FIGURE 2 Clinical, hematological and urinary oxalate effects of vitamin B-6-deficient diet on cats. Body weight and cumulative body weight gain (A), packed cell volume (B), blood hemoglobin concentration (C) and urinary oxalate excretion (D) during the experimental period. Values are means ± SEM of five vitamin B-6-deficient and four normal cats. Mean body weights did not differ significantly (P > 0.05) at any age. Mean cumulative weight differences differed significantly at d 55 and later (P < 0.05). Mean packed cell volumes differed significantly (P < 0.05) on d 2, and d 37 and later. Mean hemoglobin concentrations differed significantly at d 2 and 16 and from d 37 until the end of the study (P < 0.05). Mean urine oxalate excretion differed significantly on d 13 and later (P < 0.05).

which alternated between rarefaction and condensation. Binaural stimulation was used, and stimulus intensity was 70 dB human subjective threshold.

Recordings were made with subcutaneous 25-gauge platinum alloy needle electrodes (Grass Instruments Co., Quincy, MA) connected to an evoked response system (Model EP40A, TECA Corporation, Pleasantville, NY). Simultaneous recordings were made using
FIGURE 3 Vitamin B-6-deficient cats had prolonged 3-5N interwave intervals [INT]. A, B: Representative brainstem auditory evoked potentials (BAEP) recorded simultaneously from an experimental cat after receiving vitamin B-6-deficient diet for 91 d (A: vertex-first thoracic vertebra dorsal spinous process [V-T1] derivation, B: vertex-ear [V-E] derivation). C, D: Representative BAEP recorded simultaneously from a control cat after receiving same diet supplemented with vitamin B-6 for 91 d (C: V-T1 derivation; D: V-E derivation). Note that the 3-5N INT of the vitamin B-6-deficient cat were longer than the 3-5N INT of the control cat, but the 1N-3 and 1-3 INT of the vitamin B-6-deficient cat were shorter than the 1N-3 and 1-3 INT of the control cat. Each recording was repeated once; the second trace is printed offset slightly below the first to demonstrate replicability of the recordings. Click stimulation at 10 Hz. Interwave intervals from each pair of BAEP are shown. Calibration bars: 5 μV vertically, 1 ms horizontally.

The two most widely used electrode derivations: 1) an electrode on the vertex (dorsal midline, midway between the intercanthal line and the external occipital protuberance) referenced to an electrode on the dorsal midline over the first thoracic dorsal spinous process (V-T1 derivation); 2) the same vertex electrode, referenced to an electrode located immediately rostral to the ventral extremity of the right external ear canal (V-E derivation). The ground electrode was located on the dorsal midcervical region. Electrode impedances were < 6 kOhm, amplifier input impedance was 100 MOhm and the amplification factor was 20,000. Post-stimulus recording epoches were 10 ms; 512 epoches were averaged for each recording, and all recordings were repeated once to assure replicability of results. Epoches containing voltages exceeding the dynamic range of the system were automatically excluded from averaging. Single responses and the output of the signal averager were monitored continuously on the oscilloscope. Permanent records were stored on floppy disks and printed by an X-Y plotter (Hewlett Packard, San Diego, CA). Cursors on the oscilloscope were used to measure amplitudes, latencies and interwave intervals automatically.

Brainstem auditory evoked potentials recorded were similar to those of cats in other reports, in that the first and last waves were regularly identifiable (Buchwald and Huang 1975, Plantz et al. 1974, Shipley et al. 1980), whereas fusion of intervening waves made their identification uncertain in some recordings. Configurations of BAEP recorded simultaneously with the two different electrode derivations differed (Fig. 1). Consequently, positive or negative points were chosen in the early, middle or late periods of the potentials that were readily identifiable in every BAEP recorded. Using a widely accepted nomenclature for BAEP waves (Jewett 1970), in the V-E derivation these points were the positive peaks of waves 1 and 3 and the negative trough following wave 5; in the V-T1 derivation, they were the negative trough immediately following wave 1, the positive peak of wave 3, and the negative trough immediately following wave 5. These points are designated here as 1, 1N, 3 and 5N. The interwave intervals measured in V-E derivations were 1-3, 3-5N, and 1-5N; in V-T1 derivations they were 1N-3, 3-5N, and 1N-5N. Interwave intervals were measured in recordings made from both electrode derivations. Replicate recordings were averaged, using the evoked response system, and measurements were made on the averages.

Trends in interwave intervals data were examined by comparing the slopes of least-squares regression lines plotted through d 42 to 91 on diet. Interwave intervals of control and deficient cats decreased from d 0 to 28 (see Discussion), so those values were not used in the analysis. Mean slopes were tested for significance by Student's t test. In addition, Student's t test was used to test for differences between groups in body weight, packed cell volume, hemoglobin, urinary oxalate and interwave intervals data at individual times. In all comparisons, differences were considered statistically significant if P < 0.05.

RESULTS

Clinical, hematological and urinary oxalate data. After 11 wk of consuming the vitamin B-6-deficient diet, all experimental cats had developed a dull, unkempt haircoat with generalized fine, white scales on the skin surface. Three cats had multifocal areas of alopecia in the temporal and periauricular areas, on the dorsum of the nose, around the mouth and on the extremities. One cat had ulcers on the caudal surface of the hind limbs just below the tarsometatarsal joint. Punch biopsy specimens from areas of alopecia on one
cat showed a paucity of hair follicles. None of the control cats developed skin lesions. During wk 13, one cat in the deficient group had a generalized convolution.

Mean body weights of the vitamin B-6–deficient and control cats did not differ significantly during the experiment; however, mean cumulative weight gain of the deficient group was significantly lower than that of the control cats on d 55 and at all times thereafter (Fig. 2A).

The mean packed cell volume of the deficient cats was significantly lower than that of the control group at d 2 and from d 37 until the end of the experiment (Fig. 2B). The mean hemoglobin concentration of deficient cats was significantly lower than that of control cats at d 2 and 16 and from d 37 until the end of the experiment (Fig. 2C).

Mean urinary oxalate excretion in the deficient group was significantly higher than that of the control group from d 21 until the end of the experiment (Fig. 2D).

**Brainstem auditory evoked potentials.** The BAEP of the deficient and control cats remained similar in configuration throughout the experiment. Typical recordings from deficient and control cats are shown in Figure 3.

Although the prolongation of interwave intervals appeared to begin earlier, using Student's t test to
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FIGURE 5 Mean regression line slopes of brainstem auditory evoked potential (BAEP) interwave intervals (INT) over d 42 to 91 on experimental diets. Brainstem auditory evoked potentials were recorded simultaneously by different electrode configurations, (A) vertex-first thoracic vertebra (T1) and (B) vertex-ear. Asterisks indicate INT regression line slopes of vitamin B-6-deficient cats that were significantly longer than controls ("P < 0.01, **P < 0.05). SEM values for control and vitamin B-6-deficient groups, respectively, are: (A) 1N-3 6.58 x 10^-3, 6.22 x 10^-3, 3-5N 4.83 x 10^-3, 4.92 x 10^-3 and 1N-5N 5.59 x 10^-3, 10.55 x 10^-3 and (B) 1-3 8.34 x 10^-3, 13.01 x 10^-3, 3-5N 4.54 x 10^-3, 5.28 x 10^-3, and 1-5 (10.19 x 10^-3, 16.04 x 10^-3).

compare means at each time after beginning the diet revealed significant differences only at 91 d (Fig. 4). At 91 d, wave 3-5N of deficient cats differed significantly from those of control cats in both electrode derivations. Wave 1N-5N interwave intervals differed significantly in the V-T1 derivation. No significant differences were found in interwave intervals 1-3 or 1N-3, using either electrode derivation (Fig. 4).

Vitamin B-6-deficient cats had significantly larger slopes of regression lines plotted through 3-5N interwave intervals over wk 6-13 (Fig. 5). The mean slopes of regression lines of control and deficient cats for 1N-3 and 1-3 interwave intervals were close to 0 (0.001 to 0.007) and did not differ significantly between groups. The mean slopes of the regression lines of control cats for 1N-5N, 1N-5N, and 3-5N interwave intervals were close to 0 (0.006 to 0.005), indicating little change over time; however, the same interwave intervals of vitamin B-6-deficient cats increased at a rate of 0.027 to 0.040 ms/wk, reflecting a trend of lengthening interwave intervals.

DISCUSSION

Clinical and laboratory data indicate that vitamin B-6 deficiency developed in the cats fed the deficient diet. Growth retardation and elevated urinary oxalate excretion have been reported (Bai et al. 1989, Carvalho da Silva et al. 1959, Gershoff et al. 1959), and hypochromic, microcytic anemia has also been reported (Carvalho da Silva et al. 1959). In this experiment, packed cell volume and hemoglobin concentrations of the vitamin B-6-deficient cats remained within normal limits (Schalm et al. 1975), although they declined after d 30 and were significantly lower than those of control cats after d 37, suggesting that anemia was developing. Seizures have been reported in vitamin B-6 deficiency of many species, including cats (Dakshinamurti et al. 1990, Carvalho da Silva 1959, Loo 1980, Tower 1956). The skin lesions in the deficient cats were similar to those reported in vitamin B-6-deficient rats (Schlaepfer and Hager 1964).

The vitamin B-6-deficient group had significantly prolonged BAEP interwave intervals. The decrease in interwave intervals in both groups during the first 2 wk of the experiment probably reflected normal rapid myelination of the auditory pathways with maturation (Shipley et al. 1980), before the development of the deficient state in the experimental group. However, at 13 wk, when the deficient state was well established, early to late interwave intervals (1-5N or 1N-5N) and middle to late interwave intervals (3-5N) in deficient cats differed significantly from those of...
the controls, yet no significant difference occurred in the early to middle (1-3 or 1N-3) interwave intervals. The interwave intervals increased in ranges considered significant in disease states (Chiappa et al. 1980). In addition, the deficient group had significantly larger mean regression line slopes of 3-5N interwave intervals. Thus the prolonged interwave intervals resulted from prolongation of the late segment (3-5N interwave intervals) of the BAEP.

These results suggest the deficiency acted primarily on one or more structures that generate the later components of the BAEP: the lateral lemniscus, the preolivary region and the caudal colliculus (Achor and Starr 1980, Buchwald 1983). There was no evidence for an effect of the deficiency on the acoustic nerve or the cochlear nuclei, structures that generate the earlier stages of the BAEP (Achor and Starr 1980, Buchwald 1983).

Although vitamin B-6-deficient rats were reported to show attenuated acoustic and tactile responses (Schaeffer 1987), an effect of the deficiency specifically on central auditory pathways has not been reported previously. The mechanism producing this effect is unknown. Possible causes include deficient or delayed myelination, primary [segmental] demyelination and disorders of neurotransmission (Dakshinamurti et al. 1990, Kirksey et al. 1990, Kurtz and Kanfer 1973). The results also demonstrate that recording BAEP can be useful as a noninvasive means of detecting subtle effects of nutritional deficiencies on functional characteristics of the central nervous system.

LITERATURE CITED


