

Advances in boron essentiality research: symposium summary

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Introduction

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Boron is a trace mineral element occurring naturally in the environment and in the food supply, although it does not exist as elemental boron in foods or beverages, but rather as one or more inorganic, oxygen-containing borates, such as boric acid. Boron has long been known to be an essential nutrient for all vascular plants, and there is now considerable evidence that it may also be essential for animals and humans. In 1996, a World Health Organization Expert Committee on Trace Elements in Human Nutrition concluded that boron is probably essential. Recently, there has been a renewed interest in boron essentiality research in numerous laboratories around the world, and this symposium was designed to bring these boron essentiality researchers together to interact with each other and with experts involved with other biological studies of essential trace elements.

The overarching goal of the boron essentiality research programs underway in numerous laboratories has been to identify a biochemical function or mechanism of action of boron-containing compounds in animals and humans that may be related to the adverse effects of deficiency. Underlying this effort is the desire to increase the general scientific awareness of boron as a nutritionally beneficial, and perhaps essential, trace element found in most of the healthy foods we eat. Some of this research has also focused on ways to better define the shape of boron's U-shaped dose-response curves in various species (i.e. those intakes deemed essential vs. those exceeding acceptable upper intake levels), taking into account such factors as pharmacokinetics, bioavailability, speciation, and essentiality data.

For almost 20 years, the true pioneers in boron essentiality research are two of our current presenters from the USDA's Grand Forks, North Dakota Human Nutrition Research Center, Center Director Forrest Nielsen and Research Biologist Curtis Hunt.

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New aspects of trace element research. Eds. M. Abdulla et al.
81999, Smith -Gordon. Printed in the UK.

By 1992, the Grand Forks researchers had established evidence that boron was acting in at least two important

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general ways: (1) regulating metabolism mainly through an inhibitory action enzymes, by competing with variety of biochemical compounds in the body and controlling a number of metabolic pathways; and (2) playing an important role in cell membrane function and stability. Recent studies described in this symposium have focused on the role of boron deficiency in the reproduction, early embryonic development and growth of frogs, fish, mice and rats. Published studies now show complete U-shaped dose-response curves for boron in the frog (D. Fort) and fish (C. Eckert), establishing its essentiality for reproduction and development in these species, with similar studies underway in mice and rats (C. Keen). C. Hunt has demonstrated boron's potential role as an important modulator of immune function, and F. Nielsen has done a series of human studies on the effects of boron supplementation at a dose of 3 mg/day. In addition, boron balance study in humans has already been published (B. Sutherland and J. King), and role of boron supplementation in bone health in perimenopausal women is also planned by this group.

Besides these biological studies on boron's essentiality, two other critical areas of research will also be described in this symposium. Improved boron dietary intake data are required to more accurately assess the daily consumption of boron in the diets of population groups around the world. C. Rainey has conducted extensive studies of dietary boron intake in six countries to date and these data will be very useful in assessing the adequacy of a given population's intake of boron. Underlying both the biological and dietary intake studies is the critical importance of analytical chemistry in the determination of boron levels in foods and beverages as well as in biological tissues and fluids. G. Downing describes the analytical challenges in determining low amounts of boron in biological samples and provides many techniques that can assist analysts in minimizing sources of boron contamination and losses during sample processing.

Adverse effects of low boron exposure on reproduction, development and maturation in Xenopus laevis
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This presentation provided further evidence that boron is an essential nutrient for the South African clawed frog *Xenopus laevis*. Previous studies showed that adult frogs fed a low boron diet for either 28 d or 120 d produced a greater proportion of necrotic eggs and fertilized embryos which gastrulated at a greater rate and were substantially less viable at 96 h of development than frogs fed a boron supplemented diet. In the studies described in this presentation, complete boron concentration-response curves were established for this experimental model by using the 4-d embryo-larval developmental system called FETAX (Frog Embryo Teratogenesis Assay: *Xenopus*). Abnormal development of the embryo-larval was induced with culture media boron concentrations less than 0.3 and greater than 4980 μM . Embryo-larval development was normal with boron concentrations between 0.5 and 3320 μM .

Culture of *Xenopus* in Media containing less than 0.3 μM of boron during organogenesis (through d 4) resulted in abnormal development of the gut, craniofacial region and eye, visceral edema, and kinking of the tail (muscular and skeletal). These malformations were not seen in embryos cultured in media containing 0.5 to 3320 μM of boron. Concentrations of boron greater than 4980 μM induced abnormal development of the gut and craniofacial region. Concentrations of boron greater than 8300 μM caused kinking of the notochord, microencephaly, and pericardial edema. Based on concentration-response curves developed in this manner, boron has a wider margin of safety between nutritionally deficient and toxic intakes than either copper or zinc.

Under standard conditions, the tail of the developing frog resorbs at a consistent rate between stages 63 and 65 and is completely resorbed by stage 66. The rate of tail resorption was slower in boron deprived than boron-supplemented larvae. Addition of 100 $\mu\text{g/l}$ of thyroxine, a known enhancer of tail resorption, and to a lesser extent, 0.1% (w/v) iodine reversed the delayed tail absorption in boron deprived larvae. Tail absorption inhibition was also reversed by 100 $\mu\text{g/l}$ of boron to the same degree as that observed with the iodine addition. Initial

measurements of triiodothyronine (T₃), indicated that the concentrations of this hormone are at least 2.5-fold lower in boron-deprived than boron-supplemented larvae. Because maturation including tail resorption, is

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controlled by the thyroid axis, these findings suggest that boron has a role in the thyroid axis, perhaps in the synthesis of T₃.

In summary, findings were obtained which confirmed the nutritional essentiality of boron for *Xenopus*, and which defined the nutritionally deficient, nutritionally acceptable, and toxicological amounts of boron for frogs during early development. Boron also has an apparent significant role in the normal maturation of *Xenopus* because its lack adversely affects metamorphosis especially resorption of the tail; this role may be linked to thyroxine/triiodothyronine production of activity.

Selection for zebra fish survival in a low boron environment

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This presentation confirmed that boron is an essential nutrient for the zebra fish. Previous reports showed that the biological response of trout and zebra fish to boron exposure was characterized by a U-shaped adverse health effects dose-response curve. Now studies have been performed which show that boron deficiency has pathological consequences during two different stages of the life cycle of zebra fish. In these studies, boron deprived and supplemented zebra fish were placed in aquarium water that contained 0.1 and 45 FM of boron, respectively. The zebra fish were fed brine shrimp containing 97 FM/kg of boron.

During the early post-fertilization period, 45% of the boron deprived embryos died, whereas only 2% of the boron supplemented embryos died. A high rate of death occurred during the zygote and cleavage periods before formation of a blastula. Two morphological events preceded death. The most prevalent pathological change was extensive membrane blebbing. This usually occurred on the animal pole, but often spread to other areas of the egg with time. The other pathological change was an extrusion of cytoplasm, which usually occurred in the animal pole.

F-boron-deprived adults also exhibited photophobic behavior. The cause of this behavior was determined to be retinal dystrophy. Cone photoreceptor cell lengths were shorter in the retinas of boron-deprived fish. This shortened length was the result of reductions in the myoid and outer segments regions of the cell.

Membrane blebbing with cytoplasmic extrusion during the zygote and cleavage periods of embryogenesis, and cone dystrophy in the adult stage are changes occurring in cells that produce prodigious quantities of membrane. The membrane disruption observed was consistent with membrane alterations reported for boron deprived cyanobacteria and vascular plants. This suggests that boron is required for the synthesis or processing of membrane or cytoskeletal proteins that function in the maintenance of membrane shape and integrity for microorganisms, plants and animals.

Effects of boron deficiency and toxicity on

preimplantation mouse embryos: defining the

limits of boron nutriture using an in-vitro model

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This presentation described the use of the preimplantation embryo culture system to investigate the effects of boron deficiency on early mouse development. Results from three studies were presented. In the first study, two-cell embryos collected from mice fed a commercial stock diet high in boron were cultured for 72 h in boron deficient and supplemented media. Preimplantation development was similar for the two groups.

In the second and third studies two-cell embryos were collected from females fed 0.04 or 2.05 Fg B/g purified diet, or fed a commercial stock diet containing 11.8 Fg B/g for 10, 12, or 16 weeks. All embryos were cultured in boron-supplemented (5-20 Fg/1) medium in the second study and cultured in boron-deficient (non-detectable) medium in the third study.

Food intake and body weight of the maternal females were not affected by dietary treatment. However, the boron concentrations in tibia and liver were consistently lower in boron-deprived than boron-supplemented or stock-diet-fed mice. In study three at 16 weeks, the boron concentration in tibia and liver was significantly lower

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in boron-deprived than either boron-supplemented or stock-diet fed females.

In study two, the dietary treatment of the females did not significantly alter the in-vitro development of two-cell embryos cultured in the boron-supplemented medium. However, when the development of the two-cell embryos from the boron-deprived females was compared to that of the embryos from boron-supplemented females, a greater proportion apparently failed to differentiate into blastocysts (83.5% vs 90.1%) and a higher percentage became degenerate after 72 h (13.0% vs 8.0%).

In study three, the preimplantation development of two-cell embryos from both the boron deprived and supplemented females fed the purified diets was significantly impaired by culturing in the boron-deficient culture medium. However, the embryos from the boron-deficient females were apparently more impaired than those from the boron-supplemented females. Only 17% of the two-cell embryos from the boron-deprived mice formed morulae after one day and only 40% formed blastocysts by day 3. On the other hand, 31% of the two-cell embryos from the boron-supplemented mice reached the morula stage after one day and 57% of the embryos formed blastocysts by day 3. The number of embryonic degenerates formed after 72 h of culture in a boron-deficient medium was significantly higher with embryos from boron-deprived (57%) than boron-supplemented (20%) mice.

The results from the different paradigms tested suggest that (1) culturing embryos in boron-deficient medium exacerbates a boron-deficient state generated in utero as a result of depletion of boron in critical maternal tissue; and (2) a boron-luxuriant medium reverses embryonic boron-deficiency generated in utero.

Possible mechanisms for the impaired development of two-cell embryos caused by boron deficiency include changes in embryonic calcium regulation of development and changes in membrane function. Changes in membrane integrity could result in reduced transport capacity of nutrients and metabolites, ionic fluxes or membrane potential; any of these would be detrimental to the development of preimplantation embryos. Regardless of the mechanism/s behind the pathological effects of boron deprivation on embryo development, the findings strongly support the concept that boron is required for normal mammalian reproduction.

*Dietary boron as a physiological modulator of
immune function*
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This described evidence showing that dietary boron helps control the normal inflammatory process and may do so by serving as a signal suppressor that down-regulates specific enzymatic activities typically elevated during inflammation site. Suppression, but not elimination, of these enzyme activities by boron was hypothesized to reduce the incidence and severity of inflammatory disease.

There is emerging evidence that boron facilitates the normal inflammatory process by dampening the activity of serine proteases are major proteolytic enzymes released by activated leukocytes that, in addition to degrading structural proteins, have many essential regulatory roles in normal inflammation, including control of the blood complement and the coagulation systems. It has been found that boron compounds reversibly inhibit the activity of many serine proteases. Thus, a reasonable hypothesis is that boron helps regulate the normal inflammatory process by dampening the activity of leukocyte serine proteases and thereby reducing degradation of connective

tissue structures and membrane constituents.

Boron also probably affects hemostasis by inhibiting serine proteases in the coagulation cascade, including coagulation factors Xa, IXa, XIa, XIIa, and thrombin. This is supported by findings with rats fed a diet containing low amounts of vitamin K. These rats exhibited bleeding around the eyes and a high death rate. In this state of low blood clotting ability, boron-supplemented rats had a higher death rate than boron-deprived rats. Bleeding and death in both dietary groups ceased after restoration of dietary K. Thus, whereas boron may prevent pathologic coagulation, normal amounts of dietary boron apparently promote anticoagulation when vitamin K is limiting. A reasonable speculation is that reduced amounts of activated factor IX and thrombin, as well as the normal amounts of serine proteases higher in the coagulation cascade, were inhibited by boron to some degree.

Among the studies which show that animal models of rheumatoid arthritis respond positively to boron are those done in the presenter's laboratory. In one preliminary study, an ample (but probably not pharmacologic)

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amount of dietary boron (20 Fg/g) compared to very low amounts (<0.2 Fg/g) significantly delayed the onset of adjuvant-induced arthritis in rats. The incidence (expressed as % of animals) of arthritis at 12 d post-injection with *M. tuberculosis* (a well recognized model of rheumatoid arthritis in humans) was 41% for boron-deficient rats and 0% for the boron-supplemented rats. In another study, physiologic amounts of boron (3 Fg/g) added to a boron-deficient diet (0.2 Fg/g) more than doubled serum total antibody concentrations to an injected antigen (human typhoid vaccine) in rats.

In the most recent study, male weanling rats were fed either a boron-deficient (0.1 Fg/g) or boron-supplemented (2.0 Fg/g) diet and made arthritic on d 41 (postnatal d 63) by intradermal injection of *M. butyricum* in the subplantar region of the right hind paw. All rats exhibited signs of inflammation after the injection. Only 1 of 7, but 4 of 8 rats fed the boron-supplemented and boron-deficient diets, respectively, exhibited severe joint swelling at any time post-injection. Foot swelling remained relatively constant the first 10 d after injection regardless of dietary treatment, but then increased to a greater degree in rats fed the boron-deficient diet. After injection, supplemental dietary boron had a beneficial immunomodulatory effect on circulating concentrations of natural killer (NK) cells, the CD8a⁺CD4⁺ cells and neutrophils. For example, on d 14 after injection, the concentrations of NK and CD8a⁺CD4⁺ cells were higher in boron-supplemented than deprived rats. On d 28, but not d 14 post-injection, supplemental boron-decreased neutrophil concentrations. The findings of higher NK cells in boron-supplemented than in boron-deprived rats may be significant because there are several lines of evidence that the cytotoxic and proliferative activities of NK cells are inhibited by H₂O₂, a simple reactive oxygen species (ROS) that is released during the respiratory burst activity of monocytes specifically and leukocytes in general. Therefore, it is reasonable to conclude that boron has a beneficial effect on ROS production through the up-regulation of the cytotoxic and proliferative activities of NK cells.

The hypothesis that one of the mechanisms through which boron can influence the severity of arthritis and inflammation is through the alteration of ROS generation also is based on other lines of evidence. Findings from plant studies support the hypothesis that proper boron nutrition causes a simple reduction in leukocyte ROS generation through the down-regulation of leukocyte 6-phosphogluconate dehydrogenase, a key enzyme in the pentose-phosphate pathway, with subsequent alleviation of arthritic symptoms. There is also emerging evidence from cultured cells, animal and human studies that boron hastens the destruction of ROS that are scavenged and destroyed by defense mechanisms that employ glutathione, superoxide dismutase and catalase.

In summary, findings have been obtained that support the hypothesis that physiologic amounts of dietary boron reduce the risk for inflammatory disease by helping hold in check a system that is constantly poised to attack; in other words, boron maintains a balance that permits elimination of pathogens but avoids autoimmunity. The hypothesis is based on the concept that boron serves as a suppressive signal that down-regulates enzymatic activities typically elevated during the normal inflammatory process.

Boron supplementation of perimenopausal women affects boron, phosphorus and thyroid metabolism and counts of blood cells involved in immunity and inflammation

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This presentation described the findings from a double-blind designed experiment conducted with 43 free-living perimenopausal women who were consuming self-selected diets and experiencing discomforts associated with menopause. They were given sodium borate capsules containing 2.5 mg of boron for 60 d followed (19 women) or preceded (24 women) by 90 d of receiving a placebo capsule containing lactose powder. Blood was collected weekly after a 12 h overnight fast. Urine voided in 24 h was collected three times each week.

Urinary boron excretion during the placebo period ranged between 0.34 and 2.33 mg/d with a mean of 1.19 and

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a median of 1.15 mg/d. Of the 43 women, 2 excreted less than 0.5 mg/d, 14 excreted between 0.5 and 1.0 mg/d, 19 excreted 1.5 to 2.0 mg/d and 3 and excreted between 2.0 and 2.5 mg/d. As expected, boron supplementation significantly increased urinary boron; the mean rose to 3.29 mg/d and the median to 3.18 mg/d. Subtraction of the mean urinary value of the placebo period gives 2.1 mg, or about 84% of the 2.5 mg boron supplement. This indicates that urinary boron can be considered an excellent indicator of short-term boron intake. Thus, the urinary excretion values provide an estimation of the usual boron intake of perimenopausal women in the eastern North Dakota area in the US; that is, it ranges from about 0.3 to 2.3 mg/d with a median around 1.15 mg/d.

The Plasma boron concentrations found indicated good homeostatic mechanisms exist for the element. During the placebo period, plasma concentrations of boron ranged between 0.020 and 0.067 Fg/ml with a median of 0.033 Fg/ml. The three-fold increase in boron intake with supplementation only moderately increased the median plasma boron concentration to 0.052 Fg/ml with a range of 0.028 to 0.075 Fg/ml. The plasma boron concentrations during the placebo period were near those reported by others. Thus, it is likely that for perimenopausal women, a plasma concentration between 0.030 and 0.060 Fg/ml should be considered normal, but it remains to be determined whether a plasma boron concentration much lower than 0.030 Fg/ml is an indication of low boron status.

A large number of women reported more frequent and severe hot flashes and night sweats when they were receiving the boron supplement. Exacerbating effects of supplemental boron were reported by 46%, or 21, of the women. On the other hand, ten women, or 22%, reported a reduction in discomforts, or beneficial effect of boron supplementation. The remaining 15 women, or 33%, did not respond negatively or positively to the boron supplementation. This inconsistent response is difficult to explain, but it might have occurred because boron is similar to other substances, such as the pharmaceutical raloxifene HCl, a selective estrogen receptor modulator, which can counteract some of the negative body changes (ie bone loss) that occur postmenopausal, but which do not alleviate other symptoms. In fact, common side effects of raloxifene HCl include hot flashes and leg cramps.

When it is considered that many of the women were ingesting apparently adequate or luxuriant amounts of dietary boron during the placebo period (mean intake above 1 mg/d based on urinary excretion data), it was not surprising that only a limited number of blood and urine variables were significantly affected by the boron supplementation. However, boron supplementation significantly increased white blood cell numbers with a decreased percentage of lymphocytes and increased percentage of polymorphonuclear leukocytes.

Serum 17 β -estradiol also apparently was affected by boron supplementation. Overall the serum 17 β -estradiol concentration was higher during the boron supplementation period than during the placebo period; this increase approached significance (P=0.07) when all subjects were included in the comparison, but was significant (P=0.04) when the comparison included only those subjects whose urinary boron excretion was less than 1.0 mg/d during the placebo period. Interestingly, the most marked difference between the two periods occurred when boron supplementation preceded the placebo period. The finding that boron affected estrogen metabolism was not sur-

prising because several other studies have shown that boron can both enhance and mimic some effects of estrogen ingestion.

Another hormone whose metabolism apparently was affected by boron supplementation was thyroid hormone. When boron supplementation followed the placebo period, triiodothyronine (T₃) showed a notable decrease with boron supplementation. On the other hand, T₃ was not very different between the two periods when the boron supplementation preceded the placebo period. Moreover, an increase in serum alkaline phosphatase found with the boron supplementation, which was most marked when the supplementation followed the placebo period was suggested to be associated with thyroid hormone status.

In summary, the findings indicated the boron consumed in physiological or normal nutritional amounts is a dynamic trace element that can affect the metabolism or utilization of numerous substances involved in life processes, including hormones such as estrogen and thyroid hormone. The findings also indicated the boron homeostatically controlled in a manner that is similar to other well-established essential trace elements. Moreover, it is quite possible that some of the 16 women who were excreting 1.0 mg B/d or less were consuming inadequate boron. This is based on findings from both human and animal experiments which suggest that somewhere between 0.5 and 1.0 mg B/d is the lower limit of boron intake that assures beneficial health effects. Such a high percentage of 43 women not consuming boron in excess of 1.0 mg/d suggests that boron may be a practical nutritional concern.

Boron balance in humans
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94720, and USDA, ARS, Western Human Nutrition
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This presentation described the examination of boron metabolism, boron balance and urinary calcium excretion of seven healthy Caucasian men who were participating in a controlled metabolic study of zinc homeostasis. The 20-week study consists of four metabolic periods: equilibration, 3 weeks; baseline, 2 weeks; low zinc period, 10 weeks; and zinc repletion, 5 weeks. The three-day repeating menu diet consisted of conventional foods and beverages. Boron intake for menu day 1, 2 and 3 was 4.56, 1.87 and 4.75 mg/d, respectively. The higher boron content of menu days 1 and 3 was the result of the presence of boron-rich fruits and juices in the diet. Dietary zinc intake was 13.7 mg/d during equilibration, baseline and repletion periods, and 4.6 mg/d during the study - the second week of the baseline period, the first 2 weeks of the low zinc period, and the entire 5 weeks of the zinc repletion period. Fecal boron excretion and boron balance (dietary boron less urinary boron and fecal boron) was measured for 6 weeks during the study - the second week of the baseline period, the first 2 weeks of the low zinc period, and the last 3 weeks of the zinc repletion period.

Urinary boron excretion responded quickly to change in dietary boron over the 3 menu days. Urinary boron excretion for menu day 1, 2 and 3 averaged 3.46, 2.86 and 3.14 mg/d, respectively. Urinary boron excretion during the 8 weeks in which samples were collected averaged 3.18 mg/d, which represents 85% of the average daily intake of 3.73 mg/d. Urinary boron excretion was significantly ($P < 0.05$) higher during the third and fourth week of zinc repletion than during the third and fourth week of zinc repletion than during the baseline and the first 2 weeks of zinc repletion. Fecal boron excretion during the 6 weeks of collection averaged 0.29 mg/d, which represents 8% of average dietary boron intake. Fecal boron also tended to increase during the third and fourth weeks of zinc repletion, but this increase was not significant.

Individual urinary and fecal boron excretions were averaged over 6 d periods. Urinary boron excretion of all subjects over the entire set of collections ranged from 2.58 to 3.68 mg/d, or 69 to 99% of boron intake. The within subject coefficient of variation of urinary boron excretion ranged from 16 to 25%. The within subject coefficient of variation, over the corresponding period, for daily urinary excretion of sodium ranged from 17 to 24% and for creatinine ranged from 7 to 18%. Individual fecal boron losses over the entire set of collections ranged from

0.14 to 0.47 mg/d, or 4 to 13% of dietary boron intake. The individual variability in fecal boron excretion was 8 to 32%, which probably reflected individual differences in gastrointestinal transit time.

Boron balance ranged from 0.45 mg/d to -0.05 mg/d during the 6 weeks samples were collected. The subjects were in slight positive boron balance an average of 0.19 mg/d, over the 6 week period consisting of the second week of baseline, the first 2 weeks of the low zinc period and the last 3 weeks of the zinc repletion period. There was a higher retention of boron during the first 2 weeks of the low zinc period, and average of 0.24 mg/d, than during the last 3 weeks of the zinc repletion period, when 0.06 mg/d was retained. The lower retention reflects the increase urinary and fecal excretion of boron during the third and fourth weeks of zinc repletion.

The average daily urinary calcium excretion was higher during the 5 weeks of zinc repletion than during the last week of the baseline period and the first 2 weeks of the low zinc period. However, no correlation was found between urinary calcium and urinary boron losses. It was thought that the increase in urinary calcium excretion was not associated with boron metabolism.

The findings reported in this study confirm that boron is rapidly absorbed and excreted in the urine. A change in dietary boron from one day to the next caused a change in urinary boron excretion. However, the change in urinary boron was less than the change in boron intake; urinary boron increased only 10% with a dietary intake change from 1.9 to 4.75 mg/d. The response was similar to others who found that when dietary boron is increased, urinary boron increases rapidly over 3 d and then stabilizes at about 90% of boron intake. The findings also conform with those of other researchers which indicate that urinary boron excretion usually is about 84 to 90% of intake irrespective of the amount of dietary boron consumed. Thus, urinary boron is a sensitive indicator of recent boron in-

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take. Also, the current study like others done elsewhere show that boron homeostasis is primarily regulated by the kidney.

*Analytical challenges of low-level boron
analysis in biological matrices
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This presentation described many critical precautions that are required when undertaking analyses of boron in low amounts in the biological samples being studied in essentiality research programs. Although analytical instruments and sample handling procedures have steadily improved during the last decade in pursuit of accurate boron concentrations in biological matrices, sources of contamination and loss during sample handling must still be avoided or minimized. Boron values reported at the mg/kg concentration are now generally considered reliable; however, Fg B/kg determination still remain a challenge in many complex, biological matrices. Limiting factors include establishing boron-clean laboratory environments, the implementation of appropriate laboratory quality control, and foremost, the maintenance of proper precautions in handling and preparing samples for instrumental analysis.

The identification of significant boron contamination sources and how they travel to intersect the biological path of the animals are crucial to the success of these essentiality studies. Specific knowledge of boron's chemical pathways in the laboratory was pointed out to be essential. In addition, attention to the chemistry of boron must begin at the experimental design stage and continue through the final data analysis. Once the sample processing begins and until the material has been introduced into the instrument, encounters with contaminant sources such as air, containers, surfaces, particulate, solvents and filters can quickly compromise the accuracy and value of the results. For example, because boric acid is relatively volatile, it persists as an environmental source of contamination and as a transport mechanism for loss. Likewise, although inert Teflon containers appear boron-free, they nevertheless serve as a significant source or repository of boric acid. Furthermore, although glass surfaces, including pipettes, are easy to clean and keep sterile, they do contain boron that is subject to leaching. Vigilant measures are not only required in the laboratory environment, but uniformly maintained, prescribed amounts of boron must also be controlled, especially in the water and foodstuffs available to the animals.

The degree of difficulty in determining boron depends greatly upon the concentration range of interest. Techniques such as neutron activation-mass spectrometry (NA-MS), ion-coupled argon plasma (ICAP), and ion-coupled plasma optical emission spectrometry (ICP-OES) have detection limits approaching a few Fg B/kg. Ion-coupled plasma mass spectrometry (ICP-MS) has detection limits below 0.1 Fg/kg, and prompt gamma neutron activation analysis (PGNAA) has only slightly higher detection limits. Instrument back-grounds and various interferences do produce non-negligible problems for boron analysis and can degrade the data quality. Thus, precautionary steps are required, with efforts inversely proportional to the boron concentration in the sample and in accordance with the possibility for boron contamination.

Because boron is ubiquitous in the environment, it requires careful attention and knowledge to exclude extraneous sources from the laboratory, and more importantly, from laboratory samples. The examples presented above demonstrate a surprising number of routes for a boron compound to enter and subsequently contaminate the sample during preparation and storage. Consequently, analysts setting about to determine boron in biological matrices, especially in low Fg/kg amounts, should expect to encounter a significant learning curve as they develop the appropriate analytical protocols and skills to minimize boron contamination and losses during sample preparation and analytical procedures.

Estimation of dietary boron intake in six countries: Egypt, Germany, Great Britain, Kenya, Mexico and the United States

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Average adult dietary intakes of boron in the United States and Canada have previously been reported as between 1 and 2 mg B/d, although most of these reported studies included only a small number of subjects. In this study, available large-scale, nationwide food intake surveys were used to estimate boron intakes in the US, Germany and Great Britain, and intake survey data from three rural agricultural communities of Mexico, Kenya and Egypt were also employed. The US Department of Agriculture's Continuing Survey of Food Intakes by Individuals (1989-1991) covered the 48 conterminous United States (food records for 1-3 consecutive days for over 14,000 individuals). The Dietary and Nutritional Survey of British Adults (1986-1987) had surveyed over 2000 participants ages 16 to 64 with a full 7-day weighed dietary record. The German National Consumption Study (1985-1989) surveyed participants given food diaries, and the final data set included 4-7 days of food consumption records for over 23000 persons. The surveys from Mexico, Egypt and Kenya had been conducted during 1983-1986 as part of the Human Nutrition Collaborative Research Support Program in rural, predominately agricultural areas; in each community, 2-day dietary records were obtained monthly for one year from approximately 250 households.

A boron nutrient database was constructed to include boron concentrations for the foods and beverages consumed in each country. This database incorporated boron analytical data from various literature sources in the US, Finland, UK, Italy, Japan and China and also used these literature values to estimate the boron content of various recipes of mixed food ingredients. The analysis of the literature for boron concentrations resulted in a final analytical boron database of 527 foods. Each surveyed person's average daily boron intake was then estimated by linking the boron database values for individual foods/beverages with the survey records of foods/beverages consumed.

Estimates of mean (\pm SD) dietary intake for adults in the US, Germany, Great Britain, Mexico, Kenya and Egypt were reported as follows: 1.11 \pm 0.69, 1.72 \pm 0.47, 1.30 \pm 0.63, 2.12 \pm 0.69, 1.95 \pm 0.57 and 1.31 \pm 0.50 mg B/d for males, respectively; and 0.89 \pm 0.57, 1.62 \pm 0.76, 1.14 \pm 0.55, 1.75 \pm 0.48, 1.80 \pm 0.49 mg B/d for females, respectively. Rainey found that the top contributors to dietary boron intake in each in each of the six countries studied were as follows for the US, Great Britain, Germany, Mexico, Kenya and Egypt, respectively: coffee (6.5%), wine (14%), wine (15.4%), tortillas (56.1%), maize (35.3%) and rural breads (27.4%).

These dietary boron intake estimates provide data that will be useful for setting recommended daily intake levels when boron is confirmed to be essential for humans. These findings could also prove useful in developing survey tools for the future analyses of boron intake.

Panel discussion

The Co-chairs, J.R. Coughlin and P.J. Aggett, would like to thank the following panel members who contribute to the valuable discussion of the research findings and avenues for further research efforts were also discussed. Panel members were:

Ronald Walker, PhD, Professor Emeritus, University of Surrey, Guildford, England, UK

Forrest H. Nielsen, PhD, Center Director, USDA Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota, USA

Roger A. Sunde, PhD, Professor of Nutritional Sciences and Biochemistry, University of Missouri-Columbia, Columbia, Missouri, USA

Sean Strain, PhD, Professor of Human Nutrition, Northern Ireland Center for Diet and Health, University of Ulster, Coleraine, Northern Ireland, UK

Summation

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