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Regulation of Enzymatic Activity

One Possible Role of Dietary Boron in Higher Animals and Humans

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ABSTRACT

It is well established that vascular plants, diatoms, and some species of marine algal flagellates have acquired an absolute requirement for boron (B), although the primary role remains unknown. Discovery of naturally occurring organoboron compounds, all ionophoric macrodiolide antibiotics with a single B atom critical for activity, established at least one biochemical role of B. The unusual nature of B chemistry suggests the possibility of a variety of biological roles for B. At physiological concentrations and pH, B may react with one N group or one to four hydroxyl groups on specific biological ligands with suitable configuration and charge to form dissociable organoboron compounds or complexes. Suitable ligands include pyridine (e.g., NAD^+ or NADP) or flavin (e.g., FAD) nucleotides and serine proteases (SP). B reacts with the *cis* adjacent hydroxyls on the ribosyl moiety of the nucleotides or, in the serine proteases, the N on the imidazole group of histidine or the hydroxyl group on the serine moiety. Reversible inhibition by B of activity of SP or oxidoreductases that require pyridine or flavin nucleotides is well known. Therefore, a proposed essential role for B is as a regulator of relevant pathways, including respiratory burst, that utilize these enzymes.

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Index Entries: Boroesters; enzymatic regulation; inflammatory disease; immune function; insulin release.

Abbreviations: FBP-TP, fructose 1,6-biphosphate and triose phosphate; GGT, γ-glutamyl transpeptidase; GPD, glyceraldehyde-3-phosphate dehydrogenase; PGD, 6-phosphogluconate dehydrogenase; P-P, pentose-phosphate, ROS, reactive oxygen species; SOD, superoxide dismutase; UDPG, uridine diphosphate glucose; VDCC, voltage-dependent Ca^{2+} channel.

INTRODUCTION

There is universal agreement that vascular plants *(1)*, diatoms *(2)*, dinitrogen-fixing cyanobacteria with heterocysts *(3)*, and some species of marine algal flagellates *(4)* have acquired an absolute requirement for boron (B). However, despite extensive research efforts and the unambiguous characteristics of B deficiency in plants *(5)*, a specific biochemical role for B in any of those species remains to be elucidated. In 1981, interest in the biological effects of physiological amounts of B in animals was renewed with the report that B is a growth factor for the vitamin D-deficient chick *(6)*. The considerable progress in animal and human B nutrition made since that time has led to the development of the working hypothesis that one biological role of B in humans and higher animals is regulation of certain enzymatic activities. The hypothesis is based on the unusual nature of B chemistry as summarized below.

B CHEMISTRY

B Speciation in Biological Systems

In this discussion, organic compounds containing B–O bonds, i.e., the orthoborates $B(OR)_3$, $(RO)B(OR')_2$ and $(RO)B(OR')(OR'')$, and orthoborates of polyhydric alcohols are defined as organoboron *(7)*. Organoboron compounds include B–N compounds, because B–N is isoelectronic with C–C. Organoboron complexes are present in plants, and most likely present in animal and human tissues. Experimental evidence to date suggests these organoboron complexes are the result of interaction with either OH or amine groups as described below. The B spirochetes that function as antibiotics are well-established examples of biological organoboron polyol complexes (Structure I) *(8)*.

Boric acid is an exclusively monobasic acid and is not a proton donor, but rather accepts a hydroxyl ion (a Lewis acid) to form the tetrahedral anion $B(OH)_4^-$ (Reaction [1]) *(9)*:

$$B(OH)_3 + 2H_2O \Leftrightarrow H_3O^+ + B(OH)_4^- \; pK_a = 9.25 \; (25°C) \qquad (1)$$

Table 1
Approximate Concentrations (Wet Wt) of Inorganic B in Selected Plant, Animal, and Human Tissues, or Cell-Culture or Refined Chemical Reaction Used for System Maintenance or to Elicit Effect of B on Enzyme Activity or Product Concentration[1]

Boron Conc.[3] (mol/L)	System[2]	Remarks	Reference
0.000,000,000,1	RCR	Elastase activity, leukocyte, inhibition (synthetic boronic acids)	*(36)*
0.000,000,010	CC	Nutrient solution, maintenance, sunflower plant	*(78)*
0.000,000,600	H	Plasma, bodybuilder, male, no boron supplement	*(79)*
0.000,003,000	H	Plasma, bodybuilder, male, 2.5 mg B/d supplement	*(79)*
0.000,004,000	RCR	Lipooxygenase activity flux, cotyledon, sunflower	*(80)*
0.000,004,000	A	Plasma, rat, boron-supplemented or -deprived	*(81)*
0.000,007,100	A	Plasma, chick, cholecalciferol-, boron-deficient	*(82)*
0.000,010,000	RCR	UDPG pyrophosphorylase activity flux, pea	*(50)*
0.000,014,000	CC	Medium, maint., animal cell (Leibovitz's L-15)	*(83)*
0.000,014,000	A	Plasma, chick, cholecalciferol-, boron-adequate	*(82)*
0.000,022,000	A	Serum, bovine, fetal	*(83)*
0.000,046,000	CC	Nutrient solution, maintenance, diatom	*(2)*
0.000,071,000	H	Semen, human	*(84)*
0.000,200,000	P	Phosphoglucomutase activity (50% inhibition), pea	*(39)*
0.000,400,000	RCR	GPD[4] activity flux, yeast	*(24)*
0.000,400,000	RCR	Lactate dehydrogenase activity flux, heart, beef	*(24)*
0.003,000,000	RCR	GPD activity flux, yeast	*(85)*
0.010,000,000	RCR	Starch phosphorylase activity flux, snap bean	*(86)*
0.100,000,000	CC	Leaf, snap bean (no sign of boron toxicity)	*(86)*

[1]Amount of B in solution prior to B supplementation commonly not provided.

[2]A, animal; CC, cell culture; H, human; P, plant; RCR, refined chemical reaction. Experimental conditions vary markedly among studies and systems.

[3]Concentration of B present in nutrient media or concentration of B (not necessarily the minimum) determined to elicit change in enzyme activity or other biological responses.

[4]Glyceraldehyde-3-P dehydrogenase.

At typical physiological B concentrations (6.0×10^{-7} – ~9.0×10^{-3} mol/L) in plants, animals, or humans (Table 1), inorganic B is essentially present only as the mononuclear species boric acid $B(OH)_3$ and as borate $B(OH)_4^-$ *(10)*. Polyborate species can form near neutral physiological conditions (pH ~7.4) when borate concentrations exceed approx 0.025 mol/L *(11)*, an unusually high B concentration in biological systems, but still lower than that found in the snap bean leaf (0.1 mol/L) (Table 1). Within the normal pH range of the body, $B(OH)_3$ prevails as the dominant species (pH 1, ~100% $B[OH]_3$; pH 7.4, >98%; pH 9.25, 50%; pH 11, ~0%) *(12)*.

Uptake of physiological amounts of supplemental inorganic B (orthoboric acid and simple borates) approaches 100% *(13)*, and the bioavailability of B in foods is under investigation *(14)*. However, if plant and animal B absorption mechanisms are analogous, some organic forms of B *per se* may be unavailable to animals; the organic forms of B in soil from decomposed organic matter are not immediately available to plants and can only be absorbed after subsequent mineralization *(15)*.

Boroesters

Boroester Formation

The boroesters are chemical structures formed by reactions between B oxo compounds and certain mono or polyhydroxy compounds to form specific organoboron complexes. Boroesters probably represent the most biologically relevant B species because of the vast number of biochemicals that contain one or more hydroxy groups with suitable positions. Boric acid reacts with suitable dihydroxy compounds to form the corresponding boric acid monoesters ("partial" esterification) (e.g., Structure II) that retain the trigonal-planar configuration and no charge.

A borate monoester ("partial" esterification; monocyclic) (Structure III) with a tetrahedral configuration and a negative charge is created when borate forms a complex with a suitable dihydroxy compound. A compound of similar configuration and charge is also formed when a boric acid monoester forms a complex with an available hydroxyl group. These two types of boromonoesters can react with another dihydroxy compound to give a corresponding spiro-cyclic borodiester ("complete" esterification) that is a chelate complex with a tetrahedral configuration and negative charge (Structure IV) *(16)*. A partially esterified tridentate cleisto complex (Structure V) may be formed when a ligand contains three *cis*-oriented hydroxyl groups *(17)*. A partially esterified diborate complex of tridentate structure (not shown) is also possible *(10)*. Boric acid and trigonal-planar boroesters are neutral compounds. Borate and all other types of boroesters are characterized by a central B atom coordinated by four O atoms and a negative charge *(16)*.

Boric acid and boric acid-like structures, instead of borate, are most likely the reactive species with biological ligands, because it is probably easier for a diol to substitute for a relatively loosely bound water mole-

cule associated with boric acid or a boric acid-like structure than it is for the diol to substitute for a hydroxyl ion in borate or a borate-like structure of differences in charge *(16,18)*. The type of boroester formed depends mainly on three factors: the original structure of the ligand, the pH of the aqueous environment *(16)*, and the relative concentrations of boric acid and the ligand *(19)*.

Ligand Structure

Typically, ligands that contain adjacent and cis hydroxyl groups are the ones most likely to react with B oxo compounds to give a boroester, and the reactivity of boric acid with the ligand generally increases with an increase in the number of these *cisoid* groups *(20)*. Various boromonoesters (reaction [2]) and borodiesters (reaction [3]) are possible *(21)*:

$$\textit{cisoid} + B(OH)_3 \Leftrightarrow [\textit{cisoid}\text{-borate monoester}]^- + H_3O^+ \quad (2)$$

$$[\textit{cisoid}\text{-borate monoester}]^- + \textit{cisoid} \Leftrightarrow [\textit{cisoid}\text{-borate-}\textit{cisoid}\text{ diester}]^- + 2H_2O \quad (3)$$

The relevant *cis*-diol conformations are not present in glucose and galactose or their immediate derivatives *(21)*. However, the conformations are present in several biologically important sugars and their derivatives (sugar alcohols, onic and uronic acids), including some polymers. Common examples include mannitol, mannose, mannan, and polymannuronic acid, ribitol, ribose, erythritol, erythrose, and glycerol. Sugars often form intramolecular hemiacetals. Those with five-membered rings are called furanoses, and those with six-membered rings are called pyranoses. In cases where either five- or six-membered rings are possible, the six-membered ring usually predominates for unknown reasons *(22)*. In general, the most stable borate esters are formed when B oxo compounds react with compounds that have *cis*-diols on a furanoid ring *(4)*. Compounds in a configuration where there are *cis*-diols on a furanoid ring (e.g., ribose, apiose, and erythritan) form stronger complexes with B than do compounds configured to have *cis*-diols predominately on a pyranoid ring (e.g., the pyranoid form of α-D-glucose). Fructose in solution has a large furanose component, but glucose is virtually all in the pyranose form *(23)*.

Although furanoid-containing structures are rare in nature *(4)*, there are numerous biological compounds that contain ribose moieties, including NAD (Structure VI) and NADPH. The pyridine (e.g., NAD^+ or NADP) and flavin (e.g., FAD) nucleotides have received special attention, since they contain a ribose moiety with a *cisoid* diol conformation *(24)*. For NAD^+-requiring oxidoreductases, borate complexes with the ribosyl *cis*-hydroxy groups of coenzyme NAD^+ (with much less affinity for NADH, i.e., a factor of 15) *(24)*. The reactivity of B oxo compounds with a ligand that contains a ribosyl moiety is dependent on the total charge of the ligand, a phenomenon that has functional consequences in a biological system. Thus, when binding with NAD^+, B prefers the ribose adjacent to the

positively charged nicotinamide over the ribose adjacent to the neutral adenine *(18)*. This need for electrostatic stabilization also has the probable functional consequence of limiting, at physiological concentrations, the reaction of B oxo compounds with many ribosyl-containing compounds, including most, if not all other, nucleotides. In flavin-requiring enzymes (e.g., xanthine oxidase), B probably complexes with the ribosyl moiety on the flavin coenzyme *(20)*.

The esterification reaction that produces boromonoesters (e.g., Structure II) is easily reversible; the ester is completely hydrolyzed when transferred into water. Because boromonoesters are easily hydrolyzed when placed in aqueous solutions, it seems reasonable that boromonoesters may be isolated from hydrophobic environments (e.g., the lipid portions of the plasmalemma) where the absence of water shifts the equilibrium to the right (Reaction [4]) *(9)*.

$$B(OH)_3 + 3ROH \Leftrightarrow B(OR)_3 + 3H_2O \quad (4)$$

Substances carrying two *cis*-hydroxyl groups on adjacent C's form very stable diester complexes (Structure IV) *(18)*, which are almost undissociable in water *(25)*. The greater stability of borodiesters, compared to boromonoesters, probably explains why the B-containing antibiotics discussed above were isolated; the structure of the known B-containing antibiotics is characterized by a B atom bound to four oxy groups as a Böeseken complex of boric acid *(26)*.

pK_a

Of great importance is the fact that borate complexes often have a much lower pK_a (e.g., B-mannitol, 5.2) than free boric acid (9.25), which means that the pK_a of many B complexes is considerably below physiological pH *(21)*. This phenomenon has functional consequences and becomes the mechanism of action for some enzymatic activities. For example, boric acid is a competitive inhibitor of *Streptomyces griseus* Protease 3, and there is decreased affinity of the protein for boric acid below pH 7.0. This is directly attributable to the stability of the enzyme inhibitor complex, which is dependent on an ionizing group in the protein with an apparent pK_a of 6.6 *(27)*. This indicates that boric acid is an inhibitor of the enzyme at physiological pH by shifting the pK_a.

According to Reactions 2 and 3 above, it follows that a significant amount of boric acid is probably dissociated (i.e., present in the bound form) in most physiological environments wherever there are significant concentrations of *cis*-diols. On the other hand, the low-pH environments where B is absorbed (stomach) or excreted (kidney) should promote the undissociated (free) form of boric acid. Undissociated (and uncharged) boric acid is very soluble in water ($B[OH]_3$-saturated solution at 20°C = 0.75 mol/L) and has a high lipid solubility of the same order as urea *(21)*. It is reasonable to assume that the near 100% gastrointestinal

(GI) absorption of inorganic B and subsequent urinary excretion is closely related to the behavior of inorganic B in low-pH environments.

Because boroester formation and stability are extremely sensitive to pH, B-ligand ratios, and ligand structure *(16)*, it is not surprising that isolation and characterization of B-containing compounds in biological systems have not been particularly successful. Attempts to identify B-containing compounds formed (or possibly synthesized) in higher animals or humans logically begins with assessment of classes of compounds that contain O groups in positions likely to bond with B.

N–B Compounds

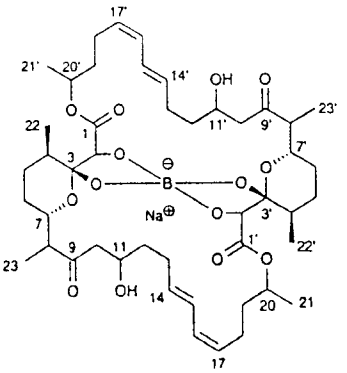
The known ability of B to form covalent bonds with the N atom of amine groups *(28)* and the observation that B binds near the coordinating Fe site of hemerythrin (the nonheme Fe-containing, O transport protein of the sipunculid worm, *Golfingia gouldii*) *(29)* suggest the possibility of a large array of biochemicals other than polyols that can react with B to form complexes. For example, there is experimental evidence *(30)* to suggest that the mechanism for inhibition of a sub-subclass of enzymes by B involves formation of a covalent bond between B and a specific N at the active site of these enzymes as described below.

B AND ENZYMATIC ACTIVITY

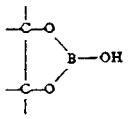
B influences the activities of at least 26 enzymes examined in various animal, plant, cell-culture, and refined chemical reaction systems by acting on the enzyme directly, by binding to cofactors (e.g., NAD) or substrates (Fig. 1), or by unknown mechanisms. The affected enzymes are distributed over four (oxidoreductases, transferases, hydrolases, and isomerases) of the six classes of enzymes.

B and Enzyme Activity Inhibition (Dampening)

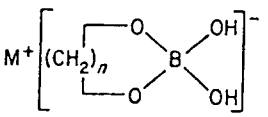
Reversible enzymatic inhibition as an essential role for an element is unusual. However, there is irrefutable evidence that B is essential for higher plants, and that B serves to inhibit or dampen several metabolic pathways in these species. For example, in plants, one substrate of the pentose-phosphate (P-P) pathway, 6-phosphogluconate, is known to complex with B, which thereby inhibits 6-phosphogluconate dehydrogenase (PGD) *(5)*, a key enzyme in that pathway. Thus, a serious problem in B-deficient plants is an increase in the amount of substrate metabolized via the P-P pathway, which gives rise to overabundant synthesis of phenolic compounds and subsequent death of plants in the subclass *Dicotyledoneae (31)*. As described below, increased activity of PGD during



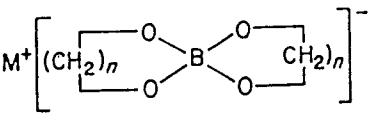
Structure I



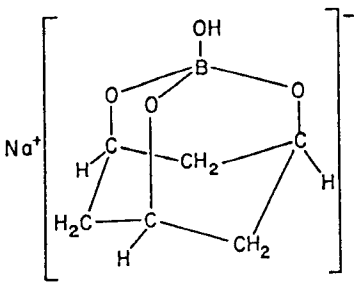
Structure II



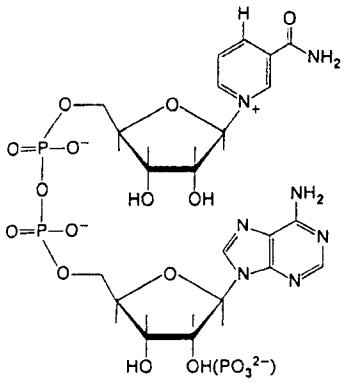
Structure III



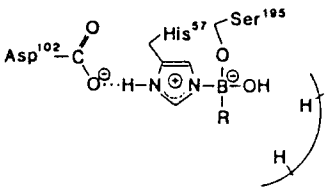
Structure IV



Structure V



Structure VI



Structure VII

Fig. 1

B deprivation in humans is hypothesized to give rise to various inflammatory diseases. Another serious outcome of B deficiency in plants is starch accumulation, a condition thought to be caused by increased activity of an enzyme in that pathway, starch phosphorylase *(5)*, as described below.

B and NAD⁺-Oxidoreductase Enzymes

Oxidoreductase enzymes that require pyridine (e.g., NAD^+ or NADP) or flavin (e.g., FAD) nucleotides are members of enzyme sub-subclasses (EC 1.1.1, 1.1.3, 1.2.1, 1.3.5, 1.6.2) that are competitively inhibited by borate or its derivatives in plant, cell-culture, and purified chemical reaction systems (Fig. 1) for the reasons explained above. For example, borate inhibits the in vitro activity of the oxidoreductase alcohol dehydrogenase (EC 1.1.1.1) by interacting with the NAD^+ cofactor *(24)*. Specific examples of NAD^+- and FAD-requiring oxidoreductase enzymes are given as they relate to control of insulin secretion and inflammatory disease.

B and Transition-State Analog Enzymes

The serine proteases (E.C. 3.4.21) represent one critical sub-subclass of hydrolases that have many essential regulatory roles, including control of the blood complement system (e.g., complement Factor I), the contact activation system (e.g., tissue kallikrein), the fibrinolytic system (e.g., thrombin), and the coagulation system (e.g., coagulation Factor Xa) *(32)*. B reversibly inhibits these enzymes as well, but by an entirely different mechanism, the transition-state analog *(33)*. The inactive (zymogenic) forms of the serine proteases are stored intracellularly or circulate

◀—Fig. 1 Examples of classes of biological organoboron complexes formed by reactions between B oxo compounds and certain mono or polyhydroxy compounds. **Structure I:** Tartrolon B, an antibiotic isolated from the myxobacterium *Sorangium cellulosum (26)*. **Structure II:** A typical boric acid monoester ("partial" esterification) with a trigonal-planar configuration and no charge formed by reaction of boric acid with a suitable dihydroxy compound *(16)*. **Structure III:** A typical borate monoester ("partial" esterification; monocyclic) complex with a tetrahedral configuration and negative charge formed by reaction of boric acid with a suitable dihydroxy compound *(16)*. **Structure IV:** A typical spiro-cyclic borodiester ("complete" esterification) complex with a tetrahedral configuration and negative charge formed by reaction of either a boric acid or borate monoester with a suitable dihydroxy compound *(16)*. **Structure V:** Scyllitol diborate, a partially esterified tridentate cleisto complex with a tetrahedral configuration and negative charge formed by reaction of boric acid with the chair conformer of cyclohexane-cis,cis-1,3,5-triol that has three equatorial hydroxyl groups *(17)*. **Structure VI:** Nicotinamide adenine dinucleotide (NAD) with ribosyl *cis*-hydroxy groups on the positively charged nicotinamide that bind to boric acid *(18)*. **Structure VII:** A serine protease-substituted boric acid complex thought to contain a negative charged boron adduct that occurs when boron completes its octet by reacting with either the O^{γ} of the active-site serine residue 195 or the $N^{\varepsilon 2}$ of the imidazole group of histidine residue 57 to form either a B–O or B–N bond, respectively *(30)*.

extracellularly, and require proteolytic cleavage for functional activity. The activated enzymes are characterized by a catalytic triad of invariant amino acid residues (histidine 57, aspartic acid 102, serine 195), which form a "charge relay" system essential for their catalytic activity *(34)*. During the course of all serine protease-catalyzed amide and ester hydrolyses, the SerOH group becomes transiently acylated *(35)*.

Nanomolar concentrations of certain synthetic peptide boronic acids effectively inhibit chymotrypsin, cathepsin G, and both leukocyte and pancreatic elastase *(36)*. The B atom in substituted boric acid compounds (e.g., arylboronic acids) is thought to inhibit the serine proteases by forming a tetrahedral B adduct that mimics the tetrahedral adduct formed during normal substrate hydrolysis (Structure VII) *(33)*. The available experimental evidence *(30)* suggests that formation of the B adduct occurs when B completes its octet by reacting with either the O^{γ} of the active-site serine residue 195 or the $N^{\varepsilon 2}$ of the imidazole group of histidine residue 57 to form either a B–O or B–N bond, respectively. Boronic acids that are analogs of serine protease substrates form the former type of complex, those that are not analogs, the latter *(30)*.

There is also evidence that natural, simple unsubstituted boric acid compounds (e.g., Na borate) that contain a trigonal B atom also bind to certain enzymes to form a reversible tetrahedral transition-state analog complex. For example, borate reversibly inactivates α-chymotrypsin by accepting the free electron pair of the N atom on the imidazole group of the histidine residue at the active site *(37)*. At least five microbial subtilisin-type serine proteases bind to simple borates to form tetrahedral transition-state analogs *(38)*. Also, phosphoglucomutase, an isomerase, and starch phosphorylase, a transferase, are two enzymes important in glycogen/starch metabolism. Neither enzyme requires NAD^+, but both are inhibited by B *(39)*, probably because they each contain an active serine residue at the active site *(40)*. Another interesting reaction between serine and borate affects the activity of γ-glutamyl transpeptidase (GGT), a membrane-bound enzyme that functions in the γ-glutamyl cycle to catalyze utilization of glutathione *(41)*. Enzyme inhibition is produced by formation of a serine–B complex, which binds at the γ-glutamyl binding site of the light subunit of GGT to act as a transition-state inhibitor *(41)*. By that mechanism, serine-borate apparently elevated the concentrations of glutathione (GSH) in cultured fibroblasts taken from individuals suffering from glutathione synthase deficiency *(42)*.

Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GPD), as an NAD^+-requiring oxidoreductase, is composed of four identical subunits and converts D-glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. ATP and NAD^+ regulate GPD activity, since the former dissociates the enzyme into dimers and/or monomers *(43)* and the latter promotes reassociation *(44)*. B also may regulate enzyme activity by forming a tetrahedral transition-state analog; there is evidence from in vitro experiments that borate binds to a specific site(s) on the enzyme, which triggers struc-

tural changes and dissociation of the tetramer *(45)*. This mechanism of action would be especially important for the process of phagocytosis, which is dependent mainly on anaerobic glycolysis as described below.

The activities of several enzymes in several plant species are inhibited by B by uncharacterized mechanisms. These include β-glucosidase *(46)* and acid phosphatase *(47)*.

B and Enzyme Activity Stimulation

In several plant species, adequate B nutriture increases the activity of certain enzymes. For example, the activity of plasmalemma NADH oxidase was inhibited in B-deficient cultured carrot cells, and under such conditions, activity could be restored by exogenous boric acid *(48)*. The activity of NADH oxidase is apparently closely related to proton secretion *(49)*. Thus, it is not surprising that proton secretion is increased when B is added to B-deficient plant medium *(48)*.

In plants, adequate B nutrition enhances uridine diphosphate glucose (UDPG) pyrophosphorylase activity, such that conversion of glucose-1-P to UDPG is increased *(50)*. Simultaneously, B inhibits the conversion of glucose-1-P to starch catalyzed by starch phosphorylase. Thus, it was proposed that starch accumulation observed in B-deficient plants is caused by decreased UDPG pyrophosphorylase activity and increased starch phosphorylase activity *(5)*.

Enzymatic Responses to Physiological B

Several of the plant cell-culture and refined chemical reaction studies described above were carried out with concentrations of B much greater (up to 100,000 μmol/L) than those expected to be encountered in most plant (~0.01–50 μmol/L) or animal (nondetectable [<1]-~14 μmol/L) tissue-culture media, or animal or human sera (~1–60 μmol/L) (Table I). For plants, it is difficult to define a physiological range of B, because the range of concentrations within which B is essential to some species overlaps the range that is toxic to others *(51)*. Furthermore, plant parts vary in B content *(15)*, and as B becomes limited, virtually all cellular B is localized in the cell wall with still further molecular compartmentalization in the form of strong association with the pectic fraction of the cell wall *(52)*. Also, findings from cell-culture or refined chemical reactions are not necessarily based on the minimum amounts of B required to elicit a change in enzyme activity. However, disregarding obvious structural differences between plant and animal species, the distribution of B in biological tissue, and the design of experimental systems, there are a remarkable number of enzymes whose activities are either inhibited or stimulated by simple borates in concentrations found in sera or plant or animal cell nutrient solutions. Most importantly, some of these changes in enzymatic activity (e.g., pea UDPG pyrophosphorylase or sunflower

lipooxygenase activity) occur at concentrations of B below those found in serum or plasma of many animals or certain human fluids (Table 1).

The types of reactions between B and biological ligands, including various enzymes, have served as guideposts in the design of experiments conducted to elucidate the roles of B in animals and humans. As described below, findings from numerous studies indicate that diets low in B (≤0.3 μg [0.028 μmol]/g supplemented with inorganic B, in amounts (~2 μg [~0.185 μmol]/g) equivalent to those found in diets luxuriant in fruits and vegetables, are sufficient to affect several aspects of animal and human physiology.

B AND ENERGY SUBSTRATE UTILIZATION

B and Glycolysis

Findings from various cell-culture and refined chemical reaction studies are not necessarily directly applicable to intact physiological systems. Nevertheless, the finding that B inhibits the in vitro activity of glycolytic enzymes stimulated efforts to determine the in vivo effects of dietary B on the glycolytic pathway. Hepatic concentrations of all metabolites in the glycolytic pathway from glycogen to pyruvate/lactate were measured in chicks fed diets low in B (~0.20 mg B/kg) or supplemented with physiological amounts of B (1.3 mg/kg) *(53)*. The effects of B on GPD activity apparently differ between in vitro (*see above*) and physiological systems; the concentration of 1,3-bisphosphoglycerate was not affected by dietary B supplementation. Most likely, the inhibitory effect of B on GPD activity is dependent on the B : ligand ratio, which was probably much higher in the in vitro system than the physiological system.

In the same in vivo study of the glycolytic pathway described above *(53)*, B supplementation of the low-B diet strongly affected the concentrations of metabolites within the three-carbon phosphorylated acid pool of the glycolytic pathway. B supplementation decreased the concentration of 2-phosphoglycerate and tended to decrease the concentrations of phosphoenolpyruvate. Furthermore, concentrations of metabolites within the fructose 1,6-biphosphate and triose phosphate (FBP-TP) pool of the glycolytic pathway were affected by physiological amounts of dietary B. For example, B supplementation decreased dihydroxyacetone phosphate concentrations. The latter findings differ from those from an earlier report where a single high oral dose of B (140 mg/kg body/wt) given to guinea pigs increased the concentration of metabolites in the FBP-TP pool *(54)*. The difference in findings is probably related to large differences in the B:ligand ratio and possible differences between species.

Regardless of the mechanism through which B influences hepatic glycolytic metabolite concentrations, the element is apparently beneficial at physiological concentrations, because chick growth was improved by dietary B *(53)*. The pronounced manner in which B influenced the hepatic glycolytic pathway provided further support for developing the hypothesis that B, present in the diet in physiological concentrations, is a modulator of energy substrate metabolism.

B and Insulin

The findings described above suggest that B has a role in energy metabolism and became the impetus to examine the influence of dietary B on insulin metabolism. It was determined that B deprivation greatly increased plasma insulin concentrations in the vitamin D-deprived rat *(55)*. Furthermore, isolated, perfused pancreata from chicks fed a low B diet exhibited a nearly 75% increase in peak insulin release, especially when the perfusate was supplemented with glucose *(56)*. This finding may be of special significance. β-cell "exhaustion" may explain the β-cell deterioration that occurs during excessive insulin demand *(57)*. β cells that are too easily induced to secrete mass quantities of insulin are more readily damaged, which eventually can cause them to stop functioning and result in diabetes mellitus *(57,58)*. Perhaps B acts to limit pancreatic β-cell stress that may occur during periods of excessive metabolic demands for insulin production.

The mechanism by which B influences peak insulin release was hypothesized *(56)* to involve the metabolism of NADPH. Changes in NADPH metabolism can induce changes in cell membrane potential, a main event leading to the release of insulin-containing secretory granules. Insulin secretion from pancreatic β cells is a complex cascade of events regulated by glucose metabolism and certain second messengers, including cyclic nucleotides, inositol phosphates, diacylglycerol, and calcium *(59)*. Because glucose passes freely across the β-cell plasma membrane, elevated extracellular glucose concentrations increase glucose metabolism in the β cell, with a concomitant increase in cytoplasmic ATP/ADP ratios. ATP binds to and closes ATP-dependent K^+ channels on the β-cell membrane, a process that decreases K^+ conductance and causes depolarization of the membrane (whose potential is typically maintained at about –80 mV). At a threshold potential of about –40 mV, voltage-gated calcium channels open, and Ca enters the cell along its electrochemical gradient, and the intracellular Ca concentration increases, a crucial step in the coupling of β-cell depolarization with insulin secretion *(60)*. Then, Ca-calmodulin-regulated proteins lead to the release of insulin-containing secretory granules. Resting membrane potential is restored mainly by opening of a second K^+ channel activated by Ca^{2+} *(59)*. In the β cell, NADPH modulates β-cell membrane depolarization ultimately to influence insulin release as discussed below.

NADPH

Within the β cell, increased NADPH concentrations precede increases in intracellular Ca concentration and insulin release. Inhibition of NADPH concentrations, via pentose shunt inhibition, decreases insulin secretion *(61)*. There is evidence that NADPH influences insulin secretion by increasing activity of the voltage-dependent Ca^{2+} channel (VDCC), such that when the pentose shunt is inhibited, less NADPH is available, resulting in decreased Ca^{2+} influx. Therefore, within the β cell, the pentose shunt and its production of NADPH play an important role in the regulation of insulin secretion.

As mentioned above, the activities of two key plant enzymes in the pentose shunt, PGD and glucose 6-P dehydrogenase are normally inhibited and thereby regulated by B *(31)*. If B controls the pentose phosphate shunt in chick β cells in a manner similar to the way it controls the shunt in many plant species, it would not be surprising to learn that an increase in peak insulin release during B-deficiency states is the result of increased activity of the pentose pathway.

Cell Membrane Ion Transport

The recent findings on B–insulin interactions provide more indirect evidence that B influences ion transport across the cell membrane. Very early research on plant B nutrition indicated that B deficiency caused a reduction in ion uptake in *Impatiens balsamina (62)*. Considerable evidence has accumulated since that time that B is involved in plasmalemma-bound transport processes for higher plants and diatomaceous species *(63)*. For example, it seems clear that B is important in plasmalemma electron transport. In B-deficient cultured carrot cells, stepwise restoration of the plasmalemma electron transport-generated H^+ secretion is obtained with the addition of physiological amounts of boric acid *(48)*.

In B-deprived rats, increases in dietary K from marginally deficient to adequate to luxuriant induced increases in the transport of Ca into platelets (454–548–586 n*M*) activated by thrombin in the presence of 1.0 mmol/L external Ca *(64)*. However, the effect of K on Ca transport into platelets was minimal in rats supplemented with physiologic amounts of B (501–501–536 nmol/L). B may have reacted directly with thrombin to somehow modify platelet activation. Thrombin (EC 3.4.21.5) is a member of the sub-subclass of hydrolases (serine proteases [EC 3.4.21]) that form reversible transition-state analogs with B and boronic acid derivatives. However, these findings are similar to those expected for plasmalemma ion transport involving regulation of the VDCC by B through inhibition of NADPH concentrations. Increases in dietary K may have increased intracellular K^+ concentrations, allowing for faster depolarization after thrombin activation, increased activity of the VDCC, and finally, increased Ca^{2+} transport into the platelets. Subsequently, the presence of B in physiological amounts may have dampened activity of the VDCC by inhibiting NADPH concentrations.

B AND INFLAMMATORY DISEASE

The Inflammatory Process and Inflammatory Disease

Under normal conditions, the defense mechanism of inflammation serves a vital function, and in most instances, elimination of antigens proceeds without evidence of clinically detectable inflammation *(65)*. Excessive inflammation, either secondary to abnormal recognition of host tissue as "foreign," or abnormal turn-off of an otherwise normal inflammatory process leads to inflammatory disease. The development of clinically apparent inflammation indicates that the immune system has encountered either an unusually large amount of antigen, antigen in an unusual location, or antigen that was difficult to digest. In some diseases, i.e., rheumatoid arthritis, the inciting agent is unknown or may be related to normal host tissue components *(65)*.

B and Inflammation Disease

B as common borax was reported to have antiarthritic activity on formaldehyde-induced arthritis in albino rats *(66)*. In a human study *(67)*, 20 patients presenting radiographically confirmed osteoarthritis received either 6 mg (0.55 mmol) oral supplements of B/d or a placebo for 8 wk in a double-blind trial. The arthritic individuals who received B supplementation self-reported substantial improvement in subjective measures of their arthritic condition (pain on movement, joint swelling, restricted movement). Rheumatoid factor, not measured in the arthritic study, is rapidly and completely precipitated in boric acid solutions (2%). This prompted the hypothesis that B reacts with sugar moeities in the rheumatoid factor to form a reversible complex *(68)*.

A recent preliminary report *(69)* suggests that luxuriant amounts of dietary B (20 μg [1.85 μmol]/g) compared to very low amounts (<0.2 μg [0.02 μmol]/g), delay the onset and severity of adjuvant-induced arthritis in rats. Addition of B in vitro over a range between 0 and 20 μg (1.85 μmol)/mL inhibited proliferation of splenic cells isolated from B-deprived rats and subsequently stimulated by 0, 5, or 50 μg phytohemagglutinin/mL. This finding suggests that dietary B may alleviate adjuvant-induced arthritis by inhibiting T-cell activity.

A search for the mechanism through which B modulates inflammation led to another recent preliminary report *(70)* that dietary B affects serum antibody concentrations. Physiological amounts of B (3 μg [0.28 μmol]/g) added to a low B diet (0.2 μg [0.02 μmol]/g) more than doubled (1.58 ± 0.04 vs 0.77 ± 0.27 as optical density at 490 n*M*, mean ± SEM) serum total antibody concentrations. The effect was probably not the result of artifactual antigen–antibody binding, because borate does not inhibit the reaction of human γ-globulin with rabbit anti

human γ-globulin *(71)*. The observed effects of B on various components of the inflammatory process as described above lead to the hypothesis that B protects against inflammatory disease, because B apparently modulates the inflammatory response. Possible mechanisms for this phenomenon are presented below.

B and the Respiratory Burst

P-P Pathway

When neutrophils and other phagocytes are exposed to appropriate stimuli, they begin to produce large quantities of superoxide, the precursor of a group of powerful oxidants that are used as microbicidal agents. During this process, the phagocytes consume much more oxygen than that needed for the generation of metabolic energy required for phagocytosis. The phenomenon is termed respiratory burst, a poor name because it is unrelated to mitochondrial electron transport. The primary electron donor for the reduction of oxygen during respiratory burst is NADPH *(72)*.

$$\mathrm{NADPH} + 2\mathrm{O}_2 \xrightarrow[\text{NADPH oxidase}]{} \mathrm{NADP^+} + 2\mathrm{O}_2^{\cdot\cdot} + \mathrm{H^+} \qquad (5)$$

The source of the NADPH for the respiratory burst comes mainly from the reduction of $NADP^+$ in the P-P pathway, which is very active during the respiratory burst. As described above, this pathway is regulated by B in plants by inhibiting the activity of two critical enzymes in the pathway *(5)*. Whether B serves as a metabolic regulator of the leukocyte respiratory burst is under active investigation.

Oxidative Damage

As described above, the major products of the respiratory burst are oxidants, including hydrogen peroxide (H_2O_2), hydroperoxy radical ($HO_2^{\cdot}$), and the hydroxyl radical ($OH^{\cdot}$). When neutrophils invade inflamed areas of the body to remove either dead or foreign components, they release, among other substances, reactive oxygen species (ROS). If not properly controlled, ROS cause severe damage to healthy tissue and lead to a myriad of inflammatory diseases. The ROS are released into a phagocytic vacuole or the extracellular space mainly to attack malignant cells, invading organisms too large to be phagocytized, and certain soluble mediators. Inflammatory disease arises from oxidant release into the extracellular space and subsequent attack on adjacent normal tissue.

Destructive ROS are scavenged and destroyed by several defense mechanisms. Superoxide dismutase (SOD) is an oxidoreductase that serves to dismutate superoxide anions that are generated during oxidative metabolism and in response to noxious stimuli. Catalase disproportionates hydrogen peroxide, and protects membrane lipids and proteins from attack by peroxy radicals *(73)*. Glutathione peroxidase reduces hydrogen peroxide by means of reduced glutathione, and the intracellular reduction of glutathione requires NADPH and glutathione reductase *(74)*.

B may be important in the oxidant scavenging process. B supplementation increased erythrocyte SOD activity in men and postmenopausal women *(75)*. SOD activity increases during increased oxidative metabolism or in response to noxious stimuli. SOD also decrease during inadequate Cu status, because SOD is a Cu-requiring enzyme. It remains to be determined whether SOD activity increased because B may have induced free radical formation (unlikely), or whether B improved antioxidative capacity. A related finding *(76)* supports the later hypothesis in that B supplementation increased plasma immunoreactive ceruloplasmin in men and postmenopausal women, and may have done so by improving Cu status through uncharacterized mechanisms.

B may exert its influence on the oxidant scavenging process through direct action on GGT. That enzyme is the major catabolic enzyme for glutathione and its derivatives. Serine–borate complex is a transition-state inhibitor of GGT *(41)*. By that mechanism, serine–borate apparently elevated the concentrations of GSH in cultured fibroblasts taken from individuals suffering from glutathione synthase deficiency *(42)*.

In another human study *(67)*, 20 patients presenting with radiographically confirmed osteoarthritis were recruited to a double-blind trial and given either 6 mg of B/d or a placebo. It is noteworthy that patients consuming the B supplement exhibited lower blood GGT concentrations (20.7 U [0.345 μkat]/L vs 26.3 U [0.438 μkat]/L) and reported substantial improvement in subjective measures of their arthritic condition (pain on movement, joint swelling, restricted movement). In rats, serine–borate was reported to increase renal glutathione content in vivo *(77)*. Thus, the available evidence is consistent with the hypothesis that B protects against oxidative damage by an unknown mechanism. Research efforts are under way to confirm the working hypothesis that B limits oxidative damage by reducing production of NADPH needed for the respiratory burst and by reducing the activity of GGT, an action that would most likely enhance body stores of glutathione and its derivatives.

CONCLUSIONS

The findings to date leave little doubt that amounts of B similar to that found in diets luxuriant in fruits, nuts, legumes, and vegetables influence a wide range of physiological variables. Changes in enzymatic activity of certain plant enzymes during B deprivation and in vitro reversible inhibition of several classes of enzymes are well documented. The findings are consistent with the hypothesis that B is a regulator of enzymatic activity in pathways closely involved with energy substrate metabolism, insulin release, and the immune system. Taken as a whole, the experimental B nutrition research data indicate an essential role for the element in animals and humans. Most likely, new advances in B nutrition research will include better characterization of the mechanisms through which B modulates immune function and insulin release.

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