

Reptile Hepatic Lipidosis

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Abstract: Hepatic lipidosis is a recognized condition that is regularly diagnosed in numerous species of reptiles. Unfortunately, there is a paucity of information concerning the pathogenesis of hepatic lipidosis in reptiles, and, until relatively recently, the collection of biopsies for definitive antemortem diagnosis presented a challenge to clinicians. Although many of the difficulties of sample acquisition have now been overcome, the practitioner is still faced with the problems of microscopic (histopathologic and electron microscopic) interpretation. There is a need to quantify the diagnosis of hepatic lipidosis. A logical case investigation is needed, with particular emphasis on histological interpretation through the grading of microscopic lesions.

Key Words: hepatic lipidosis, reptiles, liver biopsy, liver disease.

Diagnosis of hepatic lipidosis

Anamnesis (history) and physical examination

It is important to remember that hepatic lipidosis is a metabolic derangement and not a single clinical disease. There are a host of factors that can predispose to increased hepatic fat. Classically, the high fat diet (e.g., obese laboratory rats, waxworms, etc.) have been implicated in obesity, an increase in the size of coelomic fat bodies, and an increase in hepatic fat. Equally, reduction in activity, which frequently afflicts our captive reptiles, must also be considered. Therefore careful questioning of the owner with regard to exercise, food items, and feeding frequency is essential. The usual environmental information, particularly with regard to thermal provision, is obviously essential. One other common aspect to this condition, is the apparent prevalence in non breeding adult to aged females. Many female reptiles undergo seasonal cycles of lipogenesis in preparation for folliculogenesis. Those females that do not have the opportunity to breed and produce eggs, appear more prone to obesity in captivity.

Hepatic lipidosis is usually chronic and therefore observant owners who keep accurate records may report a gradual reduction in appetite, activity, fecundity and fertility, retarded weight gain or gradual weight loss, hibernation problems including posthibernation anorexia, and changes in fecal character and color. However, it may only be during episodes of increased physiological demand (e.g., hibernation, breeding, concurrent disease, etc.) that the underlying liver disease becomes clinically apparent.

Unfortunately, many owners miss these early signs of disease; as a result veterinary attention is only sought once the animal has deteriorated to a life-threatening condition. When lipidosis is advanced, most affected reptiles are in poor body condition, flaccid, and weak with poor muscle coverage. The body weight (mass) is usually below normal, often critically so, although in cases of ascites weight may be artificially maintained or even increased. Diarrhea is uncommon as most affected animals will have been anorectic for a prolonged period of time.

Acute hepatitis, an inflammatory change, is prevalent in reptiles and is most often associated with infectious agents, while acute hepatosis, a noninflammatory liver disease, is much less common. There are rare situations where toxic insult may cause acute degenerative changes; ivermectin-induced hepatic lipidosis is a well-documented example (Ji and Wang 1990). Reptiles with acute liver disease usually present in good body condition but with sudden onset depression and anorexia. If several animals are affected simultaneously, a common etiology is most likely, and infection or intoxication should be primarily considered. Diarrhea is not uncommon, and if the urates are pigmented yellow-green, then the excretion of biliverdin may indicate severe liver compromise and bile stasis. Regurgitation is frequently considered to be a poor sign. Severely affected animals will usually be depressed, lethargic, and weak; mucous membranes may be pale, hyperemic or jaundiced. As already stated, the early signs of chronic disease may be missed by the owner; this, in conjunction with the rapid onset of severe clinical signs, may confuse and convince the clinician that this is an acute hepatitis rather than the end-stage presentation of a chronic hepatopathy.

Laboratory investigations

A full clinicopathological investigation is essential to differentiate accurately between acute and chronic liver disease, and it is important that pretreatment blood samples be collected prior to the instigation of fluid therapy and other supportive measures (Divers 1999).

Reptiles with severe hepatopathies may present with diarrhea and biliverdinuria. Hypoalbuminemia can result in the production of an acellular, low protein, coelomic transudate. A more complete review of laboratory liver assessment is given by Divers (1999).

Hematologic parameters can vary with species, gender, age and seasonal status, but marked elevation of packed cell volume usually indicates dehydration. However, in cases of chronic hepatopathy, anemia may be evident and can mask serious fluid deficits. Acute inflammation or necrosis of the liver will usually result in a dramatic heterophilia and monocytosis (including azurophilia, in many species). Chronic bacterial hepatitis will usually only cause minor elevations of the total white blood cell count, although shifts in the lymphocyte/monocyte:heterophil ratio often occur. Eosinophilia may be expected in cases of parasitic disease.

Liver disease may lead to elevations of several enzymes including aspartate aminotransferase (AST), glutamyltransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). Unfortunately, most of these parameters are widely distributed in other tissues, including muscle and kidney, and therefore assessment of creatinine kinase (CK) can be helpful in distinguishing between muscle and nonmuscle sources. In many species, it appears that LDH, AST, and GGT measurements are more useful, although tissue values of GGT may be very low (Ramsey and Dotson 1995; Wagner and Wetzel 1999). In the future, isoenzyme analyses may improve the organ specificity of sensitive indicators such as LDH.

Bile acids are considered to be useful in the assessment of liver function in many birds and mammals, but their application in reptile medicine has yet to be conclusively determined. Unpublished observations at the Exotic Animal Centre (Essex, England) and Greendale Laboratories (Surrey, England) suggest normal values of less than 60mol/L using commercial enzymatic assays for 3-hydroxy-bile acids. The major bile acid varies with the taxonomic order of the reptile, and therefore concern must exist as to the universal application of a single assay across all reptiles (Skoczylas 1978). Practical protocols for bile acid stimulation tests have not

been documented but, assuming specificity and sensitivity, they may offer an accurate assessment of liver function and a means of identifying those cases of severe liver compromise where little or no hepatocellular damage exists.

Biliverdin is the major bile pigment produced by reptiles as they lack the biliverdin reductase enzyme required to produce bilirubin. To date there are no readily available commercial assays for this metabolite.

Diagnostic imaging

The radiographic hepatic shadow is often appreciable in horizontal beam radiographs of lizards, less so in snakes, and least of all in chelonians due to the superimposition of the shell. Gross hepatomegaly or microhepatica may be discernible in some lizards and snakes. Ultrasound has proved to be more useful for the assessment of hepatic size and shape in many reptiles, including chelonians. It may even be possible to detect macrohepatic changes such as the generalized hyperechoic ultrasonogram of severe hepatic lipidosis. Ultrasound also has the advantage of permitting guided biopsies of the liver parenchyma. The senior author's preferred method of imaging the liver is endoscopically using a rigid 2.7mm Hopkins telescope (67208B, Karl Storz Endoscopy, Tuttingen, Germany). Although an invasive technique that requires local, or more usually general anesthesia, endoscopy permits the examination and appreciation of the size, color, shape and contours of the liver, and allows the collection of multiple tissue biopsies under direct visual control. Some researchers have utilized magnetic resonance imaging (MRI) and computed tomography (CT) to good effect and there can be no doubting the excellent soft tissue resolution that can thus be obtained. However, such devices are beyond the capabilities of most private practices, do not readily lend themselves to the collection of tissue samples, and little has been done to correlate these images with reptilian histological structure and pathology.

Liver biopsy

At present, there appears to be nothing to surpass the diagnostic value of a hepatic biopsy for both microscopic (histology and transmission electron microscopy [TEM]) interpretation and, where infectious agents may be present, microbiological culture. In addition to facilitating a definitive diagnosis, serial biopsies offer the best means of monitoring disease progression and response to treatment, and are invaluable for providing the client with the most accurate prognosis. Tissue samples can be taken using ultrasound guided tru-cut biopsy needles (e.g., Quick-core disposable biopsy needle, Cook Veterinary Products, Queensland, Australia), via the instrument port of the rigid endoscope or surgically following a coeliotomy approach to the liver. Small biopsies must be handled with care and, where significant pressure has been applied, this fact should be relayed to the pathologist, as it can lead to confusing artifact. Handling of biopsies should, whenever possible, be with non-rat-toothed instruments in order to minimize damage. Small tissue samples are placed into histology filters before being submitted in 10% neutral buffered formalin for histopathology, in appropriate fixative for TEM, or in 10% methanol/ethanol for urate tophi demonstration. Tissue samples for microbiology should be sent in appropriate transport media depending upon whether fungal, bacterial, or viral investigations are most important (Cooper 1999).

Histopathology results

Thompson, subsequently cited in Carlton and McGavin (1995), defined hepatic lipidosis as "excessive accumulation of lipid within hepatocytes" and Elkan (1981a) drew attention to the fact that there could be both normal and pathological deposition of fat within the liver. These and

other references are a reminder of the fact that the presence of some intrahepatic lipid can be quite normal and indeed Schaffner (1998), writing specifically about the reptilian liver, states categorically that "small fat droplets normally may be found in cytoplasm". This same author goes on to emphasize that the amount of fat can vary depending upon such factors as the sex of the animal and whether the liver is examined before or after hibernation.

Thus, the presence of fat in the liver of reptiles is not, per se, indicative of lipidosis. It can be termed "fatty change" but not necessarily "lipidosis" - and nowadays never "fatty degeneration". A diagnosis of hepatic lipidosis will depend upon a number of other factors and these are discussed later. The detection of fat in a reptile's liver is not usually difficult. In a standard paraffin-embedded section the lipid will appear as "holes" - vacuoles or vesicles - and usually these can be assumed to be fat.

However, some caution is needed since vacuoles can, on occasion, contain water (hydropic degeneration; Frye 1991), glycogen, or other material as opposed to fat. Sometimes light microscopy will per se permit a distinction to be made, but often other tests are needed, such as special stains or even TEM. For this reason when describing changes in paraffin sections, the junior author reports "vacuoles in hepatocytes - probably fat" - cautionary but necessary. Whenever possible, a paraffin section stained with e.g., hematoxylin and eosin is accompanied by a cytology touch preparation of unprocessed liver stained with oil-red-O (ORO) - in which lipid stains red and the amount of coloration provides extra information on the quantity of fat present.

Once it has been ascertained by histology that fat is present in the liver of a reptile, a number of subsidiary questions need to be asked before a diagnosis (tentative or definite) of hepatic lipidosis can be made. The following is the approach followed by the junior author: it is open to criticism or comment:

1. Is the fat in the liver present in distinct lipocytes (fat cells) or is it confined to the hepatic parenchyma? (Lipocytes are sometimes found under the capsule or within portal tracts - but not commonly.)
2. If the fat is confined to the parenchyma, is it present in hepatocytes, von K  pffer cells, or both? (Usually hepatocytes are primarily affected but K  pffer cells can also be involved.)
3. If the fat is within hepatocytes, what percentage of them is affected?
4. Where, within the parenchyma, is the fat found? Is it predominantly periportal, centrilobular, or spread evenly throughout the cells? (Generally, where lipid deposition is due to metabolic disturbances rather than, say, ingested toxins, there is no zonal distribution).
5. Is the lipid present as small vacuoles (microvesicles) or as large vacuoles (macrovesicles), or a mixture of both? In the case of a mixture, what is the proportion?
6. Is there evidence that the lipid accumulation is having any effect on the ability of the hepatocyte to function? (When there are marked quantities of lipid in a cell, especially a macrovesicle, the hepatocyte nucleus is pushed to one side and flattened, giving a "signet ring" appearance. Sometimes nuclei in fat-filled stains will show changes, such as pyknosis and karyorrhexis, indicative of degeneration, and occasionally there will be a number of cells in which the nuclei have disappeared).
7. Are there other changes, such as K  pffer cell hyperplasia or inflammatory infiltrates, that may be indicative of more generalized liver disease?

It will be apparent from the foregoing that quantification is an important part of assessing whether or not the presence of fat in the liver is likely to be significant in terms of the health of the reptile. The histopathologist who is examining a slide must be prepared not only to observe but also to count and to measure. Counting should not present too many problems, usually it is best to "score" on the basis of 1 (minimal), 2 (moderate), or 3 (marked). These scores are based on the extent of the vacuolation when the liver parenchyma is viewed down the microscope. Minimal means that less than 20% of the parenchyma viewed is vacuolated whereas 3 (marked) indicates 80-100% vacuolation. In these calculations no differentiation is made between macro- and microvesicles. The area ("volume") of lipid/vacuolation in relation to nonvacuolated cytoplasm is the important criterion. Such an approach carries with it the advantage of helping to ensure some standardization and consistency in reporting; in the past, different pathologists have described and interpreted fatty change in the liver in different ways and this has served to muddle the situation - as well as being of limited value to clinician and patient.

It should be mentioned in passing that other techniques could also be used to investigate fatty change in the liver of reptiles. Transmission electron microscopy is hardly suitable for routine diagnosis but has an important role to play when working with valuable or endangered species - for instance where lipidosis is a feature of a disease "outbreak", perhaps linked with other metabolic changes, such as xanthomatosis. In such cases, TEM can provide essential information on pathogenesis, including the presence or absence of changes in intracellular organelles (Cooper and others 1999). Transmission electron microscopy is also one of the keys to more research on fat metabolism in reptiles (De Brito Gitirana 1988).

Even when the presence of what appears to be "excess" fat has been established, a diagnosis of hepatic lipidosis cannot usually be confirmed without taking into account:

- 1) The clinical history and the clinician's assessment of the case (including other laboratory results); and
- 2) The patient's details and circumstances - for example, its species, age, sex, reproductive status, hibernation, estivation, diet. These are all discussed earlier.

Postmortem features

As indicated earlier, the liver of reptiles differs in size, shape and appearance depending upon the species and certain other factors (Schaffer 1998). Generally, however, the organ is brown in color - sometimes almost black when abundant melanin is present. In hepatic lipidosis the color is pale - described by Frye (1991) as "pale yellow to light tan" - but sometimes almost white, especially when little blood is present. The color may also be affected by the natural color of fat in the species and that, in turn, can be influenced by diet. A "fatty liver" is usually swollen, with rounded nonangular edges and may weigh more than normal - organ weights can be helpful in this respect (see later). There is a tendency for the organ to sink or only to float with difficulty when placed in fixative but this again depends to a certain extent upon the degree of congestion of the organ. The general rule is to watch as the piece of liver enters the aqueous fixative - not only to see how quickly or slowly it sinks, but also because its color may change and pallor (or icterus or other features) become more prominent.

Markedly fatty livers will have a soft fatty "feel" when held for cutting and are friable - easily torn. Both the surfaces of the scalpel and the touch preparation, even before staining with specific stain (ORO, Sudan), will show greasy fat droplets (Vegad 1995).

When performing a necropsy on an animal with suspected hepatic lipidosis, care must be taken to ensure that all other body organs are meticulously examined. In particular, note should be taken of: 1) other fat deposits, including the size of fat bodies, and presence or absence of adipose tissue under the skin and around the heart (Elkan 1981b); and 2) changes in other organs, such as pancreas, that may have predisposed to fatty change.

In crocodilians, the size of the fat body is carefully assessed as part of postmortem evaluation of condition - either by weighing or by comparing its size to the animals' ventricles (FW Huchzermeyer, personal communication). Similar techniques could prove useful in other reptiles.

Treatment

While specific therapy for hepatic lipidosis will usually be reserved until the diagnosis has been confirmed histologically and the precise etiology has been identified, much can be done in general to support a reptile with hepatic dysfunction - whether infectious or non-infectious. Medical stabilization utilizing fluid therapy is essential and the provision of the species-specific preferred optimum temperature zone (within which the reptile can select a preferred body temperature) can be instrumental in effecting an improvement. Potentially useful therapies include: 1) fluid and nutritional support; 2) amino acid supplements such as carnitine, choline and methionine; 3) thyroxine; and 4) anabolic steroids including nandrolone or laurabolin.

CONCLUSIONS AND RECOMMENDATIONS

Although hepatic lipidosis of reptiles has traditionally been considered to be associated with over-feeding, obesity, and under exercise, it is now clear that this is not always true (Elkan 1981; Frye 1991). As in other animals including humans, excess lipid deposition in the liver can be due to, or follow, a number of insults, including toxins, anoxia, and impaired metabolism of carbohydrate and volatile fatty acids.

In humans and domestic animals a whole spectrum of diseases is recognized that can lead to fatty change. In *Homo sapiens*, for example, causes include alcoholism, diabetes, inborn metabolic disorders, side effects of drugs (e.g., corticosteroids), systemic disease (especially with pyrexia), pregnancy, Reye's syndrome, certain drug toxicities (e.g., tetracycline) or inborn metabolic defects of the urea cycle or fatty acid oxidation (McGee and others 1992). In domestic animals the list is shorter but a whole range of fatty liver syndromes has been reported in species as diverse as birds of prey, cats and dairy cows (Forbes and Cooper 1993; Dimski 1997).

Although hepatic lipidosis in reptiles shows in its natural history a number of significant differences from its counterpart in mammals and birds, much value can accrue from a certain amount of careful extrapolation. The first of these is in the description and classification of fatty change where the medical profession, in particular, has done sterling work in differentiating between conditions on the basis of (for example) size and distribution of vesicles. In this context, medical pathologists have been able to bring a degree of precision to the diagnosis of fatty liver in humans by proposing that the term should be used when lipid accumulation exceeds the normal 5% of body weight. Secondly, the medical profession has been able to bring to its investigations of lipidosis a weight of technical expertise and experience, much of which might, in due course, be applied to reptiles (Patrick and McGee 1980).

Research on domestic mammals and birds can also be relevant. Again, substantial work has been performed that has thrown light on the changes that occur in hepatocytes (and elsewhere) when lipid is deposited and why this can predispose to bacterial infections and endotoxemia.

Given the variability of clinical presentation, blood chemistries, and the lack of standardized reference points for ultrasonographic diagnosis compared with mammals, it appears that histopathology and TEM, in combination with the aforementioned, offer the best approach to diagnosis. Indeed, serial tissue samples will not only aid prognosis but may well help our understanding of the pathogenesis of both disease progression and recovery. To this end biopsy appears to be key in the successful diagnosis and monitoring of lipidosis and, indeed, other hepatopathies in reptiles.

Despite our improved ability to safely biopsy the reptilian liver, our understanding of hepatic metabolism and the pathogenesis of hepatic disease remains in its infancy. So much has still to be learned at the biochemical level and we should like to encourage clinicians, pathologists, and biochemists alike to become involved in this increasingly perplexing clinical syndrome. Such investigations will also be enhanced by the input of field biologists and by much needed studies on (wild) free-ranging reptiles.

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REFERENCES*

- Abdul-Raheem K, Okasha S, El-Deib S, and others. 1989. Hibernation in reptiles 3. Carbohydrate metabolism in *Eryx colubrinus* and *Eumeces schneideri*. Journal of Thermal Biology 14:133-137.
- Bush M, Teare JA. 1983. Toxicology and efficacy of ivermectin in chelonians. J Am Vet Med Assoc, 183:1195.
- Carlton WW, McGavin MD. 1995 Thompson's Special Veterinary Pathology. St Louis, Mosby:88-89.
- Cooper JE. 1999. Reptilian microbiology. In Fudge AM (ed): Laboratory Medicine, Avian and Exotic Pets. Philadelphia, WB Saunders: 223-237.
- Cooper JE, Bloxam QMC, Tonge SJ. 1999. Pathology of Round Island geckos, *Phelsuma guentheri*., some unexpected findings. Dodo, J Wild Preservation Trusts, 4:153-158.
- Cornelius LM, Jacobs G. 1989. Feline hepatic lipidosis. In Kirk RW (ed): Current Veterinary Therapy X Small Animal Practice. Philadelphia, WB Saunders: 869-873.
- De Brito Gitirana L. 1988. The fine structure of the fat-storing cell (Ito cell) in the liver of some reptiles. Z Mikrosk - anat. Forsch, 102:143-149.
- Dimski DS. 1997. Feline hepatic lipidosis. Semin Vet Med Surg (Small Anim), 12(1):28-33.
- Divers SJ. 1997. A clinician's approach to liver disease in tortoises. Proc ARAV. Houston, Texas: 75-79.
- Divers SJ. 1999. Reptilian liver and gastrointestinal testing. In Fudge AM (ed): Laboratory Medicine, Avian and Exotic Pets. Philadelphia, PA, WB Saunders: 205-209.
- Divers, SJ, Cooper JE. 2000. Reptile Hepatic Lipidosis. Seminars in Avian and Exotic Pet Medicine, 9:153-164.
- Donoghue S, Langenberg J. 1996. Nutrition., In Mader DR. (ed): Reptile Medicine and Surgery. Philadelphia, WB Saunders: 48-174.

- Elkan E. 1981a. Pathology and histopathological techniques. In Cooper JE, Jackson OF. (eds). Diseases of the Reptilia. London, Academic Press: 75-91.
- Elkan E. 1981b. Reptilian fat bodies. Br J Herpetol, 6:75-77.
- Ferrer C, Zuasti A, Bellesta J, and others. 1987. The liver of *Testudo graeca* (Chelonia), a comparative study of hibernating and non-hibernating animals. J Submicro Cytol, 19:275-282.
- Forbes NA, Cooper JE. 1993. Fatty liver-kidney syndrome of merlins. In Redig PT, Cooper JE, Remple D, Hunter B. (eds): Raptor Biomedicine. Minnesota, University of Minnesota: 45-48.
- Frye FL. 1991. Biomedical and Surgical Aspects of Captive Reptile Husbandry, 2nd ed. Melbourne Florida, Krieger: 544-545.
- Gatten RE. 1985. The uses of anaerobiosis by amphibians and reptiles. Am Zool, 25: 945-954
- Jackson OF. 1991. Reptiles Part One Chelonians. In Beynon PH, Cooper JE. (eds): Manual of Exotic Pets, 2nd ed. Cheltenham, BSAVA: 221-243.
- Jacobs G, Cornelius L, Allen S. and others. 1989. Treatment of idiopathic hepatic lipodosis in cats: 11 cases (1986-1989). JAVMA, 195(5):635-638
- Jacobs G, Cornelius L, Keene B, and others. 1990. Comparison of plasma, liver, and skeletal muscle carnitine concentrations in cats with idiopathic hepatic lipodosis and in healthy cats. J Am Vet Res, 51(9):1349-1351
- Jenkins WL. 1989. Drugs affecting gastrointestinal functions. In Booth NH, McDonald LE. (eds): Veterinary Pharmacology and Therapeutics, 6th ed. Iowa, Iowa State University, pp 657-671.
- Ji X, Wang PC. 1990. Annual cycle of lipid contents and caloric values of carcass and some organs of the gecko, *Gekko japonicus*. Comp Biochem Physiol, 96A: 267-271.
- Leclerc J, Miller ML. Inositol and choline levels in the diet and neutral lipid hepatic content of lactating rat. Int J Vitam Nutr Res, 59(2):180-183.
- McGee J, Isaacson PG, Wright NA. 1992. Oxford Textbook of Pathology. Oxford, Oxford University Press: 1325-1328.
- Patrick RS, McGee JOD. 1980. Biopsy Pathology of the Liver. London, Chapman and Hall :189-190.
- Ramsay EC, Dotson TK. 1995. Tissue and serum enzyme activities in the yellow rat snake, *Elaphe obsoleta quadrivittata*. Am J Vet Res, 56:423.
- Schaffner F. 1998. The liver. In Gans C, Gaunt AS. (ed): Biology of the Reptilia, Volume 19, Morphology G, Visceral Organs. Missouri, Society for the Study of Amphibians and Reptiles. 19:485-531.
- Skoczylas R. 1978. Physiology of the digestive tract. In Gans C, Gans KA. (ed): Biology of The Reptilia, Volume 8, Physiology B. London, Academic Press: 589-717.
- Stryer L. 1988. Fatty acid metabolism. In Stryer L. (ed): Biochemistry. 3rd edition. New York, WH Freeman and Company: 469-493.
- Vegad JL. 1995. Textbook of Veterinary General Pathology. India, Vikas, Jabalpur: 35-36.
- Wagner RA, Wetzel R. 1999. Tissue and plasma enzyme activities in juvenile green iguanas. Am J Vet Res, 60(2):201-203.

*not all references cited in text; some are provided for additional reference