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Boron

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17.1 Boron in Biology

17.1.1 Introduction

Boron is the only nonmetal in a family otherwise comprised of active metals, Group IIIA of the periodic table. As expected, boron exhibits bonding and structural characteristics intermediate to both. Like carbon (atomic number 6), boron (atomic number 5) has a tendency to form double bonds and macromolecules. In addition, there are several features that are more or less unique to boron and this group of elements. These include electron-deficient molecules (such as boron trifluoride) and bridge bonds (such as those in diborane, B_2H_6). These tendencies have formed the basis for the many hypotheses attempting to predict the mode of action of boron as a nutrient essential to the metabolism of vascular plants (Section 17.2.1), as a toxicant to animals (Section 17.1.5), and for achieving boron accumulation in cancer cells (Section 17.1.8).

In this chapter the biochemistry of boron is reviewed predominantly in vascular plants. The discussion is organized around those metabolic processes that boron nutrition repeatedly has been shown to influence: carbohydrate metabolism, hormone action, membrane structure and function, and nucleic acid biosynthesis.

17.1.2 Criteria for Essentiality

The biochemistry of boron encompasses the history of almost 75 years of research seeking to elucidate the primary role of boron in the metabolism of

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vascular plants. Few organisms, other than tracheophytes, have been demonstrated to require boron for growth. For plants, the essential nature of an element is established according to a set of criteria defined by Arnon and Stout (1939): (1) the element must be essential to the completion of the life cycle of the plant; (2) the element cannot be substituted for or replaced by any other element; (3) the element must have a distinct function (e.g., enzyme cofactor, as in the case of zinc, which is a cofactor of alcohol dehydrogenase; molecular component, such as magnesium in chlorophyll; or structural component, such as silicon in the frustules of diatoms). Two early articles demonstrated that boron met these criteria: (1) Sommer and Lipman (1926) provided evidence that boron was essential for the completion of the life cycle of a number of mono- and dicotyledonous plants; and (2) Warington (1923) demonstrated that none of the 52 elements she tested could alleviate the symptoms of boron deprivation for the several species of leguminous plants with which she worked. Thus, by 1926, the essential nature of boron to the growth of angiosperms had been established in accordance with the criteria of Arnon and Stout except for demonstration of a distinct function for boron in a plant. Although there is universal agreement that boron is essential to vascular plants, over 70 years have passed and a specific biochemical role for boron in the metabolism of a higher plant remains to be elucidated.

17.1.3 Effect of Boron on the Growth of Organisms

There is minimal evidence that boron is essential to organisms other than vascular plants. In the Monera, the only bacterium tested for a boron requirement was *Azotobacter chroococcum,* a free-living nitrogen-fixing bacterium. Boron was shown to stimulate nitrogen fixation in this prokaryote, but it could not be demonstrated that boron was essential to this process (Anderson and Jordan, 1961). Nitrogen fixation also was stimulated in three species of Cyanophyta: *Calothrix parietina, Anabaena cylindrica,* and *Nostoc muscorum.* In addition, boron stimulated the growth rate of these three species and the growth of the nonnitrogen-fixing blue-green alga, *Microcystis aeruginosa,* when nitrate was omitted from the growth medium. The increase in growth rate was not as dramatic when nitrate was provided (Gerloff, 1968). Only *Nostoc muscorum* was shown to require boron for growth. The cells deprived of boron became chlorotic in the third week of culture and were completely white by the end of 8 weeks. At this time the population of *N. muscorum* determined by cell count was only 39% of the population of the cultures supplied with boron (Eyster, 1952).

Boron is not required for the fungi *Neurospora crassa, Saccharomyces cerevisiae, Aspergillus niger,* or *Penicillium chrysogenum* (Bowen and Gauch, 1966; Gerloff, 1968). However, the morphology of the spore-forming organs

has been shown to be sensitive to variations in boron concentrations (Davis *et al.*, 1928). Species of fungi exhibited effects of boron toxicity, resulting in the aborted growth of mycelia, perithecia, and ascospores (Bowen and Gauch, 1966) and in the failure of gametes to cleave (Zittle, 1951). Foster (1949) demonstrated that penicillin production is stimulated by boron in both *Penicillium chrysogenum* and *P. notatum*, and Davis *et al.* (1928) reported that boron enhanced the growth of *P. italicum*.

Although boron was shown to enhance the growth of *Chlorella vulgaris*, with 0.5 mg boron per liter being sufficient for optimal growth (McIlrath and Skok, 1958), no requirement for boron could be demonstrated among the many species of chlorophyta tested (Gerloff, 1968). In fact, boron was found to be toxic to four species of *Chlorella* at the concentrations indicated: (1) *C. vulgaris*, greater than 0.5 mg boron per liter; (2) *C. vannielei*, 50 mg boron per liter; (3) *C. emersonii*, 100 mg boron per liter; and (4) *C. protothicoides*, 100 mg boron per liter (Bowen *et al.*, 1965). Lewin (1966a) reported an absolute requirement for boron in the marine pennate diatom, *Cylindrotheca fusiformis*, in both the light and dark. She subsequently reported an absolute requirement for boron in 16 species of marine diatoms and eight species of freshwater diatoms (Lewin, 1966b). For marine diatom species, the requirement for boron was demonstrated upon the first transfer to a boron-free culture medium. However, the boron requirement for freshwater diatoms could be shown only after a number of transfers to boron-free medium had been carried out (Lewin, 1966a,b). Preliminary studies with other phytoplankton indicated that a boron requirement is not unique to diatoms; species of Dinophyceae and Prasinophyceae do not multiply under boron-deficient conditions (Lewin, 1966b).

A requirement for boron in bryophytes has not been reported (Lewis, 1980a). In the Lycopsida *Selaginella apoda*, boron seems to be essential to the formation of spore-producing organs (Bowen and Gauch, 1965). This effect appears to be true for the fern *Dryopteris dentata*, which, when grown under boron-deficient conditions, produced no visible sori or incomplete indusia with aborted sporangia (Bowen and Gauch, 1965).

In the gymnosperms, tissue cultures derived from *Ginkgo biloba* pollen have an absolute boron requirement (Yih *et al.*, 1966); and several reports of a boron requirement in conifers are found in the literature (Ludbrook, 1942; Walker *et al.*, 1955; Blaser *et al.*, 1967). Boron deficiency in *Thuja plicata* resulted in restricted growth in meristematic regions; needles were closely bunched, giving a "rosette" appearance to shoots, and roots were short (Walker *et al.*, 1955). The symptoms of boron deficiency were most obvious during rapid growth and periods of meristematic activity (Blaser *et al.*, 1967).

Boron is considered an "apparently" nonessential inorganic constituent in the dietary requirements of the rat (McCoy, 1967). There is only one study on record that attempts to determine whether or not boron is an essential nutrient

for animals. Using the laboratory rat as the test animal, Hove *et al.* (1939) concluded that if boron was needed by the growing rat, 0.8 mg per day satisfied this requirement.

17.1.4 Plant Evolution and an Essential Role for Boron

Any unified theory attempting to explain the biochemical function of boron in plant metabolism must account for the fact that some taxonomic groups have an absolute requirement for boron while others do not. Thus, two conclusions can be drawn from the previous section: (1) there must be specific metabolic pathways common to both vascular plants and the species of diatoms, Dinophyceae and Prasinophyceae, requiring boron that are generally lacking among the other taxonomic groups; or (2) there must be at least two unique pathways requiring boron: one in tracheophytes and the other in the phytoplankton listed previously.

It has been proposed that a study of the evolution of vascular plants may provide a possible key to the role of boron in plant metabolism. Lewis (1980a) proposed that the development of an essential role for boron was a prerequisite to the evolution of vascular plants. He contends that the primary role of boron is in lignin biosynthesis and, in conjunction with auxin (indole-3-acetic acid, IAA), in xylem differentiation. This hypothesis is based on (1) the many reports in the literature that phenols accumulate under conditions of boron deficiency (Spurr, 1952; Perkins and Aronoff, 1956; Watanabe *et al.*, 1961; Watanabe *et al.*, 1964; Troitskaya *et al.*, 1970); (2) the suggestion in the literature that there is a concomitant decrease in lignin biosynthesis in boron-deficient tissue (Skok, 1958); (3) the fact that hyperauxiny is a frequent symptom of boron deficiency (Odhnoff, 1957; Neales, 1960; Jaweed and Scott, 1967; Coke and Whittington, 1968; Bohnsack and Albert, 1977); and (4) the fact that monocots, which have a much lower requirement for boron, possess an additional lignin biosynthetic pathway not possessed by dicots.

Despite this evidence, there is room to doubt Lewis's hypothesis that the primary role of boron in vascular plants is in lignin biosynthesis. In fact, lignin synthesis may be enhanced under conditions of boron deficiency (Neales, 1960); increased availability of lignin precursors and cell wall thickening are common features of boron-deficient tissues (Kouchi and Kumazawa, 1975b; Hirsch and Torrey, 1980). The relationship between boron and IAA may also be considered a secondary one. Bohnsack and Albert (1977) and Hirsh *et al.* (1982) have shown that at the biochemical and ultrastructural levels, boron deficiency and hyperauxiny are different (Section 17.3).

Lewin (1966a) proposed that the role of boron in diatoms is due to its structure as B_2O_3, which closely mimics SiO_2. This property results in the

incorporation of boron into the diatom shell. Based on the silicon to borate ratio, she concluded that boron was an important structural component of the diatom cell wall and essential to normal wall morphogenesis. In the evolution of plants, it is probable that a requirement for boron arose more than once.

17.1.5 Boron Toxicity

Economic losses due to boron toxicity are common in irrigated regions of the world; such losses arise not only through a reduction in plant productivity, but also through boron intoxication of livestock that feed on plants that have accumulated toxic levels of boron. Boron inhibits many enzymic activities (Section 17.2.9) with concomitant deleterious effects on both livestock and humans.

In vascular plants, boron is carried passively in the transpiration stream and accumulates where the transpiration stream ends (Kohl and Oertli, 1961). Because boron is relatively immobile in the phloem, very little of the accumulating boron moves out of these tissues (Oertli and Richardson, 1970; Raven, 1980). For these reasons, leaves usually exhibit the first symptoms of boron toxicity: yellowing of the leaf tip, with the chlorosis subsequently progressing along the leaf margin and then spreading into the blade. Necrosis of the chlorotic tissue follows, followed by leaf abscission (Wilcox, 1960). Thus, boron toxicity results in a loss of plant productivity.

While there are many reports in the literature relating the development of leaf symptoms to elevated levels of boron in leaves, research attempting to determine the actual manner by which boron is toxic to plants has been minimal. It is assumed that the chlorosis and subsequent necrosis of leaf tissue resulting from the accumulation of boron to a toxic level result in a loss of photosynthetic capacity that accounts for the subsequent loss in plant productivity.

Since the uptake of boron is passive through the transpiration stream in tracheophytes, boron intoxication is a function of the concentration of boron to which the plant is exposed, the length of exposure, and the rate of transpiration. Because of these phenomena, Kohl and Oertli (1961) predicted that if a leaf lived long enough, it would show symptoms of boron toxicity even when grown in the presence of a concentration of boron considered optimal. Boron concentrations that are toxic to a particular species or cultivar have proved to be nearly constant, regardless of the stage of growth (El-Sheikh *et al.*, 1971).

Some vascular plants grow well under conditions of excess boron. In some boron-tolerant species, boron fails to accumulate in the leaves, or does so at a reduced rate. In most cases, this is due to reduced boron uptake. The mechanism is unknown; reduced boron uptake may simply be due to a lower rate of transpiration in the boron-tolerant species. The sequestering of boron along the root, which occurs in some species tolerant to sodium, has not been demonstrated in

plants tolerant to boron. In other boron-tolerant species, a high concentration of boron accumulates, but the leaves do not exhibit the symptoms associated with boron toxicity (El-Sheikh *et al.*, 1971).

17.1.6 Problems Associated with Studies of Boron Metabolism

Two significant factors contribute to the difficulty of elucidating boron's mode of action. The first is the lack of a radioactive or heavy isotope of boron that would facilitate localization and transport studies. Without a radioisotope of boron, all metabolic investigations of a role for boron in plant metabolism have been comparative studies employing boron-sufficient and boron-deficient tissues.

The second is the difficulty of establishing a zero boron concentration. Boron is a common component of "hard" glass and has been shown to leach from it in a relatively short period of time. Boron is also found as a contaminant of chemicals and distilled water. Furthermore, tissue derived from boron-sufficient plants contains endogenous boron. In light of the fact that 0.1 mg boron per liter provides adequate boron for most monocots and dicots, boron sufficiency is often attained by contamination. A further complication arises from the fact that optimal and toxic levels of boron are extremely close to one another. For example, it has been demonstrated that 0.1 mg boron per liter is optimal for the growth of tomato plants (*Lycopersicum esculentum,*) while a concentration greater than 1.0 mg boron per liter is toxic to them (Davies and Addo, 1957). The micronutrient status of boron results form the fact that very low concentrations—0.01–4.0 mg boron per liter—are adequate for most gymnosperms and angiosperms grown in solution culture (Wilcox, 1960). Since boron is transported passively in the transpiration stream (Kohl and Oertli, 1961), tissue levels of boron are a function not only of the concentration of available boron but of the length of exposure to boron and the rate of transpiration. Thus, boron concentrations within the optimum range, or slightly higher, may prove toxic to some species (Kohl and Oertli, 1961; Mengel and Kirby, 1978). Unless one is careful, varying degrees of boron contamination may make it difficult to know whether one is observing the effects of boron deficiency, sufficiency, or toxicity.

17.1.7 ^{10}B (n, α) ^{7}Li Nuclear Reaction

Recently, technical progress has been made in the use of a (n, α) nuclear reaction with the stable isotope of boron—^{10}B. Initial studies on the distribution and compartmentalization of boron are beginning to appear in the literature

(Martini and Thellier, 1975; Thellier *et al.*, 1979). The distribution of boron was associated with four classical compartments: the free space (including easily dissociable borate monoesters), the cytoplasm, the vacuole, and the cell wall (as stable borate diesters) (Thellier *et al.*, 1979). The use of the ^{10}B (n, α) ^{7}Li nuclear reaction for *in vivo* studies on the localization and redistribution of boron between the cellular compartments under different physiological conditions might shed an important new light on our understanding of the biological role of boron.

17.1.8 Therapeutic Uses for Boron and Organoborates in Medicine

The idea that boron or organoborate compounds may prove efficacious in cancer therapy originated from the following observations: (1) ^{10}B is present in nature at about 20% of the concentration of ^{11}B and was not toxic to living tissues; (2) slow neutron bombardment (an average speed of 2200 m/sec with an energy of only 0.025 eV) was not harmful to living tissues; and (3) used together, the nucleus of ^{10}B absorbed slow neutrons undergoing fission to yield ^{7}Li and an α particle that share an average energy of 2.4 MeV.

The ^{10}B isotope is uniquely suited for the treatment of cancer by thermal neutron capture for several reasons. It has a large cross-section capture, 3850 barns (1 barn = 1×10^{-24} cm^2). This is significantly greater than the cross-section capture of ^{11}B and of all the common elements of normal cells (Soloway, 1964). Thus, if the malignant tissue can be enriched in ^{10}B or simply in boron compounds (which, in nature, are 20% ^{10}B), the destruction of malignant cells is possible with a minimal number of nuclear reactions occurring with those elements that are usually found in greatest abundance within a cell. Second, the size of the particles (lithium and helium nuclei) and the energy (2.4 MeV) released in the ^{10}B (n, α) ^{7}Li reaction limits the radius of action to 9 μm from the site of ^{10}B disintegration. For comparison, the sphere of travel for these particles would be about the size of a red blood cell. Thus, the radiation would be limited to the one cell containing ^{10}B and its immediate neighbor cells (Soloway, 1964). This is also important for another reason. If boron enrichment of tissue could not be achieved by an organ- or tissue-specific process, but only through the general enrichment of all tissues in a patient, this would not be a severe limitation to the use of ^{10}B-neutron capture therapy. This is because the area irradiated can be highly localized, and the reaction itself is of limited sphere (Kliegel, 1972). This makes ^{10}B more suitable than several other nonradioactive isotopes that have larger cross-section capture values than ^{10}B but produce gamma radiation when they undergo neutron capture (Soloway, 1964).

With neutron therapy in mind, researchers have synthesized and tested a number of organic and inorganic boron compounds in addition to borax and

boric acid (Kliegel, 1972). The necessary prerequisites for use of any of these compounds in cancer therapy in human patients are paraphrased from Soloway's (1964) guidelines:

1. To permit injection, the boron compound must have sufficient solubility in water, in the ideal case at pH 7.4, so that the physiology of the system is minimally disturbed.
2. The boron compound must be stable so that *in vivo* no oxidation or hydrolysis occurs.
3. Because 50 mg of boron per kg of tumor tissue is necessary for the ^{10}B (n, α) ^{7}Li reaction to be effective, the boron compound must have low toxicity or high boron content.
4. In order to obtain maximum destruction of tumor cells, it is desirable for the tumor cells to have a greater concentration of boron than the normal cells. Since cancerous cells grow at a more rapid rate than normal cells, intracellular or intranuclear enrichment can be achieved by using boron-containing pyrimidines, purines, or amino acids. Such enrichment would maximize the destruction of the chromosomes of cancer cells.
5. Because the dosage of boron or boron-containing compounds must be high to be effective, it is desirable that the source of boron or resulting complexes be colorless.

From studies employing animals, nine compounds have been identified that have low toxicity and give the desired enrichment of cancer cells with boron. One is a boric acid ester with triisopropanolamine, six are arylboric acid derivatives, and two are borohydrides of the $B_{10}H_{10}$ type (Kliegel, 1972). The latter, with their great stability, relatively low toxicity, and high boron content, are especially attractive for use in the treatment of cancer by ^{10}B-thermal neutron capture (Kliegel, 1972). One of the borohydrides, $Na_2B_{10}H_{10}$, is excreted chemically unaltered, demonstrating that this compound cannot be metabolized by mammals—a property that makes it almost perfect for ^{10}B-neutron irradiation therapy.

The idea of using boron-containing substrate analogs in cancer therapy, either in ^{10}B-thermal neutron capture or as metabolic inhibitors, has also been explored with some success. In clinical tests, the replication of herpes simplex virus type 1 was inhibited 57 and 97% by 5-dihydroxyboryl-2′-deoxyuridine, respectively, at concentrations of 200 and 800 μM (Schinazi and Prusoff, 1978).

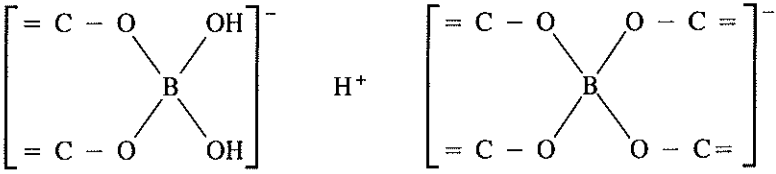
17.2 Carbohydrate Metabolism

Of the various roles that have been assigned to boron in plant growth and development, the ones that have been studied most are those that involve the

element in carbohydrate synthesis, translocation, transformation, and utilization. For vascular plants it has been proposed that boron has a role in starch synthesis, respiration, photosynthesis, carbohydrate translocation, cellulose synthesis, phenol biosynthesis, sugar-phosphate metabolism, and a number of carbohydrate-involved growth and developmental processes. In this section the various roles of boron in carbohydrate metabolism are briefly reviewed. For additional information see Dugger (1973, 1983) and Augsten and Eichhorn (1976).

17.2.1 Boron Complexes

Boron is one of the chemical elements, along with aluminum, germanium, and several others, that will form complexes with certain organic compounds. The ability of boron to complex with compounds is dependent on their having adjacent OH-groups in the cis position. Thus, sugars, sugar alcohols, and other compounds in plants having this configuration form complexes with boron; however, attempts to isolate such boron complexes from plants have failed. The type of complexes formed between boron and polyhydroxy compounds depends on the ratio of borate to the diol as well as the pH. In plants with the normal physiological level of boron, it has been proposed that the type of complex formed when the diol to borate ratio is high is the BD_2 type, while the form that exists under a low diol to borate ratio is the BD type (see diagrams).



(BD) (BD_2)

Compounds that have ring configurations other than cis may also form complexes with boron. For example, boron may complex with compounds having trans 1,2-diol groups; the important factor seems to be the angle between the OH-groups relative to the carbon axis (Zittle, 1951).

This ability of boron to complex with a large number of biologically important substances may alter the involvement of those substances in the metabolic reactions of plants. Boron might complex with substrate molecules, end products, or enzymes themselves, thereby inhibiting or stimulating a metabolic pathway involving one or more of these modified molecules. In turn, this altered metabolic pathway may cause a change in the level of a particular metabolite that could

alter a subsequent metabolic reaction. This, in turn, may alter a more obvious developmental process or the growth of the plant.

17.2.2 Sugar Translocation

Since many of the polyhydroxy compounds in plants that have the proper configuration of OH-groups are carbohydrates, much of the early research investigated the possible roles of boron in carbohydrate metabolism. There is evidence that boron deficiency in plants leads to an accumulation of sugars and starch in leaves (Gauch and Dugger, 1954; Dugger, 1973). It was pointed out that (1) such deficiency also altered the types of carbohydrates that accumulated; (2) such accumulation resulted from a breakdown of the phloem; and (3) in some fashion, boron was involved with the translocation of carbohydrates in plants. All of these proposals have been substantiated by research in a number of laboratories and over a number of years.

A primary question involves whether boron has a direct or indirect effect on sugar transport in the phloem. Skok (1958) believed that there was "some relationship between boron and sugar translocation," but since sugar-boron complexes have not been isolated from phloem sap or plant tissues, perhaps the relationship is "indirect." However, a number of reports indicate a possible direct and early effect of boron on sugar movement (Gauch and Dugger, 1954; Sisler *et al.*, 1956) and on the sugar-dependent movement of plant hormones from leaves of bean plants (*Phaseolus vulgaris*) to the hypocotyl (Mitchell *et al.*, 1953).

17.2.3 Photosynthesis

Earlier reviews on boron point out that there is little data to support a direct role for boron in photosynthesis. More recent work demonstrated that a suboptimal level of boron caused an increase in the photosynthetic rate of *Wolffia arrhiza* (Eichhorn and Augsten, 1974). It has also been reported that an increase in photosynthetic rate occurred in the marine alga *Cylindiotheca fusiformis* when the cultures contained no added boron (Smyth and Dugger, 1980). In both of these cases, there was an increase in the level of potassium in the boron-deficient cells. Subba Rao (1981) observed that nanoplankton responded to boron. During the winter months, when the temperature was low and there were more nitrates, phosphates, and silicates available, the addition of boron caused an increase in the rate of carbon assimilation. When these nutrients were lower and the tem-

perature higher during the warmer part of the year, added boron caused no positive response in the photosynthetic rate.

Augsten and Eichhorn (1976) pointed out that boron stimulated noncyclic photophosphorylation, but boron deficiency induced a reduced level of photophosphorylation in isolated chloroplasts.

17.2.4 Respiration

The earlier literature regarding the direct influence of boron on respiration is contradictory. (For review of this literature see Gauch and Dugger, 1954; Dugger, 1973, 1983.) Since boron deficiency results in the accumulation of starch and other carbohydrates, an increase in tissue respiration would be expected to result. However, when boron deficiency intensifies, respiration decreases to values below that of the controls. Augsten and Eichhorn (1976) pointed out that under boron deprivation, there is an increase in the level of phenolic compounds and an activation of phenol oxidases as the ratio of substrate metabolized via the glucose-monophosphate pathway increases.

When bean leaf tissue was infiltrated with glucose and various levels of boron from 5 to 100 μM, there was an increased level of O_2 uptake after 24 hours. In some way, boron enhanced the utilization of glucose supplied to the leaf tissue (Dugger *et al.*, 1957).

17.2.5 Starch

Winfield (1945) reported a slight inhibition of starch phosphorylase by boron. He proposed that boron reacted with sugars in the same way as phosphorus did; however, because of the low boron-to-phosphorus ratio in plants, he did not believe that boron influenced the starch $\rightleftarrows$ glucose-1-phosphate equilibrium. On the other hand, others (Dugger *et al.*, 1957; Scott, 1960) reported that boron inhibited the *in vitro* conversion of glucose-1-phosphate to starch catalyzed by starch phosphorylase. It was proposed that this enzyme was not inhibited in boron-deficient plants, and therefore starch accumulated. Scott (1960) proposed that "boron performs a protective function in plants, in that it prevents excessive polymerization of sugars at the site of sugar synthesis. . . ."

Augsten and Eichhorn (1976) proposed that starch synthesis is stimulated in boron-deficient plants by stimulation of the enzyme that converts glucose-6-phosphate to glucose-1-phosphate and by concomitant inhibition of the enzyme that synthesizes UDP-glucose from glucose-1-phosphate. Considering that the

pathway for starch synthesis is now thought to involve the sugar nucleotide ADP-glucose, this proposal seems more feasible. The authors also pointed out that steric hindrance would make the formation of a complex between boric acid and glucose-1-phosphate impossible. Regardless of the exact mechanisms involved, it is generally agreed that starch does accumulate in boron-deficient plants.

17.2.6 Cellulose and Cell Wall Glucans

Cellulose and other wall polymers constitute the largest fraction of carbohydrates in plants. If boron has a role in carbohydrate metabolism, it would seem that the synthesis of these polysaccharides would be affected. Spurr (1957) reported that the structure of the cell wall of boron-deficient celery plants was altered. He concluded that boron affected not only the rate but also the actual process by which carbohydrates were incorporated into cell walls. During *in vitro* culture of parenchyma cells from tobacco pith, boron deficiency caused a two-fold increase in the cell wall content; the largest change was noted in the galactan fraction (Wilson, 1961).

^{3}H-*myo*-inositol was incorporated at a higher rate into the D-galacturonosyl and L-arabinosyl components of pectin material in pollen tubes when the germinating medium contained boron (Stanley and Loewus, 1964). The authors suggested that boron has a role in the synthesis of pectin materials of germinating pollen, possibly related to the synthesis of D-galacturonosyl units. Others have observed (1) an increase in cell wall materials in *Lemna minor* and *Ginkgo* pollen-derived tissue cultured *in vitro*, and (2) disorganized microfibrillar structure of the cell walls of boron-deficient sunflower plants (Dugger, 1983).

In oil palm (*Elaeis guineensis*) leaves, and sunflower (*Helianthus annuus*) and soybean (*Glycine max*) plants, the level of pectic and hemicellulose substances was higher when plants were subjected to boron deficiency (Yamanouchi, 1973; Rajaratnam and Lowry, 1974). Callose, a β-1,3 glucan, accumulated in bean (*Phaseolus vulgaris*) and cotton (*Gossypium hirsutum*) plants subjected to boron deficiency (Van de venter and Currier, 1977). In addition, Timashov (1977) observed that boron-deficient sunflower root tips incorporated more ^{14}C-glucose into the total cell wall fraction than control root tissue.

Morphological changes were observed at the ultrastructural level in cell walls and cellular organelles of boron-deficient roots (Kouchi and Kumazawa, 1976). They reported an increase in the pectin and hemicellulose fractions but a decrease in the cellulose fraction under boron deficiency. It was proposed that abnormal activity of the Golgi apparatus caused the alteration in cell wall synthesis. The hydroxyproline level in the cell wall fraction of sunflower roots was observed to increase when plants were cultured with a low boron level (Shive

and Barnett, 1973); others, however, have not observed this change (Troitskaya *et al.*, 1975).

When UDP[^{14}C]-glucose was supplied to cotton fibers attached to ovules cultured *in vitro*, a large fraction of the substrate was incorporated into cell wall glucans. Although most of these glucans were not β-1,4 linked, culturing the ovules with boron in the medium did cause more of the label to appear in the cellulose fraction (Dugger and Palmer, 1980).

Augsten and Eichhorn (1976) pointed out that the growth-promoting activity of phenylboric acid derivatives results from the complexing of organic borate with cell polysaccharides. They proposed that boron controls the stabilization of the cell by forming these complexes. A correlation was observed between the complexing ability of organoborate compounds and their growth-promoting ability: the BD_2 type links the polyhydroxy-containing molecules that exist between the fibrillar chains and thus reduces elasticity, whereas the BD type forms between boron, or phenylboric acid derivatives, and wall polysaccharides and does not reduce elasticity.

17.2.7 Phenols

Boron deficiency in plants generally leads to a buildup of phenolic compounds (see reviews by Dugger, 1973, 1983; Augsten and Eichhorn, 1976). However, there is no consistent agreement as to whether this response is a primary or secondary one. Boron deficiency in celery (*Apium* sp.) resulted in an induced fluorescence of the stem tissue, a response that Spurr (1952) suggested was due to an increased level of caffeic and chlorogenic acids. Others (Dear and Aronoff, 1965) suggested that the increase in caffeic acid caused tissue necrosis; caffeic acid or its metabolite caused a breakdown of conductive tissue in the plant, bringing about death of the tissue. Others have also observed the accumulation of fluorescent products, presumably phenols, in boron-deficient plants (Watanabe *et al.*, 1961, 1964; Shkol'nik, 1974; Rajaratnam and Lowry, 1974).

This observed increase in the level of phenolic compounds in boron-deficient tissue has led Shkol'nik to propose that such accumulation is the "original cause of plant death during boron starvation" (Shkol'nik, 1974). In his hypothesis he pointed out that monocots have a lower requirement for boron than dicots and did not accumulate phenols to the same degree as boron-deficient dicots. The subsequent accumulation of auxin (IAA) in boron-deficient dicots resulted from the accumulation of phenols that inhibited IAA-oxidase. While others have observed hyperauxiny in boron-deficient tissue, some investigators (Bohnsack, 1974; Hirsch and Torrey, 1980; Hirsch *et al.*, 1982) do not agree with the

hypothesis that boron deficiency is accounted for by altered IAA levels (Section 17.3).

17.2.8 Lignin

Because in plants boron is thought to regulate the metabolic pathways leading to phenol synthesis, it would seem appropriate that the element be involved in the subsequent pathway leading to the synthesis of lignin. In fact, Lewis (1980a) proposed that boron's primary role in plants is in the biosynthesis of lignin and the differentiation of xylem tissue. In his interesting hypothesis, he points out that p-coumarate and ferulate, branch points in the biosynthesis of lignin, can be either directly reduced to two lignin precursors (p-coumaryl alcohol and coniferyl alcohol) or hydroxylated to the corresponding O-diphenols (caffeate and hydroxyferulate). With sufficient boron in plant tissues, the element complexes with these intermediates and subsequent methylation occurs in the process of lignin synthesis. However, under boron deficiency, utilization of the intermediates of the pathway is reduced, so they accumulate. This hypothesis generally agrees with published results (see reviews by Dugger, 1973, 1983; Augsten and Eichhorn, 1976). Other related compounds, such as leucoanthocyanins, flavonols, flavonones, and flavonol-3-glucosides, have been reported to accumulate in boron-deficient plant tissue (Rajaratnam and Lowry, 1974; Shkol'nik and Abysheva, 1975).

Lewis (1980a) discussed the interrelationships among boron, lignification, peroxidase enzymes, and auxin in plants. In addition, he pointed out the similarities by which wounding a vascular plant, microbial infection of the plant, and boron deficiency bring about changes in the levels of phenolic compounds and in IAA metabolism (Lewis, 1980b).

17.2.9 Boron in Enzymic Reactions

As pointed out in earlier reviews (Dugger, 1973, 1983; Augsten and Eichhorn, 1976), boron has not been shown to be a cofactor or specific component of any enzymic reaction. However, many reports, in both *in vivo* and *in vitro* systems, have shown that the element, or lack of it, alters the rates of many enzymic reactions. Reed (1947) reported that boron deficiency caused an increase in the activity of catechol oxidase. The products of this oxidation, quinones, resulted in the accumulation of phenols in boron-deficient celery plants.

It has also been shown that boron-deficient tissue had a more active polyphenol oxidase than the control tissue (MacVicar and Burris, 1948) and that boron was a competitive inhibitor of xanthine oxidase (Roush and Norris, 1950).

Later it was reported that the element also inhibited the oxidation of tyrosine (Yasunobu and Norris, 1957), was a competitive inhibitor of alcohol dehydrogenase (Roush and Gowdy, 1961), and was an inhibitor of alkaline phosphatase activity in milk and intestinal mucosa (Zittle and Della Monica, 1950).

More recent work has shown that catechol oxidase, polyphenol oxidase, tyrosinase, peroxidase, and IAA oxidase are inhibited by boron (Odhnoff, 1957; Parish, 1968, 1969; Shive and Barnett, 1973; Eichhorn and Augsten, 1974). In a technique developed by Alvarado and Sols (1957), boron was used to complex with fructose-6-phosphate, inhibiting phosphoglucose isomerase and making it possible to assay for phosphomannose isomerase. It has also been reported that boron will inhibit starch synthesis by inhibiting starch phosphorylase (Winfield, 1945; Dugger *et al.*, 1957; Scott, 1960), and Loughman (1961) reported that phosphoglucomutase is inhibited by boron.

Dugger and Humphreys (1960) reported that boron stimulated the synthesis of UDP-glucose catalyzed by UDP-glucose pyrophosphorylase. The authors suggested that the latter result was probably because boron stimulated the conversion of UTP and glucose-1-phosphate to UDP-glucose. In cell-free preparations from pea seeds (*Pisum sativa*) and sugarcane seedlings (*Saccharum officiarum*,) which contain several of the enzymes leading to sucrose synthesis, it was observed that boron in the reaction mixture stimulated the synthesis of sucrose when the substrates UDP-glucose and fructose were provided (Dugger and Humphreys, 1960). Teare (1974) has reported that the level of UDP-glucose in the roots of boron-deficient bean plants is much lower than that in the roots of boron-sufficient plants. For the growth of cotton fibers on ovules cultured in a defined medium, boron is an absolute requirement. Without boron in the medium, growth was typified by a rapid proliferation of undifferentiated cells (callus) compared to the normal growth of ovule epidermal cells into the fiber cells when boron was provided. Boron deficiency in such a system reduced the activity of the pyrimidine pathway; however, there was no reduction in nucleic acid biosynthesis, since callus production occurred. It was suggested that UDP-glucose synthesis was reduced in boron-deficient tissue; therefore cellulose synthesis from this substrate was also inhibited (Birnbaum *et al.*, 1977). The incorporation of ^{14}C-orotic acid into UDP-glucose by cotton fibers growing on ovules cultured at a suboptimal boron level was less than that in fibers cultured at an optimal level. A larger fraction of the ^{14}C-orotate was incorporated into the RNA fraction of the fibers grown at the lower level of boron (Wainwright *et al.*, 1980).

Cresswell and Nelson (1973) have reported that the level of α-amylase activity in germinating seeds of *Themeda traindra* was higher if the germinating medium contained boron, and the level of β-amylase in leaves and stems of sugarcane plants was lower if the plants were grown under a low-boron regime (Zapata, 1973).

Lee and Aronoff (1967) demonstrated that the substrate 6-phosphogluconate

complexed with boron, thereby inhibiting 6-phosphogluconate dehydrogenase. Without boron in the *in vitro* reaction, the oxidative decarboxylation of 6-phosphogluconate to ribulose-5-phosphate occurred; subsequent reactions of the pentose phosphate pathway were also stimulated. The increase in the amount of substrate metabolized via this pathway and the decrease in that metabolized via the TCA cycle resulted in the synthesis of more phenolic acids when boron was absent. Lee and Aronoff also proposed that the phenolic compounds may in turn complex with boron. This decreased the boron available for regulating 6-phosphogluconate dehydrogenase even more, thereby setting in motion the autocatalytic synthesis of phenolic acids. Shkol'nik and Il'inskaya (1975) reported an increase in the amount of this enzyme in several boron-deficient dicots. Lewis (1980a) proposed that as a result of the complexing of borate with caffeate, the caffeate $\rightleftarrows$ caffeoyl *o*-quinone reaction is prevented, and the substrate electron donor reduces the enzyme involved in the shuttle directly, thereby inhibiting catechol oxidase activity. As Lewis previously pointed out, the complexing of borate with intermediates of the lignin biosynthetic pathway regulates the conversion of p-coumarate to lignin precursors.

Smith and Johnson (1976) reported that borate inhibited alcohol dehydrogenase from yeast; borate as $B(OH)_4^-$ was competitive with NAD^+ rather than NADH. Although boron is not generally thought to be a constitutive part of any specific enzyme, acid phosphatase purified from sweet potato tubers contained boron along with manganese, magnesium, and silicon (Uehara *et al.*, 1974).

Other plant enzymes were reported to be regulated in some fashion by borate: (1) β-glucosidase activity in several dicot species was increased by boron deficiency (Maevskaya *et al.*, 1974, 1975, 1977), although not observed to increase in boron-deficient diatom cells (Smyth and Dugger, 1981); (2) the activities of ATPase and acid phosphatase were higher in boron-deficient tissue with a decrease in alkaline pyrophosphatase activity (Hinde and Finch, 1966) and reduced amino acid-dependent ATP pyrophosphate exchange (Hinde *et al.*, 1966); (3) RNAase increased due to boron deficiency (Chapman and Jackson, 1974; Sherstnev, 1974; Dave and Kannan, 1980); and (4) KCl-stimulated ATPase activity from *Z. mays* was decreased by boron deficiency (Pollard *et al.*, 1977).

17.2.10 Pollen Germination

In a previous review of the earlier literature, Gauch and Dugger (1954) referenced a large number of papers on this subject as well as on the flowering and fruiting of plants. Schmucker (1932) was one of the earlier investigators to report the several interesting effects resulting from the omission of boron from the artificial medium for germinating pollen: (1) the number of pollen grains that germinated was reduced; (2) pollen tubes that did form were short and

malformed; and (3) a high proportion of the pollen tubes burst. Schmucker proposed that the ability of boron to complex with the hydroxyl-rich compounds of the pollen tube wall (cellulose and pectic materials) in some way regulated the synthesis of wall materials in the growth of pollen tubes. Reference has also been made to the possibility that boron may regulate the uptake of water by germinating pollen (Skok, 1958; Dugger, 1973, 1983; Augsten and Eichhorn, 1976). If so, regulation may be at the cellular membrane level rather than in the development of the pollen tube wall.

O'Kelley (1957) observed that the absorption of sugars and the uptake of oxygen by germinating pollen were stimulated by boron. However, he proposed that the effect of boron on pollen tube elongation was not related to the absorption of sugar or the increase in respiration.

Kumar and Hecht (1970) reported that the addition of boron to the styles of *Oenothera organensis,* which is self-incompatible, enhanced pollen tube growth. Utilization of endogenous sugar and a decrease in callose in the styles were both influenced by boron. Stanley and Loewus (1964) reported that boron played a "definite role in pectin synthesis in germinating pollen" of *Pyrus communis;* Samorodov and Golubinskii (1978) also observed that boron stimulated pear pollen tube growth, and Dickinson (1978) observed that boron stimulated growth of *Lilium longiflorum* pollen tubes.

Plant hormones such as IAA and gibberellic acid did not enhance pollen germination or pollen tube growth. Vaughan (1977) reported that the tassels on boron-deficient *Zea mays* plants did not produce viable pollen, and the silks of these plants were not receptive to compatible pollen.

17.2.11 Conclusions

The wide array of observed plant responses to boron deficiency indicates that the element is probably involved in a number of metabolic pathways or a cascade effect; therefore, regulating metabolic processes somewhat as has been proposed for plant hormones. Thus, regulation by boron occurs because of the ability of this element to complex with the large number of OH-rich compounds in plants and not because the element is involved directly in a specific metabolic reaction (Augsten and Eichhorn, 1976; Dugger, 1983).

17.3 Hormone Action

Although the exact role of boron has not been elucidated, there persists a common premise on which many investigators agree—boron is essential for the normal growth and functioning of apical meristems (a partial list includes Chap-

man and Jackson, 1974; Kouchi and Kumazawa, 1975a; Bohnsack and Albert, 1977; Cohen and Lepper, 1977; Hirsch and Torrey, 1980; Lovatt *et al.*, 1981; also see reviews by Dugger, 1973, 1983; Augsten and Eichhorn, 1976). This led to experiments that examined the relationship between boron and plant growth regulators. The preponderance of this work has emphasized the relationship between boron and auxins for the following reasons:

1. Auxin, as IAA, is the principal growth hormone of higher plants.
2. Meristems have long been established as sources of IAA. The shoot apical meristem is the major site of free auxin formation from bound auxin precursors (free IAA is considered the metabolically active form).
3. Boron is essential for the normal growth and functioning of apical meristems.
4. Growth and geotropism have been shown to correlate with free auxin levels.
5. Normal growth and geotropic response are impaired in boron-deficient plants.
6. Adventitious root development is stimulated synergistically by the combined application of boron and auxin.

In addition to reduced growth at the root and shoot apices, boron-deficient broccoli (*Brassica oleracea*) and squash (*Cucurbita pepo*) plants exhibited a loss in geotropic response (Alexander, 1942; Bohnsack and Albert, 1977). Eaton (1940) suggested that boron-deficiency symptoms were those that would be expected in plants deficient in auxin. He successfully restored growth to boron-deficient cotton seedlings by spraying them with IAA.

A relationship between boron and auxin has also been demonstrated by other workers. Weiser (1959) reported that boron or auxin stimulated the rooting of two-node sections of *Clematis* sp., which was consistent with Eaton's hypothesis that boron-deficient plants suffered from hypoauxiny. Weiser also observed a synergistic effect when these two compounds were used together. Not only were more roots produced, but the rate of rooting was accelerated. Cuttings were soaked for 12 hours in boron (50 mg/liter) or in indolebutyric acid (IBA) (50 mg/liter) or in both, and then transferred to vermiculite. At the end of 32 days, cuttings treated with both boron and IBA had three times more roots than those treated with boron or IBA alone. It took an additional 22 days for the latter cuttings to produce as many roots. Synergism between IAA and boron on the rooting of *Phaseolus* cuttings was demonstrated by Gorter (1958). A boron concentration of 10^{-6} *M* was found to be optimal; and at this concentration, rooting increased proportionately with increased concentrations of auxin.

More recent work on adventitious root development in cuttings of *Phaseolus aureus* has provided some clarification of these earlier observations. No roots developed in cuttings of *P. aureus* without exogenous boron even when cuttings

were soaked for 24 hours in 10^{-4} *M* IBA. Provision of boron at suboptimal concentrations severely limited both root number and root length (Middleton *et al.*, 1978). Cuttings pretreated by soaking in 10^{-4} *M* IBA without added boron for 24 hours accumulated soluble sugars (sucrose, glucose, and fructose) in the hypocotyl, but no roots developed. IBA was shown to stimulate the translocation of ^{14}C-sucrose from leaves to the hypocotyls within 24 hours. Since root development does not occur without boron, the translocation of sucrose was independent of a root sink (Middleton *et al.*, 1980).

Other workers have also demonstrated that auxin stimulates sugar translocation to shoot and root apices. Using *P. vulgaris* L., var. "Black Valentine" bean plants, Dyar and Webb (1961) demonstrated that auxin applied to the apical bud restored sugar translocation to a level greater than that in the boron-sufficient plants. Auxin applied to roots increased sugar translocation to buds and roots to rates 2 and 10 times greater, respectively, than those observed for these organs in boron-sufficient plants. However, it was shown that sucrose cannot fulfill the requirement for boron in normal root growth (Whittington, 1959; Neales, 1960; Albert and Wilson, 1961).

From this work it appears that in the absence of roots, auxin maintains the polar transport of carbohydrates to the cut end of the hypocotyl. It is not clear if auxin alone, boron alone, or both are required for initiation of meristematic activity during the early stages of formation of root primordia; but it is clear that boron is required for their subsequent organization and further development.

Shkol'nik *et al.* (1964) measured the level of IAA in corn and sunflower tissues. They found a decrease in the content of free auxin in the boron-deficient shoot and root apices that paralleled the decrease in growth. In both species there was an increase in the bound auxin content of the boron-deficient plants. Bound auxin is not usually found in apices, while free auxin is localized predominantly in this region. Young leaves provide the bound form as a precursor to free auxin that is formed in the apices. Shkol'nik's results suggest that this conversion is not occurring in boron-deficient apices. The authors investigated the possibility that the low level of free auxin in boron-deficient plants was due to its destruction by increased IAA oxidase activity. However, the activity of this enzyme was found to decrease under conditions of boron deprivation.

Crisp *et al.* (1976) found no difference in the content of IAA in the leaf margin of lettuce (*Letuca sativa*) plants grown in vermiculite at 0.001 mg boron per liter relative to control plants grown at 1.0 mg boron per liter, until the plants were 66 days old.

In a very thorough investigation, Smirnov *et al.* (1977) measured both free and bound IAA in roots and shoots of sunflower, bean, corn, and wheat (*Triticum vulgare*) hydroponically cultured in Knop's nutrient solution with boron (0.5 mg per liter) and without. Plants were harvested for analysis when initial symptoms of boron deficiency appeared for a given species. The results of this study clearly

demonstrated that the influence of boron deprivation on IAA metabolism was different in the various tissues of the same plant and for the same tissues in the various species studied. Boron deficiency led to decreased levels of free IAA in shoots of sunflower, bean, and corn, but not wheat. In boron-deprived roots, free IAA decreased in corn but increased in bean and wheat. Under boron deprivation, bound IAA decreased in the shoots of sunflower, corn, and wheat, but increased in shoots of bean. Roots from the boron-deprived bean and wheat had significantly more bound IAA than the control plants.

Bohnsack and Albert (1977) demonstrated that the symptoms of boron deficiency can be induced by hyperauxiny in boron-sufficient squash plants (*Cucurbita pepo*) by the addition of 10^{-6} *M* IAA. Under conditions of boron deprivation or hyperauxiny, root elongation was inhibited, accompanied by swelling of the root tip; lateral root primordia were initiated near the root apex; a loss in geotropic response occurred; and IAA oxidase activity in apical and subapical root sections increased approximately 6–9 hours after boron was withheld, or 3–6 hours after IAA was added to the boron-sufficient medium. Thus, these authors concluded that boron-deficient plants may actually suffer from toxic effects of hyperauxiny. In addition, Bohnsack (1974) tested the possibility that boron-deficiency symptoms may actually be the result of toxic levels of ethylene synthesized in response to the state of hyperauxiny. Ethylene evolution in boron-deficient *C. pepo* roots decreased to 25% of the level in the boron-sufficient control within 24 hours after transfer of the 5-day-old plants to medium without added boron. On the other hand, boron-sufficient tissue of the same age treated with 10^{-6} *M* IAA showed a 63% increase in ethylene evolution over that in the boron-sufficient control. Thus, boron-deficiency symptoms are not due to increased ethylene biosynthesis, while auxin-induced boron deficiency-like symptoms may very well be a result of increased ethylene production. Boron deficiency and hyperauxiny both cause inhibition of root elongation, but apparently not for the same physiological reasons.

This conclusion was confirmed by Hirsch *et al.* (1982), who used electron microscopy to show that changes in sunflower root tissue characteristic of boron deprivation were not the same as those induced by IAA. The only apparent change in auxin-treated root cells was an increase in electron-dense material within the vacuoles; there was no increase in cell wall thickness, which is characteristic of boron-deficient roots. Consistent with the ultrastructural results, the levels of free IAA determined by use of a very sensitive radioimmunoassay were similar in both the boron-deprived and boron-sufficient root tips. Although boron deficiency and hyperauxiny elicit many similar responses in roots, it appears that they are not the same.

In light of the many and varied responses of auxin metabolism to boron deprivation, a single unifying hypothesis concerning a regulatory role for boron relative to the metabolism of this hormone seems unlikely.

17.4 Membrane Structure and Function

The hypothesis that was presented earlier to explain the observed effect of boron on sugar translocation in plants suggested that boron was perhaps associated with cell membranes. As a constituent of membranes, the element reacted chemically with the sugar molecules, and the resulting complex was transported across the membrane; a subsequent reaction on the inside of the membrane released the sugar into the cytoplasm (Gauch and Dugger, 1953). No experimental evidence was provided to substantiate the presence of boron in cell membranes. However, Shkol'nik and Kopman (1970) reported that the apical meristems of boron-deficient sunflower plants contained a lower level of phospholipids than control tissues. They proposed that since phospholipids occur in intracellular membranes, boron was apparently involved in the ultrastructural organization of meristem cells in roots. Hirsch and Torrey (1980) observed ultrastructural changes in the cellular membranes of sunflower roots after 6 hours in a boron-deficient medium.

Dave and Kannan (1980) observed that RNAase was enhanced in *Phaseolus vulgaris* under boron deficiency. They suggested that since RNAase is located in cellular membranes, such an enhancement indicated an alteration or disruption of membrane permeability.

Robertson and Loughman (1973, 1974a,b) carried out a series of experiments to study the effects of boron deficiency on inorganic ion flux by *Vicia faba* roots. In addition to reducing root elongation, removing boron from the growth medium reduced the uptake of ^{86}Rb. In fact, the terminal centimeter of roots showed a marked reduction in ^{86}Rb uptake. Older root tissue (2–3 cm from tip) from boron-deficient plants showed a marked increase in absorption when compared to the control root tissue. Adding boron to the growth medium before the start of the ^{86}Rb absorption period did not restore the capacity to absorb ^{86}Rb to the terminal centimeter of root tissue. In a similar study measuring the uptake of ^{32}P-labeled phosphate, these authors showed that boron-deficient root tissue exhibited a reduction in ^{32}P uptake, as had also been observed for the ^{86}Rb studies. However, the effect of boron deficiency on phosphate absorption was reversed by adding boron 1–2 hours before the ^{32}P-phosphate uptake was initiated. The authors suggested that the observed effect may relate to a rapid restoration of the phosphate carrier system in the membranes, with boron involved as a component of the system. A later study from the same laboratory (Pollard *et al.*, 1977) showed that in both *Vicia faba* and *Zea mays*, the rate of absorption of ^{32}P-phosphate was decreased by boron deficiency; but it was rapidly restored to approximately the same rate of the control by the addition of 10^{-5} *M* boron 1 hour before the absorption study began. In *Z. mays*, the boron-deficient reduction of Cl^- and Rb^+ ion absorption could also be restored by the 1-hour pretreatment with boron. There was a one-third reduction in the activity of KCl-

stimulated ATPase, a membrane-bound enzyme, in boron-deficient *Z. mays* root tissue. This activity could also be restored by the 1-hour pretreatment with boron. The authors suggested that the results support the view that boron is required for "efficient operation of the membrane transport system" and that the influence is direct, rather than an effect of the accumulation of metabolic intermediates.

In more direct studies on the influence of boron on membrane functions, Tanada (1974, 1978, 1982) reported that boron affected the bioelectric field potential of excised mung bean (*Phaseolus aureus*) hypocotyls from seedlings grown in the dark and irradiated with red light. The author suggested that this observed effect was induced by a modification of some membrane component when boron was present. Further studies also showed that boron stimulated the translocation of fluorescein in mung bean hypocotyl sections following gravitational or red irradiation stimulation. In addition, boron was necessary for the induction of the delaying action that irradiation at 710 nm has on the nyctinastic closing of *Albizzia julibrissin* pinnules. Tanada has suggested that boron was required to stabilize the positive electrostatic charge in the plasma membrane that was generated by the action of gravity and phytochrome.

It has been shown in diatom cells (*Cylindrotheca fusiformis*) (Smyth and Dugger, 1980) that a change in the influx and efflux of ^{86}Rb was an early effect of boron deficiency. After 5 hours of culture in boron-deficient medium, the ^{86}Rb influx rate was reduced 20%. However, there was an accumulation of ^{86}Rb by the boron-deficient diatoms brought about by a lower efflux rate in these cells. After 24 hours the boron-deficient cells had 30% more ^{86}Rb than the control cells. These changes could not be reversed by preincubation of the diatom cells with 50 μM boron for 3 hours. This observed effect of boron deficiency on membrane function in diatoms preceded the inhibition of cell division by 10 hours.

Roth-Bejerano and Itai (1981) reported that boron enhanced KCl-induced stomatal opening in epidermal strips of *Commelina communis*. They propose that boron combines with membrane-polyhydroxy compounds, influencing the potassium influx or efflux through guard cell membranes.

These observations led many researchers (Pollard *et al.*, 1977; Hirsch and Torrey, 1980; Smyth and Dugger, 1980; Roth-Bejerano and Itai, 1981; Hirsch *et al.*, 1982) to conclude that boron may play an important role in membrane transport or in maintaining membrane integrity.

17.5 Nucleic Acid Biosynthesis

The dependence on boron for normal meristematic activity is well documented for both angiosperms (a partial list includes Chapman and Jackson, 1974;

Kouchi and Kumazawa, 1975a; Bohnsack and Albert, 1977; Cohen and Lepper, 1977; Hirsch and Torrey, 1980; Krueger *et al.*, 1979; Lovatt *et al.*, 1981) and gymnosperms (Walker *et al.*, 1955; Blaser *et al.*, 1967). To date, some of the earliest effects of boron deficiency on plant metabolism reported in the literature were observed in root meristem tissues (Chapman and Jackson, 1974; Kouchi and Kumazawa, 1975a,b, 1976; Bohnsack and Albert, 1977; Cohen and Lepper, 1977; Krueger *et al.*, 1979; Hirsch and Torrey, 1980; Lovatt *et al.*, 1981). These effects were associated with the cessation of cell division and altered nucleic acid biosynthesis.

Determining the earliest effect of boron deficiency may be a possible key to elucidating its role in plant metabolism; the earliest symptom of boron deprivation is the one most likely to be associated with the primary role of boron in the metabolism of higher plants. The earliest effects of boron deprivation are on nucleic acid biosynthesis. The incorporation of [^{3}H]thymidine into the acid-insoluble fraction of root tips of intact 5-day-old squash plants (*Cucurbita pepo*) was decreased significantly after only 6 hours of boron deprivation and was reduced 66% when boron was withheld for an additional 6 hours. Decreased DNA synthesis correlated temporally with inhibition of root elongation (Krueger *et al.*, 1979). Similar experiments using autoradiography revealed that the incorporation of [^{3}H]thymidine into root apical meristems ceased after 20 hours of boron deprivation (Cohen and Albert, 1974). When these plants were returned to a boron-sufficient medium for 12 hours, autoradiographs showed that their incorporation was indistinguishable from that of boron-sufficient root tips. These observations suggest that DNA synthesis is impaired under conditions of boron deficiency (Cohen and Albert, 1974).

Measurements of the incorporation of radiolabeled precursors into RNA provide evidence that RNA synthesis is also impaired when boron is withheld. For example, Sherstnev and Razumova (1965) reported decreased incorporation of [^{14}C]adenine into RNA of boron-deficient sunflower leaves and roots, while other workers have demonstrated that increased incorporation of [^{14}C]orotic acid (Wainwright *et al.*, 1980) and [^{14}C]uridine (Chapman and Jackson, 1974) into RNA were early effects of boron deficiency in cotton ovules (*Gossypium hirsutum*) and mung bean (*Phaseolus aureus*) root apices, respectively.

Several workers (Albert, 1965; Jaweed and Scott, 1967; Johnson and Albert, 1967; Chapman and Jackson, 1974) have reported decreased RNA content in boron-deficient roots. When nucleic acid biosynthesis, protein biosynthesis, and respiration were measured in the same system, changes in nucleic acid biosynthesis preceded (1) a reduction in protein content (Johnson, 1971); (2) a decrease in the incorporation of [^{14}C]leucine into protein and a reduction in respiration (Krueger *et al.*, 1979); and (3) a decrease in RNA content and the observed increase in RNAase activity that accompanies boron deprivation (Chapman and

Jackson, 1974). Thus, altered nucleic acid biosynthesis appears to be a primary effect of boron deprivation and not a secondary effect resulting from inhibition of either protein synthesis or respiration, or increased ribonuclease activity. Taken together, these results strongly suggest that the availability and/or utilization of purine or pyrimidine nucleotides is altered by boron deprivation.

Of particular interest is the observation that plants growing in the absence of boron can be protected from developing the symptoms of boron deficiency by the addition of a hydrolysate of yeast RNA to the nutrient solution (Shkol'nik and Soloviyova-Troitskaya, 1961). Several workers (Johnson and Albert, 1967; Johnson, 1971; Birnbaum *et al.*, 1974, 1977) tested the effects of both purine and pyrimidine bases on plant growth to determine which component(s) of the RNA hydrolysate afforded this protection. Both intact plants and isolated organs cultured in the absence of boron were protected to varying degrees from developing the symptoms of boron deficiency when pyrimidine bases were added to the culture medium.

This result was taken as evidence that the state of boron deficiency may, in fact, be a case of pyrimidine deprivation. Such an interpretation was supported by the observations that both barbituric acid and 6-azauracil, known inhibitors of pyrimidine biosynthesis (Handschumacher and Pasternack, 1958; Ross, 1964; Potvin *et al.*, 1978; Lovatt *et al.*, 1979), produced symptoms identical to those of boron deprivation (Johnson and Albert, 1967; Albert, 1968; Birnbaum *et al.*, 1977). The accumulating evidence prompted various investigators (Lewin, 1976; Birnbaum *et al.*, 1977; Wainwright *et al.*, 1980; Lovatt *et al.*, 1981) to propose that boron deficiency results in impaired *de novo* biosynthesis of pyrimidine nucleotides. Transferring 5-day-old squash plants (*Cucurbita pepo*) to boron-deficient nutrient solution resulted in cessation of root elongation within 18 hours. However, withholding boron for up to 30 hours did not result in either impaired *de novo* pyrimidine biosynthesis or a change in the sensitivity of the *de novo* pathway to regulation by end-product inhibition (Lovatt *et al.*, 1981). A shortage in available pyrimidine nucleotides could also result from an inability of boron-deficient plants to salvage or reutilize pyrimidine bases or nucleosides, or from an acceleration of pyrimidine catabolism. Boron deprivation had no significant effect on pyrimidine salvage; catabolism was slightly increased (Lovatt *et al.*, 1981). Whether a slight increase in catabolism would cause significant perturbations in the pool size of specific pyrimidine nucleotides is not known but emphasizes the need to determine the levels of the various pyrimidine nucleotides available during these two states of boron nutrition. These results argue against the hypothesis that boron-deficient plants are lacking in uridine nucleotides collectively but leave open the possibility that boron may be essential for maintaining adequate levels of one or more specific pyrimidine nucleotide species.

Two valid areas for further investigation are the possibility that boron de-

privation leads to (1) an impairment of the utilization of uridine nucleotides for the synthesis of cytidine or thymidine nucleotides or (2) an alteration in the interconversion of nucleotides for the provision of adequate levels of pyrimidine mono-, di-, and triphosphates. Preliminary measurements have been made using HPLC to determine the pool size of available nucleotides in root apical meristems excised from boron-sufficient and -deficient squash roots. Within 24 hours after 5-day-old *C. pepo* plants were transferred to boron-deficient nutrient solution, the quantity of available UMP and UDP was less, while that of UTP was greater in the boron-deficient root apices than in the boron-sufficient controls. The pool of available CMP also was less. The latter observation is consistent with the loss of available CMP in 9-day-old chick pea roots (*Cicer arietinum*) deprived of boron for 6 days (Mamedova and Rasulov, 1977).

The shift in availability of specific pyrimidine nucleotides could be due to two possibilities that remain to be investigated: (1) that the interconversions of UMP, UDP, UTP, and UDP-glucose, or synthesis of ribonucleotides or deoxyribonucleotides of cytidine or thymidine are impaired under conditions of boron deficiency, or (2) that the utilization of a particular pyrimidine nucleotide or pyrimidine nucleotide-sugar in a specific metabolic process might be impaired under conditions of boron deficiency.

The hypothesis that boron has a fundamental role in pyrimidine metabolism, through either of the two possibilities listed previously, unifies the seemingly separate roles of boron in two very different systems: the first includes dividing cells without concomitant maturation, such as meristematic cells of root and shoot apices (Chapman and Jackson, 1974; Kouchi and Kumazawa, 1975a; Bohnsack and Albert, 1977; Cohen and Lepper, 1977; Hirsch and Torrey, 1980; Lovatt *et al.*, 1981) and DNA repair in the generative cell of pollen grains (Jackson and Linskens, 1979). The second comprises elongating cells that do not undergo cell division, such as *in vitro* cotton fiber development (Birnbaum *et al.*, 1974, 1977) and pollen tube growth (Stanley and Loewus, 1964; Yih *et al.*, 1966; Vaughn, 1977). Boron deprivation has also been shown to have a marked influence on carbohydrate metabolism (Section 17.2). Dugger and coworkers (Dugger and Humphreys, 1960; Birnbaum *et al.*, 1977; Wainwright *et al.*, 1980) have provided evidence that suggests that boron deprivation results in reduced UDP-glucose formation. This would result in reduced sucrose synthesis (Dugger and Humphreys, 1960), increased starch formation (Dugger *et al.*, 1957; Scott, 1960) (Section 17.2.5), and interference with normal cell wall formation (Kouchi and Kumazawa, 1975b, 1976; Dugger and Palmer, 1980) (Section 17.2.6), all of which typify boron deficiency. Pyrimidine nucleotides are involved directly in the biosynthesis of UDP-glucose and other nucleotide sugars. Thus, the hypothesis that boron is essential to the maintenance of adequate levels of one or more specific pyrimidine nucleotide species, or to utilization of

a particular pyrimidine nucleotide in a specific metabolic process, also unifies the seemingly disparate roles of boron in nucleic acid biosynthesis and carbohydrate metabolism.

17.6 Summary

Formation of electron-deficient molecules or bridge bonds, chemical features more or less unique to boron, have been considered the possible basis for the roles that boron has in biology. Most major taxa have been screened for boron-requiring organisms, but the only evidence demonstrating that boron is essential to a taxon is limited to the Tracheophyta and a number of taxonomically distinct marine and freshwater phytoplankton species. Although there is universal agreement that boron is essential to vascular plants, over 60 years have passed since boron was demonstrated to be essential to this group, and a specific biochemical role for boron in their metabolism still remains to be elucidated. There are, however, a number of metabolic processes in vascular plants that have been shown repeatedly to be influenced by boron nutrition: carbohydrate metabolism, hormone action, membrane function, and nucleic acid synthesis.

Several researchers have interpreted the wide array of observed plant responses to boron deficiency as evidence that the element is involved in a number of metabolic pathways or in a "cascade" effect. They contend that metabolic regulation occurs because boron complexes with a large number of hydroxyl-rich compounds and not because the element is involved directly in a specific metabolic reaction (Augsten and Eichhorn, 1976; Dugger, 1983). Other researchers argue that boron does have a single biochemical role in the metabolism of vascular plants and that the primary role of boron in plant metabolism is most likely to be associated with the earliest symptom of boron deprivation occurring at the molecular level (Johnson and Albert, 1967; Krueger *et al.*, 1979; Lovatt *et al.*, 1981).

Despite the considerable amount of research designed to determine the role(s) of boron in vascular plant metabolism, definitive evidence to support a specific role has not been obtained. Most investigations, because of the lack of a radioactive or heavy isotope of boron, have been comparative studies employing boron-sufficient and boron-deficient tissues. Some of this research is compromised by failure to establish true zero boron conditions and by failure of the researchers to distinguish between early events resulting from a primary effect of boron deprivation and the later events, which are more likely to be secondary effects. Thus the controversy surrounding the role of boron in vascular plant metabolism still awaits resolution.

With the increased use of irrigated land for food and forage crop production, a concomitant increase in the instances of boron toxicity must be expected.

Boron complexes with a number of enzymes with resulting deleterious effects to plants and the animals that consume them. Finally, the unique biochemical properties of boron are being exploited for their therapeutic potential, principally through the use of organoborate compounds in chemotherapy.

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