The Emergence of Boron as Nutritionally Important Throughout the Life Cycle

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INTRODUCTION

In 1997, it was predicted that boron would be identified as an element with practical nutritional or clinical importance by the year 2000.[1] Research findings since that time indicating that boron is needed or beneficial for many of life processes including embryogenesis, bone growth and maintenance, immune function, psychomotor skills, and cognitive functions suggest that this prediction will become true. The surprising thing about this development is that only 20 y ago students in the biological and medical sciences were being taught that boron was essential for plants but not for animals. In fact, at the time the first report about the possible nutritional importance of boron appeared,[2] boron was generally regarded as an element of only toxicologic concern, especially in the hospital setting. Now that the opinion about the nutritional importance of boron is changing, a question that has come to the fore is: Why wasn't this recognized sooner? A reasonable answer to that question is that boron apparently has a biochemical function that is very subtle. Moreover, this role apparently is one that allows optimal functioning of other nutrients or hormones and thus is overlooked as attention is directed toward altering the intake of the substance whose suboptimal metabolism is directly involved in a pathologic consequence (e.g., calcium supplementation to prevent bone loss).

In 1987, based on assorted bits of evidence, mainly from plants, it was suggested that boron, through an effect on the cell membrane, affected calcium metabolism and hormone action in higher forms of life.[3] In 1991, additional findings from plants and animals were used to change this suggestion to the hypothesis that boron has a role in cell-membrane function or stability such that it influences the response to hormone action, transmembrane signaling, or transmembrane movement of regulatory cations or anions.[4] Testing of this hypothesis has been difficult. Nonetheless, many of the recent findings showing that boron is needed or beneficial throughout the life cycle support this hypothesis.

BORON IN REPRODUCTION, EMBRYO DEVELOPMENT, AND MATURATION

Recent research has shown that boron is needed in the early stages of life; this includes the demonstration that the lack of boron adversely affects reproduction and embryo development in both the African clawed frog, *Xenopus laevis*, and the zebrafish. Studies with rats and mice suggest that low boron status may affect reproduction in mammals.

In the *Xenopus* model, dietary boron deprivation induced a marked increase in necrotic eggs and a high frequency of abnormal gastrulation.[5] The abnormal gastrulation was characterized by bleeding yolk and exogastrulation, which suggested abnormal cell-membrane function or structure. More than 80% of the embryos from boron-deficient frogs died before 96 h of development; survival of embryos at 96 h from boron-supplemented frogs exceeded 75%. Culturing of *Xenopus* in medium containing less than 0.3 μ M of boron during organogenesis (through day 4) resulted in abnormal development of the gut, craniofacial region, and eye, visceral edema, and kinking of the tail (muscular and skeletal).[6] The rate of tail resorption was slower in boron-deprived than in boron-supplemented larvae.[6] Addition of 100 μg/L of thyroxine, a known enhancer of tail resorption, and, to a lesser extent, 0.1% (w/v) iodine reversed the delayed tail absorption in boron-deprived larvae. Tail-absorption inhibition was also reversed by 100 μg/L of boron to the same degree as that observed with the iodine addition. This finding indicates that boron is needed for metamorphosis of the frog and that the need involves changing the ability of thyroxine/triiodothyronine to act, perhaps at the cell-membrane level.

In the zebrafish model, during the early postfertilization period, 45% of boron-deprived embryos died, whereas only 2% of boron-supplemented embryos died.[7] A high rate of death occurred during the zygote and cleavage periods before the formation of a blastula. Two morphologic events preceded death. The most prevalent pathologic change was extensive membrane blebbing. This usually occurred on the animal pole but often spread to other areas of the egg with time. The other pathologic change was an extrusion of cytoplasm, which usually occurred in the animal pole. These changes occurred when cells were producing prodigious quantities of membrane. It was stated that the membrane disruption observed was consistent with membrane alterations reported for boron-deprived cyanobacteria and vascular plants,[7] and it was suggested that boron is required for the synthesis or processing of membrane or cytoskeletal proteins that function in the maintenance of membrane shape and integrity.

Studies with rats and mice have not been as definitive as those with the frogs and zebrafish. However, in one study, the preimplantation development of two-cell embryos from both boron-deprived and boron-supplemented female mice was significantly impaired by culturing in boron-deficient medium. The embryos from the boron-deficient females were apparently more impaired than those from the boron-supplemented females.[8] Only 17% of the two-cell embryos from the boron-deprived mice formed morulae after 1 d and only 40% formed blastocytes by day 3. Conversely, 31% of the two-cell embryos from the boron-supplemented mice reached the morula stage after 1 d and 57% of the embryos formed blastocytes by day 3. The number of embryonic degenerates formed after 72 h of culture in a boron-deficient medium was significantly higher with embryos from boron-deprived (57%) than with boron-supplemented (20%) mice. Possible mechanisms suggested for the impaired development of two-cell embryos caused by boron deficiency included changes in embryonic calcium regulation of development and changes in membrane function.

BORON IN GROWTH AND DEVELOPMENT

Boron apparently has not been demonstrated to have a marked effect on the growth of animal models used to date. Boron was found to slightly but significantly increase the growth of embryonic trout in a dose-dependent manner.[9] In rats and chicks, growth

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has been found to be affected in a noticeable fashion by dietary boron only when a nutritional stressor is present. For example, in the presence of marginal vitamin-D deficiency, boron-deprived chicks did not grow as well as boron-supplemented chicks.[2,10]

Although growth is not markedly affected, there is much evidence that boron can affect bone formation. This effect, however, is most evident in the presence of suboptimal status of another nutrient important in bone formation or remodeling. One of the first findings reported in 1981[2] suggesting that boron is essential was that boron improved bone calcification in chicks fed a diet deficient but not completely lacking in vitamin D. At the microscopic level, boron deprivation was found to exacerbate the distortion of marrow sprouts caused by vitamin-D deficiency and delay the initiation of cartilage calcification.[11] Boron deprivation also decreased chondrocyte density in the zone of proliferation of the growth plate.[12] Luxuriant amounts of vitamin D in the diet greatly diminishes the boron effects on bone formation. The findings suggest that boron is needed for the optimal hormonal action of vitamin D, perhaps at the cell-membrane level, when vitamin D is present in limited amounts.

BORON IN THE ADULT

If boron influences hormone action, transmembrane signaling, and/or membrane function or stability, it would be expected that the lack of boron in the diet would have a variety of consequences in adult higher animals. This expectation has not gone unfulfilled. Boron nutriture has been found to affect the metabolism or utilization of numerous other substances involved in life processes including macrominerals such as calcium and magnesium, energy substrates such as triacylglycerols and glucose, nitrogen-containing substances such as amino acids and proteins, reactive oxygen species, and hormones such insulin, estrogen, calcitonin, and vitamin D.[13] Through these effects, boron has been shown to affect the function or composition of several body systems, including the eye, brain, skeleton, and immune system.

In the adult F1 zebrafish, boron deficiency caused photophobia.[7] Histologic evaluation showed that the boron-deficient adults had photoreceptor dystrophy. Compared with boron-supplemented controls, the photoreceptor cells of the boron-deficient adults were shortened because of a reduction in the myoid and outer-segment regions; these regions are characterized by the production of prodigious quantities of membrane.

The peak increase in insulin release from isolated, perfused pancreata from boron-deprived chicks was almost 75% higher than that from pancreata of boron-supplemented chicks; the difference was especially noticeable when the perfusate was supplemented with glucose.[14] It was speculated that boron influenced peak insulin release through changing nicotinamide adenine dinucleotide phosphate metabolism, which induced changes in cell-membrane potential, a main event leading to the release of insulin-containing secretory granules.[14]

Emerging evidence indicates that boron is involved in the inflammatory process or immune function in higher animals. When an antigen was injected to induce arthritis, boron-supplemented as opposed to boron-deficient rats exhibited less swelling of the paws and circulating neutrophil concentrations and higher circulating concentrations of natural killer cells and $CD8a^+/CD4^-$ cells.[15] Based on these findings, it was hypothesized that physiologic amounts of dietary boron reduce the risk for inflammatory disease by controlling the balance of the inflammatory system to permit elimination of pathogens but avoid autoimmunity. The hypothesis was based on the concept that boron serves as a suppressive signal that downregulates enzymatic activities typically elevated during the normal inflammatory process.

Assessments in both animals and humans have found that boron deprivation results in decreased brain electrical activity similar to that observed in non-specific malnutrition.[16] Boron deprivation also results in poorer performance in tasks of motor speed and dexterity, attention, and short-term memory in humans.[16] It seems very possible that these effects could have been the result of changes in membranes that affected nerve-impulse transmission.

In humans, dietary boron alters the plasma or serum concentrations of several hormones including calcitonin, 17β-estradiol, 25-hydroxycholecalciferol, and triiodothryronine. For example, estrogen therapy increased serum 17β-estradiol in postmenopausal women; this increase was depressed when the women were fed a diet low in boron.[13,17] In men older than 45 y and in postmenopausal women, feeding a low boron diet decreased the concentration of 25-hydroxycholecalciferol in serum.[17] Boron deprivation also exacerbated an increase in serum calcitonin caused by low dietary copper and magnesium.[13,17] These changes in hormone concentrations probably reflected a change in the ability of these hormones to express their actions. Because all these hormones act at the cell-membrane level, boron may have an effect at this site. The cell-membrane actions of these hormones can be exemplified by 1,25-hydroxycholecalciferol, which interacts with osteoblasts to affect their function. Non-genomic actions mediated through membrane-receptor systems include activation of voltage-sensitive Ca^{2+} channels, induction of phospholipid and sphingolipid turnover, elevation of intracellular Ca^{2+} concentrations, and activation of second-messenger systems.[18]

FUTURE CHALLENGES IN BORON NUTRITION RESEARCH

Boron is an element poised to become recognized as a nutrient clinically important for optimal function throughout the life cycle. This recognition will be made easier by the establishment of a clearly defined role for boron that explains the myriad of effects it has at physiologic intakes. Many research findings support the concept that boron has a role that affects the action or metabolism of other nutrients at the cell-membrane level. There are several possible mechanisms through which boron can have this effect; some are provided below. In boron-deficient plants, plasma membranes are highly leaky and lose their functional integrity; substantial changes occur in ion fluxes and proton pumping.[19] Attempts to define the biochemical role of boron to explain these defects in plants have been helpful for identifying possible mechanisms in higher animals.

The phosphoinositides, glycoproteins, and glycolipids of membranes contain *cis*-hydroxyl groups. Boron complexes with organic compounds containing hydroxyl groups; the formation of complexes is best when the hydroxyl groups are adjacent and *cis*. Compounds with more than two hydroxyl groups react more strongly with boron, and the intensity of reaction increases with the increase in the number of adjacent hydroxyl groups.[20] Thus, diester borate polyol complexes possibly form in membrane; these embedded complexes could act as reversible calcium chelators and affect membrane charge and integrity. A finding supporting this hypothesis is that boron deficiency decreased plasma membrane-bound Ca^{2+} in roots and leaves of plants, and this coincided with an increase in apoplastic free Ca^{2+} and K^+.[21] This finding resulted in the suggestion that less Ca^{2+} bound to the plasma membrane because of a reduction of specific Ca^{2+}-binding sites (borate esters with *vic*-diols or polyhydroxy-carboxylates), which ultimately led to the deterioration in plasma membrane integrity.

Another hypothesis is that boron is involved in redox metabolism in the cell membrane.[22] Plasmalemma NADH oxidase in carrot cells grown in culture is stimulated by boron.[23] The role of reduced nicotinamide adenine dinucleotide (NADH) oxidase in plant metabolism is unknown, but it has been speculated to be involved in the reduction of ascorbate-free radical to ascorbate. Evidence that boron affects ascorbate metabolism includes the

finding that ascorbate supplementation restores growth of the squash meristem retarded by boron deprivation.[22]

The ascorbate supplementation effect may be a reflection of the finding that boron deficiency decreases the amount of ascorbic acid, non-protein sulfhydryl compounds (mainly glutathione) and glutathione reductase, the major defense systems of cells against toxic reactive oxygen species in plants. In other words, boron may have a reactive-oxygen scavenging role in membranes. Thus, boron deficiency may affect membrane permeability in plants because it allows lipid peroxidation of the membrane. This suggestion is supported by the finding of increased malondialdehyde concentration and decreased superoxide dismutase and catalase concentrations in the roots of boron-deficient plants.[24] There is evidence that boron deprivation affects reactive oxygen species metabolism through changing erythrocyte superoxide dismutase concentration in humans.[13,17]

In summary, knowledge about the biochemical function of boron is still in the speculative stage; thus, future research should focus on determining this function. A defined function will facilitate research in other areas such as identifying boron-status indicators and whether boron is of nutritional importance in some pathologic conditions such as arthritis and osteoporosis. Even without this defined function, however, it is quite apparent that boron enhances optimal function throughout the life cycle.

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