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Interaction of Zinc and Vitamin E in the Chick[1] (40792)

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Recent dietary studies have shown a physiological interaction between zinc and polyunsaturated fatty acids (PUFA) in chicks and rats (1, 2). In zinc-deficient chicks the severity of dermal lesions is directly related to the level of PUFA in the diet. The skin lesions do not respond to treatment with prostaglandins or prostaglandin synthetase inhibitors, and it appears that the pathology results from peroxidation of PUFA. If so, vitamin E and other dietary antioxidants should have a protective effect in zinc-deficient chicks fed a diet containing PUFA.

Zinc has a stabilizing effect on biomembranes (3–5). Bettger *et al.* (5) showed that erythrocytes from zinc-deficient rats exhibit increased fragility and that extracellular zinc improves membrane integrity under conditions of peroxidative stress. Vitamin E serves both as an antioxidant (6) and as a structural component in membranes (7); it may act synergistically with zinc to maintain cellular integrity. In this regard it is notable that a portion of patients with sickle cell anemia are deficient in both zinc (8) and vitamin E (9, 10). Erythrocytes from sickle cell patients treated with zinc show increased filterability (11) and those treated with vitamin E undergo less deformation to the irreversible sickle shape (9).

The purpose of the experiments presented here is to examine the effects of vitamin E and other antioxidants on the pathology observed in the zinc deficient chick and to determine if zinc and vitamin E interact to protect skin lipids against peroxidation *in vitro*.

Materials and methods. Groups of 10-day-old broiler strain (Hubbard) chicks were housed in stainless-steel cages in an air conditioned room under constant illumination. Feed and distilled deionized water were supplied *ad libitum* except where noted in the tables. The basal diet contained 25% of EDTA-extracted soybean protein (12), 0.8% DL-methionine, 5% corn oil, and 62.5% cornstarch supplemented with vitamins and minerals.[3] By analysis this diet contained less than 1 ppm zinc and is estimated to contain 30 IU of vitamin E per kilogram.

At the end of the 3-week experimental period the chicks were weighed and judged for dermal lesions and joint abnormalities as previously described (1). Chicks with severely enlarged hocks and inability to walk or stand were given a leg score of 4 and this graded down to 0 for normal joints and gait (1, 13). Skin lesions on the toes and foot pads were similarly graded from 4, severe, to 0, normal (1). Plasma zinc concentrations were determined by atomic absorption spectrophotometry (14). Skin slices from the dorsal surface of the foot were allowed to equilibrate for 2 hr at 25°C in a buffer (1:9, w/v) containing 1 m*M* glucose, 150 m*M* NaCl, and 0.01 *M* Tris buffer, pH 7.4. The skin slices were then trans-

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[3] Minerals were supplied as (mg/kg diet): $CaCO_3$, 5000; $CaHPO_4 \cdot 2H_2O$, 38,7000; Na_2CO_3, 4200; NaCl, 5100; K_2CO_3, 6600; KCl, 1800; $MgSO_4$, 3000; Fe Citrate, 240; $MnSO_4 \cdot H_2O$, 220; KIO_3, 12; $CuSO_4$, 12; Na_2SeO_3, 0.22. Vitamin mixture in cornstarch provides (mg/kg diet): Thiamin HCl, 10; riboflavin, 10; pyridoxine HCl, 10; calcium pantothenate, 30; niacin, 50; inositol, 500; biotin, 0.4; folacin, 2.0; cyanocobalmin, 0.03; retinyl acetate, 6.9 (20,000 IU of A); cholecalciferol, 0.1 (4000 IU of D); and menadione, 25. Choline chloride added at 2000 mg/kg diet.

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ferred to a buffer (1:9, w/v) which contained 150 m*M* NaCl, 0.57 m*M* Gln, 0.07 m*M* His, 0.03 m*M* Cys, 1 m*M* glucose, and 5ppm Cu, pH 6.2. This Cu^{2+} containing buffer promotes peroxidation (5). The skin slices were incubated on a rotating wheel at 37°C for 10 or 20 hr, and the thiobarbituric acid (TBA)-reactive material released into the buffer was measured according to the method of Mengel and Kahn (15). Skin slices incubated in buffer without copper were used as blanks.

The data were analyzed statistically by the Student's *t* test and, where appropriate, by analysis of variance.

Experiment 1. Groups of chicks were fed the basal diet which was supplemented with 25 IU of vitamin E and 5 mg of zinc/kg and a series of antioxidants and compounds that protect against peroxidation. These diets were deficient in zinc but adequate in vitamin E and selenium. Supplements of butylated hydroxy toluene (BHT), ethoxyquin, propyl gallate, and ascorbate were supplied at 0.2 and 0.5% of the diet. Selenium was added at 0.5 and 2.0 ppm as sodium selenite and vitamin E at 100 and 500 IU/kg as DL-α-tocopherol. After 3 weeks, body weights, dermal scores, and leg scores were recorded.

Experiment 2. A factorial design was used in which chicks were fed the basal diet supplemented with three levels of vitamin E (none, 25 IU/kg, 525 IU/kg) and two levels of zinc (5 ppm, 100 ppm). After 3 weeks the weight, dermal score, and leg score were determined and skin samples from the foot were used for the malondialdehyde assay.

Results. The effects of vitamin E and the various antioxidants on zinc deficiency signs are summarized in Table 1. In general, supplementation with antioxidants did not significantly affect the growth rate. Leg scores, which reflect gait, hock size, and length/width ratio of the metatarsal bone, were unaffected by either ascorbate or selenium, but propyl gallate, ethoxyquin and BHT improved the leg score significantly when fed at a level of 0.5%. Vitamin E significantly improved the leg abnormality fed at either 100 or 500 IU/kg. Dermal scores were significantly improved by all antioxidants except ascorbate. Of the supplements tested and with the exception of zinc itself, vitamin E has the most dramatic effect in the alleviating the pathology of zinc deficiency. Chicks fed zinc deficient diets supplemented with high levels of vitamin E (500 IU/kg) had essentially no dermal lesions and only minimal hock disorder. The feet did not swell and there was

TABLE I. EFFECT OF VITAMIN E AND RELATED COMPOUNDS ON GROWTH RATE, DERMAL LESIONS AND JOINT ABNORMALITIES IN ZINC-DEFICIENT CHICKS

Supplements[1]	Body weight (g)[2]	Leg score	Dermal score
None	110 ± 6	2.4 ± 0.2	2.7 ± 0.3
Ascorbate, 1.02%	120 ± 4	2.6 ± 0.2	2.6 ± 0.3
Ascorbate, 0.5%	122 ± 5	2.7 ± 0.2	2.1 ± 0.3
BHT, 0.02%	104 ± 5	2.0 ± 0.2	1.8 ± 0.3*
BHT, 0.5%	98 ± 6	1.7 ± 0.2*	1.6 ± 0.3*
Propyl gallate, 0.02%	121 ± 6	1.9 ± 0.3	1.7 ± 0.2*
Propyl gallate, 0.5%	122 ± 6	1.3 ± 0.3*	1.2 ± 0.3*
Ethoxyquin, 0.02%	110 ± 4	2.4 ± 0.1	1.9 ± 0.2*
Ethoxyquin, 0.5%	104 ± 4	1.7 ± 0.2*	1.9 ± 0.2*
Vitamin E, 100 IU/kg	108 ± 4	1.8 ± 0.2*	1.9 ± 0.2*
Vitamin E, 500 IU/kg	102 ± 3	1.0 ± 0.2**	0.5 ± 0.2**
Selenium, 0.5 ppm	113 ± 5	2.4 ± 0.2	2.3 ± 0.1
Selenium, 2.0 ppm	111 ± 5	2.3 ± 0.2	1.9 ± 0.2*
Zinc, 100 ppm	526 ± 16**	0	0

[1] All diets in this experiment contained, in addition to the basal constituents, 25 IU of vitamin E, and 5 mg of Zn per kilogram.

[2] Mean ± SEM. Twenty chicks per treatment started; the mortality at 3 weeks within a group was <15%. Values with an (*) are significantly different ($P < 0.05$) from those of the chicks fed the diet designated "none." Values with an (**) are significant at $P < 0.01$.

little evidence of hyperkeratosis. However, the predominant subcutaneous redness, previously described (1), was still evident. The fact that the growth rate of the vitamin E supplemented chicks was unaffected indicates that amelioration of the dermal and joint disorders was not due to improved zinc status. As in the case of saturated fatty acids (1), vitamin E ameliorates pathology, apparently without improving zinc absorption. The results of feeding three levels of vitamin E to zinc-deficient and zinc-adequate chicks are shown in Table II. There was no evidence of vitamin E deficiency in any experimental group and the vitamin E level did not affect the plasma zinc concentration or growth rate of either zinc deficient or zinc adequate chicks. On the other hand, vitamin E markedly improved both dermal and leg scores in chicks fed the low level of zinc. Vitamin E supplementation did not affect leg score or dermal score of zinc adequate chicks regardless of whether they were fed *ad libitum* or had their feed restricted.

Data related to the production of TBA-reactive substances from skin slices incubated *in vitro* are presented in Table III. After the 10-hr incubation period all zinc-deficient chick skin had released significantly higher quantities of TBA-reactive substances than skin from zinc-adequate chicks. High levels of vitamin E in the diet of the zinc-deficient chicks significantly decreased this release. Analysis of variance of the malondiadehyde production by chick foot skin after a 10-hr incubation showed a highly significant interaction of zinc and vitamin E. By 20 hr, the release of TBA reactive products was the same for all groups, showing their same potential for peroxidation. Only the rate of release was significantly affected.

Discussion. The results of these experiments indicate that some of the pathology associated with zinc deficiency can be prevented by high levels of antioxidants in the diet and the observations are also consistent with the finding that more severe dermal lesions occur in zinc-deficient chicks fed PUFA than in those fed saturated fat (1). Ascorbate, a compound thought to promote antioxidant activity in the water soluble compartments of the cell, provided little or no protection whereas all of the lipid soluble antioxidants improved both dermal and leg scores. Vitamin E was the most potent agent in protecting against the

TABLE II. EFFECT OF DIETARY VITAMIN E LEVEL ON ZINC STATUS, GROWTH RATE, LEG ABNORMALITIES, AND DERMAL LESIONS

Dietary supplement					
Zinc (ppm)	Vitamin E (IU/kg)	Plasma zinc[3,4] (ppm)	Body weight (g)	Leg score[5]	Dermal score[6]
5	none[1]	0.4 ± 0.1[a]	112 ± 4[a]	3.2 ± 0.1[a]	2.6 ± 0.2[a]
5	25	0.4 ± 0.1[a]	109 ± 5[a]	2.6 ± 0.2[b]	2.5 ± 0.1[a]
5	525	0.4 ± 0.1[a]	103 ± 6[a]	1.2 ± 0.2[c]	0.6 ± 0.2[b]
100 (F.R.)[2]	none[1]	1.3 ± 0.1[b]	113 ± 4[a]	0	0
100 (F.R.)	25	1.4 ± 0.1[b]	110 ± 4[a]	0	0
100 (F.R.)	525	1.4 ± 0.1[b]	110 ± 5[a]	0	0
100	none[1]	1.3 ± 0.1[b]	503 ± 6[b]	0	0
100	25	1.3 ± 0.1[b]	501 ± 9[b]	0	0
100	525	1.3 ± 0.1[b]	510 ± 12[b]	0	0

[1] All diets contained 5% corn oil which is estimated to supply 30 IU of vitamin E per kilogram of diet.

[2] Feed restricted so that the weights of these chicks matched those of the chicks fed the 5 ppm zinc diet *ad libitum*.

[3] Mean ± SEM. Values having different superscripts are significantly different by Student's t test ($P < 0.05$).

[4] Twenty chicks per treatment started; the mortality within each group at 3 weeks was ≤15%.

[5] By analysis of variance the leg score data (zinc deficient vs. feed restricted controls) showed a Zn effect, $F = 992$, $P < 0.001$; a vitamin E effect, $F = 62$, $P < 0.001$, and a Zn × vitamin E effect, $F = 62$, $P < 0.001$.

[6] By analysis of variance the dermal score data (zinc deficient vs feed restricted controls) showed a Zn effect, $F = 109$, $P < 0.001$; a vitamin E effect, $F = 28$, $P < 0.001$, and a Zn × vitamin E effect, $F = 28$, $P < 0.001$.

TABLE III. EFFECT OF DIETARY VITAMIN E ON MALONDIALDEHYDE PRODUCTION BY SKIN SLICES

Dietary supplement		Malondialdehyde production[1]	
Zinc (ppm)	Vitamin E (IU/kg)	10 hr[3] (*n* moles/g·skin)	20 hr (*n* moles/g·skin)
5	none[2]	74 ± 5[a]	180 ± 10[a]
5	25	79 ± 3[a]	180 ± 10[a]
5	525	30 ± 5[b]	190 ± 20[a]
100 (F.R.)[2]	none[2]	15 ± 2[c]	190 ± 20[a]
100 (F.R.)	25	14 ± 2[c]	190 ± 20[a]
100 (F.R.)	525	17 ± 2[c]	190 ± 20[a]
100	none	18 ± 2[c]	170 ± 20[a]
100	25	16 ± 3[c]	170 ± 20[a]
100	525	18 ± 2[c]	180 ± 10[a]

[1] All values having different superscripts are significantly different by the Student's t test ($P < 0.05$). Eight samples per group.

[2] See footnotes 1 and 2, Table II.

[3] By analysis of variance the malondialdehyde data (zinc deficient vs feed-restricted controls) showed a Zn effect, $F = 3886$, $P < 0.001$; a vitamin E effect, $F = 342$, $P < 0.001$, and a Zn × vitamin E effect, $F = 150$, $P < 0.001$.

pathology of zinc deficiency. This may be due to the fact that tocopherols are taken up and retained by membranes more efficiently than the other compounds studied. Vitamin E exerts a structural stabilizing effect in biomembranes (7) and serves as a modulator of the prostaglandin synthetase complex (16) in addition to being a nonspecific antioxidant.

Skin slices from zinc-deficient chicks release TBA-reactive substances more rapidly in the presence of a peroxidative stimulus than control skin slices. This is consistent with the effect exerted by zinc at the surface of erythrocyte membranes (5). In the latter system, extracellular zinc minimized the degree of peroxidative hemolysis without affecting the degree of peroxidation. The present assay does not measure peroxidation directly but only the TBA reactive substances released into the medium. It may be that zinc-deficient membranes, undergoing the same degree of peroxidation as zinc adequate controls, lose structural integrity more quickly and thus release the peroxidation products at a faster rate.

The beneficial effect of excess vitamin E in the zinc-deficient chick suggests that defective membranes are involved in the pathology of zinc deficiency. Vitamin E is found primarily in membranes and it protects these structures against peroxidative attack. The membranes of cells in a zinc deficient environment appear to be subject to peroxidative damage analogous to those deficient in vitamin E. There is no known zinc metalloenzyme that accounts for this effect. The activity of the Zn,Cu-superoxide dismutase is unaffected by zinc status (5, 17, 18). It appears that the loss of zinc ions from vital membrane components results in a destabilized membrane. If so, membrane-bound zinc plays an analogous role to vitamin E, stabilizing membrane structure and thus reducing peroxidative damage.

Summary. Chicks fed a low zinc diet (5 ppm) based on soybean protein, cornstarch, and corn oil developed severe skin lesions on the toes and foot pads as well as gross joint abnormalities that severely impaired locomotion. Incubation of foot skin in a peroxidative buffer containing 5 ppm Cu resulted in the release of malondialdehyde or other thiobarbituric acid reactive substances. The rate of release from skin or zinc deficient chicks was 4–5 times as great as from skin of controls fed adequate zinc. Supplementation of the zinc deficient diet with fat soluble antioxidants, particularly vitamin E, decreased the severity of the skin and joint pathology. High levels of dietary vitamin E also decreased the rate of release of peroxidative products from zinc-deficient skin but had no effect

on control skin. The results show a significant physiological interaction between dietary vitamin E and zinc. It appears that cells from zinc-deficient chicks can benefit from incorporating higher than normal levels of vitamin E into their membrane structure. It is postulated that zinc protects against peroxidative damage and promotes membrane integrity.

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