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Physical and Biochemical Abnormalities Associated with Prolonged Entrapment in a Desert Tortoise

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ABSTRACT: A desert tortoise (*Gopherus agassizii*) was trapped underground without food or water for nearly 11 mo near Yucca Mountain, Nevada (USA). Physical abnormalities included weight loss, sunken eyes, and muscle atrophy. Biochemical abnormalities determined from blood sampling included marked azotemia and hyperosmolality, which were attributed largely to accumulation and retention of nitrogenous wastes. Moderate hypercholesterolemia, hypophosphatemia, and increased aspartate transaminase activity, and mild hyperchloremia, hypocalcemia, hyperbilirubinemia and anemia also were observed, compared with results obtained from other tortoises sampled at the same time. The lack of, or only mild, alterations in most laboratory data exemplified the high degree of physiological adaptation tortoises can undergo when deprived of food and water for a prolonged period.

Key words: Clinical biochemistry, desert tortoise, dehydration, entrapment, *Gopherus agassizii*, starvation.

Desert tortoises (*Gopherus agassizii*) are well-known for their ability to withstand large imbalances in water, energy, and ion budgets (Nagy and Medica, 1986; Nagy, 1988; Peterson, 1996). Because of the unpredictable availability of resources, desert tortoises have evolved a number of water- and energy-saving mechanisms that allow them to survive in the harsh desert environment. They are able to survive long periods without food and water, such as 2 to 4 mo of winter hibernation, and drought periods with little, if any, plant production. Here are described the physical and biochemical abnormalities in a tortoise trapped underground without food or water for nearly 11 mo.

An adult male desert tortoise was radiomarked on 19 April 1993 as part of a study conducted at Yucca Mountain (Nevada, USA) from 1989 to 1995 to determine factors influencing survival of desert tortoises. This tortoise entered hibernation in Oc-

tober 1993, and exited during March 1994. On 17 October 1994 the tortoise again entered hibernation at the site (36°50'N, 116°25'W; approximately 150 km northwest of Las Vegas (Nevada) but did not exit the following spring. The hibernaculum was situated under a large, fragmented boulder approximately 3.0 × 2.5 × 1.5 m. The tortoise was located weekly using radiotelemetry, but could not be seen in its burrow until 22 June 1995, when a second entrance on the side of the burrow was excavated manually by removing debris from a large crevice in the boulder. Tapping on a foreleg with a long stick elicited weak movement, indicating that the tortoise was still alive. It could be seen that the tortoise was pinned in its burrow by rock fragments that presumably fell sometime during the winter. It also was noted that the radiotransmitter, secured to the fourth pleural scute with epoxy, was not preventing tortoise movement. Attempts to free the tortoise were not pursued at that time because a survival study was being conducted and it was important to allow any events to run their course. In addition, it was potentially dangerous to dig farther into the burrow because of the danger of collapse.

The tortoise was monitored until September 1995 at which time the survival study was completed. The trapped tortoise was carefully excavated from its burrow on 11 September 1995 and transported in a well-ventilated, clean, plastic box to a field laboratory where it was examined, measured, and a blood sample taken. From 6 to 14 September 1995, 81 additional radiomarked tortoises also were brought into the laboratory for examination, measurement, and blood sampling.

The trapped tortoise was lethargic, its

limb and neck musculature had atrophied, and its eyes were sunken in the orbits. Carapace length was 226 mm, and the tortoise weighed 1849 g, 81 g less than it weighed 1 yr earlier on 13 September 1994. In contrast, five male tortoises of approximately the same size each gained >400 g during the same time period. The neck was easily extended to withdraw blood by jugular venipuncture (Jacobson et al, 1992), a procedure that usually requires a considerable amount of force. For all tortoises, 3 to 4 ml of blood were collected using a 5 ml syringe and a 23 gauge needle with a butterfly catheter assembly (Jacobson et al., 1992). Approximately 0.6 ml of whole blood was transferred to a 1 ml tube containing lithium heparin, mixed gently for 2 min, and refrigerated until shipment. The remainder of the sample was transferred to a 5 ml tube containing lithium heparin, mixed gently, and centrifuged for 5 min to separate plasma. Plasma (0.5 ml for antibody determination and 0.5 ml for blood chemistry analysis) was transferred to 1.25 ml plastic tubes and frozen at -78 C until shipment. After drawing blood, tortoises were rehydrated with 6 to 12 ml (approximately two to three times the volume of blood drawn) of saline:dextrose solution (50:50) injected subcutaneously into the axillary region. The trapped tortoise received 12 ml of the rehydration solution.

Frozen plasma and chilled whole blood samples were delivered within 10 hr to a veterinary diagnostic laboratory (APL Laboratories, Las Vegas, Nevada, USA). Plasma was analyzed using a Hitachi 747-200 automated chemistry analyzer (Boehringer Mannheim Corp., Indianapolis, Indiana, USA) for glucose (hexokinase method), blood urea nitrogen (BUN; urease method), creatinine (modified Jaffe method), uric acid (uricase method), total protein, albumin, calcium, phosphorus, total bilirubin, triglycerides (enzymatic method), cholesterol, iron (ferrene method), and magnesium concentrations; for alkaline phosphatase (ALP), aspartate aminotrans-

ferase (AST), and alanine aminotransferase (ALT) activities; and for sodium, potassium, chloride and total carbon dioxide (TCO_2) concentrations (indirect ion-selective potentiometry). Globulins, albumin/globulin (A/G) ratio, anion gap, and osmolality were calculated automatically by the analyzer. Hematologic analyses were done using the methodology of Campbell (1995), including packed cell volume (PCV; microhematocrit centrifugation, International Equipment Co., Boston, Massachusetts, USA), red blood cell (RBC) count (hemacytometer, Fisher Scientific, Pittsburgh, Pennsylvania, USA), total white blood cell (WBC) count (hemacytometer, using Nate and Herrick's solution), and hemoglobin (Hb; cyanmethemoglobin method preceded by centrifugation of lysate). Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated using RBC, PCV, and Hb values by the method in Duncan et al. (1994). Blood smears stained with modified Wright stain were examined microscopically to obtain a 100-cell differential WBC count (heterophils, lymphocytes, basophils, eosinophils, monocytes and azurophils).

Plasma samples for antibody determinations were shipped on dry ice to the University of Florida (Biotechnologies for the Ecological, Evolutionary, and Conservation Sciences, Immunological Analysis Laboratory, Gainesville, Florida, USA), where they were tested for antibodies specific to *Mycoplasma agassizii* using an ELISA test detailed in Schumacher et al. (1993).

Compared with results from other tortoises sampled (Table 1), the trapped tortoise had marked hyperosmolality and marked azotemia, with a marked increase in BUN and mild hyperuricemia. The tortoise also had moderate hypercholesterolemia, hypophosphatemia, and increased AST activity. There were mild increases in ALP and ALT activities, mild hyperchloremia and hypocalcemia, a slight decrease

TABLE 1. Comparison of blood chemistry and hematology values from a desert tortoise (*Gopherus agassizii*) trapped in its burrow for over 11 mo, with those of other desert tortoises sampled at Yucca Mountain in September 1995.

Analyte (units)	Trapped Tortoise	Population Values		
		Range ^a	Median	<i>n</i>
Osmolality (mOsm/kg)	395 ^b	253–304	271	81
Urea nitrogen (mg/dl)	259 ^b	1–7	1	81
Uric Acid (mg/dl)	7.6 ^b	0.8–5.5	2.3	81
Sodium (mEq/L)	156	129–157	139	81
Potassium (mEq/L)	4.5	2.8–5.5	4.2	81
Chloride (mEq/L)	130 ^b	99–128	109	81
Total CO ₂ (mEq/L)	23	20–35	26	81
Anion gap (mEq/L)	8	3–17	8	81
Phosphorus (mg/dl)	1.6 ^b	2.0–4.5 ^c	2.6	81
Calcium (mg/dl)	9.1 ^b	9.8–13.1 ^c	10.6	35
Glucose (mg/dl)	70	35–97	66	81
Total protein (g/dl)	2.1	1.5–5.2	3.1	81
Albumin (g/dl)	0.7	0.6–2.2	1.3	81
Globulin (g/dl)	1.4	0.9–3.0	1.8	81
Albumin/globulin ratio	0.50 ^b	0.57–0.97	0.74	81
Total bilirubin (mg/dl)	0.3	0–0.1	0.1	81
Cholesterol (mg/dl)	154.0 ^b	24.9–92.3 ^c	44.0	35
Alkaline phosphatase (IU/L)	254 ^b	27–243	95	81
Aspartate aminotransferase (IU/L)	155 ^b	10–77 ^c	29	35
Alanine aminotransferase (IU/L)	6 ^b	1–5	5	81
Iron (μg/dl)	46	20–94	49	81
Hematocrit (%)	17.5	14.5–45.5 ^c	25.5	35
Red blood cells (× 10 ⁶ /μl)	0.40 ^b	0.46–1.07 ^c	0.78	35
Hemoglobin (g/dl)	4.9	3.5–10.3 ^c	8.0	35
Mean cell volume (fl)	437.5	185.8–595.2	325.6	80
Mean cell hemoglobin (pg)	122.5	42.5–162.8	101.7	79
Mean cell hemoglobin concentration (%)	28.0	13.5–43.9	31.9	79
Total white blood cells (/μl)	4050	1055–6995	3205	80
Heterophils (/μl)	3361	557–3631	1491	80
Lymphocytes (/μl)	203	76–2409	546	80
Basophils (/μl)	486	0–3307	730	80
Eosinophils (/μl)	0	0–86	0	80
Monocytes (/μl)	0	0–131	0	80
Azurophils (/μl)	0	0–532	0	80

^a Range is mid-95th percentile when *n* > 40; minimum and maximum values when *n* < 40.

^b Value is outside the population reference range.

^c Data for males only, as values differ between sexes (Christopher et al. 1999).

in the A/G ratio, and slight anemia, the latter based on low RBC count and low normal Hb concentration and PCV. Albumin and sodium concentration were at the low and high end of reference values, respectively.

Hyperosmolality was attributed largely to the marked increase in BUN, likely resulting from a combination of decreased excretion of nitrogenous wastes, increased protein catabolism and dehydration. On a

molar basis, urea concentration was 92.5 mmol/L, sufficient to result in most of the increase in osmolality. The entrapment of this tortoise would have prevented water intake and the tortoise most likely retained urine while trapped. As urine urea concentration rises, urine production and urea excretion decrease, although complete anuria does not occur until blood osmolality is raised more than 100 mOsm/kg or exceeds 400 mOsm/kg (Dantzler and

Schmidt-Nielson, 1966). The osmolality of this tortoise (395 mOsm/kg) and the likely magnitude of increase (>100 mOsm/kg) if initial osmolality was near the median value for the population (271 mOsm/kg), suggested that this tortoise was producing minimal urine. The increase in plasma osmolality is rapid once urine osmolality has increased to about 300 mOsm/kg, and is associated with the diffusion of urea from the bladder, back into the blood. In experimentally dehydrated *Gopherus* sp., urea reabsorption through the highly permeable bladder wall contributed to blood urea concentrations of up to 123 mmol/L (Baze and Horne, 1970), similar to the level observed in this tortoise. It is possible that prolonged retention of urine could have led to subsequent urolithiasis or had adverse effects on renal function that further exacerbated the azotemia, but this could not be ascertained from these data.

Uric acid is also excreted in the urine, but is efficiently precipitated out as potassium salts, so that uric acid excretion can occur even when urine is retained, with minimal effect on blood uric acid concentration (Minnich, 1977). The mild increase in uric acid and normal plasma potassium concentration of the trapped tortoise suggested that efficient potassium excretion and storage in the urine as urate salts had occurred. Decreased potassium intake, and dilution of plasma constituents by intravascular water shifts probably also served to keep blood potassium concentration low.

The magnitude of azotemia in this tortoise was at least three times greater than that which has been described previously. When muscle and other body proteins are catabolized during hibernation or starvation, there is an increase in the production of nitrogenous waste (urea and uric acid) (Baze and Horne, 1970). Protein catabolism in this tortoise was evidenced by weight loss, muscle wasting and atrophy. Protein catabolism may have contributed, at least in part, to the low normal albumin concentration and subsequent decrease in

A/G ratio. Lipids also can be utilized as energy during starvation, resulting in increased blood concentration of ketone bodies (Christopher et al., 1994). However, a marked increase in ketones was unlikely as there was no evidence for metabolic acidosis (total CO₂ concentration was within reference values), nor was the anion gap increased.

Normal blood glucose concentration suggested that protein catabolism was sufficient to maintain glucose concentration in the absence of any food intake. Glycogen stores accumulated prior to hibernation were likely depleted, since it has been shown that liver glycogen drops to 5% of normal levels after 80 days of fasting in *Gopherus berlandieri* (Horne and Findisen, 1977). Decreased nutrient intake probably accounted for the hypophosphatemia, hypocalcemia, and mild anemia in the trapped tortoise. Blood phosphorus concentration, in particular, decreases markedly during drought and, especially, hibernation, but increases following ingestion of forage (Christopher et al., 1999). Hypocalcemia has been reported in wasting giant tortoises (Samour et al., 1986).

Dehydration was mild in the trapped tortoise probably because of efficient water retention (O'Connor et al., 1994). The tortoise's eyes were sunken, but body weight decreased by only 4.2%, remarkably little loss after 11 mo of water and food deprivation. Tortoises are able to use urine in their bladders as a water reservoir, which equilibrates with plasma during drought and dehydration (Baze and Horne; 1970; Nagy and Medica, 1986). A moist burrow environment also may have minimized evaporative water loss during entrapment. Hyperbilirubinemia, hyperchloremia and high normal sodium concentration were probably caused by dehydration, but these alterations were mild and did not contribute substantially to hyperosmolality, consistent with earlier studies on drought and dehydration in desert tortoises (Dantzler and Schmidt-Nielson,

1966; Minnich, 1977; O'Connor et al., 1994; Peterson, 1996).

Increased AST activity may have resulted from tissue and/or muscle damage associated with pressure and necrosis from rocks and debris. Skeletal muscle in most species contains a large amount of this enzyme, but AST also is found in many other tissues, including liver, kidney and RBC. Hepatic abnormalities can cause increased plasma AST, ALP and ALT activities, as well as hyperbilirubinemia and hypercholesterolemia.

It has been argued that upper respiratory tract disease, present in many desert tortoise populations, is manifested during periods of stress, such as drought (Jacobson et al., 1991). Hypercholesterolemia, hypophosphatemia, and anemia, all of which were observed in this trapped tortoise, have been reported in tortoises with upper respiratory tract disease (Jacobson et al., 1991). It is possible that these laboratory abnormalities were the result of decreased food intake rather than the direct effects of respiratory disease. Although other tortoises at Yucca Mountain have tested positive for antibodies to *Mycoplasma agassizii*, a causative agent of upper respiratory tract disease (Brown et al., 1994), the trapped tortoise was seronegative and no clinical signs of the disease were observed.

In summary, the most profound blood chemistry abnormalities in this tortoise were azotemia and resultant hyperosmolality, largely attributed to accumulation and retention of nitrogenous wastes. The lack of, or only mild alterations in other blood parameters exemplified the high degree of physiological adaptation tortoises are capable of when deprived of food and water for a prolonged period. Expiration of federal handling permits and removal of the radiotracer precluded further monitoring of this tortoise. Although its ultimate fate is unknown, this case presents the longest documented time period during which a desert tortoise underwent complete deprivation of food or water. An

evolutionary history of tolerating large changes in ionic and water balance played a major role in the survival of this tortoise trapped underground in its hibernaculum for nearly 11 mo.

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