Morphophysiologic Characterization of Peripheral Neuropathy in Zinc-Deficient Guinea Pigs (44035)

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Abstract. **Zinc-deficient guinea pigs develop a peripheral neuropathy characterized by abnormal posture and gait, hyperesthesia, slowed motor nerve conduction velocity (MNCV), and decreased sciatic nerve Na,K-ATPase activity. This study was designed to further investigate longitudinally the morphophysiologic features of the neuropathy. Weanling guinea pigs were fed a low-zinc (<1 mg/kg) diet *ad libitum* (−ZnAL), an adequate-zinc (100 mg/kg) diet *ad libitum* (+ZnAL), or the adequate diet restricted in intake ((+ZnRF). Electrophysiologic, morphologic, and biochemical parameters of peripheral nerves were examined at 2.5, 4.0, and 5.5 weeks. Serum zinc was significantly lower by 2.5 weeks and growth rate reduced by 4 weeks in −ZnAL animals. Postural abnormalities were first obvious at 4 weeks, although MNCVs were significantly slower in zinc-deficient animals at all time intervals. The conduction of sensory impulses, as measured by spinal cord somatosensory evoked potentials (sSSEP), was significantly slower in the −ZnAL animals at 5.5 weeks. Examination of teased preparations and histologic sections of sciatic nerves at 5.5 weeks revealed no degenerative lesions or differences in density of myelinated fibers (MF). The size frequency distribution of MF in all groups was unimodal, with a trend toward smaller myelinated nerve fibers in −ZnAL and +ZnRF animals. Sciatic nerve Na,K-ATPase activity in the −ZnAL animals was significantly reduced after 4 weeks of zinc deprivation. At 5.5 weeks, nerve concentrations of myo-inositol, glucose, fructose, and sorbitol were significantly decreased in −ZnAL animals compared with the +ZnRF and +ZnAL controls. The peripheral neuropathy associated with acute zinc deficiency is a parenchymatous axonal disorder characterized by slowed motor and sensory nerve impulse conduction and reduction in nerve Na,K-ATPase activity and nerve concentrations of simple sugars and their metabolites.** [P.S.E.B.M. 1996, Vol 213]

Zinc is a component of several metalloenzymes required for cell structure and function, including those of nerves (1). Manifestations of altered nervous system performance in human beings with zinc deficiency include cerebellar dysfunction (intention tremor, ataxia), dysesthesia, taste and smell dysfunction, and muscle weakness (2–6). Of the various animal models of experimental zinc deficiency, guinea pigs probably display the most obvious locomotor disorders, including abnormal gait and posture, sensory hypersensitivity manifested by skin twitches, and vocalization in response to tactile stimulation (7–10). Chicks exhibit a highly analogous neuropathy (11). A pathology common to these locomotor defects of zinc deficiency is impaired transmission of peripheral nerve impulses, manifested by slowed motor nerve conduction velocity (MNCV) (3, 8, 11). Zinc supplementation of uremic patients and zinc-deficient animals normalizes their MNCV (3, 7, 11).

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The rapid normalization of sciatic MNCV by zinc supplementation suggests a reversible metabolic abnormality. Metabolic neuropathies, such as those associated with diabetes mellitus and uremia, respond to therapy with improvement in MNCV and clinical signs when treated in the early stages of the diseases (3, 12). The structural lesions of metabolic neuropathies are those of axonal degeneration with Wallerian degeneration, myelin degeneration and regeneration, axonal atrophy, and axonal loss (3, 13, 14).

There is depressed activity of sodium, potassium–ATPase (Na,K-ATPase) activity in the sciatic nerves of zinc-deficient guinea pigs but no change in the number of sodium channels (7). The decrease in Na,K-ATPase activity may lead to a reduction of membrane potential and conduction velocity because of intracellular sodium retention (15). Reduced Na,K-ATPase activity in peripheral nerves from humans and animals with diabetes mellitus and uremia has been associated with abnormal nerve metabolism, including abnormal axoplasmic regulation of electrolytes (15), activation of nerve polyol synthesis (13), and altered concentration of myo-inositol (12, 13, 16). Free myo-inositol in peripheral nerves of streptozotocin-induced diabetic rats may be depleted by a direct or indirect inhibition of myo-inositol transport by glucose or its poly metabolite, sorbitol (12, 13); however, nerve myo-inositol levels are not significantly lower in human diabetics or in rats rendered diabetic through alloxan administration (13, 14, 17). In nerves from uremic patients, axoplasmic myo-inositol concentrations are generally increased and polyol content decreased or normal (16). The complex interrelationships among myo-inositol, carbohydrate metabolism, and Na,K-ATPase activity in the pathogenesis of abnormal nerve function has not been elucidated.

The zinc-deficient guinea pig provides a experimental model of peripheral neuropathy and was used here to study further the basis of the pathology. A longitudinal experiment was designed to assess peripheral nerve function, morphology, and metabolism. Spinal cord somatosensory evoked potentials (sSSEP) and MNCV were used to assess sensory and motor nerve functions, respectively. Peripheral nerve tissue was examined microscopically to identify any morphologic features of the neuropathy. Nerve Na,K-ATPase activity and myo-inositol and sugar concentrations were quantified.

Materials and Methods

Animals. Male and female weanling (203 ± 16 g) Hartley guinea pigs (VAF/Plus Crl:(HA)BR; Charles River Laboratories, Wilmington, MA) were singly housed in suspended stainless steel cages and maintained in a room environment in accordance with federal guidelines for animal husbandry (18). Forty-five weanling guinea pigs were randomly assigned, with equal distribution of males and females, into three experimental groups: low-zinc (<1 ppm) diet *ad libitum* (−ZnAL); zinc-adequate diet (100 ppm zinc) *ad libitum* (+ZnAL), and zinc-adequate diet restricted in intake so as to maintain body weight similar to pairmates in the −ZnAL group (+ZnRF). Upon receipt, the animals were fed the control experimental diet for seven days before the experimental period to allow the animals to acclimate to their new environment and diet formulation.

Diet. The basal soybean protein diet (−Zn) which contained <1 (0.9 ± 0.08) mg zinc/kg diet has been described (8). The control diets (+Zn) were supplemented with 100 mg zinc/kg diet provided as $ZnCO_3$. Fresh diet mixed with deionized water to provide a dry-dough consistency was provided daily either *ad libitum* or restricted. Deionized water was provided *ad libitum* and ascorbic acid (30 mg) was administered orally on a daily basis.

Body Weight and Clinical Scores. Guinea pigs were weighed three times per week and dietary intake of the +ZnRF animals was gradually reduced to maintain weight gain at the same rate as their −ZnAL pairmates. On alternate days, behavioral and physical manifestations (vocalization, gait, posture, and skin condition) of zinc deficiency were scored using a numerical system previously described (8). Clinical signs were graded on a scale of 0 to 3 with 0 being normal and 3 being severe. Scoring of clinical signs was used to monitor progression of zinc depletion and to facilitate correlation of clinical signs with the measured parameters.

Animal Use and Electrodiagnostics. Guinea pigs in each diet group were randomly assigned to one of three sample collection times: 2.5, 4.0, and 5.5 weeks after onset of the study. At each collection time, electrodiagnostic procedures were performed on animals to assess motor and sensory nerve conduction. For these electrophysiologic measurements, guinea pigs were anesthetized with a combination of 25 mg/kg of ketamine (Vetalar, Park-Davis, Morris Plains, NJ) and 13 mg/kg of xylazine (Rompun, Haver/Mobay, Shawnee, KS) administered intramuscularly into the left rear leg. All peripheral nerve stimulations for sSSEP and MNCV measurements were performed in the right rear leg. Animals were maintained in sternal recumbency on a warm water heating pad, and core body temperature was monitored periodically using a needle-type tissue probe. Body temperature was maintained between 38° and 40°C and was used in the calculation of nerve conduction velocity. sSSEP and MNCV were performed using an electrophysiology computer (Nicolet Compact Four, Nicolet Biomedical Instruments, Madison WI). sSSEP, a measure of sen-

sory nerve impulse reception and conduction, was evaluated first. A 25-mm Teflon-coated stainless steel recording needle electrode (Teca Corp., Pleasantville, NY) was introduced percutaneously into the dorsal ligament of the spinal cord at the L1-L2 vertebral space. A stainless steel reference needle electrode (E2; Grass Instrument Co., Quincy, MA) placed adjacent (5 mm) to the recording electrode served to measure background electrical (cardiac and respiratory muscular) activity. Anodal and cathodal stainless steel needle electrodes (E2, Grass Instrument Co., Quincy, MA) were placed 5 mm apart between the tibia and the Achilles tendon to stimulate the tibial branch of the sciatic nerve. A stainless steel ground needle electrode was placed subcutaneously on the lateral surface of the limb. A stimulus intensity of 1 mV and 100 ms in duration was adequate to elicit a mixture of sensory and motor responses from the nerve and produce a slight toe twitch. Recording, reference, and ground electrodes were connected to an amplifier with low- and high-frequency filters at 100 Hz and 3 kHz, respectively. A total of 500 single rectangular pulses 10 ms in duration were averaged per recording. Two reproducible recordings were collected, and for each the positive (P1 and P2) and negative (N1 and N2) deflections (waveforms) were identified. The latency and amplitude of each waveform and the duration of elapsed time from P1 to N2 were calculated.

MNCV was next examined using a previously described technique (7). Latency of the conducted nerve impulse was determined from the elapsed time from stimulation to the onset of the compound muscular action potential. Conduction velocity, expressed in meters per second (m/sec), of the sciatic nerve was calculated as the distance between the two stimulation sites (d1-d2) divided by the difference in latencies (l1-l2), corrected for body temperature.

Tissue Collection. Following sSSEP and MNCV measurements with the animal still anesthetized, the left sciatic nerve was removed, cleaned of adhering tissue, rinsed with deionized water, and placed in ice cold homogenizing buffer. Nerves of the brachial plexus were collected, snap frozen in liquid nitrogen, and maintained at −70°C until processed for nerve sugar analysis. Blood was collected to determine serum zinc levels. Anesthetized animals were then perfused with heparinized saline followed by 2% glutaraldehyde/2% paraformaldehyde fixative in cacodylate buffer. The right sciatic nerve was carefully excised according to protocols for biopsy of peripheral nerves for histologic examination (14). To prevent both wrinkling and stretching of the nerve during excision, each nerve specimen was suspended in a vial of 2% glutaraldehyde with a 0.5 g weight tied to one end and stored at 4°C for a minimum of 24 hr. After fixation, sciatic nerve specimens were rinsed in 1.0 *M* cacodylate buffer, and post-fixed in 2% osmium tetroxide for 24 hr. Osmium-fixed nerve specimens were stored in 1.0 *M* cacodylate buffer at 4°C until processed.

Sample Preparation and Microscopic Examination. Osmium-fixed nerves collected at the 5.5-week interval were processed for histologic examination. Each nerve was divided into halves. One portion was processed for teased nerve preparations, according to a protocol adapted from the method of Dyck *et al.* (14). Briefly, each sample was dehydrated in graded alcohol solutions and placed in glycerin for 24 hr to soften the connective tissue nerve sheaths. Nerve bundles were divided and subdivided under a dissecting microscope until individual nerve fibers were isolated and mounted on a dry glass slide. Slides were heated to 30°C for 2 days then permanently coverslipped for microscopic examination. Fifty nerves were examined for the presence of axonal degeneration, myelin irregularities, demyelination, remyelination, and paranodal axonal swelling (19). Remaining nerve portions were dehydrated in graded ethanol solutions, infiltrated with a methacrylate resin (JB-4, Polysciences, Inc., Warrington, PA), cut in 1-μm cross sections, and stained with hematoxylin and eosin (20). Fiber diameters and numbers were quantified with the aid of a calibrated digitizing pad and morphometry software (The Morphometer, Woods Hole, MA). Nerve cross section images were projected *via* camera lucida to the digitizing pad. Myelinated nerve fiber diameter was calculated from measurements of fiber circumference (21) from 150 fibers. The number of myelinated fibers was counted within a 0.5-mm^2 field.

Na,K-ATPase Assay. Na,K-ATPase activity was measured using a modification of the method described by Das *et al.* (22). Nerves were cut into 1- to 3-mm pieces while in cold homogenizing buffer and nerve segments were transferred to a polystyrene tube with 2 ml of homogenizing buffer. Samples were sonicated six times at full intensity (setting 5, Model VC 375, Vibra Cell, Danbury, CT) for five 10-sec bursts, and then centrifuged at 1000 *g* for 10 min to remove connective tissue. An aliquot of the supernatant was analyzed for protein using bovine serum albumin as the standard (23). Aliquots of the supernatant were added to the assay media with and without added sodium. The difference in activity with and without added sodium was calculated as Na,K-ATPase activity and was expressed on the basis of the protein value. A preliminary study showed no difference in Na,K-ATPase activity using nerves prepared in a glass homogenizer and those prepared by sonication.

Nerve Sugar Analysis. Preparation of the nerves for analysis was a modification of the procedure developed by Greene *et al.* (24). Briefly, frozen nerves of the brachial plexus were thawed, minced, boiled in deionized water for 5 min, sonicated, and centrifuged

at 1000 *g* for 10 min. An aliquot of the supernatant was removed for protein analysis and 0.1 ml of 1.5 m*M* heptose standard (0.15 μmol) was added to 2 ml of supernatant prior to derivatization to serve as an internal standard. Protein was precipitated from the sample by the addition of 0.2 ml of 5% $ZnSO_4$ and 0.3 ml of 0.3 *N* $Ba(OH)_2$. The mixture was vortexed and centrifuged, and the supernatant decanted into derivatization tubes. The sample was then frozen and lyophilized. Aliquots of the lyophilized samples (4.0 mg) were placed into 3.0 ml Reactivials (Pierce, Rockford, IL) equipped with Teflon stirbars and Teflon-faced screw caps. Then 5 ml of deionized water was added to dissolve each sample followed by the addition of 200 μl of an *O*-methylhydroxylamine oximation cocktail. The Reactivials were stirred and then heated at 70°C for 20 min. Each sample was then taken to dryness with a stream of dry nitrogen. Samples were reconstituted in 1.5 ml $CHCl_3$ and sequentially washed with dilute HCl and $NaHCO_3$, and dried, as previously described (28, 29). The resulting acetates of sorbitol, inositol, the *O*-methylaldoxime of glucose, and the *O*-methyl ketoxime of fructose were separated and quantified on a Varian (Sugarland, TX) model 3700 gas-liquid chromatograph equipped with a Quadrex (New Haven, CT) 30 m × 0.25 mm i.d. fused silica capillary column with a bonded 0.25 mm SE-30 stationary phase, and with a split-splitless injector and dual flame ionization detector. A 1-ml aliquot of the acetylated sample in $CHCl_3$ was injected at 150°C with an initial hold time of 4 min, followed by a 6°C/min temperature increase to a maximum of 280°C and a final hold time of 14 min. The analysis was done at a He (helium) flow rate of 1.5 cc/min and with a split ratio of 1:50. All solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and distilled prior to use.

Mass spectra for all carbohydrate derivatives were obtained and verified against standards on a mass spectrometer interfaced with a gas chromatograph. Mass spectra were recorded at 70 eV with an ionization current of 50 mA, a source temperature of 250°C, and a transfer temperature 218°C. All carbohydrates employed as standards were purchased from Pfanstiehl Laboratories, Inc. (Waukegan, IL).

Serum Zinc Concentration. Frozen serum was allowed to thaw at room temperature, vortexed, and an aliquot was diluted 1:4 with deionized water. Zinc concentration was determined by flame atomic absorption spectrophotometry (Model 2380, Perkin Elmer, Norwalk CT) using a known zinc standard.

Statistics. Analysis of variance followed by the post-hoc *t* test (Interactive Statistical Package, Crunch Software, San Francisco, CA) was used to statistically analyze all data obtained from the 3 × 3 factorial design except for the nerve sugar and myo-inositol data. For the latter, the GLM procedure of SAS (SAS Institute, Cary, NC) was used. Statistically significant differences between treatments were determined using the least square mean component of GLM after log transformation of the data, due to the lack of homogeneity of variances. Significance was set at $P < 0.05$.

Results

Body weights of the animals were statistically the same at the onset of the study. Comparison of the body weights and the serum zinc concentrations for the three dietary treatments and time periods are presented in Table I. Growth of guinea pigs fed the low-zinc diet essentially stopped after 2.5 weeks, and there was comparable limitation in growth of those fed the zinc-supplemented diet in restricted quantities. There was no significant difference in body weight at any time period between the −ZnAL and +ZnRF groups. Mean serum zinc concentration in the −ZnAL group was significantly lower at each time interval compared with that of the +Zn controls whose serum zinc levels were similar.

Clinical scores of the zinc deficiency signs corresponded with the progression of zinc depletion (Table II). At 2.5 weeks, guinea pigs in the −ZnAL group began to vocalize when handled and exhibited mild hair loss and wrinkled skin. At 4 weeks there was increased frequency and duration of vocalization and moderate scaliness of the skin with hair loss and subtle postural changes (arched back). At the end of the

Table I. Indices of Zinc Status: Body Weights and Serum Zinc Concentrations

Dietary treatment[a]	Body weight[b] (g)	Serum zinc[c] (μ/ml)
2.5 weeks[d]		
−ZnAL	244 ± 24*	0.47 ± 0.2†
+ZnRF	260 ± 21*	0.88 ± 0.2
+ZnAL	296 ± 32*	0.93 ± 0.2
4.0 weeks		
−ZnAL	241 ± 29*	0.32 ± 0.2†
+ZnRF	283 ± 22*	0.83 ± 0.1
+ZnAL	350 ± 39‡	0.96 ± 0.2
5.5 weeks		
−ZnAL	235 ± 25*	0.40 ± 0.1†
+ZnRF	275 ± 37*	0.76 ± 0.2
+ZnAL	374 ± 37‡	0.97 ± 0.2

Note. n = 5/group. Values (means ± SD) not sharing a common superscript symbol (*·†·‡) are significantly different; $P < 0.05$.
[a] Guinea pigs received either diet low in zinc *ad libitum* (−ZnAL), adequate diet *ad libitum* (+ZnAL), or a restricted quantity of adequate diet (+ZnRF).
[b] Average body weight ± SD at time of data collection.
[c] Average serum zinc concentration ± SD at time of data collection.
[d] Time guinea pigs were on test diets.

Table II. Average Clinical Sign Scores of Zinc-Deficient Guinea Pigs[a]

Parameters	2.5 weeks[b]	4.0 weeks	5.0 weeks
Vocalization[c]	0	1.2	2.5
Posture[d]	0	0.9	2.3
Skin[e]	0.2	1.5	2.6
Gait[f]	0	0.4	2.0

Note. n = 5/group.
[a] 0, normal; 1, mild change; 2, moderate change; 3, severe change.
[b] Time guinea pigs remained on low-zinc diet.
[c] Vocalization when picked up, held, or after being put down.
[d] Changes in posture such as elevated stance or arched back.
[e] Skin changes of hair loss, scaly skin, and amount of body affected.
[f] Changes in gait such as hop, spasm, hand stand, or refusal to move.

study, animals in the −ZnAL group consistently vocalized when handled, displayed severe scaliness of skin with extensive hair loss, and exhibited obvious gait and postural abnormalities, arched back, and little voluntary movement. The average clinical scores of the +Zn control groups remained 0, or normal, throughout.

Results of the electrophysiologic studies revealed slowed sensory and motor conduction in zinc-deficient animals. Mean MNCV from animals in each diet group for each time interval are shown in Figure 1. At each time interval, motor nerve conduction was significantly slower in zinc-deficient animals compared to zinc-supplemented animals. MNCV significantly decreased in a linear fashion and accompanied low plasma zinc and clinical signs of zinc deficiency (impaired growth, skin lesions, abnormal gait).

Stimulating the tibial nerve at the level of the metatarsus produced a polyphasic response, as has been observed in sSSEPs from mixed nerve stimulation in other species (27–30). The first pair of P1-N1 deflections were interpreted to represent presynaptic afferent sensory impulse volleys through the cauda equina and dorsal roots, and P2-N2 deflections to represent postsynaptic sink potentials in interneuronal pools within the spinal cord (28). Latencies were measured for each deflection at maximal amplitude of the action potential. The latency for the P1-N2 interval was stable in the −ZnAL for the first 4 weeks but became significantly prolonged by 5.5 weeks (Fig. 2) compared to conduction latencies in the +Zn groups. The latter were not significantly different from each other. A similar decrease was observed in the amplitude of the −ZnAL peaks at 5.5 weeks.

Histologic examination of peripheral nerves from zinc-deficient animals revealed minimal structural alterations. In teased nerve preparations, occasional myelin spheroids were observed, but at a frequency not significantly different from that in controls. The density of myelinated nerve fibers (MF) was not significantly different among the −ZnAL (6477 ± 479 MF/0.5 mm^2), the +ZnRF (6422 ± 354 MF/0.5 mm^2), and the +ZnAL (6588 ± 335 MF/0.5 mm^2) nerves. The size distribution of the myelinated fibers in sciatic nerves from animals in all diet groups was unimodal. The MF size ranged from 2.1 to 11.9 μm in diameter, with a peak range of 4–5.9 μm. Smaller myelinated fibers (<4 μm) were twice as prevalent in −ZnAL nerves compared with +ZnAL nerves, but were similar in prevalence as those in +ZnRF nerves.

Nerve Na,K-ATPase activity data are summarized in Figure 3. Na,K-ATPase activity of −ZnAL nerves dropped after 2.5 weeks of depletion and was reduced to less than 50% of that of +Zn nerves at 4.0 and 5.5 weeks.

The myo-inositol concentration in −ZnAL nerves declined over the course of the study, reaching significantly lower concentrations compared with the +Zn

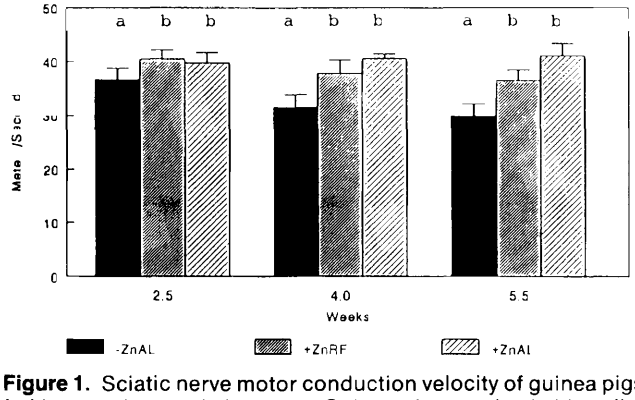

Figure 1. Sciatic nerve motor conduction velocity of guinea pigs fed low- and normal-zinc diets. Guinea pigs received either diet low in zinc *ad libitum* (−ZnAL), the adequate diet *ad libitum* (+ZnAL), or a restricted quantity of adequate diet (+ZnRF). Data was used to assess function of peripheral motor nerves. Bars, mean ± SD. Values not sharing a common superscript letter are significantly different. n = 5/group.

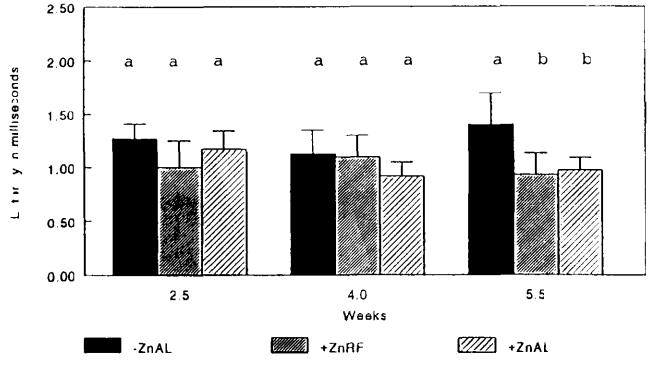

Figure 2. Latency between first positive peak and second negative peak of spinal cord evoked potentials after stimulation of the tibial branch of the sciatic nerve of guinea pigs fed a low zinc (−ZnAL) or adequate zinc diet *ad libitum* (−ZnAL), or in restricted amounts (+ZnRF). Data were used to assess afferent conduction through peripheral nerves and distal spinal cord. Bars, mean ± SD. Values not sharing a common superscript letter are significantly different. n = 5/group.

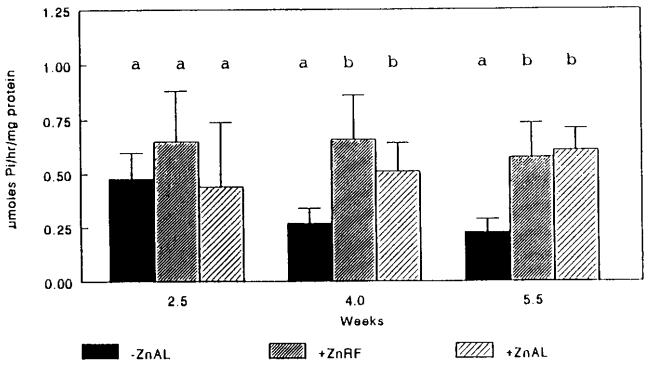

Figure 3. Na,K-ATPase activity in sciatic nerves of guinea pigs fed low- and adequate-zinc diets. Guinea pigs received either diet low in zinc *ad libitum* (−ZnAL), adequate diet *ad libitum* (+ZnAL), or adequate diet in restricted quantity (+ZnRF). Bars, mean ± SD. Values not sharing a common superscript letter are significantly different. n = 5/group.

nerve levels at 5.5 weeks (Table III). The mean levels of glucose, fructose, and sorbitol from the +Zn controls increased during the study but decreased in those fed the low-zinc diet. The latter were significantly lower than controls at 5.5 weeks.

Discussion

This longitudinal experiment confirmed an earlier study (7) showing that guinea pigs fed a low-zinc diet for 5–6 weeks develop a peripheral neuropathy characterized by clinical signs of abnormal posture and gait, and hyperalgesia. Associated with the clinical signs were slower motor nerve conduction velocity and sciatic nerve Na,K-ATPase activity.

Several previously undocumented characteristics of the neuropathy were highlighted in this study. There was a retardation of sensory conduction that corresponded to slowed motor never conduction. The conduction defects were apparently metabolic in nature since there was no histologic evidence of myelination abnormalities nor decrease in number of myelinated fibers in nerves from −ZnAL guinea pigs. There was a progressive decrease in the Na,K-ATPase activity in nerves from −ZnAL animals which was accompanied by lower myo-inositol concentrations. Also, glucose and related metabolite concentrations in nerves from the −ZnAL animals at 5.5 weeks were significantly lower than those of control animals, suggesting reduced glucose transport in the −Zn nerves. These results characterize the peripheral neuropathy of zinc-deficient guinea pigs as a sensorimotor parenchymatous axonal neuropathy.

Spinal cord somatosensory evoked potential recordings allowed evaluation of the proximal portions of the peripheral nerve and the nerve roots during afferent impulse conduction. sSSEP latencies of the afferent presynaptic volleys in −Zn nerves were increased, suggesting impaired conduction through myelinated sensory nerve fibers. This was also supported by an apparent decrease in amplitude of sSSEP peaks in the −ZnAL animals. Increased sSSEP latencies in the −ZnAL group paralleled the severity of the clinical signs of hypersensitivity to tactile stimulation. However, it is not clear whether slowed sensory con-

Table III. Concentration of Glucose, Fructose, Myo-inositol, and Sorbitol in Peripheral Nerves of Guinea Pigs Fed a Low- or Adequate-Zinc Diet

	2.5 week[a]	4.0 weeks	5.5 weeks
Glucose (μg/mg)			
−ZnAL[b]	19.44 ± 3.69[c]	21.21 ± 2.09*	13.17 ± 2.77*
+ZnRF	22.09 ± 6.13*	30.41 ± 9.39*	37.54 ± 15.5†
+ZnAL	19.86 ± 5.16*	25.78 ± 6.83*	39.89 ± 17.0†
Fructose (μg/ml)			
−ZnAL	3.74 ± 0.85*	5.86 ± 0.99*	2.39 ± 0.67*
+ZnRF	5.38 ± 1.57*	8.48 ± 3.68*	6.53 ± 2.22†
+ZnAL	3.39 ± 1.11*	7.23 ± 2.41*	6.46 ± 1.20†
Myo-inositol (μg/ml)			
−ZnAL	19.13 ± 5.00*	16.50 ± 2.59*	8.51 ± 2.32*
+ZnRF	20.37 ± 7.63*	33.24 ± 13.4*	26.27 ± 8.30†
+ZnAL	12.35 ± 3.00*	22.80 ± 7.36*	26.45 ± 7.81†
Sorbitol (μg/mg)			
−ZnAL	0.49 ± 0.11*	0.54 ± 0.08*	0.26 ± 0.06*
+ZnRF	0.45 ± 0.16*	0.93 ± 0.49*	0.62 ± 0.20†
+ZnAL	0.42 ± 0.18*	0.59 ± 0.15*	0.67 ± 0.17*

Note. n = 5/group. Values not sharing a common superscript symbol (*·†) are significantly different; $P < 0.05$.
[a] Time guinea pigs maintained on test diets.
[b] Guinea pigs received either diet low in zinc *ad libitum* (−ZnAL), adequate diet *ad libitum* (+ZnAL), or a restricted quantity of adequate diet (+ZnRF).
[c] Average sugar concentration (μg sugar/mg protein) ± SD.

duction is the cause of these clinical signs. In human metabolic neuropathies, delays in sSSEPs are frequently associated with loss of one or more cutaneous sensory modalities, and the development of paresthesia (31, 32), which represents aberrant function of unmyelinated (pain) nerve fibers. The delayed conduction of impulses in sensory and motor nerves of zinc-deficient guinea pigs supports a clinical diagnosis of sensorimotor neuropathy.

The rate of impulse conduction through myelinated nerves is dependent on myelin sheath integrity, along with the number and size of nerve fibers and condition of the axon. These parameters can be influenced by nutritional status and age (31–34). Delayed maturity of the peripheral nerves from lack of growth has been implicated as a cause of slow impulse condition times in nerves from malnourished children (35, 36) and animals (37, 38). The slowed conduction of impulses in nerves of zinc-deficient guinea pigs cannot be explained solely on the basis of lack of growth because restricted intake of the zinc-adequate diet did not cause impairment. The immature nerve is composed primarily of small myelinated fibers (<4–6 μm in diameter) (39, 40), and impulse conduction in small myelinated fibers is slower than in larger fibers (33, 41, 42). Although small myelinated nerves were predominant in the −ZnAL nerve, the myelinated nerve fiber spectrum was not different from that of growth-restricted +ZnRF nerves. Yet, nerve conduction was not impaired in the +ZnRF nerve. A similar observation was reported by Cornblath and Brown (38) in which motor and mixed nerve conduction velocities were not slowed in food-restricted juvenile rats even though nerve development was delayed. Zinc status, not retarded growth, in zinc-deficient guinea pigs appears to correlate with abnormal peripheral nerve function.

The neuropathy associated with experimental zinc deficiency exhibited minimal morphologic evidence of axonal or myelin abnormalities. The relative lack of degenerative lesions in peripheral nerves of the zinc-depleted guinea pig evokes several interpretations. There may have been inadequate time after decompensated nerve injury for development of lesions characteristic of axonal degeneration. It is also possible that zinc-depleted nerves may not cause axonal degenerative lesions, a feature occasionally noted in investigations of other peripheral neuropathies (43). Since the myelin sheath of −Zn nerves is morphologically intact, a cause of delayed impulse conduction appears to be axonal dysfunction. Further investigation of the morphologic response of the nervous system to zinc depletion is warranted.

In addition to the lower peripheral nerve Na,K-ATPase activity in acute zinc deficiency there also is reduced content of myo-inositol and glucose. A similar paradigm of nerve conduction defects has been described in the neuropathy of experimental diabetes (12). In the streptozotocin-induced diabetic rat, myo-inositol depletion is proposed to result in abnormal phosphoinositide metabolism, which in turn leads to reduced stimulation of Na,K-ATPase. This hypothesis is supported by studies showing that nerve Na,K-ATPase activity in diabetic rats is restored when the nerve myo-inositol content is normalized by supplemental dietary myo-inositol (24) or glycerophosphorylinositol (44). However, the character and progression of abnormalities in the zinc-depleted nerve is unlike those in the experimental diabetic nerve. In zinc deficiency, a significant reduction in nerve Na,K-ATPase activity precedes nerve myo-inositol depletion, suggesting that factors other than myo-inositol deficiency are involved in modulating enzyme activity. In contrast to the high nerve sugar content in diabetes, the levels of glucose and its metabolites in the zinc-deficient nerves were significantly lower than those of controls. Glucose utilization following nerve stimulation is principally used by ATPases to restore ionic gradients across nerve membranes (45). The low glucose content in the −Zn nerve may adversely affect oxidative metabolism of the nerve, leading to an energy-deficient state which may have a large negative impact of Na^+ and K^+ transport (46).

The peripheral neuropathy associated with acute zinc deficiency is an axonal disorder characterized by progressive slowing of motor and sensory nerve impulse conduction. This is accompanied by a reduction in nerve Na,K-ATPase activity and concentrations of myo-inositol, glucose, and sorbitol. These results identify metabolic perturbations that might result in decreased Na pumping and associated disturbance of the membrane potential. The zinc-deficient guinea pig provides a useful system to study nerve metabolism in peripheral neuropathy. Studies involving the central nervous system of the zinc-deficient guinea pig (47–49) have shown impairment of calcium uptake by synaptic membranes when they are stimulated by potassium depolarization, or by glutamate. This suggests a defect of calcium channels, a condition which if present at the neuromuscular junction could explain at least part of the peripheral abnormality.

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