Zinc Deficiency and Peripheral Neuropathy in Chicks (43044)

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Abstract. **Zinc-deficient chicks develop an arthritic-like neuromuscular disorder. They walk with a stilted gait and tend to remain in a squat position, bearing little weight on the legs. The purpose of this study was to determine the basis of the syndrome by making electrophysiologic measurements of nerve function. Chicks were fed low zinc (6 mg/kg) and zinc-adequate (50 mg/kg) diets, the latter *ad libitum* and pair-fed. At the end of 3 weeks, sciatic nerve function was determined *in vivo* by use of an electrodiagnostic system. Motor nerve conduction velocity was significantly lower in chicks fed the low zinc than in those fed the zinc-adequate diet. Zinc repletion of the 2-week depleted chicks was achieved by feeding the adequate diet for 2 weeks. Repletion for this period cured clinical signs and restored nerve conduction velocity to normal, but reversal did not occur within 1 week. It was concluded that the abnormal posture and locomotion of zinc deficiency are associated with peripheral neuropathy.**

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Abnormal posture and locomotion are common clinical signs of zinc deficiency in several animal species (1–7). This pathology is most severe in avian species (5–7) and guinea pigs (8). Zinc-deficient guinea pigs also exhibit hyperalgesia or increased sensitivity to touch, and they vocalize excessively when handled gently or during movement about the cage. Although zinc-deficient chicks do not vocalize, they develop an "arthritic" (7) gait and spend most of the time resting on their hocks in a squat position. Severely deficient chicks essentially refuse to stand or walk.

The physiologic basis of this locomotor disorder is unknown, but the clinical signs suggest nerve dysfunction. The purpose of this study was to evaluate peripheral nerve function electrophysiologically and to determine the rate of remission of the pathology following dietary zinc repletion.

Materials and Methods

Day-old male broiler strain chicks, obtained from a commercial hatchery, were divided into groups of 10 with equal mean weights. They were housed in electrically heated, stainless steel battery brooders with controlled temperature and a programmed light:dark cycle (14:10 hr). Feed and distilled water were supplied *ad libitum* unless otherwise designated. The food intake of one-half of the controls was restricted to the quantity consumed the previous day by those fed a low zinc diet (group pair-fed). Body weights and clinical signs were recorded weekly.

The basal diet, described in detail elsewhere (9), contained 27.3% autoclaved egg white and 5% gelatin as the source of amino acids and glucose as the source of carbohydrate. By analysis, it contained 0.9 mg of zinc/kg. To maintain a reasonable survival rate for 3 weeks, the basal diet was supplemented with 5 mg of zinc/kg as $ZnCO_3$ and this diet (6 mg/kg) constituted the low zinc diet used in the experiments described here. The zinc-adequate diet was the basal supplemented with 50 mg of zinc/kg. It was supplied *ad libitum* (AL) or pair-fed (PF) as described above.

Electrophysiologic measurements were made at the end of 3 weeks and weekly thereafter. Sciatic nerve function was evaluated *in vivo* in anesthetized chicks (60 mg/kg Xylazine and 1.5 mg/kg diazepam im) by use of a contact-electrode electrodiagnostic system (Nicolet, Madison, WI). This diagnostic system allowed measurement of the action potential and nerve conduction velocity. Basically, it consisted of two stimulator electrodes and a recording electrode which were placed

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subcutaneously. One stimulation point was caudal to the greater trochanter of the femur (proximal) and the other caudal to the distal tibia (distal). The active electrode for recording the action potential of the abductor muscle of digit IV was placed over the lateral hypotaurus (calcaneous) just distal to the tarsometatarsal joint. The nerves were stimulated once per second with a supramaximal square-wave pulse of 1-msec duration. Temperature was monitored by a skin surface probe thermometer taped to the thigh and was maintained, when needed, by a heat lamp. The amplitude of the action potential was measured in mV from the baseline to the peak of depolarization; latency was measured as the time from appearance of the stimulus artifact to the onset of depolarization. The distance between electrodes was measured and this value divided by the difference between proximal and distal latencies to calculate the nerve conduction velocity (10, 11).

A typical sign of zinc deficiency in the chick is the propensity to rest in the squat position. To evaluate this tendency semiquantitatively, we measured "squat" time, defined as the time that a chick remains standing when placed individually in its cage. Under these conditions, normal chicks remain active well beyond 10 sec, the arbitrary cut-off period used here. Essentially all deficient chicks had squat times of less than 10 sec.

Data were analyzed statistically by analysis of variance and the post hoc *t* test (Interactive Statistical Package; Crunch Software, San Francisco, CA).

Two experiments, each involving groups of 10 chicks, are reported. In each, three groups were fed for 3 weeks the respective diets, low zinc AL (designated A in Tables I and II), adequate zinc (50 mg/kg) PF (B), and adequate zinc AL (C). In Experiment 1, another group (D) was fed the low zinc diet for 3 weeks then repleted by consuming the adequate zinc diet for 1 week. A control (E) for the repleted Group D was pair-fed the adequate diet for 3 weeks, then fed the same quantity as those in Group D. During repletion, Group D was restricted to its average daily intake during the 3-day period before repletion. In Experiment 2 (design shown in Table II), two additional groups (F and G), analogous to D and E, respectively, were repleted for 2 weeks, a total experimental period of 5 weeks. Electrophysiologic measurements were made on chicks at the end of 3 weeks and on the repleted and pair-fed controls at the end of Weeks 4 and 5.

Results

Table I summarizes the results of Experiment 1. The incidence of severe zinc deficiency was evident from the low body weights and the typical clinical signs that occurred among chicks fed the low zinc diet. Deficiency signs, including poor feathering, arthritic gait, and short bones, were manifested as illustrated in Figure 1 (top). Body temperature measured in anesthetized chicks was generally not different among groups. However, the *ad libitum*-fed controls had higher body temperatures at 3 weeks than either the deficient or pair-fed groups. Squat time, the time before assuming the resting position, was substantially decreased in zinc-deficient chicks. It was improved by repletion for 1 week, but returned to normal (>10 sec) only after 2 weeks of repletion.

The amplitudes of the action potential at 3 weeks, after both distal and proximal stimulation, were lower in the deficient than in pair-fed controls. The motor nerve conduction velocity (NCV) was also significantly decreased compared with pair-fed controls of similar body weight. The difference in NCV between deficient chicks and *ad libitum* controls, whose body weight were more than four times as great only approached significance ($P = 0.07$). The reason for the low NCV in the *ad libitum* controls in this experiment is unknown, but there was no difference between pair-fed and *ad libitum*-fed controls in Experiment 2 (below) or in a preliminary experiment not reported here. Because of the large differences in body weight between zinc-deficient and *ad libitum*-fed controls, it is considered more valid to compare deficient chicks with pair-fed controls. Neither the action potential nor the conduction velocity was restored by 1 week of dietary zinc repletion. The neurologic signs had largely disappeared in surviving chicks after 2 weeks of repletion, as illustrated in Figure 1 (bottom).

Seven groups were used in Experiment 2 and the indices of zinc status at the end of Weeks 3, 4, and 5 are summarized in Table II. Growth rate and plasma zinc concentration were severely depressed in chicks fed the low zinc diet. Plasma zinc was readily restored by the adequate zinc diet, even in pair-fed controls whose growth rate was maintained at a low rate by restricted feeding. As shown in Figure 2, nerve conduction velocity was significantly reduced after 3 weeks of depletion compared with both pair-fed and *ad libitum*-fed controls. There was no difference between the two control groups. The effect of zinc deficiency persisted for 1 week after change to the adequate zinc diet. Remission of locomotor dysfunction occurred after 2 weeks of repletion, at which time there was no difference in motor nerve conduction velocity between repleted and control groups. In this experiment, there were no significant differences in muscle action potentials. The NCV data correlate well with the observed clinical signs as illustrated in Figure 1. After 2 weeks of repletion, all chicks showed greatly improved appetite and activity.

Discussion

These experiments show that motor nerve conduction velocity is decreased by zinc deprivation and

Table I. Zinc Status of Chicks and Electrophysiologic Parameters of Sciatic Nerve Function: Three-Week Depletion followed by One-Week Repletion (Experiment 1)[a]

Dietary treatment			Time on experiment (weeks)	Body weight (g)	Temperature (°C)	Squat time (sec)	DAmp[b] (mV)	PAmp[b] (mV)	NCV (m/sec)
Group	Zinc sup (mg/kg)	Condition							
A	5 (7)[c]	AL	3	165a ± 6.5	39.4a ± 0.14	4.6a ± 0.75	4.6a ± 0.71	4.3a ± 0.87	30.2a ± 0.76
B	50 (10)	PF-A[d]	3	233b ± 13	39.0a ± 0.13	>10c ± 0.0	7.1b ± 0.32	7.2b ± 0.55	37.4b ± 1.6
C	50 (8)	AL	3	738c ± 17	40.3b ± 0.18	>10c ± 0.0	5.6a ± 0.72	4.6a ± 0.59	33.9a,b ± 0.99
D	50 (7)	R-1[d]	4	184a ± 7.3	39.1a ± 0.11	8.7b ± 0.61	4.9a ± 0.84	4.5a ± 0.68	33.2a ± 1.2
E	50 (10)	PF-D	4	254b ± 15	39.2a ± 0.17	>10c ± 0.0	7.6b ± 0.55	7.5b ± 0.69	41.4c ± 1.6

[a] Mean ± SE. Values within columns not sharing a common letter are statistically different, $P < 0.05$.
[b] DAmp and PAmp indicate distal and proximal action potentials.
[c] Number of observations shown in parentheses.
[d] PF-A indicates group pair-fed to the intake of Group A; R-1 indicates repletion group fed low zinc for 3 weeks and adequate zinc for 1 week.

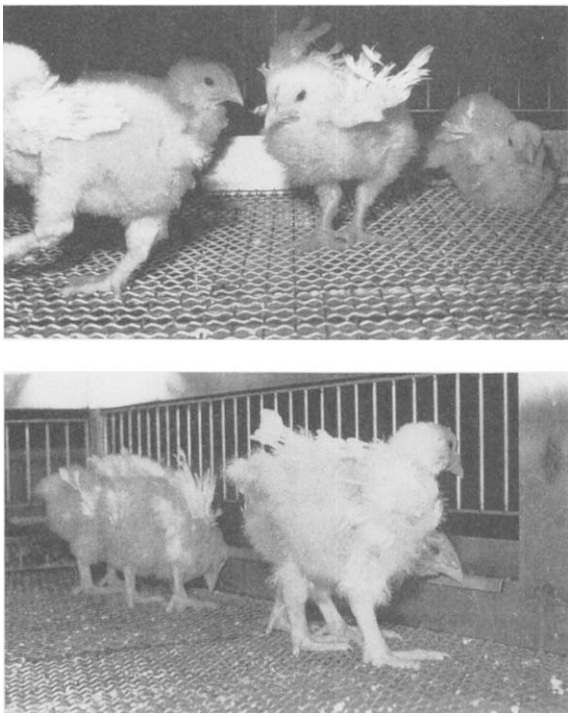

Figure 1. Photographs of zinc-deficient chicks fed the low zinc diet for 3 weeks (top) and repleted chicks (bottom) fed the adequate zinc diet for 2 more weeks. Note the abnormal stance after they were disturbed (top) and normal stance after therapy (bottom). When undisturbed, the upper group remained in a squat position.

strongly suggest that the locomotor defect observed in zinc-deficient chicks (5–7) and quail (12) is the result of defective nerve function. As in the guinea pig (8), the neurologic signs are readily reversed by zinc therapy. The effect of zinc deficiency, at least short term, on nerve conduction velocity is reversed in the chick by zinc repletion. The biochemical basis of this peripheral neuropathy is unknown, but it deserves further research in as much as elucidation of the mechanism could give insight into neuropathies of other origin.

Peripheral neuropathy occurs frequently among diabetics (13), persons subjected to heavy metal toxicity (14), in Lyme disease (15), and in end stage renal disease (16). Renal patients exhibit signs of zinc deficiency, i.e., low plasma zinc concentration, and low motor nerve conduction velocities that respond to zinc therapy. The slowed nerve conduction in experimental diabetes is associated with diminished axolemmal Na^+ potential. Both myoinositol concentration and Na,K-ATPase activity in the sciatic nerve of diabetic rats are lower than normal but can be reversed by insulin therapy (17). The reduced conduction rate may be at least partially attributable to reduced Na,K-ATPase activity but nonreversible structural changes also occur during long-term diabetes. Similar pathogenesis may occur in zinc deficiency, but it has not been studied because deficiency without zinc therapy leads to early death.

Axonal transport requires functional neurotubules (18) and zinc is involved in microtubule assembly. Hesketh (19) observed retarded microtubule reassembly in brain extracts of pigs and rats fed low zinc diets. Tubulin from zinc-deficient rat brain showed impaired ability to polymerize and assembly was stimulated by adding 10 μM zinc *in vitro* (20). These results suggest a possible mechanism for the zinc deficiency-induced neuropathy, namely, failure of neurotubular development and the consequent impairment of axonal transport.

Regardless of its pathogenesis, the neuropathy produced in the chick by zinc deficiency is associated with abnormal stance and is readily reversible by zinc therapy. Whether or not the zinc-deficient chick experiences pain in movement, similar to the diabetic patient with peripheral neuropathy, is unknown, but this animal might serve as a model for the testing of drugs and other therapy for treatment of neuropathies.

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Table II. Zinc Status of Chicks as Indicated by Body Weight and Plasma Zinc Concentration (Experiment 2)[a]

Group	Dietary treatment: Zinc sup (mg/kg)	Dietary treatment: Condition	Time on experiment (week)	Body weight (g)	Plasma zinc (μg/ml)
A	5 (9)	AL	3	154a ± 6	0.18a ± 0.02
B	50 (9)	PF-A	3	216b,c ± 11	0.99b ± 0.12
C	50 (8)	AL	3	711d ± 19	1.36b,c ± 0.13
D	50 (8)	R-1	4	195a,b ± 16	1.4b,c ± 0.07
E	50 (9)	PF-D	4	201a,b ± 11	1.37c ± 0.07
F	50 (8)	R-2	5	263c ± 26	1.17b,c ± 0.09
G	50 (8)	PF-F	5	223b,c ± 23	1.38c ± 0.09

[a] Designations as in Table I with the addition of groups F and G. Group F was fed the low zinc diet for 3 weeks and repleted for 2 weeks. Group G served as the restricted fed control, pair-fed to group F.

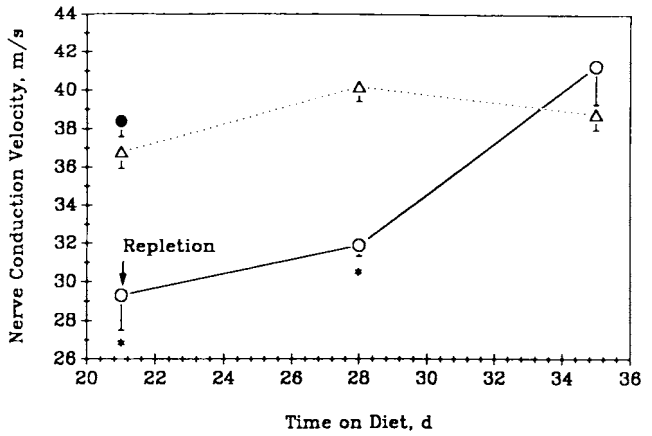

Figure 2. Effect of zinc repletion on sciatic nerve conduction velocity (Experiment 2). Chicks were fed for 3 weeks the low zinc diet AL (——) and the adequate zinc diet PF (· · ·) or AL (single point, ○). Groups of zinc-deficient chicks were then repleted for 1 or 2 weeks by consuming the adequate diet. NCV of the *ad libitum* group was measured at 3 weeks only. During the repletion period, controls were pair-fed. The data points and bars represent the means and the standard error of the means. Statistical significance ($P < 0.01$) indicated by *.

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