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HEMATOLOGY AND PLASMA BIOCHEMISTRY REFERENCE RANGE VALUES FOR FREE-RANGING DESERT TORTOISES IN ARIZONA

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ABSTRACT: Baseline values and ranges for 10 hematologic and 32 plasma chemistry parameters were analyzed for 36 free-ranging Sonoran desert tortoises (*Gopherus agassizzi*) collected in Yavapai and La Paz Counties (Arizona, USA) from 1990 to 1995. Tortoises were radio-tagged from 1990 to 1994, and attempts were made to recapture them three times a year. Tortoises were weighed, measured, and chemically immobilized to collect blood for hematology and blood chemistry assessments. Tortoise biochemistry differed ($P < 0.01$) between sites and sexes and among seasons and years. Normal reference ranges for hematologic and plasma biochemistry parameters were determined. Seasonal and annual differences in hematology and blood chemistry were related to rainfall patterns, forage availability, and physiological condition.

Key words: Blood biochemistry, desert tortoise, *Gopherus agassizii,* hematology, normal ranges.

INTRODUCTION

Desert tortoises (*Gopherus agassizii*) are long-lived reptiles found in the deserts of the southwestern USA. Sonoran desert tortoises are found south and east of the Colorado River (USA). The Sonoran population was a Category II candidate for Federal listing in 1989 (U.S. Fish and Wildlife Service, 1989). In 1995 the U.S. Fish and Wildlife Service discontinued the Category II listing (U.S. Fish and Wildlife Service, 1996), thus, the Sonoran tortoise population currently has no status under the Endangered Species Act.

Knowledge of hematologic and biochemical parameters of free-ranging desert tortoises is important for assessing and managing their populations (Nagy and Medica, 1986). Blood parameters used to diagnose chelonian diseases can be used to assess the physiologic status of a population (Jacobson et al., 1991). No physiologic information exists for free-ranging desert tortoises from the Sonoran Desert. Some physiologic information exists for free-ranging Mojave tortoises in eastern California (USA; Christopher et al., 1994, 1997), and southern Nevada (USA; O'Connor et al., 1994). Most hematologic and clinical biochemistry information has been collected from captive Mojave tortoises (Rosskopf, 1982; Nagy and Medica, 1986; Jacobson et al., 1991; O'Connor et al., 1994; Rostal et al., 1994).

Normal reference ranges of hematologic and biochemical parameters for free-ranging tortoises are important in assessing the status of tortoise populations. Deviations from expected values for "healthy tortoises" can assess the impacts of stresses such as habitat loss, forage competition with domestic livestock, off-road-vehicle use, and drought on free-ranging tortoise populations. Normal reference ranges for free-ranging tortoises have only been reported for Mojave tortoises in eastern California (Christopher et al., 1997).

To better understand the health status of free-ranging Sonoran tortoises, the hematology and plasma biochemistry of free-ranging Sonoran tortoises were investigated for 5 yr, from 1990 to 1995. The study objectives were to (1) collect baseline data on hematology and blood biochemistry of free-ranging desert tortoises and calculate normal reference ranges, (2) determine if seasonal and annual physiologic differences are present in male and female free-ranging tortoises from two sites, and (3)

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infer seasonal tortoise activities from physiologic parameter values, Sonoran tortoise nutrition studies, and local weather data.

MATERIALS AND METHODS

Two study sites in the Sonoran Desert were selected; Little Shipp Wash (Yavapai County, Arizona, USA; 34°31′N, 113°5′W) and the Harcuvar Mountains (La Paz County, Arizona; 34°6′N, 113°17′W). Both study sites were 1 km from permanent desert tortoise study plots used for population monitoring. These sites were 65 km apart, geographically separated by mountains and major roadways. Elevations ranged from 788 to 975 m. Vegetation was upland Sonoran Desert characterized by little-leaf palo verde (*Cercidium microphyllum*), saguaro (*Carnegia gigantea*), ocotillo (*Fouquieria splendens*), mesquite (*Prosopis juliflora*), cat-claw acacia (*Acacia greggii*), fairy duster (*Calliandra eriophylla*), flat-topped buckwheat (*Eriogonum fasciculatum*), and Engelmann's prickly pear (*Opuntia engelmannii*). Grasses and forbs included red brome (*Bromus rubens*), Indian wheat (*Plantago insularis*), purple three-awn (*Aristida purpurea*), big galleta grass (*Hilaria rigida*), and slender janusia (*Janusia gracilis*).

The Harcuvar Mountain site elevations ranged from 792 to 1006 m. Vegetation was upland Sonoran Desert characterized by saguaro, ocotillo, little-leaf palo verde, cholla (*Opuntia* sp.), fairy duster, flat-topped buckwheat, red brome, and Indian wheat as well as a sparse population of Joshua trees (*Yucca brevifolia*).

Most study animals were captured in 1990 in their sheltersites or out in the open. Many tortoises were located by shining a light into underground shelters. Once an adult tortoise (>208 mm median carapace length [MCL]) was found, 5-min gel epoxy was used to affix a radio transmitter (Model 125, Telonics, Mesa, Arizona, USA) to its anterior marginal scutes. The transmitters weighed 47 to 53 g, measured 4.1 cm × 2.4 cm × 2.0 cm, and had an active life of 9 to 18 mo. Both male and female tortoises were captured. For further identification, marginal scutes were notched following the system used on BLM tortoise monitoring plots in Arizona, California, and Nevada.

The sex of tortoises was determined by plastron indentation, tail morphology, and gular size (Woodbury and Hardy, 1948). A minimum of five and a maximum of 20 adult tortoises were recaptured during each sampling trip. Two sampling trips were made to Little Shipp and the Harcuvars in 1990 (September, November), and then each site was sampled three times a year (May, July, September) from 1991 through 1994.

At each recapture, tortoises were physically examined for disease, weighed with a 5 kg Pesola© scale (±0.5 kg; Ben Meadows Company, Canton, Georgia, USA), and measured with a vernier caliper (±0.1 mm; Ben Meadows Company, Canton, Georgia, USA) and 24 cm ruler. All tortoises were handled with gloves, changed between tortoises, and tortoises were kept in clean, individual cardboard boxes to minimize the probability of disease transfer among animals.

Tortoises were immobilized 4 to 6 hr after capture with 15 mg/kg of ketamine hydrochloride (Ketaset; Fort Dodge Lab., Fort Dodge, Indiana, USA) injected intramuscularly into a rear leg using a 25-gauge needle. Twenty min after immobilization, 6.0 ml of whole blood was collected by jugular venipuncture using a 22-gauge needle. A portion of the whole blood (0.6 ml) was placed in a lithium heparin microtainer (Becton Dickinson, Rutherford, New Jersey, USA), which was mixed for 5 min, kept on ice, and mailed to Animal Diagnostic Laboratory, Inc. (Tuscon, Arizona, USA) within 24 hr. Several drops of whole blood were used to fill two heparinized microhematocrit capillary tubes (Scientific Products, McGaw Park, Illinois, USA) to determine packed cell volume (PCV), hemoglobin (Hgb), and fibrinogen (FIBR). Whole blood was also used to make two air-dried blood smears. Smears were sent within two days to APL Veterinary Laboratory (Las Vegas, Nevada). In the laboratory, smears were stained with modified Wright's stain and examined for white blood cell (WBC) estimate, differential WBCs (heterophils [HETERO], lymphocytes [LYMPH], monocytes [MONO], azurophils [AZUR], eosinophils [EOS], basophils [BASO]), platelet estimate, red blood cell (RBC) morphology, hemoparasites, and evidence of anisocytosis, polychromasia, and anemia.

The remaining whole blood was placed in a lithium heparin vacutainer (Becton Dickinson) to obtain plasma, mixed for 5 min, and then centrifuged for 5 min. The plasma was pipetted off and then divided into aliquots. In 1990, the plasma was placed in red top vacutainers (Becton Dickinson) and then placed on dry ice. From 1991 to 1994, the plasma was placed in cryogenic vials (Whatman LabSales, Hillsboro, Oregon, USA) and immediately frozen in liquid nitrogen. Plasma samples were sent on dry ice within 2 days to Animal Diagnostic Lab, Inc.

Plasma was divided into three aliquots. The first aliquot (1.0 ml) was analyzed for blood biochemistry determinations with a 550 Express Analyzer (Ciba-Corning, Oberlin, Ohio, USA) at Animal Diagnostic Lab, Inc. Plasma was analyzed for 24 blood variables including glucose

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(GLU), blood urea nitrogen (BUN), creatinine (CREAT), uric acid (URIC), total protein (TP), albumin (ALB), total globulins (TG), bile acids (BILE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), cholesterol (CHOL), triglycerides (TRI), total bilirubin (TBILI), direct bilirubin (DBILI), indirect bilirubin (IBILI), sodium (Na), potassium (K), chloride (CHLR), total carbon dioxide (TCO2), anion gap (AG), and osmolality (OS). Total globulin and AG values were calculated using the following formulas: TG = TP − ALB and AG = (Na + K) − (CHLR + TCO2). The second aliquot (1.5 ml) was analyzed for vitamin A (VITA) and vitamin E (VITE), copper (Cu), selenium (Se), and zinc (Zn) by the Arizona Veterinary Diagnostic Laboratory (Tucson, Arizona). Vitamin levels were measured by high-pressure chromatography (Model 110A, Beckman, Fullerton, California, USA), and Se levels by gas chromatography (Model 5880, Hewlett Packard, Avondale, Pennsylvania, USA). Copper and Zn were measured by atomic absorption (Model Video 12, Instrumentation Lab, Waltham, Maryland, USA). The third aliquot (1.0 ml) was analyzed for testosterone, estradiol (ESTR), and corticosterone by radio immunoassay at the San Diego Zoo (San Diego, California; Lance et al., 1985).

Tortoises were rehydrated after sampling to replace any fluids voided during handling. A fluid volume equivalent to 1 to 2% body mass, of equal parts Normosol (Abbott Laboratories, Chicago, Illinois, USA) and 2.5% dextrose in 0.45% sodium chloride (Abbott Laboratories) was injected into the body cavity of each tortoise with a 20-gauge needle. Tortoises were released at the point of capture during early morning of the day following health assessment, >10 hr after injection of ketamine hydrochloride.

Permanent U.S. National Oceanic Atmosphere Administration (National Climatic Data Center, Asheville, North Carolina, USA) and automatic weather stations were used to record ambient temperature (maximum, minimum) and rainfall. Permanent weather data were collected daily at Hillside and Aguila, Arizona, from September 1990 to September 1994. The Hillside weather station was 17.7 km southeast of Little Shipp. The Aguila weather station was 20.9 km south of the Harcuvars. Automatic weather stations (Model System 10, Rainwise, Inc., Bar Harbor, Maine, USA) were installed in July 1992 at each site and data collected every hour until the study ended in September 1994. Automatic weather stations were installed to augment the permanent weather data.

Reference ranges were established for all the tortoise hematologic and biochemistry values. Using the procedure recommended by Hoffman (1971) the ranges were calculated for tortoise blood parameters as the mean ±2 standard deviations (SD). Outliers were values >2 SD from the mean and were excluded from reference ranges. Tortoises whose values were >2 SD from the mean were considered as possibly abnormal, and those with values >3 SD from the mean as probably abnormal. A program written in SPSS© (Outliers; SPSS, 1998) was used to exclude outliers and then calculate the mean and SD for each parameter by sex.

Because the same tortoises were sampled repeatedly in this study, the appropriate statistical test would have been repeated measures-multiple analysis of variance (RM-MANOVA, StatSoft, Inc., 1994). This type of analysis has three problems: (1) it is intolerant of missing values and none of the tortoises were sampled during each sampling period, (2) it simultaneously analyzes all treatment effects that are too complex to explain, and (3) it does not account for interactions among dependent variables. Instead how each treatment (site, season, sex, year) affected each blood parameter individually was tested using analysis of variance (ANOVA) with the program Statistica© (StatSoft, Inc., 1994) (significance, $P < 0.01$). Tukey's honest significant difference (HSD) test was used to identify differences between means (Statistica© Program, StatSoft, Inc., 1994).

Body mass, MCL, PCV, and Hgb were analyzed for the effects of site, sex, season, and year. Differential WBCs (HETERO, LYMPH, MONO, EOS, BASO, AZUR) were analyzed for the effects of site, season, and year. Plasma (1991–94) was analyzed for 11 biochemical parameters (BUN, CREAT, TP, ALB, Ca, CHOL, TRI, VITA, VITE, Na, K) for the effects of site, season, sex, and year. Plasma (1993–94) was also analyzed for 11 biochemical parameters (GLU, URIC, BILE, AST, ALT, ALP, TBILI, DBILI, IBILI, CHRL, TCO2) added to the health sampling in 1993 for the effects of site, season, sex, and year. Automatic weather data (rainfall, ambient temperature) were analyzed using a 2-way ANOVA for the effects of site and year and using a 1-way ANOVA for the effect of month (Statistica© Program, StatSoft, Inc., 1994).

RESULTS

General

Thirty-six tortoises were captured in the study; 13 tortoises from Little Shipp and 23 tortoises from the Harcuvars. In addi-

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TABLE 1. Significant effects of site (Little Shipp Wash and Harcuvar Mountains, Arizona), season (May, July, September), sex, and year (1991–94) on body mass, median carapace length, packed cell volume, hemoglobin, lymphocytes, and azurophils in desert tortoises (n = 36).

	F	df	P
Body Mass			
Site	27.5	132	<0.001
Median Carapace Length			
Site	14.9	132	<0.001
Packed Cell Volume			
Site	6.8	130	0.01
Hemoglobin			
Season	11.0	127	<0.001
Year	4.6	127	0.004
Lymphocytes			
Season	19.5	151	<0.001
Year	4.3	151	0.005
Azurophils			
Season	34.2	151	<0.001

TABLE 2. Significant effects of site (Little Shipp Wash and Harcuvar Mountains, Arizona), season (May, July, September), sex, and year (1990–94) on total protein, albumin, calcium, cholesterol, triglycerides, vitamin A and E, sodium, and potassium in desert tortoises (n = 36).

	F	df	P
Total Protein			
Season	4.1	127	0.01
Sex	39.3	127	<0.001
Year	23.6	127	<0.001
Albumin			
Season	5.7	129	0.00.4
Sex	22.8	129	<0.001
Year	146.0	129	<0.001
Calcium			
Sex	97.0	129	<0.001
Year	6.4	129	<0.001
Cholesterol			
Season	8.2	130	<0.001
Sex	166.1	130	<0.001
Year	3.7	130	<0.01
Triglycerides			
Season	32.6	130	<0.001
Sex	105.4	130	<0.001
Vitamin A			
Site	65.2	127	<0.001
Season	7.2	127	<0.001
Vitamin E			
Season	66.8	127	<0.001
Sex	19.8	127	<0.001
Year	14.1	127	<0.001
Sodium			
Site	9.8	129	0.00-2
Season	33.1	129	<0.001
Year	33.0	129	<0.001
Potassium			
Season	12.3	129	<0.001
Year	6.7	129	<0.001

tion to statistical analyses of the data (Tables 1–3), normal reference ranges were calculated for all data from 1990 to 1994 (Table 4). Tortoise body mass and MCL differed between sites but not among seasons, sexes, and years. Little Shipp tortoises were heavier (3.34 kg compared to 2.76 kg) and longer (264.1 mm compared to 249.9 mm) than Harcuvar tortoises.

Hematology

We determined that PCV and Hgb values were different among seasons and years and to a lesser degree between sites. Differential WBCs were different across seasons and to a lesser degree among years. Little Shipp tortoises had higher PCV in May 1992 and July 1994 compared to Harcuvar tortoises, and Harcuvar tortoises had higher PCV in May 1993 and July 1993 compared to Little Shipp tortoises. Hemoglobin values were higher in September compared to May and July for all four years combined (1991–94), and were higher in 1992 and 1993 compared to 1991 and 1994. Lymphocyte counts were higher in September compared to May and July and higher in 1992 compared to 1991, 1993, and 1994. Azurophil counts were higher in 1994 compared 1991, 1992, and 1993. No difference ($P >$ 0.01) was found for other differential WBC parameters (WBC estimate, HETERO, MONO, EOS, BASO) with respect to all other site, season, and year combinations.

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TABLE 3. Significant effects of site (Little Shipp Wash and Harcuvar Mountains, Arizona), season (May, July, September), sex, and year (1993–94) on fibrinogen, glucose, uric acid, aspartate aminotransferase, alanine aminotransferase, total bilirubin, indirect bilirubin, chloride, and total carbon dioxide in desert tortoises (n = 36).

	F	df	P
Fibrinogen			
Season	45.0	55	<0.001
Year	14.4	55	<0.001
Glucose			
Season	12.0	58	<0.001
Uric acid			
Site	9.9	58	0.001
Season	53.5	58	<0.001
Year	97.0	58	<0.001
Asparatate aminotransferase			
Sex	8.3	58	0.005
Alamine aminotransferase			
Season	12.5	58	<0.001
Year	6.9	58	0.01
Total bilirubin			
Sex	22.1	58	<0.001
Year	11.2	58	0.001
Indirect bilirubin			
Sex	20.4	58	<0.001
Year	12.6	58	<0.001
Chloride			
Season	41.5	58	<0.001
Total carbon dioxide			
Site	14.5	57	<0.001
Season	6.1	57	0.004

Plasma 1991–94

Monthly and yearly changes occurred in TP, ALB, CHOL, and VITE. Total protein and VITE were higher in July compared to May and September, and ALB, CHOL, and TRI were higher in September compared to May and July. Total protein and VITE were higher in 1992, ALB was higher in 1991, and CHOL was higher in 1994 compared to all years. Ca levels were higher in 1994, and VITA levels were higher in 1992 compared to all other years. We found that there were monthly and yearly changes in WT, VITA, and VITE for both tortoise sites (Fig. 1).

TABLE 4. Overall means and reference ranges for body mass, median carapace length, hematologic, and plasma biochemical parameters in Sonoran desert tortoises from Little Shipp Wash (n = 13) and Harcuvar Mountains (n = 23), Arizona, 1990–94. See text for seasonal, site, and year variations.

Parameter	Reference range 0 ± 2 SD (n)
Body mass (kg)	
Male	3.1 ± 0.68 (105)
Female	3.1 ± 0.63 (98)
Median carapace length (mm)	
Male	258.8 ± 22.1 (105)
Female	256.6 ± 18.1 (98)
Packed cell volume field (%)	
Male	25.0 ± 3.6 (98)
Female	24.3 ± 3.2 (85)
Hemoglobin (g/dl)	
Male	10.2 ± 1.5 (93)
Female	10.3 ± 1.3 (85)
Fibrinogen (mg/dl)	
Male	131.6 ± 32.2 (35)
Female	130.2 ± 35.1 (43)
White blood cell estimate (k/μl)	
Male	8.5 ± 3.7 (80)
Female	7.8 ± 3.7 (75)
Heterophils (k/μl)	
Male	582.3 ± 309.2 (79)
Female	483.1 ± 307.3 (74)
Lymphocytes (k/μl)	
Male	100.7 ± 62.6 (81)
Female	93.9 ± 64.8 (75)
Monocytes (k/μl)	
Male	13.0 ± 16.6 (77)
Female	9.9 ± 11.7 (72)
Azurophils (k/μl)	
Male	6.3 ± 12.3 (79)
Female	4.2 ± 7.6 (75)
Eosinophils (k/μl)	
Male	26.5 ± 33.0 (75)
Female	16.8 ± 23.4 (68)
Basophils (k/μl)	
Male	83.2 ± 46.6 (82)
Female	116.1 ± 79.4 (75)
Glucose (mg/dl)	
Male	132.6 ± 32.2 (49)
Female	127.1 ± 34.9 (59)
Blood urea nitrogen (mg/dl)	
Male	1.6 ± 2.4 (102)
Female	1.0 ± 2.0 (92)

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TABLE 4. Continued.

Parameter	Reference range 0 ± 2 SD (*n*)
Creatinine (mg/dl)	
Male	0.24 ± 0.13 (81)
female	0.25 ± 0.10 (80)
Uric acid (mg/dl)	
Male	4.8 ± 2.1 (46)
Female	5.4 ± 1.8 (57)
Total protein (g/dl)	
Male	3.4 ± 0.43 (104)
Female	3.9 ± 0.69 (96)
Albumin (g/dl)	
Male	1.7 ± 0.5 (109)
Female	1.7 ± 0.5 (91)
Total globulins (g/dl)	
Male	1.7 ± 0.5 (107)
Female	2.1 ± 0.5 (90)
Bile acids (μmol/l)	
Male	2.2 ± 3.1 (25)
Female	2.1 ± 3.3 (36)
Aspartate aminotransferase (IU/l)	
Male	73.2 ± 32.5 (67)
Female	63.5 ± 21.0 (69)
Alanin aminotransferase (IU/l)	
Male	2.9 ± 2.0 (46)
Female	2.7 ± 2.4 (59)
Alkaline phosphatase (IU/l)	
Male	72.5 ± 29.4 (34)
Female	107.3 ± 68.8 (45)
Calcium (mg/dl)	
Male	10.3 ± 1.0 (103)
Female	13.2 ± 1.5 (92)
Phosphorus (mEq/l)	
Male	1.6 ± 0.6 (86)
Female	4.4 ± 1.9 (82)
Cholesterol (mg/dl)	
Male	77.1 ± 20.8 (106)
Female	175.8 ± 56.9 (94)
Triglycerides (mg/dl)	
Male	18.7 ± 25.1 (108)
Female	237.4 ± 187.7 (92)
Total bilirubin (mg/dl)	
Male	0.1 ± 0.1 (34)
Female	0.5 ± 0.4 (44)
Direct bilirubin (mg/dl)	
Male	0.02 ± 0.01 (33)
Female	0.02 ± 0.01 (39)

TABLE 4. Continued.

Parameter	Reference range 0 ± 2 SD (*n*)
Indirect bilirubin (mg/dl)	
Male	0.1 ± 0.1 (34)
Female	0.5 ± 0.4 (44)
Copper (ppm)	
Male	0.6 ± 0.1 (53)
Female	0.5 ± 0.2 (44)
Selenium (ppm)	
Male	0.03 ± 0.01 (67)
Female	0.04 ± 0.01 (49)
Zinc (ppm)	
Male	1.9 ± 1.5 (11)
Female	2.2 ± 1.5 (11)
Vitamin A (μg/ml)	
Male	0.4 ± 0.2 (91)
Female	0.4 ± 0.2 (86)
Vitamin E (μg/ml)	
Male	4.3 ± 3.6 (90)
Female	7.9 ± 5.1 (82)
Testosterone (ng/ml)	
Male	127.0 ± 106.8 (27)
Female	1.5 ± 1.6 (33)
Estradiol (pg/ml)	
Male	
Female	113.6 ± 102.7 (46)
Corticosterone (ng/ml)	
Male	6.9 ± 5.4 (41)
Female	5.1 ± 3.4 (48)
Sodium (mEq/l)	
Male	129.2 ± 6.8 (102)
Female	130.4 ± 8.1 (92)
Potassium (mEq/l)	
Male	4.1 ± 0.6 (99)
Female	4.2 ± 0.5 (87)
Chloride (mEq/l)	
Male	104.4 ± 7.8 (36)
Female	101.9 ± 8.0 (45)
Total carbon dioxide (mEq/l)	
Male	34.6 ± 6.0 (34)
Female	33.0 ± 6.8 (44)
Anion gap (mEq/l)	
Male	−2.6 ± 10.8 (35)
Female	3.5 ± 10.0 (42)
Osmolality (mOs/kg)	
Male	268.1 ± 22.9 (82)
Female	275.8 ± 24.5 (82)

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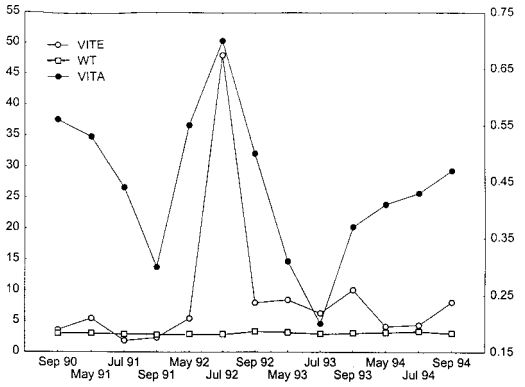


FIGURE 1. Monthly and yearly means in body mass (WT; kg), and vitamin A (VITA; μg/ml) and Vitamin E (VITE; μg/ml) in free-ranging Sonoran desert tortoises (n = 36) from Little Shipp Wash and Harcuvar Mountains (Arizona, USA) 1990–94. Left y-axis = WT; right y-axis = VITA, VITE.

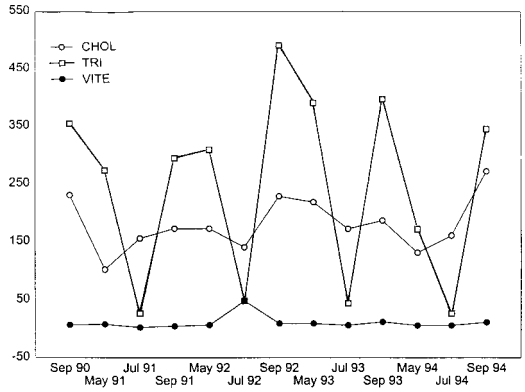


FIGURE 2. Monthly and yearly means in cholesterol (CHOL; mg/dl), triglyceride (TRI; mg/dl), and vitamin E (VITE; μg/ml) in free-ranging female Sonoran desert tortoises (n = 15) from Little Shipp Wash and Harcuvar Mountains, Arizona, USA, 1990–94.

There were also site and sex differences in blood biochemistry. Little Shipp tortoises had higher levels of VITA than Harcuvar tortoises and Harcuvar tortoises had higher levels of Na than Little Shipp tortoises. Female tortoises had higher levels of TP, ALB, CHOL, TRI, Ca, and VITE compared to male tortoises. We determined the monthly and yearly changes in CHOL, TRI, and VITE in female tortoises (Fig. 2). In this study, Ca, TP, and ALB showed little correlation to suspected vitellogenesis. Cholesterol, TRI, and ESTR values peaked in May and September in all years.

Plasma electrolytes, Na and K, showed similar seasonal and yearly changes. Sodium and K had higher levels in July compared to those in May and September and higher levels in 1993 compared to those in 1991, 1992, and 1994. There were no differences ($P > 0.01$) for other blood biochemistry values (BUN, CREAT) with respect to all other site, season, sex, and year combinations.

Plasma 1993–94

Seasonal and yearly changes occurred in FIBR, URIC, and ALT. Fibrinogen and ALT were higher in September compared to May and July, and URIC was higher in May compared to July and September. Fibrinogen and ALT were higher in 1993 compared to 1994, and uric acid was higher in 1994 compared to 1993. Only seasonal changes in GLU, CHLR, and TCO2 were observed. Glucose and CHLR levels were higher in July compared to May and September, and TCO2 levels were higher in September compared to May and July.

Sex and yearly differences were found in TBILI and IBILI. Female tortoises had higher levels of TBILI and IBILI, and there were higher levels of TBILI and IBILI in 1994 compared to 1993.

Site and sex differences in blood biochemistry were evident as well. Little Shipp tortoises had higher levels of URIC than Harcuvar tortoises, and Harcuvar tortoises had higher levels of TCO2 than Little Shipp tortoises. Male tortoises had higher levels of AST compared to female tortoises. No difference ($P > 0.01$) was found for other blood biochemistry values (BILE, ALP, DBILI) and all other site, season, sex, and year combinations.

Permanent and automatic weather station data indicated above average rainfall at Little Shipp in 1992 and 1993 and below average rainfall at Little Shipp in 1994. There was below average rainfall in the Harcuvars in 1991, 1993, and 1994. Mean annual rainfall at the Hillside (Little

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TABLE 5. Monthly rainfall (cm) data from permanent and automatic weather stations near Little Shipp Wash and Harcuvar Mountains, Arizona, 1990–94. Data from permanent and automatic weather stations were combined and averaged.

	1990	1991	1992	1993	1994
Little Shipp Wash, Arizona					
January	4.62	3.51	5.71	20.68	0.84
February	4.83	3.63	6.93	17.5	3.84
March	2.95	17.73	9.12	7.54	3.23
May	3.17	0.0	0.48	0.0	3.23
May	1.04	0.0	4.60	1.04	1.45
June	0.13	0.28	0.38	0.13	0.30
July	8.43	1.8	1.96	0.0	0.84
August	4.65	3.61	14.94	11.15	2.16
September	8.23	2.06	0.0	0.0	3.10
October	1.6	1.83	0.51	2.6	3.86
November	2.54	4.27	0.0	3.6	1.27
December	0.63	2.29	12.7	0.4	6.65
Total rainfall	42.82	41.01	57.33	64.64	30.76
Harcuvar Mountains, Arizona					
January	3.96	1.55	3.0	0.0	0.13
February	0.66	1.75	4.88	12.14	1.96
March	1.27	8.15	8.81	1.91	0.56
May	0.71	0.0	0.63	0.0	0.0
May	0.43	0.0	0.84	0.0	2.31
June	0.23	0.0	0.0	0.0	0.89
July	8.1	0.0	0.76	0.0	3.0
August	3.3	2.31	0.0	0.0	0.94
September	5.87	0.0	0.0	0.0	0.0
October	0.0	1.14	0.0	0.0	0.0
November	0.0	0.91	0.0	0.0	0.0
December	0.0	2.36	6.38	0.0	3.81
Total rainfall	24.53	18.17	25.3	14.05	13.59

Shipp) permanent weather station was 40.0 cm over a 29 yr period (1961–90). Mean annual rainfall at the Aguila (Harcuvars) permanent weather station was 24.3 cm over the same period. Automatic weather data also indicated higher rainfall in February and August compared to all other months. Monthly rainfall data per site is listed in Table 5.

DISCUSSION

Peterson (1996a) found most of the variation in field metabolic rates in free-ranging Mojave desert tortoises was due to a single climatic factor—rainfall. A similar response was found in this study as seasonal and yearly changes in rainfall were associated with variations in Sonoran tortoise blood values. The amount of rainfall determined the amount of forage available to the Sonoran tortoises and drove the "water metabolism strategy."

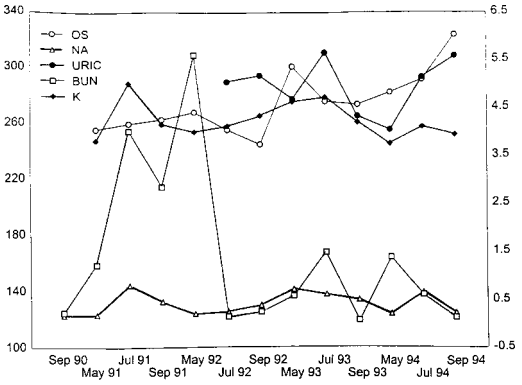


FIGURE 3. Monthly and yearly means in blood urea nitrogen (BUN; mg/dl), uric acid (URIC; mg/dl), sodium (Na; mEq/l), potassium (K; mEq/l), and osmolality (OS; mOs/kg) in free-ranging Sonoran desert tortoises (n = 36) from Little Shipp Wash and Harcuvar Mountains, Arizona, USA, 1990–94 (left y-axis = Na, OS; right y-axis = BUN, URIC, K). Above average rainfall = 1992 and 1993, below average rainfall = 1994.

Seasonal and annual changes in blood chemistry occurred in response to water and forage availability (Fig. 3)—in this study, coined the "water metabolism strategy." May samples were characterized by rising OS and BUN and low URIC levels, suggesting tortoises had not been consuming significant amounts of food or water. BUN elevations may be attributed to protein catabolism as suggested by Christopher et al. (1997). Levels of OS and especially BUN were much lower than those associated with dehydration reported by Christopher et al. (1997). During July, OS and BUN generally decreased as URIC, Na, and K increased, indicating active consumption of water and protein and electrolyte rich forage. In July 1994, a relatively dry year, OS continued to increase, suggesting tortoises were actively feeding but water availability was limited. September samples exhibited the greatest annual variation in OS, URIC, and BUN. Drier years predictably resulted in higher OS, but BUN decreased in September 1994 as

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OS and URIC increased, again indicating tortoises were feeding with restricted water availability. In September 1993, a relatively wet year, OS, URIC, BUN, K, and Na were all declining suggesting increased rates of water consumption and bladder emptying. Seasonal variations in these analyses are considerably different than that reported for Mojave populations (Christopher et al., 1997) and may be accounted for by biphasic rainfall patterns of the Sonoran Desert and the Sonoran tortoises' greater reliance on perennial forage.

The Sonoran Desert has rainfall in the winter and summer (Turner and Brown, 1994). In this study, there were above and below average years in terms of rainfall. Above average rainfall in 1992 and 1993 resulted in more available forage, while below average rainfall in 1994 resulted in less available forage (V. M. Dickinson, unpubl. data). Cable (1975) found high precipitation led to high perennial grass production and available forage is closely related to precipitation (Beatley, 1994) in the Mojave Desert. Increased body mass (WT) and elevated levels of VITA and VITE in 1992 suggests better quality forage or more foraging by tortoises. Significantly, all tortoises with elevated body mass and elevated levels of blood proteins and vitamins were observed foraging at least one week before blood collection (V. M. Dickinson, unpubl. data). From 1991 to 1993, Little Shipp tortoises had increased body mass and elevated URIC compared to Harcuvar tortoises, which also indicates foraging. Uric acid elevations have been associated with potassium and total protein intake in Mojave desert tortoises (Christopher et al., 1997) and occur in carnivorous reptiles after eating (Maixner et al., 1987). Sonoran tortoises did not exhibit reciprocal BUN and URIC seasonal fluctuations as reported for Mojave desert tortoises (Christopher et al., 1997).

A different blood biochemistry scenario occurred in 1994, the year of below average rainfall. In 1994, as tortoises became water and nutrient deprived they lost body mass and URIC levels decreased, while at the same time levels of PCV, BUN, blood proteins (TP, ALB), electrolytes (Na, K), and OS levels increased. Increased levels of CHOL during 1994 were probably a result of fat catabolism. In addition, some Harcuvar female tortoises had higher levels of TBILI and IBILI in 1993, a year of below-average rainfall. This may reflect hepatic change resulting in reduced rates of free bilirubin conjugation in years of below average rainfall in females whose physiology is affected by vitellogenesis. O'Conner et al. (1994) found higher levels of Na and K and OS in water-stressed captive Mojave desert tortoises. Similarly, Peterson (1996b) found free-ranging Mojave desert tortoises in drought conditions had increased urea, OS, and CHLR.

Tortoise sex influenced seasonal changes in clinical biochemistry. Most seasonal changes occurred when female tortoises were sampled in September and May (vitellogenesis). Recent studies reported female desert tortoises had higher levels of ALB, Ca, P, and CHOL compared to males in the fall (O'Connor et al., 1994; Rostal et al. 1994). Elevated Ca levels have been associated with vitellogenesis (Ho, 1987). Taylor and Jacobson (1982) associated higher levels of CHOL with vitellogenesis in female gopher tortoises (*G. polyphemus*). Higher levels of CHOL and lipids were observed in gravid female Mediterranean tortoises (*Testudo graeca* and *T. hermanni*) in August (time of oviposition) compared to males (Lawrence, 1987).

The observed peaks in CHOL and TRI in May and September may suggest vitellogenesis is continuous from September until May and not biphasic during the calendar year. Rostal et al. (1994) found captive tortoises in vitellogenesis in spring and fall as indicated by increased Ca levels and follicular growth. Unlike Mojave desert tortoises, Sonoran tortoises are monestrous, laying a single clutch of eggs each year (Murray et al., 1996). In addition,

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Palmer and Guillette (1990) found increased levels of ESTR during vitellogenesis in free-ranging gopher tortoises.

Higher levels of AST in male tortoises possibly resulted from increased activity and male aggression associated with mating. O'Connor et al. (1994) suggested an increase in AST levels in male desert tortoises was associated with mating or fighting in the spring. Male tortoises have been observed fighting in the fall in the Sonoran Desert (Vaughan, 1984).

For future physiologic assessment of tortoise populations, designing and implementing appropriate health monitoring programs is recommended. If the objective of the monitoring program is to compare tortoise health between two sites with different land management practices, factors such as soil type, vegetation, rainfall patterns, and incidence of disease should be similar. Otherwise, such factors must be accounted for in any analysis. Collecting health data at the same time and frequency at each study site is necessary to ensure proper statistical comparison, as is using an univariate approach to analyzing the data.

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