those times of year when such stimulation is required under natural conditions. Shortening of the photoperiod in the fall is a much more reliable cue for seasonal phasing than are changes in T_a. Therefore, photoperiodic control allows thermogenic improvement well in advance of the lowering T_a, actually demanding greater thermogenic efficiency.

The physiological background of photoperiodic control of thermogenesis seems to be closely related to photoperiodic control of other seasonally varying functions, such as reproduction and molt (*8, 13*). This suggests that there is a common neural pathway for photoperiodic time measurement, terminating at the pineal as the "neurochemical transducer" (*15*). Such a function of the pineal has been suggested for photoperiodic control of gonadotropic activity of the hypothalamopituitary system (*15, 16*). Our results suggest that this action of the pineal may be extended to control of thermoregulatory functions and corroborates conclusions about the significance of the pineal complex for thermoregulation in vertebrates (*17*). The chemical nature and the pathway of information transmission from the pineal to thermoregulatory effectors are still unclear.

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References and Notes

1. R. E. Smith and B. A. Horwitz, *Physiol. Rev.* **49**, 330 (1969); L. Jansky, *Biol. Rev. Biol. Proc. Cambridge Phil. Soc.* **48**, 85 (1973); G. Heldmaier, *Z. Vgl. Physiol.* **73**, 222 (1971).
2. D. O. Foster and M. L. Frydman, *Can. J. Physiol. Pharmacol.* **56**, 110 (1978).
3. R. A. Hoffman, R. J. Hester, C. Towns, *Comp. Biochem. Physiol.* **15**, 525 (1965); G. Heldmaier and K. Hoffmann, *Nature (London)* **247**, 224 (1974); G. R. Lynch, S. E. White, R. Grundel, M. S. Berger, *J. Comp. Physiol.* **125**, 157 (1978).
4. G. Heldmaier and S. Steinlechner, *J. Comp. Physiol.*, in press; ———, J. Rafael, P. Vsiansky, in preparation.
5. Hamsters were exposed to steadily decreasing T_a from +30 to −55°C within 8 hours until maximum thermoregulatory heat production was reached. This T_a was defined as the cold limit. Oxygen consumption and CO_2 production were monitored continuously by using an open system, and heat production was calculated from these measurements by using respiratory quotient–dependent energy equivalents [G. Heldmaier, *J. Comp. Physiol.* **102**, 115 (1975)].
6. H. J. Hohorst and J. Rafael, *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 268 (1968).
7. The four marker enzymes were cytochrome oxidase (E.C. 1.9.3.1), succinate dehydrogenase (E.C. 1.3.99.1), glycerol-3-phosphate dehydrogenase (E.C. 1.1.99.5), and glutamate dehydrogenase (E.C. 1.4.1.2) [J. Rafael and E. Wrabetz, *Eur. J. Biochem.* **61**, 551 (1967)]. Protein was determined by a modified biuret method (*6*), and DNA content of BAT was measured by a modified diphenylamine technique (J. Rafael and P. Vsiansky, in preparation).
8. K. Hoffmann, *J. Comp. Physiol.* **82**, 267 (1973); in *The Pineal Gland of Vertebrates Including Man*, J. A. Kappers and P. Pevet, Eds. (Elsevier, Amsterdam, 1979), p. 397.
9. R. J. Reiter, *Chronobiologia* **1**, 365 (1974); R. Rusak and L. P. Morin, *Biol. Reprod.* **15**, 366 (1976); C. J. Gravis, *Am. J. Anat.* **151**, 213 (1978).
10. V. B. Eichler and R. Y. Moore, *Acta Anat.* **89**, 359 (1974); A. E. Hendrickson, N. Wagoner, M. W. Cowan, *Z. Zellforsch. Mikrosk. Anat.* **135**, 1 (1972); R. Y. Moore, *Brain Res.* **49**, 403 (1973).
11. J. A. Kappers, *Z. Zellforsch. Mikrosk. Anat.* **52**, 163 (1960); R. J. Reiter and R. J. Hester, *Endocrinology* **79**, 1168 (1966); R. J. Reiter, *Ann. Endocrinol.* **33**, 571 (1972).
12. D. C. Klein and J. L. Weller, *Science* **169**, 1093 (1970); M. D. Rollag, R. J. Morgan, G. D. Niswender, *Endocrinology* **102**, 1 (1978); J. A. Romero and J. Axelrod, *Science* **184**, 1091 (1974); A. N. Taylor and R. W. Wilson, *Experientia* **26**, 267 (1970); M. Zatz and M. J. Brownstein, *Science* **203**, 358,(1979).
13. J. Figala, K. Hoffmann, G. Goldau, *Oecologia (Berlin)* **12**, 89 (1973); C. C. Rust and R. K. Meyer, *Science* **165**, 921 (1969).
14. K. Hoffmann and I. Küderling, *Naturwissenschaften* **64**, 339 (1977); R. J. Reiter, M. K. Vaughan, D. E. Blask, L. Johnson, *Endocrinology* **96**, 206 (1975); F. W. Turek, *Proc. Soc. Exp. Biol. Med.* **155**, 31 (1977).
15. J. Axelrod, *Science* **184**, 1341 (1974).
16. G. B. Ellis, S. H. Losee, F. W. Turek, *Endocrinology* **104**, 631 (1979); G. B. Ellis and F. W. Turek, *ibid.* p. 625; R. J. Reiter and L. Y. Johnson, *Horm. Res.* **5**, 311 (1974); F. W. Turek and C. S. Campbell, *Biol. Reprod.* **20**, 32 (1979).
17. C. L. Ralph *et al.*, *Biol. Rev. Biol. Proc. Cambridge Phil. Soc.* **54**, 41 (1979).
18. F. W. Turek, C. Desjardins, M. Menaker, *Science* **190**, 280 (1975).
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Cystinuria in the Maned Wolf of South America

Abstract. *Of 42 maned wolves in zoos or live-trapped in Brazil, 34 had excessive cystine in their urine. Renal clearance studies of five of the affected wolves revealed a variable defect for the reabsorption of cystine and dibasic amino acids. The renal tubular handling of other solutes including glucose, phosphate, sodium, potassium, and uric acid was considered normal. Urinary calculi composed of cystine were found in four wolves and proved fatal in three of them. With the exception of the high incidence in this species, this hereditary disease resembles the disorder described in dogs and humans.*

In recent years, considerable attention has been given to metabolic diseases in domestic and wild animals which resemble those in humans (*1*). Such diseases provide comparisons with diseases of humans and, possibly, models for studying basic disease mechanisms. Cystinuria, an inherited metabolic disease associated with excessive urinary excretion of cystine and other amino acids, has been reported in man (*2*) and dog (*3, 4*). Alteration of kidney cell transport of cystine and dibasic amino acids has been proposed as the underlying mechanism. The major clinical manifestation of cystinuria is the presence of urinary calculi composed primarily of cystine, which is highly insoluble in urine.

We previously reported the occurrence of cystine calculi in one maned wolf (*Chrysocyon brachyurus*) from a zoo (*5*). We now report the incidence and nature of this disease in a much larger number of maned wolves, both captive and wild. This rare South American canid (Fig. 1) inhabits Brazil and Argentina and is uncommonly found in zoos. Due to alteration of habitat, maned wolves are listed as vulnerable to extinction by the International Union for the Conservation of Nature Resources.

To determine the incidence of the disease we used paper chromatography, a sensitive and reliable test for detecting excessive aminoaciduria in wolves and dogs. The results were confirmed by renal clearance studies. Urine specimens were collected from adult maned wolves in zoos in six countries and from live-trapped wolves in Brazil. Of the 42 wolves tested, 34 had excessive cystine and dibasic amino acids in their urine. Nearly equal numbers of males and females were affected. Six of the eight live-trapped wolves and 28 of the 34 captive wolves were positive for cystinuria. (The captive wolves had themselves been trapped in Brazil or were their offspring.) Thus, despite the differences in diet and habitat between captive wolves and those in the natural state, the incidence of cystinuria appears to be the same for both groups. Four of the wolves had cystine stones, as determined at necropsy or by the presence of urinary obstruction. (Clinical signs of cystinuria include urinary straining, frequent urination, and urinary obstruction.) Three of the wolves died from such obstruction. Crystallographic analysis of the calculi showed them to be more than 95 percent cystine. The incidence of calculi in the other cystinuric wolves is unknown, since most of them were not radiographed.

Renal clearance studies were performed on five of the affected wolves. The wolves were deprived of food for 18 hours prior to study and were immobilized with appropriate pharmacological agents (*5, 6*). Since normal wolves were not available as controls, three normal

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dogs were used instead. The quantity of amino acids in plasma and urine was determined with amino acid analyzers (Beckman models 119 and 121). As shown in Table 1, the renal clearance of amino acids was abnormal in all five wolves. In four of the wolves the reabsorption of cystine ranged from 70 to 88 percent of the filtered load. In the fifth, cystine excretion was greater than the filtered load—a phenomenon that has been observed in some cystinuric dogs (*4*). All five wolves had minor defects in their renal clearance of dibasic amino acids. (The renal clearance of 24 amino acids was measured; those not shown in Table 1 were reabsorbed normally.) The plasma concentrations of amino acids were similar to values reported for the normal dog (*4, 7, 8*). Routine urinalyses were not remarkable, except for the presence of cystine crystals. Total cystine excretion was not measured because complete 24-hour urine collections are not possible in this species.

The renal tubular handling of other solutes was measured by standard clearance methods (*9*). Glomerular filtration rate, measured by clearance of creatinine, was 1.75 ± 0.4 ml per minute per kilogram. The reabsorption of glucose, phosphate, and sodium was 99.0, 90.6, and 99.5 percent of the filtered load, respectively. Values for potassium and uric acid, expressed as the ratio of urinary excretion to filtered load, were 0.28 and 0.40, respectively. These values are considered normal compared to those for dogs (*9*). Therefore, the renal tubular defect is limited to amino acids.

These results indicate that cystinuria in these wolves was associated with dibasic aminoaciduria and minor defects in the clearance of other amino acids. The finding that the excretion of four amino acids exceeded the filtered load in one wolf cannot be explained by reduced reabsorption alone. Therefore, it is likely that either the efflux from tubular cells or the secretion of amino acids was increased in this wolf. The apparent secretion occurred for cystine and dibasics, which supposedly share the same transport system, as in man and dog (*7, 10*). It is assumed that 98 to 100 percent reabsorption of amino acids is normal in the maned wolf, since this is the standard value in other species.

The nature of aminoaciduria in maned wolves is more similar to the abnormality in dogs than to that in humans. Human cystinurics have a profound lysinuria that exceeds cystinuria and have hyperexcretion of arginine and ornithine (*2*). This pattern has not been found in dogs

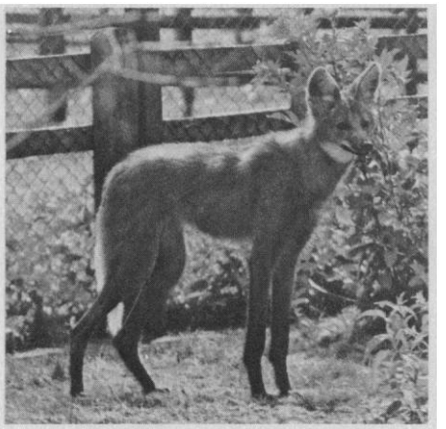

Fig. 1. Captive adult maned wolf.

or maned wolves. Lysinuria appears to be a variable defect in maned wolves, similar to that in cystinuric dogs (*4*).

The incidence of cystinuria in maned wolves is remarkable compared to that in other species. The incidence of the homozygous form of cystinuria in man is one in 7000 (*11*). The exact incidence in dogs is unknown, but presumably it is less than one in 1000. We recently studied the patterns of amino acid excretion in nine North American timber wolves and arctic wolves and found them similar to the patterns in normal dogs.

Physical examination and catheterization of the lower urinary tract of male maned wolves 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 by calculi.

The first reported search for animals with hyperaminoaciduria in zoos was conducted by Datta and Harris (*12*). The maned wolf was not included in that search. The high incidence of cystinuria in this species does not mean that it should be regarded as a readily available subject for studying cellular defects in renal amino acid transport. The importance of this report is that cystinuria may threaten the existence of this rare animal. While the genetic nature of this disease is not known, the high incidence may contribute to the vulnerability of the animal in the wild. The captive population is small, and a genetically transmitted abnormality such as cystinuria has marked implications for breeding strategy.

Table 1. Reabsorption of amino acids in five maned wolves. Negative values represent urinary excretion in excess of the filtered load. Normal dogs reabsorb 98 to 100 percent of filtered amino acids.

Amino acid	Mean reabsorption for three collection periods (%)				
	Wolf				
	1	2	3	4	5
Cystine	−229	78	70	78	88
Lysine	−275	95	94	98	98
Ornithine	−85	83	80	95	93
Arginine	−88	99	98	99	100
Glutamic acid	100	96	98	99	99
Cystathionine	43	100		92	
Serine	86	98	97	99	98
Threonine	93	100	99	99	99
Histidine	100		80	90	
3-Methyl histidine		78	91	96	

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References and Notes

1. E. J. Andrews, B. C. Ward, N. H. Altman, *Spontaneous Animal Models of Human Diseases* (Academic Press, New York, 1979).
2. S. Segal and S. O. Thier, *The Metabolic Basis of Inherited Disease* (McGraw-Hill, New York, ed. 4, 1978).
3. E. Brand and G. F. Cahill, *J. Biol. Chem.* **114**, 14 (1936).
4. K. Bovée, S. O. Thier, S. Segal, *Metabolism* **23**, 51 (1974).
5. M. Bush and K. C. Bovée, *J. Am. Vet. Med. Assoc.* **173**, 1159 (1978).
6. Renal clearance was performed under narcotic sedation and anesthesia with 1.5 percent halothane. Creatinine and insulin clearance were measured simultaneously in two wolves and were almost identical.
7. W. A. Webber, S. L. Brown, R. F. Pitts, *Am. J. Physiol.* **200**, 380 (1961).
8. B. Blazer-Yost and P. F. Jezyk, *Am. J. Vet. Res.* **40**, 832 (1979).
9. K. C. Bovée, T. Joyce, R. Reynolds, S. Segal, *Metabolism* **27**, 45 (1978).
10. C. E. Dent, B. Senior, J. M. Walshe, *J. Clin. Invest.* **33**, 1216 (1954).
11. H. L. Levy, *Genetic Screening in Progress in Human Genetics* (Plenum, New York, 1973), vol. 4, p. 1.
12. S. P. Datta and H. Harris, *Ann. Eugenics* **18**, 107 (1953).
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