HEART FAILURE ASSOCIATED WITH UNUSUAL HEPATIC INCLUSIONS IN A DECKERT'S RAT SNAKE

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HEART FAILURE ASSOCIATED WITH UNUSUAL HEPATIC INCLUSIONS IN A DECKERT'S RAT SNAKE

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Abstract: A juvenile Deckert’s rat snake, Elaphe obsoleta deckerti, was presented with a circumferential enlargement of the body in the region of the heart. The heart was enlarged approximately twice normal size. Focal mineralized lesions were present in the tunica media of the right aorta and the right atrioventricular valve. The normal sinusoid architecture of the liver was disrupted with deeply eosinophilic to lightly basophilic granules of variable size in the cytoplasm and light eosinophilic intranuclear inclusions. Similar appearing intracytoplasmic granules were seen in the glomeruli and kidney tubules.

INTRODUCTION

The causes of death of reptiles in captivity often are unidentified because they are either discarded or simply preserved as museum specimens. Most of the information relating to disease in reptiles comes from zoological collections with very few documented disease conditions in free-ranging animals. Several excellent reviews of diseases of captive reptiles are available and as more cases are reported, it is becoming increasingly evident that the same types of disease found in mammals occur in reptiles.

Heart failure associated with unusual hepatic inclusions in a juvenile Deckert’s rat snake is described. There is no known report of a similar condition for a snake.

CASE HISTORY

A juvenile male Deckert’s rat snake, Elaphe obsoleta deckerti, measuring 386 mm from snout to tip of tail was presented with a circumferential enlargement of the body in the region of the heart. This enlargement was first noted approximately one week prior to the presentation. At the time of examination the heart rate was 62 beats per min., considerably faster than that of other snakes of the same size at room temperature (20 C). The snake was observed over the next two days and during this time initiated a cycle of ecdysis manifested by a bluing of the spectacle. On the third day of observation the snake was found dead and a necropsy was performed.

The carcass contained very little fat and approximately two ml of clear fluid was present in the peritoneal cavity. The heart was enlarged approximately twice normal size, weighing 2 g and measuring 25 mm in length by 15 mm in width. The hepatic veins, post-cava and all other major vessels were distended with blood. In addition, the liver was enlarged and slightly swollen. Urate crystals were observed in the kidneys and also were seen regurgitated into the colon. All other organs appeared normal.

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MATERIALS AND METHODS

For light microscopy, 6 μm tissue sections prepared from formalin-fixed paraffin-embedded tissues taken from the heart, liver, lung, kidney and intestine were stained with hematoxylin and eosin. Additional sections of liver were stained with Shorr’s, Giemsa, Weigert’s hematoxylin, PAS, (before and after diastase), Perls’ iron stain, and Congo red for amyloid; additional sections of the heart were stained with Van Gieson’s stain.

For transmission electron microscopy (TEM), 1 mm² pieces of formalin-fixed liver were washed in three successive changes of 0.1 M sodium cacodylate (pH 7.4) and then placed for 3 h in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate. This tissue was then post-fixed for 1 h in 1% osmium tetroxide in 0.1 M sodium cacodylate. Subsequently, the tissue was dehydrated in increasing concentrations of acetone and embedded in epon. Ultrathin sections were obtained with an LKB microtome (Ultratome 111) and uranyl acetate and lead citrate stained sections were examined with a Jeol 100-c electron microscope at 80 kv.

RESULTS

Longitudinal sections of the right and left aspects of the heart revealed isolated myocardial fibers in various stages of Zenker’s degeneration and necrosis. The base of the heart, in the area of the left and right aorta, was infiltrated with lymphocytes and macrophages. Two inflammatory lesions were observed on the adventitial surface of the pulmonary artery. The first lesion was a raised fibrinous reaction involving approximately one fourth of the circumference of the vessel, while the second was a smaller, more localized lesion characterized by aggregations of lymphocytes and a moderate number of eosinophils surrounding a hyalin material. The tunica media of the right aorta was characterized by large focal vacuolations with accompanying mineralization. At times these lesions disrupted the intima. The A-V valves were edematous and the right A-V valve contained a focally mineralized lesion. The muscle bundles of the ventricle were widely separated and edematous. An organized area of hemorrhage was attached to the left epicardial surface with focal aggregates of lymphocytes surrounding a homogenous hyalin material.

The normal sinusoid architecture of the liver was disrupted. Minimal necrosis and few infiltrating inflammatory cells were present. The cytoplasm of hepatocytes contained multiple sharply-defined vacuoles (probably fatty) as well as deep eosinophilic to lightly basophilic granules of various size. Lighter eosinophilic intranuclear inclusions, with margination of the chromatin material, also were noted (Fig. 1). These granules and intranuclear inclusions were PAS positive, PAS positive after diastase digestion and negative for iron with Perls’ iron stain. Most sinusoids and vessels were filled with an eosinophilic material which was negative for amyloid.

On TEM, hepatocytes were readily identified by membrane junctions, nuclear configurations and GERL (golgi apparatus, endoplasmic reticulum, and lysosome) relationships. The most striking feature was the presence of varying sized granules within the cytoplasm and occasionally within the nucleus of hepatocytes (Fig. 2). These granules seemingly developed from vacuoles near golgi complexes which undergo progressive alterations in their size, density, and consistency. The mature or “end-stage” granules had a dense amorphous matrix containing numerous electron dense particles.

A less common hepatocellular appearance also was encountered. In these cells, dilated sacs or pooled material containing electron-dense particles of a uniform size, similar to the
FIGURE 1. Hepatocytes with vacuolar changes and deeply eosinophilic to lightly basophilic granules (G). Also, an intranuclear inclusion with margination of chromatin material. H&E × 800.

FIGURE 2. Granules of variable size within several hepatocytes. One large granule is intracytoplasmic (IC) and appears to be bulging into a nucleus while another one is intranuclear (IN). × 10,150.
particles within the cytoplasmic granules were observed (Fig. 3). This material often pushed pre-existing organelles to the margins of the cell.

Other cytoplasmic characteristics include the presence of lipid vacuoles, and an absence of glycogen rosettes. Autophagic vacuoles containing degenerating membranes occasionally were observed. Electron-dense particles were noted extracellularly.

On TEM, Kupffer cells contained engulfed degenerative cellular components of unknown origin and also cytoplasmic granules similar to those seen in the hepatocytes. Several of these cells appeared greatly distended because of the number of granules present.

Eosinophils and heterophils were observed in the parenchyma of the lung, particularly contiguous to airways and blood vessels. Focal areas of heterophils were observed in the pleura.

The epithelial cells of proximal tubules, distal tubules and collecting ducts of the kidney contained variable-sized cytoplasmic granules many of which were similar to the intracytoplasmic hepatic granules (Fig. 4). The smaller deeply staining granules in the distal tubules were compatible with sex granules. Many glomerular tufts appeared swollen and thickened. Granules similar to the intracytoplasmic hepatic granules were also observed, possibly within endothelial cells.

Cultures taken from the heart, liver, lung and kidney were all negative for bacterial growth on blood and MacConkey's agar.

DISCUSSION

The reptilian cardiovascular system is well described both anatomically and physiologically. The reptilian heart consists of two atria and a single ventricle in all major groups except crocodilians, which have evolved a complete ventricular septum. In snakes, blood in the ventricle is diverted, with little mixing, by various ridges either into the pulmonary or systemic circulation. The minimal mixing that occurs in the ventricle is left to right shunting and, functionally, before the end of systole, the heart appears to be four chambered. Although the Deckert's rat snake showed gross changes and microscopic lesions associated with heart failure, there was little systemic involvement. This may be due to the inherent morphology and physiology of the reptilian cardiovascular system.

Of 1,249 reptilian cases surveyed at the Penrose Research Laboratory of the Zoological Society of Philadelphia, 23 were saurians (lizards) with arteriosclerosis of the great vessels, some in animals with myocardial degeneration. Additional reports on arterial lesions in reptiles demonstrated that medial calcification secondary to a parasitic arteritis was the major vascular disorder in snakes. In the Deckert's rat snake the two inflammatory lesions observed on the adventitial surface of the pulmonary artery and right aorta, in addition to mineralization of the right A-V valve and Zenker's degeneration and necrosis of isolated muscle fibers, were severe enough to result in heart failure. Although there was no evidence of active parasite involvement this etiologic must be considered.

The nature of the granules is unclear. The interpretation of the ultrastructural hepatic pathology was limited by an absence of both normal and pathologic studies involving the fine structure of reptile liver. In addition, the only reference on normal cytology lacked detail. The hepatic granules probably are lysosomal in origin; however, specific lysosomal enzymatic reactions were not performed.

The electron-dense particles resemble ferritin and the amorphous matrix may be a glycoprotein. The intranuclear granules do not appear to represent cytoplasmic invaginations because they lack the additional nuclear membrane.
FIGURE 3. A hepatocyte containing pooled material (PM) of electron-dense particles partially surrounding the nucleus (N). Note numerous junctional complexes (arrows) along the plasma membranes. × 16,500.

FIGURE 4. Epithelial cells of kidney tubules and collecting ducts with variable size granules. The smaller granules (g) are consistent with sex granules while the larger granules (G) are similar to the intracytoplasmic hepatic granules. H&E × 440.
Perhaps the granules are forced into the nucleus as more granules develop in the cytoplasm. PAS-positive, nonglycogenic structures have been reported in the liver.\textsuperscript{14,18,19,21} These structures are assumed to be lysosomes which increase in number during diseased conditions. A form of hepatitis characterized by the formation of spherical, PAS-positive cytoplasmic inclusions has been reported in reptiles.\textsuperscript{4} However, little additional information was provided so it is not known if the granules in the rat snake are related.

Small deeply staining granules consistent with sex granules were seen in many epithelial cells of the distal tubules and collecting ducts of the kidneys. In snakes, as in lizards, the sexual segment of the nephron shows sexual dimorphism.\textsuperscript{9} These segments develop in males only when the testes are spermatogenically active, and have not been reported in females or in immature males.\textsuperscript{15} Histochemically, the sexual segment granules are strongly positive for phospholipid, esterase and acid phosphatase; in some species they are PAS-positive.\textsuperscript{22} Although the Deckert’s rat snake was immature, the sexual granules were well-developed. Additionally, larger eosinophilic granules were seen in proximal and distal tubules and in glomeruli and were not similar in size or staining properties (H and E) to any granules reported for the reptile kidney, but were similar to the intracytoplasmic hepatic granules. This similarity to the intracytoplasmic hepatic granules in renal tubules and glomeruli suggests that the material might arise from a lysosomal enzymatic defect much the same as a lysosomal storage disease.\textsuperscript{5,11} However, a diagnosis of a storage disease needs further investigative studies which would not be possible on an unsuspected, isolated spontaneous death.

LITERATURE CITED


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