Renal medullary AA amyloidosis, hepatocyte dissociation and multinucleated hepatocytes in a 14-year-old free-ranging lioness (*Panthera leo*)

J H Williams[a*], E Van Wilpe[b] and M Momberg[c]

ABSTRACT

A 14-year-old lioness, originating from Etosha in Namibia, and a member of a pride in Pilanesberg National Park since translocation in 1994, was euthanased due to fight-related vertebral fracture and spinal injury, incurred approximately 6–8 weeks previously. Blood specimens collected at the time of death showed mild anaemia and a leukogram reflecting stress and chronic infection. Necropsy conducted within 2 hours of death was on a dehydrated, emaciated animal with hindquarter wasting and chronic traumatic friction injuries from dragging her hindlegs. There was cellulitis in the region of bite-wounds adjacent to the thoraco-lumbar vertebral fracture, at which site there was spinal cord compression, and there was marked intestinal helminthiasis. The outer renal medullae appeared pale and waxy and the liver was macroscopically unremarkable. Histopathology and electron microscopy of the kidneys revealed multifocal to coalescing deposits of proximal medullary interstitial amyloid, which fluoresced strongly with thioflavine T, and was sensitive to potassium permanganate treatment prior to Congo Red staining, thus indicating inflammatory (AA) origin. There was diffuse hepatocyte dissociation, as well as numerous binucleated and scattered multinucleated (up to 8 nuclei/cell) hepatocytes, with swollen hepatocyte mitochondria, in liver examined light microscopically. Ultrastructurally, the mono-, bi- and multinucleated hepatocytes contained multifocal irregular membrane-bound accumulations of finely-granular, amorphous material both intra-cytoplasmically and intra-nuclearly, as well as evidence of irreversible mitochondrial injury. The incidence and relevance in cats and other species of amyloidosis, particularly with renal medullary distribution, as well as of hepatocyte dissociation and multinucleation, as reported in selected literature, is briefly overviewed and their occurrence in this lioness is discussed.

Key words: amyloidosis, dissociation, free-ranging, helminthiasis, hepatocyte, kidney, lioness, multinucleated, *Panthero leo*, trauma.

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INTRODUCTION

Pilanesberg National Park is 55 000 hectares in extent and situated in the Bolajola Region of North West Province of South Africa. It was reclaimed from farmland in the late 1970s and Operation Genesis in 1979 began its repopulation with the numerous mammalian species that had been indigenous to that area in ancient times. The over 7000 animals currently populating the park include the 'Big Five' (lion, elephant, buffalo, leopard and both black and white rhinoceros) and the diversity of mammalian species, birdlife and vegetation reflect the transition zone that the park occupies between the dry western Kalahari and the wetter eastern Lowveld regions. The park also encompasses an extinct volcanic crater which is the most perfect example of an alkaline ring complex.

Amyloidosis, hepatocyte dissociation and multinucleated hepatocytes do not appear to have been reported in lions. Each condition is briefly outlined regarding incidence and possible causes in cats (domestic and wild), other animal species and man, prior to presentation of the case history.

Amyloidosis is a systemic disease affecting humans and domestic animals with varying frequencies, and has also been reported in several wild animals, including zoo-kept Dorcas gazelles (*Gazella dorcas*) with *Arcanobacterium pyogenes* infections[43,58], a zoo-bred and kept mountain gazelle (*Gazella gazelle*)[31], zoo-bred and kept closely-related Siberian tigers (*Pantheras tigris altaica*) with no other disorders[45], hares (*Lepus europaeus*)[22], and captive cheetahs (*Acinonyx jubatus*), which had various chronic inflammatory diseases in other organs[12,36,38].

Amyloidosis is reportedly less common in cats than in dogs, with marked proteinuria being less prominent in cats due to predominantly medullary localisation of the amyloid, as opposed to glomerular deposition in dogs[35]. Amyloid deposits may be found in many organs and may be concentrated in any one or more of them, such as liver or spleen; however, renal medullary amyloidosis has been the most common site reported in Chinese Shar-Pei dogs[35], Dorcas gazelles with a high incidence of *Arcanobacterium pyogenes* infections[35,43,58], captive cheetahs[12,36,38], the inbred Siberian tigers[45], and occasionally in cattle[35].

Various hereditary forms of amyloidosis exist in humans, the best-known being the autosomal recessive disease in people of Mediterranean descent known as Familial Mediterranean Fever in which renal amyloidosis is a feature[21]. Familial amyloidosis has now also been suspected or confirmed in other species, notably Abyssinian cats (primarily renal medullary or glomerular deposition)[7,14,15,37], Siamese cats (the liver is the main target organ)[37], inbred related Siberian tigers (predominantly renal medullary amyloid resulting in end stage renal failure)[45], Chinese Shar-Pei (predominantly renal medullary)[35], beagles (especially in glomeruli, mild in medulla)[6], and English foxhounds (glomerular and interstitial)[34]. The captive cheetahs reported separately in the USA and South Africa derived from the same population so a genetic predisposition due to relative homogeneity is possible, but both groups were also subject to similar captive-management practices[36,38].

Amyloid can be specifically stained with

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Congo Red, showing green birefringence under cross-polarised light, and it fluoresces strongly under ultraviolet light when stained with thioflavine-T[5,33]. Immunohistochemical staining using antibodies to amyloid A from various species with the indirect immunoperoxidase technique[22,31,32,45] is a specific test. Potassium permanganate oxidation of Congo red staining occurs with AA amyloid but not with AL amyloid[52] or pancreatic insular amyloid[55]. Most cases of idiopathic and familial amyloidosis have been found to be of the reactive AA type. Pathology caused by amyloid is related to organ dysfunction due to compression or obstruction exerted on surrounding tissues, which may result in ischaemia and necrosis[14], or due to protein loss from affected glomeruli.

Dissociation and rounding up of hepatocytes is mentioned in Jubb *et al.*[4] as a possible lesion in cats with feline parvovirus infection (feline panleukopaenia); this was speculated to be associated with dehydration and anaemia[4,16]. Leptospirosis and to some degree *post mortem* change were also mentioned as causes[28].

Jubb *et al.*[4] refer to multinucleation of hepatocytes as 'hepatocellular fusion', thus creating a syncytial appearance with disappearance of adjacent cell membranes. It is mentioned as a rare phenomenon that might be found 'unexpectedly in cats', in which species they state that it has also been produced by experimental dioxin poisoning, but without further comment nor reference[28]. An independently found reference describing dioxin toxicity[19] claimed high mortality of domestic small animals, especially rabbits and poultry, with no specific mention of cats, after environmental contamination by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the area of Seveso in Italy. A number of rabbits had 'dystrophic lesions' of hepatic tissue but these were not elaborated upon.

One of the few reports of multinucleated/giant hepatocytes in animals is that described in protoporphyria in Limousin calves in Kansas, USA, where hepatic cords were swollen, hepatocyte cell volume and nuclear sizes varied a lot and many hepatocytes had 4–10 nuclei – these cells tended to cluster together in groups of 3 or more, or were lined up in a single cord. Large secondary lysosomes were found ultrastructurally in hepatocytes as well as in phagocytic cells in the dermis[50].

Giant cell hepatitis has also been reported in aborted equine foetuses and stillborn foals in the USA and Canada[8,40,54], where most cases were diagnosed positive for *Leptospira interrogans* serogroup *Pomona* serovar *kennewicki*[40,54]. The hepatic pathology described included hepatocyte dissociation, hepatic cord disruption and numerous multinucleated giant hepatocytes. The authors of 1 report[54] speculated that the multinucleated hepatocytes may have resulted from injury/repair processes occurring at a particular stage of organ development and may have been non-specific for leptospirosis but possibly a useful sign of its presence. One of the reports where no aetiology was found[8] concerned 3 aborted foal foetuses in which hepatic acinar architecture was lost, hepatic cords were disorganised, there was multifocal hepatocellular necrosis and most hepatocytes were large syncytial cells containing up to 10 nuclei tending to be centrally located, and some nuclei contained cytoplasmic inclusions.

More recently, in 2000, in Tokyo, Japan, giant cell hepatitis was reported in 2 young (one was 18 months and the other 2 years old) female domestic cats both suffering from mediastinal thymic lymphoma and clinical hepatic failure. Both were seropositive for feline leukaemia virus (FeLV) but negative for feline immunodeficiency virus (FIV). The livers showed destruction of lobular structure, massive necrosis around central veins and hepatocytes were generally swollen and multinucleated. Lymphoblastic cells, as found in the mediastinal tumours, plus sparse lymphocytes, neutrophils and macrophages, were found in centrilobular and periportal areas. Immunohistochemistry on the livers for FeLV, FIV, feline syncytial virus, feline herpesvirus and feline parvovirus were all negative. Electron microscopic preparations were negative for viral particles and showed no cell membrane remnants in the multinucleated hepatocytes, suggesting a failure of hepatocyte division (cytokinesis) rather than cell fusion[49].

Giant cell hepatitis, characterised by formation of multinucleated hepatocytes, is found in human neonates and infants and is called 'post-infantile giant cell hepatitis' (PIGCH), but is rare in adults. Cholestasis and autoimmunity seem to be 2 important post-infancy trigger mechanisms for syncytial hepatic giant cell formation in humans[42]. Other causes in humans include viral infection (rubella, cytomegalovirus, reovirus[8], papillomavirus[17], and HIV infection of children[26]), trisomy 17-18 syndrome[8], and drug intoxication. In many cases the aetiology is obscure[49]. Non-A non-B hepatitis in humans causes hepatocyte dysplasia, this referring to cellular enlargement, nuclear hyperchromatism, multinucleation (3–5 nuclei per cell, affected cells usually in a pericentral or periportal location) and multiple nucleoli of individual hepatocytes or clusters thereof. Some non-A non-B hepatitis patients with liver cell carcinoma also had giant cell features[30].

In South Africa a traditional Zulu remedy 'impila', which is derived from the tuber of the African ox-eye daisy (*Callilepsis laureola*) can cause acute fatal hepatocellular necrosis and severe hypoglycaemia especially in children. The toxin, atractyloside, at low doses *in vitro* in human G2 hepatocytes of the HuH-7 cell line, caused a variety of changes, including hypercondensation of chromatin, multinucleated cells, nuclear fragmentation, destruction of cytoplasmic tubulin, swelling of mitochondria and apoptosis[48]. Atractyloside causes a reduction in intracellular ATP content due to inhibition of ADP transport at the mitochondrial membrane, depletion of reduced glutathione and depressed gluconeogenesis. The ox-eye daisy occurs in the eastern and northeastern parts of South Africa, from the Eastern Cape Province to Mpumalanga[53].

CASE HISTORY

A 14-year-old lioness (*Panthera leo*) originating from Etosha in Namibia and having been translocated to the Pilanesberg National Park in 1994, was spotted in February 2004 alone near water in an emaciated condition and dragging her hindlegs. She had been injured in a fight 6–8 weeks previously between 2 warring prides of lions in the park. She was in the post-reproductive phase having last had a litter 25 months previously of which 2 males survived. Owing to suspicion of her having been bitten on the spine and her emaciation, she was immobilised by darting and 15 minutes later shot. Other pertinent history was that buffalo of Pilanesberg National Park had tested negative for tuberculosis. Lion numbers in the Park at the time were between 30 and 40.

Serum chemistry and haematology

At the time of death, blood was taken in serum tubes, serum was separated by centrifugation within 2 hours and stored at –10 °C until chemical analysis was done (RA-XT, Bayer) at 25 °C, as well as albumin, globulin, albumin/globulin ratio and electrophoresis (Sebia K20 machine, with fraction reading done on a DVSE Densitometer; distributed by Separation Scientific RSA) (see Table 1).

Electrophoresis showed a mild gradual polyclonal gammopathy although globulin levels were within high normal range; the globulin graph was slightly accentuated due to the low albumin level.

Stored serum was also used to test for FIV antibodies and FeLV antigen (Feline

Leukemia Virus Antigen/Feline Immunodeficiency Virus Antibody Test Kit, Idexx Laboratories) which were both negative. The indirect fluorescent antibody test was performed for immunoglobulin G antibodies to *Toxoplasma gondii* and this was positive at a screening dilution of 1:20.

At necropsy, peripheral blood-smear findings were corroborated by haematological values on blood drawn into an EDTA tube at the time of euthanasia (Cell-dyne 3700, Abbott) (see Table 2).

Macroscopic necropsy findings

The lioness was emaciated and dehydrated, with many ticks on the skin. There was serous atrophy of visceral fat around the heart and no solid fat reserves were found. A bullet entry site, with a 3 × 5 cm subcutaneous haemorrhage surrounding it, was present on the right midline of the skull. The left maxillary canine was fractured; all teeth were very yellow and the oral mucosa was pale. There was marked atrophy of hindquarter musculature, with traumatic alopecia and hyperpigmentation, as well as superficial degloving injury of the cranial aspects of the lower hind legs from hocks to digits, with the left hindleg being more severely affected than the right. Subcutaneously, the plantar aspect of the left hindleg was very erythematous. A large (approximately 10 × 4 cm) chronic skin ulcer was present on the belly just left of the midline, amid hyperpigmented alopecic skin (suspected self-trauma from licking). Over the thoraco-lumbar junction mid-dorsally on the back, an area of hyperpigmentation and alopecia was associated with elongated subcutaneous abscesses containing light-yellow purulent exudate, on either side. The bodies of the last thoracic and 1st lumbar vertebrae were fractured by compression so that normal architecture was lost; the intervertebral space was narrowed and devoid of disc material, and the dorsal spines were displaced towards each other and fused. The associated spinal cord segment was compressed.

The kidney medullae appeared diffusely pale and waxy, especially the proximal zone adjacent to the cortico-medullary junction. There were occasional small outer cortical cysts measuring up to 3 mm in diameter. The liver was mostly congested but some areas were an orange-brown colour. There was hypostasis of blood to the right lung, multifocal diffuse emphysema, including small bullae involving both lungs, and mild multifocal petechiation of the right lung.

The stomach contained a small amount of grass. The duodenal mucosa was congested with a few petechiae and was stained yellow with bile. The whole intestine was filled with numerous long white *Taenia regis* tapeworms. There were also scattered *Ecchinococcus granulosus* adults embedded in or protruding from the intestinal mucosa. The small amount of faeces was brown-black in colour and watery, with some hair present. A faecal flotation performed using hypertonic saline (Egg Flotation Fluid, Kyron Laboratories) and examined light microscopically at ×20 magnification, after 10 minutes, showed numerous *Ancylostoma*-like eggs.

Table 1: **Serum chemistry.**

	Lioness		Normal range (domestic cats)
Alanine transaminase (ALT) U/ℓ	25	High	2–23
Alkaline phosphatase (ALP) U/ℓ	11		T1/2 short <30
Aspartate aminotransferase (AST) U/ℓ	42	High	<22
Creatine kinase (CK) U/ℓ	262	High	20–100
Gamma glutamyltranspeptidase (GGT) U/ℓ	1		1–10
Sodium (Na) mmol/ℓ	138.0	Low	141–156
Potassium (K) mmol/ℓ	5.23	High	4.0–5.1
Chlorine (Cl) mmol/ℓ	122		115–123
Calcium (Ca^{2+}) mmol/ℓ	1.17		0.76–1.2
Urea mmol/ℓ	17.7	High	7.1–10.7
Creatinine µmol/ℓ	73		40–141
Bile acids µmol/ℓ (fasting)	2.6		1.6 ± 0.3–5.0 ± 2.6
Total serum protein g/ℓ	61.2		60–80
Albumin g/ℓ	16.5	Low	25–36
Globulin (total) g/ℓ	44.7		22–48
Albumin/globulin	0.37	Low	0.6–1.2
Alpha g/ℓ	8.4		8–16
Alpha 1 g/ℓ	2.1		
Alpha 2 g/ℓ	6.3		
Beta g/ℓ	14.3	High	6–14
Beta 1 g/ℓ	7.4		
Beta 2 g/ℓ	6.9		
Gamma g/ℓ	19.5		12–22

Table 2: **Haematology.**

	Lioness		Normal range (domestic cat)
Haemoglobin (Hb) g/ℓ	85		80–140
Red cell count (RCC)10^{12}/ℓ	5.13	Low	5.5–10.0
Haematocrit (Ht) ℓ/ℓ	0.281		0.24–0.45
Mean cell volume (MCV) fℓ	54.7		36–55
Mean cell haemoglobin content (MCHC) g/dℓ	30.4		30–36
RDW (?)%	21.0		18–25
White cell count (WCC) × 10^9/ℓ	18.9		7.0–20.5
Absolute neutrophils (mature) × 10^9/ℓ	17.07	High	2.5–15.37
Absolute neutrophils (immature) ×10^9/ℓ	0.00		0.0–0.3
Absolute lymphocytes ×10^9/ℓ	0.89	Low	1.5–7.0
Absolute monocytes ×10^9/ℓ	0.66		0.07–0.8
Absolute eosinophils ×10^9/ℓ	0.26		0.14–2.46
Absolute basophils ×10^9/ℓ	0.02		0.0–0.1
Thrombocytes ×10^9/ℓ	87.6	Low*	300–600
Anisocytosis (red cells)	1+		
Active monocytes	2+		
Parasites	*Hepatozoon* sp. Occasional *Babesia*-like sp.		

*Sample clotted/numbers appear normal.

Microscopic and ultrastructural (EM) findings

Tissues fixed in 10 % formalin for histopathological examination were routinely embedded in paraffin wax, sectioned at 6 µm and stained with haematoxylin and eosin (H&E) for light microscopic evaluation.

The most striking light microscopic finding in the kidney was accumulation of large amounts of interstitial palely-eosinophilic homogenous material in a diffuse medullary zone abutting on the cortico-medullary junction, and extend-

ing multifocally distally to involve only the proximal half of the medulla. There was no papillary necrosis. The tubules and blood vessels embedded in this material were separated from each other and slightly compressed (Fig. 1). Thioflavine T[33] staining compared with simultaneous staining of a positive control section (a dog with known glomerular amyloid) and examined using ultraviolet light, showed very strong fluorescence of the lioness' amorphous renal medullary material, no fluorescence of glomeruli, speckled intracellular fluorescence in cortical convoluted tubules and some in collecting tubule lining cells, as well as multifocally in the outer tunica media of occasional vascular walls (arterioles and occasional veins). The cytoplasm of occasional hepatic Kupffer cells, as well as the walls of most small to medium splenic arterioles and some larger veins also fluoresced. Congo Red staining alone as well as Congo Red after prior potassium permanganate treatment on sections of kidney, liver and spleen showed the amyloid deposits in the renal medulla to be sensitive to potassium permanganate, but those in the small splenic arteriolar walls (these deposits were not obvious on H&E staining) to be relatively insensitive; a similar finding was also mentioned in the article from which the staining method was obtained[52]. There was very mild occasional fibrous thickening of parietal renal Bowman's capsules, mild glomerular protein leakage without formation of distal tubular protein casts due to resorption in convoluted tubular cells, and occasional outer cortical small cystic dilatations.

Electron microscopy of the renal medulla revealed the intertubular homogenous material to consist of a meshwork of numerous, randomly-oriented non-branching amyloid filaments, about 10 nm in diameter (Fig. 2)[20]. The filaments were arranged in parallel arrays in some areas. Amorphous intra-cytoplasmic material was detected in single renal tubular cells.

Several sections were cut from a variety of liver lobes and most areas showed striking diffuse hepatocyte dissociation, with an absence of autolytic change (Fig. 3). Portal triads and central veins were easily distinguishable but most areas showed no semblance of hepatic cords nor defined sinusoids connecting the 2. Blood content varied from normal to mildly congested sinusoids in some areas, with erythrocytes surrounding many single hepatocytes due to the cellular dissociation and sinusoidal disruption that was present. There were many bi-nucleated hepatocytes in most

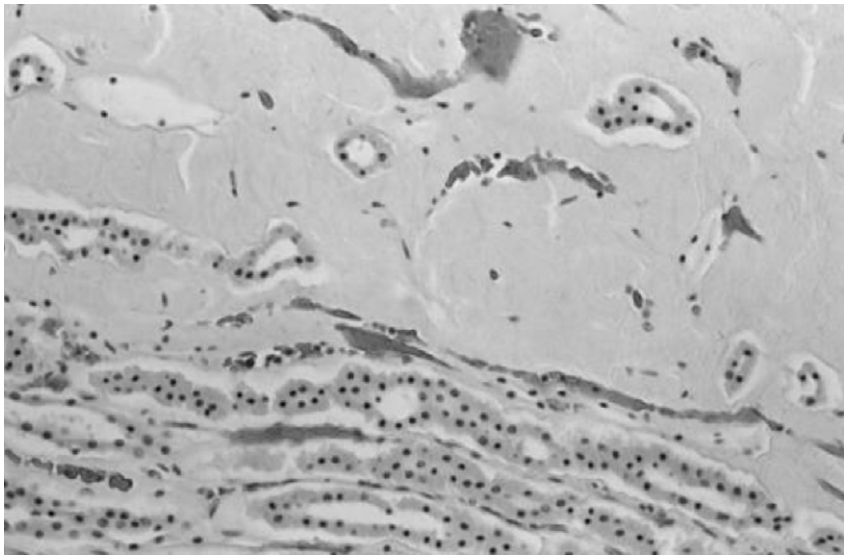

Fig. 1: **Low** (×10) **magnification of H&E-stained proximal renal medulla showing intertubular amyloid.**

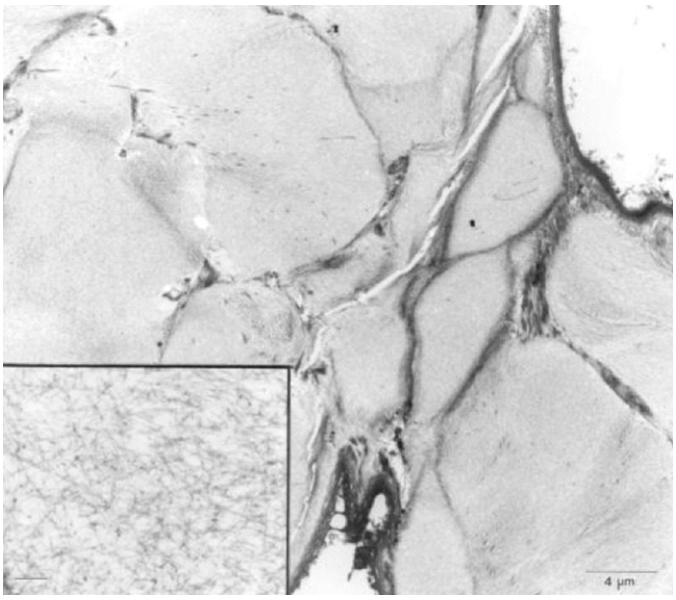

Fig. 2: **Electron micrograph of large intertubular amyloid deposits in the renal medulla. Inset: higher magnification of individual amyloid filaments.**

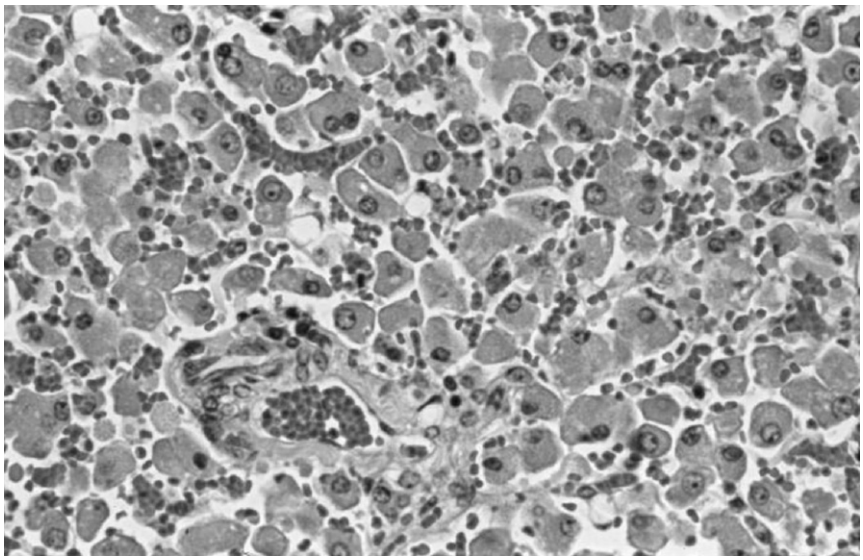

Fig. 3: **×20 magnification of H&E-stained liver showing hepatocyte dissociation.**

fields, and scattered to relatively closely-situated small groups of multinucleated hepatocytes, some with up to 8 nuclei per cell (Fig. 4). Nuclei varied in size and chromatism from larger and more vesicular/euchromatic to smaller and more heterochromatic. Ito cells containing fat vacuoles were scattered and obvious throughout. The cytoplasm of most hepatocytes was eosinophilic, with prominent, visibly-swollen mitochondria, giving a finely bubbly appearance. Small amounts of yellow-brown pigment were visible intracellularly in some hepatocytes and Kupffer cells in some areas and sections; this stained negatively with Hall's stain for bilirubin[24] and positively with Periodic Acid Schiff stain (PAS)[39] and was presumed to be lipofuscin. Occasional small clumps of extramedullary haematopoietic precursors as well as several small clumps of amorphous slightly granular eosinophilic material were found in some extracellular spaces in only few a areas (some subcapsular). Occasional extracellular hyaline droplets were present. Very few small granulomas containing fat vacuoles, lipofuscin pigment and a few macrophages were found (considered a senile change).

PAS staining showed marked glycogen depletion of hepatocyte cytoplasm; staining slightly pink however, were variable numbers of irregular-sized and -shaped areas in the cytoplasm of most hepatocytes (this was of a similar staining tone to the renal amyloid in the kidney section in the same block and PAS staining run). A reticulin stain (Gordon and Sweet's reticulin[3]) on 1 of the liver sections showed the reticulin fibre framework of all areas of hepatic lobule extraneous to central veins and portal triads to be broken up haphazardly into multiple short strands with few strands being longer than 2 or 3 hepatocytes at most, all being angled to curvilinear in shape, some showing branching, and with no continuity.

Young's modification of Warthin-Starry stain[56] on sections of both kidney and liver failed to show any spirillar silver-positive *Leptospira*-like microorganisms.

Electron microscopy of the liver tissue reflected the hepatocyte dissociation, multinucleation, glycogen depletion and the presence of lipofuscin granules seen light microscopically. Several hepatocellular mitochondria were swollen and contained electron-dense woolly densities[20]. A prominent ultrastructural feature of the hepatocytes was the presence of intracytoplasmic finely granular amorphous inclusions. These inclusions appeared to be within dilated endoplasmic reticulum and were mostly closely associated with the nucleus (Fig. 5). Similar intranuclear inclusions were apparent within single hepatocytes.

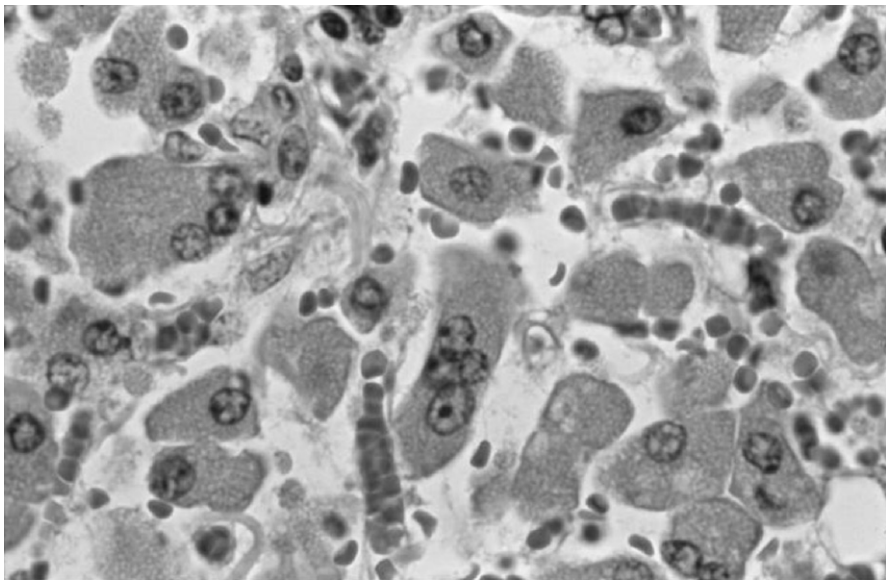

Fig. 4: **High dry** (×40) **magnification of H&E-stained liver showing multinucleation of hepatocytes.**

The spleen had occasional small deposits of hyaline-like material centri-follicularly in white matter, which did not stain with either Congo Red or thioflavine T. There was moderate red pulp congestion and extra-medullary haematopoiesis.

Sections of spinal cord from the region of macroscopic compression showed marked diffuse white matter axon and myelin degeneration, with the presence of scattered glial cells, myelinophagia and neovascularisation. Spinal nerves showed no pathological changes. Sections of cord distal to the compression lesion also had axon and myelin degeneration but involving progressively more peripheral areas of the white matter.

Several sections of small intestine revealed multifocal embedded E*chinococcus granulosus* adults in various aspects – some of them within the lamina propria and with eosinophils scattered around them.

Lung sections showed mild multifocal peribronchiolar or perivascular anthracosis as well as some distal airway macrophage phagocytosis of pigmented foreign material. Large areas of lung were partially atelectatic and there were also some areas of bullous emphysema. The right lung had mild diffuse hypostatic congestion, mild multifocal petechiae and fairly diffuse moderate alveolar wall neutrophilic vascular leucostasis.

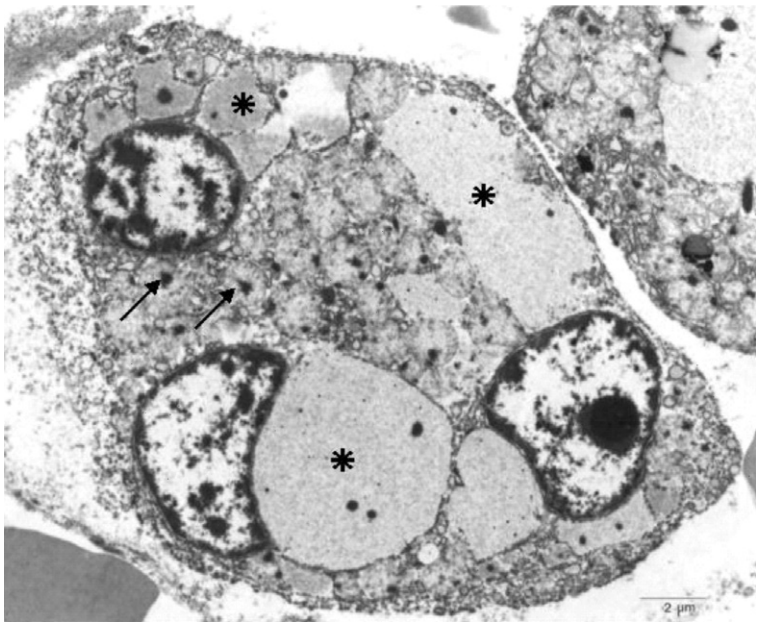

Fig. 5: **Electron micrograph of multinucleate hepatocyte showing intracytoplasmic inclusions (✱) within dilated RER. Note the intra-mitochondrial woolly densities** (↑).

DISCUSSION

Amyloid is deposited in the walls of small blood vessels and extracellularly in various sites, including spleen, liver and kidneys. The 2 most common types of amyloid protein are of either immunocytic (primary) AL type arising from immunoglobulin light chains in plasma cell dyscrasias, or of reactive systemic (secondary) AA type derived from serum amyloid A (SAA) apolipoprotein. SAA is an acute-phase immunoregulant product of mainly hepatic cells, produced in excess as a result of chronic antigenic stimulation or as part of the response to tissue injury (i.e. infectious or inflammatory diseases)[5]. SAA binds to high-density lipoproteins and in this way may enhance elimination of endotoxin or products of tissue injury, amongst other suggested protective roles[23]. SAA must be degraded initially to provide the basic constituent of amyloid fibrils; the 2nd stage of degradation of the AA intermediates to soluble peptides would protect from amyloid deposition and this 2nd step may be defective or diminished in the 5–15 % of individuals with chronic inflammatory disease that actually develop amyloidosis (this may involve genetic factors and is also associated with other factors like hypoalbuminaemia)[5,23].

Renal amyloid in this lioness was of the AA type, based on potassium permanganate and Congo red staining. Since she was suffering from infection of the bite wounds to her mid-back, had ongoing friction injuries to her hindlegs, as well as the suspected lick-associated ulcer of her belly, the amyloid was most likely associated with chronic inflammation. She originated from a healthy wild population of lions in Namibia, and was of a good age and relatively recently fecund, so familial amyloidosis is unlikely[23]. The severe hypoalbuminaemia, which is known to contribute to decreased AA-degrading activity[23] and thus amyloid deposition, most likely played a large role in amyloid fibril precipitation.

Alpha 2 globulin levels were relatively raised compared with those of alpha 1, although total levels of alpha protein were within normal range. Serum amyloid A resides within the pre-beta end of the alpha 2 range, but contributes minimally to alteration of the alpha 2 level measured since even when raised 1000-fold in acute reactions, might only increase to 1 to 5 g/ℓ at the very most (F. Reyers, Digital Veterinary Diagnostics, South Africa, pers. comm., 2004). We did not have access to direct determination of serum AA levels.

The absolute neutrophilia was interpreted as the response to the festering bite-wounds on her back plus the other traumatic injuries, as well as her stressed state, this latter being borne out also by the lymphopaenia and active monocytes. Her red cell count and haematocrit were in the low normal range but, combined with dehydration, confirmed the actual anaemia seen on macroscopic necropsy.

The slightly raised serum urea, in the presence of normal creatinine levels, could have been an indicator of digestion of blood in the intestine (melaenic faeces), pre-renal retention due to dehydration[23], as well as protein catabolism due to dehydration and starvation[18]. There may be decreased concentrating ability by renal tubules in animals with medullary interstitial amyloid deposits, leading to production of isosthenuric urine; this may have contributed to this lioness' dehydrated state but, unfortunately, urine specific gravity was not determined[23]. The lack of significant renal pathology histologically, other than the presence of amyloid, suggests renal function at the time of euthanasia was still relatively normal.

Normal hepatocytes are large polyhedral cells with round nuclei having peripherally dispersed chromatin and prominent nucleoli. Hepatocytes in a well-nourished individual store significant quantities of glycogen and process much lipid. There are numerous mitochondria in the cytoplasm, rough and smooth endoplasmic reticulum and extensive free ribosomes[57]. By contrast, this lioness' liver was markedly glycogen-depleted on PAS staining and there was neither evidence of fat accumulation nor other cytoplasmic vacuolation. Several mitochondria showed woolly densities, considered evidence of severe irreversible cell injury and cell death[20]. Most hepatocytes, including mono-, bi- and multinucleated ones, appeared on EM to be actively-producing large amounts of protein, possibly amyloid precursor; however, this is speculative. It has been suggested that Kupffer cells may synthesise amyloid by taking up amyloid precursor protein from the blood and converting and secreting it into the extracellular matrix where it polymerises into amyloid filaments[20].

The freshly-necropsied carcass, with no light or EM signs of hepatocyte autolysis, plus the fact that there were also multinucleated hepatocytes, are evidence that the hepatocyte dissociation was an antemortal change (death was instantaneous due to gunshot to the cranium). Her emaciated body condition, dehydration, mild anaemia, lack of solid fat reserves, paucity of stored glycogen in the liver, marked hypoalbuminaemia, and gastrointestinal tract filled with worms and devoid of food content, all support her malnourished state, and possibly these factors were cumulatively sufficient to result in the hepatocyte dissociation as mentioned previously in the introduction in cats with 'dehydration and anaemia'[4]. No convincing evidence of parvoviral infection-associated pathology of the gastrointestinal tract was found[16]. The multinucleated hepatocytes and mild increases in ALT and AST may have been the result of protein starvation as was noted in experimental rats[46], although extrapolation between species is not necessarily valid.

ALT is a cytosolic enzyme considered to be liver-specific for diagnostic use in cats; serum ALT levels rise with alterations of hepatocyte permeability or due to sublethal injury or necrosis, the magnitude of the increase roughly paralleling the number of cells or amount of hepatic mass affected. ALT rises within 12 hours of injury and peaks in 1–2 days after toxic insult, returning to normal over 2–3 weeks[18]. The marginal ALT increase in this lioness is difficult to interpret, but in the light of the AST and CK increases, normal ALP and GGT, the lack of histological necrosis but definite ultrastructural severe degenerative changes (woolly densities in mitochondria), marked disruption of sinusoidal supporting reticulin fibre meshwork with resultant hepatocyte dissociation, and multinucleation, liver function may have been in a near-normal early/acute stage of necrosis that was arrested at euthanasia.

AST occurs in most cells but is used as a diagnostic enzyme for liver and muscle disease due to its high activity in these tissues. It is also present in erythrocytes, so haemolysis may increase serum levels (this may be a false increase if there is haemolysis of a serum specimen)[18]. The plasma half-life is less than 12 hours in cats, and AST will also rise in sublethal or necrotic hepatocyte injury which leads to increased permeability. In cats with hepatic disease, ALT levels are usually higher than AST, which was not the case in this lioness – the AST increase may even have been from muscle breakdown, since creatine kinase (CK), which indicates primarily muscle injury[18], was moderately increased as well, and she had clinical muscle wasting and traumatic hindquarter injuries arising from her paretic state.

Fasting serum bile acid concentrations above 5 μmol/ℓ, and especially above 15 μmol/ℓ, are sensitive indicators of hepatobiliary disease in domestic cats, especially with extrahepatic bile duct obstruction, hepatic lipidosis, neoplasia

and portosystemic anomalies[9]. This lioness showed no signs macroscopically or microscopically of hepatobiliary disease, and the normal fasting bile acid level substantiates these findings.

Blue-green algal hepatotoxic cell damage, by way of disorganisation of cytoskeletal filaments, is cited as being most probably responsible for distortion of hepatocyte cell membranes *in vitro* and dissociation of hepatocytes seen early in toxicity *in vivo*[28]. Hepatocyte dissociation has been described in algal toxicity in a dog in the USA[13], outbreaks of blue-green algal poisoning of cattle and sheep in the Western Cape Province, South Africa[51], white rhinoceros newly-introduced into Borakalalo game reserve just east of the Pilanesberg National Park in South Africa in 1985[47], and in humans with renal failure in Brazil[41] via water insufficiently purified for haemodialysis.

These human cases also showed regenerative multinucleated hepatocytes between necrotic cells.

Other references on algal toxicity made no mention of either hepatocyte dissociation or multinucleated hepatocytes and these included experimental poisoning in sheep[25], and a southern African text on plant and mycotoxicoses of livestock[27]. Blue-green algal poisoning is caused by cyanobacterial microcystins, which are cyclic heptapeptides, of which more than 60 are known[41]. They are potent hepatotoxins and inhibitors of phosphatases in mammals and are known liver-tumour promoters. Water of inland lakes, reservoirs and slow-moving rivers are susceptible to blooms of *Microcystis aeruginosa* during late summer and autumn[25].

Mild or low-grade acute blue-green algal toxicity as a cause of the sinusoidal reticulin disruption cannot be excluded in this lioness, since it was the right time of the year for algal proliferation (hot, late summer/autumn weather conditions[25]). The lack of frank hepatocyte necrosis and as-yet marginally increased serum ALT level could possibly have been an indicator of a low-dose of microcystin.

Bi- and multinucleation of hepatocytes may be a normal regenerative/reparative feature of hepatocytes, for example following surgical removal of large segments of liver, or after chemically-induced necrosis[1]. Hepatocyte nuclei normally vary considerably in size and incorporate the unusual feature of, depending on species, up to more than half hepatocytes containing twice the normal (diploid) complement of chromosomes within a single nucleus making them tetraploid, and some may even contain 8 or more sets of chromosomes, these states being referred to as polyploid. Binucleate cells are also common in normal livers, with these nuclei exhibiting various ploidies. Hepatocytes, despite being differentiated, retain the capacity for mitosis should the need arise. Nuclear DNA is replicated in the synthesis or S phase of a cell cycle (at the end of interphase) and thereafter cells go into a short G2 phase where they prepare for mitotic division. Some hepatocytes, however, enter a protracted G2 phase during which they continue with their normal differentiated functions despite the presence of a duplicated (or higher ploidy) complement of DNA. Mitosis is the process whereby the duplicated chromosomes are split equally between the 2 potential daughter cells, and should be followed by cytokinesis (cytoplasmic division). Under some circumstances mitosis may occur in the absence of cytokinesis and results in the formation of binucleate and multinucleate cells[57]. Another pathway of nuclear division that may give rise to bi- and multinucleate cells is 'amitosis', whereby the nucleus stretches and the nuclear membrane invaginates, finally constricting into 2 parts lying side-by-side either attached or separate. This results in nuclei of asymmetric size, varying in amount and arrangement of heterochromatin and nucleoli. Amitosis may give rise to near haploid (aneuploid) nuclei[10,29]. No striking ultrastructural changes have been observed in the bi-, tri- and multinucleated hepatocytes resulting from mitosis without cytokinesis or amitosis of human and animal livers; also no indications of enhanced autophagocytosis or other signs of dedifferentiation were found[11]. The 3rd means of production of bi- and multinucleated hepatocytes is by cell fusion[1,2].

Adult male rats fed for 3 weeks on a high-protein diet had increased liver weight, total liver DNA content, hepatocyte volume, total mitochondrial membrane surface area and granular and smooth endoplasmic reticulum, but decreased nuclear size; serum alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) remained similar to control standard diet rats. Rats fed for 3 weeks on a low-protein diet showed decreases in all those parameters as well as in nuclear ploidy, but an increase in the number of binucleate hepatocytes; serum ALT, AST and ALP activity was mildly but significantly elevated[46].

The liver can regenerate hepatocytes in 3 ways: 1) by replication of existing hepatocytes by clonal expansion; 2) in more severe injury by replication of non-parenchymal epithelial cells in the intrahepatic biliary tree (the canals of Hering, which are the smallest biliary ducts) which have potential stem cell properties and give rise to cords of bipotential 'oval' cells which can then differentiate into either hepatocytes or cholangiolar epithelium[1]; or, 3) as demonstrated *in vivo* in mice, by cell fusion between hepatocytes and bone marrow origin haematopoietic stem cells with hepatic potential[2]. These stem cells may contribute to low rates of hepatocyte renewal and maintenance under normal conditions, but under circumstances of very strong selection pressure, may make a more significant contribution. The DNA of the bone marrow-derived cells appears to re-programme to that of hepatocytes while simultaneously 'rescuing' the DNA of the damaged hepatocyte (many of the fused nuclei have been shown to be polyploid), whether of autogenous or donor origin in mice and humans[1,2]. The fused cells, with 2 or more nuclei in the initial divisions, may with time and further divisions become mononucleate (suggesting either fusion or supernumerary nuclei being eliminated). They lose their haematopoietic markers and function exactly like hepatocytes, producing glycogen, albumin, and bile canaliculi[2]. Bone marrow cells have also been shown to have the potential to form new cardiomyocytes and cerebellar Purkinje neurons by the process of cell fusion – this has been demonstrated *in vivo* and results in the formation of multinucleated cells. It is well known that under normal conditions many hepatocytes have 2, and cardiomyocytes have 2 or more nuclei. Previous studies have shown that Purkinje neurons can be polyploid, although more than 1 nucleus has not been recorded until the recent experiment with mice[2]. Other studies have also shown that fused cells are positively selected during hepatic degeneration, thus helping survival of the damaged organ[1,2]. There is some evidence to support the possibility that at least some hepatic oval cells may be derived from a precursor of bone marrow origin[1].

Morphometric comparison of hepatic carcinoma cells arising in cirrhotic human livers with regenerative and normal hepatocytes has shown that the neoplastic population has significantly higher polymorphism, nucleocytoplasmic ratio and percentage of multinucleated cells but overall cell size is smallest. Regenerative cells have values between cancerous and normal cells; low nucleocytoplasmic ratio is associated with regeneration[44].

Multinucleation of hepatocytes in this lioness, apparent on light microscopy and ultrastructure, together with the presence

of the vacuoles containing finely granular amorphous material within both nuclei and cytoplasm of hepatocytes, might be speculated to have been largely due to the stimuli of wound-infection and trauma-induced inflammation to markedly step up production of acute-phase proteins such as SAA, although no other reference to this was found in the literature examined. However, their presence can also not be excluded as either reparative/regenerative or degenerative responses if, for instance, a low-dose of microcystin toxin from blue-green algae played any role at all in the state of her liver pathology[41]. No evidence, either serologically, histopathologically or ultrastructurally, was found of other possible causes of hepatocyte dissociation or multinucleation such as viruses (FIV, FeLV, FPV, Fel Herpes virus), neoplasia or leptospiral infection.

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