herited defect rather than a secondary event. However, it is not known whether the development of the parathyroid glands is from the neural crest or the "APUD" (amine, precursor uptake, L-DOPA decarboxylase) series of cells (*12*) which is thought to give rise to the other lesions in the MEN-II syndrome. Investigation of the G6PD content of the parathyroid tumors in black heterozygotes might help settle the question of whether these tumors follow the same pattern of mutational events as does the medullary thyroid carcinoma in these patients. We had no clinical reason to remove parathyroid tissue from three of our patients, and a hyperplastic parathyroid from the fourth was not available for study (*1*).

Patients with multiple endocrine neoplasia type I inherit defects which result in simultaneous parathyroid, pituitary, and pancreatic tumors (*13*). Studies of G6PD isoenzymes might indicate whether the series of mutational events for these neoplasms are similar to those for inherited medullary thyroid carcinoma.

STEPHEN B. BAYLIN
SUSAN H. HSU
DONALD S. GANN
*Oncology Center* and *Departments of Medicine* and *Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland 21205*
ROBERT C. SMALLRIDGE
*Kyle Metabolic Unit, Walter Reed Army Medical Center, Washington, D.C. 20012*
SAMUEL A. WELLS, JR.
*Department of Surgery, Duke University Medical School, Durham, North Carolina 27710*

### References and Notes

1. S. B. Baylin, D. S. Gann, S. H. Hsu, *Science* **193**, 321 (1976).
2. A. G. Knudson, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **68**, 820 (1971); _____ and L. C. Strong, *Am. J. Hum. Genet.* **24**, 514 (1972).
3. N. Ellis and J. B. Alperin, *Am. J. Clin. Pathol.* **57**, 534 (1972).
4. E. Beutler, *Red Cell Metabolism: A Manual of Biochemical Methods* (Grune & Stratton, New York, 1971), pp. 62–64.
5. S. H. Boyer, I. H. Porter, R. G. Weilbacher, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1868 (1962); H. N. Kirkman and E. M. Hendrickson, *Am. J. Hum. Genet.* **15**, 241 (1963); W. E. Nance, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 415 (1964). It is important to note that, in our patient, the intensity of the G6PD A band in the tumor tissues is equal to that of the B band. This fact may indicate that the diminished A band in the red cells is not due to a decreased quantity of active G6PD, but to an unequal proportion of the numbers of cells containing the A as opposed to the B form of G6PD. In the tumors, where a single clone of A cells may be represented, the intensity of the G6PD A band appears normal. Alternatively, the G6PD A in this patient's tumor tissue may be more stable than that in red cells.
6. S. B. Baylin, M. A. Beaven, K. Engelman, A. Sjoerdsma, *N. Engl. J. Med.* **283**, 1239 (1970); S. B. Baylin, M. A. Beaven, L. M. Buja, H. R. Keiser, *Am. J. Med.* **53**, 723 (1972). In calculating the activity for normal thyroid tissue we have converted the units per gram of tissue, wet weight, given for normal thyroid tissue in these references to units per milligram of protein as based on the average amount of protein per 100 μl of the 1 : 20 (weight/volume) homogenates used in the previous studies.
7. N. LeDourain, *C.R. Assoc. Anat.* **152**, 558 (1971); A. G. E. Pearse, *J. Histochem. Cytochem.* **17**, 303 (1969).
8. S. M. Gartler, L. Ziprkowski, A. Krakowski, R. Ezra, A. Szeinberg, A. Adam, *Am. J. Hum. Genet.* **18**, 282 (1966).
9. P. J. Fialkow, R. W. Sagebiel, S. M. Gartler, D. L. Rimoin, *N. Engl. J. Med.* **284**, 298 (1971).
10. H. J. Wolfe, K. E. W. Melvin, S. J. Cervi-Skinner, A. A. Al Saadi, J. F. Juliar, C. E. Jackson, A. H. Tashjian, Jr., *ibid.* **289**, 437 (1973); J. A. Carney, G. W. Sizemore, G. M. Tyce, *Mayo Clin. Proc.* **50**, 3 (1975).
11. H. Heath, G. W. Sizemore, J. A. Carney, *J. Clin. Endocrinol. Metab.* **43**, 428 (1976).
12. A. G. E. Pearse and T. Takor Takor, *Clin. Endocrinol.* **5**, 229S (1976).
13. P. Wermer, *Am. J. Med.* **35**, 205 (1963); H. S. Ballard, B. Frame, R. J. Hartsock, *Medicine* **43**, 481 (1964).
14. We thank N. Delaney for technical assistance and V. McKusick and A. H. Owens for helpful discussions and constructive criticisms. Supported in part by NIH grant 1-RO1-18404, NIH grant GM19489, NIH Outpatient Research grant 5MO1RR00722, PHS Clinical Research Centers grants RR-30 and 35, and NIH contract N01-CB-63994-39. S.B.B. is supported by NIH Research Career Development Award 1-K04-CA-000-27.

28 July 1977; revised 31 August 1977

## Ammonia Intoxication in the Near-Adult Cat as a Result of a Dietary Deficiency of Arginine

Abstract. *Near-adult cats, fasted overnight, and given a single meal of a complete amino acid diet without arginine, developed hyperammonemia and showed clinical symptoms of ammonia toxicity within 2 hours. One cat (2.7 kilograms) died 4.5 hours after ingesting only 8 grams of the diet. Since ornithine also prevented hyperammonemia, it appears that the domestic cat cannot synthesize ornithine.*

Despite the popularity of the cat as a household pet in the United States (*1*) and other Western countries, virtually nothing is known regarding its dietary requirement for amino acids (*2*). The domestic cat and presumably other felids have a much higher requirement for protein in their diet for maintenance than most other adult mammals; for example, about 20 percent of the dietary calories as protein is required by the adult cat (*3*) compared to 4 to 8 percent for other mammals, such as the rat, dog, sheep, and man (*2, 4*). This high requirement for protein appears to be due to the inability of the cat to regulate the activity of the nitrogen catabolic enzymes (such as hepatic transaminases and urea cycle enzymes) (*5*). Other peculiarities of the cat's nutrition include its inability to undertake the conversions of β-carotene to vitamin A and of tryptophan to niacin, and an inability to synthesize sufficient taurine to prevent central retinal degeneration (*6*).

In a series of experiments designed to define the indispensable amino acids for the growing kitten, we have been giving kittens the diet shown in Table 1. Individual amino acids have been sequentially deleted from the amino acid mixture, and the effect on food intake, growth, and free amino acid concentration in the plasma was measured. In the course of these studies, arginine was deleted from the amino acid mixture. When kittens were switched (without fasting) from an arginine-containing diet to one without arginine, they rapidly lost weight (about 100 g/day) compared to a gradual weight loss of about 15 g/day when any other indispensable amino acid was deleted from the diet (*7*). Vomitus was found in the cages, there was hemoconcentration and virtual complete refusal to eat. Similar observations were also made on two further groups of six kittens given an arginine-free diet.

To study further the effect of an arginine-free diet on the cat, 16 individually caged cats (6 to 8 months old) of a mean (± S.E.) body weight of 2590 ± 90 g and from four different litters were used. They had previously received a complete, purified amino acid diet (Table 1) and were divided into two groups on the basis of litter, sex, and body weight. Food was withheld from both groups from 5 p.m. the evening before the experiment. On the morning of the experi-

Table 1. Composition of the semipurified amino acid diet.

| Dietary components | Percent of diet |
|---|---|
| Amino acid mixture* | 34.7 |
| Turkey fat | 25.0 |
| Starch | 19.3 |
| Sucrose | 15.7 |
| Salt mixture † | 4.0 |
| Vitamin premix (*19*) | 1.0 |
| Choline chloride | 0.33 |
| Total | 100.0 |

*Contained the following amino acids as a percentage of the diet: L-histidine · HCl · $H_2O$, 1.2; L-isoleucine, 1.8; L-leucine, 2.4; L-lysine · HCl, 2.8; L-methionine, 1.1; L-cystine, 0.8; L-phenylalanine, 1.5; L-tyrosine, 1.0; L-threonine, 1.4; L-tryptophan, 0.4; L-valine, 1.8; L-arginine · HCl, 2.0; L-asparagine, 2.0; L-serine, 1.0; L-proline, 2.0; glycine, 2.0; L-glutamic acid, 6.0; L-alanine, 1.0; and sodium acetate, 2.5, to balance the hydrochlorides. †Hegsted's Salt Mix, Nutritional Biochemicals Corp.


0036-8075/78/0127-0431$00.50/0 

ment, a blood sample from the jugular vein was taken from each cat, and the cats in one group were then given the complete amino acid diet including arginine, while those in the other group were given the same diet except that arginine was deleted and replaced with an isonitrogenous amount of alanine. After 45 minutes, the uneaten portion of the diet was removed, and blood samples were taken at 45, 120, and 300 minutes. All blood samples were analyzed for ammonia (*8*) and glucose (*9*). Food intake in the 45-minute period from both groups of cats varied from 4 to 11 g.

All eight cats, within 2 hours of a single meal of the arginine-free diet, exhibited emesis and lethargy. The following symptoms also occurred: vocalization (moaning), frothing at the mouth, hyperactivity, hyperesthesia, ataxia, emprosthotonos, extended limbs, and exposed claws. Severely affected cats showed marked bradypnea and cyanosis. One cat weighing 2.7 kg, which ate only 8 g of diet and showed all the above symptoms died in apnea. The cats that had received the diet including arginine showed no unusual clinical signs.

The mean (± S.E.) of the concentration of ammonia in plasma from cats given the arginine-free diet (Fig. 1) shows an elevation in ammonia concentration 2 hours after being given the diet. Cats given the diet including arginine showed virtually no change in the ammonia concentration in the plasma. Plasma glucose concentration was elevated, 242 ± 30 and 271 ± 45 mg/dl, respectively, at 120 and 300 minutes after consuming the depleted diet. Cats given the complete diet had plasma glucose concentrations of 96 ± 2 and 88 ± 2 mg/dl at these times. The clinical symptoms and elevated plasma concentrations of ammonia and glucose were consistent with a diagnosis of ammonia intoxication.

In a subsequent experiment, five of the cats that previously had received the arginine-free diet were given, under the same conditions, a purified amino acid diet in which the arginine was deleted, but replaced with an equimolar amount of ornithine. These cats showed no unusual clinical signs, and their plasma ammonia (Fig. 1) and glucose concentrations were not significantly different from those of the cats fed the diet containing arginine.

These results indicate that the cat is unable to synthesize ornithine at a rate commensurate with the requirements of the urea cycle to dispose of ammonia from amino acid degradation. Since ornithine is not a constituent of protein, arginine is an indispensable amino acid for the cat not only according to the definition of Rose and collaborators (*10*) but also for the prevention of ammonia intoxication. In this respect the adult cat differs from the adult dog, which Rose and Rice (*10*) showed is able to maintain nitrogen balance on an arginine-free diet. However, young dogs (*11*) given a diet of L-amino acids minus arginine vomit immediately after eating the diet. The diet we used contained both glutamic acid and proline, the former being regarded as the precursor for ornithine synthesis in mammals, while the latter reduces the dietary requirement of arginine (*12*) in growing animals with limited ability for de novo arginine synthesis.

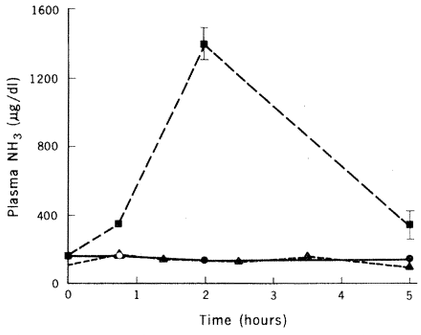


Fig. 1. Ammonia concentration (μg/dl) in the plasma of adult cats after the ingestion of a single meal of a purified diet containing arginine (○——○); no arginine (■– – –■); or no arginine plus ornithine (▲- - -▲).

The time course along with the severity of the onset of symptoms of a dietary deficiency of arginine in the cat is unique among essential nutrients. Most dietary deficiencies of essential nutrients take days, weeks, or longer to be expressed rather than a few hours. The uniqueness of the rapidity of the onset of symptoms of arginine deficiency resulting from ingestion of an arginine-free amino acid mixture presumably arises from the combination of (i) a rapid depletion of free arginine in the liver and therefore of ornithine (for adequate urea cycle function) in the postabsorptive state, and (ii) by inhibition of arginine release from protein catabolism after feeding as a result of the anabolic response of incoming amino acids. The prevention of ammonia toxicity by arginine appears to be the result of the anaplerotic effect of ornithine in the urea cycle, enhancing the detoxification of the ammonia that arises from the degradation of the excess of the other amino acids. The ability of the cat to survive long periods without food (*13*) and not suffer ammonia toxicity is undoubtedly related to the obligatory release of tissue arginine when other amino acids are released via protein degradation. A protein-free (nitrogen-free and arginine-free) diet does not jeopardize ammonia homeostasis in the cat, as it does not contain a dietary precursor of ammonia.

Hyperammonemia has been shown to produce aberrations in pyrimidine synthesis, as is evidenced by increased urinary orotic acid excretion in rats (*14*). Arginine-deficient diets have also produced elevated orotic acid excretion in rats (*15*) and other mammals (*11*) as well as aberrations in intermediary carbohydrate metabolism (*16*). Elevated urinary orotic acid excretion has also been found in man after treatment with cancer chemotherapeutic drugs (*17*) and in rare genetic disorders of the urea cycle (*18*). The cat should provide a highly responsive mammalian model for the study of hyperammonemia and the mechanism of ammonia toxicity.

JAMES G. MORRIS, QUINTON R. ROGERS
*Departments of Animal Science* and
*Physiological Sciences.*
*School of Veterinary Medicine,*
*University of California, Davis 95616*

**References and Notes**

1. L. C. Faulkner, *J. Am. Med. Assoc.* **166**, 477 (1975).
2. *Nutrient Requirements of Laboratory Animals* (No. 10) (National Research Council, Washington, D.C., 1972).
3. S. A. Miller and J. B. Allison, *J. Nutr.* **64**, 493 (1958); J. P. Greaves and P. P. Scott, *Br. J. Nutr.* **14**, 361 (1960).
4. C. F. Kade, J. H. Phillips, W. A. Phillips, *J. Nutr.* **36**, 109 (1948); D. Melnik and G. R. Cowgill, *ibid.* **13**, 401 (1937); *Nutrient Requirements of Sheep* (No. 5) (National Research Council, Washington, D.C., 1975); *Recommended Dietary Allowances* (National Academy of Sciences, Washington D.C., ed. 8, 1974).
5. Q. R. Rogers, J. G. Morris, R. A. Freedland, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 257 (1976), abstr. 285.
6. B. Ahmed, *Biochem. J.* **25**, 1195 (1931); S. N. Gershoff, S. B. Andrus, D. M. Hegsted, E. A. Lentini, *Lab. Invest.* **6**, 227 (1957); A. C. DaSilva, R. Fried, R. C. de Angles, *J. Nutr.* **46**, 399 (1952). K. C. Hayes, R. E. Carey, S. Y. Schmidt, *Science* **188**, 949 (1975).
7. A. J. Hardy, J. G. Morris, Q. R. Rogers, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 498 (1976), abstr. 1617.
8. Eskalab Reagent Kit, for the determination of ammonia in blood. We thank Dr. J. J. Kaneko and R. Mills for the measurements of plasma ammonia.
9. H. T. Yee and E. S. Jenest, Technicon Congress on the Advances in Automated Analysis, June 1969.
10. W. C. Rose and E. E. Rice, *Science* **90**, 2330 (1939).
11. J. A. Milner, R. L. Prior, W. J. Visek, *Proc. Soc. Exp. Biol. Med.* **150**, 282 (1975).
12. W. C. Rose, M. J. Oesterling, M. J. Womack, *J. Biol. Chem.* **176**, 753 (1948). Q. R. Rogers, D. M. Chen, A. E. Harper, *Proc. Soc. Exp. Biol. Med.* **134**, 517 (1970).
13. P. G. Prentiss, A. V. Wolf, H. A. Eddy, *Am. J. Physiol.* **196**, 625 (1959).
14. L. Kesner, *J. Biol. Chem.* **240**, 1722 (1965).
15. J. A. Milner and W. J. Visek, *Metabolism* **24**, 643 (1975).
16. A. J. Clifford *et al.*, *Proc. Soc. Exp. Biol. Med.* **140**, 1447 (1972).
17. S. S. Cardoso, P. Calabresi, R. E. Handschumacher, *Cancer Res.* **21**, 1551 (1961).
18. V. E. Shih and M. L. Efron, in *Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, ed. 3, 1971), p. 370.
19. C. J. Carey and J. G. Morris, *J. Nutr.* **107**, 330 (1977).
20. Supported in part by a gift from the Carnation Company, Los Angeles.

17 January 1977; revised 22 August 1977