

Cystinuria in a Maned Wolf

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SUMMARY

A renal calculus composed principally of the amino acid, cystine, was found in an 8-year-old male maned wolf (*Chrysocyon brachyurus*). Cystine crystals were found in the urine sediment. The renal clearance of 10 amino acids was abnormal, whereas reabsorption of others was normal. The renal clearance of cystine, lysine, ornithine, and arginine exceeded the filtered load. The renal tubular handling of glucose, phosphate, sodium, potassium, and uric acid was identical to that for the clinically normal dog. These findings indicated an isolated renal tubular defect for cystine and other amino acids.



Fig 1—Lateral abdominal radiograph of a maned wolf with a renal calculus.

CYSTINURIA is an inherited metabolic disease associated with excessive urinary excretion of the amino acid, cystine, and other amino acids. The disease has been reported in man¹ and the dog^{2,3} and is genetically transmitted. In both species, the major manifestation of the disease is formation of urinary calculi composed primarily of cystine. Excessive dibasic aminoaciduria usually accompanies cystinuria in man and the dog.^{1,3} Alteration of epithelial cell transport of cystine and dibasic amino acids in the kidney has been proposed as the mechanism for aminoaciduria.

A cystine calculus in the kidney of a maned wolf has been reported.⁴ In our collection of maned wolves, 1 had cystine calculi that resulted in urethral obstruction, bladder rupture, and death. To gain insight on the prevalence of this disease in the maned wolf, urine specimens from 4 other wolves in the collection were examined for cystine crystals or were tested with the cyanide-nitroprusside reaction. All wolves were found to be positive.

In this report, we describe cystinuria and cystine calculi formation in a maned wolf. Using renal clearance techniques, we investigated the pattern of aminoaciduria in the wolf.

Case History

A hand-raised 8-year-old male maned wolf (*Chrysocyon brachyurus*) was added to our collection in

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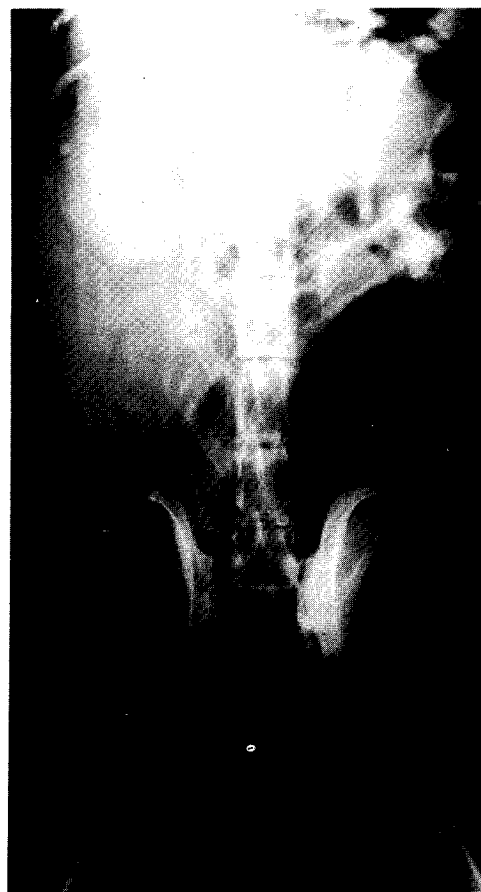


Fig 2—Ventrodorsal radiograph taken 5 minutes after the injection of 50 ml of contrast medium, seen clearly in the right kidney but not in the left kidney, which contains the calculus. Pneumocystography also was done.

TABLE 1—Results of Sequential Urinalyses

| Factor | Date | | | | |
|----------------------|--------------------|-------------------|---------------|----------|---------------|
| | 1/20 | 1/24 | 1/30 | 2/8 | 3/16 |
| Color | Yellow | Yellow | Dark yellow | Straw | Bright yellow |
| Transparency | Cloudy | Cloudy | Cloudy | Cloudy | Cloudy |
| pH | 5.5 | 5.5 | 8 | 7.0 | 8.0 |
| Sp gravity | 1.033 | 1.026 | 1.040 | 1.024 | 1.027 |
| Protein | 1+ | 1+ | 1+ | Trace | Trace |
| Blood | Trace | | Trace | | |
| Glucose | | | | | |
| Ketones | | | | | |
| Urobilinogen | 1+ | 1+ | 1+ | 4+ | 1+ |
| Bile | | | | | Trace |
| Microscopic findings | | | | | |
| RBC | 3-4/hpf | 0-1/hpf | 0-1/hpf | 200/hpf | Few |
| WBC | 5-10/hpf | 2-6/hpf | 0-1/hpf | 5-8/hpf | Many |
| Epithelial cells | 0-1/hpf | 0-2/hpf | | | |
| Casts | 2-3 (granular)/hpf | Many (granular) | | Cellular | Occasionally |
| Crystals | 1-3 (cystine)/hpf | 0-3 (cystine)/hpf | Few (cystine) | Cystine | |
| Bacteria | Moderate | | Abundant | | |

October 1977. The wolf's overall poor condition was associated with signs of gastrointestinal dysfunction. The passage of undigested food in the feces and rapid transit of ingesta through the digestive tract had been noticed since its arrival.

The wolf was dehydrated and underweight (28 kg). It had a dry, rough coat and bilateral ocular discharge due to entropion. Results of fecal examination were negative for parasite eggs and larvae. The hemogram and serum chemical values were normal, with the exception of elevated packed cell volume (PCV), total protein, serum alkaline phosphatase (SAP), and serum calcium. Dehydration was believed to be the cause of the increased values for PCV and plasma protein. The significance of the high values for SAP and serum calcium was unknown; subsequent values for these and other serum chemistries were considered within an expected normal range. Since normal base-line data are not available for this genus, values were compared with those of clinically normal dogs.

Initial attention was given to reduce dietary fat intake. After 1 month, the diet was changed from a commercial exotic feline diet^a with 12% fat and 19% protein to a commercial exotic canine diet^b with 5.5% fat and 14.5% protein. A pancreatic enzyme extract^c also was added to the diet. The response to this change was decreased gastrointestinal transit time and the passing of normal feces. However, the wolf's overall appearance remained unthrifty.

Three months later, the wolf was immobilized for diagnostic tests because of continued unthrifty appearance. The wolf weighed 29 kg and was immobilized with 150 mg of tiletamine HCl and zolazepam HCl.^d Blood values were considered within the normal range. Radiography revealed a calculus in the pelvis of the left kidney (Fig 1). Cystine crystals were seen in the urinary sediment (Table 1).

One week later, pneumocystography and intravenous urography were performed, using 50 ml of contrast medium,^e following immobilization with 100 mg of



Fig 3—Ventrodorsal radiograph taken 2½ hours after the injection of 90 ml of contrast medium. The right ureter is barely visible and contrast material can be seen around the calyces in the left kidney, indicating kidney function.

ketamine HCl^f and 10 mg of acepromazine.^g The right kidney appeared normal, but contrast medium from the left kidney could not be seen in the ureter (Fig 2). Pneumocystography showed no cystic calculi. Subsequent urography (2 wk later), utilizing 90 ml of contrast medium and abdominal compression, showed evidence of excretion from the left kidney (Fig 3). The radiographic evidence of a functional left kidney justi-

^a Nebraska Brand Chopped Frozen Feline Food, Central Nebraska Packing Co, North Platte, Neb.

^b Nebraska Brand Chopped Frozen Canine Diet, Central Nebraska Packing Co, North Platte, Neb.

^c Viokase, Viobin Corporation, Monticello, Ill.

^d Telazol, Parke, Davis & Co, Detroit, Mich.

^e Renografin 60, ER Squibb & Sons, Inc, Princeton, NJ.

^f Ketaset, Bristol Laboratories, Syracuse, NY.

^g Acepromazine Maleate, Ayerst Laboratories, New York, NY.

fied surgical removal of the calculus in an attempt to salvage the functioning kidney. Blood values at this time were again within normal limits. Using urethral catheterization during these studies, many small, fine calculi were flushed from the bladder and urethra. The calculi were found to be composed of cystine, which are not radiopaque, thus accounting for the fact that they were not seen on the pneumocystogram. Sodium bicarbonate was added to the diet to increase the urine pH in an attempt to increase the solubility of cystine.

Renal Clearance

Surgery was performed to remove the calculus from the left kidney and to measure the renal clearance of several solutes from the right kidney. The left kidney was not studied because potential urinary obstruction could produce spurious renal clearance data. The wolf had been fasted 18 hours prior to surgery. Immobilization was performed with ketamine and acepromazine, as before. Anesthesia was maintained with halothane^b and nitrous oxide. Laparotomy and cystotomy permitted catheterization of the right ureter. The catheter was advanced to the mid-ureter region and tied in place for collection of urine. A loading and maintenance infusion containing mannitol and creatinine was given into the caudal vena cava. The technique of obtaining renal clearance data has been described.³

Renal clearance studies were performed for amino acids, creatinine, glucose, phosphate, sodium, potassium, and uric acid. Preparation and analyses of specimens for measurement of plasma and urine amino acids were performed as previously reported.³ Glucose in plasma and urine was measured by a glucose oxidase method. Methods of measurements of phosphate and creatinine were previously reported.⁵ Sodium and potassium were measured on an internal standard flame photometer. Uric acid was determined by the method of Henry.⁶

The clearance of creatinine was used to estimate glomerular filtration rate. Conventional clearance formulas were used. Results for amino acids, glucose, phosphate, and sodium were expressed as percentage reabsorption of filtered load. Due to bidirectional transport, values for potassium and uric acid were expressed as urinary excretion divided by filtered load. All reported clearance values represent the mean of 3 consecutive collection measurements.

A wedge renal biopsy specimen was removed from the cortex of the right kidney before the priming infusion was given for clearance studies and was placed in 10% formalin for routine histologic examination. Urinary calculi were quantitatively analyzed by crystallography.¹

After the urine collections for measurement of renal clearance, nephrotomy was performed on the left kidney to remove the calculus. Multiple small, tan, round calculi also were removed from the bladder and urethra. Routine surgical closure techniques were used. The wolf had a prolonged recovery from anesthesia. Dur-

TABLE 2—Fractional Reabsorption of Amino Acids in a Maned Wolf with Cystinuria

| Amino acid | Mean percentage reabsorption |
|---------------|------------------------------|
| Cystine | —229 |
| Lysine | —275 |
| Ornithine | — 85 |
| Arginine | — 88 |
| Cystathionine | 43 |
| Methionine | 100 |
| Isoleucine | 100 |
| Leucine | 100 |
| Tyrosine | 100 |
| Phenylalanine | 100 |
| Histidine | 100 |
| Tryptophane | 100 |
| Threonine | 93 |
| Serine | 86 |
| Asparagine | 100 |
| Glutamic acid | 98 |
| Proline | 100 |
| Glutamine | 100 |
| Citrulline | 79 |
| Glycine | 96 |
| Alanine | 96 |
| Valine | 100 |

Glomerular filtration rate was 31 ml/min/kidney. Urine flow was 1.1 ml/min. Values are the mean of 3 consecutive clearance periods.

ing the night, the wolf attacked the abdominal incision site and completely removed and mutilated the digestive tract, necessitating euthanasia. A complete necropsy was performed.

Results

Urine specimens collected before the infusion for clearance measurements contained extremely high concentrations of several amino acids. Lysine and cystine had the highest concentration (7,313 and 5,089 nmole/ml, respectively). Urine concentration of ornithine, arginine, and cystathionine also appeared high (1,573, 409, and 360 nmole/ml, respectively). The urine concentration of 21 other amino acids ranged from 0 to 280 nmole/ml. The plasma concentration of all amino acids ranged from 0 to 490 nmole/ml. The amino acids that appeared in highest concentration in the urine were not different from others in regard to plasma concentration.

The renal clearance of several amino acids was markedly abnormal. The mean urinary excretion of cystine, lysine, ornithine, and arginine exceeded the filtered load (Table 2). The clearance of lysine and cystine was more than twofold greater than the filtered load. The fractional reabsorption of cystathionine, threonine, serine, citrulline, glycine, and alanine was reduced from normal. The reabsorption of all other amino acids was considered normal, at 98% to 100%.

The mean fractional reabsorption of other solutes was considered normal: glucose, 99.9%; phosphate, 91.8%; and sodium, 98.7%. The mean ratio of urinary potassium excretion over filtered load was 0.54. Urinary urate excretion compared with filtered load was 0.38.

The calculi were analyzed as 96% cystine and 4% mixed protein.

Pathology

The left kidney weighed less than the right one (120 g vs 90 g). Microscopically, vacuolar changes in renal tubular cells were marked. The left kidney had

^b Fluothane, Ayerst Laboratories, New York, NY.

¹ L. C. Herring Co, Orlando, Fla.

moderate hydronephrosis and hyperplasia of transitional epithelium of the pelvis.

The lamina propria of the stomach was infiltrated by plasma cells, lymphocytes, and a few polymorphonuclear cells. It had a hyalinized stroma that impinged on parietal and chief cells, with resultant generalized atrophy. There were small aggregates of lymphocytes between the lamina propria and muscularis mucosa.

The thyroid gland contained small follicles, with marked interstitial infiltrates of plasma cells and lymphocytes. The changes were similar to those of autoimmune thyroiditis in man⁷ and the dog.⁸ Other tissues were not remarkable.

Discussion

Results of renal clearance studies indicated that cystinuria in this wolf was associated with a dibasic aminoaciduria and minor defects for other amino acids. The most remarkable finding was the excretion of 4 amino acids in excess of the filtered load. Such a finding cannot be explained by reduced reabsorption alone. Therefore, mechanisms of increased efflux from tubular cells or secretion of amino acids was likely. It is interesting that the apparent secretion occurred in cystine and dibasic amino acids, which supposedly share the same transport system, as shown for man and the dog.^{9,10} It is assumed that 98% to 100% reabsorption of amino acids is normal in the maned wolf, as this is a standard value for other species.^{1,3,10} The plasma concentrations of amino acids in this wolf were similar to values reported for the clinically normal dog.^{3,10}

The renal handling of electrolytes and other important solutes was considered normal in this wolf. While such data from clinically normal maned wolves are not available, data from this wolf were identical to data for the clinically normal dog.¹¹⁻¹³ These results indicated that the renal tubular transport defect in this wolf was limited to amino acids.

It is assumed that the pathologic changes in the stomach and thyroid gland were not related to cystinuria. The atrophic gastritis may have had some relationship to the wolf's unthriftiness.

Examination of the lower urinary tract of this wolf revealed curvature of the penile urethra and a narrow urethral lumen, less than one-half the diameter of the urethral lumen in a dog of comparable size. Thus,

maned wolves may be highly vulnerable to urethral obstruction due to calculi.

Maned wolves are listed as a species vulnerable to extinction by the *International Union for the Conservation of Nature and Natural Resources Red Book* due to habitat alterations in South America. This rare South American canid inhabits Brazil and Argentina and is uncommonly found in zoologic collections. However, the captive breeding population is small and a potential genetically transmitted abnormality such as cystinuria has marked implications on breeding strategy.

The importance of this finding is that cystinuria may threaten the availability or existence of this rare animal. A screening program that can determine incidence of the disease is underway. The long-range objective would be to investigate the genetic character of the disease and limit or eliminate it with a breeding program.

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