SUMMARY AND CONCLUSIONS

Genesis and Scope of the Problem.

Boron is an essential micronutrient for plants and is invariably found to exist in animal tissues. In spite of an enormous number of publications, its biochemical role still remains a mystery, and trials to establish even its essentiality in animal systems have failed. The microelement is of great economic importance in agriculture because both its deficiency and excess in the soil are detrimental for plant life. In view of its fundamental and applied importance, the problem was undertaken for this piece of work, which is presented here in the form of a compendium containing the results obtained from a biochemical screening of various probable roles of boron. Some useful and original clues have been obtained from some of the experiments which endeavoured to reveal the biochemical role of this microelement.

The pivotal importance of this work is that it has been conducted on a material (majorly radicle and root) which is most sensitive to boron deficiency at the earliest stages of growth. The major consideration for using such a material has been that the earlier a metabolic variation due to boron deficiency is detected in the life cycle of a plant, the more fundamental it might be towards the elucidation of its function.
Most of the investigations have been conducted on cluster bean and pea radicles and plumules, which were found to be quite sensitive to boron deficiency. The biochemical investigations were majorly restricted to such plants which were not allowed to exhibit boron deficiency symptoms. This was considered important in order to study the metabolic alterations when they had been just caused due to initiation of boron deficiency and not due to secondary effects of deficiency.

1. Availability of Boron from Seed to the Embryo. Boron content of testa of cluster bean seed was not available to the embryonic tissues during the first five days of growth. About 30 per cent boron of endosperm and cotyledons was available for seedling growth. This small amount of boron was capable of maintaining growth of the seedling only for about 96 hours to a limited extent. For studies on radicle growth, 0.5 ppm boron concentration was found to be optimum for all the three plants studied.

2. Effect of Boron Deficiency on Plant Growth. Before embarking upon the biochemical studies regarding the role of boron, it was considered necessary to study its effects on cluster bean plant upon which no studies have been reported so far.

Boron deficiency significantly retarded the growth of cluster bean, pea and wheat radicles. Cluster bean radicle was most sensitive to boron deficiency as compared
to the other two. The deficiency of boron induced a curling and swelling of the radicle tip at the end of about one week after transferring the seedlings to boron-deficient solutions. One conclusion that can be drawn from the effect of boron on radicle growth is that at least one possible growth limiting and boron-requiring process in plants is concerned in root growth, before the onset of photosynthesis. The process is probably associated with cell division and cell expansion.

Statistical evaluation of the data pertaining to the effect of boron deficiency on shoot height, leaf number and flower number clearly showed that boron application significantly increased all these parameters in cluster bean.

The application of boron increased the dry weight of cluster bean axis up to about 25 per cent. This effect seems to be due to increased growth of plumule and radicle in the presence of boron, which takes place at the cost of seed reserves. In other words the seed gets depleted at a quicker rate in the presence of boron.

3. Effect of boron on cell division and cell extension. Total cell number, non-vacuolated cell number (meristematic), rate of cell division and indices of extension were measured in excised cluster bean root tips both in the presence and absence of boron. The increase in the total number of cells in boron-treated cluster bean root tips was much more in comparison to that of root tips receiving no
boron. Boron thus definitely seems to stimulate cell multiplication. The differences in the number of cells formed in the unit time might be due to corresponding differences in the number of cells available for division or to differences in the rates of division per meristematic cell or to a combination of both factors. The number of non-vacuolated cells in boron-treated root tips was higher in comparison to control tips and this bears a testimony to the fact that boron keeps more cells meristematic. Boron was also found to increase the rate of cell division in the excised root tips. Higher values of indices of extension and root tip length in the presence of boron further showed that the element favoured cell extension as well. Thus both cell division and cell extension are favoured by boron.

Application of boric acid and phenylboronic acid promoted values of indices of extension and total cell number, whereas sodium tetraphenyl boron did not. These results showed that the activity of borate as an essential plant nutrient depended upon its ability to form a biologically active complex with an in-vivo cis-diol compound.

4. Intracellular Distribution of Boron. Before undertaking detailed studies regarding the biochemical role of a bioconstituent, it is advisable to first determine its intracellular localization to facilitate subsequent studies in
those organelle/organelles in which it is found to be present in comparatively higher concentrations.

Intracellular distribution of boron was studied in plus-boron (0.5 ppm) radicles and plus-boron and boron-deficient roots, shoots and leaves + apical buds of cluster bean, pea and wheat plants.

Under optimum boron supplies, the element concentrated more in the supernatant fractions (μg/gm) in comparison to cell wall. The introduction of boron deficiency was immediately reflected in reduced levels of boron in the cytoplasmic fraction whereas in the cell wall the levels of boron remained almost constant. This showed that the cell wall did not possess any loosely bound boron which could be released for various physiological needs during a stress due to boron deficiency. Boron thus seems to play an important role in the cell wall.

Precise boron analysis of various other subcellular fractions obtained from cluster bean, pea and wheat plants by differential centrifugation reflected vast differences in the concentrations of this trace element in substructures obtained from the same plant but there were similar trends of boron distribution even in specific substructures obtained from plants belonging to different families. In nuclear fractions and non-particulate cytoplasm (dialysed supernatants), there were much higher concentrations of boron in comparison to all other fractions. Chloroplast and
ribosomal fractions contained much smaller concentrations of boron in comparison to the above fractions. Mitochondria did not contain any boron in any of the fractions obtained from various parts of different plants. It is interesting to note that absence of boron from mitochondria indicated that the trace element might not be at all involved in any of the biochemical activities of this organelle. Boron deficiency slightly affected the boron levels of chloroplasts, showing thereby that boron might be structurally or otherwise involved somewhere in the chloroplast. Prolonged deficiency of boron might decrease its concentration still more in this organelle.

Another finding is that a small fraction of boron was also retained by the ribosomal fraction. The concentration of boron fell to still lower levels in this organelle as soon as boron was withdrawn from the nutrient solution, showing that ribosomes probably received their boron supply from the cytoplasm, which was majorly affected by boron deficiency. It is however difficult to arrive at any conclusion regarding the role of boron in ribosomal activities without further detailed studies, because presence of boron in ribosomal fraction had not been found to affect protein synthesis. Although every care was taken to study the intracellular distribution of boron, even then its concentration in the organelles of an intact cell might be varying due to the dissociation of some boron complexes.
during fractionations in aqueous media and non specific adsorption of boron by certain particulates during the grinding and isolation procedures.

An intriguing observation was that withdrawal of boron from the nutrient solution most conspicuously affected the soluble (dialyzable) boron fraction. There was no major fall in the level of boron in any other fraction due to boron withdrawal as had been recorded for dialysable boron in the cell-free supernatant fraction. This suggested that soluble boron was probably serving as a boron pool and this seemed to be the major form of physiologically available boron. The depletion of the soluble boron in the cytoplasm seemed to lead to the onset of boron deficiency in plants.

Boron content of the nuclei was majorly retained by the nucleolar fraction and the nuclear sap. Boron level in nuclear sap also fell as soon as boron was withdrawn from the nutrient solution. This also demonstrated that there was a miniature buffer stock of boron which was maintained by the nucleus to meet the demands of its own physiological or structural requirements. The absence of boron in the chromatin fraction is a testimony to the fact that the element was not important in maintaining the structure of either chromatin complex or DNA in a natural intact form and the process of transcription could only be affected by boron deficiency in the nuclear sap. Little
decrease in the concentrations of boron in ribosomal and nucleolar fractions after transferring the plants to solutions lacking boron showed that the element in these fractions might be lightly bound and subject to release when the boron level in the nuclear sap declined.

5. Effect of Boron Deficiency on Cell Wall Biogenesis.

Studies on the intracellular distribution of boron suggested that the element might be playing some role in the cell wall. Considering this possibility, the following studies were conducted.

(a) Effect on neutral sugar composition of cell wall polysaccharides. Neutral sugar composition of cell wall polysaccharides of various parts of cluster bean, pea and wheat plants, receiving optimum and deficient levels of boron, was determined. Boron deficiency brought about some uniform alterations in the neutral sugar composition of cell wall polysaccharides of various plants studied. Borate did not seem to have any control on xylose content, which remained quite unaltered during its presence or absence. In all the tissues studied, deficiency of boron decreased the levels of galactose, though the magnitude of this decrease varied from tissue to tissue and from plant to plant. There was no effect of boron deficiency on glucose contents of cell walls at any stage of growth. The other two sugars, mannose and rhamnose, uniformly decreased in all the tissues obtained from boron-deficient plants.
The control exercised by borate on the incorporation of these sugars in cell wall polysaccharides seemed to be more important at younger stages than at older ones, because higher amounts of these sugars were found in younger tissues.

Sugar molecules having cis-hydroxyl groups (axial-equatorial) on adjacent carbon atoms are capable of complexing with borate, whereas trans groups, which are di-equational and presumably at the same distance from each other as the cis-groups, do not undergo complexing.

Consideration of the neutral sugar composition of cell wall polysaccharides both in the presence and absence of boron as well as the electrophoretic mobility of these sugars on borate impregnated paper (relative mobility of these sugars on borate impregnated paper decreases in the same order as the increase in their incorporation rates in the cell wall polysaccharides in the presence of borate) clearly demonstrates that those sugars, for example xylose and glucose, which have no cis-hydroxyls and are all equatorial, could not make a complex with borate and thus their incorporation into cell wall polysaccharides was not influenced by borate.

On the other hand, arabinose, galactose, mannose and rhamnose have cis-hydroxyl groups and conform to the configurational requirements described above and hence could complex with borate and their incorporation into wall
polysaccharides might have been affected.

In order to clarify the conflict arising out of Gauch and Dugger's hypothesis (7) regarding the role of borate in sugar translocation, an experiment was performed to test whether borate was getting translocated through the membrane and retained in the cell wall or not. Borate was estimated in the cell walls of plasmolysed (membrane-free) and turgid (membrane-intact) cells for this purpose. This experiment demonstrated that whatever boron was retained by cell wall, it was complexed only with the cell wall and had no relationship with the plasmalemma. In view of this observation Gauch and Dugger's emphasis that borate complexed with membrane may be acting only for the translocation of sugars and releasing them as free sugars does not seem to be plausible because if their view point is true, then no borate should be expected to be present in the cell wall.

(b) **Effect of borate on glucose-galactose interconversion.** In view of a higher concentration of galactose in cell walls of boron-treated plants as well as due to the possibility of the formation of a complex between borate and galactose, it was considered desirable to study the effect of borate on glucose-galactose interconversion. Borate was found to increase the rate of galactose synthesis most probably due to more stable complex formation between vicinal hydroxyls in galactose and ribose moieties of UDPGal and borate, thus shifting the equilibrium of the reaction
more towards galactose synthesis from UDPG.

Borate controls the incorporation of neutral sugar molecules into the cell wall polysaccharides either by complexing with such sugars which have suitable vicinal hydroxyl patterns and making their transit through the membranes quicker or by accelerating the synthesis of such sugars by stable complex formation. The situation can be studied more exhaustively and definite conclusions reached when the biosynthesis pathways of other building blocks of cell wall (mannose, rhamnose and fucose, etc) and their exact sites of synthesis are revealed.

(c) Effect of boron on lignification.

U.V. absorption spectra of lignin extracts of shoot spicas (30 mm) from both plus-boron (0.5 ppm) and boron-deficient cluster bean plants were recorded at pH 7 and pH 12.3 and their difference spectra obtained. The difference between the neutral values was exhibited only in the sharper and larger maximum at 280 nm. The difference spectra, in which the non-ionizable chromophores are eliminated, were much more descriptive. Peaks or shoulders were shown by both the curves at 250, 300 and about 350 nm. Extracts from plus-boron shoots were characterized especially by the large minimum in the region of about 350 nm. Non-conjugated phenols give the 250 and 300 peaks, while phenols with large conjugated
side chains such as the hydroxy-cinnamic acid derivatives account for peaks at wavelengths greater than 300 nm. On the basis of these spectra, lignin extracts from plus-boron plants differed from those of minus-boron plants not only in maintaining a greater amount of simple phenolic groups, but also by the amount of conjugated side chains as indicated by the major increase in the 350 nm peak.

In order to confirm whether alteration in neutral sugar composition of cell wall polysaccharides due to boron-deficiency was in any way effective in altering lignin synthesis directly, or indirectly through peroxidase, the formation of lignin-like polymers produced by ferulic acid on incubation with isolated cell wall fractions from both plus-boron and minus-boron cluster bean shoot tips was also studied. From the curves obtained by plotting values of difference spectra, it was clear that plus-boron cell wall produced more lignin-like polymers as compared to minus-boron cell wall. The reaction with ferulic acid did not occur in the presence of boiled tissues showing that the reaction was enzyme catalyzed. Lignin formation was concluded to be dependent on H$_2$O$_2$ and borate concentration by another in vitro study. These experiments suggested that borate probably controlled lignin biosynthesis through the agency of peroxidase.

(D) Effect of borate on the activity and binding of certain cell wall enzymes. Peroxidase, ascorbic acid
oxidase, pectin esterase, invertase, adenosine triphosphatase, acid phosphatase, \( \beta \)-glycerophosphatase and inorganic pyrophosphatase are some of the enzymes which have been demonstrated to be associated with cell wall by a number of authors. The roles of these enzymes in the cell wall are unknown but there could be several. For example, they might facilitate uptake of metabolites, or perhaps they function in the synthesis of wall components. Due to the capacity of borate ion to bind these enzymes ionically and covalently with the cell wall, it was considered desirable to study the effect of borate on the activity and binding of the above enzymes in the cell wall. This seems to be an interesting field of research which should be actively pursued. The results obtained from the preliminary studies are summarized below:

(1) Peroxidase. Increase in the activity of peroxidase in the cell wall seemed to be due to excessive binding of the enzyme to the cell wall in the presence of borate. Any influence of the microelement on enzyme biosynthesis should have been reflected by increased activity in supernatant due to in vivo application and on enzyme activity itself by in vitro application.

The bonding between the enzyme (sugar moiety) and borate was proved by an experiment demonstrating that borate was not permitted to dialyze out in the presence of
peroxidase to the same extent as in its absence. The possibility of a complex formation between borate and hæm prosthetic group of the enzyme was ruled out by finding no change in the absorption spectra of the enzyme solution in the visible range after adding various concentrations of boric acid. The binding between borate and sugar moiety (probably arabinose and mannose) of the enzyme was further confirmed by studying the mobility of the enzyme on paper impregnated and unimpregnated with boric acid. The mobility was reduced in the presence of borate, probably due to sugar-borate complex formation.

It thus seems that borate might exert its effect on lignification by modifying the amount of peroxidase attached to the cell wall where lignification occurs.

(ii) Ascorbic acid oxidase. The purpose of this work was to find out if there was any correlation between the high concentration of boron and ascorbic acid oxidase in growing tissues, particularly in their cell walls. Ascorbic acid oxidase was inhibited in the cytoplasmic fractions of root and shoot tips by in vivo application of boric acid whereas in the cell walls the activity increased. It showed that borate probably helped in binding the enzyme from the cytoplasm to the cell wall, at least in actively dividing cells. On the other hand, an in vitro application of borate inhibited the enzyme in the cytoplasmic fraction
from root and shoot tips but it had no effect on the cell wall enzyme, showing that borate was capable of binding the enzyme to cell wall when applied in vivo and not in vitro. Borate had no effect on the enzyme activity in leaf cell wall. Thus borate seems to help the cell walls of actively dividing cells to maintain a higher level of ascorbic acid oxidase activity, which is probably needed for cell wall growth.

(iii) Pectin esterase. Boron had no effect on the activity of this enzyme in the cell walls of non-meristematic tissues. However, in actively growing root and shoot tips cell walls, it had an inhibitory effect. This inhibition would decrease the rigidity of cell wall and hence promote cell elongation. The pectin esterase binding capacity of cell wall was also studied both in the presence and absence of borate. Borate was found to help in the binding of pectin esterase to cell wall. The binding was found to be ionic.

(iv) Invertase. Borate was found to help the binding of invertase to the cell walls of only meristematic tissues. The enzyme did not seem to be bound by borate ionically.

(v) Adenosine triphosphatase. Boron-deprived plant root and shoot tip cell walls and supernatants had more ATPase activity, about 1.5-2 times higher, than in the control plants. The application of borate, both in vivo
and in vitro, had a similar effect on ATPase activity. The results explain the marked decrease in ATP associated with boron deficiency as a consequence of ATPase activation. Another experiment performed to understand the mechanism of inhibition of ATPase by borate suggested that the inhibition was caused by complex formation between ATP and boric acid.

(vi) Acid phosphatase. When supplied in vivo, borate was found to have an activating effect on acid phosphatase in the cell wall as well as cytoplasmic fraction. In vitro addition of boron did not activate the enzyme in any fraction. The increase in the activity of the enzyme seemed to be due to its increased synthesis in the presence of borate. The activating effect seemed to be much more in meristematic tissues in comparison to non-meristematic ones. Borate was found to make an ionic binding of the enzyme to the cell wall.

(vii) Inorganic pyrophosphatase. Boron was found to have no effect on pyrophosphatase activity both in the cytoplasmic and the cell wall fractions.

(viii) β-Glycerophosphatase. Boron activated β-glycerophosphatase both in root and shoot tips, only when applied in vivo. From the data, it is suggested that like invertase, β-glucosidase, amylase and urease, which are enzymes of diagnostic value in agricultural biochemistry, the assay of β-glycerophosphatase might conveniently be used
to test the status of the available boron in a soil. Boron deficiency might result in the fall of activity of this enzyme in the root and shoot to the tune of about 50 per cent. Boron-deficient cell walls contained lesser amount of ionically bound enzyme in comparison to plus-boron cell wall samples, which clearly demonstrates that borate helps in ionic binding of \( \beta \)-glycerophosphatase, and that whatever enzyme was present in boron-deficient walls, it was probably due to covalently bound enzyme. Experiments to understand the mechanism of this activation are in progress.

5. Significance of Boron in the Metabolic Processes involved in Seed Germination.

Because radicle growth is retarded by boron deficiency at a very early stage, the major metabolic alterations brought about by this deficiency in a germinating seedling were worth studying.

(a) Role of boron in carbohydrate metabolism.

(1) Effect of boron on sugar translocation. The sugar contents of plus- and minus-boron radicle and plumule tips of pea (20 mm) were estimated. The results of the quantitative analysis in the root and shoot tips did not give any evidence that these tips were deficient in glucose and fructose due to boron deficiency. These results are contrary to Gauch and Dugger's hypothesis that the cessation
of growth in boron-deficient plants may be attributed to a carbohydrate deficiency in shoot tips and root tips. The translocation of sucrose and degradation of oligosaccharides of raffinose series in the seed were favoured by borate. Formation of more galactose from these oligosaccharides in the presence of borate will help pectin biosynthesis, which is important for primary wall formation for newly forming cells in the meristematic regions.

Another experiment was conducted to demonstrate any direct response to sucrose or galactose by excised root tips in the presence and absence of borate. Boron was found to be very important for the growth of excised root tips and the formation of lateral roots. The most interesting point is that when sufficient sucrose was present and boron was completely absent, root growth was nearly half than that in the presence of both. It was thus clear that the retarded growth of root tips was not due to lack of sucrose but due to lack of boron. When galactose was present, the growth was promoted significantly even in the absence of borate. Thus boron seemed to help the meristematic tissues to maintain higher levels of actively utilizable galactose for cell wall and membrane synthesis.

Gausch and Dugger’s hypothesis was also refuted by a third experiment in which no increase in starch content of pea and wheat seeds was obtained, even when the plants were supplied with optimum levels of boron as compared to
controls. However, boron application increased the protein content of these grains by about 7% and 12.5% in case of pea and wheat respectively.

(ii) Effect of borate on amylase activity.
Both in vivo and in vitro application of boron to germinating pea seedlings increased their amylolytic activity. Borate might be helping the germinating seed to provide more glucose due to this action on amylases.

(iii) Effect of borate on pentose-phosphate pathway.
Borate inhibits the rate of reduction of NADP by glucose-6-phosphate dehydrogenase. IMP-shunt is thus inhibited by borate even at the first step. Under boron deficiency, additional erythrose-4-phosphate is expected to be produced which, in turn, would increase synthesis of phenols. The browning of root tips at an earlier stage of boron deficiency might be thus explained.

(b) Role of boron in protein metabolism.
The study of the distribution of nitrogenous compounds during the germination of pea seeds in the presence and absence of borate showed that boron deficiency resulted in an accumulation of amino acids and decreased protein synthesis in root plus shoot tissues. The observations in cotyledons were different. Borate seemed to retard protein degradation in the cotyledons resulting in a fall of \( \alpha \)-amino nitrogen levels. It was concluded
from this observation that borate had an inhibitory action on protein splitting enzymes in the cotyledons.

In order to demonstrate whether protein synthesis was hindered due to boron deficiency through some structural or functional alteration of ribosomes or not, another experiment was done, in which protein synthesis in isolated systems was studied by using ribosomal preparations both from plus-boron and minus-boron plant tissues. It was concluded from this experiment that borate had no direct role in protein synthesis, either through ribosomal structure or otherwise. For alterations in the amounts of protein formed in boron-deficient plants, some alternative explanation should be sought.

In view of an increase in $\alpha$-amino nitrogen due to boron deficiency, it was further interesting to find whether all the amino acids increased under this condition or not. Individual amino acids were estimated in pea root tips both in the presence and absence of boron. Except aspartic acid, glutamic acid, threonine and proline, all other amino acids were found to accumulate under boron deficiency. A high concentration of the above four amino acids in the presence of borate indicated that these amino acids played an important role in protein metabolism, in particular as acceptors of ammonia for the synthesis of
amides of the plant amino acids.

In view of higher concentrations of glutamic acid and aspartic acid in the presence of borate, it was of interest to study the influence of borate on glutamate-oxaloacetate transaminase (SGOT) and glutamate-pyruvate transaminase (SGPT) which occupy a key position in amino acid metabolism of germinating seedlings. The activities of these enzymes were found to be much more in the presence of borate than in its absence both in pea and wheat seedlings. The increased activities might be due to inductive effect of higher levels of the acidic amino acids.

(c) Role of boron in nucleic acid metabolism.

A retarded protein biosynthesis due to boron deficiency might also be due to either retarded RNA synthesis or excessive RNA degradation or excessive nucleotide degradation, which will indirectly affect RNA synthesis. To elucidate these points, a number of experiments were conducted, the results of which are summarized below.

A study of the soluble nucleotide contents of embryo- axis and cotyledons of pea showed that boron-treated seedlings contained higher levels of soluble nucleotides in the axis and lower levels in the cotyledons. Boron was also found to increase RNA levels in the axis by
about 40 per cent after 2 days and 24 per cent after 12 days, but it was not found to have any effect on either DNA levels in the axis or in the cotyledons. In order to confirm increased levels of RNA under the influence of boron, some cytochemical studies were also conducted. Various parts of wheat plant such as root tips, stem tips, and spikelets from both plus-boron and boron-deficient plants were stained for RNA and DNA. Increased levels of RNA were observed in all sections stained from plus-boron plants in comparison to those from boron-deficient plants. It is clear from the microphotographs that boron promotes the formation of pollen grains and development of ovule.

The increase in the contents of RNA and soluble nucleotides clearly demonstrated that boron either promoted their synthesis, or inhibited their degradative enzymes. Further, experiments were conducted to elucidate the mode of action of borate in free nucleotide and RNA metabolism. The synthesis of new messenger RNA must presumably precede that of new proteins, so the earliest time at which a lack of boron affects the tissue could perhaps be delineated by finding an alteration in the RNA fractions. The first experiment to understand it involved the determination of various fractions of RNA (by column chromatography) from plus- and minus-boron pea plants. There was almost no difference in the concentrations of other RNA types, except in case of mRNA, between plus-boron and minus-boron
tissues. These observations clearly demonstrate that borate promotes the maintenance of higher levels of mRNA.

It was further interesting to study whether boron actually promoted nucleotide synthesis and hence RNA formation. Minus-boron plants were supplemented with nucleic acid bases alone and in combination with boron. The addition of boron along with various bases separately did not affect root elongation and RNA contents of pea seedlings in comparison to when boron was added alone. Replacement of boron by various bases also did not affect these parameters. If boron was helping in the biosynthesis of nucleotides needed for RNA synthesis, then the replacement of boron by bases in the culture solution should have affected the above two parameters. Thus an increase in root length and RNA concentration in root tips due to boron application must be due to some other reasons.

It is however interesting to note that although boron did not seem to help the biosynthesis of RNA precursors, yet either alone or in combination with a base, it increased the free nucleotide content of root tips. An increase in free nucleotide content due to boron application, even when more RNA was being synthesized was only possible if nucleotide degradation was inhibited by borate. In view of these considerations, it was decided to study the influence of boron deficiency on breakdown of acid soluble nucleotides.
in supernatant fraction of pea root tips, primary leaves and cotyledons. It was concluded that boron-deficient tissues had a greater capacity to liberate inorganic phosphorus from various nucleoside triphosphates as well as AMP. The decreased levels of ATP, UTP, CTP and GTP in boron deficiency were expected to disturb all biosynthetic reactions dependent on these nucleoside triphosphates. Further work to elucidate the mechanism of action of boron in retarding the activity of various enzymes involved in nucleotide breakdown is in progress.

Another possibility of an increase in RNA levels in the presence of borate is that ribonuclease remains inhibited. This is also possible because during boron deficiency, a higher concentration of decomplexed freely exposed RNA might lead to an increase in the activity of ribonuclease as an adaptation of plant cells resulting in excessive RNA degradation and abnormal protein synthesis. The possibility of such an action of borate was tested. First of all the effect of boron on the activity of ribonuclease in various parts of the pea seedling was studied. It was inferred that borate inhibited ribonuclease activity. The inhibition due to borate decreased in non-meristematic parts of root sections in comparison to that of meristematic sections. The enzyme activity started increasing due to boron deficiency just after a lapse of 6 hours of boron deprivation.
In order to probe into the mechanism of boron participation in inhibiting ribonuclease activity, some in vitro studies were conducted. Hydrolysis of RNA samples by ribonuclease before and after preincubation with boric acid was studied. Maximum amount of RNA was split when there was no addition of borate. Incubation of borate with RNA beforehand was as effective as its addition to the incubation mixture. Incubation of borate with ribonuclease and subsequent dialysis to leach out borate had no effect on the enzyme activity. This clearly showed that there was some interaction between borate and RNA, which resulted in the formation of a borate RNA complex. This complex seemed to be unable to act as a substrate for ribonuclease. The formation of a complex between borate and RNA was studied, first by determining any changes in V.V. spectra and then by studying the changes in pH values after mixing RNA and boric acid solutions at the same pH values. It was concluded from these studies that borate probably reacted with 2'-hydroxyl groups of ribose residues in RNA. Thus borate seems to inhibit ribonuclease by reacting with its substrate.

Another experiment was done to study whether the effects of ribonuclease and boron deficiency were similar on cell division and cell expansion in meristematic tissues. The analysis of the effects of ribonuclease in the presence and absence of borate on growth of excised pea roots has provided data giving an indication of a general influence of
borate on RNA through the agency of ribonuclease, and hence on cell division and cell expansion. The growth inhibiting effect of ribonuclease both in whole roots and segments was nullified by the presence of borate, showing thereby that the action of ribonuclease on RNA degradation was suppressed by borate. The control of borate on meristematic activity of plant tissues seemed to be through its suppressing action on ribonuclease. The suppression of ribonuclease activity by borate at the growing tips seems to be one of the primary roles of borate in controlling cell growth.

A general picture drawn from this work regarding the biochemical role of boron in plant metabolism is that the element can modify a number of biochemical processes due to its inherent property of complexing with sugars having vicinal hydroxyl groups. It participates in cell wall biogenesis by enabling the actively dividing cells to draw requisite proportions of various sugars having such hydroxyl patterns. Moreover, the complexed borate in the cell wall binds some of the cell wall enzymes covalently or ionically. The element does not seem to directly participate in mitochondrial activities and protein biosynthesis. However, it inhibits nucleotide, nucleoside triphosphate and polynucleotide degradation, which indirectly favours protein biosynthesis. The essentiality of boron to plants majorly seems to be due to its control over cell wall biogenesis and inhibition of some nucleotide and nucleic acid degrading enzymes.