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Glomerulosclerosis in Captive Cheetahs (Acinonyx jubatus)

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Abstract. The cheetah (Acinonyx jubatus) is an endangered species with low fecundity and premature death in captivity. A previous survey determined that renal failure as a result of glomerulosclerosis was a major cause of death in captive populations. This study characterizes the morphologic, histochemical, and epidemiologic properties of glomerulosclerosis in this population. Kidneys from 87 cheetahs were examined by light microscopy; kidneys from six of those cheetahs were examined by electron and fluorescent microscopy using special stains specific for collagen, glycoproteins, reticulin, and fibrin. Immunohistochemistry for the advanced glycosylation end products (AGEs), pyrraline and pentosidine; also was performed on these cases. Glomerulosclerosis was present to some degree in 82% of the population, and in 30% of cheetahs the sclerosis was moderate to severe. Affected cheetah kidneys had thickened glomerular and tubular basement membranes, culminating in glomerulosclerosis. Thickened basement membranes were positive for collagen, glycoproteins, reticulin, and AGEs. Ultrastructurally, membrane material was homogeneous and fibrillar without electron-dense deposits. This glomerular lesion in cheetahs resembles diabetic glomerulopathy in humans and chronic progressive nephropathy in rats. No cheetahs had lesions of diabetes. However, adrenal cortical hyperplasia was prevalent and highly correlated with glomerulosclerosis in this population. If cheetahs with glomerulosclerosis had hypercorticoïdism, then hyperglycemia and glomerular hypertension could lead to progressive AGE and plasma protein accumulations in membrane lesions. As in rats, daily feeding of high-protein diets and lack of genetic variation in the population may further contribute to the high prevalence of glomerulosclerosis in captive cheetahs.

Key words: Advanced glycosylation end products; cheetah; diabetic nephropathy; glomerulosclerosis; membranous glomerulopathy; rat nephropathy.

The cheetah (Acinonyx jubatus), the last surviving species of the genus Acinonyx, is endangered as a result of habitat encroachment and hunting in the wild, and from low fecundity and premature death in captivity.21,29,32 Renal failure is a major cause of death of captive cheetahs in North America, and the Cheetah Species Survival Plan (SSP) Pathology Survey determined that glomerulosclerosis and renal amyloidosis accounted for the majority of these cases.21,29,35 Eighty-four percent of cheetahs in the survey had glomerulosclerosis, and there was a significant correlation between glomerulosclerosis and membranous glomerulopathy in this population. It was postulated that these lesions represented different stages of the same disease process.29 Typical lesions of glomerulonephritis such as mesangial cell proliferation and synechia formation were not noted, suggesting that glomerulosclerosis in cheetahs was not immune-mediated as it is in domestic cats.1 Adrenocortical hyperplasia also was prevalent in the surveyed cheetah population, leading to the hypothesis that adrenal and renal lesions are associated in cheetahs. Glomerulosclerosis only rarely occurs in other species of wild felids (L. Munson, unpublished). Because of these high population prevalences and apparently primary nature of glomerulosclerosis in cheetahs, this study was initiated to better characterize the renal lesion. This report describes the morphologic, immunohistochemical, and epidemiologic characteristics of glomerulosclerosis in cheetahs.

Materials and Methods

Formalin-fixed kidneys from 87 deceased cheetahs, submitted by 29 zoological facilities in North America as part of the American Zoo and Aquarium Association Cheetah SSP Pathology Survey, were examined histopathologically. The study population included 34 males and 53 females between the ages of 12 and 204 months (mean 105 ± 50 SD months). Fixed kidneys were processed routinely, embedded in paraffin, sectioned at 4–6 μm and stained with hematoxylin and eosin (HE), Masson’s trichrome (MT), and Congo red (CR). Five kidneys with moderate to severe glomerulosclerosis and one unaffected kidney were sectioned at 2–3 μm and stained with MT, CR, and periodic acid–Schiff (PAS), Jones’ methenamine silver (JMS), and phosphotungstic acid hematoxylin (PTAH). Autofluorescence was as-
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The prevalence of glomerulosclerosis within the study population was 82% (71/87), of which 26 cheetahs (30%) had moderate to severe (Grades 2 and 3) glomerulosclerosis. Cheetahs with glomerulosclerosis ranged in age from 24 to 204 months (mean 122 ± 5 SD months). The number of cases and severity of lesions increased with age (Fig. 1). However, four relatively young cheetahs (less than 8 years old) had end-stage kidney disease from glomerulosclerosis. There was no sex predilection or geographic clustering of cases.

Histologically, glomerular basement membrane thickening was global, and glomeruli with thickened membranes were randomly interspersed with sclerotic glomeruli (Fig. 2). The obsolescent glomeruli were composed of membranous silhouettes of glomerular tufts with diffuse homogeneous basement membrane thickening and without epithelial or endothelial cells. No synechia or inflammatory changes were noted. Similar thickening was present in the basement membranes of Bowman’s capsules and surrounding some, but not all, tubules. More severely affected tubules had mildly dilated lumens with epithelial attenuation, progressing to epithelial atrophy with preservation of thickened basement membranes.

Some sclerotic glomeruli had pyrraline and pentosidine immunoreactivity in Bowman’s capsular membranes (Figs. 3, 4). Pentosidine immunoreactivity also was present in the epithelium of proximal convoluted tubules and in medullary amyloid deposits. The material expanding the basement membranes of glomeruli and tubules was eosinophilic, intensely blue with MT (compatible with collagen), positive with PAS (compatible with glycoproteins), and positive with JMS (consistent with increased basement membrane reticulum fibers [Fig. 5]). The thickened membranes were negative for amyloid and fibrin, as determined by CR and PTAH, respectively. The affected membranes also had patchy autofluorescence. In contrast, the basement membranes of unaffected cheetahs were not autofluorescent, had no pentosidine or pyrraline immunoreactivity, and were scarcely visible in HE-, PAS-, and JMS-stained sections (Fig. 5).

Ultrastructurally, the glomerular and tubular basement membranes were diffusely expanded by a homogeneous, slightly fibrillar material (Figs. 6, 7). The material expanding the basement membranes was composed of a meshwork of fibrils coated by granular deposits. No electron-dense deposits consistent with immune complex deposition were noted in glomerular basement membranes. Podocyte foot processes were unaffected in glomeruli with mild to moderate membranous changes, but they became fused and effaced.

Results
as the lesion progressed. The ultrastructure of more severely affected glomeruli confirmed the loss of endothelial and epithelial cells noted by light microscopy and demonstrated the occlusion of capillary lumens (Fig. 8). Expanded basement membranes became convoluted and folded around the remaining mesangial cells.

All cheetahs with glomerulosclerosis also had glomerular and peritubular basement membrane thickening. Many of the cheetahs with severe glomerulosclerosis also had nephrosclerosis. Five additional cheetahs had glomerular and peritubular basement membrane thickening without sclerosis. Three cheetahs had extensive peritubular basement membrane thickening without glomerular changes. Taken together, 79 of 87 cheetahs had some form of prominent renal basement membrane thickening.

Other changes noted in kidneys with glomerulosclerosis were cortical atrophy, cortical infiltration of lymphocytes and plasma cells, increased interstitial fibrosis, tubular atrophy, and mild to moderate oxalate crystal deposits. These lesions tended to be more notable in kidneys with moderate and severe glomerulosclerosis. Twenty-eight cheetahs with glomerulosclerosis had a few intraluminal protein casts, but 19 of these cheetahs also had renal amyloidosis. Seventy-six percent of glomerulosclerosis cases had mineral deposition, varying from scant cortical and medullary deposits to locally extensive regions of medullary mineralization. Forty cases of glomerulosclerosis also had hyperplasia of the collecting duct epithelium.

Of the 87 cheetahs in this study, 70 cheetahs had adrenal glands submitted to the study, and 57 of these 70 cheetahs had diffuse adrenocortical hyperplasia. This lesion was highly associated with glomerulosclerosis ($P = 0.016$). None of the 85 cheetahs in the study from which pancreases were submitted had lesions in the islets of Langerhans.

**Discussion**

The principal lesion affecting the kidneys of cheetahs was a thickening of glomerular and tubular basement membranes, culminating in glomerulosclerosis. The staining characteristics of material expanding the basement membranes was consistent with collagen, glycoproteins, and reticulin, but not compatible with amyloid or fibrin. Ultrastructurally, the material was fibrillar without electron-dense deposits and, therefore,
Fig. 2. Kidney; cheetah. Fig. 2A. A 4-year-old cheetah with severe glomerulosclerosis has thickened glomerular and tubular basement membranes, end-stage sclerotic glomeruli, and interstitial fibrosis. Masson’s trichrome. Bar = 500 μm. Fig. 2B. Progressive changes from glomerular basement membrane thickening to complete sclerosis are evident. Masson’s trichrome. Bar = 220 μm.

Fig. 3. Kidney; cheetah. Fig. 3A. Pyrraline immunoreactivity is present in Bowman’s capsules of sclerotic glomeruli in a 4-year-old cheetah. Fig. 3B. Negative control. Streptavidin-biotin-peroxidase method, Harris’s hematoxylin counterstain. Bar = 125 μm.

Fig. 4. Kidney; cheetah. Fig. 4A. Pentosidine immunoreactivity is present in Bowman’s capsules of sclerotic glomeruli and diffusely within renal tubular epithelial cells of a 4-year-old cheetah. Fig. 4B. Negative control. Streptavidin-biotin-peroxidase, Harris’s hematoxylin counterstain. Bar = 145 μm.
Fig. 5. Kidney; cheetah. Fig. 5A. The glomerulus of a 10-year-old cheetah with glomerulosclerosis and thickened basement membranes. Fig. 5B. An unaffected glomerulus from a 23-month-old cheetah. Jones’ methenamine silver. Bar = 40 μm.

not characteristic of immune-mediated membranous glomerulopathy of domestic cats.1 Also, the thickened basement membranes in cheetahs did not have the histochemical staining properties or ultrastructural characteristics of amyloid.35 Most other renal lesions noted with glomerulosclerosis were consistent with secondary changes and end-stage kidney disease.

The renal lesion in cheetahs most closely resembles the glomerulopathy of early onset diabetic nephropathy of humans11,12,40 or chronic progressive nephropathy of rats.3–5,13,28 The renal lesions of diabetic nephropathy include glomerular basement membrane thickening, diffuse or nodular glomerulosclerosis, and expansion of peritubular basement membranes. Although only the diffuse form of glomerulosclerosis was noted in cheetahs, the ultrastructural character of the affected basement membranes in diabetic humans is similar to the cheetah lesion, consisting of homogeneous fibrillar material.11,40

Diabetic nephropathy in humans is thought to be due in part to nonenzymatic glycosylation by glucose of long-lived matrix proteins to form advanced glycosylation end products (AGEs).7,12,20,25,40,41 The result is cross-linking of extracellular matrix proteins and al-

Fig. 6. Kidney, glomerular capillary loop; cheetah. The basement membrane is uniformly thickened by slightly fibrillar material, and some podocyte foot processes are fused. Uranyl acetate and lead citrate stain. Bar = 2 μm.
Fig. 7. Kidney, tubules; cheetah. Basement membranes are diffusely expanded by homogeneous fibrillar material similar to the material present in glomerular basement membranes. Uranyl acetate and lead citrate stain. Bar = 7 μm.

Fig. 8. Kidney, glomerulus; cheetah. This obsolescent glomerulus has thickened glomerular basement membranes that have folded and collapsed around the remaining mesangial cells. No epithelial or endothelial cells are present. Bowman’s capsule is thickened by the same fibrillar material. Uranyl acetate and lead citrate stain. Bar = 2 μm.
terations in the microstructure and ionic charge of affected basement membranes, leading to leakage and accumulation of plasma proteins. AGEs can stimulate extracellular matrix production and prevent normal basement membrane degradation by binding to membrane components, leading to further accumulation of basement membrane material.

Pyrraline and pentosidine are two AGEs that are present in diabetic and aged tissues of humans.

Pyrralines have been identified in glomeruli of humans with diabetic nephropathy, but the presence of pentosidine in diabetic human kidneys has not yet been examined. Both pentosidine and pyrraline immunoreactivity was present in sclerotic glomeruli of cheetahs. Autofluorescence was noted in affected cheetah kidney basement membranes, similar to the autofluorescence described for pentosidine. The presence of these AGEs in affected glomeruli of young and old cheetahs suggests that they may participate in the pathogenesis of glomerulosclerosis. Although the pentosidine immunoreactivity identified in some cheetah renal tubular epithelium and amyloid deposits could be nonspecific staining, the absence of staining in negative controls and purity of the anti-pentosidine antibody suggests that pentosidine was present. Additionally, immunoreactive AGEs have been demonstrated in renal tubular epithelium of diabetic human patients and in amyloid deposits of amyloidosis patients undergoing dialysis, further supporting our findings.

Although the glomerulosclerosis of cheetahs closely resembled diabetic nephropathy, its high prevalence was not compatible with diabetes mellitus. Also, no cheetahs in the survey had pancreatic lesions in the islets of Langerhans. Therefore, acquisition of AGEs in the renal basement membranes of cheetahs may be caused by hyperglycemia from a non-diabetic metabolic condition. Hypercorticoidism can cause hyperglycemia in the absence of diabetes mellitus.

Adrenocortical hyperplasia was noted in the cheetahs, suggesting hypercorticoidism. Hyperglycemia as a consequence of adrenocortical hyperplasia has been demonstrated in stress situations in a number of species, including cats and rats. Adrenocortical hyperplasia has been linked to prolonged stress situations such as transition from free-range to captive environments or chronic disease in other wild species, including the nine-banded armadillo, the Australian platypus, and the harbor porpoise. Hypercorticoidism also can result in glomerular hypertension, leading to membranous glomerulopathy and glomerulosclerosis by increasing the glomerular filtration rate and causing hyperfiltration of plasma proteins and proteinuria.

Therefore, stress-related hypercorticoidism with secondary hyperglycemia and glomerular hypertension leading to glomerulosclerosis cannot be ruled out in these cheetahs. The strong association between adrenocortical hyperplasia and glomerulosclerosis in our population supports this concept. Studies to assess systemic levels of glucocorticoids in the cheetah population are in progress.

The morphology of cheetah glomerulosclerosis also closely resembles rat nephropathy, in which the glomerular and tubular basement membranes are thickened by PAS- and JMS-positive material that is negative for amyloid and fibrin. Thickened basement membranes in rats also have ultrastructural characteristics similar to those in cheetahs. The predisposition of certain rat strains (Sprague-Dawley, F344, Wistar, and Milan normotensive strains) to develop glomerulosclerosis suggests that glomerulosclerosis can have a genetic basis. Furthermore, high-protein diets, especially when fed ad lib on a continual basis, accelerates glomerulosclerosis in rats and other species.

The pathogenesis of this condition revolves around the chain of events linking increased glomerular perfusion, glomerular hypertension, increased glomerular filtration rate, and hyperfiltration of plasma proteins. Leakage of plasma proteins has been demonstrated in rats by identifying deposits within the membranes of complement, immunoglobulin M (IgM), immunoglobulin G (IgG), and other plasma proteins using immunofluorescence.

Dietary or genetic factors may also explain the high prevalence of glomerulosclerosis in the cheetahs. The cheetah population lacks the genetic diversity of most species, which could result in higher prevalences of uncommon diseases, such as glomerulosclerosis and veno-occlusive disease. High dietary protein also may influence the development of sclerosis in cheetahs, because captive cheetahs are fed predominantly skeletal meat 6–7 days a week. In the wild they eat muscle meat and viscera of small antelope, rodents, and birds, but seldom eat daily. They may have evolved on high-protein diets, but the frequency of feeding in captivity may create an overall protein excess. Therefore, glomerulosclerosis in cheetahs and rats may have comparable genetic and dietary risk factors. Because cheetah-specific antibodies were not available, we were unable to determine whether our cases had accumulated immunoglobulins, as has been demonstrated in rats with this disease.

Other causes of nonimmunologic glomerular disease such as systemic hypertension and collagenofibrotic glomerulonephropathy were considered, but the typical lesions of these diseases were not present in the cheetahs. Systemic hypertension in primates, rodents, dogs, and humans results in myointimal hyperplasia of arcuate and afferent renal arteries, and cheetah arterioles were normal. Collagenofibrotic glomerulopathy of cats and humans has nodular mes-
angial collagen accumulations that stain only weakly with PAS, while the cheetah glomeruli had diffuse, strongly PAS-positive membrane accumulations.

In summary, the captive cheetah population has a high prevalence of glomerulosclerosis that resembles lesions of diabetic nephropathy or rat nephropathy. From the current understanding of the pathogenesis of these diseases, stress compounded by genetic and possibly by dietary factors could be the basis for the high prevalence of glomerulosclerosis in captive cheetahs.

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