Assessment of the neurologic effects of dietary deficiencies of phenylalanine and tyrosine in cats

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Objective—To determine the neurologic effects of reduced intake of phenylalanine and tyrosine in black-haired cats.

Animals—53 specific pathogen-free black domestic shorthair cats.

Procedure—Cats were fed purified diets containing various concentrations of phenylalanine and tyrosine for ≤ 9 months. Blood samples were obtained every 2 months for evaluation of serum aromatic amino acid concentrations. Cats were monitored for changes in hair color and neurologic or behavioral abnormalities. Three cats with neurologic deficits underwent clinical and electrophysiologic investigation; muscle and nerve biopsy specimens were also obtained from these cats.

Results—After 6 months, neurologic and behavioral abnormalities including vocalization and abnormal posture and gait were observed in cats that had received diets containing < 16 g of total aromatic amino acid/kg of diet. Electrophysiologic data and results of microscopic examination of muscle and nerve biopsy specimens from 3 cats with neurologic signs were consistent with sensory neuropathy with primary axonal degeneration. Changes in hair color were detected in cats from all groups receiving < 16 g of phenylalanine plus tyrosine/kg of diet.

Conclusions and Clinical Relevance—Findings suggested that chronic dietary restriction of phenylalanine and tyrosine in cats may result in a predominantly sensory neuropathy. In cats, the long-term nutritional requirement for phenylalanine and tyrosine appears to be greater for normal neurologic function than that required in short-term growth experiments. Official present-day recommendations for dietary phenylalanine and tyrosine in cats may be insufficient to support normal long-term neurologic function. (*Am J Vet Res* 2004;65:671–680)

Phenylalanine (Phe) is an essential aromatic amino acid in cats[1] and other mammalian species, including humans.[2] Phenylalanine is utilized in protein synthesis and also is converted to the dispensable aromatic amino acid tyrosine (Tyr) by the phenylalanine hydroxylase system. In addition to its role as a component of structural proteins, tyrosine is essential in the formation of thyroid hormones; melanin; and the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine.

In cats, dietary restriction of phenylalanine accompanied by excess tyrosine results in decreased weight gain and negative nitrogen balance, compared with cats fed unrestricted diets,[1,3] and about half the requirement for aromatic amino acids may be supplied by tyrosine.[3,4] On the basis of these findings (obtained from short-term investigations[1,4]), the **National Research Council (NRC)** recommended in 1986 that the minimal requirement for dietary phenylalanine plus tyrosine in growing kittens was 8.5 g/kg of diet, half of which should be provided as phenylalanine.[5] To account for bioavailability of amino acids in prepared diets, the Association of American Feed Control Officials increased these recommendations for growing kittens and adult cats to 8.8 g of total aromatic amino acids/kg of diet, of which 4.2 g/kg had to be supplied as phenylalanine. Results of recent studies[6,7] investigating the loss of melanin pigmentation in black cats as a result of dietary restriction of phenylalanine or tyrosine over several months have suggested that these diet concentrations may be inadequate. Similarly, nutritional deficiencies of phenylalanine and tyrosine result in altered amounts of tyrosine and catecholaminergic neurotransmitters in the CNS of rats.[8,9]

In humans, alterations in the plasma levels of aromatic amino acids may occur for several reasons. Phenylketonuria most commonly results from mutations at the gene locus that encodes L-phenylalanine hydroxylase; that enzyme is required for the conversion of phenylalanine into tyrosine.[10] In patients with phenylketonuria, alterations in plasma aromatic amino acid concentrations and their metabolism have been implicated in the pathogenesis of neurotoxicosis including development of peripheral neuropathy.[10-14] The purpose of the study reported here was to determine the neurologic effects of reduced intake of phenylalanine and tyrosine in black-haired cats.

Materials and Methods

Animals—Fifty-three specific-pathogen-free, completely black domestic shorthair kittens from the Nutrition and Pet Care Center, University of California, Davis, were used. Experimental protocols were carried out in accordance with

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National Institutes of Health laboratory animal guidelines[15] and the Animal Welfare Act and were approved by the university's Animal Use and Care Advisory Committee.

Kittens were housed either singly in stainless steel metabolic cages (60 X 60 X 60 cm) or in pairs in 1.4-m^2 cages. Room temperature was maintained at 21 ± 2°C with a regular cycle of light (14 hours) and dark (10 hours). All animals were examined daily for clinical abnormalities, including those of gait, posture, and general behavior. No specific treatments of any of the kittens were undertaken during the studies except for 1 kitten for which the dietary intake of phenylalanine and tyrosine was increased after development of clinical abnormalities, as described elsewhere.

Experimental design—Kittens were randomly allocated to 1 of 11 dietary treatment groups that supplied the following concentrations (grams of amino acid per kilogram of diet) of phenylalanine and tyrosine, respectively: 4 + 2 (n = 8); 4 + 4 (4); 4 + 6 (5); 4 + 8 (5); 10 + 0 (6); 10 + 2 (6); 10 + 4 (4); 10 + 6 (3); 10 + 8 (4); 10 +10 (4); and 24 + 0 (4). Because an insufficient number of black cats were available at 1 time to supply all cats needed for the 11 treatment groups, kittens were randomly allocated to the treatment groups in 4 subsequent time periods as fully weaned groups of kittens became available. Kittens from the same litter were allocated to different treatment groups, and an attempt was made to balance treatment groups for sex of the kittens. Kittens were accustomed to purified diets and proven to be increasing in body weight before being placed on the experimental dietary treatments. The kittens received the experimental diets for ≥ 6 months, during which time food and water were available ad libitum. Kittens were weighed at weekly intervals, and mean daily food intake was recorded for kittens housed in pairs.

Diets—Eleven purified diets with crystalline amino acids as the sole protein source were used. With the exception of phenylalanine and tyrosine, individual amino acids were supplied at 1.6 to 2.0 times the NRC dietary concentration recommendations.[5] Concentration of crude protein (N X 6.25) was maintained at 280 g/kg of diet by use of an essential amino acid mixture and a dispensable amino acid mixture. The amount of dispensable amino acid mixture added to the diet was adjusted to compensate for nitrogen contributed by the various concentrations of phenylalanine and tyrosine. The essential amino acid mixture contributed the following (grams of amino acid[a] per kilogram of diet): arginine HCl, 20; histidine HCl.H_2O, 6; isoleucine, 10; leucine, 24; lysine HCl, 16; methionine, 8; cysteine, 7; threonine, 14; tryptophan, 3; and valine, 12. The dispensable amino acid mixture contributed the following (grams of amino acid[a] per kilogram of diet): alanine, 175; glycine, 175; glutamine, 175; glutamic acid, 75; asparagine, 150; aspartic acid, 100; and proline, 150. Constant ingredients (grams per kilogram of diet) in all diets were as follows: chicken fat,[b] 240; starch,[c] 275.5; sucrose,[d] 100; cellulose, 20; complete vitamin mixture,[e] 5; complete mineral mixture,[3] 50; choline chloride,[f] 5.5; sodium acetate[g] to balance the hydrochloride moiety associated with arginine, histidine, and lysine; taurine,[h] 2.5. When adequately supplied with phenylalanine, purified diet of similar composition to that used in this study has been shown to support rates of body weight gain in kittens comparable to those achieved with commercial diets.[3,16]

Ancillary diagnostic evaluations—For CBCs and serum biochemical analyses (including free triiodothyronine (T_3) and thyroxine (T_4) concentrations), blood samples were taken via jugular puncture from 3 cats that underwent electrophysiologic and biopsy procedures prior to anesthesia. Cerebrospinal fluid was taken from the same cats via cerebellomedullary cisternal puncture (under anesthesia) after electrophysiologic evaluation. Cerebrospinal fluid was analyzed for cellular and total protein content within 1 hour of collection.

Analysis of plasma amino acid concentrations—Plasma amino acid concentrations were determined in blood samples (3 mL) obtained with heparinized syringes from the jugular vein of cats (without anesthesia) every 2 months. Immediately after collection, samples were transferred to tubes on ice prior to refrigerated centrifugation (2,500 X *g* for 10 minutes). The plasma samples were stored at –80°C until further processing. Plasma was deproteinized with an equal volume of sulfosalicylic acid (0.28 mol/L), and the precipitate was removed by centrifugation. Lithium hydroxide was added to an aliquot of the supernatant to adjust the pH to 2.2. The equivalent of 20 µL of plasma was injected into the column of the analyzer[i] for plasma amino acid concentration determination (0.4 X 10-cm column packed with spherical cation exchange resin). Samples were collected under 2 conditions: at 9 AM after food, but not water, had been withheld overnight (designated food withheld samples) and subsequently 3 hours after providing food ad libitum (designated 3-hour postfeeding samples). It has been our experience that this latter measurement provides plasma amino acid values that are more sensitive to the first limiting amino acid than the former protocol. As cats eat frequently (consuming about 16 meals/d), measurement of the postprandial plasma concentration of amino acids is considered to more accurately indicate the concentration available to the tissues, rather than measurement of the plasma concentration after food has been withheld overnight. During the period of food withdrawal, plasma tyrosine is derived from catabolism of tissue.

Electrophysiologic evaluations—Three affected cats and 1 control cat were evaluated at a single time point. The evaluations were done in the first 3 cats to develop clinical abnormalities, approximately 6 months after initiation of the study. Anesthesia was induced via IV administration of propofol[j] (4 to 8 mg/kg) and maintained with isoflurane[k] (0.5% to 3.0%) in oxygen. The recording apparatus used consisted of a 4-channel programmable evoked-response system.[l] Electrophysiologic examination consisted of electromyography, assessments of superficial peroneal **motor nerve conduction velocity (MNCV)** and **sensory nerve conduction velocity (SNCV)**, and repetitive nerve stimulation. Electrophysiologic data from the study cats were compared with data from an age-matched colony cat from the Nutrition and Pet Care Center, University of California, Davis, that was housed under the same conditions as the study cats and maintained on a proprietary cat food, as well as with published reference values[17,18] and data obtained from cats evaluated at the university's Veterinary Teaching Hospital.

Electromyographic evaluations—Evaluations were performed by use of a concentric electromyographic needle electrode[m] and a subcutaneous ground electrode.[n] Frequency bandwidth was 20 Hz to 10 kHz with a sensitivity of 50 µV.

Motor and sensory nerve conduction evaluations—Polyteflon-coated monopolar stainless steel electrodes[o] of various lengths were used both for stimulation and recording. Electrode impedance was minimized by electrolytic treatment and maintained at 0.2 to 2.0 kΩ (100 Hz). A subcutaneous needle electrode[p] was used for grounding. Compound motor nerve action potentials were recorded over the lateral short digital extensor muscle following stimulation at the level of the tarsal, stifle, and hip joints. Stimulating electrodes for sensory nerve conduction velocity assessments were inserted subcutaneously over the third and fourth metatarsal bones. Needles were placed, as described.[19]

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For recording cord dorsum potentials, exploring electrodes were placed percutaneously in the dorsal midline with the needle tip in proximity to the interarcuate ligament (ligamentum flavum) between the L4 and L5 vertebrae. For MNCV recordings, the stimulus was a rectangular, constant-current (< 4 mA), 0.2-millisecond pulse at a frequency of 1 Hz. Frequency bandwidth was 2 Hz to 10 kHz with sensitivity between 2 and 10 mV. For SNCV recordings, the stimulus was a rectangular, supramaximal constant-current (4 to 10 mA), 0.2-millisecond pulse at a frequency of 10 Hz. Frequency bandwidth was 20 Hz to 3 kHz with sensitivity between 0.5 and 10 µV. A total of 1,000 to 2,000 responses were averaged at each site.

Repetitive nerve stimulation assessments—Conditions were set as MNCV evaluations, except that frequency bandwidth was 2 Hz to 5 kHz and stimulation frequency was 1, 2, 3, 5, 7, 10, 15, or 20 Hz.

Histologic evaluations—Biopsy specimens were obtained from all 3 affected cats after electrophysiologic assessment. Specimens were taken from the distal third of the vastus lateralis and triceps brachii muscles. Fascicular biopsy specimens were obtained from the superficial peroneal nerve at the level of the stifle joint, as described.[20] Briefly, muscle biopsy specimens were snap frozen in isopentane precooled in liquid nitrogen and stored at –80°C until further processed. Fascicular nerve biopsy specimens were either snap frozen or sutured to a section of cotton tip applicator and fixed via immersion in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH, 7.4). Semithin cross sections (1 µm) of glutaraldehyde-fixed, araldite resin-embedded nerve specimens were stained with toluidine blue-basic fuchsin for light microscopy; ultrathin sections of these nerve specimens were stained with uranyl acetate and lead citrate for electron microscopy.

A standard panel of histochemical stains and reactions was applied to cryostat muscle biopsy sections (10 µm), including H&E, myofibrillar adenosine triphosphatase, modified trichrome, periodic acid-Schiff hematoxylin, esterase, oil red O, nicotinamide adenine dinucleotide tetrazolium reductase, and acid phosphatase. Longitudinal cryostat sections of nerve were stained with H&E, modified trichrome, and acid phosphatase. A complete necropsy, including the CNS, was completed on 1 cat with severe neurologic signs that was euthanatized by use of IV administration of pentobarbital and phenytoin[q] (1 mL/4.5 kg of body weight) and transcardially perfused with glutaraldehyde. Samples of radial (distal portion), ulnar (distal portion), caudal cutaneous sural, phrenic, recurrent laryngeal, vagosympathetic, optic, and trigeminal nerves from this cat were processed for plastic embedding.

Results

Clinical findings—Abnormalities in gait, posture, or behavior were observed within 6 months in cats receiving diets in which the total aromatic amino acid concentration was < 16 g/kg of diet. Neurologic signs were detected in 8 of 8 cats receiving 4 g of phenylalanine + 2 g of tyrosine/kg of diet; 4 of 4 cats receiving the 4 + 4 diet; 5 of 5 cats receiving the 4 + 6 diet; 3 of 5 cats receiving the 4 + 8 diet; 4 of 6 cats receiving the 10 + 0 diet; 3 of 6 cats receiving the 10 + 2 diet; and 2 of 4 cats receiving the 10 + 4 diet. No neurologic signs were observed in cats receiving diets in which the total dietary aromatic amino acids concentration was ≥ 16 g/kg of diet (ie, the 10 + 6, 10 + 8, 10 + 10, or 24 + 0 diets). The most severely affected cats appeared hyperactive, with frequent vocalization and ptyalism. These cats often had a kyphotic posture, with the hind limbs extended and tails held in a scorpion-like posture over the back. Trembling of the erect tail also was observed. Complete neurologic examination of severely affected animals revealed mild hind limb ataxia. No neurologic deficits involving cranial nerves, spinal reflexes, or postural reactions were apparent.

Clinical abnormalities were first observed in the 3 cats fed the 10 + 2 diet. These cats underwent detailed investigation described previously. Following diagnostic investigations, the most severely affected cat that was receiving the 10 + 2 diet was transferred onto the 10 + 6 diet. Notable clinical improvement occurred within a 1-month period. The animal was no longer hyperactive and did not vocalize; the ataxia, although still present, was less severe.

Changes in coat coloring were detected in cats from all groups receiving diets that contained < 16 g of phenylalanine + tyrosine/kg of diet. These changes included alopecia with change in hair color from black to reddish-brown to gray. These hair data have been reported previously.[6]

Mean values of food intake were recorded because some of the kittens were housed in pairs; statistical analyses were not performed on these data. However, mean food intakes of the kittens that had neurologic signs (ie, those receiving phenylalanine and tyrosine in combinations of 4 + 2 to 10 + 4 g/kg of diet) varied from 59 to 74 g/d, whereas kittens that had no neurologic signs (ie, those receiving phenylalanine and tyrosine in combinations of 10 + 6, 10 + 8, 10 + 10, and 24 + 0 g/kg of diet) had mean food intakes of 58 to 75 g/d.

Ancillary diagnostic evaluations—Results of CBCs, serum biochemical analyses (including analysis of total and free T_3 and T_4 concentrations), and cisternal CSF analyses were within reference limits in all 3 cats that were receiving the 10 + 2 diet. Plasma concentrations of tyrosine in cats receiving diets with total aromatic amino acids (phenylalanine + tyrosine) concentrations of 6 to 16 g/kg of diet were plotted graphically **(Fig 1)**. The number of samples analyzed for each dietary group after withholding of food overnight and at 3 hours after feeding were as follows: 4 + 2 group (n

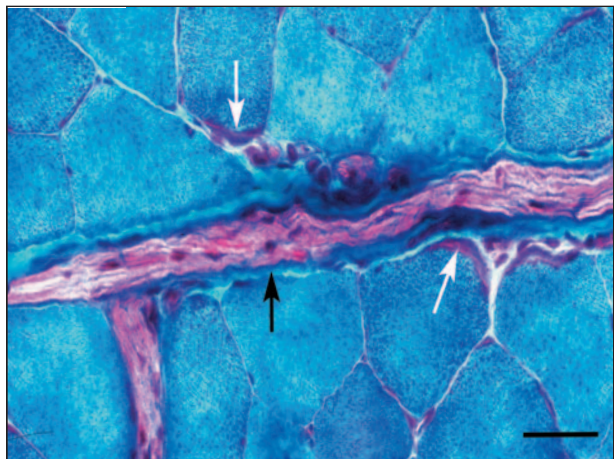

Figure 1—Mean ± SEM plasma tyrosine concentrations in cats fed diets containing various concentrations of total aromatic amino acids (phenylalanine + tyrosine) after food was withheld overnight (squares) and 3 hours after being fed (circles).

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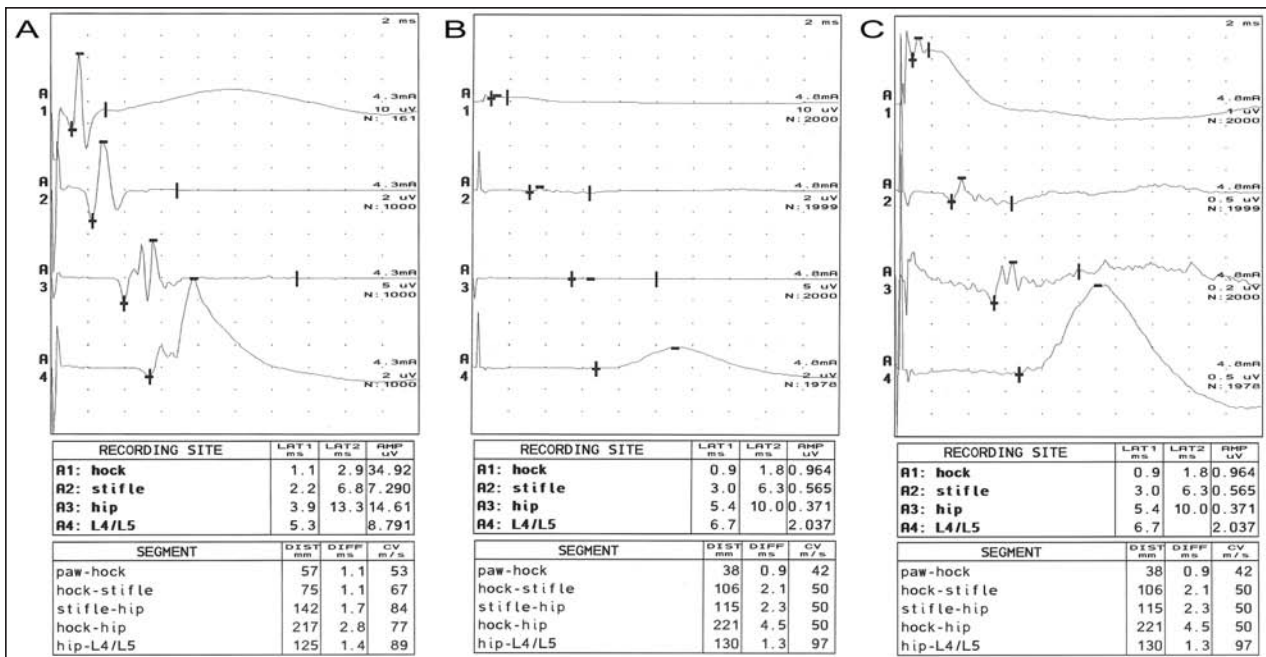

Figure 2—A—Sensory nerve conduction tracing (standard gain display) recorded from the peroneal nerve in a clinically normal cat (control). Notice that maximum conduction velocities and evoked potential amplitudes (conformation and duration) are within normal reference ranges. B—Sensory nerve conduction tracing (standard gain display) recorded from the peroneal nerve in a cat with severe neurologic signs associated with consumption of a diet containing 10 g of phenylalanine and 2 g of tyrosine/kg. Maximum conduction velocities are markedly decreased in both proximal and distal nerve segments, compared with the control cat. Notice the dispersion and low amplitude of evoked potentials. C—Sensory nerve conduction tracing (increased gain display) recorded from the peroneal nerve in the cat in panel B. Notice that the dispersion of evoked potentials is more clearly evident.

Table 1—Sensory nerve conduction velocity (m/s) and evoked potential amplitude (μV) recorded in 3 cats with neurologic signs associated with consumption of a diet containing 10 g of phenylalanine + 2 g of tyrosine/kg

		Cat			
Segment	**Variable**	**1**	**2**	**3**	**Control**
Foot–tarsal joint	Conduction velocity	53	42	45	53
	Amplitude (tarsal joint)	2.6	1.0	33.9	34.9
Tarsal joint–stifle joint	Conduction velocity	52	50	75	67
	Amplitude (stifle joint)	1.3	0.6	3.2	7.3
Stifle joint–hip joint	Conduction velocity	64	50	97	84
	Amplitude (hip joint)	0.6	0.4	2.4	14.6
Tarsal joint–hip joint	Conduction velocity	59	50	85	77
Hip joint–L4-5	Conduction velocity	54	97	108	89
	Amplitude (L4-5)	3.0	2.0	2.5	8.8

= 4 and 4, respectively); 4 + 4 (3 and 3, respectively); 4 + 6 (5 and 5, respectively); 4 + 8 (6 and 6, respectively); 10 + 0 (5 and 5, respectively); 10 + 2 (3 and 3, respectively); 10 + 4 (4 and 4, respectively); and 10 + 6 (5 and 3, respectively). At 3 hours after feeding, the concentration of plasma tyrosine increased markedly in kittens that received diets containing aromatic amino acid concentrations > 12 g/kg of diet, compared with concentration values in kittens that received diets containing aromatic amino acid concentrations ≤ 12 g/kg of diet. The data set was not expanded beyond diets containing aromatic amino acid concentrations > 16 g/kg of diet once the pattern in plasma tyrosine concentration was determined.

Electrophysiologic evaluations—In the control cat and the 3 cats with neurologic signs that were assessed, electromyography revealed no abnormalities. Motor nerve conduction velocities and motor evoked potential configurations (amplitude, waveform, and duration) were considered to be within reference limits in all cats. Sensory nerve conduction velocities were decreased in cats 1 and 2, compared with reference values (**Table 1**), and evoked potentials were of decreased amplitude and increased duration in cats 1 and 2 (**Fig 2**). These cats also had the most severe clinical signs. Cat 3, which was the least affected clinically, had normal sensory conduction velocities; however, evoked potentials were of decreased amplitude, com-

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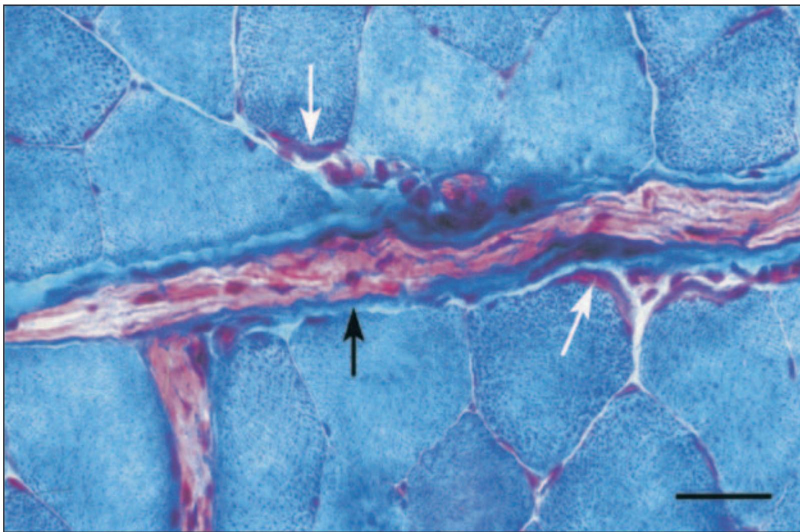

Figure 3—Photomicrograph of a transverse frozen section of vastus lateralis muscle obtained from a cat with severe neurologic signs associated with consumption of a diet containing 10 g of phenylalanine and 2 g of tyrosine/kg. Notice the apparently normal myofibers, a well-myelinated intrafascicular nerve branch (black arrow), and neuromuscular end plates in myofibers adjacent to the nerve (white arrows). Evidence of myopathic or neuropathic alteration is not apparent. Modified trichrome stain; bar = 50 µm.

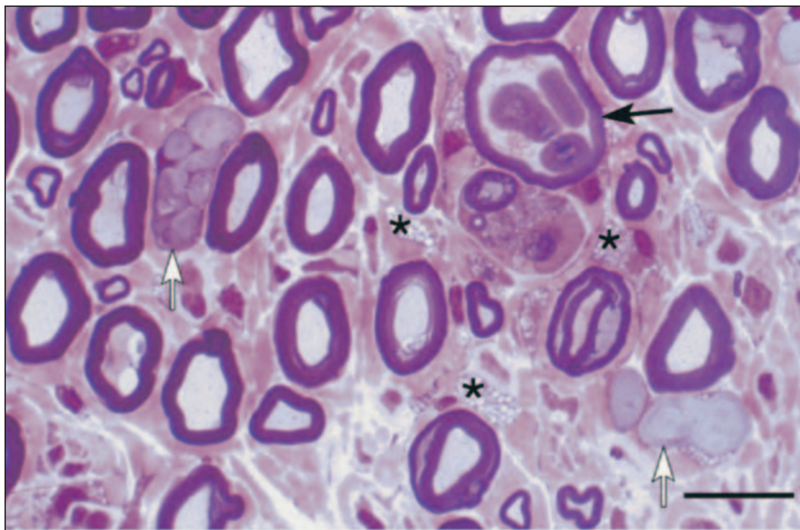

Figure 4—Photomicrograph of a section (1 µm) of a fixed, plastic-embedded biopsy specimen from the superficial peroneal nerve of a cat with severe neurologic signs associated with consumption of a diet containing 10 g of phenylalanine and 2 g of tyrosine/kg. Axonal degeneration, secondary demyelination, numerous foamy macrophages (asterisks), and myelin ovoids (white arrows) are present. Notice the swollen nerve fiber (black arrow) that is undergoing axonal degeneration. Toluidine blue-basic fuchsin stain; bar = 20 µm.

pared with reference values. Repetitive stimulation, from 1 to 5 Hz, was considered unremarkable in all cats with decrements of motor evoked potential amplitude and area ≤ 10% of the initial value at the third stimulus.

Histologic evaluation of muscle biopsy specimens—No notable abnormalities were detected in the muscle specimens obtained from the 3 cats with neurologic signs or the control cat. Several sections examined contained portions of intrafascicular or interfascicular nerve branches in which no abnormalities were detected (**Fig 3**).

Histologic evaluation of nerve biopsy specimens—In 1-µm plastic-embedded sections of biopsy specimens of superficial peroneal nerve from the 3 cats with neurologic signs, numerous degenerating axons, myelin ovoids, and foamy macrophages were detected (**Fig 4**). Myelin sheaths of apparently unaffected axons appeared to be of appropriate thickness, which suggested a primary axonopathy with secondary demyelination.

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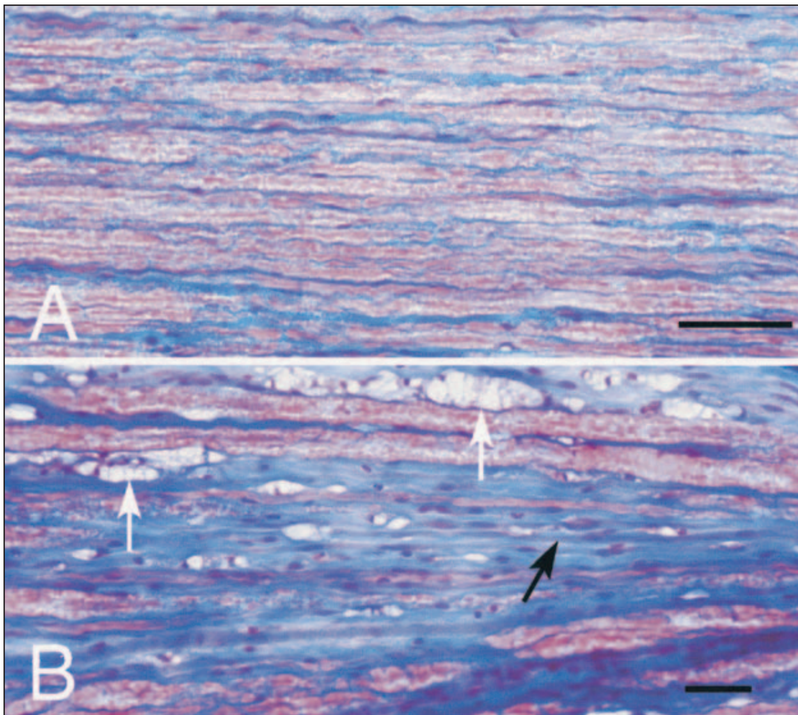

Figure 5—Photomicrographs of frozen sections of superficial peroneal nerve biopsy specimens from a clinically normal control cat (A) and a cat with severe neurologic signs associated with consumption of a diet containing 10 g of phenylalanine and 2 g of tyrosine/kg (B). In panel A, myelin is plentiful (pink staining material). In panel B, notice the marked loss of myelin in the nerve from the affected cat (black arrow); numerous chains of myelin digestion chambers or myelin ovoids (white arrows) suggest phagocytic degeneration of myelin and axons. Modified trichrome stain; in panels A and B, bars = 50 and 10 µm, respectively.

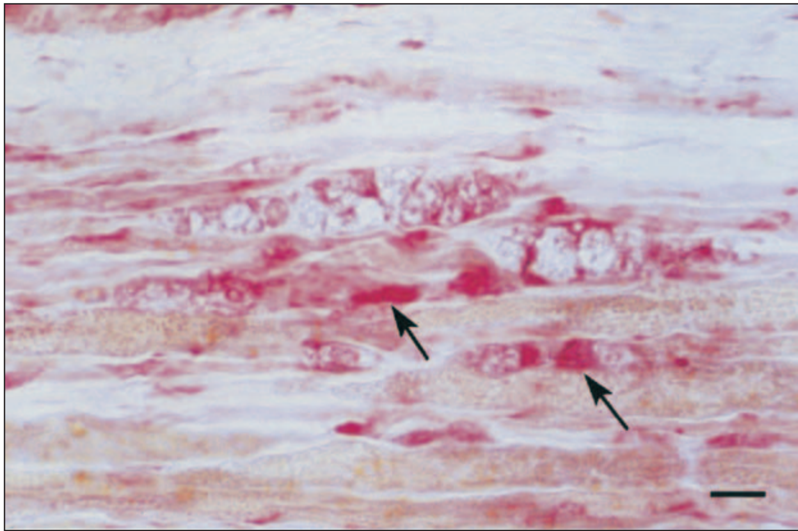

Figure 6—Photomicrograph of a section of a superficial peroneal nerve biopsy specimen from a cat with severe neurologic signs associated with consumption of a diet containing 10 g of phenylalanine and 2 g of tyrosine/kg. Notice that digestion chambers in the nerve are stained focally (arrows) indicating lysosomal activity within phagocytic macrophages. Acid phosphatase stain; bar = 10 µm.

Histochemical analysis of frozen sections of superficial peroneal nerve specimens of the 3 cats with neurologic signs revealed various stages of Wallerian-like degeneration, with phagocytic macrophage activity and loss of myelinated nerve fibers (**Fig 5 and 6**). These changes were most severe in cats 1 and 2. No abnormalities were detected in the similar sections of superficial peroneal nerve specimens obtained from the control cat. Electron microscopic findings confirmed the primary axonal loss (**Fig 7**). Axonal degeneration also was observed in plastic-embedded sections of the radial (distal portion), caudal cutaneous sural, and phrenic nerves from cat 2. No

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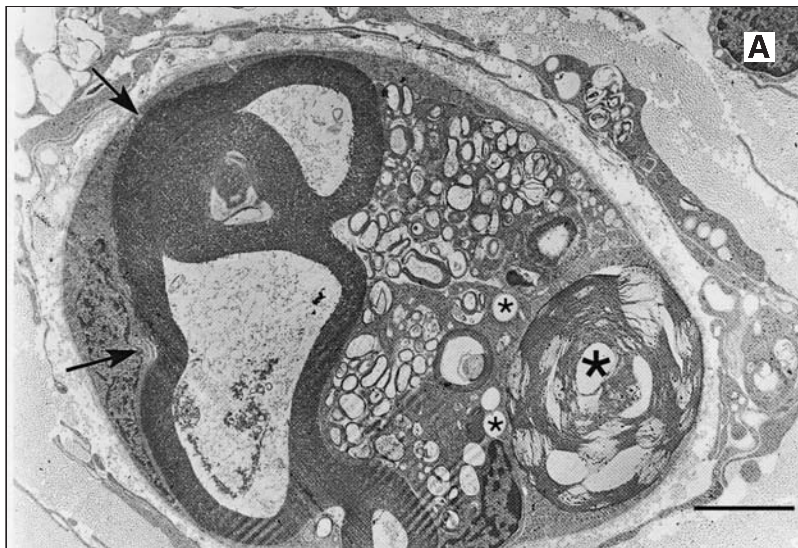

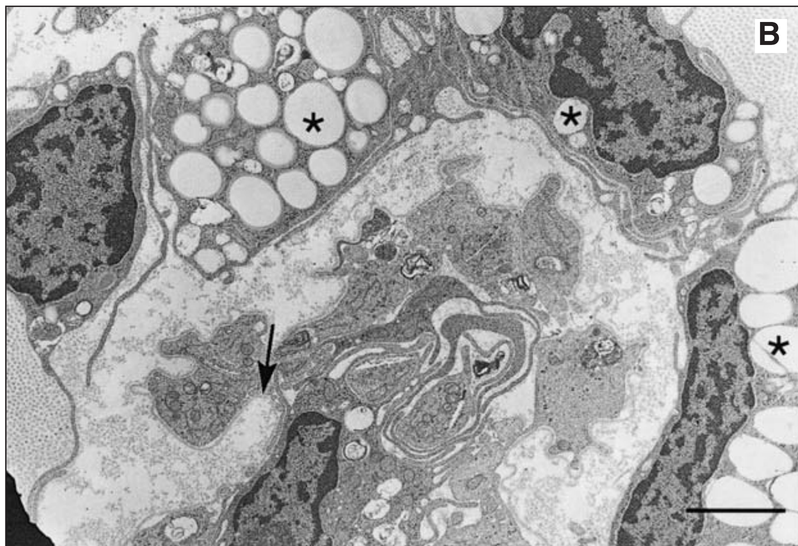

Figure 7—Electron micrographs of sections of superficial peroneal nerve biopsy specimens obtained from a cat with severe neurologic signs associated with consumption of a diet containing 10 g of phenylalanine and 2 g of tyrosine/kg. A—Wallerian-like (axonal) degeneration. The section contains a nerve fiber with an empty axis cylinder and collapsing myelin sheath (arrows). A macrophage filled with lipid debris (small asterisks) has infiltrated the neurolemmal tube, and an ovoid structure formed by myelin debris (large asterisk) is in the Schwann cell cytoplasm. B—Advanced Wallerian-like degeneration. The section contains foamy macrophages filled with lipid debris (asterisks) adjacent to Schwann cell processes that are surrounded by loosely fitting basal lamina (arrow). The axon cannot be identified. Magnification in panels A and B. bar = 2 µm.

changes were seen in sections of vagosympathetic trunk, optic nerve, trigeminal nerve, or distal ulnar nerve.

Necropsy findings—On postmortem examination of the cat with neurologic signs (cat 2), no pathologic changes were observed in any major organ systems, including the CNS. No pathologic changes were detected involving the dorsal or ventral spinal nerve roots, dorsal root ganglia, or spinal cord sensory tracts in this cat.

Discussion

Investigations of dietary amino acid requirements in domesticated species and humans usually involve short-term studies (ie, of several weeks' duration), during which time basic variables such as nitrogen balance and body weight are recorded. Information regarding the effects of prolonged dietary restriction of specific amino acids is scarce; to the authors' knowledge, long-term studies to investigate dietary deficiencies of phenylalanine and tyrosine have not been previously done. The clinical and pathologic findings of the study of this report suggest that prolonged restriction of phenylalanine and tyrosine may result in a predominantly sensory neuropathy in cats. Characterization of the neuropathy as sensory in nature was made on the basis of several findings. First, results of the motor nerve conduction velocity assessments were within

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normal limits in the 3 study cats with neurologic signs. Conduction velocities were near the lower end of reference limits for our laboratory, but evoked potential configurations were normal. By comparison, sensory nerve conduction velocity assessments were markedly abnormal in the 2 cats with the most severe neurologic signs. Conduction velocities were decreased, compared with reference values, and evoked potentials were of low amplitude and dispersed. Of the 3 cats, the cat with the least severe neurologic signs only had low amplitude of evoked potentials that was consistent with mild axonal loss. Second, examination of biopsy specimens of the superficial peroneal nerve (a mixed motor and sensory nerve) revealed axonal degeneration and secondary demyelination in all affected cats. Third, no apparent pathologic changes were evident in muscle biopsy specimens. Given the severity of the changes detected in the peripheral nerve, typical evidence of denervation (angular atrophy of type I and II myofibers and myofiber type grouping) in motor fibers over a prolonged time period would have been expected. Intrafascicular and interfascicular nerve branches (predominantly motor fibers) within the muscle biopsy specimens appeared to be unaffected. The lack of evidence of pathologic changes in motor nerve fibers in muscle specimens complemented the absence of abnormal electromyographic findings.

On the basis of findings in multiple nerve specimens from the cat that was necropsied (cat 2), the neuropathic process appeared to be limited to peripheral nerves of spinal cord origin. Absence of pathologic alterations within the dorsal root ganglia and dorsal nerve roots suggested a more distal axonal degeneration. On the basis of results of neurologic and necropsy examinations of cat 2, the cranial nerves were not affected.

To our knowledge, peripheral neuropathy as a consequence of phenylalanine and tyrosine dietary deficiency has not been reported, and the underlying pathogenesis is unclear. None of the cats were losing weight at the time of the neurologic investigations, and therefore, a catabolic process involving the peripheral sensory nerves is unlikely. Tyrosine is an essential precursor for thyroid hormones and the catecholaminergic neurotransmitters. Although peripheral neuropathies have been reported secondary to hypothyroidism in humans[21] and other animals,[22-24] hypothyroid neuropathy has not been reported in cats; furthermore, all 3 neurologically affected cats studied had normal serum T_3 and T_4 concentrations.

Several distinct groups of catecholaminergic neurons are present in the brain. These include the dopaminergic neurons of the substantia nigra and ventral tegmental area, hypothalamus, olfactory bulb, and some retinal amacrine cells. Noradrenergic neurons also are found in the locus ceruleus, subceruleus, and lower brainstem. Neurotransmitters used by the sensory neurons of the dorsal root ganglia are largely unknown; however, a subpopulation of these neurons is catecholaminergic.[25-27] Altered catecholamine metabolism has been suggested as a cause of other sensory neuropathies in humans.[28] Neurotransmitter concentrations were not measured in any of the study cats, either in the peripheral nervous system or CNS, and it is unclear whether this could explain the observed neuropathy in the affected animals.

As part of a spectrum of neurologic abnormalities, a predominantly sensory neuropathy has been described in several humans with phenylketonuria.[11-14] The neuropathy was subclinical, and electrophysiologic data supported primary demyelination; however, nerve biopsy specimens were not examined. Plasma tyrosine concentration was not reported for these individuals, but plasma phenylalanine concentration was significantly increased, compared with that of unaffected humans. Despite the inability of patients with phenylketonuria to convert phenylalanine to tyrosine, consistently low plasma tyrosine concentrations are not always detected, and a primary role for tyrosine deficiency in the neuropathology of phenylketonuria has not been proven.[10] However, experimentally, high concentrations of phenylalanine can inhibit tyrosine transport and metabolism in neuronal cells,[29] and it is possible that either absolute dietary deficiency or impaired uptake or metabolism of tyrosine may lead to a common neuropathologic end point. True dietary deficiency of tyrosine in humans is rare, particularly extending over prolonged periods. Pediatric mixtures used to provide total parenteral nutrition contain limited amounts of tyrosine because it has poor solubility, and it has been suggested that tyrosine may be a conditionally essential amino acid in newborn infants. Neuropathy has not been described as a complication of neonatal parenteral nutrition in infants; however, prolonged periods (ie, $\geq$ 6 months) of dietary deficiency are unusual. In some patients with chronic gastrointestinal tract disorders, total parenteral nutrition provided long term has been associated with decreased plasma concentrations of amino acids, including tyrosine.[30] Peripheral neuropathy has been described in some of these patients and has been associated with chromium deficiency, pathologic changes in the pancreas, and other factors such as administration of metronidazole[31-34]; however, an underlying cause was not determined in several of those cases, and plasma aromatic amino acid concentrations were not reported.[31,32]

Although anecdotal, it is interesting to note that the clinical signs in cat 1 that had developed in association with being fed a diet that had a low tyrosine concentration were markedly improved within 4 weeks after the diet was changed to one that had a greater concentration of that amino acid. This time course is probably too short to support extensive axonal regrowth as a source of the improvement and may imply other physiologic abnormalities, perhaps involving neurotransmission, in addition to axonal degeneration as a cause of the clinical signs. The notable clinical response observed after a small change in total dietary aromatic amino acid concentration (ie, from 10 g of phenylalanine + 2 g of tyrosine/kg of diet to 10 g of phenylalanine + 6 g of tyrosine/kg of diet) may be explained by the measured plasma tyrosine concentrations because the postprandial plasma concentration of tyrosine increased markedly for dietary total aromatic amino acid concentrations $>$ 12 g/kg of diet,

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compared with concentration values in kittens that received diets containing aromatic amino acid concentrations ≤ 12 g/kg of diet.

Tyrosine deficiency has been shown to result in alteration of brain concentrations of tyrosine and tyrosine-dependent neurotransmitters such as dopamine,[8,9] and although Bessman[35] proposed that an absolute tyrosine deficiency causes mental retardation in children, this hypothesis has never been proven. In cat 2, no gross pathologic abnormalities involving the CNS were observed. However, it is interesting to speculate that some of the behavioral abnormalities seen in these cats (eg, hyperactivity and vocalization) may have been the result of altered neurotransmitter concentrations in the CNS. These clinical signs are certainly not typical of those reported in dogs with sensory neuropathies,[36-42] although increased barking and growling were reported in 1 dog with a sensory neuropathy.[42]

Abnormalities of the CNS have been reported[43] in queuine-deficient germ-free mice that were fed a tyrosine-free liquid diet that was apparently adequate in phenylalanine. Queuine is a modified derivative of guanine in the first position of the anticodon of the **transfer RNA (tRNA)** for aspartate, asparagine, histidine, and tyrosine. In those mice, addition of either tyrosine or queuine to the diet resulted in abrogation of the clinical signs. In wild-type mice, queuine may be provided either in the diet or from the bacterial intestinal microflora. In the study involving the germ-free mice, plasma concentrations of tyrosine were not measured while the mice were given the tyrosine-free diet to confirm that the phenylalanine in the diet was adequate and that there was not a defect in the metabolism of phenylalanine to tyrosine. However, the enzyme tyrosine hydroxylase, which hydroxylates tyrosine to DOPA (3,4-dihydroxyphenylalanine), contains RNA, and the binding of nucleic acid causes a conformational change in the enzyme. The **Michaelis' constant (K_m)** of tyrosine hydroxylase for tyrosine is lowered on RNA binding. If the tRNA effector for tyrosine hydroxylase is a queuine-constraining tRNA, this would explain the response of these mice to either the addition of tyrosine or queuine to the diet. The addition of tyrosine to the diet (quantity not stated by authors) may overcome the low affinity of tyrosine hydroxylase for tyrosine, hence, it appears that tyrosine is an essential amino acid in the absence of queuine. Queuine concentrations were not determined in any of the cats of our study; however, concentrations were unlikely to be deficient because the cats presumably had a normal intestinal microbial population, and antimicrobials were not incorporated in the diet. A normal intestinal bacterial population is known to provide adequate queuine.

Protein and amino acid requirements of cats differ from those of humans and other small mammals. Nine essential amino acids are required by humans. In addition to these 9 amino acids, cats also require taurine and arginine because they have a reduced capacity to synthesize taurine,[44] and effects of arginine deficiency in this species are severe and can be lethal.[45] In cats, total dietary protein requirements are also high, compared with requirements of dogs and humans.[1] Consumption of diets that contain low concentrations of phenylalanine and tyrosine has resulted in a decreased melanin content in the hair of black cats, regardless of the fact that the diets had total aromatic amine contents well above NRC requirements.[6,7] These NRC requirements were based on results of relatively short-term experiments to investigate maximal growth and nitrogen balance. In the study of this report, the apparent dietary threshold above which long-term neurologic abnormalities were not observed was 16 g of total aromatic amino acids/kg of diet. This is double the current NRC requirements that were derived from the short-term studies. In cats, the K_m of tyrosinase (which is required for melanin synthesis) for tyrosine is approximately 500 times that for the acyl synthetase (required for protein biosynthesis), and this may in part explain the reduction in melanin content of hair in the black cats fed diets with low concentrations of phenylalanine and tyrosine.[6] Tyrosine hydroxylase is the rate-limiting enzyme in the conversion of tyrosine to catecholaminergic neurotransmitters. The apparent K_m value for tyrosine hydroxylase derived from rat CNS[46,47] is also greater than that for acyl synthetase,[48] albeit only 2 times. Other factors such as transport of tyrosine into neurons may also be important in the potential pathogenesis of the neurologic abnormalities seen in the cats of this study. The development of sensory neuropathy in the cats of this report supports the importance of the role of tyrosine in the metabolism of cats and suggests that the nutritional requirement for phenylalanine and tyrosine is greater for normal neurologic function than for growth.

In summary, long-term dietary restriction of phenylalanine and tyrosine in black cats appeared to result in a predominantly sensory neuropathy. The underlying pathologic mechanism is unknown, but may involve alterations in catecholaminergic neurotransmitters secondary to decreased availability of tyrosine. This model may provide insight into the relative requirements of dietary aromatic amino acids in relation to normal neuronal function.

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[a]All amino acids were obtained from Aijinomoto USA Inc, Teaneck, NJ.
[b]Foster Farms, Livingston, Calif.
[c]Melojel, food-grade cornstarch, National Food Starch and Chemical, Bridgewater, NJ.
[d]Spreckels Sugar Corp, Pleasanton, Calif.
[e]Roche Products, Nutley, NJ.
[f]DuPont, Highland, Ill.
[g]Fisher Scientific, Santa Clara, Calif.
[h]Taisho Pharmaceutical, Torrance, Calif.
[i]Model 7300 Beckman amino acid analyzer, Beckman Instruments Inc, Palo Alto, Calif.
[j]PropoFlo, Abbott Animal Health, North Chicago, Ill.
[k]Attane, Minrad Inc, Bethlehem, Pa.
[l]Viking IV D, Nicolet Biomedical Co, Madison, Wis.
[m]Nicolet Biomedical Co, Madison, Wis.
[n]Grass Medical Instruments, Quincy, Mass.
[o]Nicolet Biomedical Co, Madison, Wis.
[p]Grass Medical Instruments, Quincy, Mass.
[q]Beuthanasia-D Special, Schering-Plough Corp, Union, NJ.

References

1. Rogers QR, Morris JG. Essentiality of amino acids for the growing kitten. *J Nutr* 1979;109:718–723.

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2. Womack M, Rose WC. Feeding experiments with mixtures of highly purified amino acids. *J Biol Chem* 1934;107:449–458.

3. Williams JM, Morris JG, Rogers QR. Phenylalanine requirement of kittens and the sparing effect of tyrosine. *J Nutr* 1987; 117:1102–1107.

4. Anderson PA, Baker DH, Sherry PA, et al. Histidine, phenylalanine-tyrosine and tryptophan requirements for growth of the young kitten. *J Anim Sci* 1980;50:479–483.

5. National Research Council. *Nutrient requirements of cats*. Washington, DC: National Academy Press, 1986.

6. Anderson PJB, Rogers QR, Morris JG. Cats require more dietary phenylalanine or tyrosine for melanin deposition in hair than for maximal growth. *J Nutr* 2002;132:2037–2042.

7. Yu S, Rogers QR, Morris JG. Effect of low levels of dietary tyrosine on the hair colour of cats. *J Small Anim Pract* 2001; 42:176–180.

8. Venero JL, Herrera AJ, Machado A, et al. Changes in neurotransmitter levels associated with the deficiency of some essential amino acids in the diet. *Br J Nutr* 1992;68:409–420.

9. Hilton MA, Fonda ML, Hilton FK. The effect of tyrosine-deficient total parenteral nutrition on the synthesis of dihydroxyphenylalanine in neural tissue and the activities of tyrosine and branched-chain aminotransferases. *Metabolism* 1998;47:168–176.

10. Scriver CR, Kaufman S. Hyperphenylalanemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill Book Co, 2001;1667–1724.

11. Ludolph AC, Ullrich K, Nedjat S, et al. Neurological outcome in 22 treated adolescents with hyperphenylalaninemia. A clinical and electrophysiological study. *Acta Neurol Scand* 1992; 85:243–248.

12. Ludolph AC, Vetter U, Ullrich K. Studies of multimodal evoked potentials in treated phenylketonuria: the pattern of vulnerability. *Eur J Pediatr* 1996;155(suppl 1):S64–S68.

13. Cleary MA, Walter JH, Wraith JE, et al. Magnetic resonance imaging of the brain in phenylketonuria. *Lancet* 1994;344:87–90.

14. Villasana D, Butler IJ, Williams JC, et al. Neurological deterioration in adult phenylketonuria. *J Inherit Metab Dis* 1989;12:451–457.

15. Institute for Laboratory Animal Research. *Guide for the care and use of laboratory animals*. Washington, DC: National Academy Press, 1996.

16. Hargrove DM, Rogers QR, Morris JG. The tryptophan requirement of the kitten. *Br J Nutr* 1983;50:487–493.

17. Tuler SM, Bowen JM. Measurement of conduction velocity of the peroneal nerve based on recordings from extensor digitorum brevis muscle. *J Am Anim Hosp Assoc* 1990;26:164–168.

18. Redding RW, Ingram JT. Sensory nerve conduction velocity of cutaneous afferents of the radial, ulnar, peroneal, and tibial nerves of the cat: reference values. *Am J Vet Res* 1984;45:1042–1045.

19. Niederhauser UB, Holliday TA, Hyde DM, et al. Correlation of sensory electroneurographic recordings and myelinated fiber diameters of the superficial peroneal nerve of dogs. *Am J Vet Res* 1990; 51:1587–1595.

20. Dickinson PJ, LeCouteur RA. Muscle and nerve biopsy. *Vet Clin North Am Small Anim Pract* 2002;32:63–102.

21. Pollard JD. Neuropathy in diseases of the thyroid and pituitary glands. In: Dyck PJ, Thomas PK, eds. *Peripheral neuropathy*. Philadelphia: WB Saunders Co, 1993;1266–1274.

22. Indrieri RJ, Whalen LR, Cardinet GH, et al. Neuromuscular abnormalities associated with hypothyroidism and lymphocytic thyroiditis in three dogs. *J Am Vet Med Assoc* 1987;190:544–548.

23. Jaggy A, Oliver JE, Ferguson DC, et al. Neurological manifestations of hypothyroidism: a retrospective study of 29 dogs. *J Vet Intern Med* 1994;8:328–336.

24. Quattrini A, Nemni R, Marchettini P, et al. Effect of hypothyroidism on rat peripheral nervous system. *Neuroreport* 1993; 4:499–502.

25. Mercer EH, Hoyle GW, Kapur RP, et al. The dopamine beta-hydroxylase gene promoter directs expression of *E. coli* lacZ to sympathetic and other neurons in adult transgenic mice. *Neuron* 1991; 7:703–716.

26. Price J, Mudge AW. A subpopulation of rat dorsal root ganglion neurones is catecholaminergic. *Nature* 1983;301:241–243.

27. Son JH, Min N, Joh TH. Early ontogeny of catecholaminergic cell lineage in brain and peripheral neurons monitored by tyrosine hydroxylase-lacZ transgene. *Brain Res Mol Brain Res* 1996; 36:300–308.

28. Raw I, Schmidt BJ, Merzel J. Catecholamines and congenital pain insensitivity. *Braz J Med Biol Res* 1984;17:271–279.

29. Aragon MC, Gimenez C, Valdivieso F. Inhibition by L-phenylalanine of tyrosine transport by synaptosomal plasma membrane vesicles: implications in the pathogenesis of phenylketonuria. *J Neurochem* 1982;39:1185–1187.

30. Dahlstrom KA, Ament ME, Laidlaw SA, et al. Plasma amino acid concentrations in children receiving long-term parenteral nutrition. *J Pediatr Gastroenterol Nutr* 1988;7:748–754.

31. Gross ML, Fowler CJ, Ho R, et al. Peripheral neuropathy complicating pancreatitis and major pancreatic surgery. *J Neurol Neurosurg Psychiatry* 1988;51:1341–1344.

32. Kien CL, Veillon C, Patterson KY, et al. Mild peripheral neuropathy but biochemical chromium sufficiency during 16 months of "chromium-free" total parenteral nutrition. *J Parenter Enteral Nutr* 1986;10:662–664.

33. Jeejeebhoy KN, Chu RC, Marliss EB, et al. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am J Clin Nutr* 1977;30:531–538.

34. Verhage AH, Cheong WK, Jeejeebhoy KN. Neurologic symptoms due to possible chromium deficiency in long-term parenteral nutrition that closely mimic metronidazole-induced syndromes. *J Parenter Enteral Nutr* 1996;20:123–127.

35. Bessman SP. Genetic failure of fetal amino acid "justification": a common basis for many forms of metabolic, nutritional, and "nonspecific" mental retardation. *J Pediatr* 1972;81:834–842.

36. Jeffery ND, Smith KC, Franklin RJM, et al. Sensory neuronopathy of possible toxic etiology in a dog. *Prog Vet Neurol* 1993; 4:145–148.

37. Duncan ID, Griffiths IR. A sensory neuropathy affecting Long-haired Dachshund dogs. *J Small Anim Pract* 1982;23:381–390.

38. Cummings JF, de Lahunta A, Winn SS. Acral mutilation and nociceptive loss in English pointer dogs. A canine sensory neuropathy. *Acta Neuropathol (Berl)* 1981;53:119–127.

39. Cummings JF, de Lahunta A, Mitchell WJ Jr. Ganglioradiculitis in the dog. A clinical, light- and electron- microscopic study. *Acta Neuropathol (Berl)* 1983;60:29–39.

40. Wheeler SJ. Sensory neuropathy in a border collie puppy. *J Small Anim Pract* 1987;28:281–289.

41. Wouda W, Vandevelde M, Oettli P, et al. Sensory neuronopathy in dogs: a study of four cases. *J Comp Pathol* 1983; 93:437–450.

42. Steiss JE, Pook HA, Clark EG, et al. Sensory neuronopathy in a dog. *J Am Vet Med Assoc* 1987;190:205–208.

43. Marks T, Farkas WR. Effects of a diet deficient in tyrosine and queuine on germfree mice. *Biochem Biophys Res Commun* 1997; 230:233–237.

44. Morris JG, Rogers QR, Pacioretty LM. Taurine: an essential nutrient for cats. *J Small Anim Pract* 1990;31:502–509.

45. Morris JG, Rogers QR. Ammonia intoxication in the near-adult cat as a result of a dietary deficiency of arginine. *Science* 1978; 199:431–432.

46. Philipp E. Assay for tyrosine hydroxylase in hypothalamic homogenates using high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 1987;419:27–36.

47. Perez E, Amici R, Bacchelli B, et al. Kinetic parameters of tyrosine hydroxylase in the rat's preoptic region during sleep deprivation and recovery induced by ambient temperature. *Sleep* 1987; 10:436–442.

48. Clark JM, Eyzaguirre JP. Tyrosine activation and transfer to soluble ribonucleic acid. *J Biol Chem* 1962;237:3698–3702.

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