The effect of severe zinc deficiency on the morphology of the rat retinal pigment epithelium

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Male Sprague-Dawley weanling rats maintained on a controlled dietary zinc intake had an accumulation of osmiophilic inclusion bodies in the retinal pigment epithelium (RPE) of the zinc-deficient group. At 7 weeks of zinc deficiency there were marked ultrastructural alterations of the RPE. In some instances a deepening of the basal infoldings of the cells of the RPE were observed. There was also vesiculation and degeneration of the photoreceptor outer segments. No changes were observed in the pair-fed, weight-restricted, and ad libitum-fed controls. The possible functions of zinc in the retina are discussed. (INVEST OPHTALMOL VIS SCI 23:425-434, 1982.)

Key words: dietary zinc deficiency, inclusion bodies, retinal pigment epithelium

Zinc is an essential trace element required for normal metabolism of proteins and nucleic acids and the function of a variety of metalloenzymes. Some of the highest concentrations of zinc in animals, including man, are found in ocular tissues. Considerable interest has developed recently concerning the exact role of zinc in the eye. Studies by our group and by others have shown that zinc deficiency in chronic alcoholic cirrhotics may reversibly impair dark adaptation. Toskes et al. have demonstrated not only impairment of dark adaptation and electroretinography but also structural defects in the periphery of the retina. Their results with fluorescence angiography in patients with chronic pancreatitis suggested that zinc may play an etiologic role in these retinal abnormalities. Optic atrophy has been described in patients with acrodermatitis enteropathica, a hereditary disease of impaired zinc absorption, and in acquired cases of zinc deficiency due to malabsorption and chronic alcoholism. Administration of transitional trace metal chelators such as dithizone or 1,10-phenanthroline produces osmiophilic lipid inclusion bodies in the retinal pigment epithelium (RPE) of albino rats. The presence of these bodies cannot be ascribed solely to zinc deficiency, since these agents may chelate other transitional metals. Furthermore, the effect on the RPE might be mediated through some action shared by both compounds but unrelated to trace metal chelation.
It was the purpose of this investigation to determine the effect of diet-induced deficiency on the ultrastructure of the rat retina, using previously well-documented zinc-deficient rat models and appropriate controls. 20, 21

**Materials and methods**

*Animals and diet.* Weanling male Sprague-Dawley rats weighing 40 to 50 gm were randomly divided into four groups. All animals were fed a commercially prepared zinc-deficient, pelleted diet (Ziegler Brothers, Inc., Gardners, Pa.), which contained 0.7 ppm zinc without added phylate. This diet is complete in all other nutrients and its composition has been reported elsewhere. 20 The diet was administered with the following group variations: Zinc-deficient and ad libitum-fed controls received the diet ad libitum. Weight-restricted animals were weight-paired with zinc-deficient animals by restricting the diet so as to maintain a comparable body weight to that of the zinc-deficient rats. Pair-fed animals were given an amount equivalent in weight to that consumed by the zinc-deficient animals. Distilled, deionized water with 30 ppm zinc (as zinc acetate) was freely available to weight-restricted, pair-fed, and ad libitum groups, whereas there was no zinc replacement in the water of the zinc-deficient animals. The rats were maintained in an environment designed for trace metal studies similar to that described by Klevay et al. 21 Rats were housed individually in stainless steel wire bottom cages and maintained in a constant temperature 21° ± 2° C and under a 12 hr light/dark cycle. Stainless-steel feeders were used and the plastic water bottles

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**Fig. 1.** Growth curves for young rats subjected to different dietary zinc treatments for 5 weeks, with marked growth retardation noted in the zinc-deficient rats compared with the zinc-sufficient, ad libitum-fed controls. WR, Weight-restricted controls.

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<th>Table I. Zinc concentration in serum and femur after 7 weeks of dietary treatment*</th>
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*Values are ± SE (n = 10).
†Significantly different from zinc-deficient values, p < 0.001.
were equipped with silicone stoppers. The animals were sacrificed by decapitation for zinc determination studies or were anesthetized with pentobarbital and perfused for electron microscopy.

**Zinc determination.** Zinc concentrations were determined by standard atomic absorption technique with a Varian Techtron AA375 (611 Hansen Way, Palo Alto, Calif.) as described previously.20-22 All specimens for zinc analysis were stored in polyethylene containers that had been previously soaked in 1% EDTA in deionized water, then rinsed with deionized water to avoid zinc contamination. Tissues for zinc analysis were dry ashed and serum samples were diluted 1:3 with deionized water for atomic absorption studies.

**Electron microscopy.** Retinas from four groups of animals were fixed after 2, 4, 5, 6, and 7 weeks of controlled zinc intake by means of intracardiac perfusion with 3% glutaraldehyde (Ladd) in 0.1M cacodylate buffer (pH 7.3) containing 0.02% calcium chloride. After perfusion, the eyes were enucleated and hemisected. After the lens and vitreous were removed, the posterior halves were immersed in the same fixative for an additional 30 min. The tissues were rinsed in buffer and postfixed with 1% osmium tetroxide in 0.1M cacodylate buffer. Tissues were processed by a procedure outlined previously.23

**Results**

**Assessment of zinc deficiency.** The severity of zinc deficiency was assessed by growth rate, development of dermatologic changes typical of zinc deficiency, and serum and tissue concentrations of zinc. By the end of the first week, an obvious impairment of growth was noted between zinc-deficient and ad libitum control rats (Fig. 1). By the third to fourth week, most zinc-deficient animals had begun to manifest dermatologic signs (alopecia with crusting skin lesions around the eyes, nose, mouth, and extremities). At the end of the study period, zinc-deficient rats had a markedly retarded growth compared with the ad libitum controls and also weighed significantly less than the pair-fed rats.
Serum and femur concentrations in the zinc-deficient rats were significantly reduced compared with the values of the control groups (Table I).

**Electron microscopy.** The RPE from all three control groups revealed normal morphologic characteristics (Fig. 2). However, the RPE of zinc-deficient rats consistently contained irregularly shaped osmiophilic bodies. These cytoplasmic profiles were first observed during the third week of zinc deficiency and were quite conspicuous by the fifth week (Fig. 3). These inclusion bodies were generally located in the basal portion of the cell, and their characteristic serrated or scalloped edges were often in close association with mitochondria and smooth endoplasmic reticulum. Frequently, an inclusion body was almost entirely surrounded by mitochondria. Inclusion bodies resembled neither phagosomes (outer segment debris) (Fig. 3), lysosome-like granules, lipofuscin granules, nor microperoxisomes commonly observed in the RPE. There was a gradual overall increase in inclusion bodies, but their absolute number varied from cell to cell. Amelanotic premelanosomal granules were frequently observed in the cytoplasm of the RPE cells in zinc-deficient animals. During the sixth week of zinc deficiency, in addition to the presence of the inclusion bodies, areas of the RPE were noticeably lesioned and some of the photoreceptor outer segments were disorganized and vesiculated (Fig. 4).

The severest morphologic alterations were observed during the seventh week of zinc deficiency (Figs. 5 to 7). The opaque inclusion bodies were randomly distributed throughout the RPE cytoplasm. There was no indication that smaller inclusions had fused to form larger bodies. The irregular edges of the inclusion bodies continued to be associated with the mitochondria and the smooth endoplasmic reticulum (Fig. 6). Autophagic
Zinc deficiency affects the RPE

Fig. 4. RPE from a zinc-deficient rat maintained on the diet for 6 weeks. Large number of inclusion bodies (IB) are present. Outer segments (OS) are disrupted. The RPE is markedly lesioned. (×16,000.)

Fig. 5. RPE from a zinc-deficient rat maintained on the diet for 7 weeks. RPE is lesioned. Inclusion bodies (IB) are present. Vacuoles (V) are present in the cytoplasm of the RPE. Outer segments (OS) are disrupted. (×11,200.)
vacuoles that had been observed during the sixth week were present in most of the affected cells of the RPE in the seventh week of zinc deficiency (Fig. 5). These vacuoles were sometimes empty but generally contained sequestered material of various degrees of structural composition. In some instances aberrant cilia were seen in the RPE (Fig. 6). There was often a dissolution of nuclear material in the cells of the RPE and alterations in smooth endoplasmic reticulum, but the mitochondria generally remained unaltered (Fig. 7).

Concomitant with the accumulation of the inclusions and morphologic changes in the RPE was a noticeable decrease in lysosome-like granules and phagosomes (Figs. 5 to 7). In some instances the depth of the basal infoldings of the RPE was increased, and the plasma membranes were often deeply plicated.

The extent of degeneration in the RPE during the sixth and seventh week of zinc deficiency varied from animal to animal, but the pathologic patterns were identical.

Discussion

The results of this investigation demonstrate that when weanling albino rats are made zinc deficient, irregular osmiophilic inclusion bodies generally accumulate in the cytoplasm of the RPE cells. These inclusions are not observed in the RPE of control animals.

The inclusion bodies have a different morphologic appearance than phagosomes, lyso-

Fig. 6. RPE from a zinc-deficient rat maintained on the diet for 7 weeks. Inclusion bodies (IB) are associated with mitochondria (m) as well as the smooth endoplasmic reticulum. Aberrant cilium (arrow) is present. The plasma membrane is deeply plicated. Some outer segments (OS) appear normal, but most are degenerated. (×16,000.)
Zinc deficiency affects the RPE

Fig. 7. RPE from a zinc-deficient rat maintained on the diet for 7 weeks. RPE is severely lesioned. Inclusion bodies (IB) are present. Mitochondria are unaltered. Outer segments (OS) are vesiculated, disrupted, or degenerated. (×13,440.)

somal granules, lipofuscin granules, microperoxisomes commonly seen in the RPE. However, the osmiophilic inclusions are morphologically identical to those seen in the RPE of rats administered chelators of zinc. The inclusions are not membrane bound, are in intimate association with mitochondria and smooth endoplasmic reticulum, and are commonly located in the basal portion of the cell. They do not appear to be products of phagocytosis. These inclusions accumulate in clusters similar to the lipid droplets seen in the RPE of the mouse after the administration of retinyl acetate or retinyl palmitate. The osmiophilic inclusions seen in the RPE of zinc-deficient rats are also similar to those observed in the RPE of 14-month-old rats with hereditary retinal degeneration (Wag/Rij). Dense osmiophilic inclusions, presumably rich in lipid, are seen in the cytoplasm of zinc-deficient Euglena gracilis.

The retinas of the zinc-deficient rats initially possess morphologically intact photoreceptors, but after 5 weeks of zinc deficiency there is vesiculation, disorganization, and eventual degeneration of the photoreceptor outer segments. These alterations are most marked in areas where the cytoplasm of the RPE contain a significant accumulation of osmiophilic inclusions.

Although there is a significant number of degenerated photoreceptor outer segments after 5 weeks of zinc deficiency, there is not an observable increase in phagosomes and lysosomes. Rather, with continued zinc defi-
iciency, these organelles of catabolism are not readily observed in the RPE. This may be the result of the marked ultrastructural alterations in the RPE, which would create a diffusion barrier. Prevention of the exchange of metabolites and nutrients between the RPE and the photoreceptors would result in subsequent degeneration of the latter.

The mechanism(s) for these morphologic changes is as yet unclear. Numerous lines of evidence support the concept of a major interaction between zinc and vitamin A. Zinc deficiency may interfere with vitamin A metabolism by decreasing retinol-binding protein (RBP) production and/or release from the liver, as reported in experimental animals. RBP, the carrier protein for vitamin A, is a rapid-turnover protein with a half-life of 12 hr. We recently reported that zinc deficiency in man decreased the serum level of rapid-turnover proteins such as prealbumin and transferrin (with similar unpublished data on RBP), and zinc repletion increased or normalized these levels.

Zinc is also an integral part of alcohol dehydrogenase, the metalloenzyme that catalyzes the interconversion of vitamin A alcohol (retinol) to vitamin A aldehyde (retinal). Huber and Gershoff have demonstrated altered vitamin A metabolism in the eyes of zinc-deficient rats as manifested by decreased activity of alcohol dehydrogenase and decreased conversion of retinol to retinal. They also reported that alcohol dehydrogenase in the retina was much more sensitive than hepatic alcohol dehydrogenase to the effects of zinc deficiency. Raskin et al. administered pyrazole (an inhibitor of alcohol dehydrogenase) to rats and demonstrated decreased retinaldehyde levels in the retina and altered functional activity of the retina as assessed by electoretinography.

In the present investigation, the RPE of zinc-deficient rats may have an inadequate supply of utilizable zinc necessary to maintain enzyme activity, thus impairing the conversion of retinol to retinal. If the conversion of retinol to retinal is inhibited during zinc deficiency and the primary lesion is in a decrease in alcohol dehydrogenase activity, the photoreceptors may not be able to obtain as much retinal as they require. This could result in increased synthesis of high-affinity receptor for the uptake of vitamin A by the RPE, and therefore an increase in vitamin A uptake. A "futile cycle" might continue for as long as there is insufficient retinal reaching the photoreceptors, manifested by an accumulation of retinyl esters in lipid-containing inclusion bodies in the RPE. This process could interfere with the normal biosynthesis of the visual pigment and the metabolism of the RPE.

It has also been suggested that excess retinol and its esters are toxic to cells and are membranolytic. If the lipid-containing inclusion bodies in the RPE of zinc-deficient rats contain vitamin A derivatives such as retinyl esters, they could cause lysosomal instability and the release of lytic enzymes. For example, cathepsin D (hydrolytic enzyme) release is mediated by retinol. The release of proteolytic enzymes into the cytoplasm of the RPE could mediate the degenerative changes seen in the RPE of zinc-deficient rats. This damaging effect on membranes also could be augmented in zinc deficiency. Zinc plays a vital role in membrane stability, as recently reviewed by Bettger and O'Dell. Zinc deficiency enhances lipid peroxidation and damage to lipid membranes, and thus the combination of retinyl esters and zinc deficiency might produce a synergistic deleterious effect on the retina.

Lastly, zinc deficiency impairs the normal metabolism of amino acids, nucleic acids, and proteins, and these alterations may play a role in the retinal damage observed in the zinc-deficient rats. One of the earliest manifestations of zinc deficiency in animals is decreased activity of thymidine kinase, an integral enzyme in DNA synthesis. Decreased DNA synthesis in the liver of zinc-deficient rats has been reported. Decreased brain DNA, RNA, and protein is observed in infant rats deprived of zinc. Utilization of amino acids for protein synthesis in zinc-deficient animals is impaired, probably due to both decreased synthesis and increased degradation.
mechanisms whereby zinc deficiency may produce a deleterious effect on the RPE, and we are currently investigating several postulated causes of zinc deficiency–induced retinal damage.

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REFERENCES


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