Investigations on the Role of Boron in Plants[1] III. Anatomical Observations[2]

Sung G. Lee and S. Aronoff
Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50010

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Summary. Electron microscopic examination of leaf mesophyll cells of boron-deficient sunflower plant reveals that chloroplasts degenerate and cell walls undergo profound structural changes before any visual deficiency symptoms become apparent. Mitochondria, which increase in number as deficiency develops, frequently show myeline figures, while nuclei may develop dense rhombohedral structures.

Although various anatomical boron deficiency symptoms have been described in the past, none has been reported at the subcellular level. In this paper we report changes as revealed by electron microscopy.

Methods and Materials

Sunflower seedlings, germinated in moist sand, were grown in Hoagland's complete nutrient solution. Plants were made boron-deficient by transfer from complete nutrient to a minus-boron solution. This transfer was done when the first leaves were 40 to 50 mm long. Young leaves, 10 to 20 mm long, were sampled from boron-sufficient (B+) and boron-deficient (B−) plants. Plants were grown in B− solution up to 7 days. The first visual deficiency symptom, the browning of leaves, was apparent on the fifth day. Young (expanding) leaves were prefixed for about 16 hours with 3 % glutaraldehyde, adjusted to pH 7.3 with 0.1 M phosphate buffer. One mm-cubed mesophyll tissues were cut from the prefixed leaves under the dissecting microscope and the tissue blocks were fixed for 4 hours with 1 % OsO_4, adjusted to pH 7.3 with Palade's buffer (3) at 4°. The tissue blocks were dehydrated in a graded series of aqueous ethanol (30 %, 50 %, 70 %, 95 %, 100 %) and the alcohol then replaced by 4 changes of propylene oxide. The tissue blocks were embedded in an Epon resin mixture as described by Luft (2), the resin heat-polymerized, and 600Å tissue sections made with an LKB ultra-microtome equipped with a diamond knife. The tissue sections were stained with 2 % uranyl acetate. The stained sections were examined with an RCA EMU F-3 electric microscope.

Observations. Changes in cellular structures were observed as early as 3 days after the removal of boron from nutrient medium. At this time no visual deficiency symptoms were recognizable. The damaged cells were localized in groups of a few to several cells near the basal margin of leaves. These damaged cells were seen as copper-brown spots under the dissecting microscope.

One of the earliest changes appears to occur in chloroplasts. B− chloroplasts appear to contain larger amounts of starch, supporting the contention of Dugger et al. that boron decreases the enzymatic conversion of glucose-1-phosphate to starch (1). Also observed in boron-deficient chloroplasts is unidentified osmophilic material (product X) (fig 2a and 2b). Product X appears to be confined to individual chloroplasts since the entire stromae of some chloroplasts are filled with this material while other chloroplasts in the same cell appear to be devoid of it. As X is confined within the chloroplast membrane, it may be a polymer incapable of diffusing through the chloroplast membranes. Its deposition appears to be localized in individual cells. Nearly all of the chloroplasts in 1 cell may have X while adjacent cells have none.

B− cells appear to contain a greater number of mitochondria with well developed cristae. Myelin figures are commonly observed to be associated with B− mitochondria (fig 3). Mitochondrial development appears to occur prior to chloroplast changes.

In advanced B− stages, 6 to 7 days after transfer to B− nutrient, changes also appear in the nucleus. Angular, heavily osmophilic rhombohedral structures, may be observed (fig 4). The cell walls also undergo changes. B− leaf mesophyll cell walls

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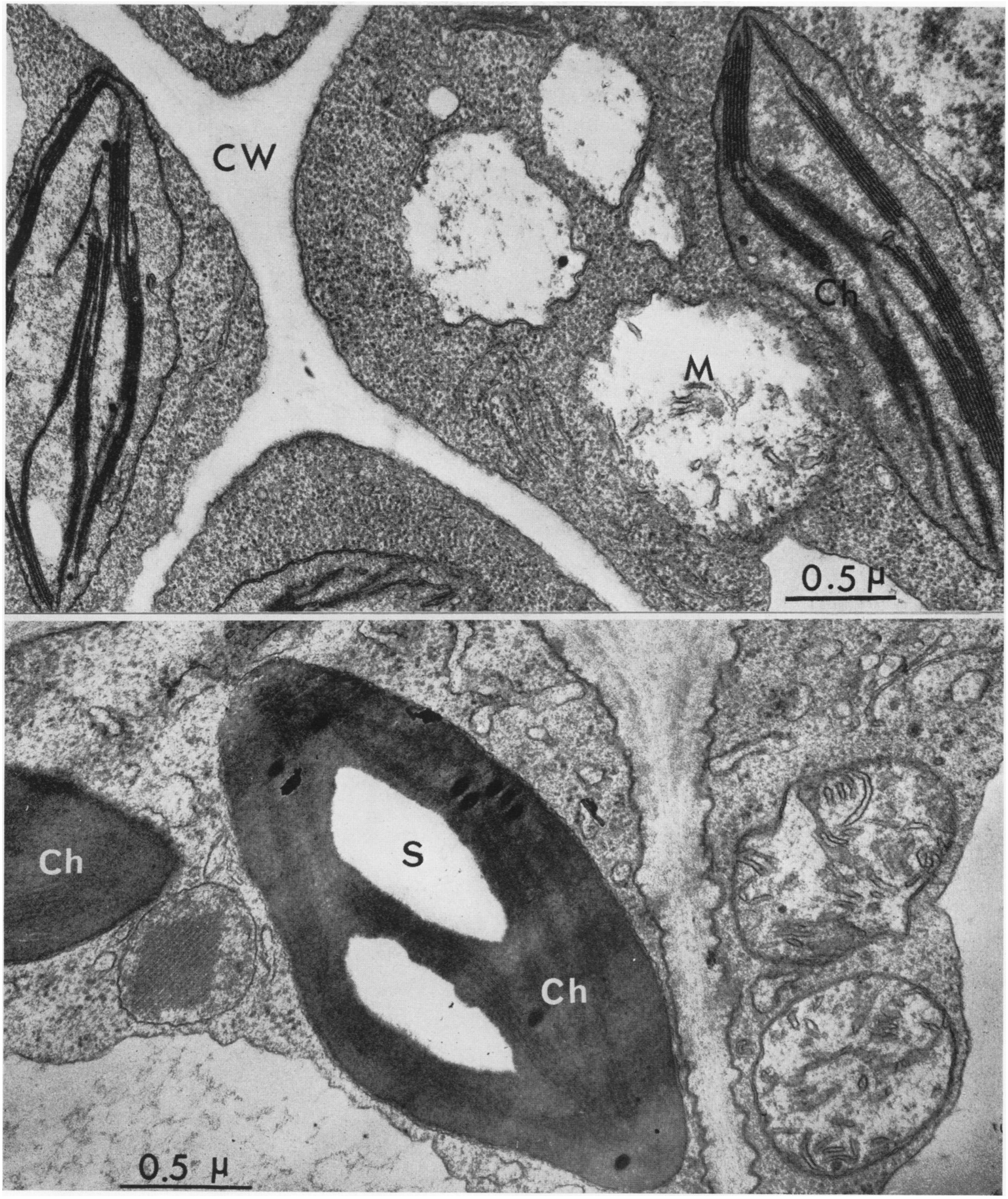
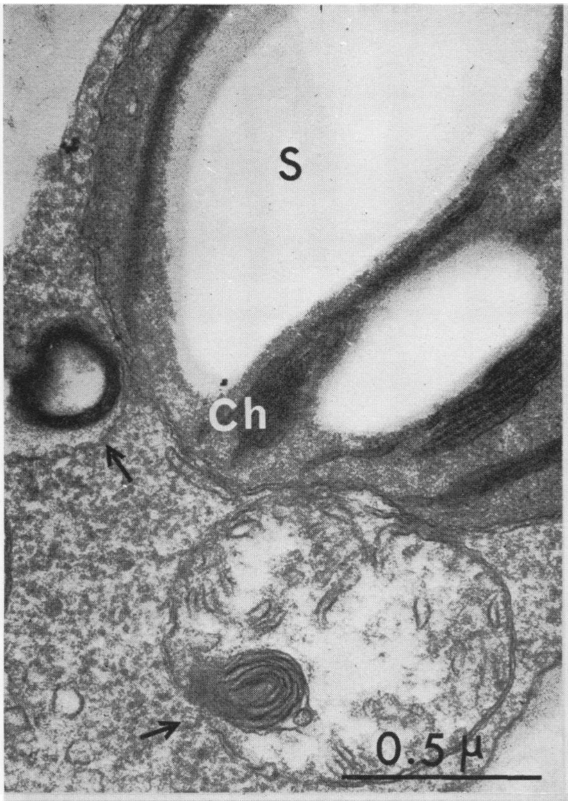
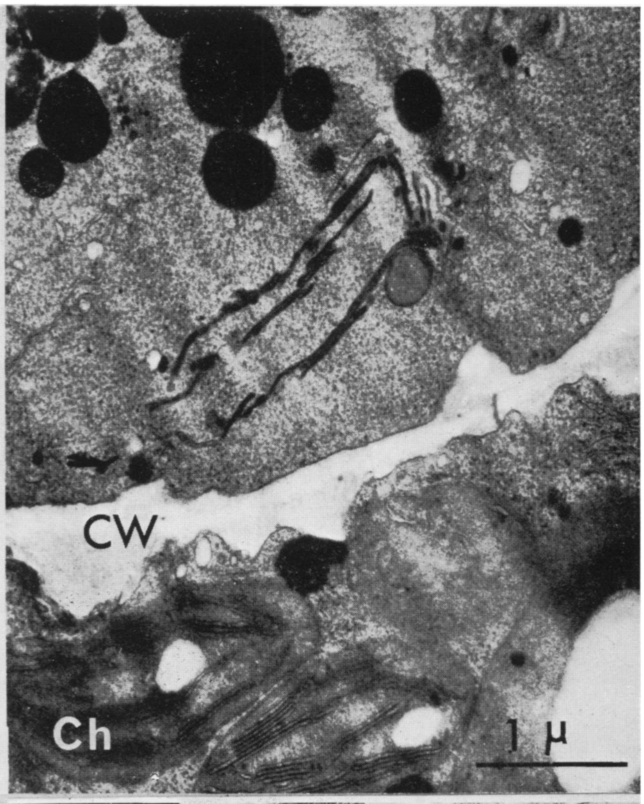
FIGS. 1a (upper) Normal (B+) and 1b (lower) (boron-deficient (B−) mesophyll cells showing chloroplasts (Ch), mitochondria (M), cell wall (CW) and nucleus (N). 9800×.



FIGS. 2a (upper) Normal (B+) and 2b (lower) boron-deficient (B−) mesophyll cells showing deteriorating chloroplasts with dark, osmophilic product X, (uniformly dispersed throughout the chloroplast) starch and oil droplets, and cell wall (CW). Note that grana are still recognizable. (2a, 42,100×; 2b, 43,000×.)





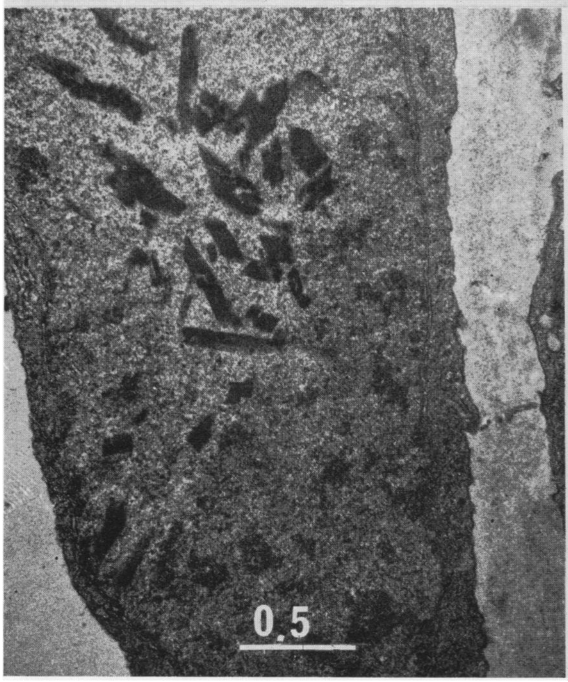


FIG. 3. (top, left) A 3-day-boron-deficient mesophyll cell showing mitochondria with myelin figures (arrows). (49,600×)

FIG. 4. (bottom, left) Six-day-boron-deficient cell showing a nucleus with rhombohedral-like structures. (18,400×)

FIG. 5. (top, right) Seven-day-boron-deficient cells showing numerous osmophilic bodies (arrow) in the cytoplasm. Note rough cell wall (CW) structure. (28,600×)

FIG. 6. (bottom, right) Seven-day-boron-deficient cell showing deposition of osmophilic material in cytoplasm (arrow). Note also that mitochondria are undergoing disintegration. (23,700×)

appear to be thicker and have a rough, serrated appearance (fig 5), in agreement with light microscopic studies (4).

In extremely deficient cells (7 days after transfer), no subcellular structures are recognizable. Such cells contain a large number of heavily osmophilic bodies which have the appearance of oil droplets (fig 5). As the deficiency becomes even more severe, these osmophilic bodies fill the entire cell (fig 6).

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