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Communication

Metabolic Requirement of *Cucurbita pepo* for Boron[1]

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ABSTRACT

Lateral roots of intact summer squash seedlings (*Cucurbita pepo* L.) were used to quantify the effects of boron deficiency on DNA synthesis, protein synthesis, and respiration. The temporal relationship between changes in these metabolic activities and the cessation of root elongation caused by boron deprivation was determined. Transferring 5-day-old squash seedlings to a hydroponic culture medium without boron for 6 hours resulted in a 62% reduction in net root elongation and a 30% decrease in the incorporation of [^{3}H]thymidine into DNA by root tips (apical 5-millimeter segments). At this time, root tips from both boron-deficient and boron-sufficient plants exhibited nearly identical rates of incorporation of [^{14}C]leucine into protein and respiration as measured by O_2 consumption. After an additional 6 hours of boron deprivation, root elongation had nearly ceased. Concomitantly, DNA synthesis in root apices was 66% less than in the boron-sufficient control plants and protein synthesis was reduced 43%. O_2 consumption remained the same for both treatments. The decline and eventual cessation of root elongation correlated temporally with the decrease in DNA synthesis, but preceded changes in protein synthesis and respiration. These results suggest that boron is required for continued DNA synthesis and cell division in root meristems.

Demonstration that B is a micronutrient essential to vascular plants (16, 20) has resulted in 60 years of research seeking to elucidate the primary role of B in the metabolism of higher plants. Despite the considerable amount of research conducted to identify the metabolic event dependent on B, definitive evidence to support a specific biochemical role has not been obtained. The dependence of normal meristem activity on B is well documented for both angiosperms (3, 9–11, 16, 17, 20) and gymnosperms (2, 19). For example, a reduction in root elongation within 6 h of transferring plants to culture conditions without added B is routinely observed for varieties of *Vicia faba* (14), *Lycopersicon esculentum* (1), *Phaseolus aureus* (3), *Cucurbita pepo* (5, 11), and *Helianthus annuus* (13). Total root elongation is the result of two processes, cell division and cell elongation. While a number of early researchers suggested that B is necessary for cell division in meristems (17, 20), definitive evidence establishing the greater sensitivity of cell division *versus* cell elongation to B deficiency was obtained more recently (5, 9).

Experiments using autoradiography revealed that the incorporation of [^{3}H]thymidine into root apical meristems of 5-d-old squash plants ceased after 20 h B deprivation (4). When these plants were returned to B-sufficient medium for 12 h, autoradiographs showed a pattern and intensity of incorporation indistinguishable from that of B-sufficient root apices (4). Taken together, these observations suggest that DNA synthesis is impaired under conditions of B deficiency.

This study was undertaken to define the relationship between inhibition of root elongation and DNA synthesis in root apices of *Cucurbita pepo* specifically to determine if impaired DNA synthesis is a primary effect of B deprivation or a secondary response resulting from decreased respiration and/or protein synthesis.

MATERIALS AND METHODS

Chemicals. All radiolabeled chemicals were purchased from New England Nuclear. Mineral salts for Shive's nutrient solution were of analytical reagent quality from Fisher Scientific Co. All other chemicals were from Sigma.

Plant Materials. Summer squash seeds (*Cucurbita pepo* L., cv Early Prolific Straightneck), supplied through the courtesy of the Joseph Harris Co., Inc., were imbibed in distilled H_2O for 24 h at room temperature. They were then spread evenly between two sheets of paper toweling placed in a plastic box (33 × 23 × 9.5 cm), and moistened with 7 ml H_2O. The covered box was placed in a growth chamber where the seeds germinated in the dark for 48 h at 30°C. After germination, the terminal 5 mm of the primary root was pinched off to promote lateral root formation, and the seedlings were inserted into holes in the lid of a polyurethane breadbox (38 × 14 × 13 cm, 5.5 L) covered with aluminum foil and containing Shive's nutrient solution. The box was transferred to a growth chamber where the plants were hydroponically cultured in aerated solution at 30°C under continuous illumination at 310 $\mu E/m^2 \cdot s$. After 3 d, plants of uniform appearance were selected for treatment. The lateral roots of several plants were marked with India ink 10 mm from the tip to determine the rate of root elongation. The plants were then transferred to soft glass (B-free) jars (1.8 L) containing either complete Shive's nutrient solution (0.1 mg B/L) (+B)[4] or modified Shive's nutrient solution with the boron omitted (−B). The plants were transferred to a growth chamber (this was designated as T_0) and allowed to grow for specified periods in aerated solutions at 30°C under continuous illumination of 310

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[4] Abbreviations: +B, 0.1 mg boron/L hydroponic culture solution, boron-sufficient; −B, boron withheld from the hydroponic culture solution, boron-deficient.

$\mu E/m^2 \cdot s$. At the end of the treatment period, net elongation was determined and the plants were used as described below.

Respiration. Fifty 5.0-mm root tips were excised from 6 to 8 plants for each treatment (+B or −B) after 6, 12, 18, and 24 h. The tips were then placed in the main compartment of a Warburg flask and respiration was measured by determining O_2 consumption over a 1-h period.

DNA and Protein Synthesis. Five-d-old seedlings were grown for 2 to 23 h in +B or −B solutions as described above, and three similar plants were transferred to graduated cylinders containing 60.0 ml of fresh nutrient solution supplemented with [^{3}H]thymidine (0.69 μCi/ml, 1 μM) and L-[^{14}C]leucine (0.083 μCi/ml, 10 μM). Cylinders containing the three plants were placed in the growth chamber at 30°C under continuous light, at 310 $\mu E/m^2 \cdot s$. At the end of the 1-h labeling period, the roots were washed and the apical 5.0 mm were excised from five roots per plant. The 15 tips were pooled and homogenized in 3.0 ml ice-cold water using a Polytron tissue homogenizer (Brinkman Instruments, PCU-2; speed 6). To determine precursor uptake, the homogenate was mixed thoroughly with a vortex mixer and 2 ml of the suspension was immediately transferred to a scintillation vial containing 2 ml Hydrofluor liquid scintillation cocktail (National Diagnostics). To determine incorporation of [^{3}H]thymidine and [^{14}C]leucine, the homogenate was divided into three aliquots; 1.0 ml ice-cold 10% TCA was added to each to precipitate the acid-insoluble fraction, and each sample was filtered through a 0.45 μm glass filter (6). Each precipitate containing labeled DNA and protein was then washed with 5.0 ml of cold (2°C) 5% TCA three times and aspirated. The samples were dried for 20 min under a heat lamp and immediately placed in a scintillation vial containing 5.0 ml of scintillation fluid, PPO-POPOP toluene concentrate (New England Nuclear Corp.), diluted in scintillation grade toluene. Simultaneous counting of [^{3}H]thymidine and [^{14}C]leucine was accomplished by a dual-counting channel. Quenching was determined by an automatic external standardization system of quench analysis. A standard quench curve was determined for each isotope by using a series of samples containing a known quantity of ^{3}H or ^{14}C radioactivity and increasing amounts of chemical quencher.

Determination of Cell Number. Apical 5.0-mm root segments of +B and −B plants were harvested after 0, 6, 12, 18, and 24 h of treatment and immersed in 2.0 ml of 5% chromic acid (w/v) in 1 N HCl and stored at 5°C. Immediately before counting, the cells were separated by repeatedly forcing the sample through a No. 22 needle. A sample was withdrawn and placed in a Levy-Houser deep well (0.2 mm) counting chamber with a Fuchs-Rosenthal grid. The number of cells in an area 0.2 × 1 × 1 mm was counted at × 400. Cell number was determined for four samples for each root and two roots were examined in each experiment (11).

Significance levels were determined by Student's t test.

RESULTS

Squash seedlings require B for continued root elongation (Fig. 1). Net root elongation in −B plants was 62% less than that of the +B control plants after only 6 h of B deprivation. Root elongation for +B plants was approximately 1.25 mm/h for the first 18 h after transfer to fresh +B Shive's nutrient solution and approximately 2.5 mm/h over the next 30 h. For plants transferred to −B Shive's nutrient solution, root elongation essentially ceased 18 h after transfer (Fig. 1). After 24 h of B deprivation, symptoms of B deficiency were clearly visible: root tips were brown, hooked, and larger in diameter than comparable +B root tips; stubby lateral roots, having been initiated but failing to elongate, were present almost to the root apex. The apical 5-mm segment excised from roots of −B plants was significantly heavier than that of +B plants after 24 h of treatment: Compare 7.0 mg/

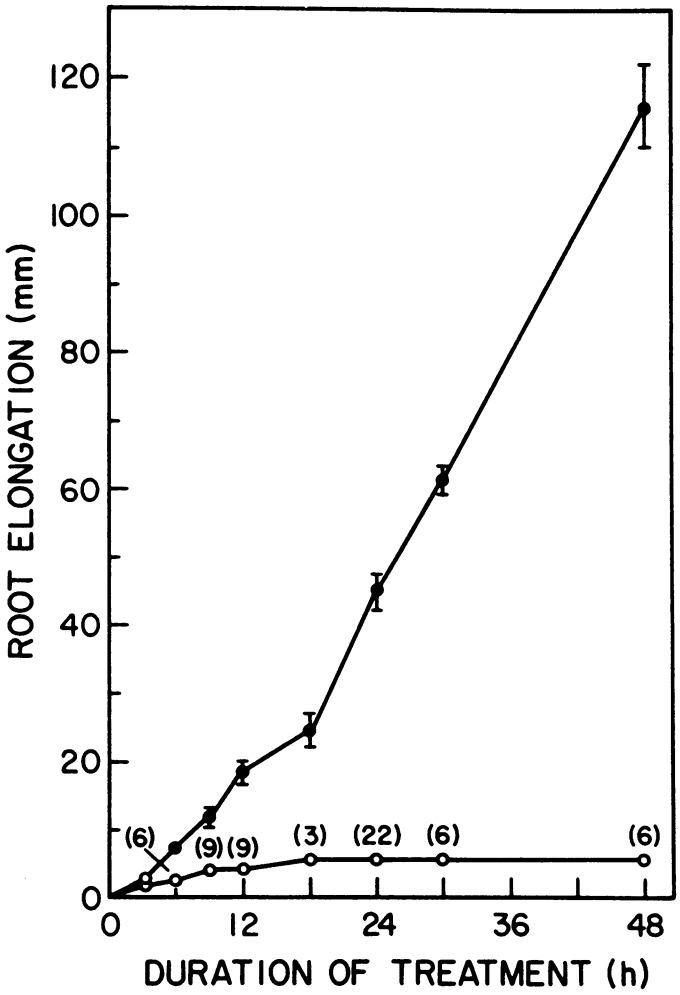

FIG. 1. Average root elongation in millimeters for 5-d-old squash plants transferred to +B nutrient solution (0.1 mg B/L) (●) or −B nutrient solution (B withheld) (○). Vertical bars indicate SE. When bars are absent, SE is less than ± 0.7 mm. The number of experiments is given in parentheses.

10 +B root tips to 9.3 mg/10 −B tips ($P < 0.01$). There was no significant difference at the 5% level in the number of cells per 5 mm root tip from +B or −B plants at the end of 24 h of treatment (Table I). However, after 30 h of treatment, the number of cells per root tip was significantly less for roots from −B plants ($P < 0.001$; Table I).

Root apices of plants deprived of B for 6 h incorporated 29% less [^{3}H]thymidine into DNA than +B roots ($P < 0.05$) (Fig. 2A). Reduced incorporation of thymidine into DNA by −B root apices became dramatically lower as boron deprivation continued. At the end of 12 h, incorporation of thymidine into DNA in −B root tips was 66% less than that of root apices of the +B control plants ($P < 0.05$). The incorporation of [^{3}H]thymidine into DNA increased in the +B root apices during the period from 18 to 24 h after transfer corresponding with the increased rate of root elongation observed for the +B control plants for this same period (Figs. 1 and 2A).

In contrast, incorporation of [^{14}C]leucine into protein was not significantly different ($P < 0.05$) between +B and −B root apices until 12 h of B deprivation. At this time, protein synthesis was reduced 43% by B deprivation ($P < 0.05$) (Fig. 2B). Respiration was not significantly different ($P < 0.05$) in +B and −B roots even after 18 h of B deprivation (Fig. 2C).

Reduced incorporation of [^{3}H]thymidine into DNA or [^{14}C]leucine into protein observed for −B root apices was not due to

Table I. *Cell Number for 5-mm Apical Root Segments Excised from +B and −B Squash Plants*

Plants were 5 d old when treatment was initiated: +B (0.1 mg B/L); −B (boron withheld).

Time	Treatment Condition	5-mm Apical Root Segment	Significance (Student's *t* Test)
h		*cell no.* × 10^{-4}	
6	+B	6.05 ± 0.90 (4)	NS
	−B	6.23 ± 1.54 (4)	
12	+B	6.96 ± 1.83 (4)	NS
	−B	6.65 ± 1.42 (4)	
18	+B	6.13 ± 0.09 (2)	NS
	−B	6.31 ± 0.71 (2)	
24	+B	6.36 ± 1.83 (6)	NS
	−B	6.33 ± 1.72 (6)	
30	+B	6.73 ± 0.46 (4)	$P < 0.001$
	−B	4.26 ± 0.59 (4)	

decreased availability of either precursor. Uptake of [^{3}H]thymidine by +B and −B root apices during the last hour of the 24-h treatment period was in two separate experiments, 46 and 48 pmol/15 +B root tips *versus* 42 and 50 pmol/15 −B root tips. Uptake of [^{14}C]leucine was in two separate experiments conducted in the same manner 2.9 and 2.9 nmol/15 +B root tips and 3.2 and 3.4 nmol/15 −B root tips.

The ability of −B root apices to synthesize DNA and protein was further tested by measuring the incorporation of the appropriate precursor at 15-min intervals for 1 h immediately following 3 or 18 h of +B and −B treatment (Figs. 3 and 4). Consistent with the results summarized in Figure 2, there was no difference in the incorporation of [^{3}H]thymidine into DNA or [^{14}C]leucine into protein in +B and −B root apices after only 3 h of treatment (Figs. 3A and 4A). However, after 18 h of −B treatment, the incorporation of both precursors was dramatically reduced during the following 1-h labeling period (Figs. 3B and 4B).

DISCUSSION

In seeking to identify the essential role of B in the metabolism of vascular plants, we have sought to determine the earliest effect

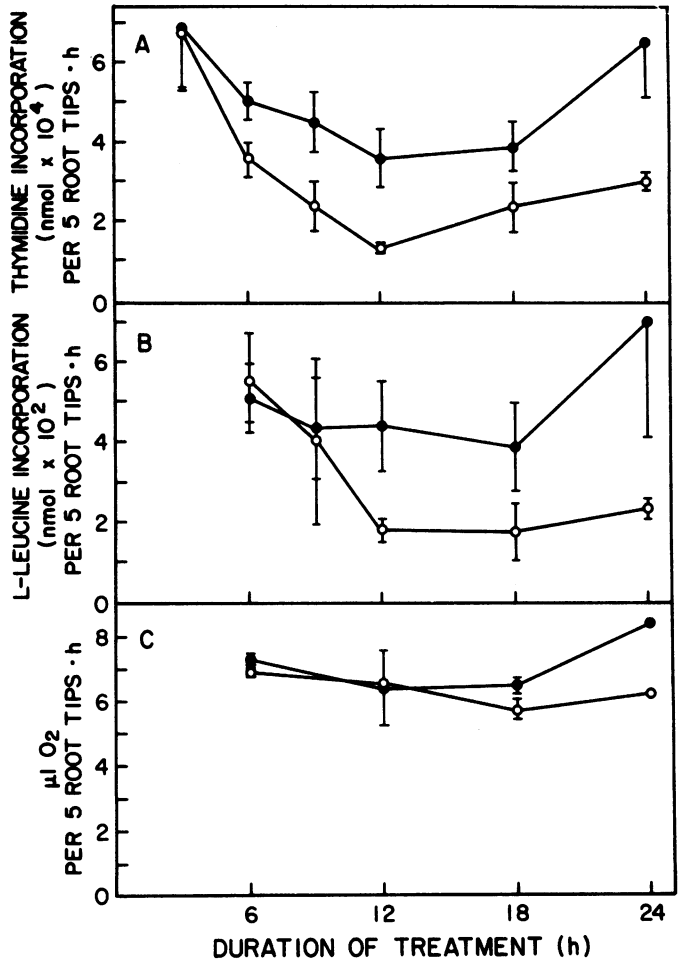

FIG. 2. Incorporation of [^{3}H]thymidine (A) and [^{14}C]leucine (B) into DNA and protein, respectively, in apical 5-mm root segments of intact squash plants during a 1-h labeling period at the end of each treatment period. Respiration rate (C) was determined, using a Warburg apparatus, for root tips excised at the end of each treatment period. In each case, the data are the average of three separate experiments. Vertical bars indicate standard error.

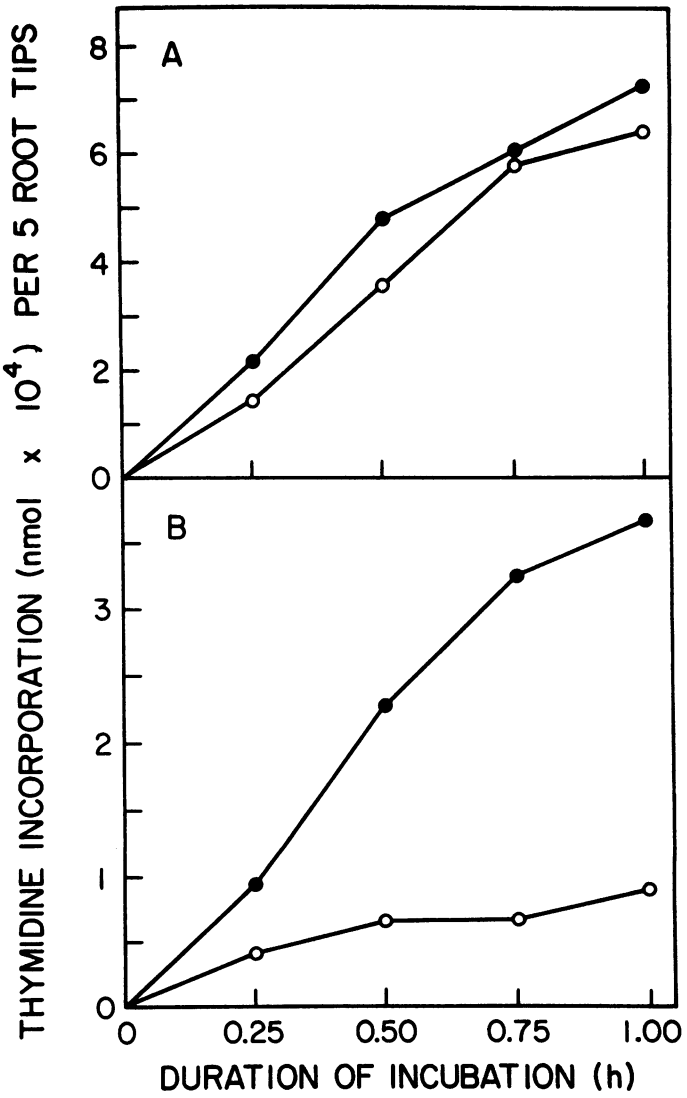

FIG. 3. Effects of B on [^{3}H]thymidine incorporation into DNA in apical 5-mm root segments of intact squash plants during a 1-h labeling period following 3 h (A) or 18 h (B) of +B (●) and −B (○) treatment. Points are the mean of three samples with five roots per sample.

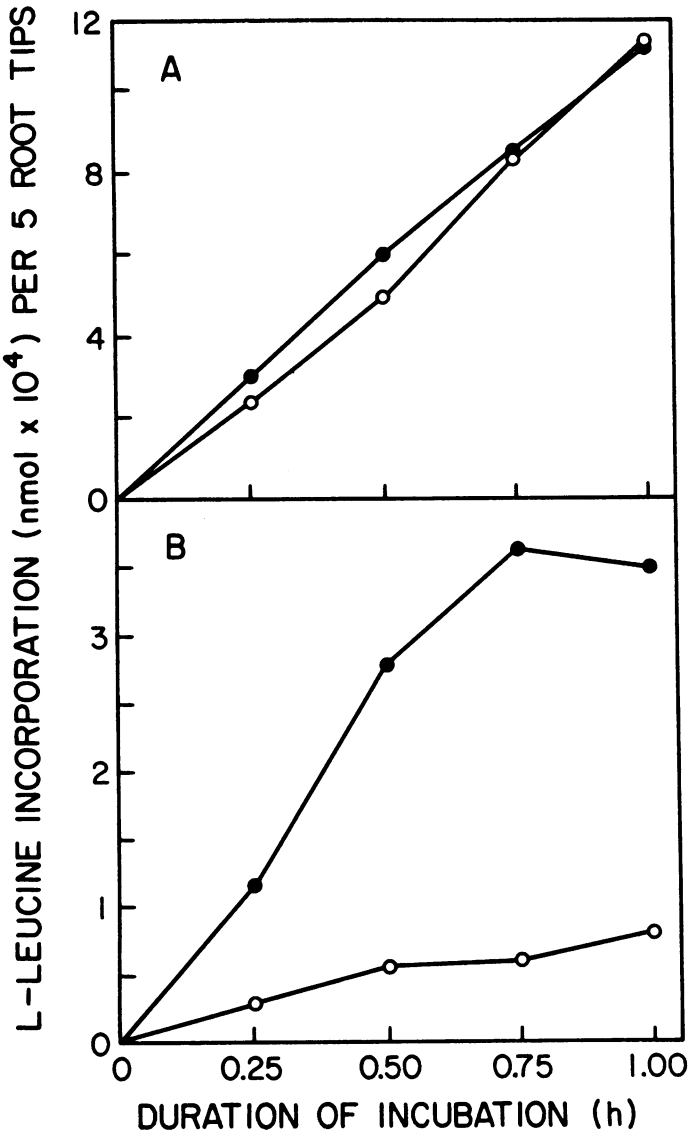

FIG. 4. Effects of boron on [^{14}C]leucine incorporation into protein in apical 5-mm root segments of intact squash plants during a 1-h labeling period following 3 h (A) or 18 h (B) of +B (●) and −B (○) treatment. Points are the mean of three samples with five roots per sample.

of B deprivation at the biochemical level, at this is the one most likely to be associated with the metabolic requirement of higher plants for B. The reduced incorporation of [^{3}H]thymidine into DNA, which occurred within 6 h after transfer of +B squash plants to Shive's nutrient solution without B, is the earliest effect of B deficiency reported in the literature. DNA synthesis was significantly reduced a full 6 h before protein synthesis was reduced; respiration was not significantly different after 12 h of B deprivation.

Since DNA synthesis is inhibited 66% 6 h prior to the cessation of root elongation, it is tempting to speculate that decreased ability to synthesize DNA during B deprivation is the cause of reduced root elongation characteristic of −B plants. It is clear from the results of this study that the early failure of −B roots to grow at a normal rate is not due to inhibition of protein synthesis or respiration. However, loss in ability to synthesize protein, which occurs after 12 h of B deprivation might be a factor contributing to total inhibition of root growth after 18 h of deprivation.

The reduced incorporation of precursor into DNA at 6 h and into protein at 18 h of −B treatment is not due to a reduction in precursor uptake, which was normal in −B roots for 24 h, nor to a decrease in the number of cells per 5-mm apical root segment. Since the length of root excised was constant regardless of the total length of the root, no difference in cell number would be expected between the two treatments unless cells failed to undergo enlargement was inhibited during B deficiency prior to the cessation of cell division, yielding a root tip with many small cells, or unless the actual destruction of cells occurred. It is well established in the literature that cell enlargement and xylem differentiation continue for at least 72 h despite the fact that B deprivation inhibits cell division in the root tip within 20 h (5, 14, 15). As a result, differentiation of the stele proceeds into the apical region of the root and the area occupied by meristem (9, 14). At this time, an actual loss in cell number would be expected. This may explain the lower number of cells in apical 5-mm root tips excised from plants deprived for B for 30 h.

Moore and Hirsch (13) observed continued incorporation of [^{3}H]thymidine by B-deficient root apices of sunflower (*Helianthus annuus*), consistent with a reduced, but linear, rate of elongation throughout the 72-h −B treatment period. In their study, it took 72 h of B deprivation to cause a 60% reduction in root elongation in sunflower. This is 2.75 d more than was required to effect a 60% reduction of root growth in squash. These results suggest that the hydroponic culture system employed by Moore and Hirsch (13), contained a low level of B or that cell division in root apices of sunflower seedlings is not as sensitive to B deprivation as it is in squash.

Whether B is directly involved in DNA synthesis or only secondarily through maintenance of an adequate supply of nucleotide precursors remains to be determined. These two possibilities are both supported by several reports in the literature demonstrating that the utilization or the level of available purine or pyrimidine nucleotides, or both, is altered by boron deprivation (3, 4, 7, 8, 11, 12, 18).

In either case, our results, taken together with those appearing in the literature, support the possibility that the biochemical role of B may be at the level of DNA synthesis.

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