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EFFECTS OF THE ABSENCE OF BORON AND OF SOME OTHER ESSENTIAL ELEMENTS ON THE CELL AND TISSUE STRUCTURE OF THE ROOT TIPS OF *PISUM SATIVUM*

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(WITH FIVE PLATES)

Introduction

While the rôle of the various elements in the growth of the plant has attracted the attention of a large group of workers since the beginning of modern botany, the real function of certain of these elements still remains one of the least understood phases of plant physiology.

Relatively early in the history of the investigation of the growth of plants in culture solution, certain elements were found to be "essential" while others appeared to be non-essential for the normal development of the organism. Obviously, however, it is far easier to demonstrate that an element which is required in relatively large quantities is essential than to prove that elements are not necessary at all. In consequence of the difficulty of establishing the need of plants for certain elements which are required in very small quantities, the list of the "essential" elements was very short, while by implication the list of "non-essential" elements included all the rest.

At the present time the more refined purification of the so-called "chemically pure" salts, and more carefully controlled methods of physiological research, permit a study of the effects of the absence from culture solutions of certain elements with greater exactness than before, with the result that elements once considered unnecessary are being shown to be indispensable for the normal development and functioning of the plant.

The present study forms a part of the work on the essential nature of boron and certain other elements for higher green plants which one of the

writers (**7, 8, 9**) has been carrying on for the past three years. It represents, however, a cooperative extension of this work into a relatively new field.

The great bulk of the research on the influence of the different essential elements on the development of the plant has employed, as a sole criterion, the mass of tissue produced by plants grown in solutions of varying composition and concentration. This represents only a limited front of attack on the general problem. An ultimate goal should be the determination of the actual rôle of each of the elements in the metabolism of the plant, and the consequent influence of these elements on the morphology of the cell and of the tissues.

While a study of the structure of the cell and the tissues can not furnish final criteria of the rôle of the various elements in the metabolism of the plant, it may represent the first practicable steps toward this end. The work upon which this paper was based was undertaken with this end in view.

Boron seems an unusually favorable element to be employed in a first study of this kind. It was early noted (**8**) that many dicotyledonous plants when grown in solutions without boron developed short thick roots with enlarged apices. It seemed, therefore, that a study of the pathological changes which take place in these cells and tissues might give some clue as to the function of boron in plant growth.

Both the chemical and cytological phases of such a problem demand refined, delicate and specialized technique. The interpretation of the results requires a knowledge of two fields of literature. At the suggestion of Dr. J. Arthur Harris, whose interest in and encouragement of this work we have greatly appreciated, we therefore undertook a cooperative investigation of the problem.

Pisum sativum (variety Golden Vine) was chosen as the experimental plant because it was found to show the effects of the absence of boron within a few days and also to produce root tips of a very suitable size for cytological work.

In first experiments plants were grown in a satisfactory culture solution to which boron was or was not added. This solution was the same as that described later as the control solution.

As work on the problem progressed it became evident that a study of the effect of the absence from the culture solutions of some other of the essential elements would be extremely desirable, since such experiments might throw some light on the problem involved. Therefore in a series of cultures most of the elements essential for plant growth were excluded one at a time from the solutions, and the effects of the absence of the various elements in the absence and presence of boron were studied.

Methods

Culture methods

All plants were grown in solutions of highly purified salts. Quart Mason jars were usually employed as containers. In a few instances pyrex breakers were used. The exceptional cases will be discussed later.

This investigation included cultures with and without each of the following elements: boron, magnesium, sulphur, manganese, potassium, nitrogen, iron, phosphorus, and calcium. Each of the last eight groups included cultures with and without boron.

Although zinc was shown by Mazé (**3**) to be essential for maize and by one of us (**7, 9**) to be essential for several other plants, it was not included in this investigation which deals primarily with the histology and cytology of the root, because of the fact that, in the absence of zinc, the roots appear normal even after the tops are apparently dead. The results caused by the absence of calcium were so striking and so different from anything published so far that they are being reserved for another paper which is ready for publication.

The cultures without potassium were divided into two subgroups, one in which potassium was replaced by sodium and one in which no sodium was added. Pyrex beakers were used as containers in experiments omitting sodium to preclude the possibility of the introduction of this element by solution from soft glass. The purpose of this division was to determine whether any effect of the replacement of potassium by sodium could be observed. In other experiments without potassium (where the effect of sodium was not being studied), Mason jars were employed.

Solutions used were:

Control solution

	Per liter		Gm. per liter
KNO_3	0.80 gm.	Mn (as $MnSO_4$)	0.0015
KH_2PO_4	0.15 gm.	Al (as $Al_2(So_4)_3$)	0.0005
$MgSO_4 \cdot 7H_2O$	0.50 gm.	Cu (as $CuSo_4$)	0.000125
$CaSO_4$ saturated solution	300 cc.	I (as KI)	0.00025
B (as H_3BO_3)	0.0005 gm.	F (as KF)	0.00025
		NaCl	0.0127

Iron (as $FeSO_4$) was added at intervals as required by the plants. Manganese, iron, and boron were omitted from the solution given above in studies concerning the absence of these elements.

Solution without magnesium

	Per liter	
KNO_3	1.20 gm.	B, Mn, Al, Cu, I, F and NaCl as in control solution.
KH_2PO_4	0.15 gm.	
$CaSO_4$ saturated solution	300 cc.	

SOLUTION WITHOUT SULPHUR

	Per liter	
KNO_3	1.00 gm.	Traces of the various elements were added as chlorides or nitrates instead of sulphates. Fe as citrate.
$Ca(NO_3)_2$	0.60 gm.	
$MgHPO_4$	0.20 gm.	

SOLUTIONS WITHOUT POTASSIUM

I

	Per liter	
$NaNO_3$	1.00 gm.	Traces of various elements as in control excepting that I was added as NaI.
$CaSO_4$ saturated solution	300 cc.	
$MgHPO_4$	0.30 gm.	

II

	Per liter	
$Ca(NO_3)_2 \cdot 4H_2O$	1.2 gm.	Traces as above.
$CaSO_4$ saturated solution	300 cc.	
$MgHPO_4$	0.30 gm.	

SOLUTION WITHOUT NITROGEN

	Per liter	
KH_2PO_4	0.15 gm.	Traces of various elements as in control solution.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	
$CaSO_4$ saturated solution	300 cc.	
K_2SO_4	0.80 gm.	

SOLUTION WITHOUT PHOSPHORUS

	Per liter	
KNO_3	0.80 gm.	Traces of various elements as in control solution.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	
$CaSO_4$ saturated solution	300 cc.	

PURIFICATION OF SALTS

KNO_3, $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$, Na_2SO_4, $MnSO_4$ and H_3BO_3 were repurified by repeated recrystallization from pure distilled water. $CaSO_4$ was prepared by dissolving chemically pure $Ca(NO_3)_2$, filtering and precipitating the $CaSO_4$ with chemically pure H_2SO_4. The $CaSO_4$ was then washed free from acid. $FeSO_4$ was prepared by dissolving iron wire (as used for standardization) in chemically pure H_2SO_4, filtering and crystallizing. $MgHPO_4$ was prepared from purified K_2HPO_4 by precipitating with purified $MgSO_4$. The precipitate was then washed many times with pure distilled water. With the exception of $MnSO_4$ and H_3BO_3 none of the substances used only in very small amounts was repurified. NaCl was used in relatively larger quantities but P. W. R. (Powers-Weightman-

Rosengarten) analyzed salts appeared to be pure enough, so this salt was not purified further. $NaNO_3$ was prepared by evaporating pure NaCl with redistilled HNO_3 until all the chlorine was driven off. The $NaNO_3$ was then crystallized. In the investigations without sulphur it was very difficult to exclude boron when this was desired. $FeSO_4$ seemed to be the only soluble salt easily freed from boron, and the lack of boron was shown in sulphur-free solutions only when a minimum amount of iron as the citrate was added.

No test of the purity of the salts was made other than that shown by the ability or failure of plants to develop in solutions made with them but without the addition of boron. This procedure was followed because we have found that many plants respond to smaller quantities of boron than can be determined chemically and because, according to AUER-WELSBACH (**1**), small amounts of this element are very difficult to detect spectroscopically in the presence of relatively large amounts of other elements.

HISTOLOGICAL AND CYTOLOGICAL METHODS

In obtaining the material for the present investigation, two important precautions were observed. First, environmental factors which may influence the structure and general appearance of the cell components were made as nearly alike as possible and, second, an effort was made to take the tissues for fixation under the most normal conditions during periods of most active nuclear division of the meristematic cells. The material was, therefore, always taken on bright days, in the morning between eight and twelve o'clock. In all cases, with the exception of those mentioned separately, the roots were killed after the plants had been in corresponding culture solutions for from two to three weeks. The majority of the experimental series were repeated during different times of the year.

As a killing reagent, NAWASCHIN'S modification of FLEMMING'S killing solution was used throughout the investigation. As previous experience of one of the writers indicates, this fixing agent is very reliable and gives splendid results with tissues and cell components. Subsequent treatment was that usually required by and given to cytological preparations. Longitudinal sections of the roots were cut 12 μ and 15 μ thick, and were stained with safranin and aniline blue or with HEIDENHAIN or DELAFIELD haematoxylin.

The photomicrographs were taken with a Zeiss achromatic objective [8 mm. ap. 0.20 (A)] and a projection ocular 2. A combination of Wratten B and E filters was employed. All photomicrographs were taken with exactly the same magnification (about 45 diameters).

Results of histological and cytological studies

Plants grown in culture solutions with and without boron are shown in fig. 5, plate VIII. The three plants to the left were grown with boron for about three weeks; those to the right were grown without boron for the same length of time.

PLANTS GROWN IN STANDARD CULTURE SOLUTIONS WITH AND WITHOUT BORON

WITH BORON, FIG. 1, PLATE VII.—The apical portion of roots of *P. sativum* grown in culture solutions in the presence of boron show the typical differentiation of the primary meristem into three distinct regions, plerome, periblem and dermatogen. In the region of contact with the promeristem, the root cap is very well developed and is usually about 20 cells in width. The plerome is about 12 to 14 cells in diameter, the cells being 11 μ x 18 μ, and the nuclei about 10 μ x 10 μ. The nuclei occupy a central position in the cell and each contains a conspicuous nucleolus. Mitotic figures are very numerous in both the plerome and the pleriblem. The dermatogen is usually not very much differentiated from the periblem. The diameter of the root tip in the middle of the region of the primary meristem is approximately 28 to 32 cells. Starting with the beginning of the promeristem, the region of intensive cell division extends for a distance of about 20 to 25 cells to the beginning of the region of elongation. The region of maturation usually does not begin at a distance of less than from 60 to 70 cells from the tip.

WITHOUT BORON, FIGS. 2–4, PLATE VII.—The apical portions of roots grown in culture solutions containing the elements usually considered essential, but without boron, show remarkable changes from those described above. The region which corresponds to the primary meristem of the normal root can hardly be called meristem. Progressive changes (in the sense in which this term is used by students of pathological plant anatomy) take place, which entirely modify the appearance as well as the structure of this region. Since the term meristem cannot be properly applied to the region under discussion, it would be logical to avoid the terms plerome and periblem in indicating that portion of the root in which pathological changes take place. But because the terminology of pathological plant anatomy lacks uniformity in general and is entirely inadequate for this special case, we will refer to the regions found in the apical part of roots grown without boron as plerome, periblem, and dermatogen, always keeping in mind the fact that these names are used here, not for regions of primary meristem, but for those homologous with the primary meristem in the normal root.

In the absence of boron the root cap is considerably deformed or even entirely lacking. In the region of the plerome and periblem progressive

changes take place which result in an enormous enlargement of the whole apical portion of the root. Distinct hyperplasia is developed in the plerome, while in the periblem hypertrophy usually obtains. In the central portion of the apical part of the root the plerome region usually extends from about 30 to about 32 cells in diameter, which is about twice as many cells as are present in the corresponding region of roots grown with boron. In the early history of the cultures the cells divide by mitosis but soon lose their ability to divide further by typical mitosis. In the absence of boron the regularity in the arrangement of the cells is lost. The mechanism regulating the divisions of the cells is apparently disturbed, with the result that the cells appear in irregular uncoordinated rows or even without the formation of definite rows. The cells are extremely irregular in shape and size. They range from 10 μ x 15 μ to 11 μ x 18 μ, and the nuclei are about 6 μ x 7 μ.

The rows of cells in the periblem regions are more regular than in the plerome and correspond in number to those of the normal root. The cells, however, are much larger than normal, being 50 μ x 50 μ with nuclei 7.5 μ x 8 μ. It is interesting to note that although these cells are much larger than the cells of the normal root, their nuclei are smaller. Returning to the plerome we note that besides the lack of regularity of arrangement and form of the cells, another interesting abnormality appears. The cells of this region undergo premature development which is manifest in the appearance of isolated elements of xylem as far down as the level of the twentieth cell from the apex. These first occur at irregular intervals and show unequally thickened walls which do not take the stain typical for lignified cell walls. The elements occurring slightly farther up stain red with safranin but are extremely irregular in appearance. Incompletely scalariform or scalariform-reticulate xylem elements usually predominate. Besides the abnormal occurrence of these xylem units, the premature development of the tissues is further manifest in the formation of isolated meristematic regions of branch root primordia in different parts of the apical portion of the root. These regions are sometimes formed at a distance of no more than 10 to 13 cells from the apex. However, they very rarely continue their development for any length of time, but are apparently suppressed shortly after they have been formed. This is illustrated in fig. 4, plate VII. The photograph presents a tangential section in which a series of new meristematic regions is shown, most of which were suppressed soon after they were formed. Only the fifth of these incipient lateral roots developed far enough to grow through the periblem. A careful examination of the material shows that typical mitoses are usually absent. In these newly formed meristematic regions, slightly abnormal mitoses are observed.

Another important characteristic of the tissue found in the plerome region is the formation of thick strands which apparently represent the collapsed and thickened walls of cells. These are shown clearly in fig. 3, plate VII. Farther away from the apical portion, a region occurs in which the lignified elements become more prominent and are even connected for more or less regular distances. These, however, may be interrupted by portions in which the connection between the individual elements is lacking. Pitted vessels begin to be more prominent in this region.

Recovery from injury due to the lack of boron

Root tips in the process of recovery from injury due to the lack of boron present a very interesting picture. If boron be added to solutions which have produced plants showing typical symptoms of the lack of boron, but whose tissues are not too badly disintegrated, evidences of recovery appear very quickly.

In a series of experiments boron was added to solutions in which the plants showed an extreme degree of injury due to the lack of this element, and root tips of these cultures were fixed after 6, 24, and 48 hours. A definite zone of truly meristematic cells seems to appear as early as 6 hours after the addition of boron. A newly formed meristematic region is apparent in the tissues fixed at the end of 24 hours. After 48 hours a new long slender growth may be seen at the end of the enlarged apex. This newly formed root apex sometimes grows more than a centimeter in 48 hours, and acquires the diameter and general appearance of the normal root.

Plants grown in solutions without magnesium

With boron, fig. 6, plate VIII.—The root apices of plants grown in solutions lacking only magnesium do not show any noticeable deviation from the normal. Mitotic figures are numerous and regular.

Without boron, fig. 7, plate VIII.—Rather distinct peculiarities were observed when both boron and magnesium were absent which were not seen when boron alone was lacking. We cannot say that these were real differences, and not variations of the effects of the absence of boron alone, since sections of only three root tips were available for study. It is, however, desirable to describe these differences which are as follows: the plerome and periblem regions are less definitely marked off in the most apical portion of the root than those of cultures grown without boron alone. Hypertrophy sometimes occurs in the plerome as well as in the periblem. There are also some other deviations from the typical conditions for the absence of boron. The isolated regions of branch root primordia are not so prominent. The cells of the periblem region appear less hypertrophied than

those of the corresponding region of roots of plants grown without boron only, their size being 25 μ x 25 μ instead of 50 μ x 50 μ. The size of the nuclei is, however, the same in both cases.

Plants grown in solutions without sulphur

With boron, fig. 8, plate VIII.—The roots appear more or less normally developed with the exception of the occurrence of rather large intercellular spaces in the periblem region, where whole rows may be missing. A similar phenomenon sometimes occurs normally in certain species of plants but we have not observed it in roots of *P. sativum*. Sometimes single cells are lacking, making the rows discontinuous. This apparently does not occur in the plerome region where the cells appear very normal, and where mitotic figures occur. The size of the cells in the plerome corresponds to those for plants grown in the control solutions.

Without boron.—In our first experiments, where an attempt was made to exclude both boron and sulphur we failed to produce the typical effects due to the lack of boron. Contamination of the solution by boron was suspected, since $FeSO_4$ is the only soluble iron salt easily purified and this could not be used because it would add sulphur which we also wished to exclude.

In earlier experiments in which there was probably some contamination by boron, the roots did not differ much from those grown in solutions to which boron had been added, but the intercellular spaces observed in the periblem region when only sulphur was lacking were even more conspicuous. This is well shown in a tangential section of a root, fig. 9, plate VIII. In a later experiment, in which only a very limited supply of iron (as ferric tartrate) was used, and the contamination by boron thus greatly reduced, an approach to the typical effects due to the absence of boron is easily observed.

Plants grown in solutions without manganese

With boron, fig. 11, plate IX.—The size of the root and the regions of meristem is normal. Mitotic figures are numerous and normal. In the region of elongation the cell walls are, however, abnormal, being irregularly thickened in certain parts and loosely coherent in others. A considerable thickening of the transverse cell wall is particularly noticeable.

Without boron, fig. 10, plate IX.—In contrast with other cultures grown without boron, roots grown without this element and without manganese have well developed root caps in all cases which were studied. In the meristematic region of the primary root, the nuclei divide by mitosis, but some irregularities in the process of division are observed. The metaphases appear very much deformed. An orientation of the spindle nor-

mally parallel to the longitudinal axis is seldom observed. Figures in which anaphases and telophases can be recognized are almost entirely lacking in the root apices, but reduced anaphases and telophases are present in the secondary roots. These stages seem, however, to occur only during the early period of the development of the laterals. There is some evidence that, after the plants have been growing for a considerable length of time in the absence of manganese and boron, the nuclei of the cells of the principal root apex begin to divide by pseudoamitosis, or fail to divide at all. This may, however, be a general phenomenon common to the absence of several of the essential elements.

The pathological changes of the tissues consisted in considerably developed hyperplasia of the cells of the plerome, and hypertrophy of the cells of the periblem. The premature development of the cells is very pronounced, the isolated and incompletely developed xylem elements appearing early. The formation of secondary roots occurs very close to the meristematic region. The cell walls appear to be extremely thick in certain cells, which is clearly shown in the periblem of the upper part of the photomicrograph, fig. 10, plate IX.

Plants grown in solutions without potassium

As mentioned in the paragraph describing the solutions used in this investigation, groups of the plants in the work with potassium were employed in order to determine whether there was any visible effect of the replacement of potassium by sodium. The cytological and histological pictures were the same in both cases, so what is said for one solution holds for the other also. Pyrex containers were used for these cultures to prevent the introduction of sodium from the glass. Since pyrex is a borosilicate, enough boron was supplied to the boron free solutions from the containers to prevent the symptoms of the lack of boron from appearing for some time. Within a month, however, plants growing in solutions in pyrex containers to which no boron had been added began to show the characteristics produced by the absence of boron. In experiments in which ordinary Mason jars were used, the effects of the absence of boron appeared in the early stages of growth.

With boron, fig. 12, plate IX.—The roots exhibited normal differentiation of the meristematic tissue into plerome, periblem, and dermatogen. The root cap was very well developed and typical mitoses occurred frequently in the meristem. It differed from the control in that there was a somewhat loose arrangement of the cells in certain parts of the roots and the occasional dropping out of entire rows. These results differ from those obtained by Reed (5) for *Spirogyra* which was cultured in the absence of potassium and in which mitotic divisions were not observed. His plants

were kept in potassium free solutions for thirty-five days, while ours were without potassium other than what was stored in the seed for less than three weeks. Whether mitotic division in his *Spirogyra* plants ceased because other processes had been too adversely affected, whereas ours had not yet reached this stage, or whether these very different types of plants respond differently to the absence of potassium is a matter for further investigation.

The rôle of potassium in the metabolism of the cell has been studied by MACALLUM (**2**), and WEEVERS (**11**). See MOLISCH (**4**), pp. 63–64. These authors succeeded in demonstrating, by microtechnique, the presence of this element in the cytoplasm of cells of all plants investigated except in those of the *Cyanophyceae*. Their reaction for potassium in the nucleus was negative, and from this fact they concluded that potassium as a rule is absent from the nuclei. Their test, however, would not show potassium in organic combination, and although we do not know that such compounds exist in the plant, we still have to consider this possibility.

Laws of physical chemical equilibrium make it appear impossible that the potassium ion would be entirely absent from the nucleus when it is present in the surrounding cytoplasm, and furthermore, a colloidal substance with such elasticity as SEIFRIZ (**6**) has shown the nucleus of *Cryptobranchus* to have, would very probably prevent the precipitation of the potassium compound in particles large enough to be seen with an ordinary microscope.

In addition to the results in the papers mentioned above, WEEVERS (**11**) found that potassium was especially abundant in the cell vacuole and absent in the chromatophores. Potassium was conspicuous in the growing points and storage organs of the phanerogams. The reaction showed plainly in the sieve tubes, but poorly in the other vascular elements. A considerable amount of potassium was demonstrated in the cambium and medullary rays. MOLISCH (**4**), p. 64, comes to the general conclusion that, although it is difficult as yet to decide what the exact rôle of potassium in cell metabolism may be, there are many indications that this element plays a part in the building up of the plasma of the growing points, and that it is important in the development of turgor in the cells.

WITHOUT BORON, FIGS. 13, PLATE IX, and 14 and 15, PLATE X.—The typical pathological changes due to the lack of boron in the series of experiments from which both boron and potassium were excluded were not obtained at once in the first experiment. The difficulty may be attributed to the fact that in attempting to grow plants in pyrex containers sufficient boron may be introduced from the glass for normal development during the early stages of growth. Another difficulty was that the salts used in this experiment were not quite free from boron. Finally both difficulties were over-

come and a typical demonstration of the abnormalities due to the absence of boron was obtained.

A longitudinal section of the apical portion of a root is represented in fig. 13, plate IX. The meristematic region is abnormal but not entirely abortive. It is represented in the photomicrograph as a very dark area. The nuclei show division by mitosis, in which all of the phases were observed, but there is a deviation from normal mitosis in the somewhat irregular appearance of the chromosomes. Hypertrophy and hyperplasia of the periblem and plerome respectively were distinct, but not as extensive as in the cultures lacking boron only. The region showing hyperplasia was only 22 cells in diameter as compared with 30 to 32 cells in the corresponding region of root tips grown in the absence of boron only. The premature development of the root regions was not as distinct as in the cultures lacking boron alone. The lignified elements of the xylem, as represented in fig. 13, are at a region less than two thirds of the distance from the apex as compared with one sixth of the distance where boron alone was excluded. The abnormal formation of branch root primordia was not observed. Root apices from plants grown in solutions from which both boron and potassium were excluded appeared less deformed than those of plants in which only boron was absent. Here a slight contamination of the culture medium may explain the differences.

A longitudinal section of the apical portion of a root grown in a pyrex container for two weeks is shown in fig. 14, plate X. There is practically no difference between this root and those grown in the control solutions. A root from the same culture which was grown for a month and a half is represented in fig. 15, plate X. Although it is not a typical picture of a root grown in the complete absence of boron there is considerable resemblance. Pyrex glass evidently yielded sufficient boron for normal development in the early stages of growth but not enough to supply the plants for continued development.

Plants grown in solutions without nitrogen

With boron, figs. 16 and 17, plate X.—The typical characteristics of roots of pea plants grown in solutions without nitrogen but in the presence of all other essential elements is that they are comparatively long and of small diameter. Sections of the same root are shown in figs. 16 and 17. The section represented by fig. 16 is not in immediate continuation of that given as fig. 17, there having been omitted from between these two sections another part as long as that in fig. 17 and similar in structure to the upper part in fig. 17.

The root cap is very poorly developed. The meristematic region is present and the mitotic figures are distinct, but the cells appear disarranged,

especially in the periblem region, where large intercellular spaces may be observed. The general orientation of the rows is apparently very much affected by the absence of nitrogen. The region of mutation appears to be present at a normal distance from the root apex. The elements of the xylem appear to be connected in the regular manner. The secondary roots, when formed, are deflected downward to an unusual degree and destroy the cortex not only where they protrude but also in the region immediately surrounding this part. The cells of the plerome region are 10 μ x 20 μ; the nuclei are 10 μ x 10 μ. The nuclei are regular and spherical and appear normal. In the periblem region, the cells are about 30 μ x 15 μ, very irregular and in sections often without visible connection with the neighboring cells. The nuclei, however, are not diminished in size and are similar to the nuclei of the plerome region.

WITHOUT BORON, FIGS. 18 and 19, PLATE X.—The differentiation of the regions which are more or less distinct in case of plants grown without nitrogen and with boron, is less obvious in the roots of plants grown without boron. As a rule the progressive tissue changes which occur in these roots generally take the form of hyperplasia, which in this case occurs in the region corresponding to the plerome as well as the periblem. The cells are very small and their number correspondingly great. In certain parts of the root apices the diameter is more than 50 cells in width as compared with 28 to 32 cells in the normal root and 46 to 50 cells where boron only is absent.

The cells which undergo hyperplasia are very small and extremely irregular in size, ranging from 5 μ x 7 μ to 10 μ x 10 μ. Many of the cells could not be measured because their outlines were so indefinite. The nuclei are very small in the region of hyperplasia, being about 4 μ x 5 μ. In the parts where a slight differentiation into regions of primary meristem have taken place, the cells of the periblem are 15 μ x 22 μ and the nuclei are 6.25 μ x 6.25 μ.

In contrast to the roots of plants grown without nitrogen but with boron, the cells of the periblem region, when such a region becomes differentiated, occur in more or less regular rows. The tissue of the plerome, however, shows extreme irregularity in the arrangement of the cells. The typical rows could be distinguished very rarely and the cells varied greatly in size and shape. In a few cases, however, as represented in fig. 18, plate X, the hyperplasia was not developed to so great an extent, and hypertrophy was more pronounced.

PLANTS GROWN IN SOLUTIONS WITHOUT IRON

WITH BORON, FIG. 21, PLATE XI.—The root tip did not differ from the control; mitotic figures were numerous and normal. The size of the cells and of the nuclei was the same as that of the controls.

WITHOUT BORON, FIG. 20, PLATE XI.—Root tips of plants grown in solutions without iron and without boron showed the same characteristics as those of plants grown in solutions from which boron only was absent. No mitoses were observed in the apical part of the root, but well developed mitoses were present in the meristematic region of the secondary roots, as shown in fig. 20, plate XI. A few well developed metaphases and other stages of nuclear division are present even in the central cylinder of the primary roots.

PLANTS GROWN IN SOLUTIONS WITHOUT PHOSPHORUS

WITH BORON, FIG. 22, PLATE XI.—The external form of the roots of plants, grown for from two to three weeks in solutions without phosphorus but with boron, does not appear very much modified in comparison with normal roots. A study of the tissues showed that the arrangement of the cells was regular. Within the cells, however, the abnormalities were pronounced. The same fixing fluid which proved to be so excellent for all other cases studied apparently gave sections exhibiting plasmolysis. We have as yet no means of telling whether this condition was caused by the fixing agent or whether it occurred before the tissue was killed.

The nuclei are the structures most affected by the absence of phosphorus. They lose their typical more or less spherical shape and assume an extremely irregular outline. Amoeboid, elongated, spindle shaped and constricted nuclei are very often found in the meristematic region. Some of the nuclei are much smaller than those of normal cells. Instead of occupying a position in the center of the cell, as is characteristic of nuclei in meristematic tissue, they often appear flattened against the transverse cell wall. Normal mitotic figures were not observed. The nuclei sometimes appeared more normal in the region of elongation of the root.

Somewhat similar effects on the cells of lower plants have been described by REED (5). He found that not only were mitoses absent in plants of *Spirogyra* which were growing for three weeks in phosphorus free solutions, but that the cells grown for a certain length of time in such solutions were difficult to stimulate to divide when they were placed in solutions containing phosphorus. According to REED, cells transferred to phosphorus free solutions first lose the soluble phosphorus compounds and later show injury in the living parts of the cells.

In our experiments changes in the root tips of the peas described above took place in plants which were kept in phosphorus free solutions for from two to three weeks. Plants kept under similar conditions for a shorter time showed mitotic divisions of the nuclei which were normal or only slightly abnormal. The fact that peas can be grown in phosphorus free solutions for as long as four or more weeks is difficult to explain in

view of the fact that plants with comparatively minor injuries to the cells caused by the absence of boron die in so short a time.

It must be remembered, however, that the influence of the absence or presence of a given element can not be judged wholly by its morphological effects on the cell and tissue structure. The effect of boron on the cells and tissue structure may not be fully represented by abnormalities of structure. This influence may be merely secondary, and its real function concerned directly or indirectly with some very vital and more continuous process, such as, for example, respiration.

The whole problem of the effects of the absence of phosphorus, as well as of the absence of many of the other essential elements, requires much additional investigation.

WITHOUT BORON, FIG. 23, PLATE XI.—Pathological changes in the tissues of plants grown in the absence of both phosphorus and boron resemble those described for plants lacking boron only. The cells of the central cylinder seem to be most injured. Their protoplasts disintegrate. The process of disintegration ultimately results in the complete dissolution of the nucleus and cytoplasm. The disintegration of certain individual cells results in an irregularity of the arrangement of the cells of the central part of the root. The nuclei which retain a nucleolus, even though it may be very inconspicuous, remain alive. These nuclei, however, differ from those typical of meristematic tissues in that they resemble a coarse precipitate instead of the finely granular structure of the typical resting nucleus.

Discussion

In conducting the present investigation our primary object was to determine the changes in the cells and tissues of the root tips of *P. sativum* induced by the absence of boron from the culture solutions. To make these tests more critical, certain other elements recognized as essential, namely magnesium, sulphur, manganese, potassium, nitrogen, iron, phosphorus, and calcium, were omitted one at a time from the media. These studies, in which elements other than boron were excluded, were carried out only to determine whether or not their absence would influence the typical effects induced by the absence of boron. They are, therefore, very incomplete in so far as the specific effect of their absence is concerned. Each of these elements deserves an extensive, independent investigation from the cytological and histological view point, which, because of the purpose of this problem, was not included in this paper. We feel, however, that the results obtained here incidental to the study of the absence of boron, are of sufficient importance to warrant their being recorded as a basis for future special investigations of the structural changes occurring in the absence of these several elements.

Studies of the effects of the absence of boron in the presence and absence of certain of the other essential elements have shown the structural modifications to be roughly similar for all the elements with the exception of calcium. The absence of calcium caused such marked and rapid changes of the tissues, and its effect anticipated that of the absence of boron, so that if the absence of boron caused any abnormality it could not be observed when both elements were wanting. The remarkable effects of the absence of calcium will be treated separately in a subsequent paper.

Meristematic tissues are the first to show the effects of the absence of boron. On the exclusion of boron from the medium, the factors regulating the process of cell division and the normal orientation of the cells in the tissues no longer function normally. In a relatively short time after the plants are transferred to boron free solutions the cells cease dividing and a general arrest of growth follows. Thus the absence of boron influences not merely the structure of the cell but the organization of the tissue. Although more or less regular mitotic divisions are present in the early stages of root development of plants grown without boron, some modifications of this process occur very soon and result in pathological tissue changes. Hyperplasia becomes very pronounced in the region which corresponds to the plerome of the normal root while a distinct hypertrophy is observed in the periblem. The suppression of mitotic divisons in the absence of boron is not typical for the cells of the whole root. Secondary meristematic regions may be formed in the more apical part of the root. These are, however, usually suppressed during an early stage of development, and true secondary roots seldom develop. Normal mitoses occur at the beginning of these new meristems but soon disappear.

The changes in the anatomical structure of the roots of *Vicia faba* induced by the absence of boron were studied by Miss WARINGTON (**10**). She found that the cells of the cambium were affected first. These cells were shown to lose their regular outline, and peculiar dark streaks were found between them. Their development was also irregular, a few large cells often occurring in place of a number of small ones. This was particularly noticeable in the region of the cambium, and according to Miss WARINGTON'S interpretation, was partly due to the breaking down of some individual cells rather than to any enlargement of existing cells. She also noticed the suppressed development of branch root initials, which, owing to the injury sustained from the lack of boron, failed to develop normally. She interpreted the thickened appearance of the roots as due to the irregular development of the pericycle and the formation, but suppressed development, of the root initials above mentioned.

We cannot fully agree with Miss WARINGTON'S explanation of the cause of the thickened appearance of the root. The principal cause, ac-

cording to our investigation is the hypertrophy of the periblem and hyperplasia of the plerome regions. The formation of the suppressed root initials may, of course, add some additional thickness, which is usually seen as spherical enlarged areas in the stunted root apex.

The changes in root tips of *P. sativum* induced by the complete or, perhaps better, the nearly complete absence of boron from the culture solution, take place so rapidly that our study is chiefly one of final results. This makes interpretation difficult. It is obvious that a factor or factors regulating growth have been disturbed, but it is impossible, with only this final morphological picture to decide whether this is a direct or an indirect effect. Studies of root tips taken at relatively short intervals after the plants have been transferred to boron free solutions may help in solving the problem by providing a more or less complete series of the intermediate stages.

Another line of attack is suggested by the fact that certain plants, namely the grasses (**8**), (**9**) respond quite differently to the absence of boron from the nutrient media. We are inclined to believe that such differences in response cannot be due only to the amount of boron stored in the seed, but that they are probably concerned with some marked difference in the metabolism of the different plants. We hope that a more extensive comparative study of different forms will give a clew as to what part boron plays in the development of the organism.

Summary

1. Plants of *Pisum sativum* grown in solutions in the absence of boron exhibit short, thick, and stunted roots.
2. Plants grown in culture solutions in the absence of boron and of certain of the other essential elements showed pathological changes roughly similar to those found in the absence of boron only.
3. The enlargement of the root apices is due to the hyperplasia of the plerome and hypertrophy of the periblem regions.
4. The meristematic region of root tips grown without boron becomes abnormal. The cells cease dividing normally and existing cells undergo premature development or pathological changes. Isolated xylem elements appear in regions occupied by the meristem in normal roots, or in the region of elongation.
5. The primordia of the secondary roots begin to be formed abnormally close to the root tip. Usually, however, they are soon suppressed and secondary roots seldom develop.
6. In a general way, we may state that the absence of boron causes a disturbance in the regulation of growth and development.

7. The profound structural changes here observed make it quite clear that physiological investigations of the various elements must be accompanied by morphological, histological and cytological studies to attain their fullest significance.

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EXPLANATION OF PLATES

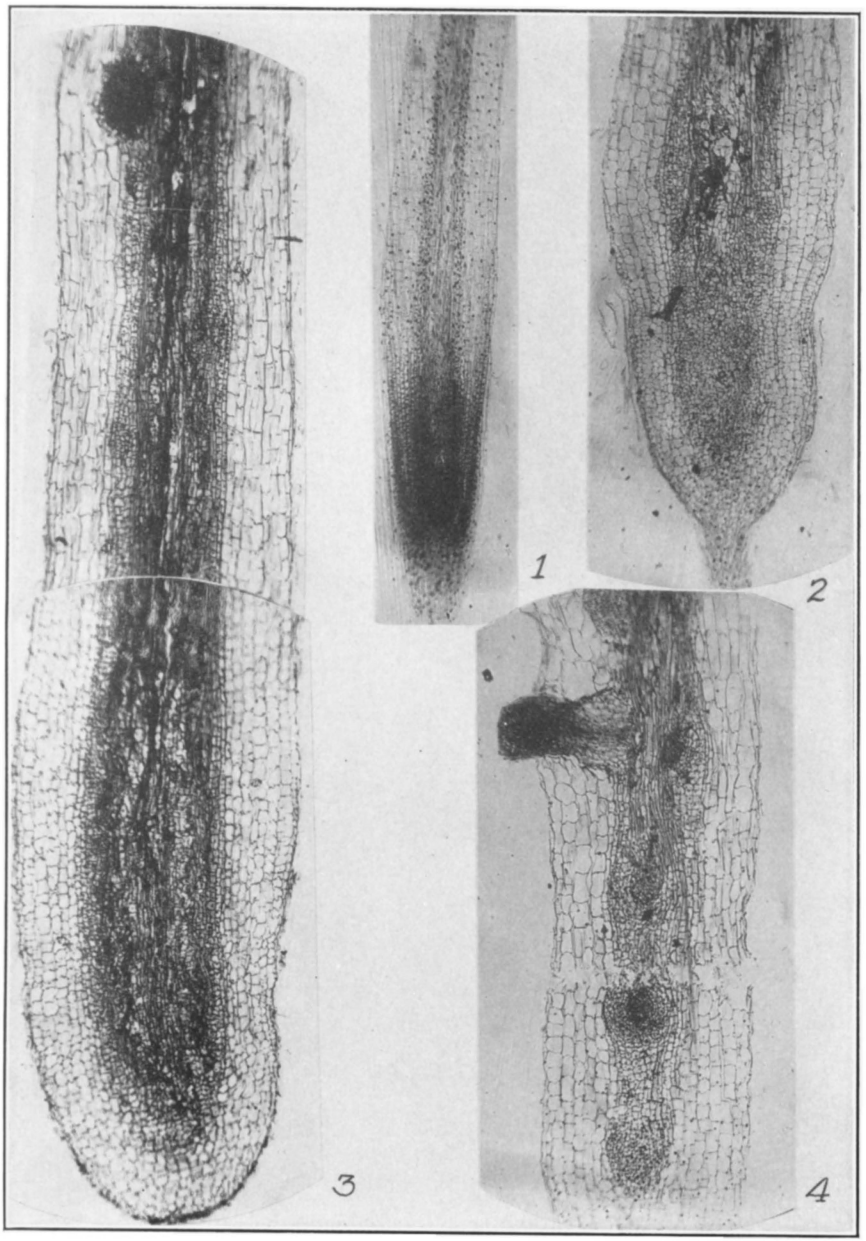
All photomicrographs were taken with a Zeiss achromatic objective [8 mm. ap. 0.20 (A)] and a projection ocular 2. A combination of Wratten B and E filters was employed. All photomicrographs were taken at the same magnification. The experimental plant was *Pisum sativum* (variety Golden Vine). All figures excepting 5, plate VIII, represent longitudinal sections of root apices.

FIG. 1. Grown in control solution; all elements present.

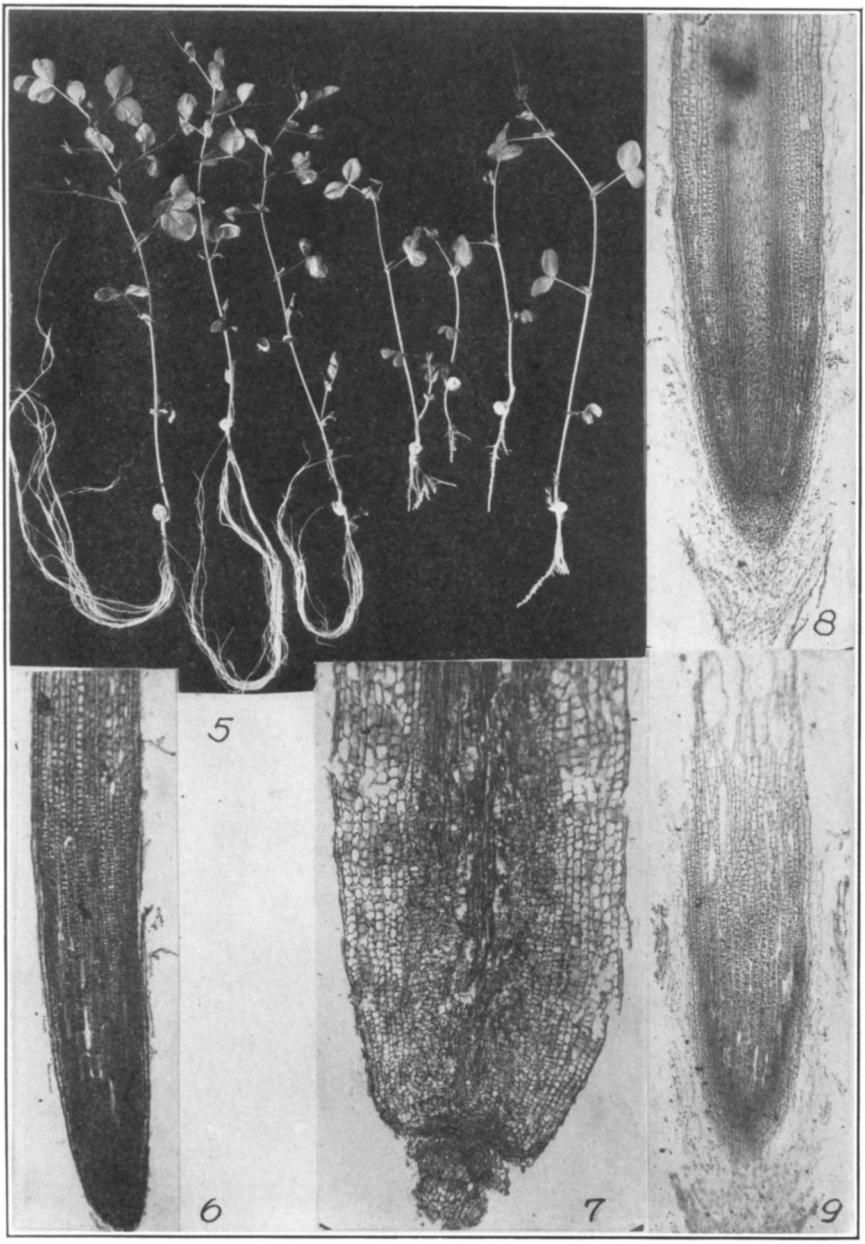
FIG. 2. Grown in the absence of boron.

FIG. 3. As in figure 2. Shows hyperplasia of plerome and hypertrophy of periblem.

FIG. 4. Tangential section of a root grown in the absence of boron. Secondary root primordia are formed very close to the root tip.



SOMMER AND SOROKIN—ABSENCE OF BORON



SOMMER AND SOROKIN—ABSENCE OF BORON

FIG. 5. The four plants to the right were grown in the absence of boron for three weeks. They have very short stunted roots, secondary roots made very little growth. The three plants (controls) to the left were grown in the same kind of solution with the exception that boron had been added.

FIG. 6. Grown in the absence of magnesium and in the presence of boron.

FIG. 7. Grown in the absence of both magnesium and boron. The pathological changes in the tissues are typical for the absence of boron.

FIG. 8. Without sulphur. A slight irregularity may be observed in the periblem. The rows of cells are loosely connected.

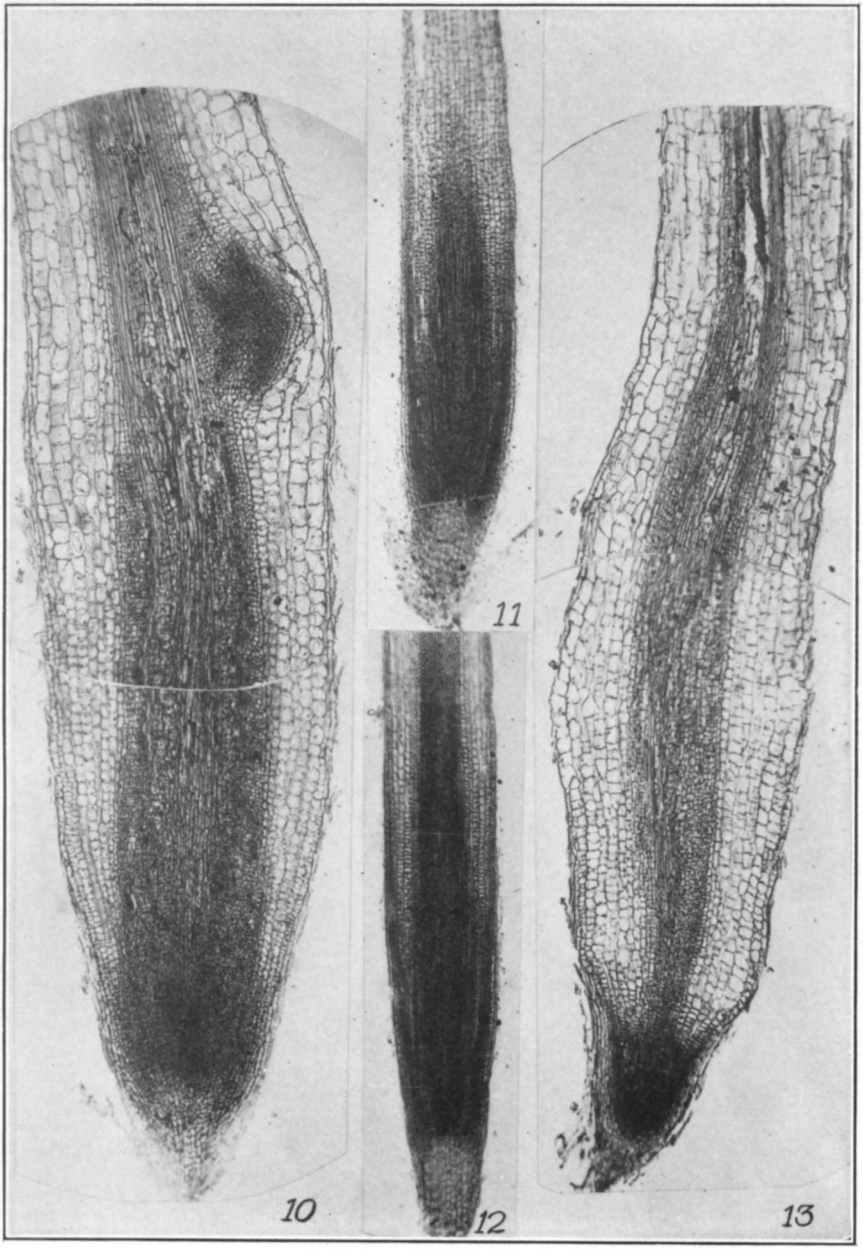
FIG. 9. Without sulphur and with no boron excepting a small amount occurring as an impurity in the salts. The rows of cells are very loosely connected. (A tangential section).

FIG. 10. Without manganese and without boron. Hypertrophy of the periblem and hyperplasia of the plerome are well developed. A branch root primordium may be seen in the upper part of the figure. Irregularly thickened cell walls are present in the periblem.

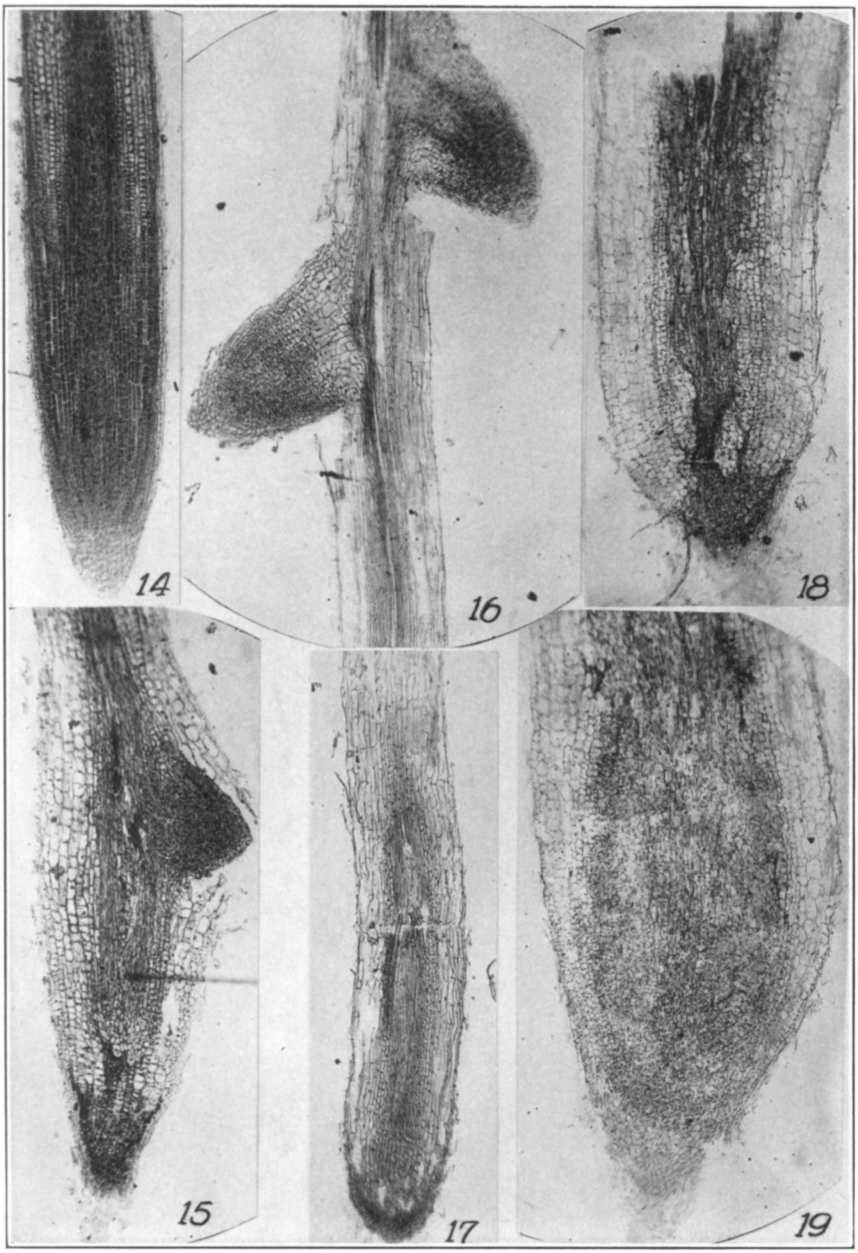
FIG. 11. Without manganese, with boron.

FIG. 12. Without potassium, with boron.

FIG. 13. Without potassium and without boron. Typical effects of the absence of boron are present.



SOMMER AND SOROKIN—ABSENCE OF BORON



SOMMER AND SOROKIN—ABSENCE OF BORON

FIG. 14. Without potassium and without boron. Grown for two weeks in pyrex containers. Pyrex yielded enough boron to supply the plant during the early stages of growth.

FIG. 15. As in fig. 14 but grown in pyrex for six weeks. The root shows some abnormality, but the typical effect of the absence of boron was not yet obtained.

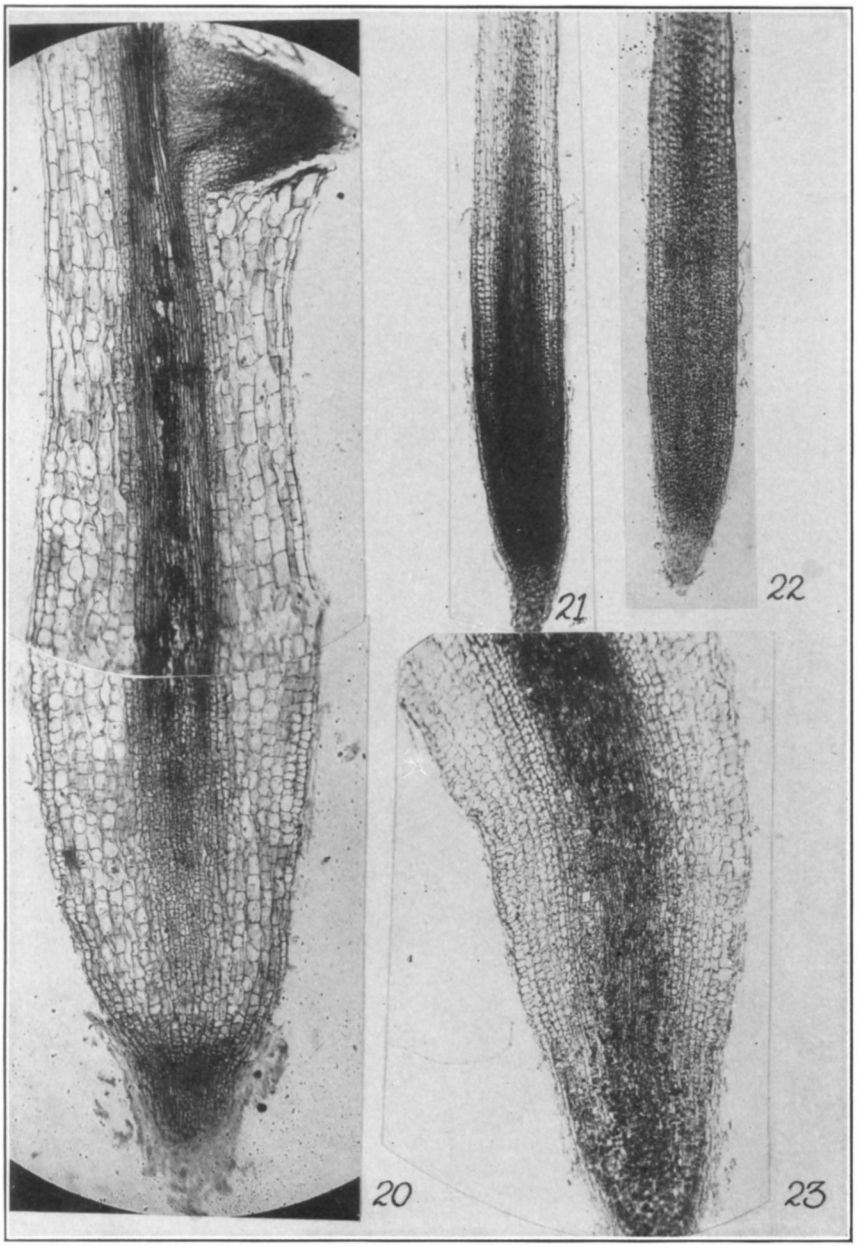
FIG. 16. Without nitrogen, with boron.

FIG. 17. Apical portion of root shown in fig. 16.

FIG. 18. Without nitrogen and without boron.

FIG. 19. As in fig. 18. Hyperplasia of the plerome is very well developed.

FIG. 20. Without iron and without boron. Typical effects of the absence of boron.
FIG. 21. Without iron, with boron.
FIG. 22. Without phosphorus, with boron.
FIG. 23. Without phosphorus and without boron.



SOMMER AND SOROKIN—ABSENCE OF BORON