Biochemical Effects of Physiological Amounts of Dietary Boron

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Research on human and animal boron nutrition has progressed sufficiently over the past decade to develop working hypotheses for biochemical roles of the element. It is well established that vascular plants, diatoms, and some species of marine algal flagellates have acquired an absolute requirement for boron although the primary role remains unknown. Discovery of naturally-occurring boron oxy compounds, all ionophoric macrodiolide antibiotics with a single boron atom critical for activity, established at least one biochemical role of boron. Recent findings suggest that physiological amounts of supplemental dietary boron (PSB) affect a wide range of metabolic parameters in chick and rat model systems as well as humans. Cholecalciferol (vitamin D₃) regulates energy substrate utilization; current findings indicate that boron modifies that regulatory function. For example, in chicks, PSB substantially corrected vitamin D₃-deficiency-induced elevations in plasma glucose concentrations. Boron also alleviates perturbations in mineral metabolism characteristic of vitamin D₃ deficiency. In rachitic chicks, PSB alleviated distortion of the marrow sprouts in the proximal tibial epiphysial plate. Boron may help prevent inflammatory disease as several key regulatory enzymes in the inflammatory response are inhibited by PSB. The findings to date support the hypothesis that boron is essential for animals and humans. Also, boron and vitamin D₃ have the same overall effect on the utilization of energy substrates found in plasma. Further advances in boron nutrition research will probably include characterization of the mechanisms through which boron modulates immune function, insulin release, and vitamin D metabolism. © 1997 Wiley-Liss, Inc.

Key words: vitamin D; energy substrate utilization; bone; mineral metabolism

INTRODUCTION

Dietary boron was identified as a growth factor for the vitamin D-deficient chick 15 years ago [1], a finding that renewed interest in the biological effects of physiological amounts of boron. There were no reported attempts to determine the biological effects of physiological amounts of boron in any animal species during the 37 years prior to this finding except for a brief description of a small effect of dietary

Abbreviations: FBP-TP, fructose 1,6-biphosphate and triose phosphate; GGT, gamma-glutamyl transpeptidase; GPD, glyceraldehyde-3-phosphate dehydrogenase; IAA, indole acetic acid; NMR, nuclear magnetic resonance; P-P, pentose-phosphate, ROS, reactive oxygen species; SOD, superoxide dismutase; UDPG, uridine diphosphate glucose.

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boron deprivation on liver RNA synthesis in rats [2]. The earliest reported attempts
to induce a boron deficiency in rats were unsuccessful [3–5]. A report in 1945 [6] that
supplemental dietary boron was beneficial to potassium-deficient rats was not con-
*confirmed* 2 years later under different dietary conditions and boron supplementation [7].
All of the findings described in the 1940s were confounded by the use of basal diets
that were either nutritionally inadequate or supplemented with excessive amounts of
boron [100–2,200 mg (9.25–200 mmol)/kg].

protocols accounts for most of the advances in boron nutrition research made in the
last 12 years. The refinements reflect greater appreciation for characteristics of boron
distribution within the class Angio-
*spermae* (most common source of plant foodstuffs), and boron anthropomorphic
sources. These characteristics were manipulated to *improve* analytical detection of
boron, reduce environmental boron *contamination*, obtain boron-low drinking water
[≤0.015 μg (0.001 μmol)/mL] and construct boron-low diets [<0.1 μg (0.009 μmol)
B/g for animals; 0.36 mg (0.033 mmol) B/8400 KJ for humans]. Supplemeting these
diets with inorganic boron, in amounts [–2 μg (–0.185 μmol)/g] equivalent to that
found in diets luxuriant in fruits and vegetables, was sufficient to affect several
aspects of animal and human physiology. This progress in boron nutrition research
over the last decade has led to the development of certain working hypotheses de-
scribed below.

The earlier unsuccessful attempts to observe any consistent biological effect of
physiological amounts of boron in animals is remarkable in view of convincing
evidence, first described in 1923, that boron is essential for plants [15]. There is now
universal agreement that vascular plants [16], diatoms [17,18], dinitrogen-fixing cyan-
obacteria with heterocysts [19], and some species of marine algal flagellates [20]
have acquired an *absolute* requirement for boron. Boron is involved in exceptionally
diverse plant cellular processes including sugar transport, cell wall synthesis and
lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respi-
ration, indole acetic acid (IAA) metabolism, phenol metabolism, membrane function,
DNA synthesis, and possibly redox reactions through *involvement* in ascorbate me-
tabolism [21]. Even so, a specific biochemical role for boron in any of those species
remains to be elucidated despite extensive research efforts and the unambiguous
characteristics of boron deficiency [22]. Perhaps, then, it is not altogether surprising
that progress in elucidation of the role of boron in animal and human physiology has
been exceptionally slow.

To date, five naturally occurring biological boron *oxy* compounds, as natural boron
esters1 synthesized by various bacteria, have been identified and they are all antibo-
iotics with at least four *functioning* as ionophoric macrodiolides. The structure of

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1Definitions of boron esters follow that of Thellier et al. [39] and van Duin et al. [35] where “boro-
monoester” denotes a compound formed when boric acid or borate react with a suitable dihydroxy
compound to give the corresponding boric acid ester or borate monoester (“partial esterification”) and
“borodiester” denotes a compound *formed* when these boromonoesters react with another dihydroxy
compound to give a borate diester.
tartrolon B (Structure I), isolated from the myxobacterium *Sorangium cellulosum*, is the latest in this class of compounds to be described [23]. Aplasmomycin A, B, and C are closely related antibiotics isolated from strain SS-20 of *Streptomyces griseus* obtained from a shallow sea sediment [24–28]. Boromycin, synthesized by certain strains of *Streptomyces antibioticus*, has the ability to encapsulate alkali metal cations and reduce the permeability barrier of the cytoplasmic membrane toward potassium ions [25,29–31].

**BORON CHEMISTRY**

**Boron Speciation**

At physiological concentrations, inorganic boron is essentially present only as the mononuclear species boric (orthoboric) acid B(OH)₃ (with a trigonal-planar bonding arrangement) and as borate B(OH)₄⁻ (with a tetrahedral bonding arrangement) [32]. Trigonal-planar bonding is preferred because it represents the stabilized form [32]. 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%) [33]. Boric acid is an exclusively monobasic acid and is not a proton donor but rather is a Lewis acid, accepting a hydroxyl ion to form the tetrahedral anion B(OH)₄⁻ [Eqn. (1)] [34]:

\[
\text{B(OH)}_3 + 2\text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{B(OH)}_4^- \quad \text{pK}_a = 9.25 \quad (25^\circ\text{C})
\]  \hspace{1cm} (1)

**Boron Esters and Complexes**

Boric acid and borate can react with a suitable dihydroxy compound to form the corresponding boric acid ester and borate monoester (partial esterification) (Structure II). These two types of borumonoesters can react with another dihydroxy compound to give a corresponding borodiester (Structure III) [35]. A tridentate complex (Structure IV) may be formed when a ligand contains three *cis* oriented hydroxyl groups [32]. A diborate complex of tridentate structure (not shown) is also possible [32].
Experimental data suggest that boric acid and boric acid-like structures, instead of borate, are the reactive species in the esterification process [35,36].

The type of borooester formed depends in large part upon the original structure of the ligand, the pH of the aqueous environment [35] and the relative concentrations of boric acid and the ligand [37]. For example, the nuclear magnetic resonance (NMR) signal from an aqueous solution of boric acid (0.1 mol/L) and glycol (1.0 mol/L) [35] indicated the presence of boric acid and borate only at pH <8. At pH >8, the signal indicated a marked decrease in the concentration of boric acid and borate species but a marked increase in the concentration of borate monoester and a much smaller increase in the concentration of borate diester. In contrast, the NMR signal from an aqueous solution of boric acid (0.1 mol/L) and glycolic acid (1.0 mol/L) [35] indicated the presence of boric acid and boric acid monoester at low pH. A rise in pH induced the formation of borate diester species (peak at pH = 3) and a large decrease in boric acid and boric acid monoester concentrations (which minimizes near pH = 3). Further increases in pH induced a rebound in boric acid and boric acid monoester concentrations (through pH = 11), extinction of the borate diester species (near pH = 7), and maxima (near pH = 7), and then extinction (near pH = 11) of the borate monoester species. Because borooester formation and stability are extremely sensitive to pH, boron-ligand ratios, and ligand structure, it is not surprising that isolation and characterization of boron-containing compounds in biological systems has not been particularly successful.

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 [Eqn. (2)] [34].

\[
\text{B(OH)}_3 + 3\text{ROH} \rightleftharpoons \text{B(OR)}_3 + 3\text{H}_2\text{O} \tag{2}
\]

Substances carrying two cis-hydroxyl groups on adjacent carbons form very stable diester complexes (Structure III) [38] which are almost undissociable in water [37,39]. The greater stability of borodiesters, compared to boromonoesters, probably
explains why the boron-containing antibiotics discussed above were isolated: the structure of the known boron-containing antibiotics is characterized by a boron atom bound to four oxy groups as a Böeseken complex of boric acid [23].

**Biological Boroesters and Boron Complexes.** Attempts to identify boron-containing compounds formed (or possibly synthesized) in higher animals or humans logically begins with assessment of classes of compounds that contain oxygen groups in positions likely to bind with boron. For example, data from refined chemical reactions [40] indicate that the reaction of boric acid with various dihydroxy (e.g., riboflavin) and polyhydroxy (e.g., mannose) biological ligands gives rise to boroesters of potential biological importance. Typically, the pertinent ligands contain adjacent hydroxyl groups in the cis position and the reactivity of boric acid with the ligand generally increases with an increase in the number of these cisoid groups [40]. As described above, the exact form of the complex varies according to pH and ligand: boron ratio. The relevant cisoid diol conformations are also present in several biologically important sugars and their derivatives (sugar alcohols, -onic, and -uronic acids). Examples include mannose, ribose, galactose, and fructose [41]. The pyridine (e.g., NAD\(^+\) or NADP) and flavin (e.g., FAD) nucleotides have received special attention as they contain a ribose moiety with a cisoid diol conformation [42].

A borate complex is sometimes formed without the presence of a cisoid -diol group. For example, certain ring conformations in trans -1,2-diols favor complex formation with boron [40]. Also, hydroxyl groups arising from lactol formation (in glucose, alpha-D-form only), hydration of COOH groups (in alpha-hydroxy, e.g., lactic; and aromatic o-hydroxy acids, e.g., salicylic acid), or hydration of ketone groups (in benzil or alloxan) are reactive with B(OH)_3 [40]. In some situations, the stereochemistry is correct for the reaction of borate with the hydroxyl groups in 1,3 positions (e.g., in pyridoxine) [43]. Substitution of one or more of the hydroxyl groups coordinated with boron, or substitution of individual hydrogens in those hydroxyl groups gives rise to many classes of cyclic coordination complexes.

Reactions between boron and other elements with electron pair donors would also be important in biological systems. Compounds with B-S, -Se, -P, -As, -Sb, -C, or -N bonds have been synthetically produced [34] and the existence of biological organo- or amino-boron compounds would be of special interest.
BORON AND ENZYMATIC ACTIVITY

Boron is known to influence the activities of over 15 enzymes examined in various animal, plant, cell culture, and refined chemical reaction systems. The affected enzymes are distributed over four (oxidoreductases, transferases, hydrolases, and isomerases) of the six classes of enzymes. The following discussion is arranged according to the stimulatory or inhibitory (or dampening) effect of boron on these activities.

Enzymatic Inhibition by Boron

The majority of enzymes known to be influenced by boron exhibit decreased and reversible activity in the presence of the element. Conversely, decreases in the concentration of boron typically, but not always, enhances the activity of these enzymes. Enzymatic inhibition as an essential role for an element is unusual. However, there is irrefutable evidence that boron is essential for higher plants and that boron is an inhibitory or damping factor in several metabolic pathways in these species as described below.

Boron 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 (E.C. 1.1.1., 1.1.3., 1.2.1., 1.3.5., and 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 oxidoreductases, the inhibitory mechanism is related to reactions between boron and various hydroxy-containing components of the enzyme system. Thus, for NAD*-requiring oxidoreductases, borate is thought to complex with the ribosyl cis-hydroxy groups of coenzyme NAD* (with much less affinity for NADH [42]). In binding with NAD*, borate apparently demonstrates preference for the ribose adjacent to the positively charged nicotinamide over the ribose adjacent to the neutral adenine [38]. For example, borate inhibits the in vitro activity of a key oxidoreductase in the glycolytic pathway, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GPD), an enzyme that converts D-glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. Boron is thought to interact with the NAD* cofactor associated with GPD [42]. In flavin-requiring enzymes (e.g., xanthine oxidase), boron probably complexes with the ribosyl moiety on the flavin coenzyme [40]. Examples of NAD*- and FAD-requiring oxidoreductases that are competitively inhibited by borate with respect to NAD* or FAD are listed in Figure 1.

Boron and transition state analog enzymes. Other enzymes are reversibly inhibited by boron as they form transition state analogues with borate and boronic acid derivatives. For example, one sub-subclass of hydrolases, the serine proteases (E.C. 3.4.21.), are known to be inhibited by boron. These enzymes 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) [44]. The inactive (zymogenic) forms of these proteins are stored intracellularly or circulate extracellularly and require proteolytic cleavage for functional activity. The activated enzymes are characterized by a catalytic triad of invariant amino acid residues (histidine, aspartic acid, and serine) that form a “charge relay” system essential for their catalytic activity [45,46]. During the course of all serine protease-catalyzed amide and
Fig. 1. Schematic of selected biochemical pathways (intermediate substrates not always shown) containing selected substrates, products, or enzymes whose concentrations or activities are decreased (● or hatched rectangle) or increased (○ or open rectangle) by simple borate supplemental (but not necessarily the inverse by borate depletion) in humans (H), animals (A), plants (P), cell culture systems (CC), or refined chemical reactions (RCR). See individual citations for specific form and molar concentrations (some far above expected physiological range) of boron used to elicit the reported response. Enzyme nomenclature follows EC recommendations [109]. Exact enzyme classification is typically not provided in the literature such that the listed EC numbers serve only as a general classification guide. FAHP, fatty acid hydroperoxides; FR-6-P, fructose 6-P; FR 1.6-P, fructose 1,6-P₂; X 5-P, xylulose 5-P; GAD 3-P, glyceraldehyde 3-P; 2P Glyc, 2-P glyc erate; 1,3-P₂ Glycer, 1,3-P₂ glycerate; (OH)₂ acetone P, dihydroxyacetone P; GSSG, oxidized glutathione; GSH, reduced glutathione; identification of letters A through I is provided in listing below:

SUBSTRATE OR PRODUCT CONCENTRATION

Inhibitory effect of boron

Human

A. Glucose, serum [12].

Animal

B. Dihydroxyacetone-P, liver [71].
C. Fructose 1,6-diphosphate, liver (high boron concentrations) [72].
D. Glucose, plasma (in cholecalciferol deficiency model that induces elevated concentrations) [74–76, 78].
E. 2P-Glycerate, liver [71].
F. β-Hydroxybutyrate (in cholecalciferol deficiency model that induces elevated concentrations) [78].
G. Pyruvate (in cholecalciferol deficiency model that induces elevated concentrations) [78].

Plant

H. Starch [57].
Fig. 1. Continued

**Stimulatory effect of boron**

*Huexan*

I. Estradiol-17β [14].
J. 25-hydroxycholecalciferol [11,14].
K. Triglyceride, serum [14].

*Animal*

L. 1,25-dihydroxycholecalciferol (in cholecalciferol deficiency model that induces depressed concentrations) [80].
M. RNA, liver [2].
N. Triglyceride, serum/plasma (in cholecalciferol deficiency model that induces depressed concentrations) [75,79,84,85].

*Plant*

O. RNA [110–112].
P. ATP [112].

**SELECTED ENZYMES STIMULATED (in italics) OR INHIBITED BY BORATES**

(presented according to EC classification)

1. Oxidoreductases
   1.1. Acting on the CH-CH group of donors
      1.1.1. With NAD⁺ or NADP⁺ as Acceptor
             Q. [1.1.1.1] Alcohol dehydrogenase; requires NAD⁺; RCR [42,113,114].
             R. [1.1.1.27] Lactate dehydrogenase; requires NAD⁺; RCR [42].
             S. [1.1.1.44] Phosphogluconate dehydrogenase (decarboxylating); requires NADP⁺; RCR [115].
             T. [1.1.1.49] Glucose 6-P dehydrogenase; requires NADH⁺; P [116].

      1.1.3. With Oxygen as Acceptor
             U. [1.1.3.22] Xanthine oxidase; contains FAD; RCR [117].

   1.2. Acting on the Aldehyde or Oxo Group of Donors
      1.2.1. With NAD⁺ or NADP⁺ as Acceptor
             V. [1.2.1.12] Glyceraldehyde-3-phosphate dehydrogenase; requires NAD⁺; RCR [118].

   1.3. Acting on the CH-CH Group of Donors
      1.3.5. With a Quinone or Related Compound as Acceptor
             W. [1.3.5.1] Succinate dehydrogenase, ubiquinone; contains FAD; RCR [119].

   1.6. Acting on NADH or NADPH
      1.6.2. With a Cytochrome as Acceptor
             X. [1.6.2.2] Cytochrome-b₅ reductase; requires NADH; contains FAD; CRC (not shown) [120].

      1.6.99. With Other Acceptors
             Y. [1.6.99.3] NADH oxidase (NADH dehydrogenase); requires NADH; P, CC (not shown) [64,67].
1.13. Acting on Single Donors With Incorporation of Molecular Oxygen (Oxygenases)
   1.13.11. With Incorporation of Two Atoms of Oxygen
     Z. [1.13.11.12] Lipoygenase; P [83].

1.14. Acting on Paired Donors with Incorporation of Molecular Oxygen
   1.14.18. With Another Compound as One Donor, and Incorporation of One Atom
             of Oxygen
     AA. [1.14.18.1] Monophenol monooxygenase (tyrosinase) (catechol oxidase) (polyphenol oxidase); P [63,121].

2. Transferases
   2.3. Acyltransferases
      2.3.2. Aminoaacyltransferases
      BB. [2.3.2.2] Gamma glutamyltranspeptidase (GGT); CC [59,96].

2.4. Glycosyltransferases
   2.4.1. Hexosyltransferases
      CC. [2.4.1.1] Starch phosphorylase; P [56,57].

2.7. Transferring Phosphorus-Containing Groups
   2.7.7. Nucleotidyldtransferases
      DD. [2.7.7.9] UTP-glucose-1-phosphate uridylytransferase, UDPG pyrophosphorylase; P [68].

3. Hydrolases
   3.1. Acting on Ester Bonds
      3.1.27. Endoribonucleases Producing Other Than 5’- Phosphomonoesters
      EE. [3.1.27.5] RNAase; P [112,122].

3.2. Glycosidases
   3.2.1. Hydrolyzing o-Glycosyl Compounds
      FF. [3.2.1.21] β-Glucosidase; P [123–125].

3.4. Acting on Peptide Bonds (Peptide Hydrolases)
   3.4.21. Serine Proteinases
      3.4.21.14. Microbial serine proteinases
               Streptomyces griseus Protease 3; RCR [53].
               Thermitase; RCR [54].
               Subtilisin C; RCR [54].
               Alkaline proteinase; RCR [54].
               Proteinase K; RCR [54].

3.6. Acting on Acid Anhydrides
   3.6.1. In Phosphorus-Containing Anhydrides
      GG. [3.6.1.3] Adenosinetriphosphatase (ATPase); P [126].
      GG. [3.6.1.3] Adenosinetriphosphatase (ATPase), KCl-stimulated; P [127].

5. Isomerases
   5.3. Intramolecular Oxidoreductases
      5.3.1. Interconverting Aldoses and Ketoses
      HH. [5.3.1.8] Mannose-6-phosphate isomerase; RCR [128].

5.4. Intramolecular Transferases
   5.4.2. Phosphotransferases
      II. [5.4.2.2] Phosphoglucomutase; P [55,129].
ester hydrolyses, the SerOH group becomes transiently acylated [47]. It is well established that the trigonal boron atom in substituted boric acid compounds (e.g., arylboronic acids) forms a tetrahedral adduct with the active-site serine in these proteases which mimics the tetrahedral adduct formed during normal substrate hydrolysis [48–50]. The slow-binding, tight-binding behavior of various boronic acid derivatives with their cognate serine proteases is well documented [50–52].

There is also evidence that simple unsubstituted boric acid compounds (e.g., sodium borate), which contain a trigonal boron atom, also bind to certain enzymes to form a reversible tetrahedral transition state analogue complex. At least five microbial subtilisin-type serine proteases bind to simple borates to form tetrahedral transition state analogues [53,54]. Phosphoglomutase, an isomerase, and starch phosphorylase, a transferase, are two enzymes important in glycogen/starch metabolism. Neither enzyme requires NAD*, but both are inhibited by boron [55–57] probably because they each contain an active serine residue at the active site [58]. Another interesting reaction between serine and borate affects the activity of gamma-glutamyl transpeptidase (GGT), a membrane-bound enzyme that functions in the gamma-glutamyl cycle to catalyze utilization of glutathione [59]. Enzyme inhibition is produced by formation of a serine-borate complex which binds at the gamma-glutamyl binding site of the light subunit of GGT to act as a transition-state inhibitor [59].

GPD, described above as a 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 as the former dissociates the enzyme into dimers and/or monomers [60] and the latter promotes reassociation [61]. Boron also may regulate enzyme activity by forming a tetrahedral transition state analogue; there is evidence from in vitro experiments that borate binds to a specific site(s) on the enzyme that triggers structural changes and dissociation of the tetramer [62]. This mechanism of action would be especially important for the process of phagocytosis, which is dependent mainly upon anaerobic glycolysis as described below.

The activities of several enzymes in several plant species are inhibited by boron by uncharacterized mechanisms. It seems reasonable that at least some of these enzyme activities (e.g., tyrosinase [EC 1.14.18.1] [63]) are reduced in the presence of adequate boron nutriture by the indirect action of boron on upstream metabolite concentrations.

**Boron and Enzyme Activity Stimulation**

In several plant species, adequate boron nutriture increases the activity of certain enzymes. For example, the activity of plasmalemma NADH oxidase was inhibited in boron-deficient cultured carrot cells and that under such conditions activity could be restored by exogenous boric acid [64]. The activity of NADH oxidase is apparently closely related to proton secretion [65,66]. Thus, it is not surprising that proton secretion is increased when boron is added to boron-deficient plant medium [64,67].

In plants, adequate boron nutrition enhances uridine diphosphate glucose (UDPG) pyrophosphorylase activity such that conversion of glucose-1-P to UDPG is increased [68]. Simultaneously, boron inhibits the conversion of glucose-1-P to starch by starch phosphorylase. Thus, it was proposed that starch accumulation observed in boron deficient plants is caused by decreased UDPG pyrophosphorylase activity and increased starch phosphorylase activity [22].
Enzymatic Responses to Physiological Boron

Several of the plant cell culture and refined chemical reaction studies described above were carried out with concentrations of boron 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 boron because the range of concentrations within which boron is essential to some species overlaps the range which is toxic to others [69]. Furthermore, plant parts vary in boron content [70], and, as boron becomes limited, most all cellular boron is localized in the cell wall with still further molecular compartmentalization in the form of strong association with the pectins (Patrick Brown, personal communication). Also, findings from cell culture or refined chemical reactions are not necessarily based on the minimum amounts of boron required to elicit a change in enzyme activity. However, disregarding obvious structural differences between plant and animal species, the distribution of boron in biological tissue and the design of experimental systems, there is 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.

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

BORON AND ENERGY SUBSTRATE UTILIZATION

Boron and Glycolysis

As discussed above, it seems clear that findings from various cell culture and refined chemical reaction studies are not necessarily directly applicable to intact physiological systems. Nevertheless, the findings that boron inhibits the in vitro activity of glycolytic enzymes stimulated efforts to determine the in vivo effects of dietary boron 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 boron (~0.20 mg B/kg) or supplemented with physiological amounts of boron (1.3 mg/kg) [71]. The effects of boron on GPD activity apparently differ between in vitro (see above) and physiological systems; the concentration of 1,3-bisphosphoglycerate was not affected by dietary boron supplementation. Most likely, the inhibitory effect of boron on GPD activity is dependent upon the boron: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 [71], boron supplementation of the boron-low diet strongly affected the concentrations of metabolites within the three-carbon phosphorylated acid pool of the glycolytic pathway. For example, boron supplementation decreased the concentrations of 2-phosphoglyc-
<table>
<thead>
<tr>
<th>Boron conc. (mol/L)</th>
<th>System</th>
<th>Remarks</th>
<th>Reference</th>
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<tbody>
<tr>
<td>0.000,000,010</td>
<td>CC</td>
<td>Nutrient solution, maintenance, sunflower plant</td>
<td>130</td>
</tr>
<tr>
<td>0.000,000,100</td>
<td>CC</td>
<td>Bioreactor field flux, hypocotyl, mung bean</td>
<td>131</td>
</tr>
<tr>
<td>0.000,000,600</td>
<td>H</td>
<td>Plasma, bodybuilder, male, no boron supplement</td>
<td>132</td>
</tr>
<tr>
<td>0.000,001,000</td>
<td>CC</td>
<td>Nutrient solution, maintenance, low range, gym/angio</td>
<td>133</td>
</tr>
<tr>
<td>0.000,001,000</td>
<td>RCR</td>
<td>Adrenaline-B complex flux</td>
<td>134</td>
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<tr>
<td>0.000,003,000</td>
<td>H</td>
<td>Plasma, bodybuilder, male, 2.5 mg B/day supplement</td>
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<tr>
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<td>RCR</td>
<td>Lipooxygenase activity flux, cotyledon, sunflower</td>
<td>83</td>
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<tr>
<td>0.000,004,000</td>
<td>A</td>
<td>Plasma, rat, boron-supplemented or deprived</td>
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<tr>
<td>0.000,004,600</td>
<td>CC</td>
<td>Nutrient solution, maintenance, sunflower protoplast</td>
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<td>0.000,005,000</td>
<td>A</td>
<td>Serum, bovine, calf</td>
<td>8</td>
</tr>
<tr>
<td>0.000,007,000</td>
<td>CC</td>
<td>Medium, maint., animal cell (MEM w Hanks' salts)</td>
<td>8</td>
</tr>
<tr>
<td>0.000,007,100</td>
<td>A</td>
<td>Plasma, chick, cholecalciferol-, boron-deficient</td>
<td>76</td>
</tr>
<tr>
<td>0.000,010,000</td>
<td>CC</td>
<td>Nutrient solution, maintenance, Elodea densa</td>
<td>67</td>
</tr>
<tr>
<td>0.000,010,000</td>
<td>CC</td>
<td>Resting membrane potential flux, cell, root, Helianthus</td>
<td>67</td>
</tr>
<tr>
<td>0.000,010,000</td>
<td>CC</td>
<td>Phosphate uptake flux, Z. mays</td>
<td>127</td>
</tr>
<tr>
<td>0.000,010,000</td>
<td>RCR</td>
<td>UDPG pyrophosphorylase activity flux, pea</td>
<td>68</td>
</tr>
<tr>
<td>0.000,012,000</td>
<td>A</td>
<td>Serum, porcine</td>
<td>8</td>
</tr>
<tr>
<td>0.000,014,000</td>
<td>CC</td>
<td>Medium, maint., animal cell (Leibovitz’s L-15)</td>
<td>8</td>
</tr>
<tr>
<td>0.000,014,000</td>
<td>A</td>
<td>Plasma, chick, cholecalciferol-, boron-adequate</td>
<td>76</td>
</tr>
<tr>
<td>0.000,016,000</td>
<td>CC</td>
<td>Nutrient solution, maintenance, plant</td>
<td>137</td>
</tr>
<tr>
<td>0.000,022,000</td>
<td>A</td>
<td>Serum, bovine, fetal</td>
<td>8</td>
</tr>
<tr>
<td>0.000,028,000</td>
<td>P</td>
<td>Water, soil (toxicity threshold for B-sensitive lemon)</td>
<td>69</td>
</tr>
<tr>
<td>0.000,046,000</td>
<td>CC</td>
<td>Nutrient solution, maintenance, diatom</td>
<td>18</td>
</tr>
<tr>
<td>0.000,050,000</td>
<td>CC</td>
<td>Medium, Gamborg’s B-5, plants</td>
<td>138</td>
</tr>
<tr>
<td>0.000,060,000</td>
<td>A</td>
<td>Serum, rabbit</td>
<td>8</td>
</tr>
<tr>
<td>0.000,060,000</td>
<td>A</td>
<td>Femur, chick (calculated from dry weight)</td>
<td>78</td>
</tr>
<tr>
<td>0.000,100,000</td>
<td>CC</td>
<td>NADH oxidase activity flux, pflasmalemma, carrot</td>
<td>139</td>
</tr>
<tr>
<td>0.000,100,000</td>
<td>CC</td>
<td>Proton release flux, cell, carrot</td>
<td>140</td>
</tr>
<tr>
<td>0.000,100,000</td>
<td>CC</td>
<td>ATPase activity, flux, root, sunflower</td>
<td>135</td>
</tr>
<tr>
<td>0.000,190,000</td>
<td>P</td>
<td>Water, soil (tox. threshold for B-semi tolerant corn)</td>
<td>69</td>
</tr>
<tr>
<td>0.000,200,000</td>
<td>P</td>
<td>Phosphoglucomutase activity (50% inhibition), pea</td>
<td>55</td>
</tr>
<tr>
<td>0.000,350,000</td>
<td>CC</td>
<td>Cytoplasm, leaf, clover leaf</td>
<td>137</td>
</tr>
<tr>
<td>0.000,370,000</td>
<td>CC</td>
<td>Nutrient solut., maintenance, upper range, gym/angio</td>
<td>133</td>
</tr>
<tr>
<td>0.000,400,000</td>
<td>RCR</td>
<td>Alcohol dehydrogenase activity flux, yeast</td>
<td>42</td>
</tr>
<tr>
<td>0.000,400,000</td>
<td>RCR</td>
<td>GPD activity flux, yeast</td>
<td>42</td>
</tr>
<tr>
<td>0.000,400,000</td>
<td>RCR</td>
<td>Lactate dehydrogenase activity flux, heart, beef</td>
<td>42</td>
</tr>
</tbody>
</table>
TABLE I. Continued

<table>
<thead>
<tr>
<th>Boron conc.(^a) (mol/L)</th>
<th>System(^b)</th>
<th>Remarks(^c)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000,600,000</td>
<td>RCR</td>
<td>6-P gluconate dehydrogenase activity flux</td>
<td>115</td>
</tr>
<tr>
<td>0.000,930,000</td>
<td>P</td>
<td>Water, soil (tox. threshold for B-tolerant asparagus)</td>
<td>69</td>
</tr>
<tr>
<td>0.003,000,000</td>
<td>RCR</td>
<td>GPD activity flux, yeast</td>
<td>118</td>
</tr>
<tr>
<td>0.005,000,000</td>
<td>RCR</td>
<td>GPD hybridization flux, rabbit/horseshoe crab</td>
<td>62</td>
</tr>
<tr>
<td>0.007,640,000</td>
<td>CC</td>
<td>Cell wall, leaf, clover</td>
<td>137</td>
</tr>
<tr>
<td>0.010,000,000</td>
<td>RCR</td>
<td>Gamma-glutamyl transpeptidase activity flux</td>
<td>59</td>
</tr>
<tr>
<td>0.010,000,000</td>
<td>RCR</td>
<td>Starch phosphorylase activity flux, snap bean</td>
<td>57</td>
</tr>
<tr>
<td>0.016,000,000</td>
<td>RCR</td>
<td>Xanthine oxidase activity flux, milk, whole, raw</td>
<td>117</td>
</tr>
<tr>
<td>0.020,000,000</td>
<td>RCR</td>
<td>Cytochrome b(_5) reductase activity flux, microsomal</td>
<td>120</td>
</tr>
<tr>
<td>0.050,000,000</td>
<td>RCR</td>
<td>Protease 3 activity flux, S. griseus (serine protease)</td>
<td>53</td>
</tr>
<tr>
<td>0.100,000,000</td>
<td>CC</td>
<td>Leaf, snap bean (no sign of boron toxicity)</td>
<td>57</td>
</tr>
<tr>
<td>0.100,000,000</td>
<td>RCR</td>
<td>Serine protease activity flux, bacterial</td>
<td>54</td>
</tr>
</tbody>
</table>

\(^a\) Amount of boron in solution prior to boron supplementation commonly not provided.
\(^b\) Concentration of boron present in nutrient media or concentration of boron (not necessarily the minimum) determined to elicit change in enzyme activity or other biological responses.
\(^c\) A, animal; CC, cell culture; H, human; P, plant; RCR, refined chemical reaction. Experimental conditions vary markedly among studies and systems.

\(^d\) See reference for experimental conditions including substrate concentrations and reactions times.

\(^e\) Compared to a lower experimental boron concentration.

\(^f\) Gym/angio, gymnosperms, and angiosperms.

\(^g\) Glyceraldehyde-3-P dehydrogenase.

erate 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 boron. For example, boron supplementation decreased dihydroxyacetone phosphate concentrations. The latter findings differ from those from an earlier report where high doses of boron (240 mg/kg body weight) increased the concentration of metabolites in the FBP-TP pool [72]. The difference in findings is probably related to large differences in the boron:ligand ratio.

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

**Boron and Blood Glucose**

There is now considerable evidence that blood glucose concentrations respond to physiological amounts of dietary boron. In animal models, the response is especially
marked during concomitant vitamin D₃ deficiency. Vitamin D is known to influence tissue energy substrate utilization in addition to mineral metabolism. For example, rachitic chick bone that was incubated aerobically consumed more glucose and released more lactate than normal bone. When the rachitic bone was pretreated 48 hours with vitamin D₃, the rate of glycolysis returned to normal [73].

In the vitamin D-deficient chick, physiological amounts of supplemental boron [3 μg (0.277 μmol) B/g] added to a boron-low diet [0.04 μg (0.004 μmol)/g] decreased the abnormally elevated plasma glucose concentrations by 29% [74]. Supplemental boron decreased these concentrations by only 6% in the vitamin D₃-adequate control group. Further physiological stress, introduced as magnesium deficiency, reversed the effect of dietary boron in the vitamin D₃-deficient animals. The ability of physiological amounts of dietary boron to attenuate the rise in plasma glucose concentrations induced by vitamin D₃ deficiency has been demonstrated repeatedly in the chick model (Table II) [75–78]. In postmenopausal women fed a low magnesium (115 mg/2,000 kcal), marginal copper (1.61 mg/2,000 kcal) diet [12], a daily dietary intake of 3.23 mg (0.299 mmol) boron for 49 days, compared to a daily intake of 0.23 mg (0.021 mmol) for 63 days, decreased fasting serum glucose concentrations within the normal range approximately 6% [88 ± 3 vs. 94 ± 3 mg/dL (mean ± SEM)]. Although the magnitude of the response varies between experiments, species, and dietary treatment, the findings to date indicate that dietary boron apparently modulates circulating glucose concentrations.

**Boron and Insulin**

The findings described above suggest that boron plays a role in energy metabolism; there is also evidence that dietary boron modifies insulin metabolism. For example, boron deprivation greatly increased plasma insulin concentrations in the vitamin D-deprived rat (Table III) [79]. Furthermore, isolated, perfused pancreata from chicks fed a low boron diet exhibited a nearly 75% increase in peak insulin release, especially when the perfusate was supplemented with glucose [80]. This finding may be of special significance. β-cell “exhaustion” may explain the β-cell deterioration that occurs during excessive insulin demand [81]. 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 [81,82]. Thus, it is hypothesized that boron acts to limit pancreatic β-cell stress that may occur during periods of excessive metabolic demands for insulin production.

**Boron and Lipids**

Boron affects lipid metabolism in plants, animals, and humans. For example, the addition of boron to sunflower cotyledons induces an increase in the concentration of free fatty acids and phospholipids and also delays triglyceride degradation [83]. This phenomenon was thought to be related to the decreased activity of lipoxygenase because findings from a subsequent in vitro investigation indicated that 4 μmol/L boron inhibited lipoxygenase activity by 30% [83]. Lipoxygenase catalyzes the oxygenation of polyunsaturated fatty acids containing a cis-1, cis-4-pentadiene structure (e.g., linoleic acid) to fatty acid hydroperoxides. The reaction takes place concomitantly with the β-oxidation of fatty acids during the germination process in oil seeds.

With the above findings on plant boron-lipid interactions in mind, it is not sur-
### TABLE II. Effects of Dietary Boron, Vitamin D₃, and Their Interaction on Selected Indices of Energy Substrate Utilization, Mineral Metabolism, and Growth Cartilage Development in Chicks [78]a

<table>
<thead>
<tr>
<th>Treatmentsa</th>
<th>Inadequate Vitamin D₃</th>
<th>Adequate Vitamin D₃</th>
<th>P valuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mg B/kg diet</td>
<td>1.4 mg B/kg diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 mg B/kg diet</td>
<td>1.4 mg B/kg diet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(14)c</td>
<td>17.7 ± 2.0d</td>
<td>(14) 16.3 ± 1.7</td>
<td>(15) 15.9 ± 1.0</td>
</tr>
<tr>
<td>Chondrocyte density, count/μm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(15)</td>
<td>4.18 × 10⁻³ ± 5.4 × 10⁻⁴</td>
<td>(14) 4.50 × 10⁻³ ± 2.1 × 10⁻⁴</td>
<td>(11) 4.46 × 10⁻³ ± 4.6 × 10⁻⁴</td>
</tr>
<tr>
<td>Femur calcium, mol/kg e</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(13)</td>
<td>2.97 ± 0.30</td>
<td>(14) 3.02 ± 0.25</td>
<td>(13) 2.79 ± 0.22</td>
</tr>
<tr>
<td>Femur phosphorus, mol/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(13)</td>
<td>1.84 ± 0.17</td>
<td>(14) 1.90 ± 0.15</td>
<td>(13) 1.75 ± 0.13</td>
</tr>
</tbody>
</table>

*aCockerel chicks aged one day were fed respective diets for 26 days and then killed.

bAmount of boron (orthoboric acid, mg/kg) and vitamin D₃ (vitamin D₃ powder in corn endosperm carrier) supplemented to the basal diet (−0.18 mg B/kg diet). Inadequate and adequate vitamin D₃ were 3.13 and 15.6 μg vitamin D₃/kg diet, respectively.

Data were analyzed by using 2 × 2 ANOVA.

Number in parentheses represents number of samples per dietary group and varies according to availability, integrity, and/or variability of individual samples.

Group mean ± standard deviation.

All mineral concentrations expressed on dry weight basis.
<table>
<thead>
<tr>
<th>0 mg B/kg diet</th>
<th>2.4 mg B/kg diet</th>
<th>0 mg B/kg diet</th>
<th>2.4 mg B/kg diet</th>
<th>Analysis of variance</th>
<th>t tests or contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma glucose, mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11)^c</td>
<td>8.27 ± 1.28^d</td>
<td>(10)</td>
<td>7.44 ± 1.22</td>
<td>(9)</td>
<td>38.5 ± 16.3</td>
</tr>
<tr>
<td><strong>Plasma insulin, pmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td>151 ± 103</td>
<td>(10)</td>
<td>32 ± 20</td>
<td>(5)</td>
<td>19 ± 27</td>
</tr>
<tr>
<td><strong>Plasma triglycerides, mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>0.508 ± 0.113</td>
<td>(11)</td>
<td>0.723 ± 0.282</td>
<td>(8)</td>
<td>3.00 ± 2.00</td>
</tr>
<tr>
<td><strong>Heart calcium, mmol/kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>3.70 ± 0.30</td>
<td>(11)</td>
<td>4.00 ± 0.01</td>
<td>(8)</td>
<td>3.70 ± 0.25</td>
</tr>
<tr>
<td><strong>Heart phosphorus, mmol/kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12)</td>
<td>0.313 ± 0.014</td>
<td>(11)</td>
<td>0.326 ± 0.016</td>
<td>(11)</td>
<td>0.287 ± 0.027</td>
</tr>
</tbody>
</table>

*Weanling rats were fed respective diets for 65 days and then killed.

^a Amount of boron (orthoboric acid, mg/kg) supplemented to the basal diet (~0.06 mg/kg). All rats were injected with either 1 ml citrate buffer/kg body weight or 75 mg streptozotocin/ml citrate buffer/kg body weight 3 days before kill.

^b Data were analyzed by using Student's t test for unequal variances caused by streptozotocin injection or 2 × 2 ANOVA with a priori comparisons.

^c Number in parentheses represents number of samples per dietary group and varies according to availability, integrity, and/or variability of individual samples.

^d Group mean ± standard deviation.
prising that numerous studies indicate that boron affects lipid metabolism in chicks, rats, and humans. Dietary boron increased serum/plasma triglyceride concentrations that may have been too low because of the influence of other nutritional stressors. For example, in chicks, the response is usually more pronounced with concomitant severe vitamin D-deficiency. Severely depressed plasma triglycerides were increased by physiological amounts of supplemental boron in chicks (aged 40 days) deprived of boron, fed inadequate amounts of vitamin D, and exhibiting severe vitamin D-deficiency (as evidenced by extremely poor body weight) (Table IV) [75]. The results of three studies of rats (two vitamin D-adequate [84,85]; one vitamin D-deprived [79]) also indicate that dietary boron increases circulating amounts of triglycerides within a range of normal values (Table III). In postmenopausal women [14], a daily dietary intake of 3.23 mg (0.299 mmol) boron for 49 days, compared to a daily intake of 0.23 mg (0.021 mmol) for 63 days, increased fasting serum triglyceride concentrations (in the normal range) approximately 9% [122 mg/dL (1.38 mmol/L) vs. 111 mg/dL (1.25 mmol/L); P<0.01]. Further research is needed to determine whether boron exerts this relatively consistent effect on animal and human triglyceride metabolism through an effect on, for example, lipoygenase activity.

No unifying hypothesis has been developed to explain the effects of dietary boron on serum glucose, insulin, and triglyceride concentrations. However, the increase in serum triglycerides and decrease in serum glucose during boron supplementation suggests that boron induces a shift in the consumption, or at least distribution, of endogenous fuels.

**TABLE IV. Effect in the Vitamin D Deficient Chick of Dietary Boron on Indices of General Physiology [71]**

<table>
<thead>
<tr>
<th>Dietary treatment boron (mg/g diet)</th>
<th>Body weight g</th>
<th>Triglyceride mmol/L</th>
<th>Ionized calcium mmol/L</th>
<th>Alkaline phosphatase μkat/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>490</td>
<td>0.553</td>
<td>1.17</td>
<td>206</td>
</tr>
<tr>
<td>0.28</td>
<td>663</td>
<td>0.711</td>
<td>1.31</td>
<td>190</td>
</tr>
<tr>
<td>0.44</td>
<td>948</td>
<td>1.129</td>
<td>1.31</td>
<td>123</td>
</tr>
<tr>
<td>0.66</td>
<td>798</td>
<td>0.937&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37</td>
<td>98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.01</td>
<td>942</td>
<td>1.119&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.40</td>
<td>72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.90</td>
<td>1090&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.163&lt;sup&gt;d&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.42</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.33</td>
<td>1151&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.660&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.37</td>
<td>82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Linear Regression—P values

- Boron: 0.0001, 0.0001, 0.0001, 0.04
- r²: 0.24, 0.19, 0.15, 0.04
- Slope: 341, 0.37, 0.067, -25
- Standard error: 58, 0.08, 0.017, 122

<sup>a</sup>Cockerel chicks aged 1 day were fed respective vitamin D-inadequate diets (3.13 μg vitamin D₃/kg) for 40 days and then killed.

<sup>b</sup>Adjusted to pH 7.0.

<sup>c</sup>Linear regressions based on the natural log of the dietary boron concentrations. Data from 114 chicks used in regression analysis. Values in a column not sharing common letters are different (P < 0.5; ANOVA).
BORON 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 [86]. 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 [86].

Boron and Inflammation Disease

Boron as borax was reported to have anti-arthritic activity on formaldehyde-induced arthritis in albino rats [87]. In a human study [88], 20 patients presenting radiographically confirmed osteoarthritis received either 6 mg (0.55 mmol) of boron/day or a placebo for 8 weeks in a double-blind trial. The arthritic individuals who received boron supplementation self-reported substantial improvement in subjective measures of their arthritic condition (pain on movement, joint swelling, or 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 boron reacts with sugar moieties in the rheumatoid factor to form a reversible complex [89].

A recent preliminary report [90] suggests that luxuriant amounts of dietary boron [20 μg (1.85 μmol)/g] delay the onset and severity of adjuvant-induced arthritis in rats. Addition of boron in vitro over a range between 0 and 20 μg (1.85 μmol)/mL inhibited splenic cell proliferation stimulated by 0.5 or 50 μg phytohemagglutinin/mL. This finding suggests that dietary boron alleviates adjuvant-induced arthritis by inhibiting T cell activity. A search for the mechanism through which boron modulates inflammation led to another recent preliminary report [91] that dietary boron affects serum antibody concentrations. Physiological amounts of boron [3 μg (0.28 μmol)/g] added to a boron-low 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 nM, 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 gamma globulin with rabbit anti human gamma globulin [92].

The observed effects of boron on various components of the inflammatory process as described above leads to the hypothesis that boron protects against inflammatory disease. At least, it seems clear that boron modulates the inflammatory response. Possible mechanisms for this phenomenon are presented below.

Boron and the Respiratory Burst

Pentose-phosphate 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 [Eqn. (3)] [93].

$$\text{NADPH} + 2\text{O}_2 \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + 2\text{O}_2^- + \text{H}^+$$

(3)

The source of the NADPH for the respiratory burst comes mainly from the reduction of NADP$^+$ in the pentose-phosphate [P-P] pathway, which is very active during the respiratory burst. Glucose 6-P dehydrogenase and P-gluconate dehydrogenase are two key enzymes in the P-P pathway and are oxidoreductase enzymes that require NAD. In plants, one substrate of the P-P pathway, 6-phosphogluconate, is known to complex with boron, which thereby inhibits 6-phosphogluconate dehydrogenase. Thus, a serious problem in boron-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 [22]. Whether boron 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 ($\text{H}_2\text{O}_2$), hydroperoxy radical ($\text{HO}_2^-$), and the hydroxyl radical (OH$^-$). 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 per oxy radicals [94]. Glutathione peroxidase reduces hydrogen peroxide by means of reduced glutathione and the intracellular reduction of glutathione requires NADPH and glutathione reductase [95].

Boron may be important in the oxidant scavenging process. Boron supplementation increased erythrocyte SOD concentrations in men and post menopausal women [12]. SOD concentrations increased during increased oxidative metabolism or in response to noxious stimuli. They also decrease during inadequate copper status because SOD is a copper-requiring enzyme. It remains to be determined whether SOD concentrations increased because boron may have induced free radical formation (unlikely), or whether boron improved antioxidant capacity. A related finding [14] supports the later hypothesis in that boron supplementation increased plasma immunoreactive ceruloplasmin in men and post menopausal women and may have done so by improving copper status through uncharacterized mechanisms.

Boron 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 [59]. By that mechanism, serine-borate apparently elevated the concentrations of GSH in cultured fibroblasts taken from individuals suffering from glutathione synthase deficiency [96].

In another human study [88], 20 patients, presenting with radiographically confirmed osteo-arthritis, were recruited to a double-blind trial and given either 6 mg of boron/day or a placebo. It is noteworthy that patients consuming the boron 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, or restricted movement). In rats, serine-borate was reported to increase renal GSH content in vivo [97]. Thus, the available evidence is consistent with the hypothesis that boron protects against oxidative damage by an unknown mechanism. Research efforts are underway to confirm the working hypothesis that boron 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.

BORON AND BONE/MINERAL METABOLISM

**BORON and Cartilage/Bone Structure**

There is considerable evidence that dietary boron modulates growth cartilage structure. Calcification of growth plate cartilage matrix normally begins distal to the tips of marrow sprouts that invade the hypertrophic zone of growth cartilage from the metaphysis as a parallel array of straight excavations. The distance between the tips of the marrow sprouts and the first appearance of calcified matrix is a convenient measure of the mineralization rate.

Boron supplementation of a boron-low diet reduced gross bone abnormalities in the vitamin D-deficient chick [78,98]. At the microscopic level, physiological amounts of dietary boron inhibited the initiation of cartilage calcification in chicks compromised with both magnesium and vitamin D deficiencies (Fig. 2) [76]. When chicks were supplied with adequate dietary magnesium, dietary boron enhanced initiation of cartilage calcification. The findings indicate that physiological amounts of boron may function to modify mineral metabolism in vitamin D₃ deficiency by suppressing bone anabolism in magnesium deficiency and bone catabolism in magnesium adequacy. The effects of boron on cartilage calcification apparently are beneficial in both magnesium inadequacy and adequacy because the vitamin D₃ deficiency-induced mortality was substantially reduced by dietary boron. Furthermore, supplemental boron alleviated distortion of the marrow sprouts, a distortion characteristic of vitamin D₃ deficiency.

Other findings [99] indicate that boron has an undefined role in the maturation of

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**Fig. 2.** Photomicrographs (all at same magnification) depicting marrow sprout (MS) orientation and initiation of cartilage calcification (ICC) in the chick proximal tibial epiphysial plate. ICC beyond a frame is denoted by slashed arrows. Boron supplementation to a boron-low diet corrected the cholecalciferol deficiency-induced disorientation of the MS [compare (a) and (b), and (c) and (d)]. ICC is inhibited by boron in magnesium inadequacy [compare (a) and (c)], but enhanced in magnesium adequacy [compare (c) and (d)]. Dietary supplements (mg per kg of diet): (a) B, 0; Mg, 300; (b) B, 3; Mg, 300; (c) B, 0; Mg, 500; and (d) B, 3; Mg, 500 [76].
the growth plate. In ovo injections of boron or 1,25-dihydroxycholecalciferol reduced the abnormal height of the growth plate of 1-day-old chicks hatched from vitamin D₃-deficient eggs. Also [78], physiological supplements of boron to a boron-low diet increased chondrocyte density in the proliferative zone of the growth plate in vitamin D-deficient chicks (Table II). These findings indicate that boron also influences metabolic events not directly related to extracellular matrix calcification.

**Boron and Vitamin D Metabolism**

There is an apparent close relation between vitamin D and boron metabolism. In vitamin D-deficient chicks fed low dietary boron (~0.29 mg/kg), boron supplementation (~1.36 mg/kg) markedly improved plasma 1,25-dihydroxycholecalciferol concentrations (145 ± 60 vs. 66 ± 30 nmol/L, mean ± SD), whereas in the vitamin D-adequate chicks, the boron supplementation decreased these concentrations (126 ± 32 vs. 201 ± 74 nmol/L, mean ± SD) [80]. Whether boron improves vitamin D absorption from the gut, increases the half-life of vitamin D metabolites, or enhances hydroxylation of metabolically active precursors is under active investigation.

Boron supplementation of a boron-low diet decreased the incidence of mortality in vitamin D-deficient chicks (0 vs. 26%) [76]. Furthermore, dietary boron stimulated growth in vitamin D-deficient, boron-deprived chicks but did not markedly affect growth in chicks receiving adequate vitamin D nutrure [1,75,98]. In a community-based study [11,14] with mixed groups of volunteers (men, and women on or not on estrogen therapy), boron supplementation (3 mg/day for 49 days) after consumption of a low boron diet (63 days) increased serum 25-hydroxycholecalciferol (62.4 ± 7.5 vs. 44.9 ± 2.5 mmol/L, mean ± SEM), an effect that may be especially important during the winter months where those concentrations normally range between 35 and 105 mmol/L [100].

**Boron and Mineral Metabolism**

Dietary boron ameliorates the deleterious effects of vitamin D deficiency on several aspects of mineral metabolism through uncharacterized mechanisms. For example, vitamin D deficiency induces abnormally elevated concentrations of plasmatal alkaline phosphatase activity in the chick [101,102]. Vitamin D-deficient chicks fed 3.33, compared to those fed 0.16 mg boron/kg diet, exhibited a 60% reduction in plasma alkaline phosphatase activity (Table IV) [75]. In the same animals, the boron supplement improved low plasma ionized calcium concentrations.

In the vitamin D-deficient rat fed a low-boron diet, supplemental dietary boron enhanced the apparent absorption and retention of calcium and phosphorus and increased femur magnesium concentrations [103]. Also, dietary boron increased femoral calcium and phosphorus concentrations in vitamin D-adequate, but not inadequate chicks (Table II) [78]. These findings indicate that further research is needed to ascertain whether an interaction between boron and vitamin D affects calcium retention and possibly mobilization. Also, in the vitamin D-deprived, boron-deprived rat, supplemental boron elevated cardiac calcium and phosphorus concentrations (Table III) [104]. Dietary boron probably affected cardiac mineral metabolism indirectly through unknown mechanisms because it did not affect cardiac boron concentrations.

Mineral metabolism in humans is influenced by dietary boron and the influence is modulated, at least in part, by magnesium nutrure. For example, in postmenopausal
women housed in a metabolic unit and fed low amounts of magnesium [109 mg (4.48 mmol) Mg/day] and boron [0.36 mg (0.033 mmol) B/d], supplemental boron [2.87 mg (0.265 mmol) B/day] decreased the percent of calcium intake lost in the urine (17.5 vs. 18.7% total Ca intake) [105]. In the same study, boron increased the percent of calcium intake lost in the urine of postmenopausal volunteers fed slightly more than the recommended amount of magnesium [340 mg (14.0 mmol) Mg/d] [105]. A similar phenomenon occurred in either free-living sedentary or athletic premenopausal women consuming self-selected typical Western diets: boron supplementation increased urinary calcium loss (athletic: 0 months of B supplementation, 1.3 ± 1.1 mmol Ca/day. 10 months of B supplementation, 2.7 ± 1.0 mmol Ca/day; sedentary: 0 months of B supplementation, 1.6 ± 0.9 mmol Ca/day. 10 months of B supplementation, 1.9 ± 1.0 mmol Ca/day) [106]. In a different study [11] of older volunteers fed a low-magnesium (115 mg/2,000 kcal), marginal copper (1.61 mg/2,000 kcal) diet (men, and women on or not on estrogen therapy), boron repletion after boron depletion decreased calcitonin and increased ionized calcium but not total calcium concentrations in serum.

CONCLUSIONS

The findings to date leave little doubt that amounts of boron similar to that found in diets luxuriant in fruits, nuts, legumes, and vegetables influence bone structure and function. Many of the findings are consistent with the hypothesis that boron enhances the uptake or hydroxylation of vitamin D or extends the functional half life of vitamin D metabolites. The apparent effects of boron on energy substrate utilization may be more related to uncharacterized influences on the metabolism of vitamin D, the metabolites of which participate directly in the process of insulin release. Because 1,25-dihydroxycholecalciferol is known to inhibit antigen- or mitogen-stimulated lymphocyte proliferation, lymphokine production, and antibody production [107,108], it would not be surprising to find that boron influences the immune system by modulating vitamin D metabolism.

Taken as a whole, the experimental boron nutrition research data indicate an essential role for the element in animals and humans that involves energy substrate metabolism, bone structure and function, and the inflammatory response. Most likely, new advances in boron nutrition research will include better characterization of the mechanisms through which boron modulates immune function, insulin release, and vitamin D metabolism.

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