Zinc-Deficient Embryos: Reduced Thymidine Incorporation

Abstract. *The 12-day-old embryos of rats with a deficiency of zinc showed a reduced uptake of tritiated thymidine when compared with controls, as shown by liquid scintillation and autoradiography. The high incidence of gross congenital malformations resulting from zinc deficiency may thus be caused by impaired DNA synthesis.*

Maternal zinc deficiency during pregnancy results in a high incidence of congenital malformations in the developing rat fetus (*1*, *2*). When normal adult females were subjected to a zinc-deficient diet throughout pregnancy, almost all the full-term young had gross congenital malformations, encompassing every organ system. Shorter periods of deficiency were also teratogenic; for example, when pregnant females were fed the zinc-deficient diet from day 6 through day 13, about half of the full-term young were malformed. The profound effects on the embryo led us to postulate that zinc played a role in development at a very fundamental level. Impairment of DNA synthesis would be consistent with the observed effects of zinc deficiency.

Requirement of zinc ion for DNA synthesis has been demonstrated (*3*) in liver perfused with ethylenediaminetetraacetate (EDTA) of partially hepatectomized rats. Injections of small amounts of zinc stimulate the incorporation of ^{3}H-thymidine into regenerating liver of zinc-deficient rats, but have no effect on normally fed controls (*4*). Dietary zinc deficiency also decreases the incorporation of thymidine into nuclear DNA of liver cells in young growing rats that have not been hepatectomized (*5*). We now report that a dietary zinc deficiency during pregnancy results in decreased incorporation of ^{3}H-thymidine by varied cells of embryonic rats *in situ*.

Table 1. Incorporation of ^{3}H-thymidine into 12-day-old-embryos of zinc-deficient and zinc-supplemented rats 1 hour after injection. Because of the small body size, three zinc-deficient or two control fetuses were pooled for each sample. Incorporated radioactivity is expressed as mean disintegrations per minute (dpm) ± standard error.

Group	Litters (No.)	Samples (No.)	dpm/embryo	dpm/mg of embryo (wet weight)
Zinc-supplemented (controls)	5	7	131,233 ± 19,272	7,243 ± 1,167
Zinc-deficient	12	18	11,100 ± 2,108*	1,889 ± 302*

* $P < .001$ as compared with controls.

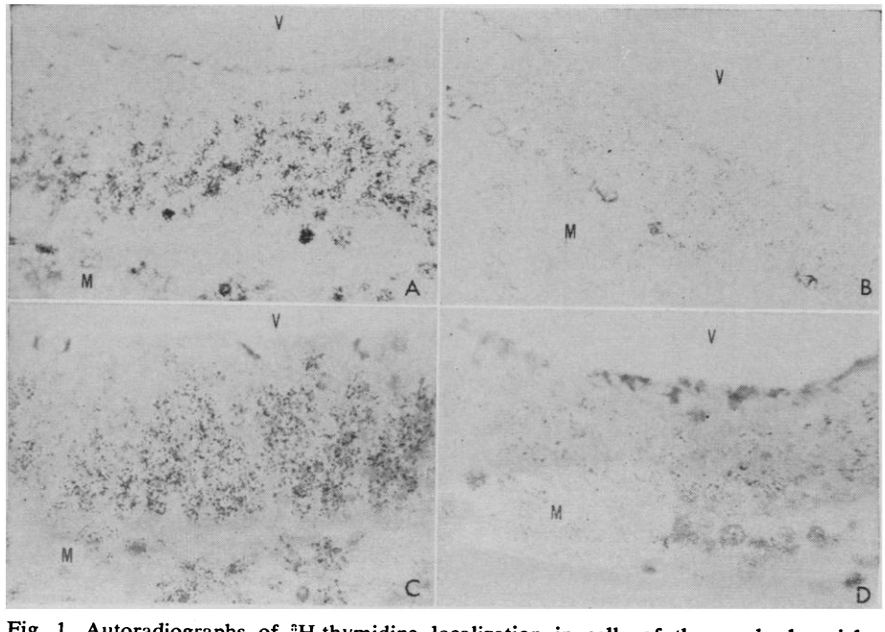

Fig. 1. Autoradiographs of ^{3}H-thymidine localization in cells of the cerebral vesicles of 12-day-old embryos from zinc-supplemented (left) and zinc-deficient (right) female rats. (A) and (B) Unstained sections (× 140). (C) and (D) Hematoxylin-stained tissues showing mitotic activity (× 224). Abbreviations are: *V*, ventricular lumen; *M*, mesenchyme.

Female rats of the Sprague-Dawley strain (body weight, 210 ± 10 g) were purchased from a commercial source and given a purified ration (containing added zinc, 100 ppm) for 5 to 28 days before breeding. At estrus they were caged with males for 2 hours so that copulation time could be determined precisely. At the beginning of pregnancy (day 0, as determined by the presence of sperm in vaginal smears), females were fed either a zinc-deficient diet [with 0.35 to 0.40 ppm of zinc (*6*)] or continued receiving the zinc-supplemented control diet (100 ppm of added zinc). The diet had the following composition, in percent: isolated soybean protein (washed in EDTA), 30.0; sucrose, 53.7; corn oil, 8.0; salt mix (*7*), 4.0; and DL-methionine, 0.7 (*8*). Crystalline vitamins were given separately (*9*). Care was taken to eliminate sources of zinc contamination in the environment as well as in the purified diet (*8*).

Experiments on ^{3}H-thymidine uptake were performed on 5 control and 12 zinc-deficient rats on day 12 of gestation. All the zinc-deficient litters showed gross malformations. One control and two zinc-deficient pregnant females were used for each day's experiment (with one exception when a pregnant control was not available), and tissues were treated simultaneously. Females were anesthetized with sodium pentobarbitol and injected by tail vein with 0.5 mc of thymidine-methyl-^{3}H (6.7 c/mmole). One hour later, some embryos were removed and transferred into vials for liquid scintillation counting. At the same time, littermates were fixed in Bouin's solution for autoradiography.

Samples for scintillation counting were dissolved in Nuclear-Chicago Solubilizer and diluted with PPO scintillation fluid (2,4-diphenyloxazole in toluene). Radioactivity was determined on a Nuclear-Chicago Mark I liquid scintillation counter with the channel ratio method (*10*) to correct for quenching. Tissues embedded in paraffin for autoradiography were serially sectioned at 6 μ, coated with Kodak NTB-2 liquid emulsion, and exposed in the dark for 6 weeks. After being developed, rep-

resentative sections were stained with Mayer's hematoxylin for demonstration of nuclei.

The results of the scintillation counting of embryonic tissues are shown in Table 1. Incorporation of labeled thymidine into the embryos of zinc-deficient rats was significantly lower than in controls, whether based on the count per whole embryo or per unit weight.

Autoradiographs showed that cells of zinc-deficient embryo tissues contained less ^{3}H-thymidine than did those of controls. In both groups the highest concentration of label was found in the outer half of the primitive ependymal zone of the cerebral vesicles (Fig. 1, A and B), the undifferentiated mesenchyme, embryonic blood, and liver cells. Heart and somatic muscle, and lining cells of the primitive gut, trachea, and bronchi were less heavily labeled in both deficient and control tissues. Developing central nervous system was examined in detail because of the high incidence of congenital malformations in this tissue in the young of zinc-deficient rats (*1*). More mitotic figures were present in the surface neuroepithelium of deficient embryos than in controls. However, in both groups, ^{3}H-thymidine was not incorporated into these dividing cells (Fig. 1, C and D) since these are cells in which DNA synthesis had occurred prior to introduction of the label (*11*). The apparent paradox of increased mitotic figures with decreased DNA synthesis cannot be explained on the basis of present information and is being investigated further.

Both the autoradiographic and the quantitative data presented demonstrate that the incorporation of ^{3}H-thymidine into embryonic tissues is reduced by zinc deficiency. This suggests that DNA synthesis may be impaired when zinc is insufficient. Although more detailed studies are necessary to prove that DNA synthesis is depressed under these conditions, the data presented are in agreement with earlier findings relating to DNA synthesis and zinc in other systems. Thus, our study suggests that the gross congenital malformations resulting from zinc deficiency may be caused by a basic defect in the synthesis of DNA.

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6. Determined by atomic absorption spectrophotometry.
7. Composition of basal salt mix (grams): $CaCO_3$, 600; $Ca(H_2PO_4)_2 \cdot H_2O$, 220; K_2HPO_4, 650; NaCl, 336; $MgSO_4 \cdot 7H_2O$, 250; $FeSO_4 \cdot 7H_2O$, 50; $MnSO_4 \cdot H_2O$, 4.6; KI, 1.6; and $CuSO_4 \cdot 5H_2O$, 0.6. Zinc-supplemented salt mix included the addition of 9.8 g of $ZnCO_3$.
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9. A mixture of crystalline vitamins in glucose was given three times each week, in small glass dishes in amounts to provide the following intake (μg) per day: Ca-pantothenate 500; *p*-aminobenzoic acid and riboflavin, each 100; thiamine-HCl, pyridoxine-HCl, and nicotinic acid, each 300; menadione, 250; folic acid, 6; biotin, 2.5; vitamin B_{12}, 0.3; and choline chloride, 10 mg; inositol, 5 mg; ascorbic acid, 1 mg; α-tocopheryl acetate, 1.2 international units (I.U); vitamin D_3, 15 I.U.; and vitamin A palmitate, 150 I.U. During pregnancy the vitamin supplement was doubled.
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12. Supported in part by NIH grant HD-01743. H.S. was predoctoral trainee under Health Sciences Advancement Award FR06138.

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Silica in Developing Epidermal Cells of Avena Internodes: Electron Microprobe Analysis

Abstract. *Silica is present in significant amounts in mature guard and subsidiary cells of stomata, in trichomes and silica cells, and in the walls of long epidermal cells of* Avena. *No silica was detectable in cork cells of cork-silica cell pairs. Silica must be accumulated in these specialized epidermal cells quite rapidly.*

Grasses, sedges, and *Equisetum* absorb large quantities of silica from the soil and accumulate it as amorphous silica ($SiO_2 \cdot nH_2O$) in specialized cells in aerial portions of the plant (*1*, *2*). This silica may also infiltrate the cellulose framework of the cell walls and thus contribute to maintaining erectness of the plant body (*3*).

Most of the methods employed to measure amounts of silica and to show its pattern of distribution in plant tissue involve chemical analysis. The structure of silica-containing bodies (phytoliths), as revealed by the scanning electron microscope, can be related to models postulated from light microscopy studies (*2*). We now report on the use of the electron microprobe for detecting and quantifying silica in biological material. Here, we specifically refer to silica in the epidermal cells of the oat *Avena* internode.

For the electron microprobe analysis, the following procedures were employed for tissue preparation: (i) pieces of tissue two to four cell layers thick were sliced from different positions of next-to-last internodes (located just below the peduncular or last-formed internode) and placed in aluminum foil envelopes; for this purpose, tissue pieces were removed from top to bottom of mature and young elongating internodes; (ii) the tissue pieces in the foil were frozen in liquid nitrogen, then dried at 0°C in a Virtis automatic freeze dryer (No. 10-010); (iii) the dry pieces were then mounted on polished stainless steel with butyl acetate–silver preparation, coated with carbon, and stored in a desiccator until ready for microprobe analysis.

The electron microprobe (model EMX-SM, Applied Research) was operated at 20 kv, giving an electron beam penetration into the tissue of about 20 μm. The sample current was 0.004 μa.

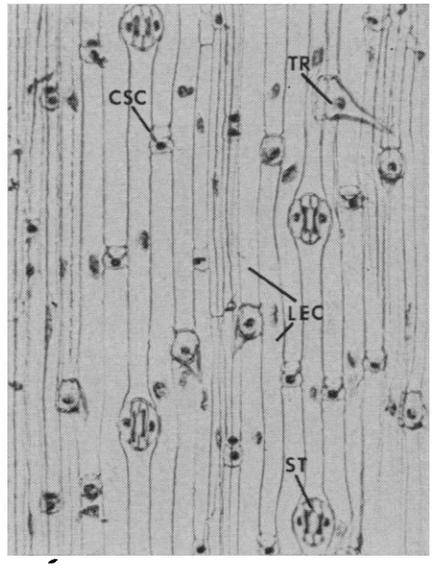

Fig. 1. Light micrograph, illustrating types of epidermal idioblasts in internodes of the oat plant that were analyzed for silica with the electron microprobe. It shows long epidermal cells (*LEC*) with various types of idioblasts intercalated between the long cells. These include stomata (*ST*), cork-silica cell pairs (*CSC*), and trichomes (*TR*) (× 328).