EVOLUTION OF XYLEM RESULTED IN A REQUIREMENT FOR BORON IN THE APICAL MERISTEMS OF VASCULAR PLANTS

By CAROL J. LOVATT

Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA

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Summary

Critical review of the literature regarding the influence of boron on the metabolism of vascular plants shows that the cessation of cell division in the apical meristem is the earliest and most prevalent result of boron deficiency in vascular plants. Furthermore, the literature, in general, can be interpreted to support the view that perturbations in auxin metabolism, increased lignification, phenol accumulation and reduced sucrose transport are probably secondary effects of boron deprivation resulting from the decrease or complete cessation of plant growth. It is proposed that the acquisition of a role for boron essential to the normal functioning of the apical meristem was a consequence of the evolution of xylem and the subsequent passive transport of boron in the transpiration stream. This resulted in the accumulation of boron at the transpiration terminus, i.e. the shoot apex of a primitive vascular plant. Thus, it was in the shoot apex that boron, for the first time in the evolution of life, reached a concentration sufficient to influence the metabolism of an organism. In support of this hypothesis, evidence is presented that demonstrates that boron probably was not available in the environment at a concentration great enough to result in its being a primordial essential nutrient. The accumulation of boron in the shoot apex resulted in the acquisition of an essential role for boron in vascular plants, thereby imparting the selective advantage of preventing boron toxicity. In addition, the accumulation of boron in the shoot apex to a concentration sufficient to influence the metabolism of a eucaryotic cell presents the possibility that boron may be required by vascular plants in a metabolic pathway that is not unique to them, but one which they may have in common with non-vascular plants and, potentially, all living organisms. A possible role for boron in maintaining an adequate supply of one or more species of pyrimidine nucleotide or in facilitating their utilization is proposed.

Key words: Boron, evolution, vascular plants, apical meristems xylem.

Introduction

Among the several early articles which demonstrated that boron was essential to vascular plants, Sommer and Lipman (1926) provided evidence that boron was essential for several monocotyledonous and dicotyledonous plants, and Ludbrook (1942) established a requirement for boron in conifers. Earlier, Warington (1923) had shown that this requirement was specific for boron by demonstrating that the symptoms of boron deprivation for several angiosperms could not be alleviated by any of the 52 elements she tested. For ferns and their allies, Bowen and Gauch (1965) reported that boron was required by *Selaginella apoda* and *Dryopteris dentata*. With the exception of several diatom species (Lewin, 1966a, b), there is no evidence confirming a requirement for boron in a taxon other than the Tracheophyta. Most major plant taxa have been screened, some more rigorously than others, for boron-dependent species (Dugger, 1983); a noteworthy exception

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is the Bryophyta. Although there is universal agreement that boron is essential to vascular plants, over 60 years of research have failed to elucidate its specific role.

EVOLUTION OF BORON ESSENTIALITY AND THE PROPOSED ROLES FOR BORON IN PLANT METABOLISM

A unified theory to explain the biochemical function of boron must account for the fact that two taxonomic groups have an absolute requirement for boron while others do not. Thus, it can be concluded from the previous section that either there is a specific metabolic pathway common to both Tracheophyta and the species of Bacillariophyta requiring boron, which is generally lacking among the other taxonomic groups, or there must be at least two pathways requiring boron – one in vascular plants and another in diatoms. Existing evidence supports the second possibility. Lewin (1966a) has shown that the role of boron in the metabolism of diatoms is indeed unique to this group. Based on the silicon-to-boron ratio of the diatom frustule and the structural similarity between B_2O_3 and SiO_2, Lewin concluded that boron was an important structural component of the diatom cell wall. In vascular plants, several metabolic processes potentially unique to this group are influenced by boron nutrition; these include lignin synthesis, indole-3-acetic acid (IAA) metabolism, sucrose transport and the normal functioning of apical meristems. The essentiality of boron is critically evaluated in relation to each of these metabolic capacities.

Boron and lignin synthesis

Lewis (1980) developed a very interesting hypothesis which unified the evolution of an essential role for boron with the origin of vascular plants and with three metabolic capacities unique to tracheophytes. He proposed that fungi and algae contain large concentrations of compounds which complex with borate. This prevented boron from exerting a regulatory role in the metabolism of these organisms. After sucrose had been adopted as a major carbohydrate in the Chlorophyta, phloem developed in land plants, which evolved from this group, as a tissue in which the complexing of boron with soluble carbohydrates was minimal. Since boron was not sequestered by complexing with carbohydrate, it was free to acquire a regulatory role in plant metabolism. This acquisition, according to Lewis, was a role necessary to the synthesis of lignin, a role which subsequenly catalyzed the evolution of lignified land plants and, in conjunction with auxin, the differentiation of xylem.

This hypothesis is supported by: (a) the many reports in the literature that phenols accumulate under conditions of boron deficiency (Spurr, 1952; Perkins & Aronoff, 1956; Watanabe *et al.*, 1961, 1964; Troitskaya, Dranik & Shkol'nik, 1970); (b) the suggestion that there is a concomitant decrease in lignin synthesis in boron-deficient tissue (Skok, 1958) and (c) the observation that hyperauxiny is a frequent symptom of boron deficiency (Odnoff, 1957; Neales, 1960; Jaweed & Scott, 1967; Coke & Whittington, 1968; Bohnsack & Albert, 1977).

Despite the accumulation of phenols during boron deprivation, there is little evidence demonstrating a concomitant decrease in lignification. In fact, lignin synthesis may actually be enhanced under conditions of boron deficiency as first suggested by Neales (1960) and later confirmed by McIlrath & Skok (1964). Normal xylem differentiation continues in a number of vascular plants species despite the fact that boron deficiency results in the cessation of cell division in the

apical meristem. As a result, differentiation of the stele proceeds into the region occupied by the meristem (Neales, 1960; Kouchi & Kumazawa, 1975b, 1976). This is accompanied by the accumulation of phenols and lignification of walls of the cells in the meristem (Perkins & Aronoff, 1956; Neales, 1960; Albert & Wilson, 1961; Kouchi & Kumazawa, 1975b; Hirsch & Torrey, 1980).

Taken together, these results suggest that altered phenol and lignin metabolism are secondary effects caused by degeneration of some metabolic function in the root apical meristem under conditions of boron deficiency which leads to cessation of cell division, to failure of the apical meristem to move ahead of the differentiating stele, to eventual thickening of the cell walls of the meristem and to accumulation of phenols.

Role of boron in auxin metabolism

IAA is synthesized in apical meristems of both shoots and roots (Greulach, 1973; Leopold & Kriedemann, 1975). The observation that boron is necessary to maintain the normal functioning of apical meristems led to experiments that examined the relationship between boron and auxins. A loss of geotropic response, typical of IAA-deficient plants, occurs in boron-deficient broccoli (*Brassica oleracea*) (Alexander, 1942) and squash (*Cucurbita pepo*) (Bohnsack & Albert, 1977). Eaton (1940) tested the hypothesis that boron deprivation resulted in hypoauxiny and successfully restored growth to boron-deficient cotton plants by spraying them with IAA. The observation that boron and auxins have a synergistic effect on promoting the adventitious rooting of cuttings (Gorter, 1958; Weiser, 1959; Middleton, Jarvis & Booth, 1978) is consistent with the hypothesis that boron-deficient plants suffer from hypoauxiny.

Further work on adventitious root development has provided insight into the relationship between boron and auxin in the rooting of cuttings. Using *Phaseolus aureus*, Middleton *et al.* (1978, 1980) demonstrated that boron is not required for root initiation but that subsequent root growth from the very early stages of development is dependent on an adequate supply of boron. This confirmed the previous work of Hemberg (1951). Cuttings pretreated for 24 h in 10^{-4} M indolebutyric acid (IBA) without added boron accumulated soluble sugars (sucrose, glucose, and fructose) in the hypocotyl but no roots developed. It was subsequently demonstrated that IBA stimulted the translocation of [^{14}C]sucrose from leaves to the hypocotyl within 24 h. Thus, it appears that sugar transport is facilitated by added auxins in the absence of sink activity usually provided by roots but that neither auxins nor sugars can replace the requirement for boron for root growth. While high concentrations of supplied auxins are necessary to initiate rooting of cuttings, they are inhibitory to subsequent root growth (Torrey, 1953; Jarvis, Ali & Shaheed, 1983). Recently, Jarvis *et al.* (1983, 1984) proposed that boron, which is essential to cell division and normal development of the root primordia, acts by reducing auxin to concentrations optimal for root growth. They are currently investigating the possible role of boron in enhancing IAA oxidase/peroxidase activity.

Torrey (1953) demonstrated that IAA at a concentration of $5{\cdot}7 \times 10^{-6}$ M, like boron deficiency, completely inhibited root elongation of *Pisum sativum* but markedly accelerated the differentiation and lignification of xylem into the region occupied by the meristem. Bohnsack & Albert (1977) also demonstrated that symptoms indistinguishable from boron deficiency could be induced by hyperauxiny in boron-sufficient squash plants (*C. pepo*) by the addition of 10^{-6} M IAA to

the hydroponic culture medium. Under conditions of boron deprivation or hyperauxiny, root elongation was inhibited. Concomitantly, root tips became swollen, 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 approx. 6 to 9 h after boron was withheld, or 3 to 6 h after IAA was added to the boron-sufficient medium. Hyperauxiny is often accompanied by increased ethylene production (Abeles, 1973). Roots of 5-d-old boron-sufficient *C. pepo* plants transferred to hydroponic culture medium containing 10^{-6} M IAA showed a 63% increase in ethylene production over that of the boron-sufficient control plants within 24 h. However, ethylene evolution decreased 75% when the boron-sufficient plants were transferred to hydroponic culture medium without boron for 24 h. Thus, boron-deficiency and hyperauxiny both cause inhibition of root elongation but apparently not by the same physiological mechanism.

This conclusion was confirmed by Hirsch, Pengelly & Torrey (1982) who used electron microscopy to determine if changes in sunflower root tissue characteristic of boron deprivation were the same as those induced by IAA. The only apparent change in auxin-treated root cells was an increase in electon-dense material within the vacuoles; there was no increase in cell wall thickness of the meristem cells which is characteristic of boron-deficient roots. Consistent with the EM results, use of a very sensitive radioimmunoassay confirmed that the IAA concentration of boron-deficient root tips was not greater than that of boron-sufficient root tips. Thus, during boron deficiency, roots from intact plants exhibited increased IAA oxidase activity but had normal levels of IAA; this is opposite to the situation proposed to occur when boron is lacking during the rooting of cuttings.

Boron and sugar transport

A relationship between boron and translocation of sugar has been suggested by a number of researchers (Gauch & Dugger, 1953; Sisler, Dugger & Gauch, 1956; Skok, 1958). Gauch & Dugger (1953) proposed that boron might be a structural component of the plasma membrane. A molecule of sucrose or another sugar would react with the borate molecule at the surface of the membrane to form a borate–sugar carrier-complex which would then transport the sugar molecule across the membrane. This mechanism is compatible with observed changes in membrane permeability in response to boron nutrition (Pollard, Parr & Loughman, 1977; Roth-Bejerano & Itai, 1981). Although boron can increase the basipetal translocation of [^{14}C]sucrose applied to leaves of bean hypocotyl cuttings, sucrose does not alleviate the symptoms of boron deficiency (Whittington, 1959; Neales, 1960; Albert & Wilson, 1961) nor replace boron in the rooting of cuttings (Middleton *et al.*, 1978, 1980). Increased translocation of sugar resulting from the addition of boron-deficient plants is probably an indirect effect owing to the restoration of sink activity. No sucrose-borate complexes have been isolated or localized in plants, and boron is not redistributed by lateral or basipetal transport in plants (Albert & Wilson, 1961).

Boron and the normal functioning of the apical meristem of vascular plants

The dependence of normal meristematic activity on boron is well documented for both angiosperms and gymnosperms (a partial list of historic and modern references includes: Warington, 1923; Sommer & Sorokin, 1928; Jolivette & Walker, 1943; Palser & McIlrath, 1956; Whittington, 1957, 1959; Walker, Gessell & Haddock, 1955; Neales, 1960; Albert & Wilson, 1961; Yih & Clark, 1965; Blaser, Marr & Takahashi, 1967; Chapman & Jackson, 1974; Kouchi & Kumazawa, 1975a,

b; Bohnsack & Albert, 1977; Cohen & Lepper, 1977; Hirsch & Torry, 1980; Lovatt, Albert & Tremblay, 1981). Apical meristems of roots are extremely sensitive to boron deficiency (some recent references include: Chapman & Jackson, 1974; Cohen & Albert, 1974; Kouchi & Kumazawa, 1975a, b, 1975; Bohnsack & Albert, 1977; Cohen & Lepper, 1977; Krueger *et al.*, 1979; Hirsch & Torrey, 1980; Lovatt, Albert & Tremblay, 1981). The transfer of 5-d-old squash plants (*Cucurbita pepo*), grown in the presence of an optimal concentration of boron (0·1 mg l^{-1}), to a medium without boron results in the complete cessation of root growth a full 24 h before shoot growth is affected (Cohen & Albert, 1974; Bohnsach & Albert, 1977; Krueger *et al.*, 1979; Lovatt *et al.*, 1981). Lateral roots, like adventitious roots, do not require boron for initiation (i.e. organization of the meristem) as initiation of lateral root primoridia almost to the root tip is a characteristic symptom of roots deprived of boron (Neales, 1960; Albert & Wilson, 1961; Chapman & Jackson, 1974; Kouchi & Kumazawa, 1975a; Hirsch *et al.*, 1982). However, all roots require boron for normal cell division and growth once the root meristem is organized. It is important to note that an analogous situation occurs in the shoot apex. Under conditions of boron deficiency, the organization of new leaf primordia occurs but their subsequent development, which is dependent on cell division, is inhibited (Kouchi & Kumazawa, 1975a).

The length of time that root growth will proceed at a linear rate is directly proportional to the concentration of boron available in solution and a continuous supply of boron is necessary for unrestricted root growth. These observations suggest that once boron enters the root cortex, it must be utilized rapidly as it soon becomes unavailable to the dividing meristemic cells presumably owing to rapid removal in the transpiration stream. Movement of boron other than in the transpiration stream to effect the redistribution of boron laterally from one tissue to another is either lacking or of insignificant extent since the transfer of plants growing in the presence of boron to a medium without boron results in the cessation of root growth within a matter of hours for a number of vascular plants (Neales, 1960; Albert, 1965; Kouchi & Kumazawa, 1975a; Hirsch & Torrey, 1980; Lovatt *et al.*, 1981). Split root experiments have shown that boron is not translocated basipetally from leaves or laterally from roots growing in the presence of boron to roots that are in a boron-free medium; the latter cease to grow (Albert & Wilson, 1961).

A possible way of elucidating the biochemical role of boron in the metabolism of vascular plants is to determine the earliest effect that withholding boron has on the physiology of the plant. This earliest symptom is likely to indicate its primary metabolic role. The earliest effects of boron deprivation that have been determined are on nucleic acid biosynthesis in apical meristems of roots. When 5-d-old squash plants (*C. pepo*) are transferred to a nutrient medium with no added boron, the incorporation of [^{3}H]thymidine into the acid-insoluble fraction is significantly less than that in the boron-sufficient control plants after only 6 h. The incorporation of [^{3}H] thymidine is reduced 66 % when boron is withheld for an additional 6 h. The decrease in DNA synthesis correlated temporally with inhibition of both cell division and root elongation, which ceased after 18 h of boron deprivation (Krueger *et al.*, 1979; Dugger, 1983). When boron-deficient squash plants were returned to a boron-sufficient medium for 12 h, autoradiographs showed that incorporation of [^{3}H]thymidine was indistinguishable from that of boron-sufficient root tips (Cohen & Albert, 1974). These observations suggest that DNA synthesis is rapidly, but reversibly, inhibited under conditions of boron deficiency.

Measurements of the incorporation of radiolabelled precursors into RNA

provide evidence that RNA synthesis is also impaired when boron is withheld. For example, Sherstnev & 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, Palmer & Dugger, 1980), [^{14}C]uridine (Chapman & Jackson, 1974), and [^{32}P] (Cory & Finch, 1967) into RNA were early effects of boron deficiency in cotton ovules (*Gossypium hirsutum*) and in root apices of mung bean (*P. aureus*) and broad bean (*Vicia faba*), respectively.

Several workers (Albert, 1965; Jarweed & Scott, 1967; Johnson & Albert, 1967; Chapman & Jackson, 1974) have reported decreased RNA content in boron-deficient roots. When nucleic acid biosynthesis, protein biosynthesis and respiration were measured in the same species, changes in nucleic acid biosynthesis in each case preceded (a) the reduction in protein content (Johnson, 1971); (b) the decrease in the incorporation of [^{14}C]leucine into protein and the reduction in respiration (Krueger *et al.*, 1979) and (c) the decrease in RNA content and the observed increase in RNAase activity that accompanies boron deprivation (Chapman & Jackson, 1974). From these results, it appears that altered nucleic acid biosynthesis is a not secondary effect resulting from inhibition of either protein synthesis or respiration, or increased ribonuclease activity. With one exception, altered nucleic acid synthesis is the earliest reported response to boron deprivation. The exception was observed with sunflower roots which continued to grow, albeit at a reduced rate, when hydroponically cultured in one-quarter strength Hoagland's nutrient solution without boron but the authors (Moore & Hirsch, 1983) suggest that the plants may not be totally boron-deficient. Nevertheless, a loss in membrane integrity was observed 6 h after withholding boron (Hirsch & Torrey, 1980) and prior to any changes in DNA synthesis (Moore & Hirsch, 1983). These authors proposed that a loss in membrane integrity was the earlier effect of boron deficiency.

Attempts have been made to determine the mechanism which results in altered nucleic acid synthesis during 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 additon of a hydrolysate of yeast RNA to the nutrient solution (Shkol'nik & Soloviyova-Troitskaya, 1961). Several workers (Johnson & Albert, 1967; Johnson, 1971; Birnbaum, Beasley & Dugger, 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 boron may be essential for maintaining adequate levels of pyrimidime nucleotides and that the state of boron deficiency might actually be a case of pyrimidine starvation. Such an interpretation was supported by the observations that both barbituric acid and 6-azauracil, known inhibitors of pyrimidine biosynthesis (Handschumacher & Pasternack, 1958; Ross, 1964; Potvin *et al.*, 1978; Lovatt *et al.*, 1979), produced symptoms identical to those of boron deprivation (Johnson & Albert, 1967; Albert, 1968; Birnbaum *et al.*, 1977).

The hypothesis that boron has a fundamental role in pyrimidine metabolism unifies the seemingly separate roles of boron in the two very different systems which require boron. The first includes dividing cells without concomitant maturation such as meristematic cells of root and shoot apices (Chapman &

Jackson, 1974; Kouchi & Kumazawa, 1975a; Bohnsack & Albert, 1977; Cohen & Lepper, 1977; Hirsch & Torrey, 1980; Lovatt *et al.*, 1981) and DNA repair in the generative cell of pollen grains (Jackson & Linskens, 1979). The second comprises elongating cells which do not undergo cell division such as *in vitro* development of cotton fibres (Birnbaum *et al.*, 1974, 1977) and pollen tube growth (Stanley & Loewus, 1964; Yih, Hille & Clark, 1966; Vaughn, 1977).

Boron deprivation also has a marked influence on carbohydrate metabolism. Dugger and coworkers (Dugger & Humphreys, 1960; Birnbaum *et al.*, 1977; Wainwright *et al.*, 1980) have provided evidence that suggests that boron deprivation results in reduced formation of UDP-glucose. This would result in reduced sucrose synthesis (Dugger & Humphreys, 1960), increased starch accumulation (Dugger, Humphreys & Calhoun, 1957) and interference with normal cell wall formation (Kouchi & Kumazawa, 1975b, 1976; Dugger & Palmer, 1980), all of which typify boron deficiency. Finally, the cytidine nucleotides are of central importance in the synthesis of the major phosphoglycerides which serve as components of membranes (Lehninger, 1977) linking pyrimidine nucleotide metabolism with the putative role of boron in maintaining membrane integrity (Roth-Bejerano & Itai, 1981; Hirsch *et al.*, 1982).

Thus, the hypothesis that boron is essential to the maintenance of adequate concentrations of one or more specific pyrimidine nucleotide species unifies not only the seemingly separate roles of boron in cell division and cell elongation but also the seemingly disparate roles of boron in nucleic acid biosynthesis, carbohydrate metabolism, and membrane permeability.

This hypothesis has suggested three valid areas for investigation: (a) that boron deficiency results in impaired *de novo* biosynthesis of pyrimidine nucleotides (Lewin & Chen, 1976; Birnbaum *et al.*, 1977; Wainwright *et al.*, 1980; Lovatt *et al.*, 1981) or reduced provision of pyrimidine nucleotides by altered activity of the salvage pathways (Lovatt *et al.*, 1981); (b) that the interconversions of UMP, UDP, UTP, and UDP-glucose or other nucleotide-sugars or synthesis of ribonucleotides or deoxyribonucleotides of cytidine or thymidine are impaired under conditions or boron deficiency (Dugger & Humphreys, 1960; Birnbaum *et al.*, 1977; Mamedova & Rasulov, 1977; Wainwright *et al.*, 1980; Lovatt *et al.*, 1981) and (c) that the utilization of a particular pyrimidine nucleotide or pyrimidine nucleotide-sugar in a specific metabolic process might be altered by boron deprivation (Lovatt *et al.*, 1981).

Although the transfer of 5-d-old squash plants (*C. pepo*) to boron-deficient nutrient solution resulted in cessation of root elongation within 18 h, the withholding of boron for up to 30 h 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; however, 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 the need to determine the concentrations of the various pyrimidine nucleotides available during these two states of boron nutrition is emphasized. Although these results argue against the first hypothesis, they leave open the second and third possibilities.

If either one of these two possibilities proves correct, boron will be essential to a metabolic pathway that is probably not unique to vascular plants. Thus, it is

necessary to address the question of how vascular plants alone came to require boron for the normal functioning of a pathway that they either share with all living organisms or, at least, with non-vascular plants.

BORON AVAILABILITY

As no element present at a concentration less than 2 nM in the ocean or 20 μmol kg^{-1} in the earth's crust is essential to life, it has been proposed that elemental abundance was an important factor in the origins of mineral nutrient requirements in organisms (McClendon, 1976). Such elements fall under one of four categories related to their abundance and biochemical suitability: (a) a unique requirement dating from the origin of life; (b) a unique requirement that was acquired later; (c) a primodial requirement satisfied by a number of elements with evolutionary selection favouring the most abundant and (d) a later acquisition satisfied by a number of elements, but the most abundant was selected (McClendon, 1976). McClendon assigned boron to the second group. There is considerable indirect evidence to support the hypothesis that boron was of limited availability during the early evolution of organisms.

The hypothesis that life evolved in ancient oceans and primeval pools that lacked appreciable amounts of boron is consistent with many reports that most extant organisms have no requirement for boron. This hypothesis also provides a reasonable explanation as to why those organisms that did eventually come to require boron are from very different taxa, i.e. they probably acquired an essential role for boron independently and long after the groups diverged.

Boron availability must have remained low throughout the evolution of vascular plants for none have a mechanism which regulates the amount of boron taken up by the plant. In vascular plants, boron is carried passively in the transpiration stream and accumulates where the transpiration stream ends (Kohl & Oertli, 1961). Near transpirational termini, boron moves into the phloem, i.e. from high boron concentration to low. A short distance away from the terminus, the situation is reversed and boron moves out of the phloem. Thus, transport of boron in the phloem is ineffective and very little of the accumulating boron moves out of those tissues it reaches (Oertli & Richardson, 1970; Raven, 1980). Since the uptake of boron is passive through the transpiration stream in tracheophytes, boron intoxication is a function of the concentration of boron in the soil solution to which the plant is exposed, the length of exposure and the rate of transpiration. Any tissue or organ located at the end of the transpiration stream that lives long enough will eventually accumulate boron to a toxic level even when grown in the presence of a concentration of boron considered optimal (Kohl & Oertli, 1961). The sensitivity of vascular plants to very low concentrations of boron, their inability to regulate the uptake of boron and the observed accumulation of boron at the end of the transpiration stream suggest that boron was not present in the soil solution to any appreciable degree during the evolution of vascular plants and supports the hypothesis that boron is a late addition to the oceans and to the water table.

Concentrations of boron that are toxic to vascular plants are low relative to the concentrations that other essential nutrients must achieve to cause toxicity. For example in *Citrus* spp. the accumulation of boron to concentrations between 100 and 2500 $\mu g\ g^{-1}$ leaf d. wt results in boron toxicity symptoms from slight to acute. Sulphur produces only slight effect of toxicity in *Citrus* at concentrations greater

than 5000 μg g^{-1} leaf d. wt whereas potassium must exceed 25000 μg g^{-1} leaf d. wt to cause toxicity (Chapman, 1968).

Finally, consistent with the hypothesis that boron is of limited availability in the earth's waters are the facts that the concentration of boron in modern oceans is only 4·6 mg l^{-1} as undissociated H_3BO_3, which is less than 0·02 % of the total dissolved elements (Sverdrup, Johnson & Fleming, 1946), and that the maximum concentration of boron is only 1·6 mg l^{-1} in fresh waters of the northern hemisphere (Raven, 1980), approx. 1 % of the total constituents (Goldman & Wetzel, 1966). This is probably due to the fact that boron is found in very low concentrations in the earth's crust. The more common elements of the lithosphere, O, Se, Al, Fe, Ca, Na, K, and Mg, comprise 98·59 % by weight of the earth's crust to a 10-mile depth (Epstein, 1972). Of the 18 elements most frequently required by organisms, only three are available in the earth's crust at concentrations lower than boron (930 μM); they are copper (870 μM), cobalt (430 μM), and molybdenum (15 μM). The last three were each assigned to two of McClendon's groups but all were included in the same group as boron, i.e., each had a unique requirement that was acquired later (McClendon, 1976).

Boron deficiency is common in the United States; 41 states have soils with concentrations of boron limiting to plant growth (Berger, 1965). This is a significant occurrence as plants require so little boron for optimal growth. General leaching has not made boron increasingly available to plants via the water table. Even now, the occurrence of toxic levels of boron in the water table or in the soil profile is of limited geographic distribution and usually restricted to areas which rely heavily on irrigation (Wilcox, 1960). The assumption that boron has not reached a level of availability that might be toxic to organisms is confirmed by the fact that neither plants nor animals have evolved specific physiological mechanisms that afford protection from boron excess.

Acquisition of a Requirement for Boron in Phytoplankton

The utilization of boron as B_2O_3, at first probably interchangeably with SiO_2, is not surprising; the structure of B_2O_3 closely mimics that of SiO_2. This property probably resulted in the incorporation of boron into the frustule of diatoms. Eventually, boron became essential to the process of cell wall morphogenesis in these organisms. Whether boron is replacing silicon or silicon is replacing boron as an element essential to cell wall morphogenesis in diatoms is not clear. McClendon(1976) assigned silicon to the fourth group of essential elements, i.e. a requirement that developed later in the evolution of organisms and could be satisfied by a number of elements of which the most abundant was favoured. The historical relationship between the abundance of boron and silicon is not known. Present concentrations of these two elements in the earth's oceans are: Si, 0·2 to 0·4 mg l^{-1}, B, 4·6 mg l^{-1} (Sverdrup *et al.*, 1946), or 107 μM silicon, 430 μM boron (McClendon, 1976).

Evolution of an Essential Role for Boron in the Normal Functioning of the Apical Meristems of Vascular Plants

The indirect evidence presented earlier suggests that boron was not available in sufficient concentration during the early history of life to result in its being a

primordial essential nutrient. It is proposed that the later acquisition of a requirement for boron by vascular land plants was not due to the eventual accumulation of boron in the earth's waters to the necessary critical concentration but was instead the result of the unique situation which evolved in vascular plants that permitted boron to accumulate in a specific tissue to a concentration sufficient to influence metabolism. This situation arose as a consequence of the evolution of xylem and the subsequent passive transport of boron in the transpiration stream. It resulted in the accumulation of boron at the end of the transpiration stream at a concentration sufficient to influence metabolic processes. The plant body of primitive vascular plants was dominated by stem tissue (both aerial and subterranean). It follows that the shoot apex itself was probably a significant site of boron accumulation, especially since functioning xylem tissue ends in the zone of differentiation just a few millimeters behind the apex. Boron, like O_2, CO_2, NH_3, N_2, urea and IAA, is a biologically important solute whose transmembrane movement can generally be accounted for by concentration differences across the membrane (Raven, 1980). Thus, boron arriving at the end of the functional xylem would easily move into the growing cells in the zone of elongation and from those cells into the dividing cells of the apical meristem. The maintenance of the boron gradient is insured by the lowering of the concentration of boron in the cells of the meristem by the ongoing processes of cell division and cell expansion.

Thus, the shoot apex evolved as the only tissue in primitive vascular plants that received a continuous supply of boron potentially maintained at a concentration greater than that of the environment and thus greater than that experienced by other organisms. This situation was conducive to the acquisition of a role for boron in the apical meristem of an early vascular plant essential to a pathway potentially common to other organisms. The acquisition of an essential role for boron in the normal functioning of the apical meristem may have provided the advantage of preventing boron toxicity in vascular plants. The accumulation of boron in the shoot apical meristem to a critical level that 'promoted' cells to divide, acts as a safety valve that insures that the meristem will move ahead of the differentiating xylem, which is indiscriminantly carrying boron to the shoot apex. The idea that meristem cells are capable of division and will do so once boron reaches a threshold concentration is consistent with evidence demonstrating that inhibition of DNA synthesis and cell division and elongation resulting from boron deprivation are soon reversed after the addition of boron. In view of the fact that borate forms complexes with D-fructose, D-mannose, D-galactose, and with the α forms of D-xylose and D-glucose (Zittle, 1951), with mononucleotides and nucleosides (Khym, 1967), and with several vitamins (Zittle, 1951), one might expect boron to be toxic. The acquisition of a role for boron in the meristematic cells of the shoot apex would result in its inheritance by all other cells of the plant. Whether the requirement for boron is expressed in other tissues within the plant depends on whether or not the metabolic process requiring boron is expressed.

Since roots of early vascular plants originated from stems, it is logical that root apical meristems also require boron to function normally. Roots are more sensitive to boron deprivation because they do not receive boron through the transpiration stream, but directly from the surrounding medium by diffusion (Raven, 1980). The removal of boron from the surrounding medium results in the cessation of root growth within a matter of hours, while the shoot is affected at a somewhat later point in time, presumably when the transpiration stream becomes void of boron.

Conclusions

An evolutionary approach to the acquisition of an essential role for boron in the metabolism of vascular plants forces us to examine critically the many observations regarding boron nutrition, i.e. deficiency and toxicity, in all organisms and to integrate this information into a coherent whole. Critical to this approach is an understanding of boron availability. McClendon (1976) proposed that boron was not available in sufficient concentration during the early evolution of life and, thus, was a requirement acquired at a later time. This hypothesis is supported by: (a) the demonstration that organisms do not have mechanisms for preventing the uptake of excess boron; (b) the observation that boron at low concentrations is toxic to both plants and animals; (c) the fact that boron is presently only a minor constituent of the earth's crust, modern oceans and the water table; (d) the evidence suggesting that only two taxa require boron and for two distinctly different metabolic processes.

Here, it is proposed that a requirement for boron was acquired as a result of the evolution of xylem through which boron was passively carried to the end of the transpiration stream, i.e. the shoot apex, and accumulated to a concentration sufficient to influence metabolism. The exposure of the cells of the apical meristem to significant quantities for boron was a unique situation, which resulted in the acquisition of an, as yet unknown, role for boron essential to the normal functioning of the apical meristems of vascular plants. A requirement for boron in the processes of cell division and cell expansion possibly provided a mechanism for preventing boron intoxication of the apical meristem by insuring that the meristem cells moved ahead of the differentiating xylem and the accumulating boron because cell division, providing no other factors were limiting, occurred once the critical level of boron was reached.

Critical review of the metabolic processes unique to vascular plants in relation to boron nutrition has provided convincing evidence that disruption of the normal functioning of the apical meristem is the earliest and most prevalent effect of boron deficiency in vascular plants *in vivo*, the influence of boron on auxin metabolism, lignification, phenol accumulation and sucrose transport probably being secondary effects. A testable hypothesis to encompass the observed effect of boron deficiency on nucleic acid synthesis, membrane integrity and normal carbohydrate metabolism has been sought. Analysis of available evidence suggests to this author, and others, that boron is essential to maintain adequate concentrations of one or more specific nucleotide species or to facilitate their utilization.

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