M.Sc. Plant Pathology.

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PATHOLOGICAL ANATOMY OF BORON-DEFICIENT PLANTS.

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CONTENTS.

INTRODUCTION.

Boron is an element definitely known to be essential for the normal growth of higher plants but its true rôle in the physiology of the plant is still a matter for conjecture. Doubtless its metabolic functions will ultimately be revealed by physiological and biochemical studies but it was thought that a thorough investigation of the influence of boron on the anatomy of the plant would constitute a desirable precursor to such researches and, by giving some indication of the rôle played by boron in the life of the plant, should indicate other pertinent studies most likely to yield constructive results. Accordingly the studies described below were undertaken in order to obtain a full comprehension of the anatomical features which result from an inadequate supply of boron.

The swede was chosen as the subject for the greater part of these studies, due to the fact that in this plant symptoms of boron deficiency are strikingly definite, and therefore the anatomical responses to a lack of boron should be more readily determinable. In addition, the swede lends itself to a study of the effects of different concentrations of boron in nutrient solutions since its boron requirement is relatively high.

Apple and celery were also included in this study because they, too, show pronounced symptoms of boron deficiency. The diseases studied in connection with these species were "internal cork" and "cracked stem", respectively. In the former instance naturally diseased fruit was the only part of the plant studied because of the relative impractibility of growing apple trees under greenhouse conditions. Each of the three diseases investigated is of common occurrence and of considerable economic importance throughout North America, which further warrants a study of the type suggested above.

REVIEW OF LITERATURE.

The essential nature of boron for the growth of higher plants has been proved by several workers, the species studied representing several different families. The first claim for the discovery of boron as a plant constituent was made by Wittstein and Apoiger (1857) who obtained boric acid from the ashes of the seeds of Maessa picta. Agulhon (1916) demonstrated by field and sand culture experiments that boron was beneficial to several species but he gave no evidence that growth was impossible in the complete absence of boron. This was left to Mazé (1915, 1919) who worked with corn. However, definite and conclusive proof was given by Miss Warington (1933) that boron is not merely a stimulant but an element necessary to the normal growth of members of the Leguminosae.

Since then this statement has been confirmed by a number of workers including the following; Sommer and Lipman (1926) working with cotton, sunflower, buckwheat, barley, castor bean, flax and mustard; Johnston and Dore (1928) and Johnston (1928) with potato and tomato; McMurtrey (1929) with tobacco; Haas (1929, 1930) and Haas and Klotz (1931) with citrus trees; Dennis and O'Brien (1937) with oats; Martin (1934) with sugar cane; Hoagland (1933) with strawberry; McHargue and Calfee (1932) with lettuce; Sommer and Sorokin (1928) with peas; Warington (1937) with carrot; Ferguson (1938) with cauliflower, cabbage, celery and beets; Weinberger (1936) with peach; Dehlert and Shive (1936) with blueberry.

The interest in boron as related to plant life had almost a purely academic interest until 1931 when Brandenburg (1931) demonstrated that boron was important to agriculturists by proving that boron deficiency occurs under field conditions and is the cause of the heart and dry rot of sugar beet.

Up to the present day several economic plants have been found to be affected by physiological disorders due to a boron deficiency. On the tobacco in the Deli district of Sumatra a disease called topziekte (top-rot) occurs the symptoms of which for the most part correspond with those obtained when the plants are grown in boron free cultures. Mes (1930) stated that experiments at Deli have proven that top-rot is due to boron deficiency. McMurtrey jr. (1935) reports also that boron deficiency on tobacco occurs under field conditions.

The lettuce is said by McHargue and Calfee (1933) to be affected under field conditions by a physiological disease caused by a deficiency of boron. This disease was described by Stone and Smith (1897) under the name "top-burn" of lettuce. Current literature refers to this disease as "tip-burn".

Purvis and Ruprecht (1937) have recently described on celery a disease known as "cracked stem" which is destructive in the United States and seems quite common in Quebec. On the same plant Gram (1936) has described a heart rot similar to that of the sugar beet; this disease is also present in Canada.

Dearborn, Thompson and Raleigh (1936) have noted a browning of cauliflower due to boron deficiency, which disease has been shown to be of economic importance.

On lemon a disease called "hard fruit" is reported to be cured by application of borax (Hall, 1938), (Anonymous, 1937).

The apple has been known for a long time to be affected by a group of physiological disorders which are now attributed to boron deficiency as demonstrated by Askew, Chittenden and Thomson, (1936). These are corky core, drouth spot, internal cork and a form of die back of the twig. These diseases have been described by McLarty (1928), Mix (1916) and Carne and Martin (1937). McLarty, Wilcox and Woodbridge (1937) noted a yellowing of alfalfa due to a boron deficiency.

In eastern Canada brown heart of turnips and swedes is the most important of the diseases due to boron deficiency. A good description of this disease is given by Dennis and O'Brian (1937).

Relatively few anatomical studies have been made in connection with the effect of boron deficiency in plants and many of them are incomplete. Almost invariably one or more parts of the plant have been omitted or studied superficially. This is most unfortunate, since different plants may show striking differences in their reactions to boron deficiency.

Brenchley and Thornton (1925) showed that the absence of boron inhibits the development of the nodules of _Bacillus radicicola_ on the broad bean. They described sheaths of blackened tissues enveloping the abortive or incompletely developed root nodule. Cell proliferation, often followed by a locally dispersed necrosis, occurs in the inner layers of the root cortex.

A certain amount of cell proliferation, associated with nodule formation, occurs also in the pericycle causing a slight swelling. A few tracheids may be present among this proliferated tissue, but these do not extend into the incipient nodular tissue in the cortex. This absence or restriction of the development of tracheids is the most significant feature of the atypical nodules associated with boron deficiency.

Brenchley (1926) states in another paper that anatomical changes, disintegration and blackening occurs in the tissue of certain plants and especially in many of the Leguminosae when they are grown in the absence of boron.

Warington (1926) studied in more detail the changes induced in the anatomical structure of the broad bean by the absence of boron from the culture solution. Referring to stems, she found that the tissues of the vascular bundles are the most severely affected. Hypertrophy of the cells of the cambium and immediate derivatives makes the xylem appear to be far removed from the phloem. This hypertrophy is usually followed by degeneration with discoloration, but direct disintegration without previous enlargement may also occur. Frequently disintegration of the phloem and ground parenchyma occurs as well as a poor development of the xylem, while this last tissue which is sometimes scattered in small groups, may ultimately break down.

The large elongated cells in the cambium region tend to be directed radially but they may be quite irregular and in longitudinal section follow no rule at all relative to arrangement. The growing points suffer before more mature tissues and it seems that elongation of cells is the first symptom of boron deficiency, disintegration and displacement of xylem and phloem occurring later. The cortical fibrovascular bundles are usually the first to be affected. Pith and ground parenchyma, particularly that in association with the xylem, often show cellular disorganization or disintegration which results in patches of blackened tissue. The lumen of the tracheids is relatively small and frequently blocked by some unknown substance.

Corresponding tissues of the root are affected similarly. Cells of the root tips have blackened contents, but the form of the cells remains unchanged.

Sommer and Sorokin (1928) studied carefully the effects of the absence of boron on the structure of the root tips of Pisum sativum. They noted that the region which corresponds to the primary meristem of the normal root can hardly be called meristem; The root cap is considerably deformed or even entirely lacking and the apical portion of the root is enormously enlarged. Distinct hyperplasia is evident in the plerome and hypertrophy of the periblem occurs. In the early stages of the culture cells divide by typical mitoses but they soon loose this ability. Smaller nuclei, lack of regularity in arrangement and form of cells, premature development of some cells into xylem and abortive formation of branch-root primordia in the apical portion of the root are other interesting features noted by these workers.

In the periblem the rows of cells are more regular than in the plerome and correspond in number to the normal root; the cells, however, are much larger than normal. The appearance of thick strands which apparently represent the collapsed and thickened walls of dead cells was also noted.

The pathological anatomy of boron-deficient tomatoes has been described quite fully by Van Schreven (1935). The first symptoms generally appear in the procambium of the stem tips. The growing points are killed and secondary root and axillary buds are stimulated to grow. Many of the young rootlets do not emerge through the cortex or if they do they remain very short.

The stelar structure in particular is affected severely although necrotic cells are found in other tissues. The first change is a discoloration of the cell wall followed by the death of the cell contents. Cells of the cambium, phloem and ground parenchyma are often deformed by distention or compression. The phloem may be much enlarged while the xylem is usually poorly developed. These degeneration symptoms were also found in the flower and flower buds when one is formed, which is not of frequent occurrence. In the leaf veins and petioles it is chiefly the phloem on the under side that is affected by the lack of boron; this explains the curving downward of the veins and the petioles.

The leaf tissue is considerably thickened owing to enlargement of the individual cells. The plastids in the chlorotic tissue degenerate but their number does not decrease although they may decrease in size.

Johnstone and Dore (1929) and (1928) have examined the anatomy of the same plant and state that the failure of boron deficient plants to remove sugars from their leaves is a consequence of the breaking down of the phloem which becomes compressed or displaced, while the xylem may degenerate and the tracheid lumina become completely blocked. These authors state that the conditions in the broad bean as described by Warington (1926) seem to be very similar to those occurring in the tomato when grown in boron deficient solutions. They thought that the peculiar brittleness of the petioles might be due to a lack of pectic substance in the middle lamella but microscopic examinations using customary pectic stains and chemical analyses revealed no difference between normal and boron-deficient material. Their suggestion to explain the brittleness of petioles is the lack of proper lignification. Brenchley (1927), has demonstrated that boron occurs most abundantly in the bark and in the lignified parts.

Two other solanaceous plants have been studied anatomically; they are the tobacco and the potato. Shive (1936) working with tobacco states that boron deficiency is most pronounced and very destructive in the regions of the physiologically most active tissues, involving apical meristems, cambium and phloem. In the growing stem tips the first symptom is necrosis of the cells at the apex. The necrotic layer follows the procambium strands backward to the youngest leaves. As the disease progresses the somewhat thickened highly refractive cell walls become yellow and later completely broken down.

In the stem the vascular tissues are affected. An unusual development of thick-walled strongly lignified fibers occurs in the outermost layer of phloem. In young stems small areas of necrotic tissue appear in the phloem underlying these fibers, and in the course of development this necrosis involves more extensive regions of phloem and cambium, the affected tissues appearing as disorganized masses of amorphous material. Extreme irregularities of cell division in the meristems, subsequent abnormal enlargement of some cells and not of others and abnormal thickening of cell walls in unusual places occur as well.

Van Schreven (1934) reports similar studies on the same plants and notes about the same internal features except that the cortex, ground parenchyma and epidermis may also be involved. Many cells containing crystals of calcium oxalate are found. The thickening of the leaf blade is a result of the enlargement of individual cells. The number of chloroplasts is increased but they are smaller than in healthy plants so that the relative amount of chlorophyll is less. The nucleus may enlarge in the diseased cells and disintegration of the mesophyll cells sometimes takes place.

Mes (1930) has also made anatomical studies of boron-deficient tobacco but they are far from being as complete as those already mentioned.

The pathological anatomy of potatoes grown in boron free sand as described by Van Scheven (1935) is essentially similar to that which he described for the tomato and the tobacco. However, in the tubers he found brown discoloration of the cortical cells and of cells of the vascular region, generally at the stem end, and in some cases parts of the medullary region were affected as well. Symptoms on the same plant are also described by Johnston (1928).

Haas and Klotz (1931) studied the anatomical changes produced by boron deficiency on citrus trees. They found that the midribs or veins become conspicuously corky and split open without gum exudation. Cork formation may take place covering the palisade tissue for some distance from the vein but it does not involve the ventral surface of the leaf. Cork tissue develops completely in and about the fibrovascular system.

In the shoot, the cambium region shows disintegration and accumulation of gum. Longitudinal sections of the stem show that this gum separates the cortex from the woody cylinder, and oozes out of splits in the bark. In rare instances, gum deposits occur in the xylem and appear to have been produced prior to cell maturation. The individual xylem vessels do not contain gum. Some of the gum is impregnated with a dark substance which is probably a disintegration product.

Martin (1934) in his experiments with sugar cane found the first internal symptom of boron deficiency to be a change in the lignified fibers on both the upper and lower portions of the chlorophyll-bearing bundle sheath. Enlargement of the lower cells of the bundle sheath in the leaves was sometimes so marked as to cause the formation of elongated gall-like bodies on the lower leaf surface. The bundle fibers appeared small, poorly developed, loosely arranged and lacking in the normal silica content. No abnormality in the phloem or xylem was noted other than a crowding together as a result of the enlargement of the bundle sheath cells. On the other hand, sections of young leaves showing deficiency symptoms revealed deep constrictions in the injured regions.

Eltinge (1936) working on Zea Mays found that the first internal symptoms of boron deficiency appeared as disintegration of parenchyma cells. In later stages of injury the entire cross-section of the leaf collapsed into a thin band which stained red. Further modifications of lamina cells were observed in some instances, namely: failure to differentiate, hypertrophy - especially of the lower epidermis -, cell enlargement and formation of a multiseriate palisade. The last two features resulted in a greater thickness of lamina. Cellular disintegration eventually occurred in the stem-tip. The root tips, which were of "cheesy" consistency, showed considerable enlargement, while development of branch roots unusually close to the tip was also noted.

An interesting piece of work along this line is that of Rowe (1936) who investigated the anatomical changes in the sugar beet due to a boron lack. As far as disintegration and hypertrophy of the tissues themselves are concerned there is nothing very different from what has already been said for other plants. She notes however that some hypertrophied cells become cross divided by extremely thin walls. She also observed that the tracheids in process of differentiation become thick-walled, but not rigid, since hypertrophy of the surrounding cells causes them to assume irregular shapes. The plugging of sieve tubes with presumably callose is much more frequent in diseased than in healthy tissue. The root tips show about the same characteristics as noted by Sommer and Sorokin (1928); the root tip, however, becomes diseased much later than the shoot apex in contrast to the majority of the other plants studied. When diseased plants were fed with boron they would show a measure of recovery.

In the recovery process she found that the affected buds and embryonic leaves could not show any renewal of growth but that recovery involves the activation of new axillary buds, each of which develops its own system of secondary vascular elements in the bulb independant of the others. It is suggested by Rowe that plugging of the sieve tubes is the first indication that the tissue is suffering from boron deficiency.

Relative to the turnip the only work is that of Jamalainen (1935). The xylem parenchyma cells in the brown hearted areas become distended and much enlarged leaving no intercellular spaces. This is the cause, according to this author, of the dark appearance of the diseased portions. In the lightly diseased tissue the cells become elongated in one definite direction but in the severely affected tissue the direction of elongation of the parenchyma cells seemed to follow no rule at all. Very often in such places the cells press against and crush one another giving rise to dried up masses of dead cells. In the diseased tissue normal parenchyma cells occur. In special and very severe cases the cells of the cambium bordering the cortex may be abnormally enlarged.

With regard to the cell wall Dennis and O'Brien (1937) state that "under conditions of boron deficiency in turnip the middle lamella of affected cells is partially disorganized".

Mix (1916) was an early investigator of the various physiological disorders affecting the apple known as cork, corky core, internal cork, drouth spot and die back. He studied the histological pathology of these diseases and he states: "The brown spots of cork (internal cork of to-day) appear as aggregations of cells with brown shrunken contents. A number of cells though not all are shrunken and collapsed. Around the corky portion the healthy cortex cells form a ladder like arrangement of smaller more nearly rectangular cells. It is as though they had been stimulated to rapid division. Outside this zone the pulp cells are normal in size and form. The close relation of the dead spots to the vascular system is very evident under the microscope."

With regard to the drouth spot the trouble in the early stages is confined to two or three layers of the hypodermal parenchyma. The cells retain their normal shape but their contents become brown and amorphous. But in later stages the cortex cells immediately beneath the dead-cell layers become nearly rectangular in outline and are apparently dividing in a plane parallel with the surface of the fruit. The two outermost layers of this tissue develop suberized cell walls.

Sections of twigs affected with the die-back show a discolored zone consisting of what Sorauer calls "Parenchymholz". This is a tissue in which no normal wood fibers or vessels are recognizable. It consists of irregular but nearly isodiametric cells with comparatively thin pitted walls and large lumina. The cells themselves appear healthy and contain large quantities of starch. The brown color of the parenchyma zone is due to a brown intercellular substance of unknown nature.

Suit (1930-32) made histological examinations of the spots of internal cork. He found that each spot was surrounded by a cork layer. The roots of diseased trees were also studied. The vascular rays of both Fameuse and McIntosh diseased roots were plugged with some sort of material while the sections from the normal roots showed only a very small amount of plugging in a few cases.

Young and Bailey (1936),however, questioned the finding of Suit as being applicable as a general rule. They reported that as much plugging may be found in healthy as in diseased trees and that the amount of plugging varies at different points along the same root.

Jamalainen (1936) states that in the diseased spots the cell walls are crinkled and the cells more or less pressed against each other.

MATERIALS AND METHODS.

The following specific boron-deficiency disorders were investigated on plants growing in the field: (a) internal cork of apples, (b) cracked stem of celery, and (c) brown heart of swedes. In addition the anatomical changes induced in celery and swedes when grown under conditions of inadequate boron supply, as described below, were studied by comparing the anatomy of diseased plants with that of normal plants.

The normal plants were in each case grown in sand cultures to which a nutrient solution was added that contained 2 p.p.m. of boron, this quantity having been shown by previous experiments to be about optimum for the species studied under the other conditions of the experiment. The same culture technique was used for both swedes and celery. Seed of the swede variety Laurentian and of the celery variety Golden Self-Blanching were sown in flats of pure quartz sand and as soon as the seedlings had developed two leaves they were transplanted into one-and-a-half inch pots filled with the same quartz sand.

When the plants had five leaves they were removed and thoroughly washed in tap-water, rinsed in several changes of distilled water and transplanted into twelve-inch two-gallon butter-crocks provided with a half-inch drainage hole at the bottom, filled with finely ground, pure quartz sand. The crocks, glazed inside and outside, were thoroughly washed and rinsed with distilled water. The sand was washed several times by draining distilled water through the filled pots before planting.

At the time of transplanting and at weekly intervals thereafter each pot received 500 c.c. of nutrient solution to which boron was added as specified below. The plants were watered daily with distilled water and great care was taken to avoid contamination from any source. All glassware (of boron-free type) was cleaned in chromic acid cleaning solution and thoroughly rinsed in many changes of distilled water before use. The nutrient solution was made up with the following ingredients for five liters:

$MgSO_4.\ 7H_2O$	4.881 gm
KH_2pO_4	2.694 "
$CaCl_2$	5.546 "
KNO_3	10.012 "
NH_4NO_3	18.249 "
$MnSO_4.2H_2O$	0.00268"
$FeCl_3.\ 6\ H_2O$	10 cc of a 0.5% solution.

The boron was supplied as boric acid in the nutrient solution at the rate of parts per million as specified below. The chemicals used were C.P.Baker's analyzed and were not repurified.

Different series of test swede plants received proportions of boron as follows: (a) 2 p.p.m.; (b) 0.25 p.p.m.; (c) 0.0 p.p.m.. Only two treatments were applied to the celery plants namely: 2 p.p.m. added and no boron. Examination of both healthy and diseased plants were made when the buds in boron free plants were dying, and as soon as symptoms of severe diseased conditions were apparent in those plants receiving 0.25 p.p.m. Death of the buds of the former plants occurred after about 10 weeks, and the latter condition was reached in about four months.

The studies on the internal cork of apple were made with fruit of Fameuse variety obtained from orchards in the district of Montreal where this disease has been present for a number of years.

Most of the observations on the swede, were made with free hand sections stained in 1% neutral red, and when the material was found worthy of more careful and detailed study it was stored in a fixing solution.

For the purpose of histological studies on swedes the material was killed and fixed in F.A.A. No.1(Rawlins 1933) which was found satisfactory except for root tips which were killed in Flemming's weaker solution. The material was dehydrated with normal butyl alcohol (Riker and Riker 1936) using the same as a solvent for paraffin or tissue-mat sold by Fisher Sci. Co, which was used with much satisfaction. At times Dowson's quick method (1922) was used and found satisfactory for embedding. Sections were cut 10 microns thick and stained with safranin and fast green which gave good differentiation except for the root tips which were stained in Flemming's triple stain.

For histological studies on the apple and celery both F.A.A. and Flemming's weaker solutions were used for fixation. The stains were of three types: safranin and fast green, Flemming's triple stain, and Sudan IV and Haemalum, following

Barton Wright's schedule (1938). This was found much more satisfactory than either Rawlin's (1933) or Conn's (1929) method, and proved very reliable for paraffin sections when tested against free hand and freezing microtome sections stained in this same stain and against the zinc-chlor-iodide test of Rawlin's (1933).

Drawings were made with the aid of a camera lucida or with a projecting apparatus using an ordinary microscope the objective of which was replaced by a special camera lens. Photomicrographs were taken with a Bausch & Lomb apparatus.

RESULTS.

I Studies in the Comparative Anatomy of Healthy and Boron-Deficient Swedes.

Quite definite macroscopic symptoms of boron deficiency were evident in roots, stems and leaves of those plants growing with insufficient boron. Accordingly, comparative histological studies were carried out on each of these organs, the results of which are described below.

The plants grown in sand without boron in the nutrient solution for eight weeks were smaller than those grown under the same conditions with 2.0 p.p.m. of boron in the nutrient solution and the foliage looked unhealthy (Fig. 1). The deficiency symptoms began to show about one week earlier, first in the outermost leaves and worked progressively inward. However, before the innermost leaves were involved the terminal bud became necrotic so that at a certain stage the plant would show strong symptoms in the outer leaves and necrosis in the terminal bud while some of the inner young and partly grown leaves appeared normal. Finally all leaves became involved and this was soon followed by death of the plant.

In the development of the symptoms the blades of the outer leaves first became lighter green in color, thickened considerably and became brittle and leathery; they usually curved downward both at the tip and along the margin, (Fig. 2) the midrib becoming prominent and somewhat water-soaked.

Fig. 1. Healthy plant at left grown in sand with 2.0 p.p.m. of boron in nutrient solution and diseased plant at right grown without boron at the end of eight weeks. Note the small size of the diseased plant its chlorotic appearance and discoloration at the leaf margins.

Fig. 2. Healthy leaf at extreme right and diseased leaves showing various degrees of curling and chlorosis from plants grown with a subminimal supply of boron.

Later appeared a yellowish color along the margin which later became red by the development of anthocyanin pigments (Fig. 1 and 8). Very frequently also occurred the formation of galls on the petioles sometimes accompanied by splitting of the epidermis and underlying tissues (Fig. 3, 4 and 5). The galls appeared near the base of the petiole (Fig. 5), higher up in the midrib (Fig. 3 and 4) and in the veins. Ruffling and mottling also occurred but these were less constant symptoms. Necrosis sooner or later set in. It commenced at the tips of the blades and along the margins, beginning at the terminal points of the veins and progressing inward. There was no formation of new buds after the death of the terminal bud as is the case for most other plants grown in boron deficient solutions.

The "root" did not develop to any appreciable extent before the plant died. It showed a brown rough and deeply ridged skin (Fig. 6). In cross section it presented the peculiar aspect of water-soaked and brown tissue, excepting for a small central part which was lighter in color. Outside this central part the tissues were soft and fleshy throughout. In some instances small dark spots could be seen in the cortical region. The majority of the root tips were much enlarged stunted, knobby and usually blackened especially at the very apex. The branch root primordia formed profusely near the tip and most of them did not develop to any extent. They appeared as black dots on the surface of the root. The whole root system was not much developed and appeared brown and water-soaked (Fig. 7).

Fig. 3. Leaf showing galls on the midrib of a leaf of a boron-deficient plant.

Fig. 4. Midribs showing galls and splitting of epidermis and underlying tissues.

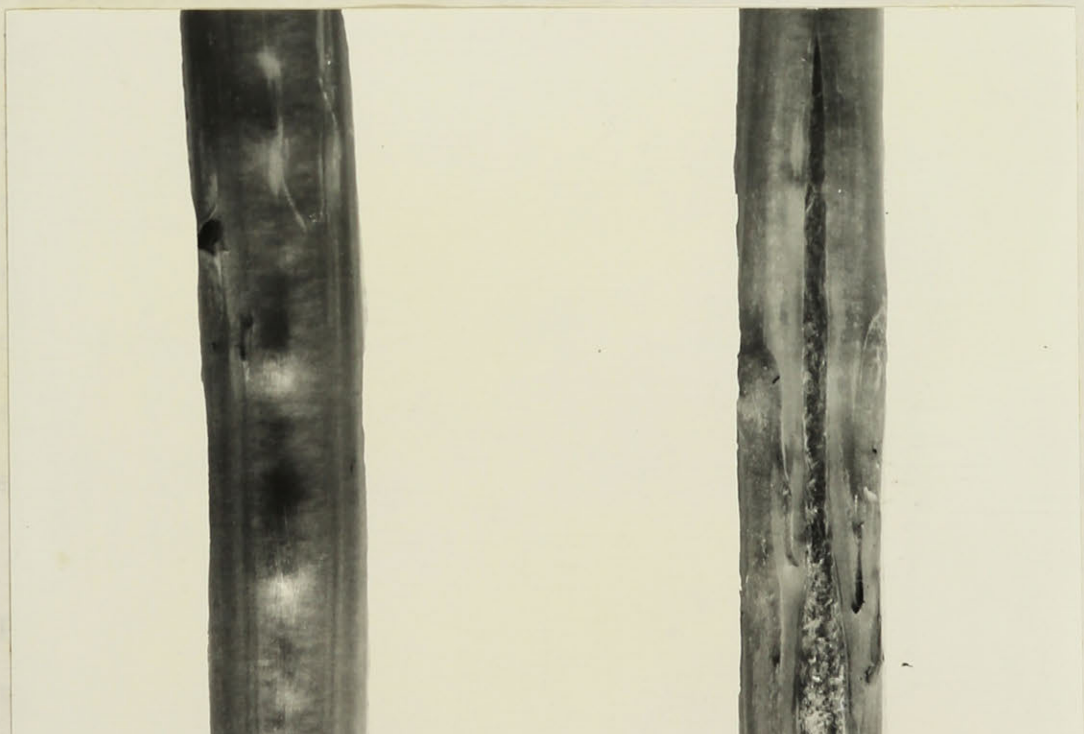

Fig. 5. Petioles showing galls and splitting of epidermis and hypodermal tissues.

The plants grown in sand with the nutrient solution containing 0.25 p.p.m. of boron grew healthy for several weeks and kept pace with the plants fed 2.0 p.p.m., but at the end of four months they had developed the foliage symptoms described above. Their "roots" were about three inches in diameter while the healthy plants grown with 2.0 p.p.m. of boron had "roots" four to five inches across.

The foliage symptoms were quite similar to those described previously for the plants grown without boron in the nutrient (Fig. 8). The outermost leaves were very brittle, curled downward with prominent midribs and veins (Fig.2). The blades were thick and rough with purplish coloration more intense at the margin (Fig. 8 and 9). The leaves were, however, as large and well developed as those of normal plants. Quite frequently there occurred on the midribs and on the petioles very conspicuous galls accompanied sometimes by splitting of the epidermis and underlying tissues (Fig. 3, 4 and 5). The inner leaves though somewhat brittle and chlorotic did not show the purplish coloration on the margin and were not curled downwards to the same extent as the outer ones.

Externally the short stem looked normal but when the swedes were cut longitudinally or radially in the lower portion the central part, which is the pith, was dark brown and water-soaked in appearance, (Fig. 10c) while the pith of a stem of a healthy plant was much lighter in color and lacked the water-soaked appearance.

Fig. 6. Healthy (left) and diseased (right) "roots" of plants shown in Fig. 1. Note the brown color and the deeply ridged skin.

Fig. 7. Healthy and diseased plants also shown in Fig. 1. and 6. Note the difference in the development of the root system and the dark color of the diseased one at right.

Fig. 8. Swede plant grown in nutrient solution with 0.25 p.p.m. of boron for four months. Note curling downwards of the leaves and discoloration of the margins.

Fig. 9. The same plant as in Fig. 8 showing the root system which is brown in color.

The fleshy hypocotyl and root was rather small and malformed tending to be tap-root shaped instead of bulbous (Fig. 10a and 12). The skin was rough with very small ridges (Fig. 11).

The whole root system was brownish yellow instead of white (Fig. 9) and a high proportion of the root tips were enlarged, knobby and swollen with secondary and abortive root tips near the primary one in the very same way as was found on the plants fed no boron. The proportion of healthy root tips and rootlets was, however, much greater in the former than in the latter.

Internally the "root" had developed a great deal of brown-hearted tissue in both the root and hypocotyl portions, and even in the stem (Fig. 10 a b c and 12). A conspicuous band of brown water-soaked tissue varying in thickness had developed in the cambium region (Fig. 12). In severely diseased plants the cambium region everywhere might be involved in this manner but in early stages only parts of the cambium of the fleshy root showed this trouble. The plants fed 2.0 p.p.m. of boron remained perfectly healthy throughout.

A. Comparative Anatomy of Diseased and Healthy Roots.

Preliminary examinations indicated that particular attention should be devoted to those regions of the root where meristematic or other intense physiological activity was centred. Such regions are localized particularly at the root tips, at the regions of cambial activity of the roots and in the rapidly growing storage "root". The term root tip is used to include the root cap and

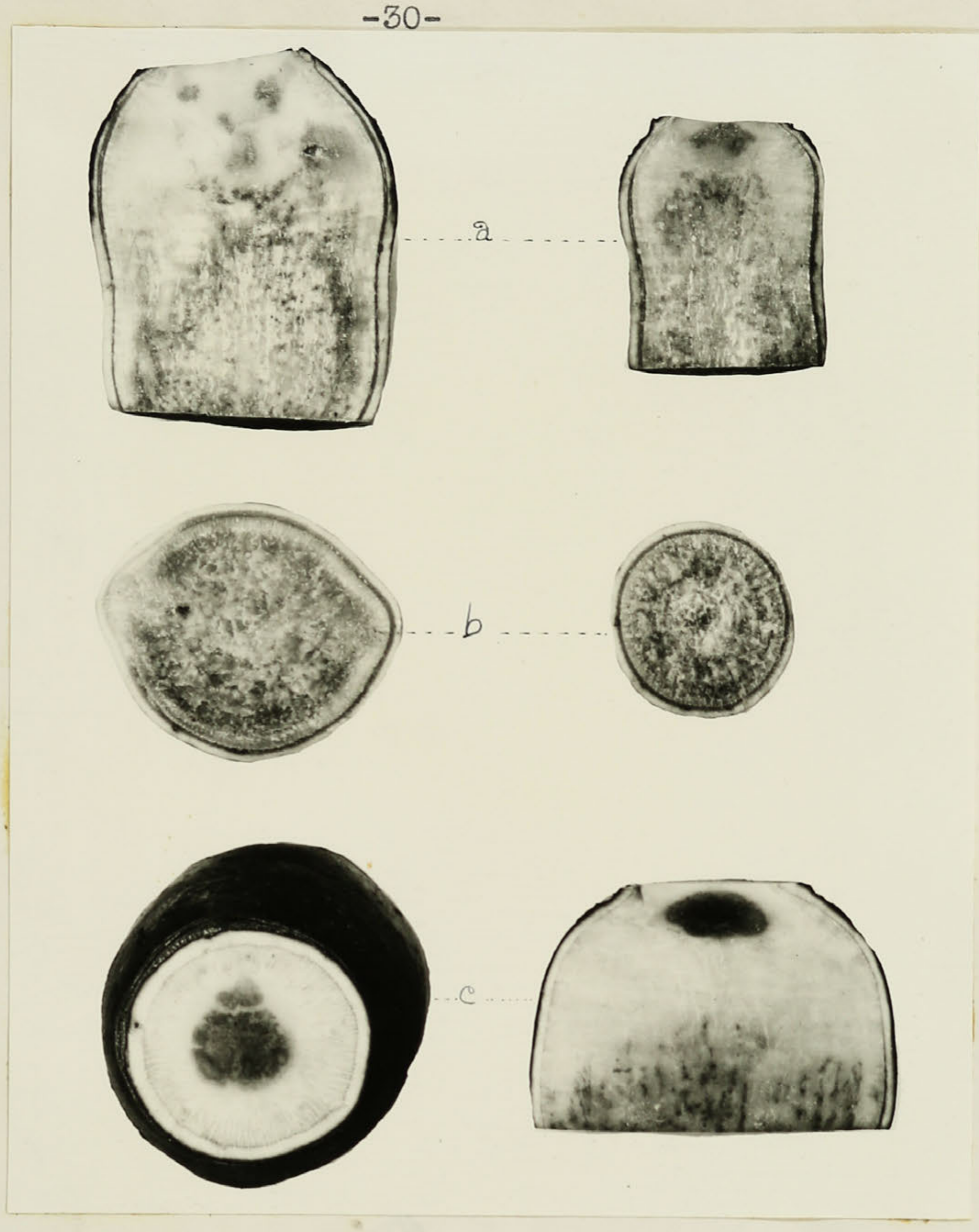

Fig. 10. "Roots"of swedes grown in nutrient solutions with 0.25 p.p.m. (a) Longitudinal section showing injury in the cambium region and brown-heart in the centre. (b) Cross section showing the same features. (c) Longitudinal and cross section showing injury in the pith of the stem part of the bulb.

Fig. 11. "Roots"of swedes grown in nutrient solution with 0.25 p.p.m. Note the rough skin. These plants were grown for about four months.

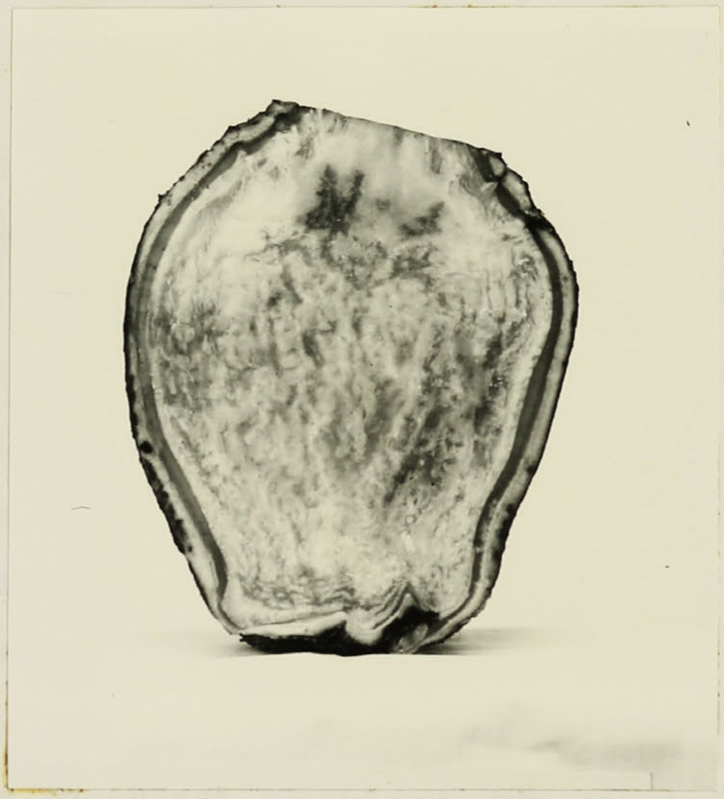

Fig. 12. Swede "root" grown in nutrient solution with 0.25 p.p.m. showing the water-soaked appearance of the cambium region and brown-hearted areas.

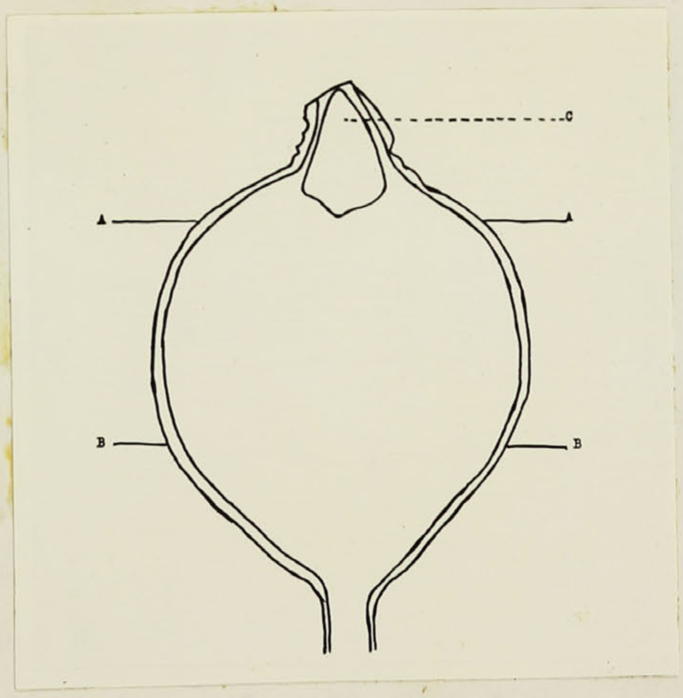

Fig. 13. Sketch to show regions as seen in longitudinal section of a typical Laurentian swede. Below BB- root portion. Between AA and BB- hypocotyl portion. Above AA. Epicotyl or stem portion of the storage "root." At C. pith.

terminal meristem, as well as the more mature tissues which have been formed by the histogens or differentiated from the procambium strands prior to initiation of vascular cambium. The term storage "root" or merely "root" is used, for want of a better one, to refer to the enlarged, bulbous food storage organ which is comprised of modified tissues of root, hypocotyl and lower portions of the true stem (epicotyl) (Fig. 13). Comparative anatomical studies were carried out on the root tips, storage "roots" and branch roots in the zone where some secondary thickening had occurred. These studies are presented separately.

1. Root tips.

a. Healthy root tips from plants grown in a medium containing 2.0 p.p.m.

The root tips of plants grown in a culture medium containing 2.0 p.p.m. of boron showed the tissue regions of plants of this type (Cruciferae). There is a well-developed root cap differentiated from a calyptrogen-dermatogen which also gives rise to the epidermis. A clearly defined periblem and plerome are the parent meristems of the cortex and stele respectively (Fig. 14). The region of cell division and cell elongation was quite extensive and no xylem or phloem elements could ever be found in longitudinal sections of root tips one centimetre long. The meristematic

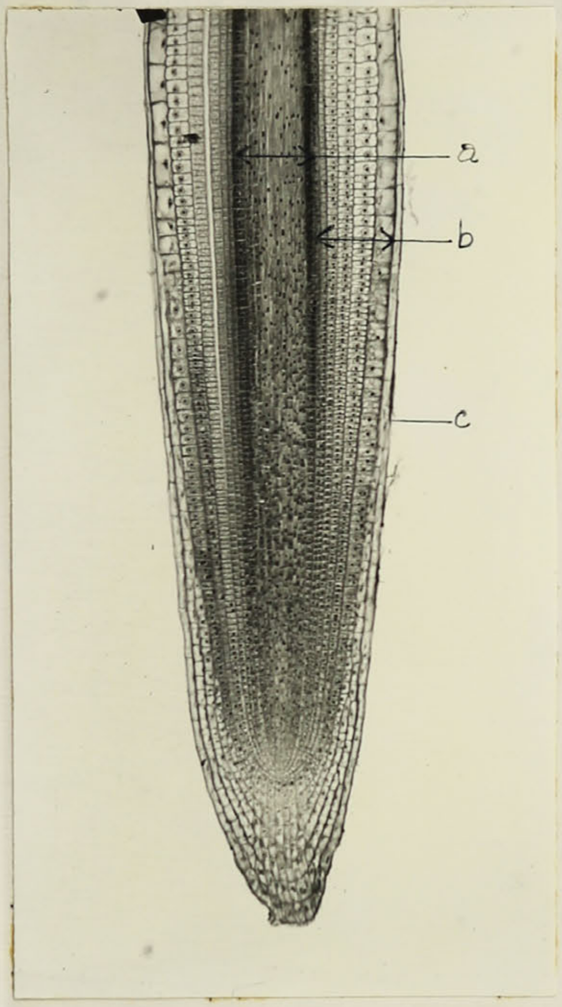

Fig. 14.

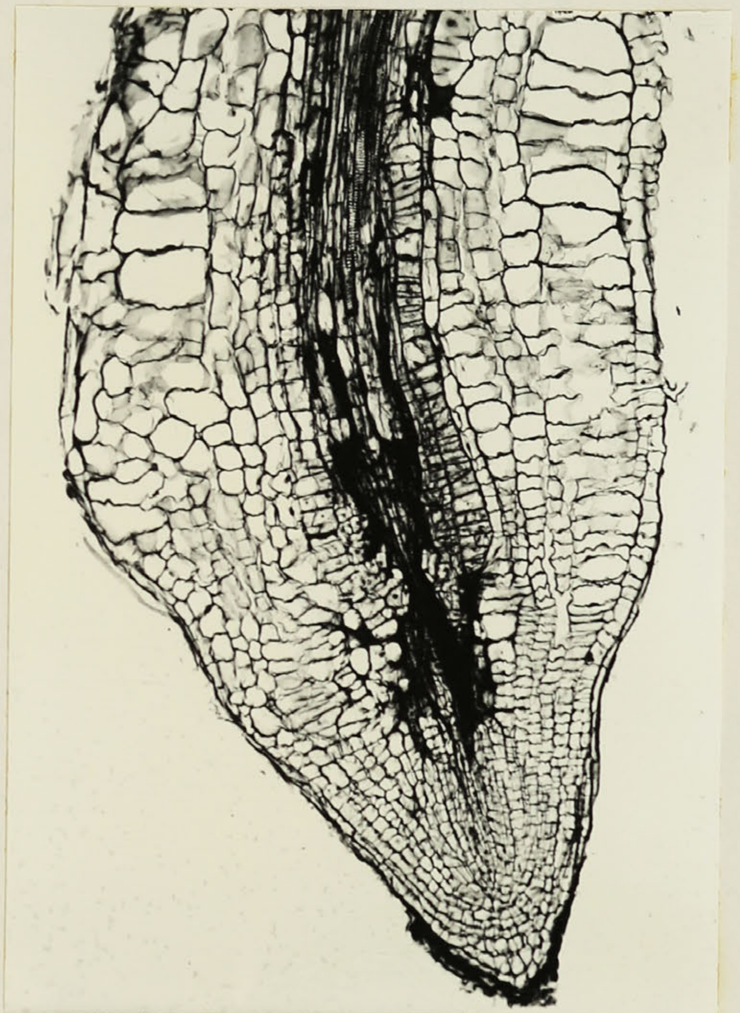

Fig. 15.

Fig. 14. Median longitudinal section of a root tip from a normal swede: (a) plerome, (b) periblem and (c) dermatogen.-x52

Fig. 15. Median longitudinal section of a root tip of a plant grown without boron. Note the wanting root cap, disintegration in the plerome, hypertrophy in the periblem, early differentiation of some xylem vessels in the plerome and the general absence of nuclei and protoplasm in the cells.-x52.

regions of the root tips mentioned in this description are referred to in the sense of Eames and MacDaniels (1925) whose concept of the plerome is a tissue region which extends from and includes that part of the terminal meristem which gives rise to the stele together with the resultant stelar cylinder to a zone at which tracheal differentiation is manifest. The periblem according to these workers is the cylinder of occasionally meristematic cells surrounding the above central core, exclusive of the epidermis or dermatogen from which the true epidermis is derived.

b. Diseased root tips from plants grown in a medium free from boron.

The root tips of plants grown in a culture medium free from boron at the end of eight weeks showed a marked response to the absence of this element. The terminal regions of most root tips were much enlarged and usually blackened. The entire organ was relatively stunted and covered with small knob-like protuberances which indicated abortive attempts at branch root emergence. The branch root primordia formed profusely and unusually close to the root apex where their presence was often indicated macroscopically by small black dots on the surface.

Microscopic examination revealed that the meristematic region of the root tips did not present the characteristics of normal meristems (Fig. 15, 16 and 17). The cells had no definite

Fig. 16.

Fig. 17.

Fig. 16. Median longitudinal section of a root tip of a plant grown without boron. Note the presence of a branch root primordium, early differentiation of plerome into xylem vessels not connected with one another, hypertrophy of plerome and periblem cells, and some disintegration.-x52

Fig. 17. Median longitudinal section of a root tip of a plant grown without boron. Note the abundance of branch root primordia.-x32

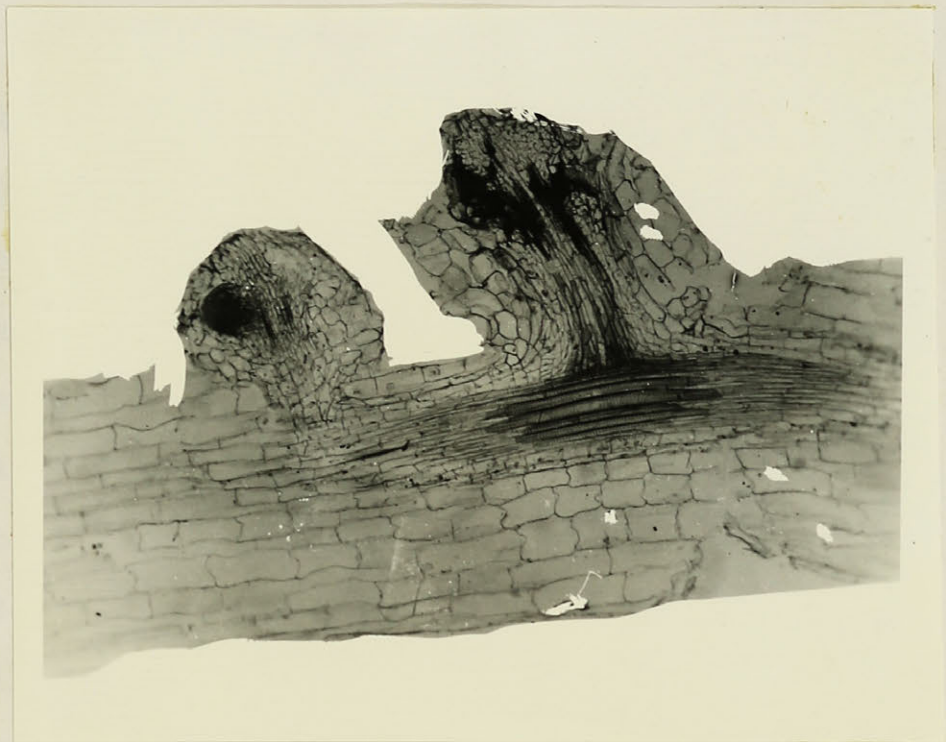

Fig. 18. Longitudinal section of a root tip of a plant grown without boron showing secondary abortive root primordia with tertiary primordia two of which are abortive X320.

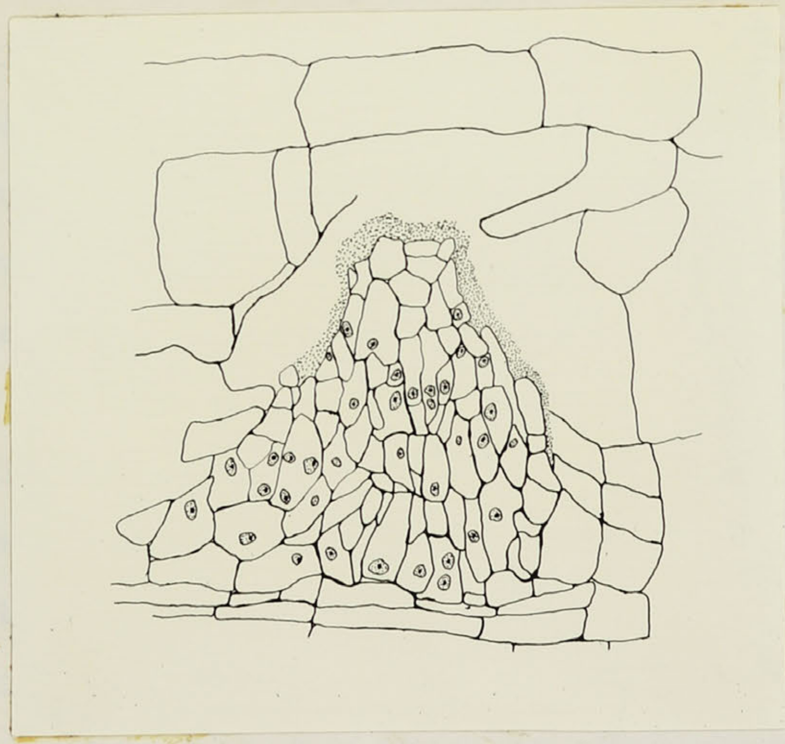

Fig. 19. Abortive branch root primordia. Note the abnormal shapes of the cells and the sheath of disintegrated tissue surrounding the primordia X500.

arrangement. At the very tip there was no distinction between the plerome and the periblem. The cells were hypertrophied and very variable in shape with nuclei smaller than normal (Fig. 16). The cells were not in definite rows and cell division appeared to have ceased, or if division took place it was in an abnormal fashion. A little way from the apex there was hypertrophy in the periblem which had matured very quickly (Fig. 15). Some cells were almost giant as compared with the normal ones. Nuclei in many cells had disintegrated as well as the protoplasm (Fig. 15). Early maturing of daughter cells of the plerome occurred resulting in the formation of tracheids unusually near the tip (Fig. 16). Tracheids were observed only twelve cell lengths removed from the tip. This feature was similarly met with, and particularly commonly so in branch root primordia, which were formed profusely and unusually near the tip (Fig. 16 and 17). The abnormal branch root primordia described above presented a squarish form with cells irregular in shape and size. (Fig 19), differing greatly from the rounded conical shape and size of normal branch root initials whose cells are arranged with precision and show a progressive increase in size from the apex backward. Sooner or later the diseased primordia became abortive, some of them ceasing growth before penetrating the cortex. They were usually surrounded by a sheath of disintegrated tissue, which stained intensely with safranin and gentian violet (Fig. 19 and 17).

Many examples were seen where an original root tip had stopped developing, and on each side two other branch roots had arisen to become quickly abortive in turn. This process was observed to occur to a tertiary degree (Fig. 18). A true root cap was usually lacking. (Fig. 16 and 17).

c. Diseased root tips from plants grown in a medium containing 0.25 p.p.m. of boron.

A large proportion of the root tips of plants grown in a culture solution to which 0.25 p.p.m. of boron had been added were definitely diseased, though from one-quarter to one-third of the total number of root tips on each plant of this series remained apparently quite healthy. Those root tips which were diseased, however, very closely resembled those from plants grown in absence of boron. Normal histogen arrangement was lacking. The region normally occupied by the meristems contained groups of cells irregular in shape and arrangement. These cells were larger than those of similar location in a healthy root, so that cell enlargement did not show the usual progressive trend from the apex to the region of vascular differentiation. Severe hypertrophy was apparent in the region normally occupied by the periblem. The unusual frequency of initiation of new branch root primordia was again strikingly evident, though cessation of growth of individual primordia often occurred at a later stage of development as compared with the same phenomenon in roots grown in absence of boron. A greater proportion of the branch roots attained complete emergence from the parent root before finally becoming abortive. The irregular formation of tracheids relatively close to the apex of diseased roots of this series was also apparent and to about the same degree as noted in plants grown without boron. The reader is referred to the photomicrographs taken from roots of plants grown without boron, since the features exhibited by both series of plants are similar (Fig. 15 to 19).

2. Branch roots above the point where secondary thickening occurs.

The roots examined for this phase of the present study were chosen randomly from any of the lateral roots whether arising from the primary root or hypocotyl. Sections were cut from a zone intermediate between the parent organ and the point where secondary thickening had commenced. In other words sections were cut in a region where secondary thickening had occurred.

a. Healthy roots from plants grown in a medium containing 2.0 p.p.m.

A great number of roots from plants grown in 2.0 p.p.m. of borón were examined individually. The roots were all healthy and orientation of tissues was essentially constant. The primary xylem of such roots was arranged in diarch fashion. A cambium layer developed on either side of this band of primary xylem and became continuous by extending through the pericyclic parenchyma adjacent to the protoxylem at the ends of the two primary rays. Secondary xylem matured in two groups, one on each side of the primary xylem tissue. In between the two groups of the xylem broad parenchymatous rays developed, being continuous from the ends of the primary xylem to the pericycle. A typical phloem

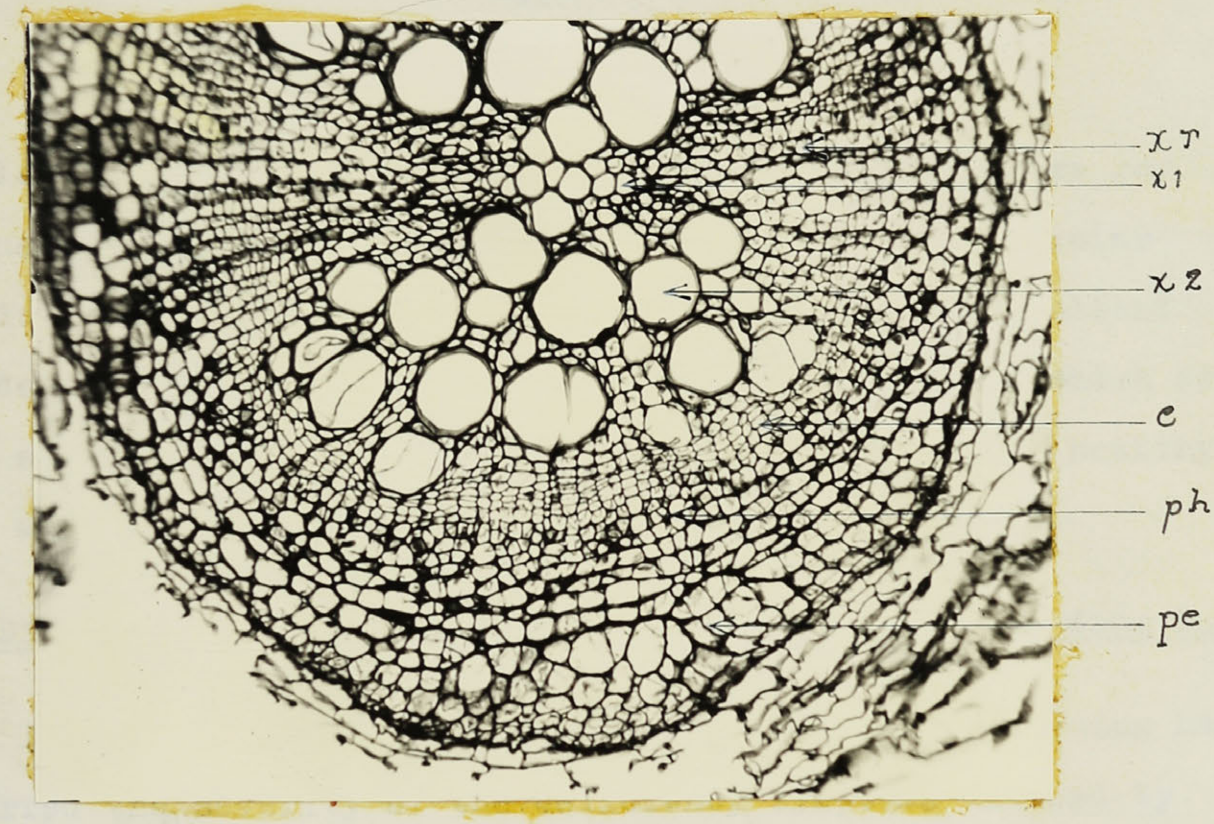

Fig. 20. Photomicrograph of a cross section of a healthy root: (x1) primary xylem, (x2) secondary xylem, (c) cambium, (ph) phloem, (pe) pericycle and (xr) xylem ray.-x100

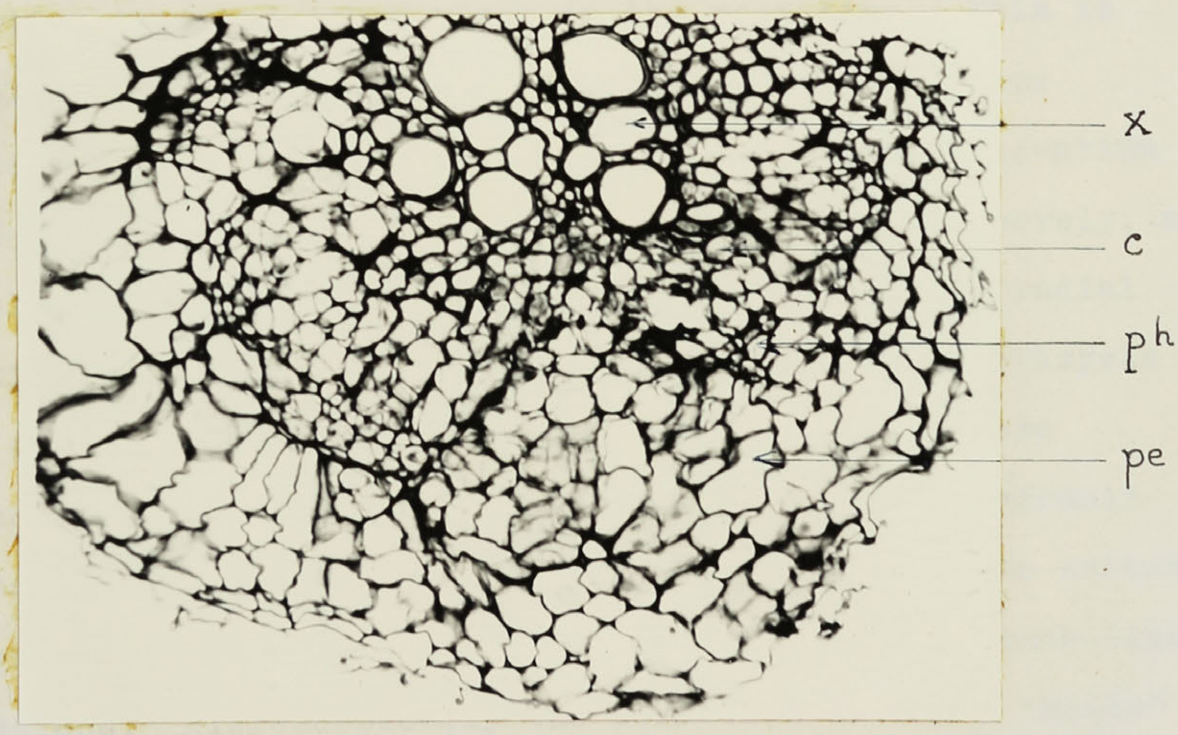

Fig. 21. Photomicrograph of a diseased root grown without boron in the nutrient: (x) secondary xylem, (c) cambium region, (ph) phloem and (pe) pericycle.-x100

developed on the outer side of the cambium except where ray tissue was formed. Shortly after initiation of a vascular cambium a phellogen arose in the pericycle. The resultant periderm leads to death of the cortex and endodermis which are soon sloughed off. A transverse section of a typical healthy root at this stage of development is shown in fig. 20.

b. Diseased roots from plants grown in a medium free from boron.

In those parts of the root where secondary thickening had occurred the severity of the deficiency symptoms induced by growth in a boron-free medium were not as great as might be expected, when we consider the extensive malformation and distortion that had taken place in the root tips. This is probably due to the fact that the more mature parts are meristematically much less active. The cells of the cambium and of the phloem were hypertrophied but not very severely, and cell elongation of these tissues tended to be in the radial direction. The injury was much more severe in the pericycle where strands of collapsed and disintegrated cells were frequently found. These strands stained purple in safranin and fast green in spite of continued washing to remove excess fast green. Frequently, also, the root had several knob-like protrusions. Microscopically the formation of these "knobs" was found to be due to a combination of hyperplasia and hypertrophy of groups of either phelloderm or pericycle cells. The hypertrophied cells of the phelloderm were elongated radially, while those of the pericycle were usually elongated tangentially. Sometimes restricted regions of disintegrated cells were found in the cambium and phloem regions but this was infrequent (Fig. 21). Commonly vessels of both primary and secondary xylem were partially or completely blocked.

c. Diseased roots from plants grown in a medium containing 0.25 p.p.m. of boron.

The roots from plants of this series did not show any boron-deficiency symptoms until they had attained a diameter of more than two millemetres. Roots of three millemetres in diameter indicated an abnormal and extensive proliferation of cells in the cambium region. This was the most striking feature. The cambium was replaced by a cylinder composed of from eight to ten layers of cells with their long axis orientated radially. The xylem appeared normal and resembled that of healthy roots except for the blocking of a large proportion of the vessels, the absence of thin-walled newly formed vessels, and for the smaller size of the vessels near to the cambium region. The phloem and pericycle were normal.

3. Storage "roots".

a. Healthy "roots" from plants grown for eight weeks in a medium containing 2.0 p.p.m. of boron.

The stage of development, size and general appearance of these plants are shown in fig. 1, 6 and 7. The "root" was somewhat thickened but had not yet started to become bulbous in shape. The thickening was due entirely to the formation of the primary and secondary plant bodies in a manner common to all plants.

The main anatomical difference between the storage "root" and the true root, just described, is that in the storage "root" the two segments of xylem vessels alternating with the broad parenchymatous rays are no longer visible. The cambium ring is complete and the xylem vessels are produced in definite rows alternating with xylem rays (Fig. 22). Opposite each row of xylem vessels there is a fascicle of phloem which consists of sieve tubes, companion cells and phloem parenchyma. The pericycle which is usually referred to as the cortex encloses the vascular stele and is composed of large regular cells. The epicotyl portion is similar to the other parts excepting for the presence of pith in the centre. The pith is made up of more or less isodiametric cells with conspicuous intercellular spaces.

b. Diseased "roots" from plants grown for eight weeks in a medium free from boron.

A cross section under the microscope showed that the xylem was practically normal excepting for the rather frequent reduction in size of the vessels and the crushing inwards of the walls of others. At times the vessels showed partial or complete blocking by some substance the nature of which is not known (Fig.23). Between the differentiated phloem and xylem, and occupying the cambium zone, was an extensive band all of which looked like abnormal parenchyma cells continuing to carry on cell division (Fig. 24). Little on no normal phloem could be found as the old phloem had been crushed (Fig. 24), and there was apparently no new normal phloem being formed. There was also a great deal of hypertrophy in the cortex. When the hypertrophy was very extensive there was no definite direction of elongation due apparently to crowding of cells against each other, but when it involved a comparatively few cells they usually elongated radially. Sometimes a band of disintegrated tissue more or less complete circumferentially was formed which seemed to result from the collapsing of many cells due very likely to the pressure exerted by the surrounding tissues; as well patches of disintegrated tissues occurred in cortex and to a less extent in the phloem and cambium (Fig. 24 and 23).

These conditions were to be found in the root, hypocotyl and stem portions of the storage "root", but were most pronounced in the root and hypocotyl.

c. Healthy "roots" from plants grown for four months in a medium containing 2.0 p.p.m. of boron.

These plants had bulbous "roots" four to five inches across, bore several large leaves and were very healthy in appearance. The "roots" had developed by this time a great deal of tertiary plant body.

The method of thickening of the hypocotyl and root which go to form the enlarged succulent portion of the swede is

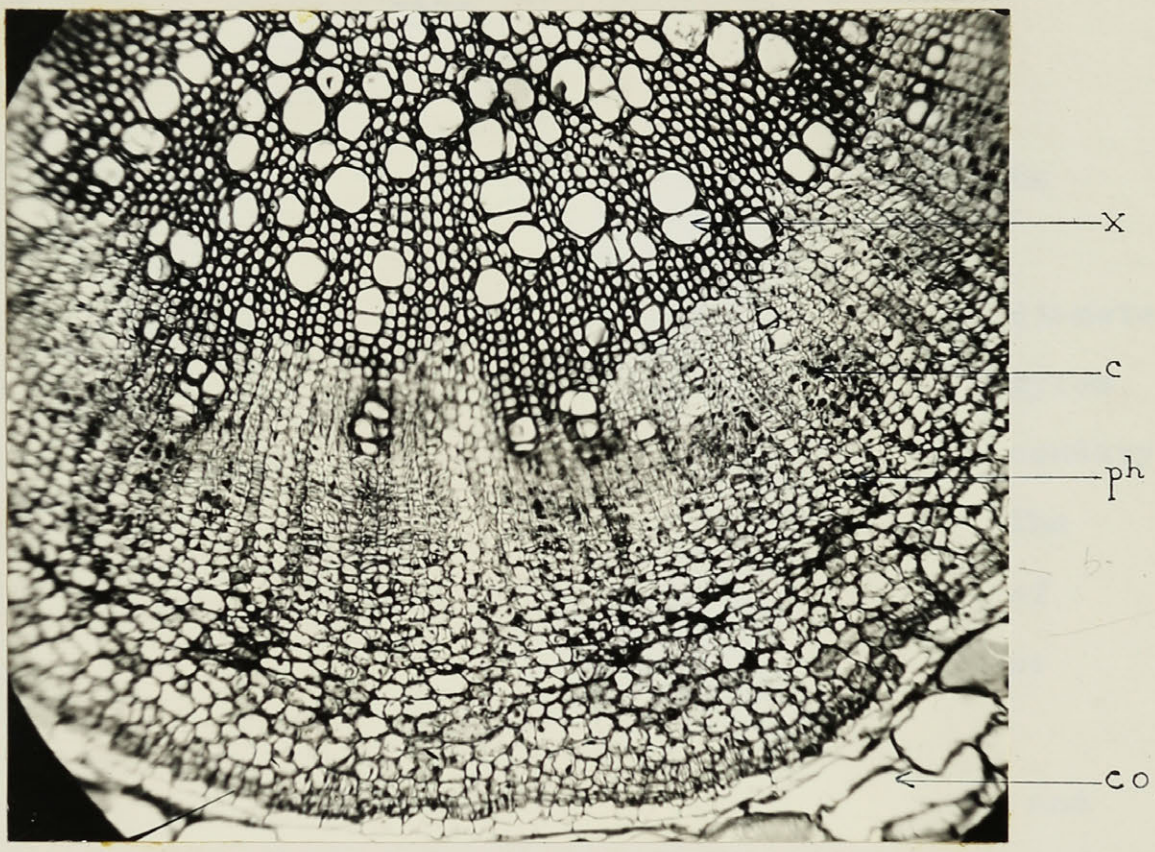

Fig. 22. Photomicrograph of a cross section of a healthy storage root: (x) xylem, (c) cambium, (ph) phloem, (co) cortex, and (p) periderm.-x65

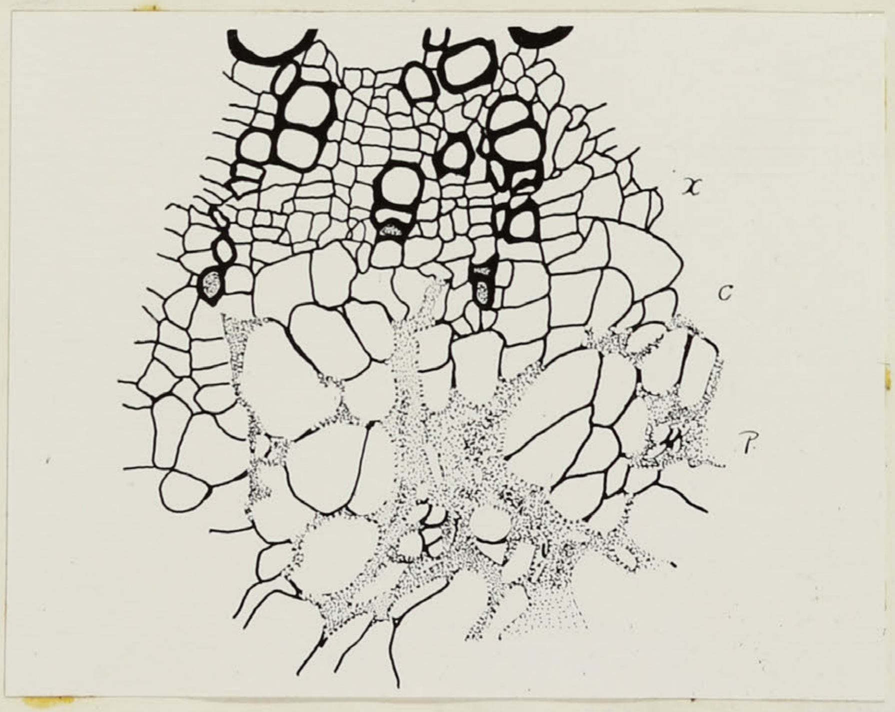

Fig. 23. Camera lucida drawing of a transection in a diseased storage root showing the small malformed and blocked vessels(x), and a severe stage of hypertrophy and disintegration in the cambium and phloem regions (c) and (p).-x215

unusual in many respects. Besides the normal action of the cambium ring forming secondary phloem on the outside and secondary xylem on the inside (Fig. 25), the increase in diameter is also due to cell divisions occurring in the secondary xylem parenchyma and to the formation of many small rings of secondary cambium from and within the secondary xylem parenchyma. The fascicular cambium forms on the inside a high proportion of xylem parenchyma with vessels in rows standing rather close together. For a time the newly formed cells of the xylem parenchyma remain small but later they become very active and cell divisions and rapid cell enlargement become a feature of this tissue. This results in a marked increase in the proportion of the xylem parenchyma in the vascular bundles and a much wider separation of the vessels. In this way the vessels become widely scattered through the xylem parenchyma singly or in very small groups. At the same time the parenchyma of the xylem portions of the rays laid down by the interfascicular cambium increases in extent in a similar manner causing a wider separation of the secondary xylem bundles (Fig. 27).

In addition the xylem parenchyma tissues give rise to small secondary cambium rings. These may begin to form anywhere in the xylem parenchyma and particularly within the newly formed parenchyma. They may originate within parenchyma cells located only a few cells inside the cambium. The secondary cambium ring gives rise to concentric bundles of which there may be very many (Fig. 25 CB and 28). Both amphivasal and amphicribral

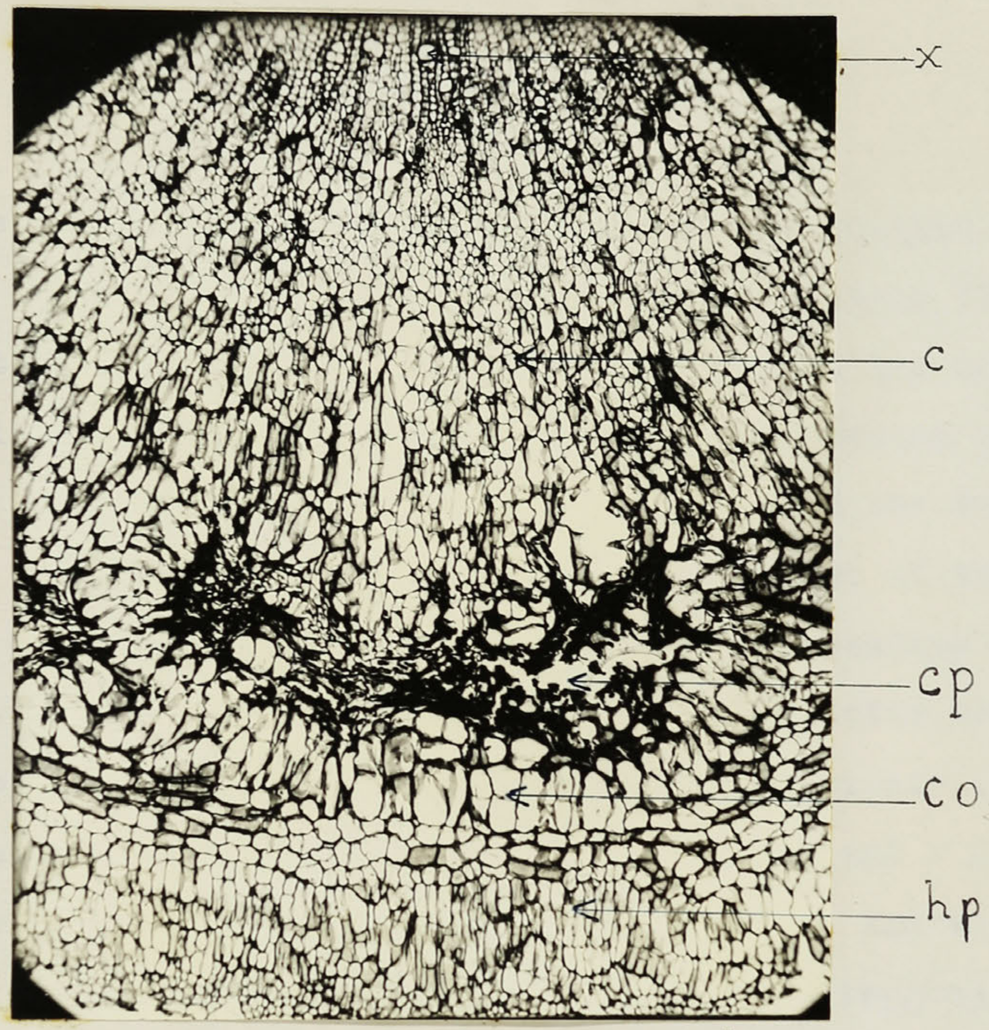

Fig. 24. Photomicrograph of a cross section in a diseased storage root eight weeks old: (x) xylem, (c) cambium region, (cp) crushed phloem, (co) cortex and (hp) hypertrophied phelloderm.-x65

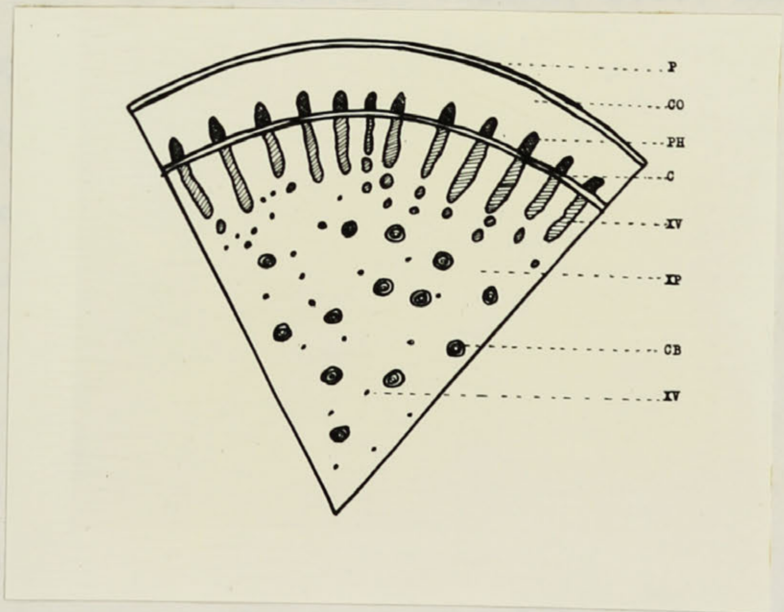

Fig. 25. Diagrammatic drawing of sector of swede "bulb" or "root" in cross section:(P) Periderm and bark, (CO) cortical region, (PH) phloem, (C) cambium, (XV) xylem vessels, (XP) xylem parenchyma and (CB) collateral bundle.

bundles are formed but the former are much more abundant. The phloem and xylem of the bundles are of course tertiary tissues. The concentric bundles show up in cross section to the naked eye as small whitish areas usually with a darker centre scattered throughout the flesh of the swede (Fig. 26). Thus the tissues inside the cambium ring, excluding the small amount of the primary plant body are made up of a high proportion of secondary parenchyma (Fig. 28 a) greatly increased by its own mitotic activities, a small proportion of scattered secondary xylem vessels (Fig. 28 xv), some tertiary phloem, some tertiary xylem vessels and a good deal of tertiary xylem parenchyma due to both secondary and tertiary thickening of the fleshy axis. Thickening in the regions outside the cambium is normal. Secondary and tertiary thickening are also features of other members of the mustard family or Cruciferae such as the turnip, radish, etc., and has been described by Hayward (1938) and Soeding (1924). The formation of the concentric bundles in swedes does not occur until the eighth week of growth.

The fleshy edible portion of the swede is made up partly by the epicotyl, partly by the hypocotyl and partly by the root. It is difficult to decide how much each part contributes to the formation of the fleshy axis, since this varies with varieties and with environmental factors. The hypocotyl,however, is usually considered to be more root-like than stem-like though it is a transitional region. The part devoid of lateral roots, or practically so, is usually considered as the hypocotyl while

Fig. 26. "Roots"of swedes showing various patterns of brown-heart as it occurs in the field, and the presence of concentric bundles in between which brown-hearted areas occur, which gives the mottled appearance.

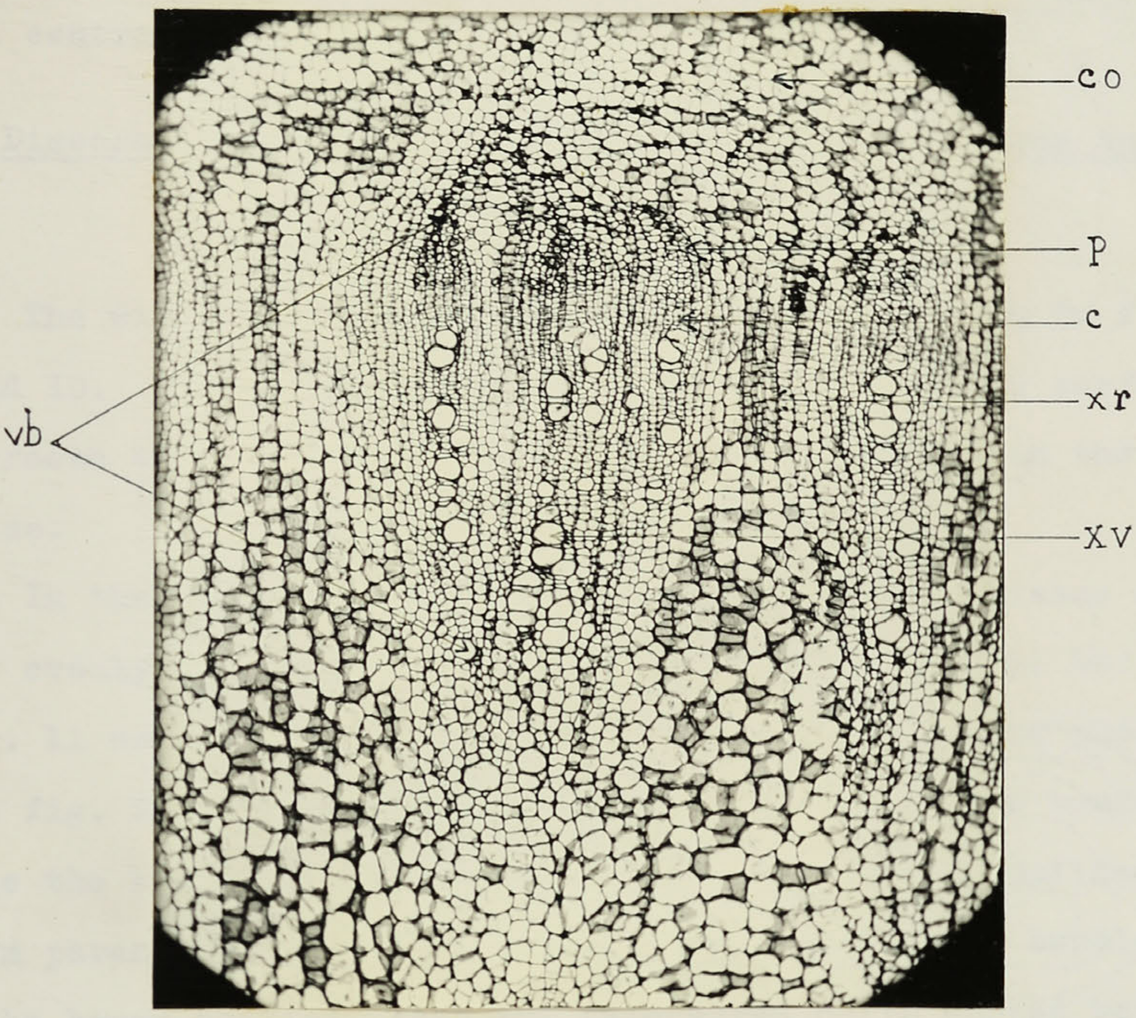

Fig. 27. Photomicrograph of a cross section in the storage root of a normal swede four months old: (xp) xylem parenchyma, (vb) vascular bundle, (xv) xylem vessels, (xr) xylem rays, (p) phloem, (c) cambium and (co) cortical region. This section shows the cambium and the tissues formed from it (secondary thickening).

the lower part from which arise lateral roots is the true root. The stem or epicotyl portion is determined easily by the presence of a central pith (Fig. 13).

d. Diseased "roots" from plants grown for four months in a medium containing 0.25 p.p.m. of boron.

The external features of these plants are shown in fig.8, 9 and 10. They showed reduced growth, rough cracked surfaces of the roots and strong foliage symptoms particularly in the outer leaves.

In the "roots" a great deal of brown-hearted tissue occurred very evenly distributed throughout the root hypocotyl and stem. (Fig. 11 and 12). It is interesting to compare these photographs with fig. 26, which shows brown heart in field grown swedes. While the brown heart was very severe it remained confined to the xylem parenchyma and did not involve the concentric bundle tissues. In the brown-hearted areas the parenchyma cells showed both hyperplasia and hypertrophy, the latter being more common (Fig. 29 a and b). The hypertrophy usually resulted in elongated cells though not always by any means. Usually it was found that groups of cells were elongated in the same direction often forming a more or less regular but abnormal tissue (Fig. 29 a). Almost as frequently, however, the cells had elongated in any direction whatsoever. It was frequently observed that in a rectangular-shaped area the long axes of the hypertrophied cells were at right angles to the long axis of the brown-hearted area.

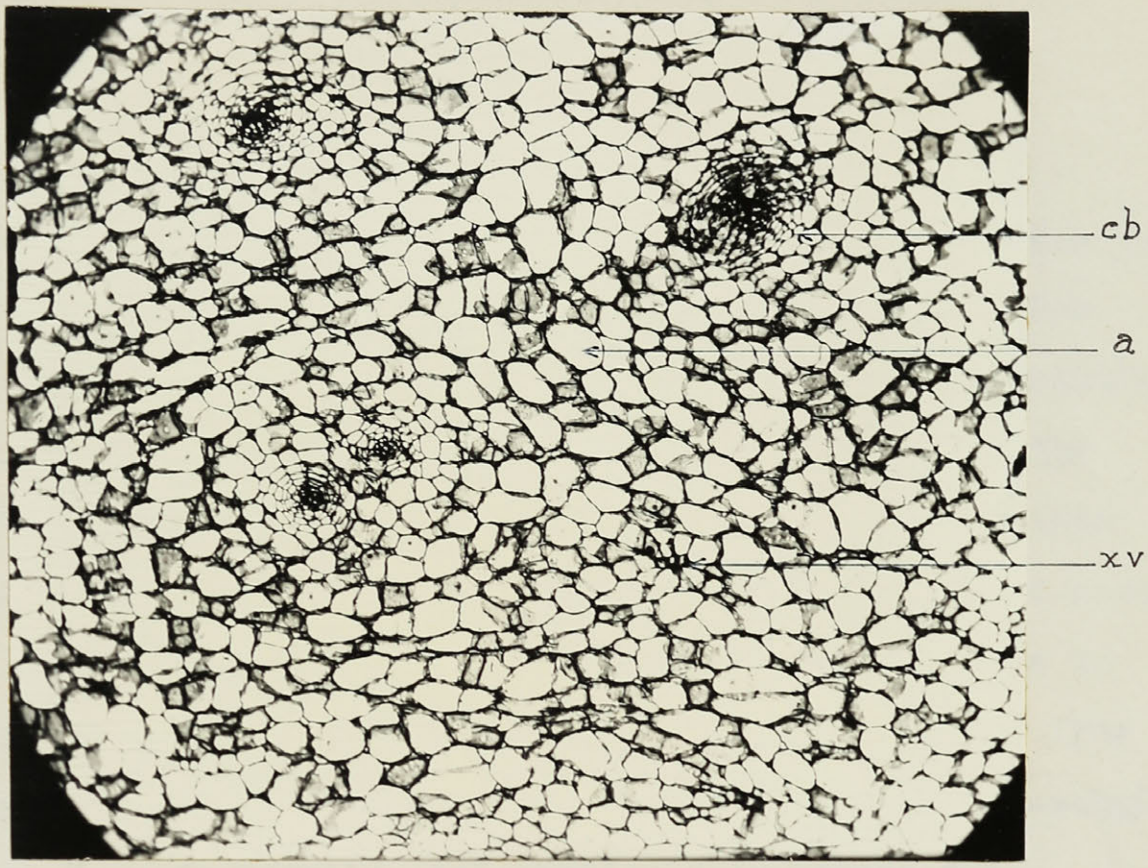

Fig. 28. Photomicrograph of a cross section in the central part of a normal fleshy root grown for four months with 2.0 p.p.m. in the nutrient solution: (a) xylem parenchyma, (cb) collateral bundle and (xv) secondary xylem vessels.-x40

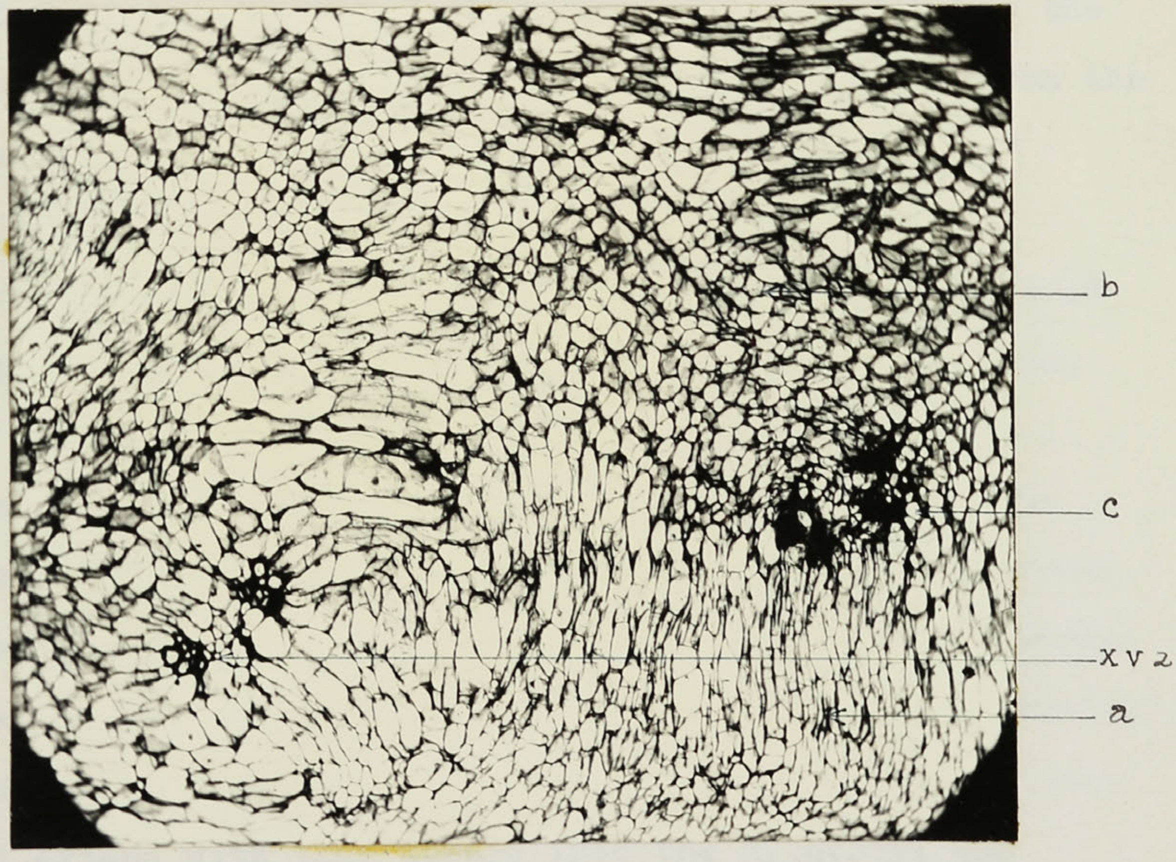

Fig. 29. Photomicrograph of a cross section in central part of a fleshy root grown for four months with 0.25 p.p.m. in the nutrient solution showing a brown-hearted area: (a) group of cells elongated in the same direction, (xv2) secondary xylem vessels, (b) hyperplastic parenchyma and (c) blocked secondary xylem vessels surrounded by disintegrated cells.-x40

On the other hand if the diseased area was irregular in shape the cells did not show this definite directional elongation. The hyperplasia and hypertrophy caused occlusion of the intercellular spaces, which accounts for the water-soaked appearance of the brown-hearted areas. The concentric bundles were smaller than those found in the normal plants, but otherwise they looked normal. There was some plugging of secondary xylem vessels but none in the tertiary xylem. The rows of xylem vessels last formed from the cambium were normal and surrounded by normal xylem parenchyma (Fig. 30a). Very frequently, and particularly around the groups of secondary xylem vessels which were blocked, there were present small groups of disintegrated cells, which stained densely (Fig. 29c). Another feature commonly met with was the crushing together of several cells resulting apparently from the pressure exerted by the surrounding hypertrophied cells of hyperplastic tissue. Secondary xylem vessels were usually embedded in these groups of crushed cells. The crushed groups of cells appeared to be quite extensive and often formed long streaks in the xylem parenchyma.

A broad, sharply delimited band of hypertrophied tissue was found replacing the cambium (Fig. 30c). This band appeared brown and water-soaked to the naked eye (Fig. 12). The cambium was at times completely altered in this way both circumferentially and longitudinally. It seemed always to begin in the true root portion and extend with time upward into the hypocotyl and finally the epicotyl portions. The cells of this zone, seen in

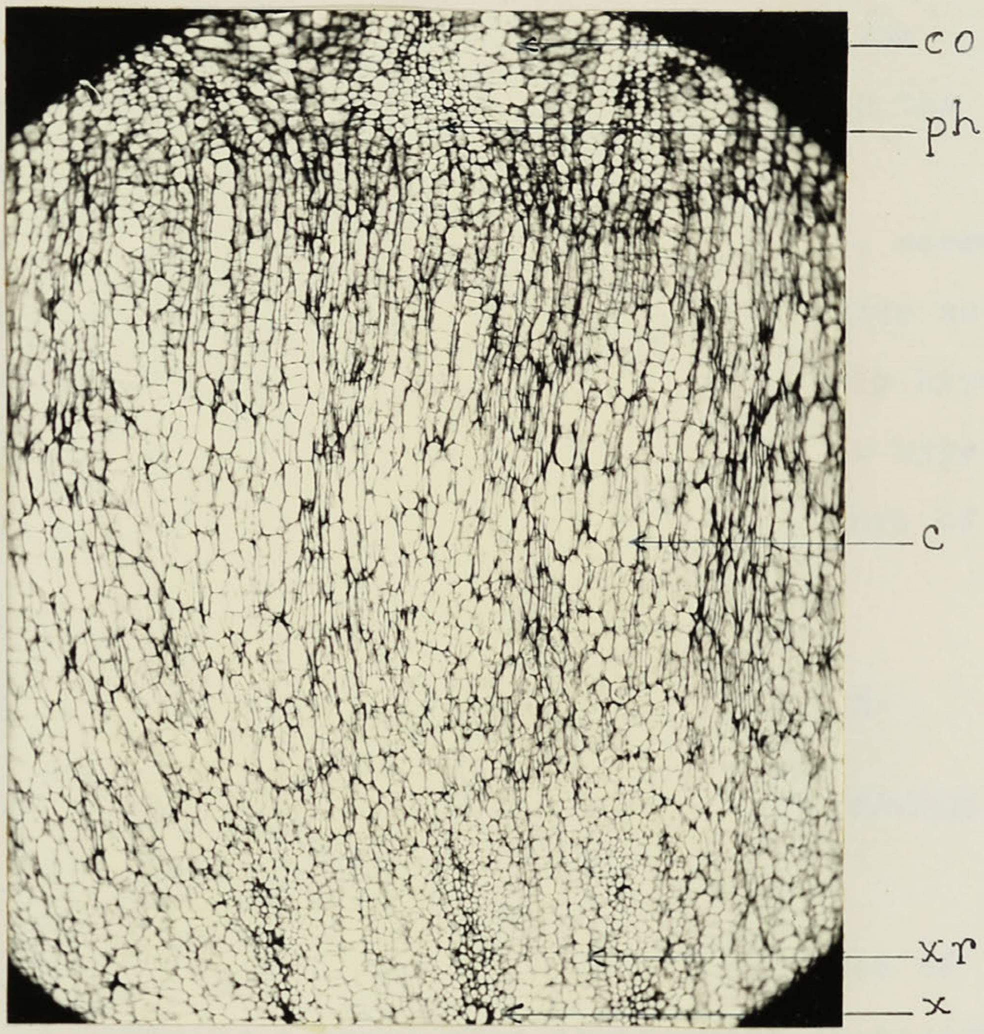

Fig. 30. Photomicrograph of a cross section in a storage root of a swede grown for four months in a nutrient solution with 0.25 p.p.m. of boron: (x) xylem vessels, (xr) xylem rays, (c) hypertrophied cambium region, (ph) phloem and (co) cortex.-x40

transection were elongated radially. In tangential view they were roundish in shape instead of being elongated in the longitudinal direction of the axis of the "root" as in the normal plant (Fig. 30).

The cortex and the phloem bundles embedded in it, seemed normal (Fig. 30 co and ph). In addition to the features noted above the pith cells in the stem were also considerably hypertrophied leaving almost no intercellular spaces. This hypertrophy probably accounts for the water-soaked appearance of the pith.

B. Comparative Anatomy of Diseased and Healthy Leaves.

a. Healthy leaves from plants grown in a medium containing 2.0 p.p.m. of boron.

The petioles had a vascular system which consisted of collateral bundles having a horse-shoe arrangement in accordance with the form of the petiole (Fig. 31). Each bundle was made up of four to eight units separated by parenchymatous rays. The bundles were crescent-shaped with the open arc directed toward the adaxial surface of the petiole (Fig. 34A). In the upper part of the midrib and the smaller veins the vascular system was reduced to one large bundle. The cambium was well defined in the larger veins and gave rise to some secondary vascular tissues. The phloem contained sieve tubes, companion cells and parenchyma, and was bounded on the outside by a zone of mechanical tissue, the bundle cap (Fig. 36 bc). The parenchyma

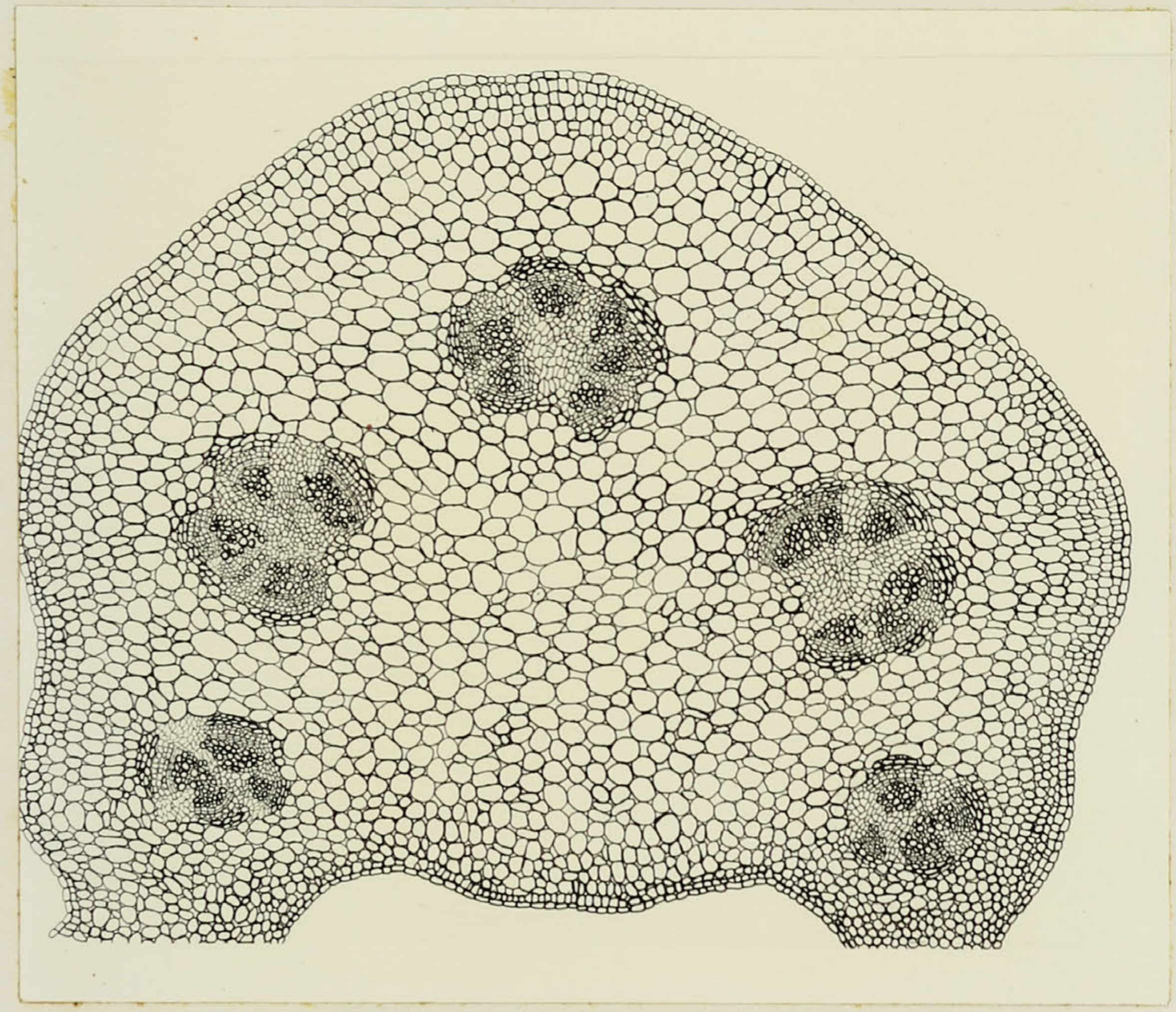

Fig. 31. Projection drawing of a cross section in a normal petiole showing the location of the sets of vascular bundles in the ground parenchyma.-x25

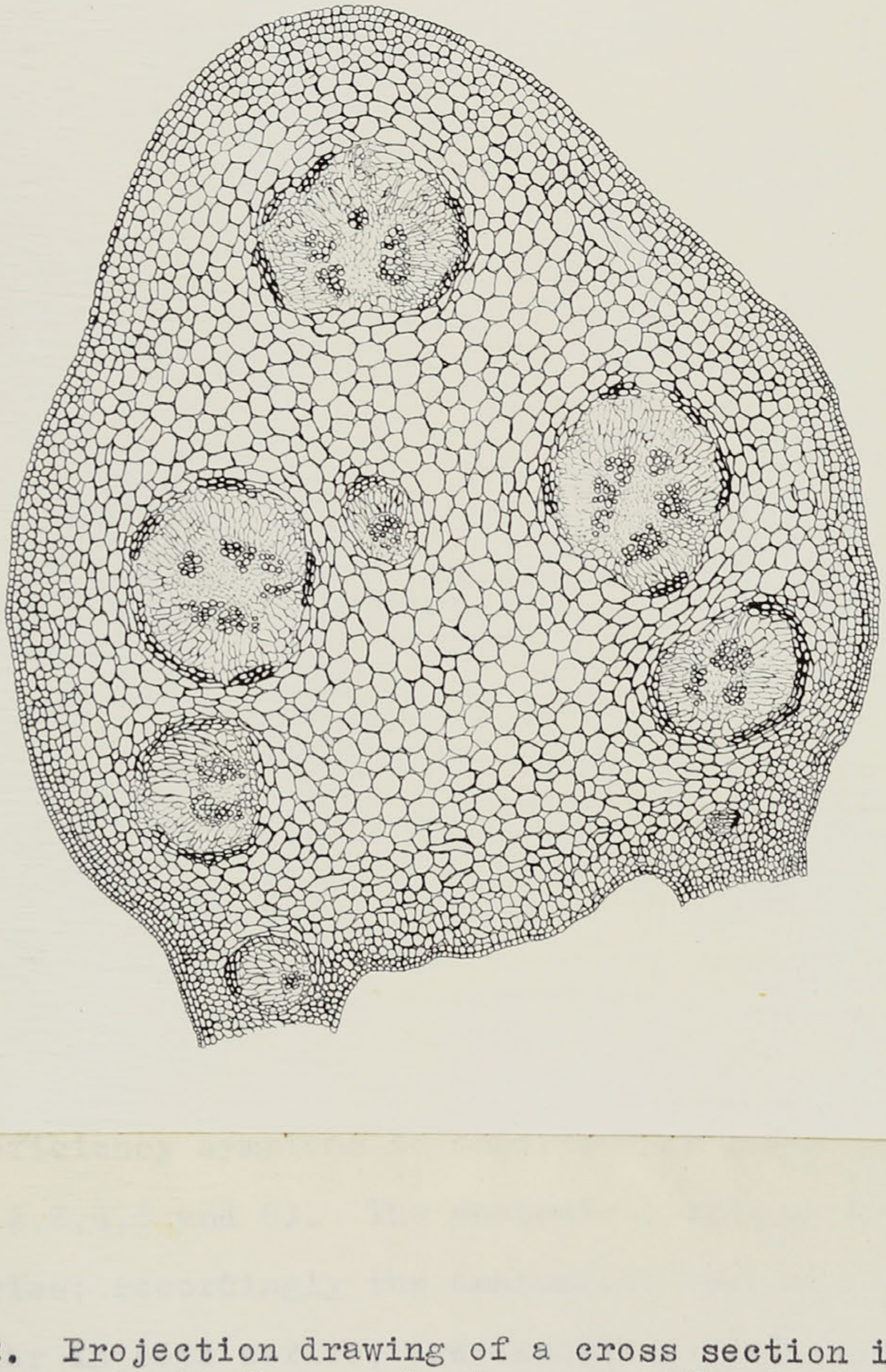

Fig. 32. Projection drawing of a cross section in a diseased petiole from a plant grown without adequate supply of boron. Note the hypertrophy of the vascular bundles and the radial elongation of cells.-x25

cells adaxial to the bundle may also be considered as mechanical tissue, for they were thick-walled. The vessels were large 10 to 30 in number, with 20 to 25 as a mean for each bundle, and round or somewhat angular.(Fig. 36).

The remaining tissues of the petiole were parenchymatous. The subepidermal layers of cells contained chloroplasts; the cells were round with very conspicuous intercellular spaces (Fig. 31).

The upper epidermis of the normal blade was made up of regularly shaped rectangular cells, while the lower epidermis included a certain number of almost square cells, as seen in transection. The palisade parenchyma was composed of two or three layers of cells most of which were distinctly cubical. The spongy parenchyma consisted of roundish cells and was provided with large air spaces (Fig. 33).

b. Diseased leaves from plants grown in a medium free from boron for eight weeks, and from plants grown in a medium containing 0.25 p.p.m. of boron for four months.

The foliage of these plants showed very pronounced boron-deficiency symptoms as described on pages 21 and 23 (Fig. 1,2,3,4,5 and 8). The anatomical changes were similar in both series; accordingly the anatomical changes, which proved to be similar in both series, are described below together.

There was a direct correlation between the degree of development of the external and internal symptoms. The first and most

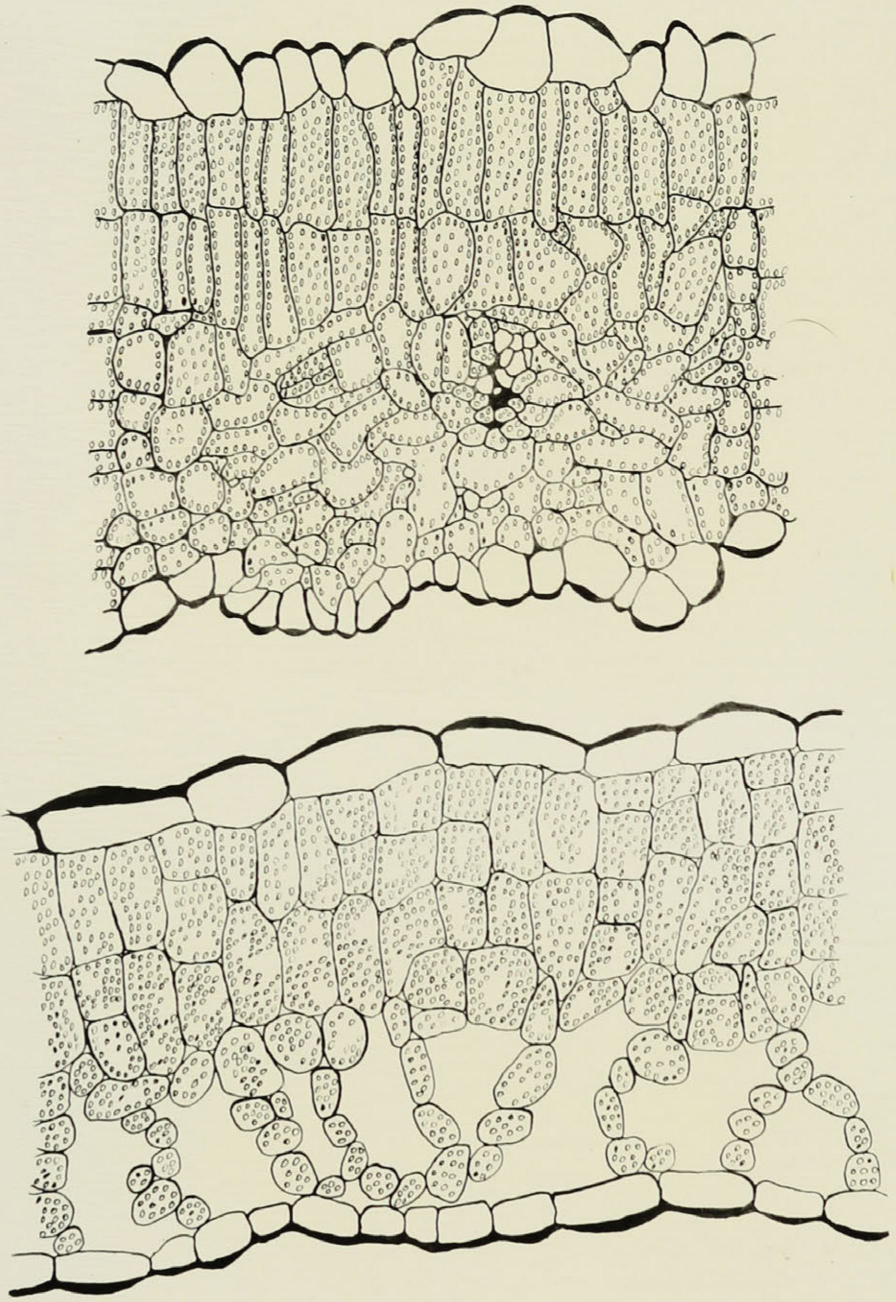

Fig. 33. Camera lucida drawing of a cross section in the leaf blade of a diseased plant (above) and healthy (below). Note the irregular epidermis, the elongation of the palisade cells, the spongy parenchyma made up of closely packed cells and the absence of intercellular spaces in the diseased leaf.-x175

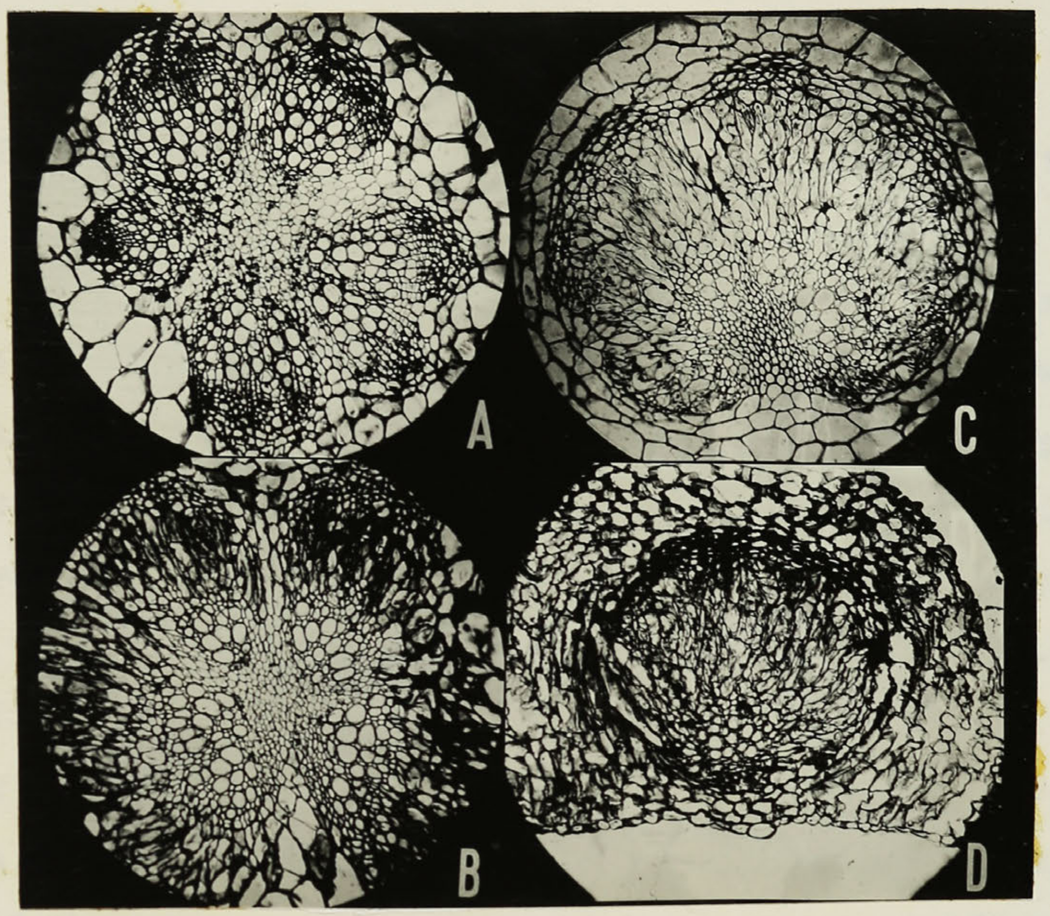

Fig. 34. Photomicrograph showing similar features as fig. 32, 36 and 37. (A) Normal vascular bundles in midrib, (B) corresponding diseased vascular bundles in midrib, (C) diseased vascular bundle in large vein and (D) diseased vascular bundle in small vein.-x32

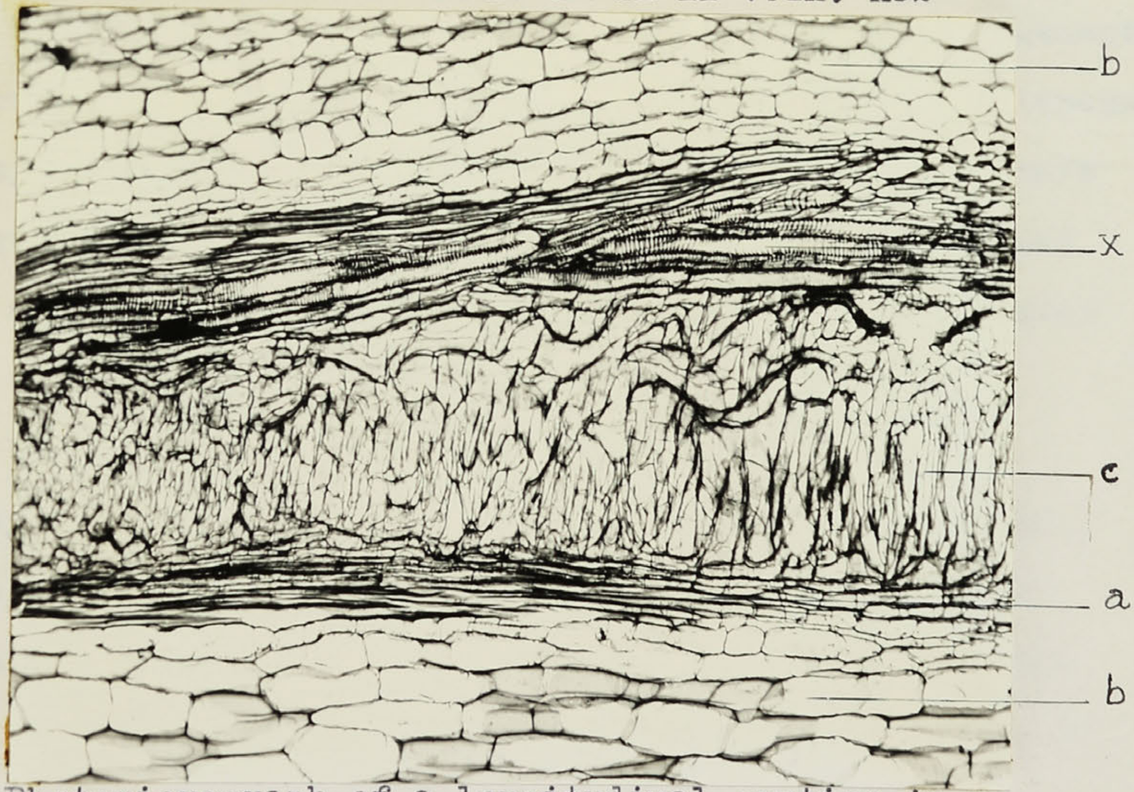

Fig. 35. Photomicrograph of a longitudinal section in a diseased vascular bundle showing the hypertrophied area in the cambium and phloem region. Note the radial elongation of the cells: (a) bundle cap (c) cambium and phloem region, (x) xylem vessel and (b) ground parenchyma.-x50

severely involved leaves, for at least quite a time, were the outermost ones. The leaves became involved progressively inward to the growing point. Thus on some plants the inner and younger leaves might be normal while the outer leaves were very heavily deranged with the intermediate leaves in the process of developing the various anatomical changes. Thus on a single plant it was possible by examining the leaves of various ages to follow the development of these various anatomical changes.

The first easily observed anatomical alteration was a thickening of the leaf blades. This was due to an increase in the length of the palisade cells and an increase in the number of cells of the spongy parenchyma, which was composed of closely packed cells irregular in shape leaving a few small intercellular spaces. Some of the cells were very large and elongated in one direction, but there did not seem to be any definite arrangement. These alterations probably accounted for the unusual brittleness and rigidity of the diseased leaves. The epidermal cells were quite irregular, some being sunken, some being elevated above the others and some with their outside walls at various angles (Fig. 33).

It is believed that the thickening of the lamina was preceded by a certain amount of blocking of the sieve tubes. There was always an accumulation of starch in the thickened leaves which indicates that something had happened to prevent the movement of carbohydrates away from the leaf. Blocking of the sieve tubes might account for this.

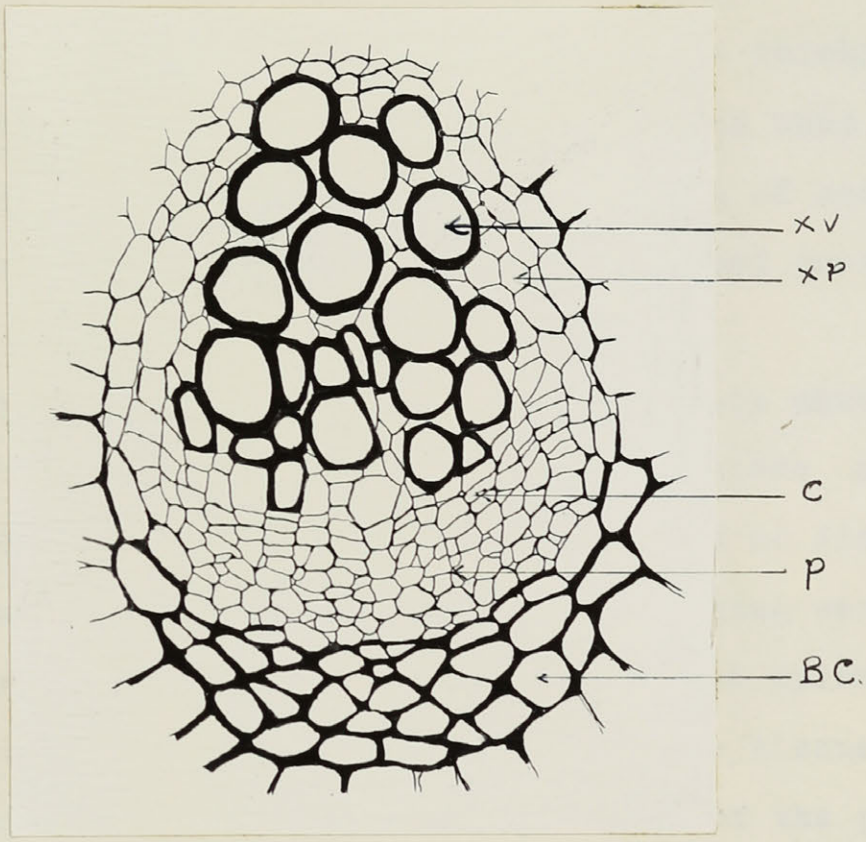

Fig. 36. Camera lucida drawing of a normal vascular bundle; (xv) xylem vessel, (xp) xylem parenchyma, (c) cambium, (p)phloem and (bc) bundle cap.-x215

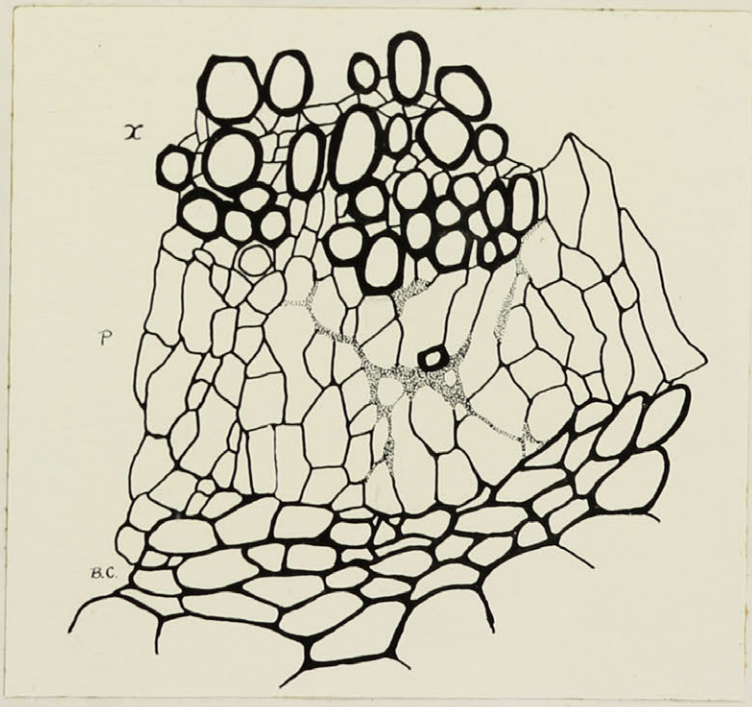

Fig. 37. Camera lucida drawing of a vascular bundle in a petiole of a plant grown without boron; (x) xylem vessel, (p) cambium and phloem region showing cell enlargement and small patches of disintegrated tissue, and (bc) bundle cap.-x215

Measurements revealed that the smallest thickness of healthy leaves was 275 microns while diseased ones measured 372 microns. The largest measurements taken of healthy leaves was 421 microns while the largest for diseased ones was 550 microns.

In a very slightly diseased leaf the only anatomical changes to be observed were thickening of the leaf blade and some blocking of sieve tubes. Such leaves showed no discoloration or any abnormal appearance. The next happening was development of hyperplastic tissue between the phloem and xylem of the vascular bundles. Most of this hyperplastic tissue soon became hypertrophied and caused a wide separation of the phloem and xylem. Later the phloem parenchyma might also become hypertrophied. (Fig. 32, 34B, C, and 37). These events often lead to crushing of the sieve tubes. In the heavily diseased bundles disintegration in the hypertrophied tissues was quite common. Usually disintegration followed hypertrophy (Fig. 37 and 39), but also it did occur in cells which had previously not enlarged (Fig. 40). In a few exceptional cases hyperplasia and hypertrophy occurred in the xylem causing an unusually wide and scattered distribution of the vessels (Fig. 38).

Disintegration was observed to begin with a breaking down of the cell walls, followed by breaking down of the protoplasm (Fig. 37). The final result was a mass of formless deep-staining material with no discernable parts of the cells remaining (Fig. 39 and 40).

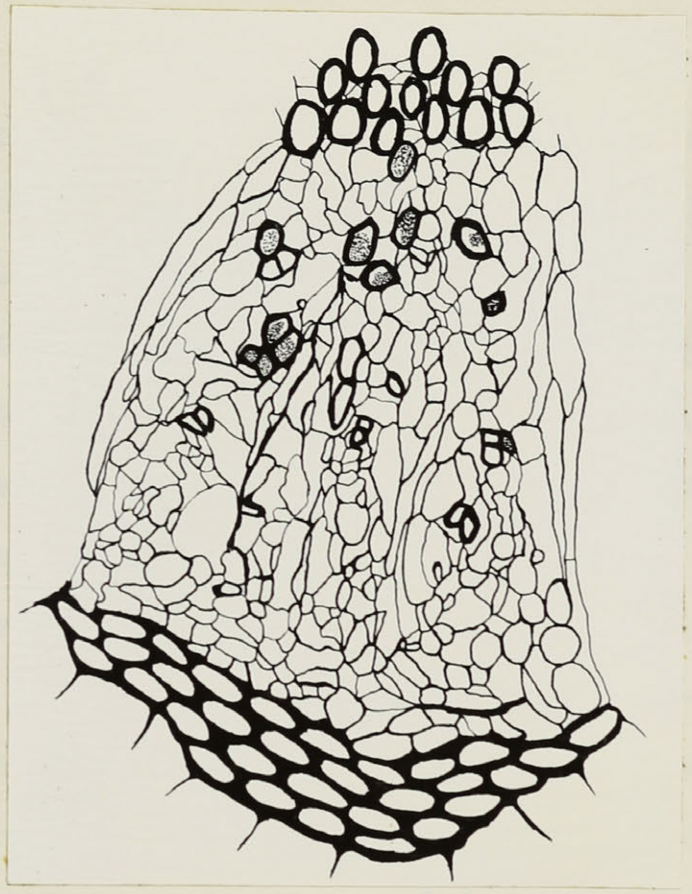

Fig. 38. Camera lucida drawing of a diseased vascular bundle in a midrib showing hyperplasia and hypertrophy. Note the xylem vessels scattered in the abnormal tissue. Some are malformed and blocked with some substance.-x215

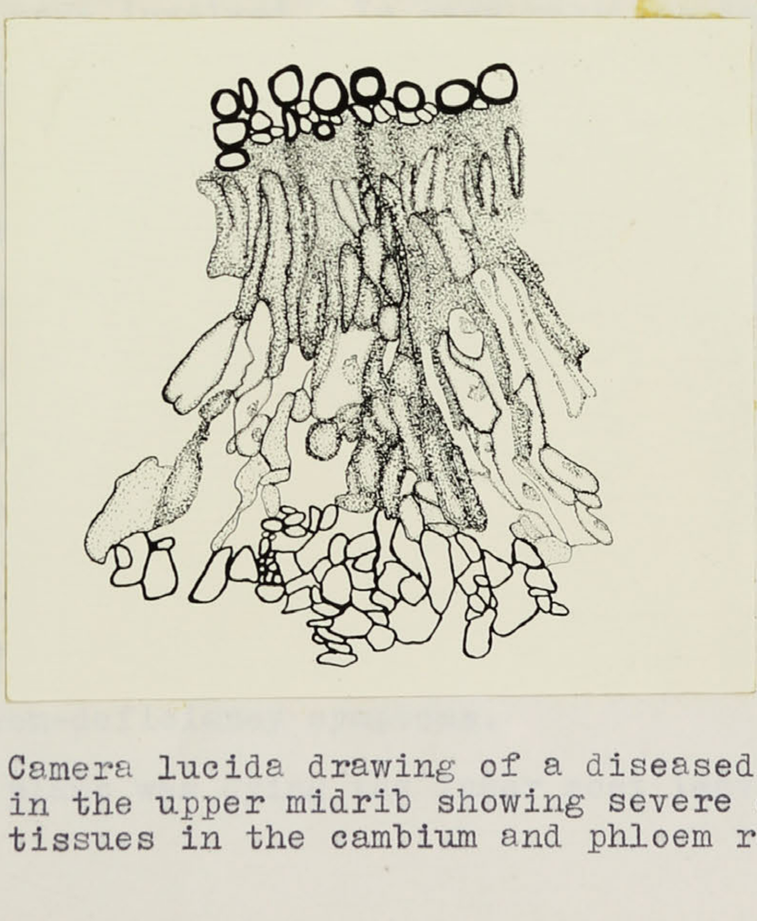

Fig. 39. Camera lucida drawing of a diseased vascular bundle in the upper midrib showing severe disintegration of tissues in the cambium and phloem region.-x215

In transections of the diseased bundles it was observed that the hypertrophied cells were at first radially elongated (Fig. 34B and C), but later this regularity was lost due apparently to their continual growth leading to extreme crowding against each other resulting in the crushing of some (Fig. 34D and 37) The radial elongation of cells is well evidenced by a longitudinal section of a diseased vascular bundle (Fig. 35). The points at which these alterations in the bundles began to show were in the midribs and veins always from about one-quarter to one-half an inch back from the tips. From this point they developed downwards towards the lower parts of the veins and midribs, and finally in the severely diseased leaves extended downwards to the base of the petioles. However, frequently not all of the bundles at the same level in a large vein, in the midrib, or in the petiole were involved. In many petioles the two opposite corner bundles were the only ones showing any abnormalities. These are the bundles leading to the two lowermost lobes (or leaflets) of the blade. Whenever this situation was found these two leaflets were always very much diseased. Symptoms in the veins were apparently the same as in the midribs for a corresponding length measured from the tips of each.

It seems evident that the three stages, that is hyperplasia, hypertrophy and disintegration develop in this order of sequence and that disintegration represents the most advanced stage of internal boron-deficiency symptoms.

When a plant was dying the inner most leaves and the bud turned black and withered. Internally these parts show the most severe stage of injury, that is disintegration which had occurred without any previous hyperplasia or hypertrophy. At the base of the petiole a crescentic band of disintegrated tissue was complete across the petiole and involved all the vascular bundles and the parenchyma tissue adaxial to them (Fig. 40). In the higher parts of the midrib the whole leaf was reduced to a thin band of disintegrated tissue. The bud presented the same appearance as the innermost leaves. No normal cells could be distinguished in these buds excepting for a few vessels which had kept their general outline.

The xylem tissues as a general rule did not seem to suffer very much from the lack of boron. The xylem vessels in the great majority of the cases looked normal with regard to their situation. Where hypertrophy had occurred they were as a general rule smaller near the cambium and generally very much so. No young unlignified vessels could be seen as in healthy bundles. This situation also held for the basal part of the petioles where no hypertrophy had occurred. The vessels were also less in number, the range found being 5 to 25 with a mean of 12 to 18. It seems probable that when boron-deficiency occurred no more normal vessels were formed and that the normal differentiation ceased.

Another common happening, but usually of later development, was the formation of galls due to hypertrophy of cells of the ground parenchyma in restricted areas in the petioles, midribs and

Fig. 40. Photomicrograph of a cross section in a diseased inner leaf which was brown. Note the complete band of disintegrated tissue involving all the vascular bundles. Disintegration took place without hypertrophy.-x64

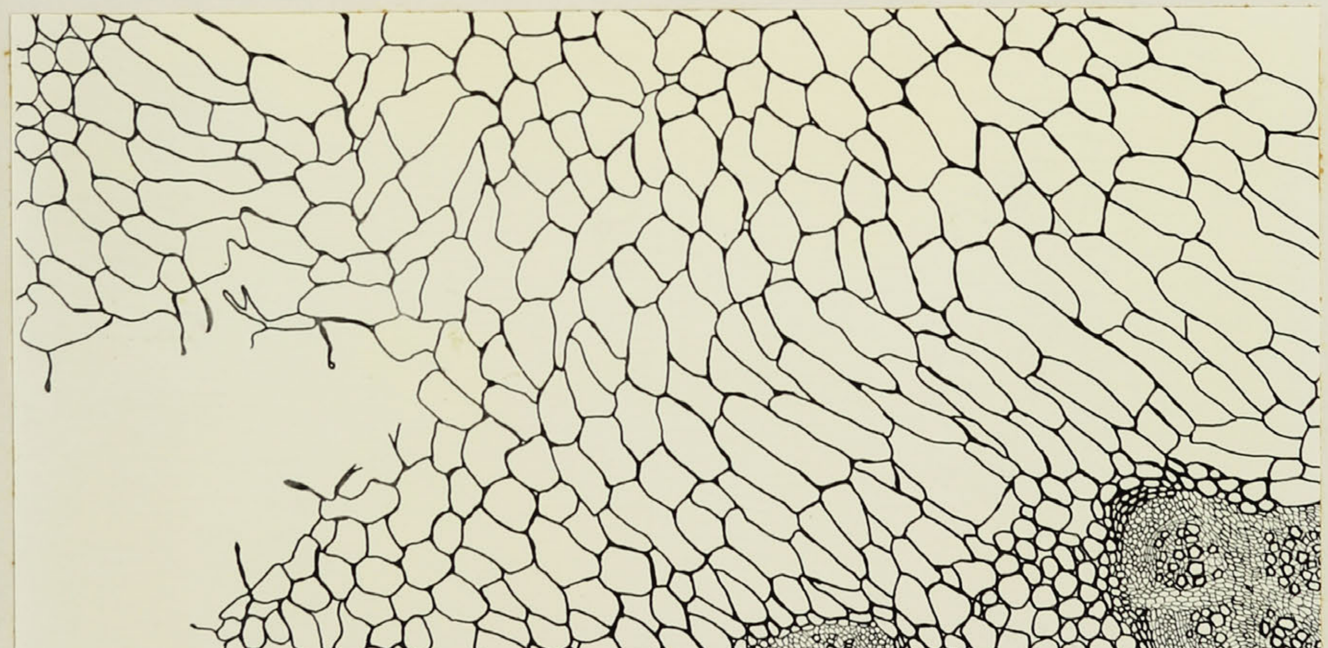

Fig. 41. Projection drawing of a cross section in a petiole at a level where a gall accompanied by splitting was present. Note the hypertrophied cells of the ground parenchyma, the splitting of the epidermal and hypodermal tissues and the apparently healthy condition of the vascular bundles.

veins. The cells became elongated being more or less rectangular and leaving no intercellular spaces or very little (Fig. 41). Disintegration of the cell walls resulted in brownish patches. The galls and splitting along the petioles and midribs were due to the hypertrophy of the pith and cortex cells only and their formation was not related to the condition of the vascular bundles. These galls occurred at the base of the petiole where no hypertrophy within the bundle was apparent, while in other places where there were no galls the phloem was much hypertrophied and the pith was normal. Even one millimetre above a gall where no hypertrophy of the pith was evident there might be typical enlargement of the phloem cells.

II Studies in the Comparative Anatomy of Healthy and Boron-Deficient Celery.

Celery plants were grown in the greenhouse according to the methods already described. At the end of about 10 weeks the plants grown in boron-free sand were beginning to die and had withered buds the plants had a chlorotic appearance and were small. The petioles remained small, had brown streaks along the ridges on the abaxial surface, and small brown flecks were found between the ridges. Typical lesions of cracked-stem similar to those found under field conditions had also developed on the petioles but they were not numerous. The lesions were crosswise cracks formed by longitudinal strips of epidermal and subepidermal tissue curling outward.

The blades of the leaflets were chlorotic and brittle but not to the same extent as the leaves of swedes suffering from severe boron-deficiency. The innermost embryonic petioles became dark brown, withered and died in the characteristic manner of boron-deficient plants. The roots presented the typical brownish water-soaked and stunted appearance observed on other plants. The root tips were dark brown, enlarged and stunted, and developed branch roots abnormally near the apex.

The plants grown in sand with 2.0 p.p.m. of boron. though not much larger, were green and looked healthy.

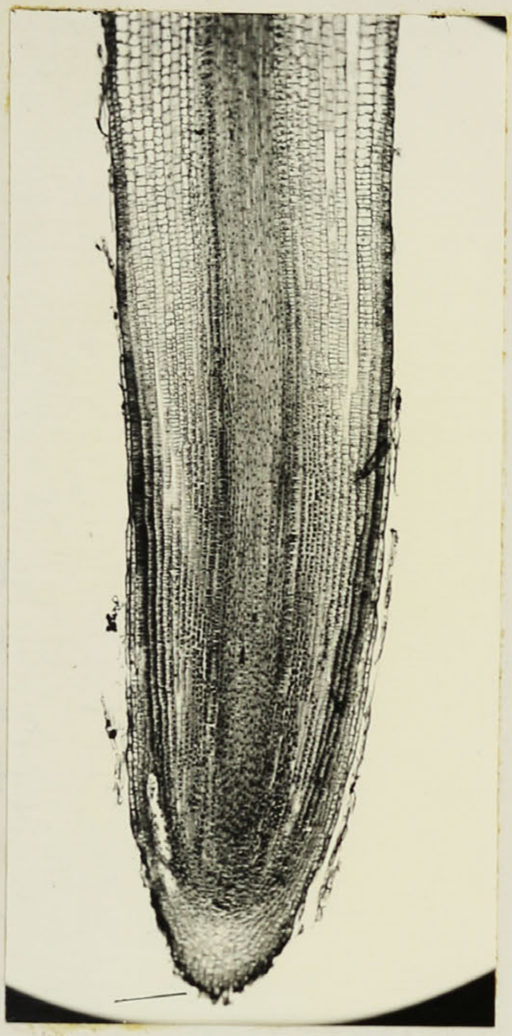

Fig. 42.

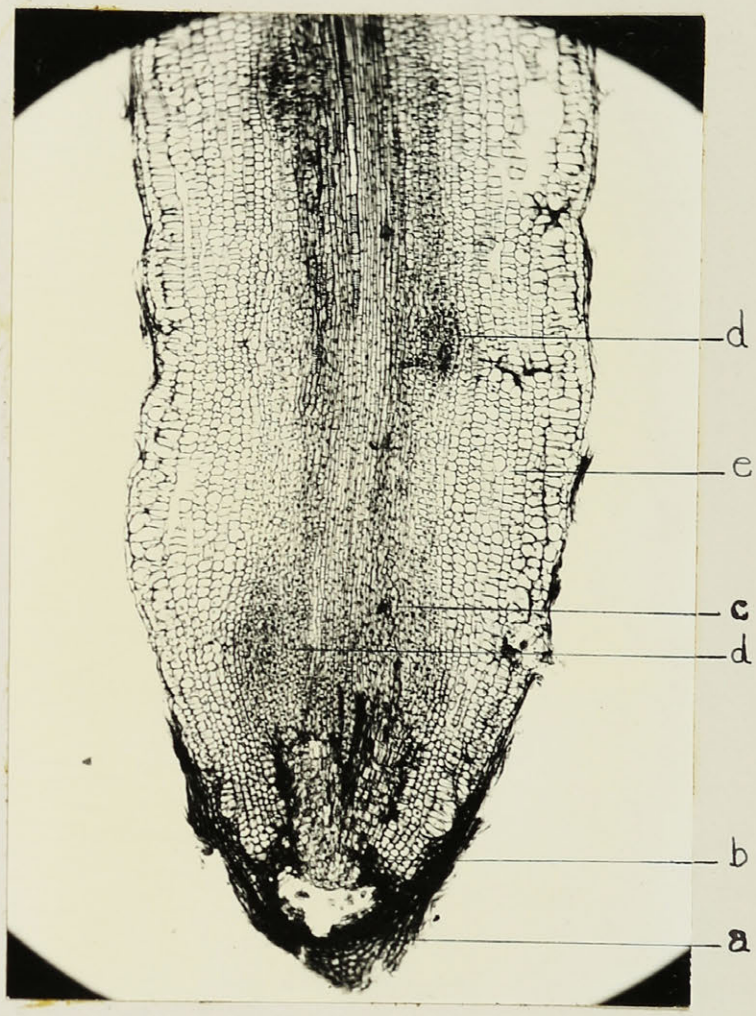

Fig. 43.

Fig. 42. Median longitudinal section in a root tip of a normal celery plant grown with adequate supply of boron.-x35

Fig. 43. Median longitudinal section in a root tip of a plant grown without boron. Note(a) the disintegrating root cap, (b) patches of disintegrated cells in the plerome near the tip, (c) early differentiation of xylem vessels, (d) the presence of branch root primordia near the tip, (e) disintegration in the periblem and general hypertrophy of the periblem cells.x35

I. Anatomy of celery plants grown in a medium containing 2.0 p.p.m. of boron.

In the healthy plant the slender primary root has a diarch protostele. The two strands of primary xylem alternating with the primary phloem elements are separated from them by ground parenchyma. The pericycle is well defined and is surrounded by the endodermis; the cortex is made up of large thin-walled cells. In longitudinal section the root tips show three definite regions, the plerome, the periblem and the dermatogen (Fig. 42). The root cap consists of several layers of cells ending in a point and overlaps the epidermis for some distance. Where secondary thickenings have occurred there can be seen two definite rays of parenchyma cells running along the same radius as does the protoxylem strands and two alternating sectors of xylem vessels and parenchyma. The vessels are produced in radial rows. The phloem is extensively developed and contains many oil ducts. The pericycle which surrounds the phloem is also very extensive and is limited by a periderm which at the same time limits the root, for by that time the cortex and endodermis have been sloughed off.

The fleshy crown stem is made up of a central pith in which are seen oil ducts and medullary vascular bundles. The pith is bordered by an almost complete cylinder of vascular tissue. In the cortex are found numerous strands of collenchyma.

The petioles are made up of a set of collateral vascular bundles and parenchyma tissue (Fig. 44 and 45). The phloem and

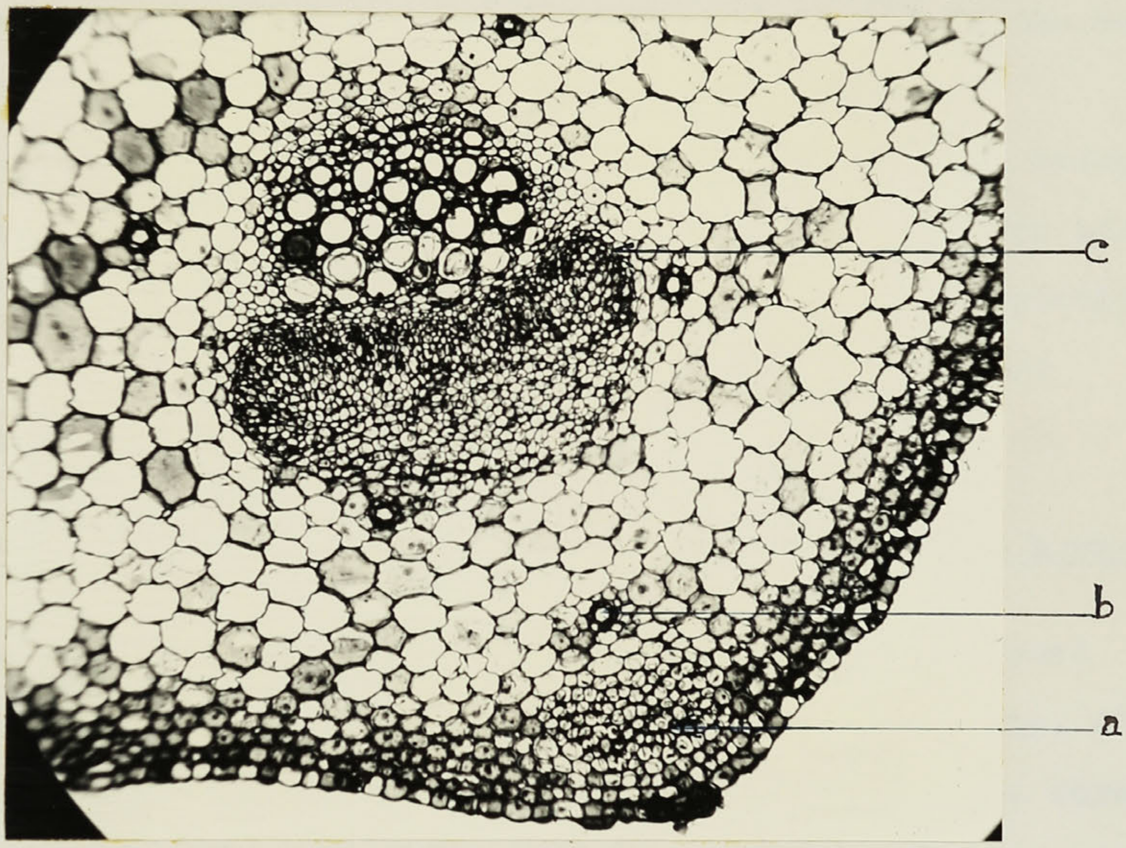

Fig. 44. Cross section of a vascular bundle from a plant grown with an adequate supply of boron: (a) collenchyma strand, (b) oil gland, (c) vascular bundle.-x75

the bundle cap are considerable in area. Opposite every bundle near the abaxial epidermis is a strand of collenchyma (Fig. 44 and 49). Near the strand and adaxial to it is an oil duct (Fig. 44). Several oil ducts are also present in the phloem and in the central parenchyma tissue.

The blades of the leaflets are relatively thin, the mesophyll consisting of a single layer of palisade cells and four to six layers of spongy parenchyma whose cells are somewhat compactly arranged (Fig. 51). The vascular bundles in the veins are similar to those of the petioles.

2. Anatomy of celery plants grown in a medium free from boron.

In boron-deficient plants the root tips had a collapsed root cap reduced to a thin band of blackened material (Fig. 43a). In some instances the root cap was entirely lacking. The three histogens were usually completely disintegrated at the very tip (Fig. 43b) and occasionally the plerome for a distance extending sometimes 25-30 cells from the tip, the cell walls being more or less completely broken down and the protoplasts blackened. In the plerome some cells had differentiated into xylem very near the tip (Fig. 43c); the xylem elements could be seen within the disintegrated tissues. The tracheids were frequently isolated from each other so that they did not join end to end to give continuous tubes in the normal manner. Branch root primordia had originated abnormally near the tips (Fig. 43d) but these had

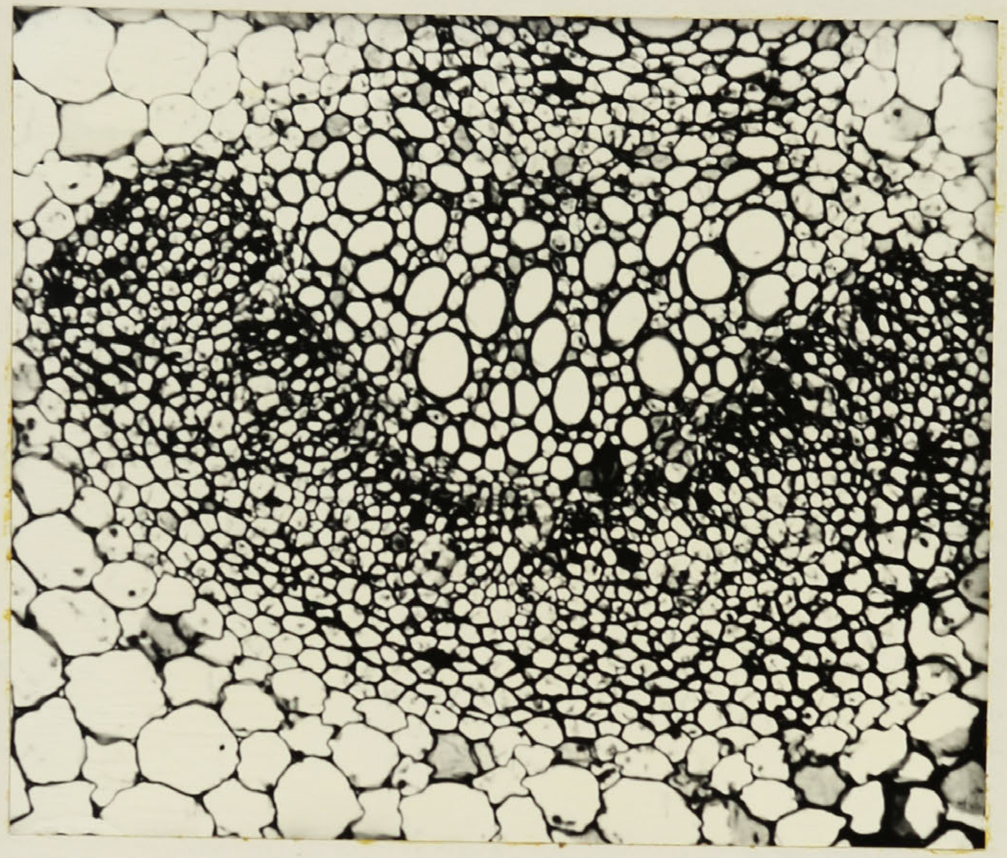

Fig. 45. Cross section of a vascular bundle from normal plant grown with an adequate supply of boron. The dark appearance of the cells in the cambium region is due to heavy staining and not due to any abnormality of these cells.-x115

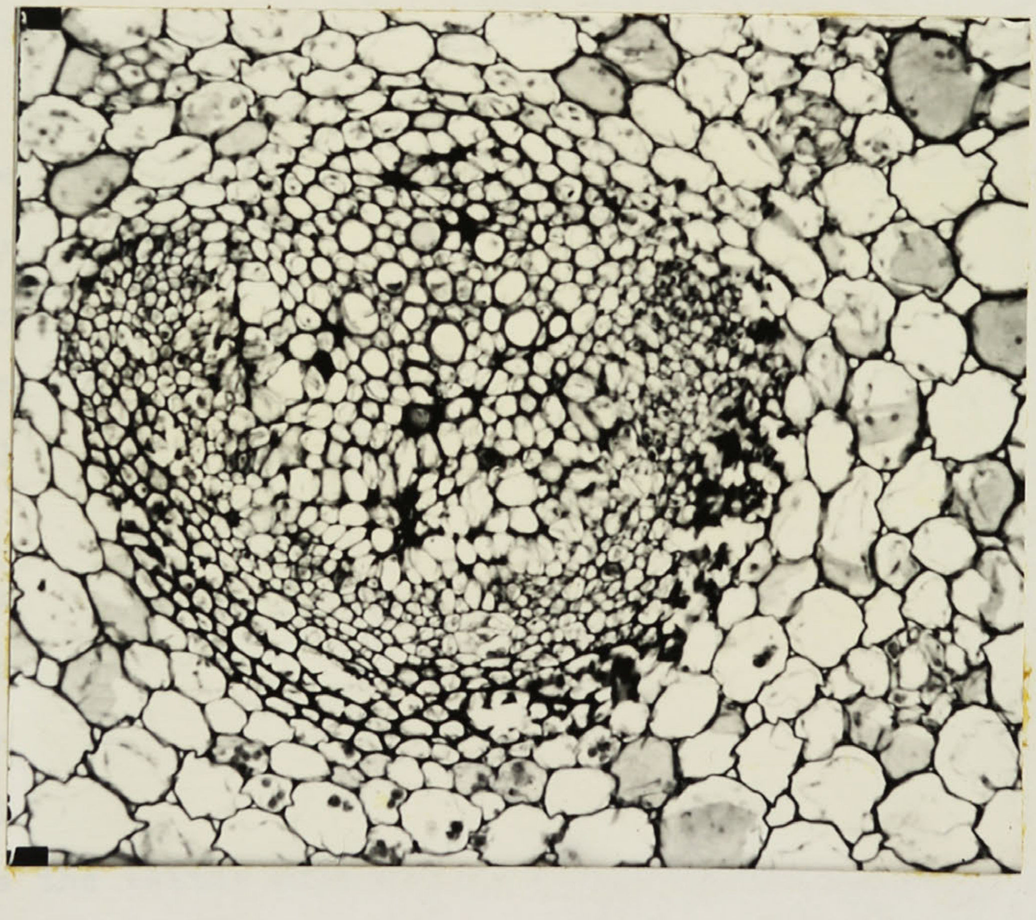

Fig. 46. Cross section of a vascular bundle from a plant grown without boron. Note hypertrophy of cells of the cambium and phloem region with few patches of disintegrated cells. Base of the petiole.-x115

usually not progressed very far before having ceased growth. They frequently failed to penetrate the cortex and almost invariably were surrounded by a sheath of blackened collapsed cells which evidently belonged to the root primordia and were not cortical cells which had been crushed by the advancing root tip. In the plerome in the region of elongation there was little abnormality to be noted besides the presence of proto- and metaxylem vessels.

In the periblem the hypertrophy was general, and patches of disintegrated tissue involving four to six cells also frequently occurred (Fig. 43c). The lack of regularity of arrangement of cells, was another abnormality commonly met with in the periblem.

The disturbance created by boron-deficiency in the roots where secondary thickening had occurred was slight and infrequent. Injury was seen as patches of disintegrated cells in the pericycle. As in the primary root only a few cells were involved. In a few instances xylem vessels were found blocked by some material similar to that found in the swede.

In the stems and petioles the tissues which showed the most severe symptoms of boron lack were the vascular bundles and the collenchyma strands. There could be distinguished two types of abnormalities, namely, hypertrophy and disintegration; the former was rarely found without being followed by disintegration, while the latter occurred at times without any apparent antecedent hypertrophy. At the base of the petioles and in the stems, the

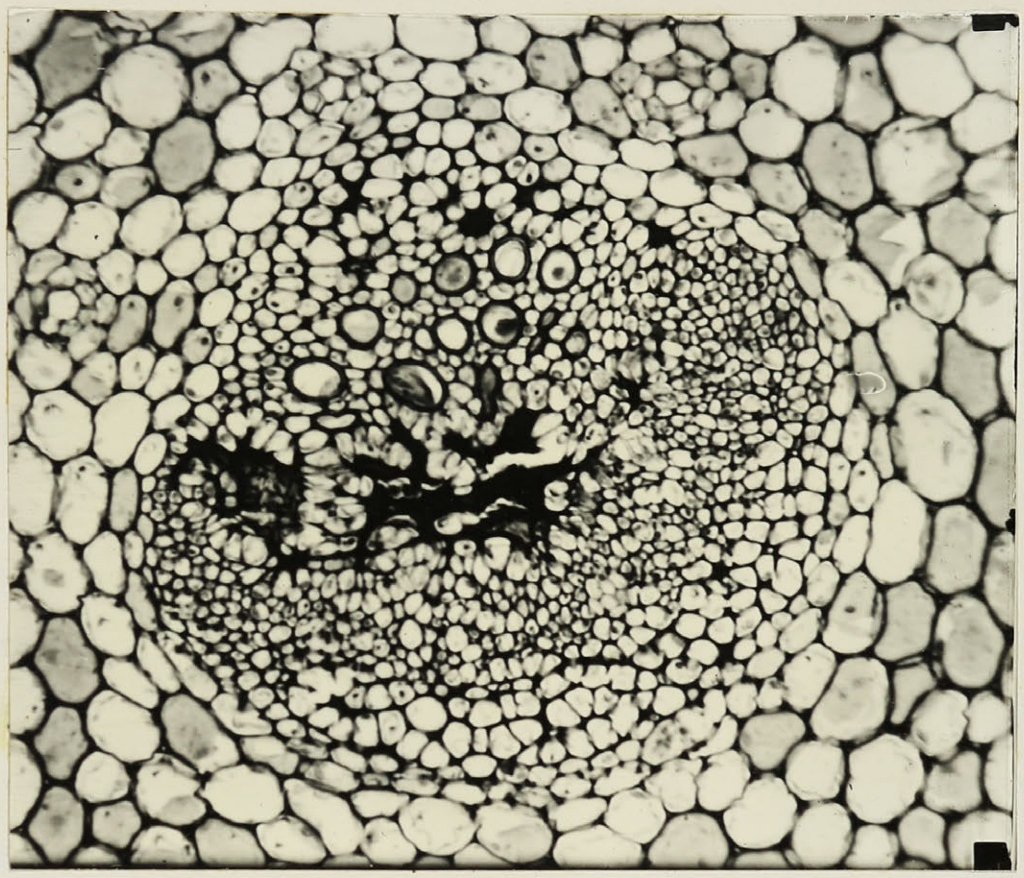

Fig. 47. Cross section of a vascular bundle from the middle portion of a celery petiole grown without boron. Disintegration is more severe than in fig. 46. Note some xylem vessels blocked with some substance and others small and malformed.-x115

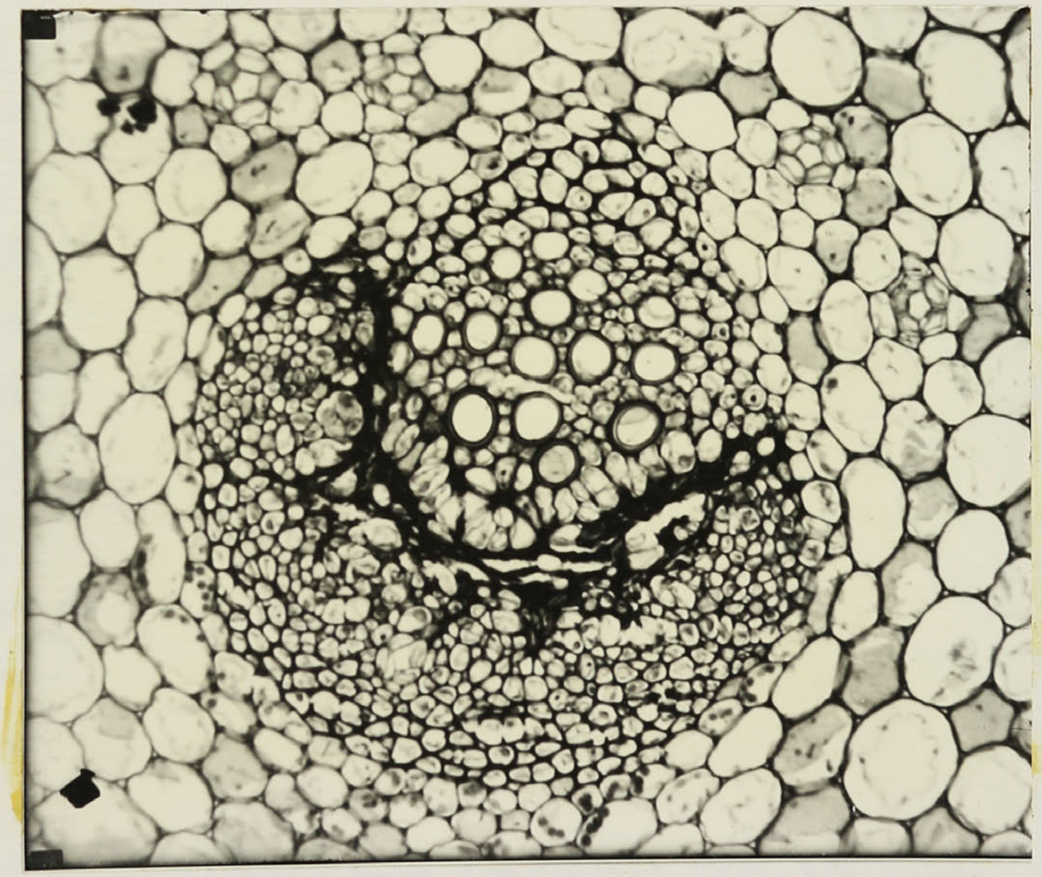

Fig. 48. Cross section of a vascular bundle from leaflet stalk showing severe disintegration together with hypertrophied xylem parenchyma cells. Some vessels are blocked.-x115

cambium cells and two or three adjacent rows of phloem cells often showed definite hypertrophy although the cell enlargement was far from being as extensive as in the swede (Fig. 46). Usually strands of disintegrated tissues were present around the hypertrophied groups of cells as well as in other parts of the phloem where hypertrophy was not noticeable. It appeared evident that these strands of disintegrated tissue were the result of the crushing of a few cells even when the hypertrophy of the surrounding cells was not noticeable. Some isolated phloem cells contained a densely purple-stained substance which was considered to constitute a plugging material. The xylem vessels near the phloem and cambium were small. Frequently they had one wall pushed inward, and several were blocked with a substance the nature of which is unknown. In some instances cells of the xylem parenchyma were slightly hypertrophied. In the middle parts of the petioles the hypertrophy of the phloem cells was not so great as at the base but it was still noticeable. The prominent feature was the disappearance of the cambium and its replacement by a band of disintegrated tissue more or less complete (fig. 47). Some vessels were blocked and strands of collenchyma showed more severe disintegration than at the base of the petioles. Some of the collenchyma cells had their lumina blocked with some substance.

In the stalks of the leaflets the epidermal cells bounding the collenchyma strands were frequently hypertrophied. The area of disintegrated tissue in the vascular bundles was greater than

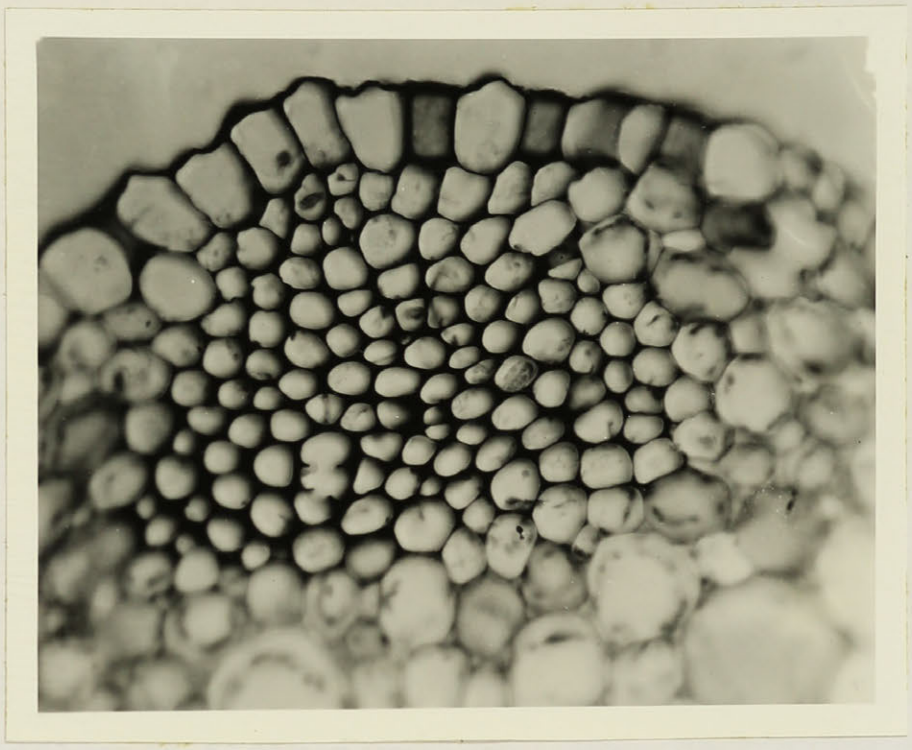

Fig. 49. Cross section of a celery petiole showing normal collenchyma strand.-x64

Fig. 50. Cross section in a celery petiole from a plant grown without boron showing disintegration of the collenchyma strand.-x64

either at the base or in the middle portion of the petiole (Fig. 48). The phloem cells were less regular but they were not hypertrophied to any appreciable extent. The vessels were malformed and blocked but the xylem tissue was never found displaced with vessels scattered in the phloem as was noted sometimes for other plants. In the parenchyma the patches of disintegrated cells were much more abundant.

In the ground parenchyma of the petioles the cells bordering the oil ducts had densely purple-stained protoplasts which may be an indication of disintegration. When the fast green stain was used alone these cells appeared yellow. In the epidermal and subepidermal layers of cells numerous patches of disintegrated cells were present. The cells usually kept their general outline, but sometimes they were hypertrophied. In every case, nevertheless, the cell walls and the protoplasts were in a state of disintegration. The intercellular spaces in these spots were sometimes filled with a yellowish brown substance; also, quite frequently the epidermis had collapsed and formed a thin dark band.

The strands of collenchyma on the abaxial surface of the petioles exhibited more severe symptoms of boron-deficiency than the vascular bundles (Fig. 50). Disintegration reached a severe stage in the upper parts of the petioles. Collapsing of the cells was so severe as to cause the strands to separate from the epidermis or from the ground parenchyma. All gradations occurred in severity and in the number of cells involved.

The leaf blades in the diseased plants showed severe anatomical symptoms. They were not thicker than the normal ones,

Fig. 51. Cross section of a normal celery leaf blade.-x145

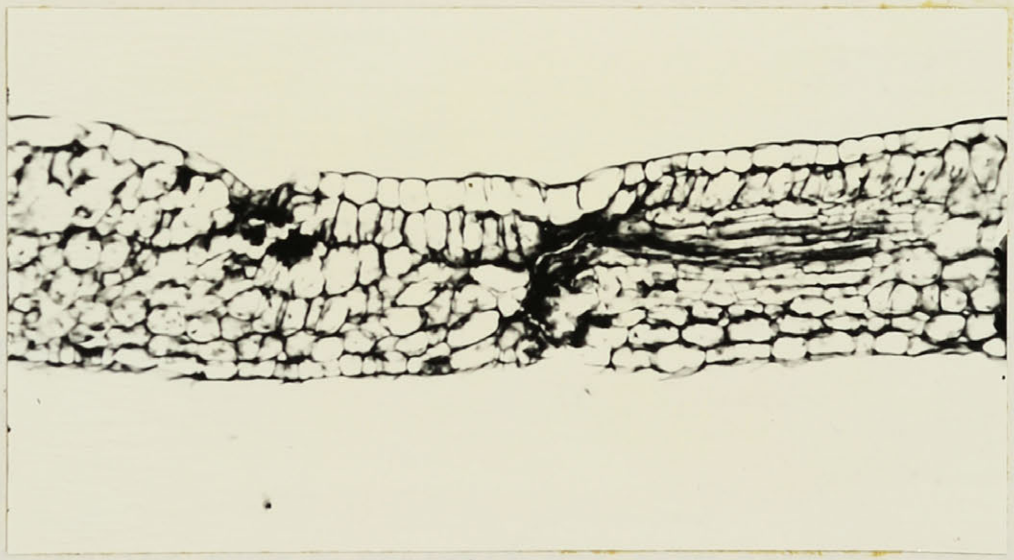

Fig. 52. Cross section of a leaf blade from celery plant grown without boron. Note the spongy parenchyma made up of cells more closely packed than in normal blades, and a few areas of collapsed cells resulting in constriction of the blade.-x145

but the spongy parenchyma was made up of cells more closely packed than in normal plants, and very few intercellular spaces remained. The vascular bundles were in an advanced stage of disintegration which appeared to have taken place without previous enlargement. The cell contents as well as the cell walls were densely stained purple with the safranin and fast green combination. Frequently there were present in transections areas of collapsed cells involving either the spongy parenchyma alone or the spongy parenchyma, the palisade cells and the epidermis. This usually resulted in some constriction of the blade (Fig. 52).

3. Anatomy of lesions of cracked-stem produced under natural conditions and in sand culture without boron in the nutrient solution.

The lesions of cracked-stem presented an interesting situation. Sections through these lesions showed that the outermost part was a mass of collapsed and broken down cells (epidermal, collenchyma and parenchyma cells) (Fig. 53). Beneath this area was a rather thick layer of hyperplastic cells in which a fairly distinct cambium-like region could be seen suggesting that a periderm had been formed, but tests with sudan IV and zinc-chlor-iodide revealed no evidence that cork had been produced. The outermost layers of cells were dried and pushed away by the growth and continual production of new cells below.

The vascular bundles and collenchyma strands of naturally diseased plants showed some, though not all, of the typical

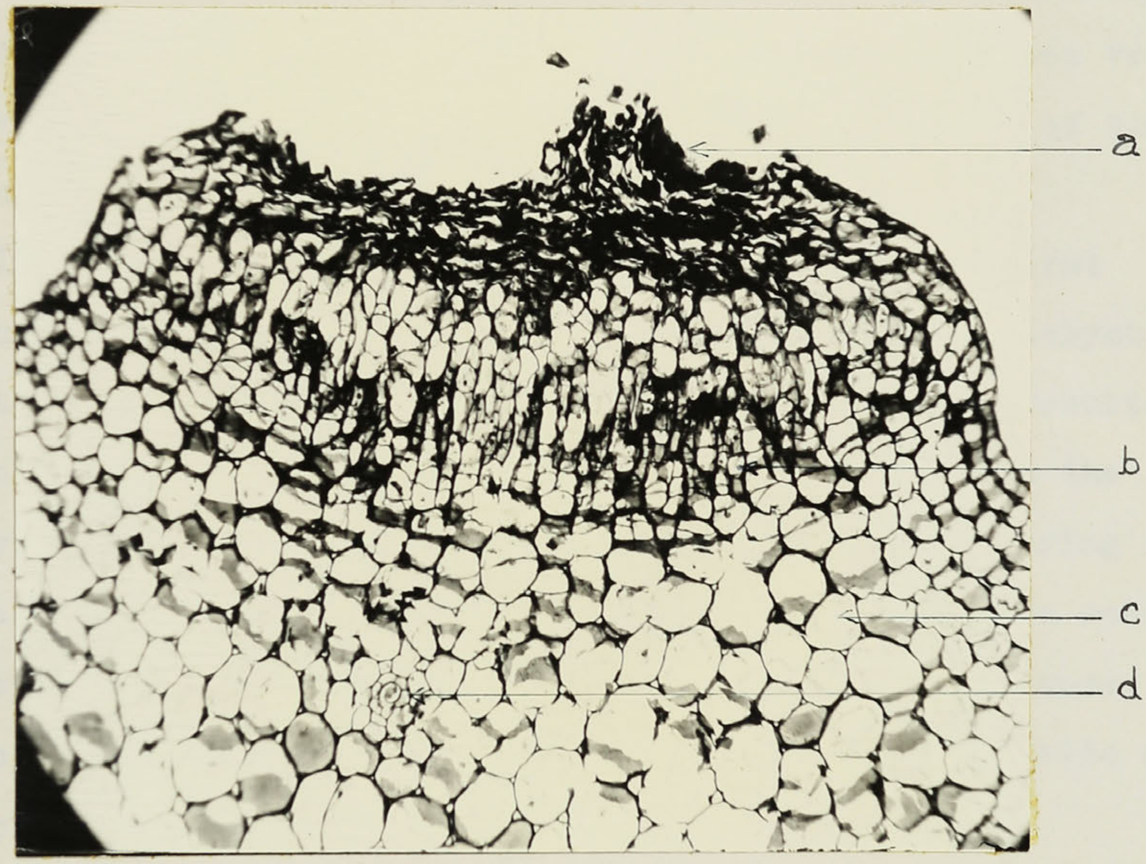

Fig. 53. Cross section of a celery petiole through a cracked stem lesion: (a) disintegrated collenchyma strand, (b) meristematic tissue, (c) normal ground parenchyma and (d) oil gland.-x72

symptoms of boron-deficiency as produced under artificial conditions. These were the blocking of a certain number of xylem vessels, malformation of others which included both blocked and unblocked ones, and more or less complete disintegration of the collenchyma bundles. This latter abnormality was very frequent. Very often also the blocking of the lumina of some collenchyma cells was observed.

In the development of cracked-stem lesions the first happening was initiation of cell division in the parenchyma cells beneath the collenchyma strands of the ridges. This resulted in the formation of a mass of abnormal cells which pushed the overlying tissues outward causing eventually the rupturing of the tissues, hence the cracked-stem lesions. This was also accompanied by disintegration of the epidermal and collenchyma tissues. Some spots were found where cells were beginning to divide and the epidermis was still intact.

The cracked-stem lesions produced under field conditions and in the greenhouse in nutrient solutions without boron were found to present the same anatomical disturbances in the celery petioles.

III Studies in the Comparative Anatomy of Normal Apple Fruits and of Apple Fruits Affected with Internal Cork.

The specimens used for the studies on internal cork were secured from orchards where this disease was prevalent.

On the apple there are quite a number of related troubles due to a lack of boron. On the fruit the writer is aware of three distinct diseases; drouth spot, corky core and internal cork. Specimens could be secured of only the last mentioned disease. As far as external symptoms were concerned the specimens used fitted very well with the descriptions given for this disease by Carne and Martin (1937) in Australia Askew, Chittenden and Thomson (1936) in New Zealand and by Jamalainen (1936) in Finland.

Externally effected fruits presented an uneven surface of depressions and elevations, and they were much smaller than normal fruits. Cross, or longitudinal sections in the apple revealed the presence of numerous dark brown areas having a corky appearance (Fig. 54). These spots were distributed throughout the flesh of the fruit but were most abundant near the periphery although they were frequently found within the core. Corky areas near the periphery of the fruit were frequently surrounded by green tissue. Light yellow areas differing from the dark brown ones described above in being larger and not so definite, were sometimes present in the core.

Fig. 54. Appearence of internal cork spots in Fameuse apple.

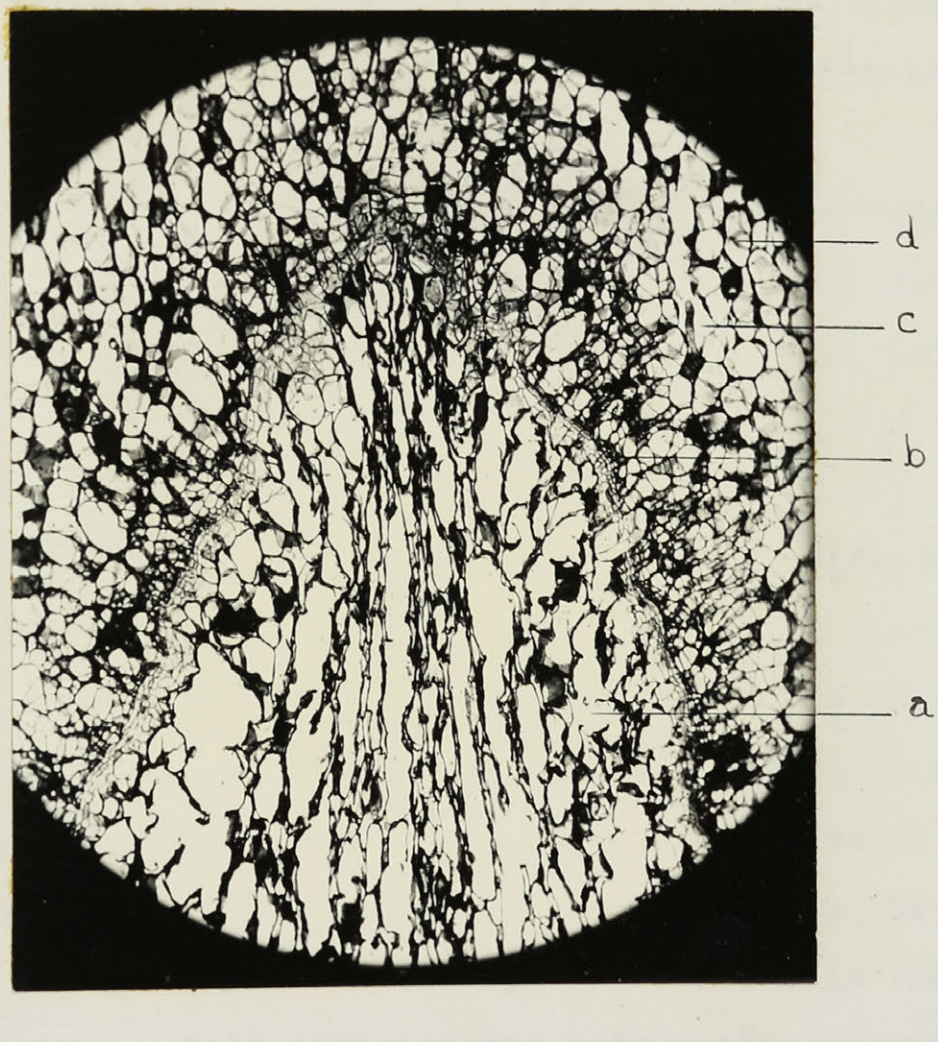

Fig. 55. Internal structure of the lesions of internal cork; (a) central part with collapsed cells, (b) cork layer, (c) mycelium-like cell and (d) normal parenchyma.-x32

The mature apple fruit is made up of large parenchyma cells, which appear rectangular in shape, and of small vascular bundles distributed throughout the flesh. Near the epidermis the cells are roundish and much smaller than in the central part.

The internal cork areas were more or less spherical with a central part made up of collapsed and dried up cells. (Fig. 55a). These collapsed cells had no suberized cell walls but were cellulosic in nature as revealed by the zinc-chlor-iodide test and by staining reactions with sudan IV. Abundant starch grains were found in these cells.

A complete layer of cells, more or less isodiametric, which had suberized cell walls, was formed around the central area of collapsed cells (Fig. 55b and 56a). This cork layer was two to five (mostly three) cells thick and suberization was complete. This cork layer had originated from a conspicuous phellogen made up of small rectangular thin-walled cells (Fig. 56b). It appeared certain that this anatomical structure was a periderm. In the largest spots a goodly number of small cubical phelloderm cells were present outside the phellogen. (Fig. 56c). These were usually in more or less regular rows and appeared to have originated from the cork cambium. The situation did not appear so clear in the smaller lesions. Cell division had been active in the tissue surrounding the cork. The parenchyma cells not far from

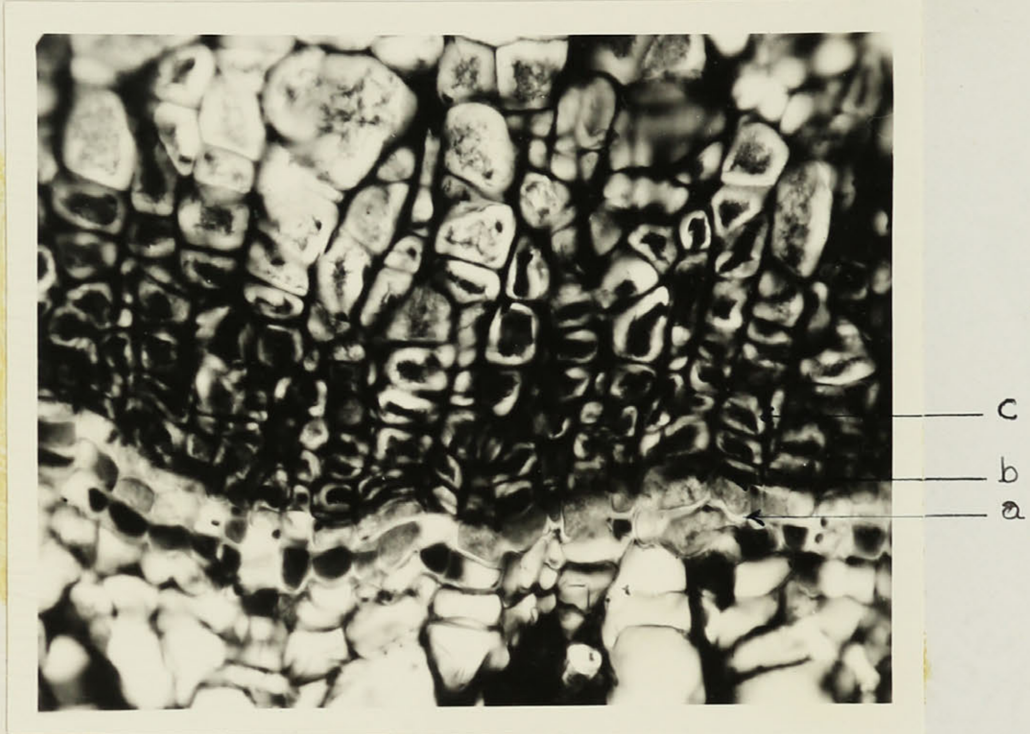

Fig. 56. Section in a lesion of internal cork showing details of the periderm: (a) phellem, (b) phellogen, (c) phelloderm.-x320

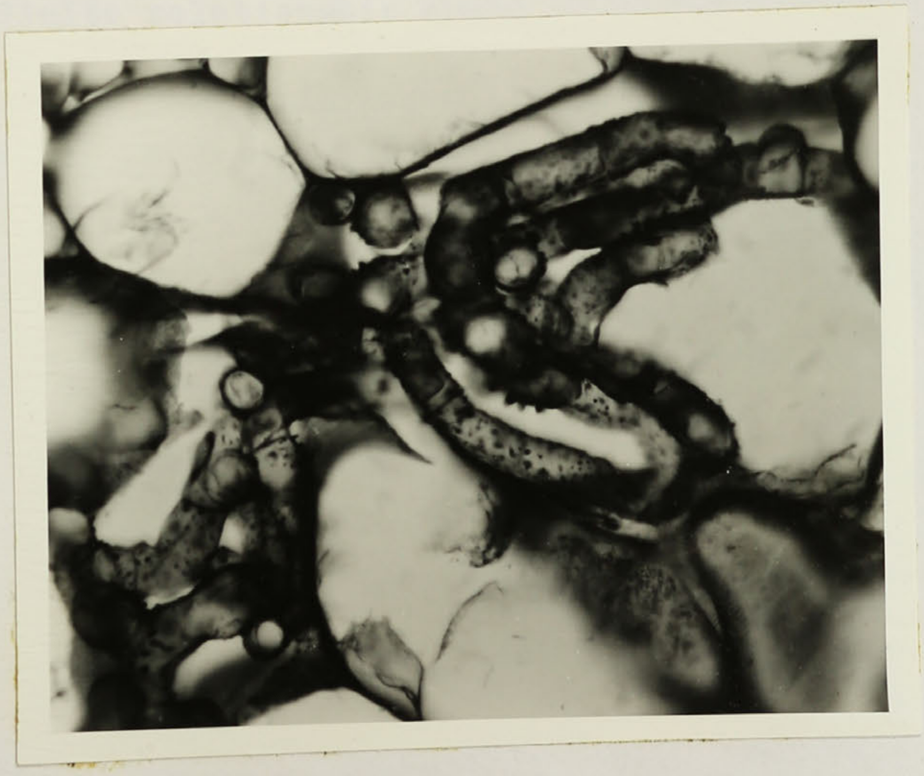

Fig. 57. Mycelium-like cells inside large parenchyma cells of the apple not very far from the cork layer. Note the echinulate thickenings of the walls.-x320

the spots had become meristematic and formed within themselves numerous small cells resembling a multiseptate, stout mycelium (Fig. 57). These cells were attached to the wall of their mother cells. The mycelium-like cells had walls variously and unusually thickened. The walls frequently showed echinulate as well as very irregular knob-like thickenings. Other parenchyma cells on dividing formed a thin dividing wall. In several instances cells were found with two nuclei. As a general rule the cells surrounding the internal cork lesions had dense protoplasts that had readily taken up safranin and appeared more or less purple with the safranin and fast green combination.

No definite relationship could be found between the occurrence of the cork lesions and the distribution of the vascular bundles, as stated by Mix (1916).

DISCUSSION.

All three plants studied proved to be seriously affected in various but essentially the same ways by the lack of boron. These anatomical studies seem to indicate that the effect of a complete lack or inadequate supply of boron is not so much a matter of starvation as of a condition which interferes with the vitality of the cells and their ability to perform their normal functions which are essential to the life of the plant. Furthermore, the very small quantities necessary for the normal growth of the plants tested under greenhouse conditions leads to the conclusion that boron is not a food at least in the ordinary sense of the word. That it is essential for normal cell division cannot be doubted.

That boron is particularly needed in the most actively growing tissues is shown by the severe disturbances noted in (a) the apical meristems of both roots and shoots, (b) lateral meristems such as cambium of the branch roots, hypocotyls, petioles, midribs and veins and (c) other actively dividing tissues such as the meristematically active tissues in the xylem of the storage "root" of swedes, which play an important part in its enlargement.

In this connection the swede is the most interesting of the plants studied. Up until the time of the initiation of concentric bundle formation the swede behaves very much like other plants of about the same age and stage of development when fed an insufficiency of boron. During that time the most sensitive parts are the meristematic tissues of the growing points and the cambium. After tertiary plant body formation begins the most sensitive tissues, as demonstrated by the development of brown-heart with no symptoms elsewhere, are to be found in the xylem. The cells of the xylem parenchyma inside the cambium ring in the storage "root" are actively dividing during the bulbous enlargement of this structure, and concentric bundle formation is also considerable, which may explain the presence of boron-deficiency in the xylem of this organ before any disturbances develop in any other part of the "root" or in any other organ.

There is to be observed a sequence of events and a progressive development in the establishment of the diseased conditions in the plants suffering from boron-deficiency. The studies on swedes grown without boron or with 0.25 p.p.m. of boron in the nutrient solution serve to illustrate this point. At a certain time all the leaves of one plant do not present the anatomical boron-deficiency symptoms to the same degree. The outer leaves, which usually show the most severe external symptoms, are those in which anatomical symptom expression is the most pronounced. The inner ones, on the contrary, at least for a time, remain quite healthy or show slight anatomical disturbances. There is also a progressive development of anatomical changes in a single leaf. In the vascular bundles of the leaves disturbances commence to show near the vein tips and develop downward until the whole vascular bundle from vein tips to base of the petiole becomes involved. In studying separately all the leaves of one plant it is possible to get different stages of symptom expression and find, for example in the higher midrib of one small leaf a situation analogous to that which is found at the base of the petiole in a larger and older one. From this it seems reasonable to infer that once the situation in the large leaf was similar to what it is actually in the smaller one and similarly the condition at the base of the petiole has once existed only in the higher parts of the midrib and veins.

It was found that the first symptoms of boron-deficiency, at least in the above ground parts, were the plugging of some sieve tubes of the vascular bundles and thickening of the leaf blades due to elongation of the palisade cells and proliferated spongy parenchyma. This thickening of the leaf blade is no doubt a response of the plant brought about by the hindrance to food translocation away from the leaf due to the inability of the phloem to function properly. It is a known fact that in the boron-deficient plants there is accumulation of carbobydrates in the leaves. The development of anthocyanin pigments in the diseased leaves is a further proof of this fact since it is a common response of plants to the accumulation of carbohydrates in the leaves.

The next event in the development of symptom expression in the vascular bundles of the leaf is essentially similar to what has happened in the mesophyll; the cambium and its immediate derivatives become hyperplastic so that repeated cell division in time causes the phloem to appear far removed from the xylem. Eventually some of the cells, apparently the ones adjacent to the xylem vessels, start to elongate at right angles to the long axis of the bundle itself; that is the long axes of these cells run from the xylem to the bundle cap, or radially. The result of this is a crushing of the phloem against the bundle cap. Longitudinal radial sections of diseased vascular bundles show that the abnormal cells are elongated only radially. In tangential view they appear as round cells. While they are abnormally long in radial direction, in actual volume they do not exceed much that of normal cambium cells.

As the individual cells of this abnormal tissue continue to enlarge they eventually become irregular in shape and of various forms due to the fact that they become strongly pressed against each other. They tend to increase the size of the whole bundle. Later the walls of some of these cells become yellow or brown and break down; the protoplasts disintegrate in turn, and groups of cells may be crushed into amorphous masses of material which form dark streaks between the living cells. Other adjacent cells become involved in the same way until finally all the hypertrophied cells in the bundle may disintegrate and form an amorphous mass of brown material.

This state of affairs seems to begin at the tip of the midrib and at the tip of the veins at the same time and works progressively downward into the midrib and to the base of the petiole. All the veins behave as the higher part of the midrib for a corresponding length measured from the tips of each. Reference has already been made to cases where the abnormal condition of the corner vascular bundles was more severe than that of the others. It was found in these instances that these bundles are the ones which pass into the blade to constitute the vascular bundles of the veins of the lower lobes of leaflets of the blade.

Progressive development of symptoms is also displayed in other parts of the swede in plants grown without boron in the nutrient solution. In the roots, the root tips are the first to show defects. These include mainly hypertrophy of the periblem, disintegration of parts of the plerome, early maturation of tracheids near the tip, profuse production of abnormal branch root primordia unusually near the tip, and absence of root cap. Later the cambium is replaced by a broad band of cells elongated radially and hypertrophied tissues may also develop in the parenchyma of the cortex and phloem. The root tips become abnormal before the leaf thickens which indicates that the root tips are the most sensitive parts of these plants to a lack of boron.

Plants produced with 0.25 p.p.m. grow normally for several weeks before the deficiency occurs and not until a "root" of considerable size has been produced. In these plants brown-heart is the first symptom to appear and this can be found before any other disturbances have shown. This is similar to the development of brown-heart in the field. With the progress of time other deficiency symptoms appear. The order of development of the various disturbances in general is believed to be as follows: brown-heart in the root portion of the "root", brown-heart in the hypocotyl and epicotyl, abnormal root tips, blocking of sieve tubes, disturbances in the foliage, hypertrophy of the cambium in the root, hypertrophy of the cambium in the hypocotyl and finally hypertrophy of the cambium in the stem. The advanced stages involve death of the growing points, general necrosis in the foliage and death of many of the fine roots. The final stage is death of the entire plant.

The most sensitive part of the celery plant to a boron-deficiency appears to be the leaves which show under field conditions the lesions known as cracked-stem on the petioles without any concomitant injury being evident in other parts of the plant. Further proof of this is given by the fact that plants grown without boron in the nutrient solution develop first cracked-stem. Later the root tips become abnormally thick and diseased. The branch roots become very slightly affected.

In the leaves, in the main, the same trend of events is observed as in the swede. Usually the first anatomical derangement is to be found in a region of the parenchyma just inside the collenchyma strand and between it and the oil gland adaxial to it.

Both hyperplasia and hypertrophy are to be found resulting in a proliferation of the parenchyma of this region. This proliferation causes the collenchyma strand to collapse and disintegrate. The broken down collenchyma and the epidermis are eventually pushed outward and burst open to form the crosswise cracks typical of the cracked-stem lesions. Thus the cracked condition is formed in a manner quite similar to that which results in galls and splitting of the petioles of the swede which are due to hypertrophy of the ground and cortex parenchyma in localized areas.

At the same time or shortly after, the effects of the boron-deficiency start to show in the vascular bundles of the veins of the leaflets and gradually develop downward into the stalks of these leaflets, into the higher parts of the petiole and finally are to be found in the vascular bundles at the base of the petioles and even in the stem.

The details of the sequence of events in the establishment of the diseased conditions in the vascular bundles are essentially similar as in the swede though they show minor differences. The hyperplasia noted in the vascular bundles of the swede has not been noticed. There is the possibility, however, that it was present being not sufficiently extensive to be noticed. The first symptom is the hypertrophy of some cambium cells. This hypertrophy is, however, relatively slight and soon results in the crushing of surrounding cells. The number of hypertrophied cells in the vascular bundle increases gradually resulting in a crushing of cells. This continues until all the cells of the cambium and its immediate derivatives are either hypertrophied or crushed. Apparently xylem parenchyma cells eventually become hypertrophied and then through disintegration contribute to the formation of a thick band of amorphous brown material which has replaced the cambium region. The phloem appears to suffer little injury except for the plugging of some sieve tubes. The process of cell disintegration presents no material difference from that which usually occurs in the swede.

The order in which the different tissues of an organ or parts of the plant degenerate may be taken to be in general directly correlated with the relative degree of susceptibility of each to a subminimal supply of boron or in other words to indicate roughly their relative boron requirements. However, there is the possibility that one tissue may give up of its boron, to its own detriment, to another tissue. This is a possible explanation for the occurrence of brown-heart while the meristems remain healthy, and for the development of corky core in apples while the rest of the tree remains sound. Further support is given to the hypothesis of the possibility of translocation of boron from one part to a more actively growing one by the constant production of new growing points in the roots of both swedes and celery. In other plants as noted in the review of literature the tendency to form new growing points is general in both roots and shoots.

While complete lack of boron causes rapid death of cells, subminimal supplies of this element stimulate the meristematic activities of the meristems and of other actively dividing tissues. The latter is evident from a study of plants affected with boron-deficiency diseases as they occur in the field or produced under controlled conditions in greenhouses. Brown-heart is the result of both hyperplasia and hypertrophy of xylem parenchyma; cracked-stem of celery is also the result of proliferation of parenchyma tissue which pushes out strips of epidermal and hypodermal tissues. This stimulation to cell division is still more evidenced in the lesions of internal cork of apple where are found large cells which may form in their inside a dividing wall or a tremendous amount of small cells of all sizes and shapes ranging from round to mycelium-like. Here also is produced a very regular and well organized periderm around the internal cork areas. Periderm formation was not found in the other plants studied. In the swede hyperplasia is usually found, especially at the base of the petioles and of the midribs, where is sometimes observed a mixture of hyperplasia and hypertrophy. Where galls are formed on the petioles or midribs the parenchyma of these parts sometimes shows hyperplasia as well as hypertrophy.

Though an inadequate supply of boron stimulates the activity of the meristems it prevents the differentiation and proper maturation of the cells so formed. This most likely accounts for the fact that no large thin-walled immature xylem vessels can be found in vascular bundles of either root petioles or midribs, and also for the fact that both the number and size of the vessels are smaller in the diseased than in the healthy vascular bundles. Also in the vascular bundles where the tissues are hypertrophied the cells being elongated mostly in the radial direction all the cells are parenchymatous with thin walls and none of them can be considered to be either xylem, phloem, cambium or normal parenchyma. There seems to be a contradiction when the situation in the root tips is considered. Apparently some cells of the plerome differentiate abnormally near the tip into either protoxylem with spiral or annular vessels or into metaxylem with reticulated vessels. It must be kept in mind that the tissues of the root tips of diseased plants in which elongation has practically ceased are much older than corresponding ones in the normal plant and therefore that differentiation and maturing of xylem vessels considered in this light is very likely normal. It is to be observed, however, that differentiation of these xylem vessels is far from normal. Instead of forming continuous vessels, the tracheids most frequently are isolated one by one and do not mature properly for they take the safranin stain very faintly.

Improper maturation is also indicated by the fact that the xylem vessels do not seem to be completely lignified. Very frequently in roots and hypocotyls and in the vascular bundles of the petioles and midribs vessels are found which are malformed with distorted walls mostly pushed inward due to pressure caused by hypertrophy of surrounding tissue.

Another effect which is probably indirect is the plugging of sieve tubes and xylem vessels with some substance or substances of unknown nature. This is very likely a product of the disintegration of the surrounding cells.

These studies indicate that boron plays an all important rôle in cell division, enlargement and maturation. In its relative absence or with subminimal supplies of boron cell division is considerably activated but the cells produced by this meristematic activity usually cannot differentiate or mature properly.

Finally the writer desires to draw attention to the fact that the tissues which were found to be most susceptible to a boron-deficiency were those which are actively meristematic. These include the primary meristems, the cambiums and the parenchyma cells in apple fruits, in the petioles of celery and in the xylem of the bulbous portion of the swede all of which take an active part in the formation of these structures.

SUMMARY.

1- These investigations are concerned with the histopathological features of (1) the following plants suffering from a lack of boron and (2) the following specific boron-deficiency disorders: (a) swedes grown from seed in sand culture without boron in the nutrient solution, (b) swedes grown from seed in sand culture with 0.25 p.p.m. of boron in the nutrient solution, (c) brown-heart of swedes, (d) celery grown in sand culture without boron in the nutrient solution, (e) cracked-stem of celery and (f) internal cork of apples.

2- The swedes grown without boron feeding became very heavily diseased and death set in before tertiary thickening had occurred. In these plants the meristematic regions such as root tips and cambium suffered severe disturbances while the xylem remained sound.

3- The swedes produced with 0.25 p.p.m. of boron in the nutrient solution developed "roots" of considerable size with much tertiary thickening before the effects of an insufficiency of boron began to show. The first disturbance to develop in these plants was brown-heart which is due to hyperplasia and hypertrophy of the xylem parenchyma. Later the meristems became involved as in the swedes grown with no boron in the nutrient.

4- The first symptom to develop in celery when grown with an insufficiency of boron was always cracked-stem and trouble in the meristems appeared later. Cracked-stem is essentially due to disturbances in parenchyma and collenchyma tissues.

5- A true cork layer developed around the margins of internal lesions in the flesh of the apples. Cork formation was never found in the other plants and is apparently an unusual happening.

6- In general the meristems have been found to be the tissues most susceptible to a lack of boron. Plugging of sieve tubes was also usually an early happening with the onset of boron-deficiency. Disturbances in other tissues seemed to be largely if not entirely secondary. Cracked-stem of celery, internal cork of apples and brown-heart of swedes, at first thought, do not seem to agree with this statement but it must be remembered that the parenchyma cells which in each case become involved are actually highly meristematic due to the methods of growth of the organs in which each trouble is found.

It may also be argued that the course of events displayed in these leaves indicate a reutilization of boron by the plant. These troubles result from an increased activity on the part of mature cells while the younger and meristematic regions remain sound. The theory may be advanced that this may be due to a movement of boron from these cells to the normal meristematic tissues in an effort to keep these tissues healthy as long as possible.

A knowledge of the actual concentration of boron in the various tissues, of the movement of boron within the plant and of the form in which it exists in the plant is badly needed for a complete understanding of the rôle of this element in the life of the plant.

7- Normal cell division, enlargement and maturation were dependent upon a sufficiency of boron.

8- Apparently the cells most active in a physiological sense were the ones most susceptible to a lack of boron.

9- The primary histological effects were hyperplasia, hypertrophy and cell disintegration; and they often happened in this order, but all the three occurred independently as well.

10- Cell disintegration was observed to begin with the cell wall followed by breaking down of the protoplast. The final result was a formless mass of deep staining material.

11- There was imperfect xylem differentiation and abundant branch root primordia formation unusually near to the diseased root tips. These attempts at branch root root development were in all cases sooner or later abortive.

12- A well developed periderm in the internal cork of apples is interesting since the formation of a well organized tissue from the stimulated meristematic activity resulting from a boron-deficiency is very unusual. Usually the tissues formed have no regularity, differentiation or organization of any sort.

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