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OXALOSIS IN WILD DESERT TORTOISES, *GOPHERUS AGASSIZII*

Elliott R. Jacobson,[1,4] Kristin H. Berry,[2] Brian Stacy,[1] Louis M. Huzella,[3] Victor F. Kalasinsky,[3] Michelle L. Fleetwood,[3] and Mark G. Mense[3]

[1] College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610, USA
[2] U.S. Geological Survey, Western Ecological Research Center, Moreno Valley, California 92553, USA
[3] Armed Forces Institute of Pathology, Washington, D.C. 20306-6000, USA
[4] Corresponding author (email: jacobsonE@mail.vetmed.ufl.edu)

ABSTRACT: We necropsied a moribund, wild adult male desert tortoise (*Gopherus agassizii*) with clinical signs of respiratory disease and elevated plasma biochemical analytes indicative of renal disease (blood urea nitrogen [415 mg/dl], uric acid [11.8 mg/dl], sodium [>180 mmol/l] and chloride [139 mmol/l]). Moderate numbers of birefringent oxalate crystals, based on infrared and electron microscopy, were present within renal tubules; small numbers were seen in colloid within thyroid follicles. A retrospective analysis of 66 additional cases of wild desert tortoises was conducted to determine whether similar crystals were present in thyroid and kidney. The tortoises, from the Mojave and Sonoran deserts, were necropsied between 1992 and 2003 and included juveniles and adults. Tortoises were classified as healthy (those that died due to trauma and where no disease was identified after necropsy and evaluation by standard laboratory tests used for other tortoises) or not healthy (having one or more diseases or lesions). For all 67 necropsied tortoises, small numbers of crystals of similar appearance were present in thyroid glands from 44 of 54 cases (81%) and in kidneys from three of 65 cases (5%). Presence of oxalates did not differ significantly between healthy and unhealthy tortoises, between age classes, or between desert region, and their presence was considered an incidental finding. Small numbers of oxalate crystals seen within the kidney of two additional tortoises also were considered an incidental finding. Although the source of the calcium oxalate could not be determined, desert tortoises are herbivores, and a plant origin seems most likely. Studies are needed to evaluate the oxalate content of plants consumed by desert tortoises, and particularly those in the area where the tortoise in renal failure was found.

Key words: Calcium oxalate, desert tortoise, *Gopherus agassizii*, renal failure.

INTRODUCTION

The desert tortoise (*Gopherus agassizii*) is a federally listed threatened species in the southwestern United States (US Department of the Interior, 1990). In 1989, a long-term research program was established to determine causes of illness and death in desert tortoises (Christopher et al., 1999). Part of this program involved salvaging ill, moribund, or severely injured tortoises for necropsy to determine causes and contributors to death (Berry and Christopher, 2001). Through this program, several new diseases in wild desert tortoises have been described, including mycoplasmosis, cutaneous dyskeratosis, shell necrosis, urolithiasis, sarcocystosis, renal and polyarticular gout, and mycotic pneumonia (Jacobson et al., 1991, 1994; Brown et al., 1994; Homer et al., 1998; Christopher et al., 2003).

Here, we report renal and thyroid oxalosis in a wild adult male desert tortoise from western San Bernardino County, California, USA. The tortoise was observed in the field in 2003, was lethargic, and had clinical signs of upper respiratory tract disease typical of mycoplasmosis (Jacobson et al., 1991; Brown et al., 1994). It was determined to have been in renal failure by a plasma chemistry panel (blood urea nitrogen [BUN], 415 mg/dl; uric acid [UA], 11.8 mg/dl; sodium [Na], >180 mmol/l; and chloride [Cl], 139 mmol/l). Crystals in the renal tubules were seen and were identified as oxalates by infrared spectroscopy. A retrospective analysis of histologic samples from 66 additional and previously necropsied desert tortoises was conducted to determine effects of age, health status, and geographic location on presence of oxalate in thyroid and kidney.

MATERIALS AND METHODS

Study area and tortoise evaluations

The study area encompassed parts of the Mojave and Colorado/Sonoran deserts, USA (Fig. 1; clockwise from the NW corner:

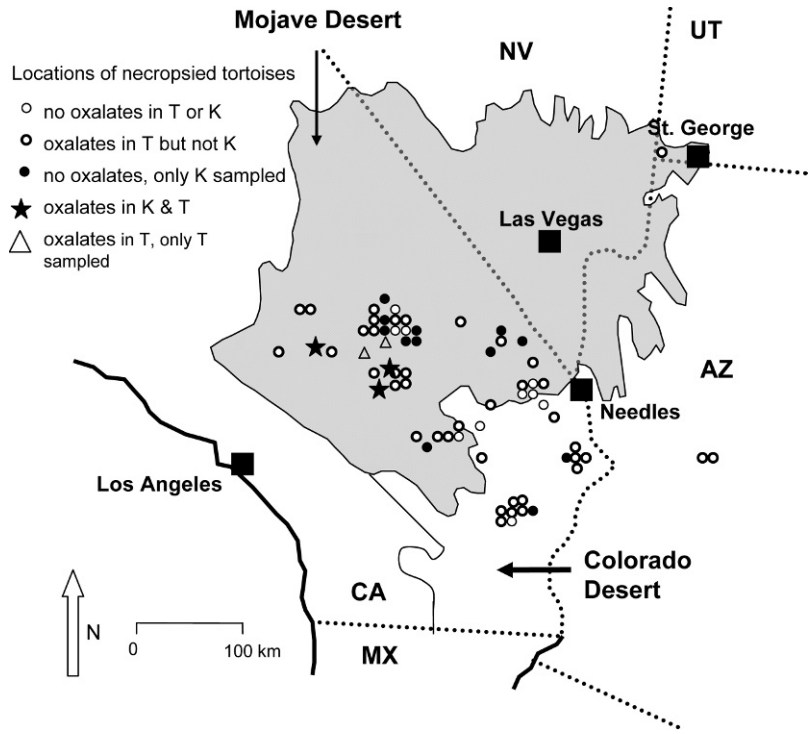

FIGURE 1. Map of southern California, and adjacent areas of Nevada, Arizona, and Utah showing the locations of tortoises having oxalates in thyroid (T) and kidney (K) and those lacking oxalates in T, K, or both.

35°13′N, 117°50′W; 36°59′N, 113°54′W; 33°32′N, 115°30′W; 34°28′N, 113°04′W). The tortoises were primarily from southern California; two tortoises were from western Arizona and one tortoise was from southwestern Utah. The tortoises were salvaged from the wild and were submitted for pathologic evaluation due to a variety of health problems, including shell necrosis, cutaneous dyskeratosis, respiratory disease, starvation or dehydration, and trauma (Homer et al., 1998; Berry et al., 2002). Techniques used to necropsy desert tortoises have been described previously (Homer et al., 1998; Berry et al., 2002). As part of health assessment, hematologic and plasma biochemical analytes were evaluated against standards developed for wild, healthy adult tortoises in the western, northeastern, and eastern Mojave Desert regions (Christopher et al., 1999). After the light microscopic observation of the crystals in one tortoise (no. 75), a retrospective study was performed on 66 additional desert tortoises that were necropsied between 1992 and 2003. Of these, thyroid glands from 54 tortoises and kidneys from 65 tortoises were available and in suitable condition for histologic examination. We used Fisher's exact test (SPSS Inc., 2004) to compare the presence of oxalates between age classes (juveniles and adults), health categories (healthy or not healthy), and origins (Mojave or Colorado/Sonoran desert). For these tests, we used a smaller subset of tortoises (n=52), where histologic slides were available for both sets of tissues from the same tortoise. Healthy tortoises were those that died due to trauma and where no disease was identified after necropsy and evaluation by standard laboratory tests used for other tortoises. Oxalate crystals were categorized based on the following relative assignment by number of crystals (Stacy et al., 2008): none (0), rare (1-plus), small (2-plus), moderate (3-plus), and large (4-plus).

Crystal analysis

To confirm the chemical composition of the crystals in tortoise 75, we followed the methods described by Murakata et al. (2006). In brief, specimens approximately 5 μm thick were cut from paraffin-embedded formalin-fixed tissue and mounted on aluminum-coated glass slides for infrared analysis or on carbon disks for scanning electron microscopy. Infrared spectra were obtained using a Nicolet Continuum infrared microscope attached to a Nicolet model 860 Fourier transform infrared spec-

trometer (ThermoNicolet, Madison, Wisconsin, USA). The measured infrared spectra were compared with those of authentic samples and to spectra stored in a digital spectral library. A Hitachi model S-3500N scanning electron microscope (Hitachi, San Jose, California, USA) equipped with a ThermoNoran energy-dispersive X-ray spectroscopy accessory (ThermoNoran, Madison, Wisconsin, USA) was used to determine the elemental composition of the materials in the tissue.

RESULTS

Desert tortoise 75

In the field (5 June 2003), tortoise 75 was lethargic, had dried yellow exudate over its nares, ocular discharge, with edema and crusting of the lower palpebrae. At necropsy (13 June 2003), both nares were occluded with exudate. Blood urea nitrogen (415 mg/dl), UA (11.8 mg/dl), Na (>180 mmol/l), and Cl (139 mmol/l) exceeded mid-95% reference intervals for healthy tortoises in the western Mojave Desert in spring, the time of salvage (Christopher et al., 1999: BUN, 1–13 mg/dl; UA, 1.8–8.9 mg/dl; Na, 125–160 mmol/l; and Cl, 94–122 mmol/l). These analyte levels supported a diagnosis of renal failure. Throughout the kidney, renal tubules were obstructed or obliterated by moderate numbers of birefringent crystals that resembled those of calcium oxalate (Fig. 2A). Spicules and arrays of crystals were surrounded by inflammatory cells that ranged from loosely arranged pigment-laden macrophages to distinct granulomas with multinucleated giant cells. Many macrophages contained phagocytized crystal fragments. Leukocytes and thin bands of fibrous connective tissue expanded the surrounding interstitium. A flattened layer of cells surrounded some crystals with little or no additional tissue response. The crystals were birefringent with polarization microcopy (Fig. 2B). Additional findings included small numbers of urate tophi scattered throughout the kidneys and moderate amounts of melanin granules within the cytoplasm of renal epithelial cells. Light microscopic examination of the thyroid of tortoise 75 revealed minimal numbers of similar crystalline material within thyroglobulin (Fig. 3A). Using polarizing microscopy, the crystalline material also was birefringent (Fig. 3B). In contrast to the kidney, there was no inflammation associated with these intracolloidal crystals.

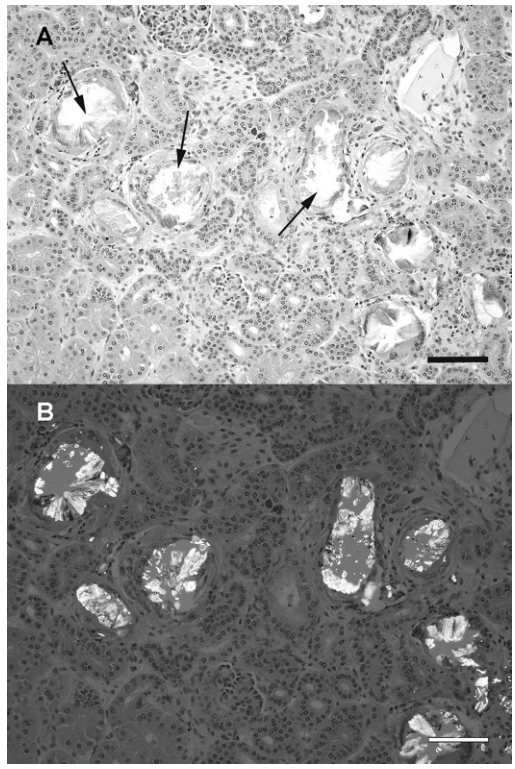

FIGURE 2. Light microscopic image of the kidney of a desert tortoise. A. Crystalline material (arrows) is scattered throughout renal tubules. H&E stain. Bar=100 μm. B. Crystalline material is birefringent. H&E stain. Bar=100 μm.

The infrared spectra of the anisotropic crystalline material seen in the kidney exhibited all of the peaks (Fig. 4) associated with an authenticated sample of calcium oxalate monohydrate and differed from an adjacent area of kidney lacking this material. In addition, the scanning electron microscopy with energy dispersive X-ray analysis confirmed the presence of calcium. The crystalline morphology and results from spectroscopy supported a diagnosis of calcium oxalate.

Tortoise 75 also exhibited changes in the nasal cavity consistent with upper

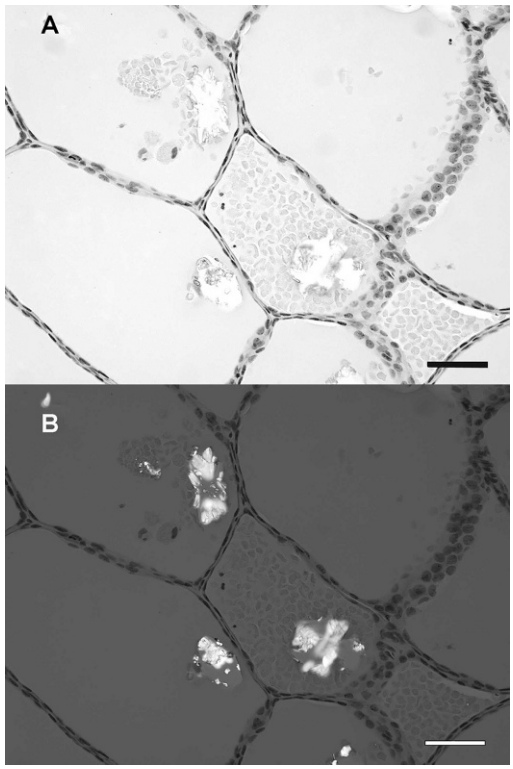

FIGURE 3. Light microscopic image of the thyroid of a desert tortoise. A. Crystalline material is seen within thyroglobulin. H&E stain. Bar=50 μm. B. Crystalline material is birefringent. Bar=50 μm.

respiratory tract disease (Jacobson et al., 1991) caused by *Mycoplasma agassizii* (Brown et al., 1994). There was diffuse hyperplasia of nasal cavity mucosal epithelial cells with infiltrates of heterophils, lymphocytes, and some macrophages. *Mycoplasma agassizii* was not indentified in the nasal cavity using nasal cultures and a polymerase chain reaction test (Brown et al., 1995). However, the tortoise was seropositive when tested for exposure using an enzyme-linked immunosorbent assay (Schumacher et al., 1993).

Results of retrospective analyses of all 67 cases

Forty-eight cases were from the Mojave Desert and 19 were from the Colorado/Sonoran Desert (Fig. 1). The 54 tortoises with thyroid available were five juveniles, 18 adult females, and 31 adult males, ranging in carapace length at the midline from 56 mm to 320 mm. Forty-four of these 54 tortoises (81%) had similar looking birefringent crystals scattered within the colloid of thyroid follicles. Those having thyroid crystals consisted of three juveniles, 17 adult females, and 24 adult males. All had amounts that were categorized as minimal and were considered incidental findings. In addition to tortoise 75, two other tortoises (one adult male and one adult female) had similar looking intratubular crystals scattered throughout the kidney. As with the thyroid, the crystals were categorized as minimal in number and, in contrast to tortoise 75, were considered incidental findings in both cases and unlikely to affect renal function.

Of the 52 tortoises with both thyroid and kidney available, 10 were healthy and 42 were ill, with one or more of the following primary diseases or lesions: cutaneous dyskeratosis (17); fungal infection and colonization (three); inflammation of chin glands, eyelids, and salivary glands (one); mycoplasmosis (10); osteopenia or malnutrition (three); pneumonia (three); renal gout (one); renal oxalosis (two); shell necrosis (one); thyroid dysplasia or atrophy (two); and trauma associated with burns (one). Pathology of diseases of the first 32 tortoises in this retrospective study has been published previously (Homer et al., 1998; Berry et al., 2002). Tortoises with oxalates in the thyroid were common among both ill and healthy groups, whereas tortoises with oxalates in the kidney also had oxalates in the thyroid and occurred only in the ill group. Presence of oxalates in the thyroid and kidney did not differ significantly between healthy and unhealthy tortoises, between age classes, or between desert regions (Fisher's exact test, $P=0.382$, $P=0.242$, and $P=0.467$, respectively).

DISCUSSION

Renal oxalosis, previously unreported in desert tortoises, was seen in desert tortoise

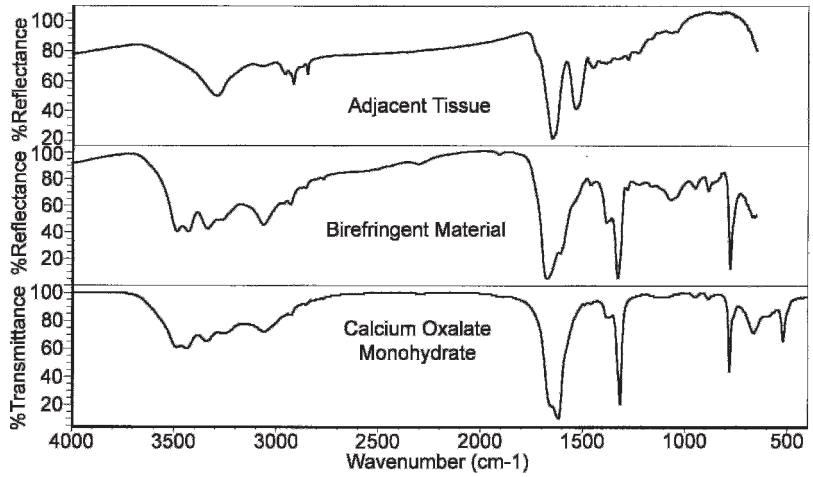

FIGURE 4. Infrared spectrum of birefringent material (center) within the kidney of a desert tortoise was compared with adjacent renal tissue (top) and to an authenticated sample of calcium oxalate monohydrate. The crystalline material exhibited all of the peaks associated with an authenticated sample of calcium oxalate monohydrate and differed from an adjacent area of kidney lacking this material.

75, which had renal tubules that were obstructed by crystalline material. Infrared spectral and scanning electron microscopic analysis of kidney identified the crystals as calcium oxalate. The changes seen in the kidney associated with crystals of calcium oxalate that were observed by light microscopy were considered sufficient to compromise renal function. In a retrospective study of necropsied desert tortoises where kidney was evaluated, two additional cases were found having similar-looking crystals in the kidney. However, the amount of crystals in the kidney was categorized as minimal and was considered an incidental finding and unlikely to affect renal function.

In domestic animals, oxalate toxicosis can be caused by ingestion of ethylene glycol or oxalate-accumulating plants (Maxie and Newman, 2007). Congenital renal oxalosis has been reported in calves (Gospal et al., 1978). In humans, oxalosis also may result from systemic *Aspergillus* infections (Nime et al., 1973). Given the unlikely exposure of wild desert tortoises to ethylene glycol and no evidence of *Aspergillus* infection, we believe that a plant origin seems the most likely source of the renal calcium oxalate seen in desert tortoises. In a recent study in green turtles (*Chelonia mydas*), a herbivorous species, renal oxalosis was seen in 18 turtles from two different geographic locations (Stacy et al., 2008). Consumption of oxalate-accumulating plants also was thought to be the source of the renal oxalosis. Desert tortoises often experience extended periods of dehydration and starvation during or after droughts (Berry et al., 2002); dehydration and starvation may influence oxalate deposition in the kidney. Many native plants in the Mojave and Colorado deserts of California where tortoises occur contain oxalates, particularly in the families Cactaceae (Shirley and Schmidt-Nielsen, 1967), Brassicaceae (*Brassica* sp.), Chenopodiaceae, and Poaceae (*Pennisetum* spp.; Burrows and Tyrl, 2001). The desert tortoise has evolved with desert species in these plant families. Unfortunately, in the last few decades, many alien species, such as the Saharan or Moroccan mustard (*Brassica tournefortii*) and fountain grass (*Pennisetum setaceum*) have invaded the Mojave and Colorado deserts (Lovich, 2000; Minnich and Sanders, 2000). Although tortoises have not been observed to forage on *B. tournefortii* or *P. setaceum*, no efforts have been made to

determine whether they will consume them under drought or experimental conditions, and no research has been conducted on the oxalate content. Studies are needed to evaluate the oxalate content of alien species of mustards such as *Brassica* and the commonly eaten native cactus *Opuntia basilaris*.

Although spectral analysis was not performed on the crystalline material in the thyroid, we assume that the crystals also represent calcium oxalate because the morphology of the material observed by light microscopy was the same as that seen in the kidney. Intracolloidal deposition of calcium oxalate crystals in the thyroid is documented in many mammalian species, including greater than 50% prevalence in humans, chimpanzees, goats, ewes and sows (Reid, 1991). Based on our findings, desert tortoises have a similarly high prevalence. Furthermore, oxalate crystals in human thyroids seem to dissipate within hours after death (Katoh et al., 1993b); thus, prevalence and numbers of crystals may be underestimated in our study due to the prolonged postmortem interval in many cases. Intracolloidal oxalate crystals are observed in the absence of exogenous oxalate exposure, disturbances in oxalate metabolism, or significant histologic changes in affected glands (Katoh et al., 1993b). Crystals have been observed in normal thyroid glands, as well as goiters, follicular adenomas, and carcinomas, and there is no apparent association with concurrent disease (Katoh et al., 1993a, b). In our study, no correlation was found between health status and presence of crystals in the thyroid. In all cases, minimal amounts of crystals were observed in the thyroid, and their presence was considered an incidental finding. In humans, crystal formation increases with age and it is hypothesized that formation is associated with a low functional state of affected follicles (Katoh et al., 1993b). Age, and thus accumulation of oxalates, may not be factors because there was no statistically significant difference between juvenile and adult tortoises in presence of oxalates. However, the sample size of juveniles was so low that the subject should be revisited when a larger number of samples from juveniles becomes available.

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