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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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.

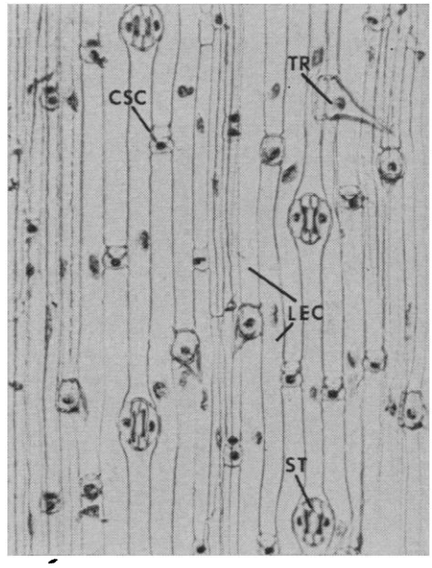

Fig. 1. Light micrograph, illustrating types of epidermal idioblasts in internodes of the oat plant that were analyzed for silica with the electron microprobe. It shows long epidermal cells (*LEC*) with various types of idioblasts intercalated between the long cells. These include stomata (*ST*), cork-silica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

The x-ray spectrometer was peaked for silicon, and an ammonium dihydrogen phosphate crystal was used. Secondary electron-scanning images were used to examine the tissue and select cells for analysis. Qualitative determinations of silicon distribution were made by raster and line scanning, while quantitative measurements were made by point counting an average of five locations per cell. Photographs were taken of x-ray and secondary electron-scanning images with a Polaroid Land camera.

Electron microprobe analysis for silica in mature *Avena* internodal epidermis, obtained from upper or mature portions of the internode, revealed silica in each of the three idioblast types. In the unicellular hairs or trichomes (Fig. 1), it occurred throughout the lumen of the cell (Fig. 2G). In the stomata (Fig. 1), it was found in both the guard and subsidiary cells (Fig. 2A). In contrast with these cells, in cork-silica cell pairs (Fig. 1), most of the silica was confined to the lumen of the silica cell with little silica present in the cork cell below it (Fig. 2C). In long epidermal cells which separate the various types of idioblasts (Fig. 1), silica was primarily found in cell walls (Fig. 2F). That silica was found in each of these types of epidermal cell or cell complex confirms earlier studies by light microscopy (*4*). However, microprobe analysis reveals that its location in the idioblast is quite specific. As indicated above, it may occur in the cell lumen or in the cell wall, depending on the cell type; moreover, in two cells derived from a common initial (cork-silica cell pairs), one member specifically accumulates silica, and the other does not to any appreciable extent. The physiological basis for these differences in silica deposition patterns in different types of cells in the same tissue has not been investigated. Different kinds of cells or cell complexes in the epidermal system may have different mechanisms by which they accumulate silica.

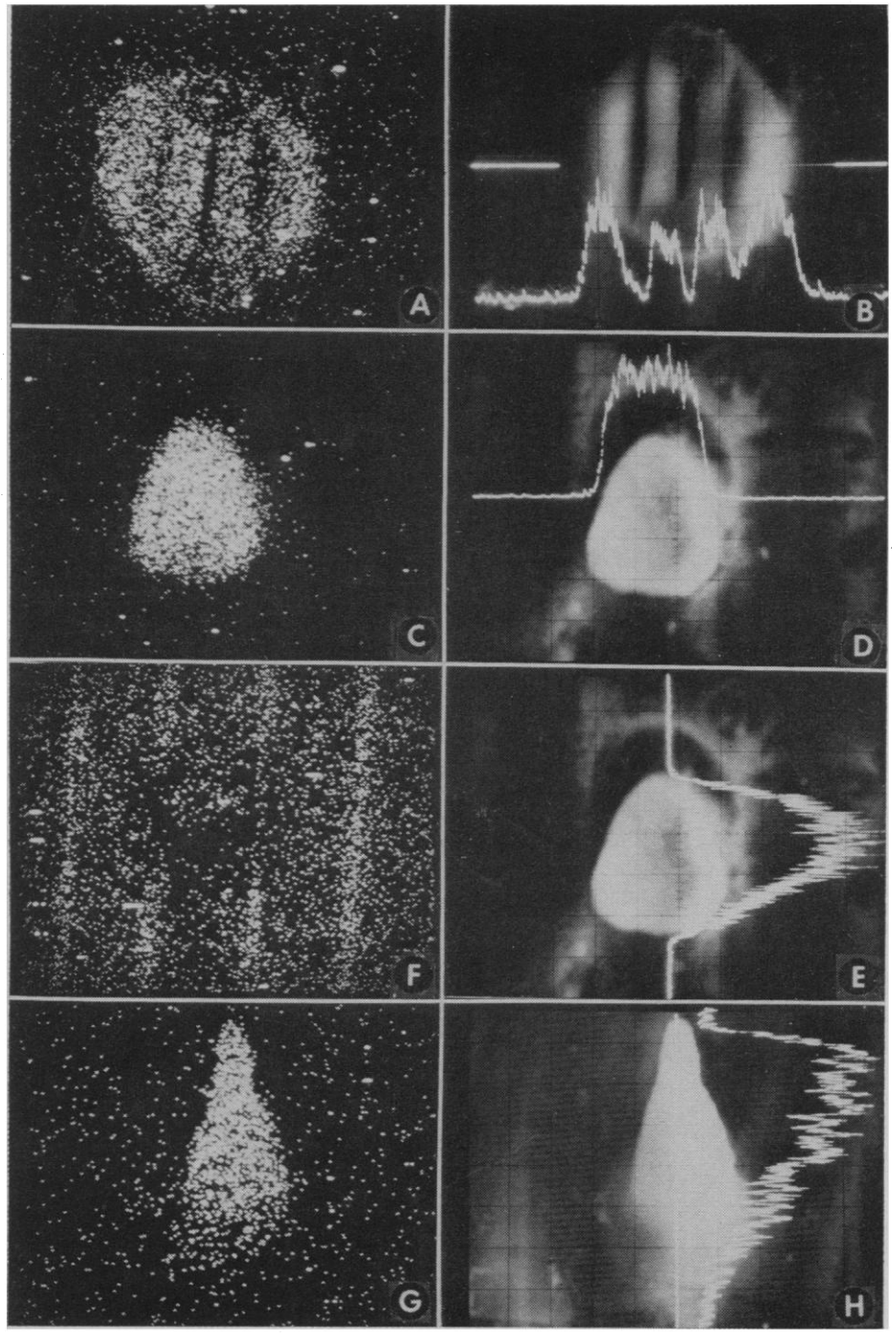

Fig. 2. Silicon distribution patterns and silica profiles in oat epidermal idioblasts as revealed by electron microprobe analysis. A, C, F, and G are x-ray images indicating silica distribution in these cells. B, D, E, and H are secondary electron images of the same cells with silica x-ray profiles superimposed. Profile in B was lowered so that stomatal apparatus could be seen. A and B, stomatal apparatus (× 1000); C, D, and E, cork-silica cell pair. In D and E cork cell outline is delineated by light colored cell wall above the silica cell (× 1055). F, long epidermal cells (× 1200); G and H, trichome or hair (× 1000).

The distribution pattern for silica was analyzed with the electron beam by line scanning through mature idioblasts at the top of a 6-cm internode. The first scans were made transversely through a silica cell (Fig. 2D) and longitudinally (Fig. 2E) through the same cell and the cork cell above it. The amount of silica across the silica cell was remarkably uniform; however, the longitudinal scan through this same cell showed that silica was highest in the center portion and dropped steeply at the upper and lower ends of the cell. The scan also indicated that the adjacent cork cell had little silica in it. A scan across the median portion of a stomatal apparatus (Fig. 2B) indicated that the silica was fairly uniform across each of the guard and subsidiary cells, with essentially comparable amounts in both types of cells. A longitudinal scan through a trichome (Fig. 2H) revealed that silica was distributed much less uniformly. The greatest amount of silica was found in the apical part of this cell, decreasing in more or less linear fashion toward the basal portion of the cell. From these data, it appears that silica is not distributed uniformly in silica cells or in trichomes, at least in the longitudinal direction. We can also conclude that the

cork cells of cork-silica cell pairs are probably devoid of silica.

Analyses were made to determine the amount of silica present in cork-silica cell pairs in immature and mature internodes. In an immature 6-cm internode, where the uppermost centimeter was exposed to the atmosphere (sheath absent), no silica could be detected with the electron microprobe in silica cells, except in the uppermost centimeter of the internode. The cork cells had no silica present; even the cork cells at the top of the internode had silica present at a level only slightly above background. In a mature 9-cm internode, where again only the uppermost centimeter was exposed to the atmosphere, silica was present in significant amounts in silica cells in the uppermost 5 cm with none detectable in the lowermost 4 cm. The amount of silica in cork cells was insignificant. Silica in silica cells in these upper levels of the mature internode, compared with a sample of pure SiO_2 (776 count/sec), averaged 83 percent silica. Both sets of data indicate that silica deposition in silica cells must take place quite rapidly, because at one level, silica is totally absent, and at the next highest level, it occurs in significant amounts ~ 500 to 600 count/sec. The data also indicate that silica is accumulated by silica cells in both exposed and ensheathed portions of the internode. Thus, exposure of the internode to the atmosphere by its emergence from the sheath, together with the expected increase in amount of transpiration from the exposed portion of the internode, may not be essential for the silica accumulation process.

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β-Hydroxybutyrate Dehydrogenase: Lack in Ruminant Liver Mitochondria

Abstract. *The enzyme* D(−)-β-*hydroxybutyrate dehydrogenase has been reported to occur in all mitochondria isolated from mammalian tissues and is especially high in liver. By contrast only a very low concentration of the enzyme is detectable in mitochondria from bovine and sheep liver. The amount of the enzyme in mitochondria from other ruminant tissues such as kidney and heart are comparable with those from mammalian sources. The very low activity of* β-*hydroxybutyrate dehydrogenase is not referable to the inability of substrates to penetrate mitochondria or to decreased electron transfer activity but rather to a lack of the enzyme in ruminant liver mitochondria.*

The enzyme D(−)-β-hydroxybutyrate dehydrogenase (BDH) (E.C. 1.1.1.30) catalyzes the reversible NAD-linked oxidation (*1*) of D(−)-β-hydroxybutyrate (BOH) to acetoacetate (*2*). The enzyme is stereospecific for the D(−)-stereoisomer, has a specific and absolute requirement for lecithin (*3*), and is found tightly bound to the mitochondrial membrane (*4*). The enzyme can be released from the membrane by treatment of mitochondria with phospholipase A (*5*). Earlier studies concerning the distribution of this dehydrogenase in animal tissues have shown that it is present in all tissues examined (*4*); the activity is usually greatest in liver. However, we find that liver mitochondria from ruminants, both beef and sheep, have very low BDH acivity. The BDH activity in heart and kidney mitochondria from the same two ruminant sources is in the same range as that from nonruminants (20 to 25 times higher than that of ruminant liver).

Bovine, rat, and sheep mitochondria were isolated as described (*6*). The bovine heart mitochondria were prepared on a large scale as reported (*6*), except that 0.25*M* sucrose containing 0.01*M* HEPES (*1*), *p*H 7.55 (sucrose-HEPES), was substituted for the "sucrose-Tris" buffer used originally. The preparation of mitochondria from rat, sheep, and bovine liver and kidney cortex was, however, scaled down so that 200 g of tissue could be processed within approximately 2 hours. The tissue was trimmed free of connective tissue, minced with scissors, and then suspended in three volumes (volume to weight) of cold sucrose-HEPES buffer. The minced tissue was homogenized with a glass homogenizer of the Potter-Elvehjem type. Two loose fitting pestles were used sequentially with the clearance between the pestle and the homogenizer wall being 0.026 and 0.018 inch, respectively. The pestles were rotated at 1000 rev/min. The homogenate was sedimented for 10 minutes at 1650 rev/min in a refrigerated centrifuge (MSE Mistral 6L) in a six place swinging bucket rotor (Cat. No. 62301). The resulting supernatant was decanted through a double layer of cheesecloth and then sedimented for 10 minutes at 18,000 rev/min in a Spinco preparative centrifuge (No. 30 rotor). Two distinct layers were obtained. The upper layer was discarded after being separated mechanically from the lower layer with a glass rod. The lower layer, which was a rich brown color, contained the purified mitochondria. The purified mitochondria were suspended and centrifuged twice more, the first time in one-half and the second in one-fourth of the original volume of buffer. Each time any residual upper layer was discarded.

Total mitochondrial protein was analyzed using a modification of the method of Lowry (*7*). Crystalline bovine serum albumin served as the protein standard for the biuret method (*8*). Mitochondria, whose protein content had previously been determined by the biuret method, served as a secondary standard for the Lowry procedure.

Livers from frog, cat, monkey, and a variety of rodents all have been shown to have substantial BDH activity (*4*). In agreement with these observations, we also found that rat liver mitochondria have high BDH activity (Table 1). Bovine heart and kidney mitochondria also had a substantial amount of the dehydrogenase activity. Consequently, it was surprising when a very low BDH activity was observed in bovine liver mitochondria. Moreover, the BDH activity of bovine liver mitochondria was so low that it was necessary to use the more sensitive assay reaction, β(−)-hydroxybutyrate to cytochrome *c* reductase, to obtain an accurate measurement of BOH oxidation (Table 1). Because the low BDH activity in the bovine liver mitochondria suggested that ruminant liver might be exceptional in this respect, mitochondria were prepared from calf and sheep liver. Mitochondria from these two sources also had very low BDH activity.