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Morphology and Histology of the Digestive System of the Desert Tortoise, *Gopherus agassizi*. Linnaeus Fund Research Report

ROBERT M. WINOKUR[1], MARTIN HICKS[1], AND NAOMI CHANEY[2]

[1]*Department of Biology, University of Nevada, Las Vegas, Nevada 89154 USA [E-mail: winokur@ccmail.nevada.edu];*
[2]*School of Medicine, University of Nevada, Reno, Nevada 89557 USA*

Little is known about the details of structure of the digestive tract of chelonians. Chelonians have often been included in broader surveys where a variety of different groups of reptiles are examined. In such studies, (e.g., Luppa, 1977), the chelonians included are often only a few European species. This results in a substantial underestimate of the variation in structure that has evolved in this reptilian order. Our study focused on the American desert tortoise, *Gopherus agassizi*. In spite of considerable official concern for the survival of this species, there are few detailed studies of the anatomy and histology of this or any other species of tortoise. Some general information on the chelonian digestive system and histology (including tortoises) may be found in the veterinary literature (e.g., Frye, 1991; Boyer and Boyer, 1996). Barboza (1995) studied the digestive tract of the desert tortoise and provided a drawing showing the major named regions. Some histologic content may be found in reports that focus on specific pathology and parasitism (e.g, Mader, 1996). Information on the normal histology of the digestive system, however, is an important component of studies on diet, nutrition, and general biology, which in turn are important in tortoise conservation.

Our overall objectives were to study the details of anatomic and histologic structure and proportion in various regions of the digestive tract of both adult and juvenile desert tortoises.

Methods. — The digestive tracts of three juvenile and two adult *G. agassizi* were utilized for histologic studies. Specific regions of the gut examined were the esophagus, esophagus-stomach junction, stomach, pyloric sphincter, duodenum, ileum, ileocecal valve, large intestine, cecum, large intestine-rectum juncture, and rectum. No clear distinction could always be made between regions of the small intestine in juvenile specimens.

Thirty-five blocks of tissue were fixed in 10% neutral buffered formalin, or Bouin's fluid, embedded in paraffin, and sectioned at 6 to 10 µm. Stains utilized were Ehrlich's hematoxylin and eosin (H & E), periodic acid Schiff's (PAS), Van Gieson's picrofushin, Lillie's modifications of Masson's trichrome, alcian blue (pH 2.5) and PAS with Groat's hematoxylin, and Goldner's modification of Masson's trichrome.

Twenty-three preserved hatchlings and juvenile specimens were used to study the proportional relationships and gross morphology of the gut. Gut length was determined by using a piece of string to measure the entire length of the gut and then measuring the length of the string to the nearest mm. Gut mass was determined by first removing all gut contents by dissection and gentle washing, then weighing the entire wet but drained intestinal tract to the nearest mg, and then weighing individually dissected segments of the gut to obtain the mass of the esophagus, stomach, small intestine, and large intestine. The vestige of the yolk sac was removed before weighing because it varied greatly in size and was an uncontrolled variable. Dry weight was then determined by drying the specimens in an oven until no significant weight loss occurred.

Results. — The gut of the desert tortoise is anatomically simple without blind diverticula and largely conforms in structure to a generalized vertebrate gut. Considerable variation between adult and juvenile age classes with regard to gut layers and morphology was observed. Most of this variation occurs in the mucosa, including its component smooth muscle layer, the muscularis mucosa. Variation was largely absent between age classes in general staining properties of the mucosal epithelium, however, morphological differences were evident. The esophageal mucosa of the juvenile had elongated, occasionally branching papillae that were absent in adults. In the esophagus, the muscularis mucosa was well developed in adults but thin and single layered in juveniles. A pyloric sphincter was found in all specimens examined and consisted of a thickening of the muscularis externa when compared to adjacent regions. The stomach of juveniles contained numerous tubular acinar glands that were represented as elaborate branched villi-like projections in the adult specimens. The small intestine of both juveniles and adults had regional variations in mucosal structure. The duodenum of the adult had numerous, deep, complex branching invaginations. This changed distally with the appearance of long, broad papillae. The frequency and shape of the papillae varied regionally in adults with the ileum containing longer and larger numbers of papillae. The small intestine of juveniles, however, did not show this pattern and papillae were denser and better developed in the duodenum. The large intestine of adults had regularly occurring folds that contained extensive, elongated tubular glands. This mucosal structure was seen throughout the upper and lower large intestine, although the necks of the glands were more elongated in the lower (more distal) regions. The morphology of the large intestine of juveniles was relatively simple and devoid of convolutions and tubular glands. The rectum of adults had regional variations between the upper and lower portions. In the lower rectum, adjacent

Table 1. Mass (g) and length (mm) distributions of the digestive tract of hatchling and juvenile desert tortoises, expressed as % of total gut. Carapace length of tortoises examined = 63.5 ± 2.8 mm, range 48.1–102.6, $n = 23$; mass (wet weight) of tortoises examined = 44.35 ± 8.46 g, range 18–136, $n = 15$.

	wet weight % $n = 15$	dry weight % $n = 10$	length % $n = 12$
Esophagus	6.08 ± 0.52	5.75 ± 0.55	7.36 ± 0.53
Stomach	22.98 ± 1.14	27.23 ± 1.14	15.34 ± 1.02
Small Intestine	21.62 ± 1.25	23.03 ± 1.35	28.33 ± 1.15
Large Intestine	49.32 ± 1.48	44.00 ± 1.28	48.23 ± 1.48

Gut wet weight as % of tortoise wet weight = 7.95 ± 0.31, $n = 15$
Ratio of total gut length to carapace length = 4.2 ± 0.13, $n = 23$

to the circular layer of the muscularis externa, there was a third layer of smooth muscle composed of discrete fascicles that were longitudinally oriented.

The cells of the mucosal lining of the gut showed positive staining for neutral carbohydrates using PAS stain and also contained cells that stained positively with alcian blue, which stains acidic mucus. Alcian blue positive staining most often occurred in the apical regions of the elongated columnar cells found lining the intestinal lumen.

Table 1 records the mass and length relationships between different regions of the digestive tract. It also records the total length relationships between the entire digestive tract and the size of the tortoise. These data suggest that about one half the gut is composed of large intestine with a little under a quarter each being composed of stomach and small intestine. The tortoise digestive tract makes up a little less than 8% of the mass of the entire tortoise and is slightly in excess of four times the length of the carapace.

Discussion. — The significance of the morphologic and histologic variations seen in different regions of the tortoise gut and those between different age classes remains to be explained. Qualitative differences in diet between hatchlings and adults might be expected on the basis of size alone, but have not been adequately studied in natural populations. Detailed studies by Barboza (1995) and Nagy et al. (1997) have revealed much about desert tortoise water and digestive physiology. However, any relationships between specific diet and gut morphology have yet to be studied. Further analysis using histochemical techniques should reveal more about the kinds of epithelial cells found in the mucosal lining. We are currently examining gut mass in subadults and adults and data derived from these studies will be compared with those already available for juveniles.

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