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## Veno-occlusive Disease of the Liver in Captive Cheetah

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**Abstract.** Liver tissues from 126 captive cheetah were evaluated by light microscopy and histochemistry; eight animals were evaluated by electron microscopy. The main hepatic lesion, a vascular lesion resembling veno-occlusive disease (VOD) of the liver and characterized by subendothelial fibrosis and proliferation of smooth muscle-like cells in the central veins, was seen in 60% of the sexually mature cheetah. Although this hepatic vascular lesion was seen in cheetah as young as 1 year of age, the most severe lesions, usually associated with liver failure, were found in cheetah between the ages of 6 and 11. There was no sex predisposition, and in approximately 40% of the VOD cases, liver disease was not suspected clinically or at necropsy. VOD was found in other felidae, especially in the snow leopard. High levels of vitamin A in livers, as well as in diets of the cheetah, could be a contributing factor in the development of VOD in some groups of cheetah.

Liver diseases of undefined nature have been reported as one of the major causes of death in captive cheetah since the late 1960's.<sup>41</sup> An attempt to identify hepatitis B-like virus as a potential cause of liver disease found that only 3.7% of the cheetah sera tested had reactive antibodies against the virus surface antigen.<sup>43</sup> The nature of the histologic changes is also poorly defined; separate studies, carried out in 1970 and in 1981, described veno-occlusive disease (VOD) of the liver in a limited number of captive cheetah.<sup>6,40</sup> Clinical and biochemical evidence of liver damage usually appeared in the late stage of the disease.<sup>6</sup> Clinical signs included ascites, anorexia, lethargy, and occasionally, icterus. Elevation of liver enzymes was not proportional to the severity of the hepatic lesions. There has been no report in the literature of cheetah in the wild with VOD.

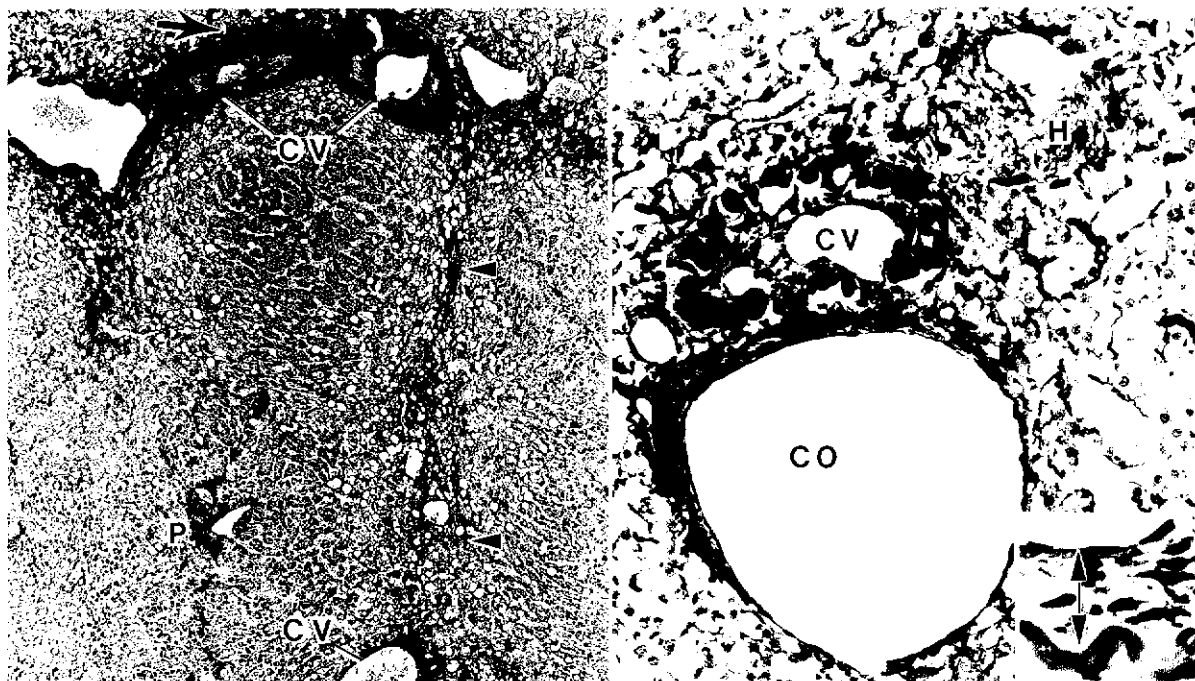
Dietary factors can be suspected in the etiology of a non-infectious disease with high incidence in groups of animals from a wide geographical range and across felid species boundaries.<sup>6,24,29,32,40,41</sup> Cheetah in the wild prey mostly on small antelope,<sup>8</sup> while those kept in North American zoos are fed commercially prepared feline diets, sometimes supplemented with raw liver, or zoo prepared diets. These diets are usually based on the nutrient requirements of the domestic cat, which may or may not be adequate for the cheetah.<sup>42</sup> This study characterizes, by light and electron microscopy, the main hepatic lesions in the cheetah kept in captivity. Livers from other exotic cats were evaluated in order to verify if these hepatic lesions are seen exclu-

sively in the cheetah, which may indicate a possible genetic basis for the disease. There is also an attempt to identify dietary factors which may play a role in the pathogenesis of these liver lesions.

### Materials and Methods

Liver tissue from 126 captive cheetah that died between 1945 and 1986 were evaluated by light microscopy. Diseases that most probably contributed to their death ranged from liver disease, which was the most often diagnosed, to renal failure, salmonellosis, pneumonia, neoplasia, feline infectious peritonitis, septicemia, diabetes mellitus, pancreatitis, anesthesia, surgery, blastomycosis, toxoplasmosis, tuberculosis, and unknown. Most animals were from North American zoos (22 zoos in the United States and Canada); five were from Great Britain, and four were from Australia. Ten liver biopsies taken from eight of the 126 cheetah were also evaluated by electron microscopy. There were 104 sexually mature cheetah, ranging from 3 to 16 years of age; 22 were juvenile cheetah, aged 1 day to 13 months. Cheetah livers were compared with livers from 42 other exotic cats with or without clinical signs of liver disease. They represented 16 of the 37 species of felidae and included the black-footed cat, clouded leopard, cougar, desert cat, European wildcat, flat-headed cat, jaguar, jungle cat, leopard (African and Persian), lynx, marguay, ocelot, rusty-spotted cat, sand cat, snow leopard, and tiger (Bengal and Siberian).

Liver sections from all animals were stained with hematoxylin and eosin (HE) and Masson trichrome. Silver stain for Type III collagen and reticulin fibers (reticulin fibers consist of Type III collagen, fibronectin, and at least one additional non-collagenous glycoprotein),<sup>19,39</sup> congo red for amyloid fibers, phosphotungstic acid-hematoxylin for fibrin, and



**Fig. 1.** Liver section from 6.5-year-old female cheetah with VOD. Lesion characterized by partial occlusion of central vein (CV), perivenular fibrosis (arrow), and bridging (arrowheads) of adjacent central veins. Parenchyma surrounding portal triad (P) appears normal. Masson trichrome.

**Fig. 2.** Liver section from 6-year-old male cheetah with VOD. Lumen of central vein (CV) partly occluded by fibrous connective tissue. Focal hemorrhage (H) and collateral (CO) vessel adjacent to central vein. Masson trichrome. Inset: Intima of central vein thickened (arrow); also spindle-shaped cells with elongated nuclei. Normal-appearing endothelial cells line the lumen. HE.

periodic acid-Schiff-alcian blue for mucopolysaccharides were used on selected tissue sections.

Sections (1–2 mm thick) of liver tissue (eight animals) collected at necropsy or at laparoscopy, were immediately fixed in 4% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate or 3% glutaraldehyde in the same buffer. At a later time, 0.5 mm cubes were trimmed, washed twice in cacodylate buffer, and post-fixed in 1.33% osmium tetroxide in 0.2 M S-collidine at pH 7.4. Tissues were dehydrated through ascending concentrations of ethyl alcohol, transferred to propylene oxide, and embedded in epon. Semi-thin sections (2  $\mu$ m) were cut, stained with 1% toluidine blue, and evaluated for suitable areas for electron microscopy. Thin sections were cut at 60 to 80 nm, stained with uranyl acetate and lead citrate, and examined with a Jeol 100 CX-II transmission electron microscope.

Three diets from three different food manufacturers and nine cheetah livers were analyzed for vitamin A levels. Diets A (main ingredients include frozen horsemeat with horsemeat by-products, bone meal, liver, fish meal, soya meal, vitamins, and minerals) and B (main ingredients include frozen beef with bone, chicken, beef by-products, vitamins, and minerals) are commercially prepared diets for exotic carnivores, while diet C (canned product with liver as the primary component), is not specifically formulated for exotic cats but has been used to stimulate the appetite of anorexic felids. Vitamin A analyses of liver and diet samples were done by

high performance liquid chromatography at the Animal Health Diagnostic Laboratory, Michigan State University.<sup>36</sup>

## Results

### Histopathological evaluation

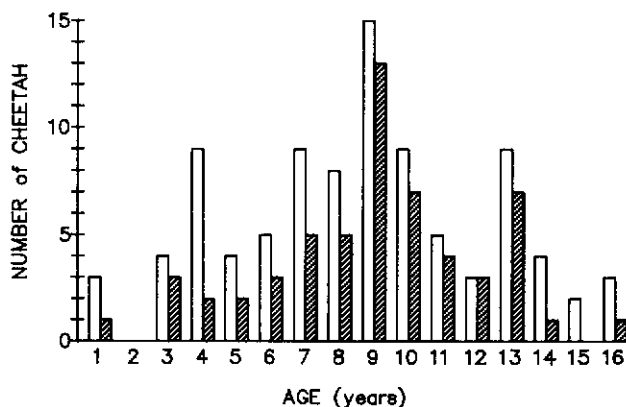
The main hepatic lesion, a vascular lesion resembling veno-occlusive disease of the liver (VOD), was found in 62 out of 104 sexually mature cheetah. The detailed description of this lesion was based on 14 animals exclusively with VOD, and was characterized by intimal thickening and partial or total occlusion of a few to the majority of the central veins, with loosely arranged to dense fibrous connective tissue (Figs. 1, 2). There was slight to severe perivenular and sinusoidal fibrosis, with occasional bridging of adjacent central veins (Fig. 1). The surrounding sinusoids were usually congested, and occasionally there was extensive central hemorrhagic congestion. Recanalization of the occluded veins and formation of collaterals were noted in the more severe cases (Fig. 2). The parenchymal lesion varied from necrosis of individual hepatocytes to focal areas of degeneration and necrosis. Inflammatory cells (polymorphonuclear cells to, in most cases, macrophages with some lymphocytes and plasma cells) were



**Fig. 3.** Liver section from 10-year-old male cheetah with VOD. Large fibrin deposit (F) in sinusoid. Intimal thickening (arrow) in one part of central vein. Masson trichrome.

present, usually in response to the necrotic tissue. Vacuolization of individual cells or mild to severe diffuse vacuolization of cells in the centrilobular region was frequently associated with VOD. The majority of these cells, at least in cheetah livers evaluated by electron microscopy, were positively identified as hypertrophic and hyperplastic Ito cells, fat-storing cells within the space of Disse. The capsule of the liver was occasionally thickened, due to the proliferation of fibrous connective tissue and/or to the accumulation of several layers of fibrin. Very small to large fibrin deposits were occasionally seen free in the sinusoids or they were admixed with degenerate/necrotic liver cells in focal areas at the proximity of the central veins (Fig. 3). Bile stasis was noted in several sexually mature cheetah with VOD.

Proportionally, there were as many male as female cheetah with VOD. The earliest observation of this lesion was in a 1-year-old cheetah and was the only case found in the juvenile group (22 cheetah). The lesions in cheetah between 1 and 5 years of age were not severe enough to be the primary cause of death, while in cheetah aged 6 to 11, death could be attributed in most cases to liver failure (Fig. 4). Between 12 and 16 years of age, renal failure was the most frequent debilitating syndrome, followed by liver failure. VOD was seen in other exotic cats, including snow leopard, Siberian tiger, African leopard, jungle cat, ocelot, and



**Fig. 4.** Prevalence of veno-occlusive disease (VOD) of the liver in captive cheetah. □, total number of cheetah per age group evaluated by light microscopy. ▨, number of cheetah with VOD.

rusty-spotted cat. The lesions were not as severe as those described in the cheetah, except in the snow leopard and the rusty-spotted cat.

Clinical history and pathological reports were available in 52 out of 62 animals with VOD. Clinical signs characterized by ascites, icterus, anorexia, weight loss, vomiting, and lethargy were based on 14 animals exclusively with VOD. An additional 17 animals with VOD, as well as other diseases (excluding cases of feline infectious peritonitis, hepatic amyloidosis, cholangiohepatitis, and bile duct carcinoma), had ascites and/or icterus. In the remaining animals with VOD, clinical signs, and, in some cases, gross findings associated with VOD, may have been obscured by concurrent, more life-threatening, conditions (tuberculosis, feline infectious peritonitis, septicemia, renal failure, etc.) or the hepatic lesions were too mild to be diagnosed clinically or even at necropsy. The course of the disease varied from a few years to sudden death.

Perisinusoidal fibrosis, usually radiating from the central veins, and often associated with sinusoidal cell vacuolization (Ito cells), was present in an additional 21 cheetah that did not have VOD (Fig. 5). Other hepatic lesions in the livers of sexually mature cheetah with or without VOD, were peliosis hepatis (11 out of 104), amyloidosis (ten out of 104), and cholangiohepatitis (two out of 104). Inflammation of the hepatic parenchyma was usually secondary to fungal (*Blastomyces*), protozoal (*Toxoplasma*), viral (feline infectious peritonitis), or bacterial (especially salmonella) infections. By light microscopy, less than ten sexually mature cheetah had normal-appearing livers.

#### Ultrastructural evaluation

On semi-thin sections, six out of eight cheetah livers collected for electron microscopic evaluation had marked hypertrophy and hyperplasia of Ito cells (Fig.

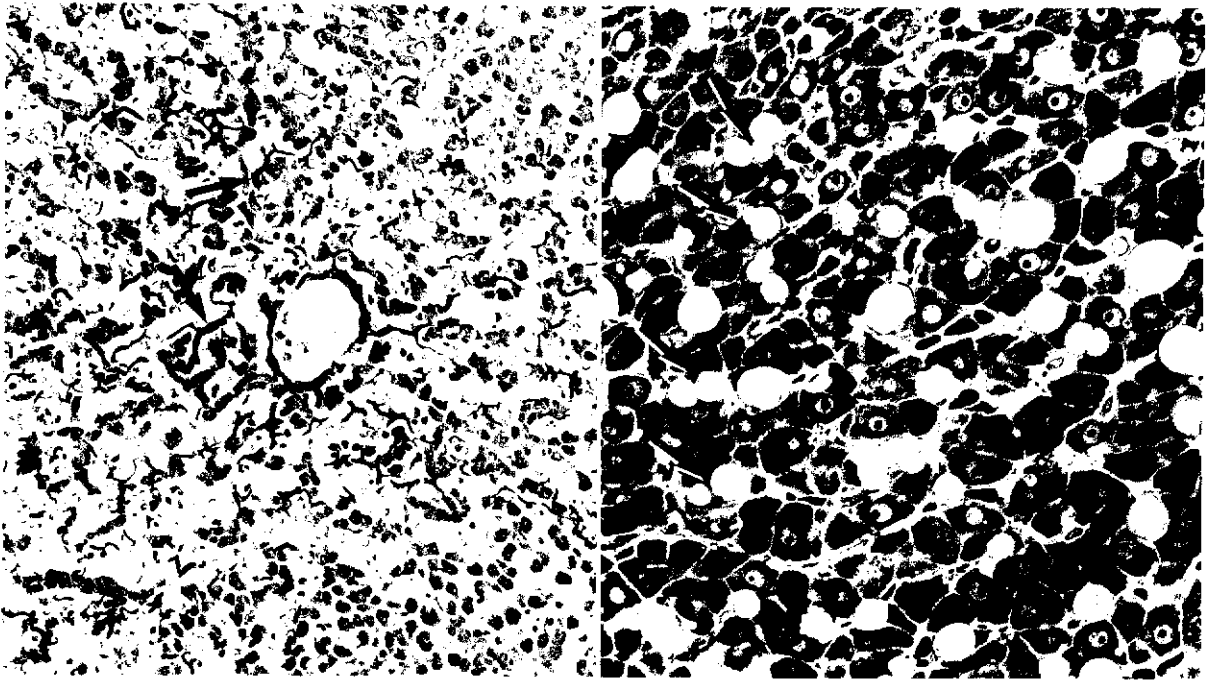


Fig. 5. Liver section from 10-year-old female cheetah with perisinusoidal fibrosis (arrows). Masson trichrome.

Fig. 6. Semi-thin section of liver from cheetah A-7, hypertrophy and hyperplasia of Ito cells (arrows). Toluidine blue.

6). Four of these eight cheetah were diagnosed with hepatic VOD, while perisinusoidal fibrosis was found in three, and amyloid deposits in one (Table 1).

By electron microscopy, the wall of the central vein was thickened, due to the presence of spindle-shaped cells widely dispersed between bundles of microfibrils and mature collagen fibers (Fig. 7). The subendothelial proliferation of these cells with matrix deposition eventually led to the occlusion of the vascular lumen. Only a few endothelial cells were degenerated. Spindle-shaped cells were considered to be smooth muscle-like cells because their cytoplasm contained microfilaments and dense plaques, and a basal lamina surrounded their periphery. In the area adjacent to the central vein, there was disruption of the normal structure of the hepatic cord due to the excessive accumulation of large bundles of microfibrils and mature collagen fibers. Hepatocytes in the area were atrophic, some of them undergoing degeneration and necrosis. The space of Disse was markedly dilated and filled with individual or bundles of microfibrils (diameter: 13 to 17 nm) that could represent Type III collagen (A. Martínez-Hernández, personal communication), granular or floccular material, and occasional bundles of mature collagen fibers (Fig. 8). These microfibrils were forming basement membrane or were included within the mature collagen fibers. By light microscopy, the material present in the space of Disse was positive for mature collagen fibers with Masson trichrome, and for Type III collagen or reticulin fibers with silver stain, but was negative for

amyloid fibers with congo red or Masson trichrome (with Masson trichrome, amyloid has a purple, waxy appearance). Thickened basement membrane-like material, entrapped red blood cells, and hypertrophic Ito cells would often contribute to the dilatation of the space of Disse (Fig. 9). Endothelial cells had widened fenestrae with occasional cytoplasmic blebs, and the sinusoidal border of the hepatocytes had lost their microvilli.

Ultrastructural changes in the liver of two cheetah with perisinusoidal fibrosis were limited to the space of Disse and had the same characteristics as those already described for VOD. The hepatic lesions of cheetah A-7, fed a diet formulated for anorexic animals and rich in liver, differed from the other two cases with perisinusoidal fibrosis. Large bundles of mature collagen fibers were present in the space of Disse instead of the abundance of microfibrils (Fig. 10). This was again associated with enlarged fenestrae, loss of microvilli on the sinusoidal surface of neighboring hepatocytes, and hypertrophic Ito cells, some of which protruded into the sinusoidal lumen. In all three cheetah livers with perisinusoidal fibrosis, Ito cell proliferation was associated with the production of a thin basement membrane-like material between the villi of the hepatic cells.

In the group of cheetah evaluated by electron microscopy (Table 1), obvious liver function abnormalities and clinical signs of liver failure were seen in three out of four cheetah with VOD. The animal with hepatic

**Table 1.** Clinical summary and hepatic histology of cheetah chosen for electron microscopy evaluation.

Animal	Diet*	Clinical Signs†		Serum Chemistry				Liver Histology		
		Ascites	Icterus	Albumin (g/dl)	Bilirubin (mg/dl)	AST (U/liter)	ALT (U/liter)	Veno-occlusive Disease	Peri-sinusoidal Fibrosis	Ito Cell Proliferation
A-1	A, Li	+	+	ND	0.4	123	153	5	4	5
A-2	A, Li	+	+	2.1	0.6	287	226	5	3	5
A-3	A, Li	-	-	4.0	0.1	74	90	4	4	5
A-4	A, Li	-	-	3.2	0.3	39	83	-	2	4
A-5	A, Li	-	-	4.1	0.1	23	56	-	1	5
A-6	A	-	+	2.6	6.3	311	492	5	4	1
A-7	C	-	-	3.4	0.1	34	63	-	2	4
A-8‡	A	-	-	4.4	0.5	98	157	-	1	2
Domestic cat§				2.5-4.2	0-0.9	14-46	11-37			

\* Commercial diets A and C contained 38,550 and 95,820 IU of vitamin A/kg on a dry basis, respectively; Li, diet A replaced by raw beef liver (+545,000 IU of vitamin A/kg, dry weight) 1 day/wk.

† Clinical signs present (+) or absent (-).

‡ This animal had amyloid deposits (positive with congo red) in the periportal region; no tissue from this area was available for electron microscopic evaluation.

§ Normal ranges for domestic cat.

AST = aspartate aminotransferase.

ALT = alanine aminotransferase.

Severity code for liver pathology: 1 = very mild; 2 = mild; 3 = moderate; 4 = moderate to severe; 5 = severe.

ND = not determined.

amyloidosis (A-8) and the remaining animal with VOD had some increase in liver enzymes, while cheetah with perisinusoidal fibrosis had a slight, questionable elevation of one liver enzyme, alanine aminotransferase.

#### Vitamin A levels in commercially prepared diets and cheetah livers

Cheetah livers and their diets were analyzed for vitamin A content because there was a significant proliferation of Ito cells, a vitamin A-storing cell, in the livers of several cheetah evaluated in this study. The vitamin A concentrations in the commercial diets were: Diet A—38,550 IU/kg, dry weight or 15,882 IU/kg, wet weight; diet B—10,950 IU/kg, dry weight or 3,438 IU/kg, wet weight; and diet C—95,820 IU/kg, dry weight or 30,758 IU/kg, wet weight. Diets A and C were dramatically higher, while diet B was slightly higher, than the recommended vitamin A allowance for the domestic cat (10,000 IU/kg, dry weight).<sup>25</sup> Diet D (primarily frozen horsemeat and horsemeat by-products) (Table 2) was a commercially prepared diet for exotic carnivores and was unavailable for vitamin A analysis because it has not been commercially available for a few years.

Seven out of nine cheetah livers had vitamin A levels five to 19 times higher than the upper limit of the normal range for vitamin A level in the liver of the domestic cat (Table 2). By light microscopy, high hepatic vitamin A concentration was associated, in all seven cheetah, with obvious proliferation of Ito cells and some fibrosis of the space of Disse. VOD was seen

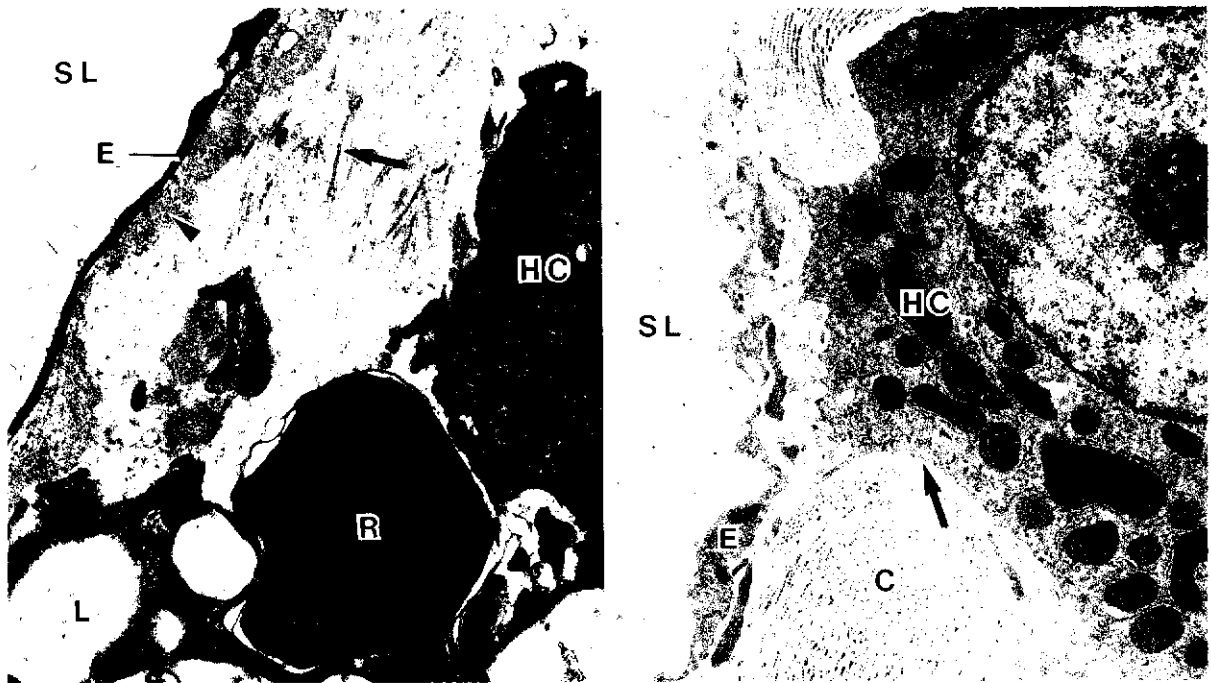
in the three cheetah with the highest vitamin A levels. Every week, these three animals were fed diet A (38,550 IU of vitamin A/kg, dry weight) for 5 days, followed by a day of fast, and the next day their diet consisted exclusively of raw beef liver (+545,000 IU of vitamin A/kg, dry weight). Cheetah B-5 was given diet B (10,950 IU of vitamin A/kg, dry weight) four times a week. On the other 2 days, this animal received entire carcasses from small animals, such as chicken and rabbit, or a portion of the animal carcass (excluding viscera), such as goat or calf. This cheetah was fasted for 1 day. The vitamin A concentration in the liver of this animal was 3.5 times higher than the upper limit of the normal range for vitamin A concentration in the liver of the domestic cat (2,000-4,000 IU of vitamin A/kg, dry weight), and it was not associated with significant swelling or proliferation of Ito cells. In the domestic cat, signs of toxicity, such as deforming cervical spondylosis and Ito cell changes, are noted with hepatic vitamin A concentrations of 15,442  $\mu\text{g/g}$ , dry weight and above.<sup>25</sup> Cheetah B-5 did not have signs of toxicity, possibly because the animal was relatively young or because the vitamin A concentration was normal for cheetah. Cheetah B-4, a juvenile, was the only animal with a vitamin A level within the range seen in domestic cats.

In the 104 sexually mature cheetah that were surveyed, the increase in number and size of Ito cells was noticeable in 68. It was usually associated with perisinusoidal fibrosis, and 44 out of 68 of these animals had VOD. There was a good correlation between the



**Fig. 7.** Vascular wall of central vein from cheetah with hepatic VOD (A-2). Spindle-shaped cells (S), basal lamina (arrowhead), and cytoplasmic extensions containing dense plaques (d) within a matrix of mature collagen fibers (C) and bundles of microfibers (arrow). Normal endothelial cells (E) line lumen (L) of central vein.

**Fig. 8.** Space of Disse from cheetah with hepatic VOD (A-3), widened and filled with abundant microfibers, some arranged in bundles (arrowheads), some attempting to form a basement membrane (arrow), and others mixed within a bundle of mature collagen fibers (C). Portions of an endothelial cell (E), hepatocytes (HC), and sinusoidal lumen (SL). Bar = 1  $\mu$ m.



**Fig. 9.** Space of Disse from cheetah with hepatic VOD (A-1). Thick layer of basement membrane-like material (arrow-head), several individual mature collagen fibers (arrow), an Ito cell with large lipid droplets (L), and a trapped red blood cell (R). Portions of endothelium (E), a hepatocyte (HC) and sinusoidal lumen (SL) also apparent.

**Fig. 10.** Liver of cheetah with perisinusoidal fibrosis (A-7). Space of Disse widened with bundles of mature collagen fibers (C) causing loss of microvilli (arrow) on neighboring hepatocytes (HC). Endothelial cell cytoplasm (E) and sinusoidal lumen (SL).

increase in the number of Ito cells and the severity of VOD in 16 out of 62 cheetah with VOD. However, 18 out of 62 cheetah with VOD did not have significant Ito cell proliferation, even though more than half of them had severe liver lesions.

### Discussion

Veno-occlusive disease (VOD) of the liver was diagnosed, by light microscopy, in 60% of the captive adult cheetah population evaluated in this study and was considered a major cause of liver disease in this endangered species. The earliest vascular lesion was an intimal thickening of the central vein due to the proliferation of smooth muscle-like cells and the presence of excessive amounts of fine fibers, which progressed eventually to partial or total occlusion of the lumen. This lesion could result from a direct injury to the endothelial cells lining the central vein; however, no significant lesion was consistently apparent ultra-structurally. Alternatively, it could be a compensatory reaction to a decrease in blood inflow from the sinusoid due to perisinusoidal fibrosis. Budd-Chiari syndrome, generally the result of thrombosis of the large hepatic veins,<sup>4</sup> cannot be excluded, since in most cheetah, the large hepatic veins were not evaluated at necropsy; however, no recent or organized thrombi could be found

in the lumen of the central veins by light microscopy. Changes in the space of Disse were consistent with an increased sinusoidal pressure following blood outflow impairment and subsequent fibrosis (A. Martinez-Hernandez, personal communication). The abundance of immature collagen in that space or in the wall of the central vein, could also indicate active fibroplasia,<sup>3,19</sup> possibly resulting from the continuous presence of one or more injurious agents within the liver. The loss of microvilli on the sinusoidal border of hepatocytes, and, in some cases, the formation of a thick basement membrane-like material, could interfere with the normal absorption of nutrients and diffusion of oxygen from the sinusoid.<sup>28</sup> This could lead to liver function impairment and, eventually, to cellular degeneration and necrosis, as was noted in some of the animals. A diminution of the sinusoidal diameter, caused by widening of the space of Disse, could also result in an increase in portal pressure and ascites.<sup>21</sup>

Chronic vitamin A ingestion in humans has been associated with hepatic lesions, characterized by an increase in number and size of Ito cells and by perisinusoidal fibrosis.<sup>9,15</sup> Central vein fibrosis, fibrosis of portal areas, and progression to cirrhosis have been mentioned in later stages of the lesion.<sup>17,33</sup> Storage of toxic doses of vitamin A in Ito cells, which are thought



to be facultative fibroblasts, could stimulate them to form collagen, leading to fibrosis of the space of Disse and the central vein.<sup>9,10,15,17,20,33</sup> In the nine cheetah evaluated for liver vitamin A content, an increase in severity of hepatic lesions paralleled the increase in concentration of vitamin A, suggesting that vitamin A excess could play an important role in the development of VOD in cheetah. When the entire adult population surveyed was considered, the etiopathogenesis of VOD became more complex. Because of discrepancies between the increase in the number of Ito cells and the severity of the hepatic lesions, vitamin A excess, as a possible contributing factor in the pathogenesis of VOD, could account for only 26% of the VOD cases. In addition, in approximately 30% of the cheetah with VOD, there was no Ito cell proliferation associated with the lesion, eliminating vitamin A excess as a potential cause in this particular group. It is evident that other factors besides vitamin A excess play a role in the pathogenesis of VOD in cheetah.

Although VOD is a distinctive lesion, it does not suggest any specific etiologic mechanism. The best known cause of VOD is pyrrolizidine alkaloid poisoning. It has caused economically important livestock losses in pasture areas containing a high density of pyrrolizidine-alkaloid containing plants such as *Senecio* or *Crotalaria*;<sup>18</sup> it has also caused fatal liver disease in humans ingesting food contaminated with the alkaloids.<sup>5,30,35,37</sup> Megalocytosis, a common feature in pyrrolizidine toxicity, was not present in any of the cheetah livers evaluated in this study, nor could any source of pyrrolizidine alkaloids be identified in their diet or environment. Aflatoxicosis has occasionally been associated with occlusion of the central veins;<sup>26</sup> however, this mycotoxicosis is primarily characterized by biliary proliferation and marked variation in the size of liver cell nuclei, neither of which were noted in the liver of these cheetah. Tumors originating from the liver should be expected after long-term exposure, since aflatoxin is a potent carcinogen in several animal species.<sup>26</sup> However, only one cholangiocellular carcinoma was diagnosed in this study. Other well-established causes of VOD are irradiation and antineoplastic or immunosuppressive drugs.<sup>31,44</sup> Based on their clinical history, none of the cheetah received any of these treatments.

A lack of genetic variation, already demonstrated in the wild and captive cheetah population,<sup>27</sup> could have explained the high prevalence of VOD seen in this study. However, VOD is not confined to the cheetah. It has been described in other felidae,<sup>6,29,32,40</sup> especially in the snow leopard, where the prevalence and severity are as high as in the cheetah,<sup>24</sup> suggesting that VOD in cheetah was probably not the result of a genetic disorder. The most obvious common denominator be-

**Table 2.** Comparison of vitamin A levels and pathological changes in the livers of cheetah on different diets.

Animal	Age (years)	Diet*	Liver Vit A ( $\mu\text{g/g}$ dry weight)	Ito Cell Proliferation	Veno-occlusive Disease
B-1	6.0	A, Li	57,580	5	5
B-2	6.5	A, Li	45,258	5	5
B-3	13.0	A, Li	74,026	5	4
B-4	1.1	D	2,149	—	—
B-5	4.5	B	14,034	—	—
B-6	10.0	A	20,257	3	—
B-7	7.0	C	39,190	4	—
B-8	12.0	A	28,039	4	—
B-9	10.0	A	28,669	4	—
Domestic cat†			2000–4000		

\* Commercial diets: diet A contained 38,550 IU of vitamin A/kg, dry weight; diet B contained 10,950 IU of vitamin A/kg, dry weight; diet C contained 95,820 IU of vitamin A/kg, dry weight; diet D, vitamin A level unknown; Li, diet A replaced by raw beef liver (+545,000 IU of vitamin A/kg, dry weight) 1 day/wk.

† Normal range for vitamin A in the liver of domestic cat at the Animal Health Diagnostic Laboratory, Michigan State University.

Severity code for liver pathology: 1 = very mild; 2 = mild; 3 = moderate; 4 = moderate to severe; 5 = severe.

tween different species of felidae and groups of cheetah from a wide geographical range is the diet. Animals could have been exposed to different toxic compounds from the diet, or ingredients fed to captive animals could have changed in quality, quantity, and sources. As an example, fish meal was used in the early 1970's in the commercially prepared exotic feline diet.<sup>13</sup> However, because of difficulties in the availability of the product, soybean products partly or completely replaced fish meal as one of the constituents in the commercial diets fed to exotic carnivores. Fish meal has been associated with nitrosamine intoxication, resulting in vascular lesions resembling VOD of the liver.<sup>22,23,38</sup> Another example is the non-steroidal plant estrogens found in high concentrations in a commercially prepared feline diet that has been fed to cheetah in several American zoos.<sup>34</sup> The source of these biologically active plant estrogens was soybean products added to the diet. Ingestion of these plant estrogens by cheetah was associated with a state of hypercoagulation, one of the suggested causes of hepatic vascular lesion following thrombosis of the large hepatic vein in women using oral contraceptives.<sup>2,7,14</sup> As with other estrogens,<sup>1,11,12,16</sup> plant estrogens could also have a direct effect on the endothelium by forming gaps in the endothelial lining, resulting in increased vascular permeability and leakage of plasma into the subendothelium. Subsequent stimulation and proliferation of mesenchymal cells, particularly the smooth muscle cells, could lead to intimal thickening of the vessels.<sup>12,16</sup>

The specific cause for VOD in each individual animal cannot be identified. However, based on correlations between morphologic findings and dietary analyses, it is suggested that at least one dietary factor may, at the present time, play a role in the pathogenesis of hepatic vascular lesions. Hypervitaminosis A may be responsible mainly for Ito cell proliferation and perisinusoidal fibrosis, which could progress to central vein sclerosis, as has already been demonstrated experimentally in the rat.<sup>20</sup> Vitamin A excess in cheetah could also increase the severity of the lesion and accelerate the clinical manifestation of VOD from other causes. By simple manipulation of the diet, this factor can be corrected, allowing future opportunity to identify other possible factors that are not obvious at this time.

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### References

- Almen T, Hartel M, Nylander G, Olivecrona H: The effect of estrogen on the vascular endothelium and its possible relation to thrombosis. *Surg Gynecol Obstet* **140**: 938-940, 1975
- Alpert LI: Venocclusive disease of the liver associated with oral contraceptives: case report and review of literature. *Hum Pathol* **7**:709-718, 1976
- Bornstein P, Sage H: Structurally distinct collagen types. *Ann Rev Biochem* **49**:957-1003, 1980
- Bras G, Brandt KH: Vascular disorders. *In: Pathology of the Liver*, ed. MacSween RNM, Anthony PP, and Scheuer PJ, pp. 315-334. Churchill Livingstone, New York, 1979
- Brooks SEH, Miller CG, McKenzie K, Audretsch JJ, Bras G: Acute venocclusive disease of the liver. Fine structure in Jamaican children. *Arch Pathol* **89**:507-520, 1970
- Dinnes MR, Henrikson R: Liver disease in cheetahs: preliminary report. *In: Proc Am Assoc Zoo Vet*, pp. 110-117, 1970
- Dugdale M, Masi AT: Hormonal contraception and thromboembolic disease: effects of the oral contraceptives on hemostatic mechanisms. *J Chronic Dis* **23**:775-790, 1971
- Eaton RL: The Cheetah. *The Biology, Ecology and Behavior of an Endangered Species*, pp. 41-87. Robert E Krieger Publishing Co, Malabar, FL, 1982
- Forouhar F, Nadel MS, Gondos B: Hepatic pathology in vitamin A toxicity. *Ann Clin Lab Sci* **14**:304-310, 1984
- Fraser R, Day WA, Fernando NS: Review: the liver sinusoidal cells. Their role in disorders of the liver, lipoprotein metabolism and atherogenesis. *Pathology* **18**: 5-11, 1986
- Friederici HHR: The early response of uterine capillaries to estrogen stimulation. *Lab Invest* **17**:322-333, 1967
- Gammal EB: Intimal thickening in arteries of rats treated with synthetic sex hormones. *Br J Exp Pathol* **57**:248-254, 1976
- Herdman R: Cheetah breeding program. *In: The World's Cats*, ed. Eaton RL, vol. 1, Ecology and Conservation, pp. 255-263. World Wildlife Safari, Winston, OR, 1973
- Hoyumpa AM, Schiff L, Helfman EL: Budd-Chiari syndrome in women taking oral contraceptives. *Am J Med* **50**: 137-140, 1971
- Hruban Z, Russell RM, Boyer JL, Glagov S, Bagheri SA: Ultrastructural changes in livers of two patients with hypervitaminosis A. *Am J Pathol* **76**:451-468, 1974
- Irey NS, Norris HJ: Intimal vascular lesions associated with female reproductive steroids. *Arch Pathol* **96**:227-234, 1973
- Jacques EA, Buschmann RJ, Layden TJ: The histopathologic progression of vitamin A-induced hepatic injury. *Gastroenterology* **76**:599-602, 1979
- Kelly WR: The liver and biliary system. *In: Pathology of Domestic Animals*, ed. Jubb KVF, Kennedy PC, and Palmer N, 3rd ed., vol. 2, pp. 240-312. Academic Press, New York, 1985
- Kent G, Gay S, Inouye T, Bahu R, Minick OT, Popper H: Vitamin A-containing lipocytes and formation of type III collagen in liver injury. *Proc Natl Acad Sci USA* **73**:3719-3722, 1976
- Kim CJ, Park CI, Kim CS, Lee YB: Histochemical and ultrastructural studies of hepatic fibrogenesis; its initiation and the effect of dexamethasone in rats. *Yonsei Med J* **23**:89-97, 1982
- Kluge T, Sommerschild H, Flatwork A: Sinusoidal portal hypertension. *Surgery* **68**:294-300, 1970
- Koppang N, Helgebostad A: Toxic hepatosis in fur animals. III. Conditions affecting the formation of the toxic factor in herring meal. *Nord Vet Med* **18**:216-225, 1966
- Koppang N, Helgebostad A, Armstrong D, Rimeslätten H: Toxic and carcinogenic effects of dimethylnitrosamine (DMNA) in the blue fox (*Alopex lagopus*). *Acta Vet Scand* **22**:501-516, 1981
- Munson L, Worley MB: Comparative hepatic histopathology in snow leopards and cheetahs. *In: Proc Am Assoc Zoo Vet*, 1987
- National Research Council: *Nutrient Requirements of Cats*. National Academy Press, Washington, DC, 1978
- Newberne PM, Butler WH: Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: a review. *Cancer Res* **29**:236-250, 1969
- O'Brien SJ, Roelke ME, Marker L, Newman A, Winkler CA, Meltzer D, Colly L, Evermann JF, Bush M, Wildt DE: Genetic basis for species vulnerability in the cheetah. *Science* **227**:1428-1434, 1985

- 28 Orrego H, Medline A, Blendis LM, Rankin JG, Kreaden DA: Collagenisation of the Disse space in alcoholic liver disease. *Gut* **20**:673-679, 1979
- 29 Reynolds TB, Peters RL: Budd-Chiari syndrome. *In: Diseases of the Liver*, ed. Schiff L and Schiff ER, pp. 1622-1630. JB Lippencott Co, Philadelphia, 1982
- 30 Ridker PM, Ohkuma S, McDermott WV, Trey C, Huxtable RJ: Hepatic venoocclusive disease associated with the consumption of pyrrolizidine-containing dietary supplements. *Gastroenterology* **88**:1050-1054, 1985
- 31 Rollins BJ: Hepatic veno-occlusive disease. *Am J Med* **81**: 297-306, 1986
- 32 Ruedi D, Heldstab A, van den Ingh TSGAM: Liver cirrhosis in snow leopards—further results. *Int Ped Book of Snow Leopards* **2**:195-204, 1980
- 33 Russell RM, Boyer JL, Bagheri SA, Hruban Z: Hepatic injury from chronic hypervitaminosis A resulting in portal hypertension and ascites. *N Engl J Med* **291**:435-440, 1974
- 34 Setchell KDR, Gosselin SJ, Welsh MB, Johnston JO, Balistreri WF, Kramer LW, Dresser BL, Tarr MJ: Dietary estrogens—a probable cause of infertility and liver disease in captive cheetah. *Gastroenterology* **93**:225-233, 1987
- 35 Stillman AE, Huxtable R, Consroe P, Kohnen P, Smith S: Hepatic veno-occlusive disease due to pyrrolizidine (Senecio) poisoning in Arizona. *Gastroenterology* **73**:349-352, 1977
- 36 Stowe HD, Goelling B: Experience with HPLC for diagnostic serum and tissue vitamin A assays. *In: Proc Am Assoc Vet Lab Diagnos*, pp. 385-395, 1981
- 37 Tandon BN, Tandon RK, Tandon HD, Narndranathan M, Joshi YK: An epidemic of veno-occlusive disease of liver in Central India. *Lancet* **2**:271-272, 1976
- 38 Ungar H: Venooocclusive disease of the liver and phlebotatic peliosis in the golden hamster exposed to dimethylnitrosamine. *Pathol Res Pract* **181**:180-187, 1986
- 39 Unsworth DJ, Scott DL, Almond TJ, Beard HK, Holborow EJ, Walton KW: Studies on reticulin. I: Serological and immunohistological investigation of the occurrence of collagen type III, fibronectin and the non-collagenous glycoprotein of Pras and Glynn in reticulin. *Br J Exp Pathol* **63**:154-166, 1982
- 40 van den Ingh TSGAM, Zwart P, Heldstab A: Venooocclusive disease (VOD) of the liver in cheetahs and snow leopards. *Schweiz Arch Tierheilkd* **123**:323-327, 1981
- 41 van de Werken H: Preliminary Report on Cheetahs in Zoos and in Africa, pp. 1-10. Royal Zoological Society, Amsterdam, 1967
- 42 Wittmeyer Mills A: A comparative study of the digestibility and economy of three feline diets when fed to lions and tigers in confinement. *In: The Comparative Pathology of Zoo Animals*, ed. Montali RJ and Migaki G, pp. 87-91. Smithsonian Institution Press, Washington, DC, 1980
- 43 Worley MB: The continuing role of a feline hepatitis B-like virus in the pathogenesis of liver disease in exotic felids. *In: Proc Am Assoc of Zoo Vet*, pp. 17-20, 1983
- 44 Zafrani ES, Pinaudeau Y, Dhumeaux D: Drug-induced vascular lesions of the liver. *Arch Intern Med* **143**:495-502, 1983