublished).

Energy is required to maintain such gradients, to preserve the structural integrity of the cell, and to keep hemoglobin in the reduced form (Bishop, 1964). Energy production by reptilian red cells is much higher than that of mammalian red cells but somewhat lower than that of birds. Oxygen consumption at 25°C varies from about 30 mm^3/ml of cells/hour in *Alligator, Emys, Chelydra,* and *Pseudemys* to approximately 50 mm^3/ml of cells/hour in *Terrapene, Natrix,* and *Thamnophis.* Rate of oxygen utilization increases in *Alligator* and *Pseudemys* directly with temperature up to 38°C; *Alligator* cells survive only briefly above 45°C. *Alligator* reticulocytes consume more oxygen than do mature red cells. Glucose and high levels of saline do not affect rates of oxygen consumption (Tipton, 1933). Energy rich phosphate compounds which result from metabolic reactions are present in higher concentration in the red cells of reptiles than in those of most

mammals. The cells of lizards and snakes contain twice the level of adenosine triphosphate than those of any other animals examined (McCay, 1931; Kerr and Daoud, 1935; Rapoport and Guest, 1941).

The glycolytic pathway, tricarboxylic acid cycle, and probably the glucose-6-phosphate pathway are involved in energy production by red cells of reptiles. Tracer experiments using the lizard *Egernia cunninghami* showed that red cells oxidize glucose and acetate readily, yielding a large number of metabolic intermediates of the glycolytic and citric acid cycles. Succinic dehydrogenase, however, is probably absent from red cells of *Egernia* (Barwick and Bryant, 1966). The respiratory system of turtles furnishes the energy to maintain electrolyte gradients between red cell and plasma. In the absence of oxygen, or in cells poisoned with cyanide, gradients are maintained for short periods only because of the presence of stores of high energy phosphate (Maizels, 1956).

Stores of polysaccharides have been demonstrated histochemically in red cells of certain reptiles (Gerzeli, 1954). Diphosphoglyceric acid, an intermediate of the glycolytic cycle and of major importance in the mammalian red cell, was not found in the erythrocytes of snakes and turtles (Rapoport and Guest, 1941) but was detected in *Egernia* (Barwick and Bryant, 1966). Lactic dehydrogenase (LDH), a glycolytic enzyme, is present in reptiles (Wilson *et al,* 1964; Kaplan, 1965). Its activity in cells of the snakes *Coluber constrictor* and *Drymarchon corais* is only slightly less than that of human red blood cells (Vesell and Bearn, 1961/1962; Vesell and Brody, 1964). Electrophoretic patterns usually exhibit five LDH isozymes for iguanid lizards (Gorman and Dessauer, 1966) and alligators and one to three for snakes (Vesell and Beam, 1961/1962; Dessauer, unpublished). Glucose-6-phosphate dehydrogenase occurs in the red cells of *Anolis.* Active acid and alkaline phosphatases have been found in a turtle, a water snake, and a moccasin. The activity of the alkaline phosphatases is enhanced by magnesium and inhibited by oxalate and fluoride. Turtle cells contain a phytase, a phosphatase which utilizes phytic acid as substrate and does not require magnesium. Both phytic acid and phytase appear to be limited to the erythrocytes of turtles and birds (Rapoport *et al,* 1941, 1942; see also Bishop, 1964, p. 159).

V. **Blood Composition and Reptilian Systematics**

A. INTRODUCTION

Blood chemistry, especially the red cell antigens and protein structure, reflects genetic affinities of the reptiles. Structures of homologous proteins exhibit hierarchies of variation that parallel degrees of divergence of different taxa (Dessauer and Fox, 1964). Homologous proteins such as the transferrins

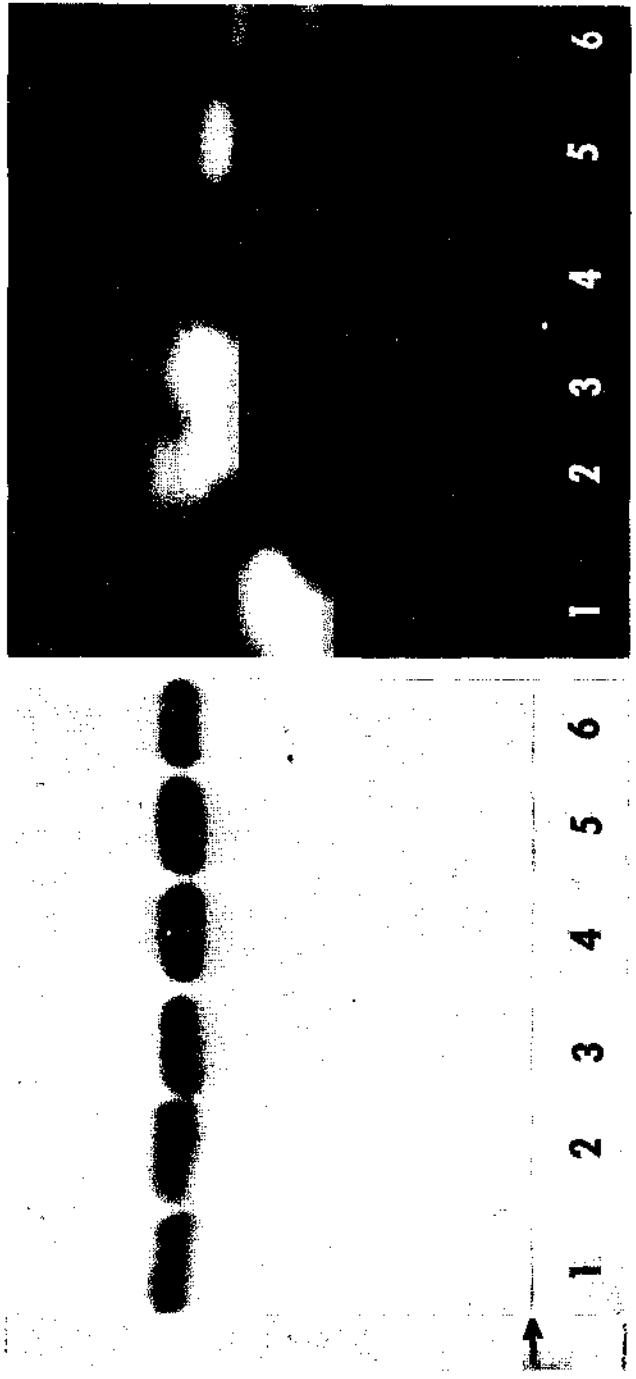


FIG. 14. Leucine naphthylamidases (Left) and transferrins (Right) of snakes of the genus *Thamnophis,* illustrating a conservative and a highly variable protein. Plasma samples to which radioactive iron was added were subjected to starch gel electrophoresis in borate buffer, pH 8.6. After electrophoresis amidases were localized by means of a histochemical method; transferrins were localized by radioautography. Arrow indicates site of sample application; anode is toward the top. (1) *T. sumichrasti,* (2) *T. eques,* (3) *T. elegans,* (4) *T. couchii hammondii,* (5) *T. sirtalis,* (6) *T. sauritus.*

(Figs 10 and 14) exhibit extensive variation. Such proteins and blood group systems demonstrate biochemical individuality and are useful in studies of population dynamics. Other proteins such as the leucine naphthylamidases (Fig. 14) are remarkably conservative structures, exhibiting little change in widely divergent species. Such molecules aid in grouping divergent forms (Dessauer, 1966).

B. DEFINING THE SPECIES

The presence in different individuals of cellular antigens and structural variants of homologous proteins emphasizes the population aspect of the species, the presence throughout the populations of a given species of many proteins of constant structure indicates the distinctness of the species. Variant forms of red cell antigens and of homologous proteins, probably inherited as allelomorphic genes, have been demonstrated in numerous reptiles. Systems of blood groups probably occur in all species (Section III C, 5 above). They have been examined systematically in turtles of both the Pleurodira and Cryptodira (Frair, 1963). Polymorphic forms of transferrin (Dessauer *et al,* 1962 a and b; Gorman and Dessauer, 1965, 1966; Fox and Dessauer, 1965), esterases (Dessauer *et al,* 1962b; Gorman and Dessauer, 1966), albumins (Zweig and Crenshaw, 1957; Dessauer and Fox, 1958; Crenshaw, 1965), leucine naphthylamidases (Pough and Dessauer, unpublished), and hemoglobins (Gorman and Dessauer, 1965; Manwell and Schlesinger, 1966) are known.

The number of structural variants may be extensive enough to identify individual animals. Frair (1963) was able to distinguish each of 24 tortoises *Testudo hermanni* on the basis of patterns of red cell antigens. The polymorphism of transferrins among the racer *Coluber constrictor* collected in the sand dune area of northern Indiana was great enough to identify five of a sample of six snakes (Dessauer *et al.,* 1962a). Some variant forms occur in high frequency in a population, but others are rare and emphasize the hidden resources of variation in a species. Only one specimen out of a sample of 500 of the garter snake *Thamnophis elegans* had a variant form of leucine naphthylamidase (Dessauer, 1966).

Comparison of frequencies of individual proteins in adjacent populations shows the extent of interbreeding; comparisons of frequencies in populations throughout the range of the species may suggest clines and indicate the direction of gene flow. Frequencies of transferrins, esterases, and other variant proteins gave a measure of the extent of introgression of two subspecies of the lizard *Cnemidophorus tigris* and indicated that transferrins are inherited on allelomorphic, autosomal genes (Dessauer *et al,* 1962b). Crenshaw (1965), studying a hybrid population of slider turtles (*Pseudemys),* found evidence for gene exchange between species known to be isolated over most of their range and was able to suggest a possible mechanism of albumin inheritance. The distribution of transferrins among (U.S.) west coast garter snakes showed that aquatic and terrestrial populations are distinct, do not intergrade, and are different species (Dessauer *et al.,* 1962a; Fox and Dessauer, 1965). Of two variant hemoglobins found in the turtle *Chrysemys picta* the type with a very low oxygen affinity is frequent in turtles from the warmer states, Arkansas and Louisiana. The type with a high oxygen affinity is more abundant in turtles in the colder states, Wisconsin and Minnesota (Manwell and Schlesinger, 1966). Certain populations of a species are characterized by high frequencies of an apparently unique form of a protein. In *Anolis* from the islands of the Caribbean, specific transferrins indicate the island of origin for the lizards, though they presumably belong to the same species (Gorman and Dessauer, 1965). Certain subspecies of *Pseudemys scripta* (Zweig and Crenshaw, 1957), *Natrix sipedon, Thamnophis sirtalis, Coluber constrictor,* and *Lampropeltis getulus* (Dessauer and Fox, 1958) are readily distinguished by differences in plasma albumins.

Proteins often suggest genetic affinities of species and species groups. Lactic dehydrogenase isozymes of the red cells distinguish the species of the lizard genus *Anolis* indigenous to the islands of the Lesser Antilles and tentatively assigned to the roquet-species group, Specimens of *Anolis aeneus* from three islands and the mainland of South America were classified as three named forms until study of blood proteins substantiated suspicions that populations had been recently introduced onto two of the islands and the mainland (Gorman and Dessauer, 1965, 1966). The snakes, *Regina grahami* and R. *septemvittata,* until recent years included in the genus *Natrix,* have similar plasma protein patterns, quite different from those of other species of *Natrix* (Dessauer and Fox, 1964). Numerous electrophoretic studies have demonstrated the distinctness of the protein complement of particular species (Bird, 1955; Plagnol and Vialard-Goudou, 1956; Dessauer and Fox, 1964; Latifi *et al,* 1965; Kmetova and Paulov, 1966; Maldonado and Ortiz, 1966; Newcomer and Crenshaw, 1967; Voris, 1967; Rider and Bartel, 1967).

C. RELATIONSHIPS WITHIN MAJOR GROUPS

1. *Crocodilia*

Proteins of a few members of the Crocodylidae and Alligatoridae have been compared by physicochemical and serological methods. Low serological correspondence was found between *Crocodylus niloticus* and *Alligator sinensis;* however, close correspondence was indicated for *Alligator sinensis* of China and *A. mississippiensis* of North America (Graham-Smith, 1904) and between the latter and *Caiman latirostris* (Lewis, 1964). Electrophoretic evidence

is in agreement with serological findings. Plasma albumins of the Nile and American crocodile have identical electrophoretic mobilities. Mobilities of the transferrins of *Caiman* and *Alligator* are equal, but different from those of the crocodiles. Hemoglobins of the four species are electrophoretically distinguishable (Dessauer and Fox, 1964; Coulson and Hernandez' 1964).

2. *Testudines*

Immunological correspondence of serum proteins (Frair, 1964) and physicochemical properties of plasma proteins (Dessauer and Fox, 1956, 1964; Cohen and Stickler, 1958; Tondo, 1958; Leone and Wilson, 1961; Crenshaw, 1962; Frair, 1964; Lewis, 1964; Newcomer and Crenshaw, 1967) and hemoglobins (Dessauer *et al,* 1957; Sullivan, 1966; Sullivan and Riggs, 1967a, b and c) largely support current schemes of classification and suggest affinities of living forms. Turtles of the suborder Cryptodira show low immunological correspondence to species of the suborder Pleurodira. Globins of species of the Chelidae, a family of the Pleurodira, show unique properties. Based upon blood studies the (1) marine, (2) softshell, and (3) fresh water and land turtles of the Cryptodira appear to be divergent forms. Families of sea turtles exhibit closer immunological correspondence to each other than to non-marine species. The genera *Chelonia* and *Caretta* show very high correspondence and have similar hemoglobins. Softshell turtles have unique hemoglobins. Further, antisera against *Trionyx ferox* plasma proteins interact appreciably with serum of other softshell turtles but give practically no reaction with serum of species from other families. Species of fresh water and land turtles divide into two groups on the basis of the electrophoretic behavior of plasma albumin (Fig. 9). Albumins of *Dermatemys, Staurotypus, Sternotherus* (3 species), and *Kinosternon* (5 species) have relatively fast migration rates. Plasma proteins of these genera also exhibit high immunological correspondence. The only other turtle known to have a "fast-albumin" is the pleurodire *Chelodina longicollis.* Hemoglobins of *Chelydra, Staurotypus, Claudius, Kinosternon* and *Sternotherus* show some similarities in physicochemical properties. Emydine turtles are apparently a heterogenous group; *znú-Pseudemys* sera exhibits 55% serological correspondence to *Chrysemys,* 45% to *Terrapene,* but only 7% to *Kachuga tecta.*

3. *Squantata*

Immunological evidence, although almost non-existent at the subordinal level, reveals a remote relationship between the Varanidae and lizards of the Gekkonidae, Scincidae, and Agamidae, but almost no affinity between Varanidae and snakes of the Boidae, Colubridae, Crotalinae, and Viperinae (Graham-Smith, 1904; Cohen, 1955). Electrophoretic mobilites of the albumins, transferrins and hemoglobins of a wide variety of Squamata (Dessauer and Fox, 1964) form overlapping series, not allowing differentiation of the suborders (Figs 9 and 10). Fingerprints of arginine peptides of tryptic digests of hemoglobins of iguanid lizards and of snakes have a number of common features (Fig. 12; Dessauer and Fox, 1963; Dessauer and Sutton, 1964). Blood of lizards generally contains more glucose than blood of snakes (Table II); snake plasma contains a higher concentration of sialic acid than that of other reptiles, including iguanid lizards (Seal, 1964).

Among sub-families of snakes the Boinae display high immunological correspondence with the Pythoninae; the Elapinae with the Hydrophiinae; and the Crotalinae with the Viperinae, though some authors consider members of these pairs distinct on the family level. The Colubridae show coldest and about equal affinity to the Crotalidae and Elapidae. The Typhlopidae are remote from all families tested (Boidae, Colubridae, Viperidae, and Elapidae), though they exhibit some similarity to the Boidae (Graham-Smith, 1904; Kellaway and Williams, 1931; Kuwajima, 1953; Cohen, 1955; Pearson, 1966). Leucine naphthylamidase is absent from the plasma of the Boidae, exhibits weak activity among the Crotalidae and Elapidae, but is exceptionally active in plasma of the Colubridae (Dessauer, 1967; Ehrensing, 1964). Fingerprints of tryptic peptides of hemoglobins of Boidae, Colubridae, and Crotalidae are remarkably similar (Dessauer and Sutton, 1964, and unpublished).

Recent findings may help unravel relationships of genera of snakes presently grouped in the Colubridae and Crotalidae. Within the Crotalidae, *Sistrurus* is biochemically closer to *Crotalus* than to *Agkistrodon.* Moccasins are a more divergent group of species than rattlesnakes (Cohen, 1955; Pearson, 1966). *Agkistrodon* and *Trimeresurus* on Formosa are near relatives (Kuwajima, 1953). Pearson (1966) classified 11 genera of the Colubridae into four subgroups on the basis of immunological correspondence of serum proteins. One group includes *Farancia, Diadophis,* and *Heterodon.* Species of the second group, including *Lampropeltis, Pituophis, Rhinocheilus,* and *Elaphe,* and the third group, including *Coluber, Masticophis,* and *Opheodrys,* are more closely related to each other than to species in the other groups. Pearson's fourth group, the Natricinae, also appears to be a natural category on the basis of physicochemical evidence. Plasma of natricine genera from Africa, Asia, Europe, and North America contains a unique esterase, which is absent from the plasma of other colubrid snakes. One of the polypeptide subunits of their globins is electrophoretically unique (Dessauer, 1967)

D. CHARACTERISTICS AND AFFINITIES OF MAJOR GROUPS

Both physicochemical and immunological evidence emphasize the evolutionary divergence of major groups of the Reptilia. Serology demonstrates

the fairly close relationship of some lizards and snakes, indicates a very remote affinity between turtles and crocodiles, and shows the wide divergence of the Squamata from the Testudines and Crocodilia. Such conclusions find their basis in the work of Graham-Smith (1904). Utilizing antisera against serum proteins from two species of tortoise and a sea turtle, a Chinese alligator, an agamid and a varanid lizard, and a boid and a colubrid snake, he compared homologous and heterologous precipitin reactions between sera from about 40 species of reptiles. The forms sampled by him are presently classified in 4 families of turtles, 2 families of crocodilians, 6 families of lizards, and 4 families of snakes. Graham-Smith's conclusions, although obtained with the crudest of techniques, have been supported by investigators using modern methods (Wolfe, 1939; Cohen, 1955; Lewis, 1964).

Species of the three major groups of living reptiles are easily distinguished by the electrophoretic behavior of their blood proteins (Figs 9 and 10). Albumins and transferrins of the Testudines migrate at slower rates in alkaline buffers than do the albumins and transferrins of the Crocodilia and Squamata. Red cell hemolysates from turtles resolve into complex patterns of multiple hemoglobins. Hemolysates from snakes, lizards, and crocodiles resolve into simple patterns of one or two components. Hemoglobins of the Squamata have uniquely slow mobilities. Their plasma usually lacks a fraction with the mobility of mammalian gamma-globulin.

Even low molecular weight blood constituents are useful in distinguishing these forms, reflecting the many gains and losses in metabolic potential which accompanied divergent evolution. Thus, the high concentration of urea in the blood of turtles reflects the presence of active urea-cycle enzymes. The lack or low concentration of urea in blood in other reptiles is the result of the loss or suppression of the urea-cycle (Table II). Other metabolic specializations (Section IV C above) are probably responsible for the uniquely high concentration of high energy phosphates in red cells of the Squamata, the presence of phytic acid in erythrocytes of turtles, and the high levels of 3-methylhistidine in cells of the Crocodilia.

VI. Summary

The blood of reptiles is a rich source of evidence on their heritage and on the ways in which they have adapted to metabolic challenges. The composition of the blood of all major reptilian groups is typical of that of vertebrates in general. It contains most of the same components of low molecular weight. The macromolecules, though of unique and highly specific structure, include proteins analogous to and probably homologous with the volume expanders, transport proteins, clotting factors, and immune bodies of mammals. The nucleated red cells, which make up 20 to 30 per cent of their blood volume, contain approximately the same amount of hemoglobin, but have more complete systems of metabolic pathways and longer life spans than the unnucleated erythrocytes of mammals.

Concentrations of many blood constituents, however, fluctuate to a much greater extent than one would expect from a knowledge of mammalian physiology. Daily events such as feeding, diving, and changes in the temperature or availability of water in the environment often lead to drastic shifts in blood levels of salts and metabolites. Certain constituents undergo marked cyclic changes in association with reproduction. During estrus in females the yolk precursor, plasma vitellin, appears in the blood causing increased protein, calcium, and magnesium levels. Other seasonal shifts in metabolic capabilities result in dramatic cyclic changes in blood levels of metabolites such as glucose.

The wide evolutionary divergence of the three major groups of living reptiles is emphasized by blood chemistry. Major structural differences exist between the hemoglobins, albumins, transferrins, and other blood proteins of turtles, crocodiles, and squamates. Blood composition of one group of the Reptilia often responds to a physiological event quite differently from that of another group, reflecting marked differences in metabolic potentials. The pattern of changes in the blood during a dive is different in fresh water turtles than in other reptiles; the effect of insulin on blood sugar is less marked in squamates than in turtles and crocodiles; feeding leads to drastic shifts in the electrolytes of crocodiles but has little effect on salt balance of lizards. Divergence of metabolic potential is so great that one even observes differences in certain low molecular weight metabolites. Thus, only turtle blood contains phytic acid and moderate amounts of urea, and lizard blood characteristically contains a high concentration of glucose.

Homologous blood proteins exhibit hierarchies of variation that parallel degrees of divergence of taxa of close and distant relationship. Comparative evidence on proteins can clarify difficult problems of relationship. Such evidence illustrates the individuality of the organism and emphasizes that species may be composed of differing populations. It has been useful in detecting hybrids, in comparing degree of divergence of island populations, and in grouping and estimating degrees of divergence of species, genera, and higher taxonomie categories.

Presumably natural selection for mutations of adaptive value has led to the accumulation of these many differences between proteins. Variations in the structure and concentration of plasma albumin appear to correlate with the availability of water, metabolic rate and diving physiology. The structure of the hemoglobin molecule often relates to the availability of oxygen in the environment and the activity of the organism. Such meager evidence represents about all the information as yet available relating structural differences

between homologous blood proteins of reptiles to physiological and ecological factors.

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CHAPTER 2

Morphology of the Circulating Blood Cells

MARIE-CHARLOTTE SAINT GIRONS

Museum National d'Histoire Naturelle, Brunoy, France

I. Introduction

The earliest works on the blood of reptiles described only the structure of its various elements, often comparing them with those of other vertebrates. Treatises on hematology generally contain sections on reptilian blood; unfortunately these are often based on the study of far too few, principally European species. A few recent monographs consider single species and include, besides descriptions of the different circulating blood cells, other observations on various problems - parasites of the blood, seasonal or sexual variation in the numbers of corpuscles, hematopoiesis, and the like.

The earliest published works are those of Mandl (1839) and Gulliver (1840) concerning the erythrocytes of crocodilians and turtles. Important studies of comparative morphology of the blood include those by Gulliver (1842, 1875), Milne-Edwards (1856, 1857), Hayem (1879), Pappenheim (1909), Werzberg (1910), Schulz and Krüger (1925), Loewenthal (1928, 1930), Babudieri (1930), Jordan (1938), Ryerson (1949), Altman and Dittmer (1961), and Pienaar (1962); the last work contains an important bibliography. See Efrati *et al.* (1970) for a study of hemopoiesis in a lizard.

Many questions remain unanswered, and the difficulty in determining the different cellular lineages appears to be one of the principal obstacles to a comparative study of reptilian blood. Only mature cells are considered in this chapter although various stem cells are found in the circulating blood. Pienaar (1962) gives a synonymy of the nomenclature used by the major previous authors (pp. 29-33) and attempts to standardize the names given to the cells of the circulating blood. I will use a slightly simplified version of his system of nomenclature.

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Classical reptiles and amphibians, with their three chambered hearts, cannot have a high blood pressure, and therefore cannot achieve endothermy, will pretty much always be at the mercy of the environment for their body temperatures, and cannot sustain high performance for very long periods. This may sound like a disadvantage, but it has some very distinct advantages of its own. Most notably a much lower metabolism, they would require far less energy and oxygen just to simply maintain themselves (a very general measure is that a reptile of same mass as say a mammal or a bird has about 1/40th t Most insects, reptiles, fish, and amphibians, are not able to maintain a regular core temperature from within, and are therefore more dependent on the temperature of their surroundings. They are therefore called ectotherms (ecto = outside + therm = heat).Â There are advantages and disadvantages to being either cold-blooded or warm-blooded. In particular, since the efficiency of chemical reactions in the cell is dependent on the core temperature, being warm-blooded allows for more activity in colder environments.Â Since temperature is one of many physiological parameters that must be controlled to maintain life, shouldnâ€™t evolutionary biologists have to describe each of these thermoregulatory mechanisms and how they became more sophisticated? The morphology of the reptilian heart results in the mixing of oxygenated and deoxygenated blood (cardiac shunts). In birds and mammals cardiac shunts are detrimental, but in reptiles this condition is often considered a derived trait, conveying important physiological functions and favored by natural selection. Alternative views are advanced suggesting that, in reptiles, cardiac shunts represent either an ancestral condition or an embryonic trait.