AN ABSTRACT OF THE THESIS OF

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Title: ALUMINUM TOXICITY IN THE PRIMARY MERISTEM OF
WHEAT ROOTS

Abstract approved: Redacted for privacy (David P. Moore)

The progression and consequences of aluminum toxicity were studied microscopically in the root tips of four wheat varieties representing different classes of Al tolerance. The root tips studied were collected from seedlings grown in nutrient solution cultures containing either sublethal or lethal Al treatments which were unique for each variety. The lethal treatment resulted in the destruction of both cytoplasm and nuclei throughout the root primary meristem of each variety. Some damage was sustained by root tips exposed to the sublethal treatment but if an adequate number of viable cells remained throughout the primary meristem, apical root growth could be reinitiated in an Al-free recovery solution. Reinitiation of apical root growth occurred in spite of the death and subsequent loss of the quiescent center following most sublethal Al treatments.
The movement of Al into the root tip could be followed during the Al treatment by using dyes that either combined with Al or fluoresced in ultra violet light when chelated with Al. There was strong evidence that Al penetrated the boundary between the root apex and rootcap and then, during a lethal treatment, ascended into and throughout the meristematic cells of the central cylinder. Al had also penetrated the epidermis and cortex but was prevented from entering the central cylinder by the endodermis which apparently constituted an additional barrier to the movement of Al. Ascension of Al into the central cylinder from the root apex would by-pass the barrier at the endodermis.

Microscopic examination of Al injured root tips revealed that the primary effect of Al was the death of cells. Death occurred rapidly, usually within the first 24 to 48 hours of exposure to Al. The mitotic cycle, however, was affected almost at the onset of the Al treatment. Evidence in this study suggested that the entire mitotic cycle was impeded by Al although the process of mitosis was least affected. The mitotic cycle proceeded again if the Al stress was removed before the cell's cytoplasm was completely displaced by vacuoles.

The cause of varietal tolerance to Al could not be attributed to any structural feature observed through a microscopic examination of root tips from the several wheat varieties grown in this study.
Aluminum Toxicity in the Primary Meristem of Wheat Roots

by

Stanley John Henning

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Date thesis is presented  

Typed by Ilene Anderton and Lyndalu Sikes for Stanley John Henning
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ALUMINUM TOXICITY IN THE PRIMARY MERISTEM OF WHEAT ROOTS

I. INTRODUCTION

Aluminum (Al) can seriously injure many plants growing in acid soils while some other plants thrive in this environment. Within single plant species, many varieties tolerate Al and others are damaged by it. There has been much speculation about the mechanism responsible for this. Wheat has received much of this attention because of the wide differences in Al tolerance among its varieties. Under one set of conditions, the most tolerant wheat varieties known can tolerate up to 50 times as much soluble Al as the most sensitive varieties.

Reaction to Al among wheat varieties appears to be relative. Toxicity can be induced in both the most sensitive and tolerant varieties if experimental conditions are properly adjusted. All evidence indicates that inhibition of root growth is the primary symptom of Al toxicity noted in susceptible plants. Many hypotheses explaining the physiological effects of Al on plants have not taken into account the actual damage done to the root. The severe root damage caused by Al can adequately explain such phenomena as poor top growth and reduced yields. Other phenomena such as reduced
uptake of P, Ca, Mg and K, nutrient deficiency and pH changes can also be related to the toxic effects of Al on roots.

Roots that have been exposed to a toxic level of soluble Al fail to reinitiate apical root growth when placed in an Al free system. This observation suggests that irreparable damage has been done to the cell division process in the primary meristem. However, there have been few reported histological and cytological studies of such effects of Al in plants. Less is understood about the mechanism responsible for differential Al tolerance within plant species. The wide differences in tolerance among wheat varieties afford a unique opportunity to study this mechanism. Comparison of the effects of Al on root tissues and cells of several differentially tolerant varieties of wheat is an appropriate starting point for such an investigation.
II. LITERATURE REVIEW

This study was initiated as a histological examination of Al toxicity in wheat roots. Specifically, it was the intent of this study to report findings that pertain to the effect of Al on plant roots and inferences concerning the action of Al within the root. Factors that may contribute to differential tolerance to Al among species and among varieties within a species were of special interest. The role of Al in relation to plant nutrition on acid soils has been extensively reviewed by Jackson (1967). Recently, Foy (1974) has characterized the inhibitory effects of Al as gross symptoms, cytological effects, and physiological-biochemical effects.

**Gross Effects**

The gross effects of Al on plants were first described by McLean and Gilbert (1927). These symptoms included discoloration and stunting of main roots and laterals. Roots failed to develop and the whole root system appeared dwarfed. These symptoms were apparent long before top growth was visibly depressed. Fleming and Foy (1968) presented several photomicrographs of an Al sensitive wheat variety which demonstrated inhibition and thickening of lateral roots caused by Al. Foy et al. (1969) presented photographs of entire soybean root systems that were Al injured. The stubbiness of lateral roots
and limited root development were shown in good detail.

**Cytological Effects**

There were no detailed papers reporting the histological and cytological changes in roots from onset of Al treatment to cessation of growth in damaged plants. Fleming and Foy (1968) have shown some work but the photomicrographs in their paper showed very little cytological detail. They treated a sensitive wheat variety with a toxic amount of Al and observed that root elongation ceased within 24 hours. Injury after five days Al treatment was characterized by disorganization of the root cap, root apex and vascular elements. The injured root appeared longitudinally compressed and lateral roots were initiated one to two centimeters from the tip. Lateral roots were shapeless and without organization. It was suggested that Al did not effect initiation of lateral roots or their penetration through the cortex. Although they did not provide a photomicrograph or indicate the location, these workers suggested the presence of polynucleated cells in Al injured tissue. Other workers have suggested the occurrence of polynucleated cells in Al injured cotton roots. Rios and Pearson (1964) found a large number of enlarged, binucleated cells in the meristematic region but provided no photographic evidence. Huck (1972) also reported polynucleated cells but found that they were confined to cortical tissue. In addition, he
indicated there was a breakdown of cells in the endodermis and that Al injured tissue was extremely fragile and collapsed during fixation and paraffin-embedding.

Levan (1945) found that many metals induced colchicine mitosis in onion roots but did not mention Al as one of those metals. A colchicine mitosis lacks spindle formation and cell division will not occur although the chromosomes have doubled. In his work, Levan did find that Al caused sticky chromosomes but cell division was nevertheless accomplished.

### Physiological-Biochemical Effects

**Nutritional**

Physiological-biochemical effects of Al can be classed as nutritional and effects on general metabolism, enzyme activity and protoplasm. Al-induced calcium (Ca) and phosphorus (P) deficiency were the major nutritional effects suggested. Generally, evidence for Al-induced Ca deficiency was based on visual symptoms and chemical analysis of plant tops. Symptoms in plant tops occurred several days after Al treatment and this led Kerridge (1969) to conclude the primary effect of Al toxicity was cessation of root growth. Many of the observed effects of Al on plants may be simply an indirect effect caused by lack of root growth in sensitive plants.

In a series of experiments studying the effects of polyvalent cations (Al and Sc) in barley, Clarkson and Sanderson (1971) showed
reduced uptake of Ca per plant. However, inhibition of Ca uptake by Al did not cause cessation of root growth. These workers found that Al treatment caused root weight to be reduced by 46% but Ca uptake per unit root weight was not depressed and possibly could have been greater.

Ali (1973) studied the influence of cations on Al toxicity in wheat. He concluded Al toxicity in nutrient solution could be prevented by extra amounts of any one of the cations Ca, Mg, K, or Na. These findings suggested a nonspecific, non-nutritional cation phenomenon and that Al toxicity could not be due to Al-induced deficiency of Ca, Mg, K, or P. It is worthwhile noting that Ali's work was unique in that a precise measure of Al injury can be assessed by the reinitiation of root growth or lack of it in a recovery solution after Al treatment (see also Moore, 1974).

If Al induced a P deficiency, the site of action had to lie within the plant according to Munns (1965). He presented data that disallows the conclusion Al toxicity inhibited growth primarily by inducing Ca or P deficiency. Munns suggested the term "deficiency" would have to be stretched to mean something more than an inadequate supply of nutrients in plant tissue. Wright (1943) proposed one such mechanism which was internal precipitation of P by Al. Electron microprobe analysis by Rasmussen (1968) did show Al and P localized together at the surface and within epidermal cells of *Zea mays*. However,
Waisel et al. (1970) found no such combination in bean and barley roots, but they induced Al toxicity in an alkaline (pH 9.3) solution where Al and P would not be expected to co-precipitate.

**Effects on General Metabolism, Enzyme Activity and Protoplasm**

Inhibited mitotic activity was the primary effect of Al causing root growth to stop in onion roots (Clarkson, 1965a). Very few mitotic figures could be observed in root squashes seven hours after exposure to a toxic Al concentration. It was at this time that all measureable root growth had likewise ceased. However, the root squash technique could not identify changes in mitotic activity in specific tissues during that seven hours. In some studies on DNA synthesis in Al treated barley roots, Sampson et al. (1965) noted abnormalities caused by Al. DNA synthesis continued following Al treatment but the DNA synthesized had an unusual base composition and was metabolically labile. The labile DNA was light weight and its base composition differed from that of heavy weight, genetic DNA (Sampson et al., 1963). Because no abnormalities were noted in the mitotic process, Clarkson (1968) speculated Al exerted a toxic influence on DNA replication.

Effects of Al on phosphorylated compounds in whole barley roots was studied by Clarkson (1966a). He found that $^{32}$P incorporation into phosphorylated hexose sugar was much reduced, while there was
an apparent accumulation of the nucleotide triphosphates, ATP and UTP. This implied inhibition of hexose sugar phosphorylation and should reflect a decrease in respiration. Although several barley varieties did show depressed respiration, it was noted only after extended Al treatment. These observations led Clarkson to conclude that impaired respiratory metabolism was not the cause of mitotic inhibition.

Huck (1972) pulsed $^{14}$C into the ambient atmosphere of untreated and Al treated cotton seedlings and observed the fate of labelled sucrose in roots. Untreated cotton seedlings assimilated most of the radioactivity into insoluble cell wall materials within 4 to 6 hours after the pulse. Roots of Al treated seedlings accumulated $^{14}$C labelled free sucrose. Huck speculated that a "metabolic block" was present in the latter roots which may be associated with polymerization of cellulose. It must be noted that the quantity of radioactive sucrose assimilated in lateral roots of treated plants 10 hours after the pulse was greater than in the controls.

Plasmolysis of cells treated with trivalent cations caused adhesion of protoplasm to side walls while the plasmalemma became detached from the end walls (Scrath, 1923). Using Spirogyra, a green, fresh water algae, Scarth was able to induce this trivalent cation type of plasmolysis with $10^{-4}$ M Al. The procedure he used consisted of washing the algae in distilled water, exposure to Al for
one hour and plasmolysis with 0.2 M KNO₃ to which was added a trace of CaCl₂ to delay penetration. Al alone could have caused the same plasmolysis but M/100 would be required. KNO₃ without Al pretreatment induced a plasmolysis where the protoplasm separated smoothly and uniformly from cell walls with little adhesion. Bohm-Tuchy (1960) presented very good microphotographs of this phenomenon described by Scarth. She also demonstrated that Al can induce adhesion of protoplasm to side walls of plasmolyzed onion cells.

**Differential Tolerance to Al**

**Plant Species and Varieties**

Differences in tolerance to Al among plant species would be expected simply because of natural selection. McLean and Gilbert (1927) reported these differences among many crop species. Mutation and natural selection have occurred within species to yield varieties possessing differential tolerance to soluble Al. Varietal differences have been reported in alfalfa (Dessureaux, 1969), cereals (Neenan, 1960), barley (MacLean and Chiasson, 1966; MacLeod and Jackson, 1967 and Reid et al., 1969), wheat (Foy et al., 1965a and Kerridge and Kronstad, 1968, Kerridge et al., 1971), soybean (Armiger et al., 1968), sunflower (Foy et al., 1974) and dry bean, snapbean and lima bean (Foy et al., 1967b).
Although soil can be used to test Al tolerance, it is difficult to observe a plant's roots which are injured. Nutrient solution culture has lent itself to these studies because roots can easily be observed and the composition of the culture medium can be rigorously controlled.

Observation of Al damage to roots grown in nutrient solution allowed Kerridge and Kronstad (1968) to demonstrate inheritance of Al reaction in wheat. Further work by Kerridge et al. (1971) showed that three reaction types existed in 50 wheat varieties. Moore (1974) modified the procedures developed by Kerridge (1969) to increase the sensitivity of the screening method. His modification utilized regrowth in an Al-free solution after a 48 hour exposure to Al as an index of damage. He found that four distinct classes among 50 varieties could be identified in this way.

pH Change in the Root Zone

Several hypotheses to explain the occurrence of differential Al tolerance between plant species and varieties within a species have been proposed (Brown et al., 1972). Foy et al. (1965b, 1967a) suggested that Al sensitive varieties of wheat and barley lower the pH in the root zone whereas resistant varieties created a more alkaline environment. But among Al sensitive and tolerant soybean varieties, Foy et al. (1969) could find no differential pH changes. McLean and
Gilbert (1927) tested twelve crops and found that the tendency of these crops to change the reaction of the solution culture either more acid or alkaline was not correlated with the sensitiveness toward Al. Dodge and Hiatt (1972) presented data showing differential pH changes in several solution cultures used to grow wheat. It is interesting to note in these Al free nutrient solutions, the induced pH change between Al sensitive and tolerant varieties did not differ markedly in the first 48 hours. The questionable practice of allowing pH to fluctuate in solution culture is two fold. First, the form of Al in solution is pH dependent (Moore, 1974). Secondly, the growth differences between sensitive and tolerant crops can be extremely large. A fluctuation in pH can reflect either Al injury in the sensitive plant or greater metabolic activity in the tolerant plant.

**Al Uptake and Transport**

Of the plant species that show symptoms of Al toxicity, chemical analysis data in the literature indicated very little Al is translocated from the roots. No differences in Al accumulation in the tops of sensitive and tolerant wheat was found by Kerridge (1969) even after 20 days exposure to Al. There was a greater concentration of Al in the roots of sensitive varieties; however, the weight of roots was markedly reduced. The lesser concentration in tolerant roots could reflect a dilution effect caused by continued growth.
Locating Al in a plant's roots has not met with a great deal of success. Advantage has been taken of large, negatively charged molecules of organic dyes (e.g. hematoxylin) which combine with trivalent cations. McLean and Gilbert (1927) found that Al treated roots of corn and cabbage were strongly stained but redtop, the most tolerant species in their study, was lightly stained. Hematoxylin stained the protoplasm and nuclei of the epidermis and outer cortex. These workers noted that none of the roots were severely injured by Al. Wright and Donahue (1953) used hematoxylin to show that Al did not penetrate beyond the endodermis in barley roots even when treated for four weeks. The roots were described as being few, much shortened, with stubby tips and somewhat brittle.

Lack of a suitable radioactive isotope of Al prompted Clarkson and Sanderson (1969) to substitute scandium (Sc) for Al to utilize $^{46}$Sc in autoradiographic studies. Sc had been shown to cause toxicity in onion similar to Al. The rate of Sc uptake in the apical 3.0 millimeters of the axis was more rapid than elsewhere in the root and proceeded in two phases. Phase 1 was a rapid, initial uptake little affected by low temperature. This was attributed to superficial absorption onto the mucigel of the root cap. Phase 2 was slower but remained constant for 24 hours and was highly temperature dependent. The movement of $^{46}$Sc in Phase 2 uptake appeared to be between root cap cells and directly into meristematic cells. Little $^{46}$Sc
became associated with root cap cells. Radial movement into meristematic cells was greatest in the apical 1.5 millimeter of the root. This region contains mostly primary cell walls whereas secondary walls occur in more differentiated cells. \(^{46}\)Sc movement into the vascular cylinder appeared to be effectively stopped by the endodermis. The endodermis was thought to become differentiated about 0.5 to 0.6 millimeter from the apex of the root tips.

Recent work utilizing an electron microprobe has allowed the detection of Al from its characteristic \(x\)-radiation given off when excited by an electron beam. Rasmussen et al. (1968) found Al precipitated on the surface of epidermal cells. Movement into the cortex occurred only when the root surface was broken, usually by penetration of a lateral root. Entry of Al into cortical cells of barley and beans was detected by Waisel et al. (1970). They found Al concentrated in cell lumens and not in cell walls. These workers contended the major difference between their work and Rasmussen et al. was in sample preparation. The former allowed their samples to thaw before microanalysis whereas Waisel et al. kept theirs frozen at all times.

**Objectives**

The literature is lacking a detailed histological description of Al injured roots. Present evidence suggests the primary meristem
is irreversibly damaged by a toxic treatment of Al. Although attempts have been made to locate Al in damaged roots, little success has been achieved. Dyes and electron microprobe analysis appear to be the best means available to locate Al since no suitable radioactive isotope of this element is available.

Al toxicity reaction among wheat varieties expressing differential tolerance has not been studied histologically. Reaction to Al appears to be relative, meaning that the symptoms of toxicity are identical but induced by different Al levels in the culture medium. A microscopic examination of Al injury in each reaction class could show the mutual effects and perhaps define the mechanism of toxicity.

The goals of this study were:

1. to make a detailed study of the progression of Al toxicity among various wheat reaction types to ascertain similarities and differences in response to Al,

2. to identify how Al treated roots become irreversibly damaged, and

3. to locate Al in the roots of Al treated plants.
III. MATERIALS AND METHODS

This study involved a microscopic examination of wheat root tips. To obtain slides for this examination, roots had to be grown and prepared. These two steps will be discussed separately.

Plant Growth

Plants were grown in a growth chamber with either 16 hour light periods or continuous lighting as will be noted. Continuous lighting was used to overcome any possible temperature fluctuation in the nutrient solution between light and dark periods. The procedure used to grow plants from which root tips were harvested is given in the following sequence:

a. Seeds were soaked in aerated tap water for 24 hours.

b. Sprouted seeds were placed on polyvinylidene chloride resin screen mounted in an acrylic cover. Greater uniformity of root elongation during the initial growth period was attained if all seed was aligned "seam side down". The acrylic cover is mounted on top of a 25 liter black polyethylene waste basket as described by Kerridge et al. (1971).

c. The seedlings were grown in 24 liters of full strength nutrient solution for 48 hours prior to Al treatment. This solution was adjusted to, and maintained at pH 4.0 ± 0.1 with $\text{H}_2\text{SO}_4$. 
or KOH. The solutions were continuously aerated and placed in a water bath in a growth chamber where the solution temperature was maintained at $25^\circ C \pm 1^\circ C$. Table 1 gives the composition of a full strength nutrient solution.

d. The acrylic covers with seedlings were transferred to Al treatment solutions which were also maintained at pH 4.0 $\pm$ 0.1. Al as $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$ was added to either one-tenth or one-half strength nutrient solutions as will be noted in the discussion. A one-tenth or one-half strength nutrient solution consisted of the appropriate fraction of the major plant nutrients given in Table 1 but omitting P. P was omitted to avoid precipitating Al from the nutrient solution. Micro-nutrients were supplied at full strength except the iron source was changed to FeCl$_3$ to avoid chelation of Al. Further detail describing the composition of the Al treatment is given by Ali (1973).

e. After an appropriate number of hours in the Al treatment, the seedlings were transferred back to the full strength nutrient solution, which is now called the recovery solution. Seminal root tips about $\frac{3}{16}$ of an inch long were cut from 10 to 12 plants at designated times. About 30 root tips were harvested during each collection. Preparation of these root tips will be discussed in the next section.
Table 1. Composition of the full strength nutrient solution.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>4 mM</td>
</tr>
<tr>
<td>Mg SO$_4$</td>
<td>2 mM</td>
</tr>
<tr>
<td>K NO$_3$</td>
<td>4 mM</td>
</tr>
<tr>
<td>(NH$_4$)$_2$ SO$_4$</td>
<td>0.435 mM</td>
</tr>
<tr>
<td>KH$_2$ PO$_4$</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>Mn SO$_4$</td>
<td>2 µM</td>
</tr>
<tr>
<td>Cu SO$_4$</td>
<td>0.3 µM</td>
</tr>
<tr>
<td>Zn SO$_4$</td>
<td>0.8 µM</td>
</tr>
<tr>
<td>Na Cl</td>
<td>30 µM</td>
</tr>
<tr>
<td>Fe-CYDTA$^a/$</td>
<td>10 µM</td>
</tr>
<tr>
<td>Na$_2$ MoO$_4$</td>
<td>0.1 µM</td>
</tr>
<tr>
<td>H$_3$BO$_3$</td>
<td>10 µO</td>
</tr>
</tbody>
</table>

$^a$/ CYDTA: Cyclohexanediamine Tetraacetic Acid
Preparation of Slides

Root tips were prepared for microscopic examination according to the botanical microtechnique procedures described by Johansen (1941). Material prepared for energy dispersive analysis of x-rays (EDAX) under the scanning electron microscope (SEM) beam was prepared according to the procedures outlined by Feder and O'Brien (1968). Details of the former procedure are given by Jensen (1962) pp. 78-90. A brief outline of these procedures follows:

a. Fixation: Root tips were placed in 60 ml bottles containing 20-30 ml of FAA. The bottles were placed in a vacuum desiccator and evacuated. The tissue was kept at reduced pressure in FAA for at least four hours. FAA is a mixture of formalin, glacial acetic and alcohol and its composition is given by Jensen, p. 79. The pH of this mixture was adjusted to 4.5 with sodium acetate where noted for some experiments; otherwise it was not adjusted.

b. Dehydration: The FAA was replaced with the first tertiary butyl alcohol (TBA) dehydrating mixture without washing the root tips. Washing was not needed since the root tips were collected from nutrient solution culture. The formula for the various TBA mixtures used for dehydration are given by Jensen, pp. 80-81.
c. Paraffin infiltration and embedding: Many deviations from Jensen's procedure were used during this step. After the third change of 100% TBA, it was replaced by a 1:1 mixture of TBA and paraffin oil. After 12 hours, one-half of this mixture was poured off and replaced with pure paraffin oil for an additional 12 hours. 30 ml glass vials were filled with 20 ml of melted paraffin and allowed to solidify. The root tips and paraffin oil - TBA mixture were poured onto the solid but warm paraffin. Excessive paraffin oil - TBA mixture was poured off before the vials were placed in a warming oven, about 70°C. As the paraffin melted, the root tips settled to the bottom of the vial. This step is vital to remove TBA which would otherwise crystalize during the embedding process and mechanically damage the cells. Excessive melted paraffin was poured off before the root tips were transferred to a second vial of paraffin and the infiltration process repeated. A third infiltration was done but "Paraplast" was substituted for paraffin. Paraplast is a commercial tissue embedding medium, composed of purified paraffin and plastic polymers of regulated molecular weight, melting point 56°C - 57°C. Embedding was done in melted Paraplast in paper embedding boats according to the method outlined by Jensen, pp. 82-83. The paper boats were not coated with glycerine.

Embedded material was stored at -5°C.

1/ Obtained from Van Waters and Rogers, Inc., Portland, Oregon.
d. **Sectioning:** Serial longitudinal sections were cut 10 microns thick on a rotary microtome. Jensen, pp. 83-88, discusses the principles used for microtoming at some length.

e. **Affixing sections to slides:** The serial sections of one root tip were mounted with Haupt's adhesive to a single glass slide by the water floatation method. The formula for Haupt's adhesive and the water floatation method are given by Jensen, pp. 88-90. All glass slides were etched with a diamond tip pencil to identify the specimens.

f. **Staining:** Prior to staining, paraffin must be dissolved and the sections rehydrated. These steps are discussed by Jensen, p. 90.

The primary stain was hematoxylin and the counter stain was fast green. Both dyes are discussed extensively by Johansen, (1941) pp. 50-53 and p. 59. As a dye, hematoxylin has little or no affinity for tissues, unless a trivalent cation is present, consequently mordanting in some form is necessary. Roots exposed to an Al solution were mordanted as this element was absorbed. Iron mordanting could be carried out on other sections to stain the nuclear component in the cells. Fast green stains the cell wall and cytoplasm as well as intensifying the appearance of hematoxylin.

The schedule for staining generally followed that given by Jensen, pp. 90-92, but with some modifications. Heidenhain's iron haematoxylin procedure was followed by fast green counterstaining.
rather than orange G. After bringing the slides to water, the following schedule was used:

1. Sections to be mordanted were placed in a 4% ferric ammonium sulfate solution for at least 1/2 hour. Slides that were "self mordanted" by Al were placed directly into hematoxylin stain after being brought to water.

2. Mordanted slides were washed with 3 changes of distilled water over a 15 minute period.

3. Slides were stained for at least 1/2 hour in 0.5% aqueous hematoxylin. This was prepared by dissolving 0.5 gram of hematoxylin in 100 ml of distilled water. A trace (ca. 10-50 mg) of sodium bicarbonate was added to the solution to "ripen" the dye. The solution should acquire a rich wine red color and will be useable for about one week or until the solution turns brown.

4. The sections were washed in running tap water for 15 minutes.

5. Destaining excessive hematoxylin was done by the picric acid method described by Tuan (1930). Approximately five minutes treatment at 45°C in distilled water saturated with picric acid removed the excessive hematoxylin which would otherwise turn the specimens gray.
6. The sections were again washed in running tap water for 15 minutes.

7. The sections were dehydrated for 3 minutes in each of 50, 70, 95 and 100 percent ethanol solutions used in that order.

8. Counterstaining with fast green followed for 3 minutes.
   A 0.5% solution of fast green in 50% clove oil and 50% absolute ethanol was used.

9. The slides were removed from the fast green stain, dipped in absolute ethanol to remove excessive stain and differentiated for 3 minutes in a mixture of 50% clove oil, 25% absolute alcohol and 25% xylene. The slides were removed from the first differentiating solution, dipped in absolute ethanol and placed in a second container of differentiating solution for another 3 minutes.

10. After differentiation, the slides were transferred through two changes of 1:1 absolute ethanol and xylene for 3 minutes.

11. Finally the slides were transferred through six changes of xylene with each transfer requiring 3 minutes. The many changes were required to remove any clove oil and 100% alcohol adhering to the slides.
g. Preservation: The slides were removed from xylene and a drop of "Permoun 1/2 was placed over the wet specimens before a cover slip was mounted on the slide. The cover slip was permanently mounted by warming the slide overnight at about 50°C.

Root tips prepared for EDAX in the SEM were killed and fixed with 10% Acrolein in tap water. Acrolein is a potent tear gas and should be handled with care. Dehydration was done by transferring the root tips through 3 changes of each of the following compounds: 2-methoxyethanol, absolute ethanol, n-propanol and n-butanol. The root tips were kept refrigerated in each change of solution for at least 24 hours.

The root tips were infiltrated with 3 changes of a 1:1 glycol methacrylate and n-butanol mixture with at least 24 hours allowed for each change. This was done at room temperature. Embedding was accomplished with glycol methacrylate, a monomer mixture which polymerizes into a solid, plastic medium. Polymerization occurs when the mixture is heated in the absence of oxygen. Melted paraffin was poured over a dish containing the monomer mixture and this served as an oxygen barrier. The warming plate holding the dish of monomer mixture was set at about 40°C. so the paraffin became solidified. Directions for preparation of the monomer

1/ Obtained from Van Waters and Rogers, Inc., Portland, Oregon.
mixture and sources of the constituents are given by Feder and O'Brien (1968).

After the monomer mixture had polymerized, about 5 days, the root tips were cut out of the plastic blocks and cemented to plastic stubs. One-half of a root tip was cut away longitudinally and the remaining half was used for the EDAX. The feed rate of the microtome was 3.5 micron per cut.
IV. RESULTS AND DISCUSSION

Effects of Al on Roots

Slides were prepared to study the effects of Al on the roots of wheat varieties representing four different levels of tolerance to Al (Ali, 1973). The design to obtain root tips was to subject these varieties to lethal and sublethal doses of Al according to the procedure described by Moore (1974). A lethal dose of Al was defined as the minimum concentration needed to prevent reinitiation of apical growth in seminal roots after recovery from a 48 hour Al treatment. The conditions of both Al treatment and recovery were given previously. Most seminal roots reinitiated apical growth within 48 hours after a sublethal dose of Al. Table 2 gives the wheat varieties and the respective sublethal and lethal Al dosages used for each.

Table 2. Al levels lethal and sublethal to four wheat varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Al level (ppm)(^a/)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sublethal</td>
</tr>
<tr>
<td>Brevor</td>
<td>0.1</td>
</tr>
<tr>
<td>Druchamp</td>
<td>1.0</td>
</tr>
<tr>
<td>Chinese Spring</td>
<td>2.0</td>
</tr>
<tr>
<td>Atlas 66</td>
<td>20.0</td>
</tr>
</tbody>
</table>

\(^a/\)Al treatment in one-tenth nutrient solution, 48 hour exposure, pH 4.0 and 25°C.
Atlas 66 and Brevor root tips were studied in detail because these varieties represented the known extremes of Al tolerance in wheat. Several series of slides were prepared from seminal root tips collected from each variety before Al treatments were begun and every 12 hours during the 48 hour Al treatments. After wheat seedlings were transferred back to an Al free nutrient solution, root tips were collected at 12, 24 and 48 hours of recovery. Five slides of root tips from each collection taken before recovery were stained with hematoxylin without iron mordanting. Ten to fifteen slides of root tips from each collection taken throughout the experiment were stained with hematoxylin after iron mordanting. All slides were counterstained with fast green. After a cover slip was permanently mounted, the slides were examined by both bright field and phase contrast microscopy.

Microscopic examination of these slides required the identification of tissues and cells throughout the root tip. For the most part, the anatomical nomenclature given by Esau (1967) was used throughout the discussion of this thesis. According to Esau, primary meristems are those cells which give origin to the fundamental body parts of plants which include root and shoot axes, their branches and appendages. The root primary meristem produces cells which give growth in two directions. First there are divisions which increase root length and secondly, divisions which form a rootcap
that precedes the growing root. The meristematic cells within the root, but not the rootcap, constitute the apical meristem. Those meristematic cells in the rootcap are designated as rootcap initials. Daughter cells from these meristematic cells differentiate into the several tissues shown in longitude and transverse sections of root tips in Figure 1.

The root epidermis in wheat consists of a uniseriate layer of cells. These cells are closely packed, radially elongated and thin walled. In wheat, the epidermis does not persist throughout the life of a root. Generally root hairs develop from epidermal cells but in the roots collected in this study, they were hairless. Root hairs do not develop on most plants grown in water culture.

The rootcap is a mass of living parenchyma cells covering the apical meristem. Parenchyma cells are variable throughout a plant but generally they have thin walls, a polyhedral shape and are concerned with vegetative activities. A mucilage layer separates the rootcap from the apical meristem or root apex. The outermost layers of rootcap cells are also covered by mucilagel. This layer of mucilagel is thought to aid in root penetration through the soil. Although rootcap cells are sloughed off as the root grows through the soil, the departing cells remain turgid because they are surrounded by mucilagel. When plants are grown in water, the rootcap can become reduced in size and structurally loosened.
Figure 1. Anatomy of wheat root tips.
The cortex in wheat is a relatively simple tissue mostly composed of parenchyma cells. Only the innermost layer of cortical cells differentiate into a specialized tissue, the endodermis. In wheat longitudinal sections, the cortex is arranged in orderly rows of cells whereas in cross section, it is formed of concentric layers. This arrangement results from periclinal cell divisions increasing the number of layers while anticlinal divisions increase the circumference of each layer. Many intercellular spaces develop in the cortex and can be detected 50 to 100 microns from the boundary between the apical meristem and rootcap in wheat. As a cortical cell matures, it develops large vacuoles which contain cell sap, there is no cytoplasm in vacuoles.

An endodermis is characterized by casparian strips on its anticlinal walls. The casparian strip is common to nearly all plant roots but is narrower than the cell wall. It is usually located closer to the inner tangential wall and is considered to be an effective barrier against the movement of solutes into the central cylinder. Formation of the casparian strip begins after centripetal growth of the cortex is completed. In wheat, this can be within 200 microns of the rootcap. The casparian strip develops in the primary cell wall before any secondary wall development is evident. Cytoplasm in the endodermis becomes attached to the casparian strip and will not separate from it even when the tissue is plasmolyzed.
The central cylinder contains the vascular elements, phloem and xylem, and associated parenchyma cells. The layer of cells immediately within the endodermis is the pericycle. It is generally an uniseriate layer of parenchyma cells. In wheat the pericycle may be interrupted by the differentiation of xylem and phloem elements. The pericycle remains potentially meristematic even in the differentiated portions of the root. It is from the pericycle that lateral roots are initiated.

Differentiation of the vascular elements generally occurs beyond the meristematic region of the root tip. Only the central metaxylem vessel extended into the apical meristem. The central location of these vessel elements aided in finding the best median longitudinal section on each slide. Most of the root tissues except the vascular elements could be identified within a few hundred microns of the boundary between the apical meristem and rootcap. Before these tissues became fully differentiated, they went through a period of cell division and elongation. The epidermis and cortex became differentiated much closer to the rootcap than tissues in the central cylinder. Formation of vacuoles in cells was one of the first steps in tissue differentiation. Cortical cells developed vacuoles within 100 microns of the boundary between the apical meristem and rootcap. In the central cylinder, vacuoles developed in the central metaxylem vessel about 400 microns from the boundary while other
tissues developed vacuoles somewhat less than 1000 microns from the rootcap. Even at 1000 microns, the pericycle was only slightly vacuolated. Secondary wall development did not occur in the root tip to a great extent outside of the central cylinder. The vascular elements developed secondary walls about the same time vacuoles became prominent.

In wheat, there is a clear boundary between the rootcap and the rest of the root. On the rootcap side lie the rootcap initials and it would seem logical to designate the cells on the other side as apical meristem initials. But these latter cells rarely divide and certainly do not initiate all the meristematic cells throughout the root tip. Most of the nomenclature for cells opposite the rootcap reflect a type of development (See Esau, 1967, p. 116-124). Instead of using that nomenclature, the cells opposite the rootcap were identified as apical cells and formed what will be designated as the root apex throughout the discussion in this thesis. Esau had used this same term to identify the apical meristem in any root but she included all plant species. Many plant species do not have such a clear boundary between the rootcap and the rest of the root like wheat.

**Atlas 66: Sublethal Treatment**

Figure 2 shows a series of Atlas 66 root tips sections collected before and during the 48 hour sublethal (20 ppm) Al treatment. These
Figure 2. Atlas 66 root tips through 48 hours of sublethal A1 treatment - mordanted by absorbed A1.
slides were stained without iron mordanting. In this series of slides, hematoxylin was attracted to the Al already present in the root tip section because of its affinity for a trivalent cation. However, the Al - hematoxylin stain represented only the Al remaining sufficient to attract a detectable amount of dye. Al could be present in unstained portions of the root tip in amounts too low to be detected. In addition, loses of Al could have occurred either during fixation or preparation of the root tips. The fixative, FAA, was an acidic solution of formalin, acetic acid and alcohol which could dissolve Al salts. During preparation of the root tips, there were 40 or so changes of solution, several of which were capable of dissolving Al salts.

The control root tip in Figure 2 retained very little hematoxylin and that could have been attracted to the iron normally found in the plant tissue. A sufficient amount of iron was present in the nutrient solution for adequate plant nutrition. After 12 hours exposure to Al, the root tip still resembled the control except for Al - hematoxylin staining of most nuclei in epidermal cells and several cortical cells adjacent to the epidermis. Al - hematoxylin staining extended into several cells of the epidermis between the root apex and rootcap but did not completely surround the root apex. Some rootcap cells immediately across the root apex-rootcap boundary had stained nuclei too. Hematoxylin also stained the cytoplasm of most epidermal
cells and some cortical cells as well as the intercellular space between these cells.

After 24 hours of Al treatment, Al-hematoxylin completely surrounded the root apex along the boundary with the rootcap. The stain could be observed in epidermal cells and rootcap cells adjacent to the boundary between these two parts of the root tip. Some of the rootcap initial cells immediately opposite the root apex showed signs of swelling. Other cells in the rootcap and root apex appeared to have collapsed, perhaps from the pressure exerted by the swelling cells. There was further penetration of Al into the cortical tissue but the nuclei of these cells were not as darkly stained with hematoxylin as the nuclei in the epidermis. In addition, both epidermal and cortical cells were swollen and only a small portion of the cell was occupied by cytoplasm. The remainder of the volume was vacuolated although the nucleus was still evident. Similar vacuolation also occurred in swollen rootcap cells too.

Swelling of both epidermal and cortical cells had advanced greatly after 36 hours of Al treatment. Further displacement of cytoplasm in both epidermal and cortical cells continued from what was noted at 24 hours. Very little hematoxylin could be detected in the swollen cells because little of the cytoplasm matrix remained and the cell sap was most probably lost during fixation and preparation of the roots. Dense Al-hematoxylin stain could be detected in the
least swollen cells, in collapsed cells and between the boundary of the root apex and rootcap. There were, however, many swollen cells in both the root apex and rootcap close to the collapsed cells. Most meristematic cells appeared intact although the vacuoles, which are usually small in these cells, were somewhat enlarged.

After 48 hours of Al treatment, swelling had occurred throughout the cortex but did not include the endodermis. Very little Al-hematoxylin could be detected throughout these swollen cells but there was virtually no cytoplasm remaining. There was a great deal of Al-hematoxylin about the root apex but that was located only in normal and collapsed cells. Hematoxylin staining indicated that Al had ascended in the meristematic cells from the root apex. A semi-spherical group of cells at the root apex has been identified as the "quiescent center" by Clowes (1954). The importance of the quiescent center will be discussed later in a separate section. Entry of Al into the meristematic cells seemed to be through the boundary separating the root apex and rootcap and then upward. Although Al appeared to be ascending into the meristematic cells, most remained intact even though vacuoles had enlarged significantly.

Another sequence of Atlas 66 roots treated with sublethal Al for 48 hours are shown in Figure 3. These root tip sections were stained with hematoxylin after being mordanted with iron which made stainable the chromatin in the nucleus so the stages of cell division
Figure 3. Atlas 66 root tips through 48 hours of sublethal Al treatment - mordanted with iron ammonium sulfate.
could be seen. In the control section, mitotic figures could be observed. About 10 percent of the meristematic cells appeared to be in some phase of cell division. However, after 12 hours of Al treatment, the occurrence of mitotic figure was greatly reduced in the meristematic cells of the central cylinder and none were evident in the epidermis. The nuclei in the epidermis were heavily stained with hematoxylin and the stain had advanced to about the same extent as noted in Figure 2 at 12 hours. Nuclei in other cells were stained with hematoxylin and nearly all were simply large, metabolic nuclei with one to several nucleoli. Again it could be noted that Al appeared to have begun to move into the root along the boundary between the root apex and rootcap.

Twenty-four hours after Al treatment had begun, Al-hematoxylin completely surrounded the root apex along the boundary with the rootcap. Several cells were collapsed in the epidermal layer about the root apex while some rootcap initial cells immediately across the root apex-rootcap boundary were swollen. Additional swelling had occurred in epidermal and cortical cells during the previous 12 hours. The cytoplasm in several of these swollen cells had formed bodies about the size of nuclei and these were heavily stained with hematoxylin. No swollen or collapsed cells had any mitotic figures which would indicate that Al did not prevent the completion of mitosis during and stages of cell division. Within the
undifferentiated central cylinder, occasional mitotic figures were present but most nuclei were large and in interphase with one to several nucleoli present.

After 36 hours of Al treatment, very few cell divisions could be observed in the central cylinder and most sections did not contain any. These divisions occurred only in cells that had most of their cytoplasm intact. Most epidermal and cortical cells had continued swelling and their cytoplasm was displaced by vacuoles. The displaced cytoplasm became adhered to cell walls or concentrated in the corners of the cell. Nuclei remained within the vacuolated cell although in some instances, there were bridges of cytoplasm linking them to the material adhered to the cell walls.

At the end of the 48 hour sublethal Al treatment, several cells in stage of cell division were found throughout the central cylinder. It would be doubtful if root length could have increased significantly by those divisions. Several cell divisions could be seen just a few cells removed from the root apex-rootcap boundary. Other divisions near the differentiated portion of the root did not constitute lateral root initiation. Swelling usually extended through the cortex but not to the endodermis which seemed to remain unswollen although the cytoplasm was drawn up to the casparian strip. There were swollen and collapsed cells in both the root apex and rootcap adjacent to the boundary separating these two parts of the root. It appeared that
cells collapsed opposite swollen cells although swelling probably occurred in either the root apex or rootcap as a matter of chance.

**Atlas 66 : Lethal Treatment**

Figure 4 shows a sequence of Atlas 66 root tips collected from the lethal (36 ppm) Al treatment. The root tip sections in this series of photographs were stained with hematoxylin but without being mordanted in iron. The control is the same root section shown in Figure 2. Twelve hours after Al treatment was begun, the root apex was surrounded by Al-hematoxylin stain. Nuclei and cytoplasm in most epidermal cells were stained with hematoxylin. In the rootcap, hematoxylin stain was in and around rootcap initial cells. There was likewise some hematoxylin staining of cortical cells and intercellular spaces in the cortex.

After 24 hours of Al treatment, the root apex was surrounded by a dense amount of Al-hematoxylin stain. There appeared to be a few cells swelling in the rootcap next to the boundary between it and the root apex and the cytoplasm was being displaced by large vacuoles. Some epidermal and cortical cells just beyond the rootcap boundary had swollen and became vacuolated too.

Thirty-six hours of Al treatment resulted in a greater accumulation of Al-hematoxylin in the boundary between the root apex and rootcap. Some cells were swollen while others were collapsed in
Figure 4. Atlas 66 root tips through 48 hours of lethal Al treatment - mordanted by absorbed Al.
each part of the root. The swelling in both epidermal and cortical tissues was not as great as noted after 36 hours of sublethal Al treatment.

After 48 hours of Al treatment, the meristematic cells in the central cylinder had been greatly affected by Al. The cell walls in this region were wrinkled like those of swollen cortical cells. Within the meristematic cells, the nucleus remained but the cytoplasm had been displaced by vacuoles and was adhered to the cell walls. In many instances, the nucleus was attached to this cytoplasm and held in place next to the cell wall. Occasionally, the nucleus was attached to cytoplasm adhered to opposite cell walls and that resulted in its deformation. The cytoplasm in the endodermis was mainly attached to the casparian strip and the nucleus was generally attached to it. Unlike other cells in the cortex, the endodermis did not swell markedly. Although both epidermal and cortical cells were swollen after 48 hours of lethal Al treatment, these cells were not swollen as greatly as those subjected to the same period of sublethal Al treatment. More hematoxylin was detected in these less swollen cells probably because the cytoplasm was denser rather than due to the higher level of Al.

Figure 5 shows another series of Atlas 66 roots treated with lethal Al except these were mordanted with iron before hematoxylin staining. After 12 hours of Al treatment, a few cells were in stages
Figure 5. Atlas 66 root tips through 48 hours of lethal Al treatment - mordanted with iron ammonium sulfate.
of division but only within the central cylinder. Most meristematic cells were in interphase and their nuclei were large and had one to several nucleoli. The nuclei in cortical cells were usually surrounded by cytoplasm although a large portion of the cell would be empty.

Only an occasional mitotic figure could be observed in meristematic cells within the central cylinder after 24 hours of Al treatment. Other than that, the nuclei in meristematic cells appeared like normal, interphase nuclei before cell division was initiated. The nuclei in the epidermis and some cortical cells were heavily stained with Al-hematoxylin.

After 36 hours of Al treatment, the terminal portion of the apical meristem, about 300 microns, was greatly affected by Al. The walls of meristematic cells in this portion of the root tip were wrinkled and the cytoplasm had become adhered to cell walls. The nuclei in these meristematic cells appeared granular and their nucleoli were not as prominent. Beyond this affected portion of the root, the cells and their cytoplasm were intact and the nuclei appeared normal. No mitotic figures were seen in the primary meristem at this time.

At the end of the 48 hour Al treatment, further degeneration of the cytoplasm had occurred throughout the apical meristem. All the nuclei appeared granular and in many instances were embedded in the cytoplasm matrix adhered to the cell wall. The chromatin did
not appear diffuse as is the case in normal interphase but were not initiating mitosis either. In fact, these nuclei appeared to be degenerating as a result of Al toxicity.

Atlas 66: Recovery

Recovery of Atlas 66 roots from both sublethal and lethal Al treatments is shown in Figure 6. Many cell divisions were evident throughout the meristematic cells in the central cylinder after the first 12 hours of recovery from the sublethal treatment. No tissues or structures could be identified in these actively dividing meristematic cells except the central metaxylem vessel. The orientation of many of the planes of cell division was periclinal. Esau (1967) has found that the sequence of periclinal divisions is centripetal in most roots. The inner daughter cell continues to divide whereas the outer cell begins to differentiate or undergoes anticlinal division. This sequence of divisions would result in increased meristematic diameter and the outer cells would differentiate sooner than the inner cells.

After 24 hours of recovery, the diameter of the meristematic region had increased markedly and there was a new root apex formed. The outer cells of this new apex were forming what appeared to be a new rootcap about 300 microns behind the old rootcap which was still attached to the root. Between these was a region of damaged
Figure 6. Recovery of Atlas 66 root tips following sublethal and lethal Al treatments.
cells that constituted the old root apex and included cells that were previously the quiescent center. The older daughter cells at the margin of the new meristematic region were becoming clearly delineated from the old, swollen cells, but these cells did not constitute a complete epidermis yet.

Besides a new rootcap, a new epidermis was well formed after 48 hours of recovery. The old rootcap and most of the Al-injured cells comprising the old epidermis, cortex and endodermis had sloughed off by this time. Differentiation of the new epidermis had arisen from the outermost daughter cells originating from the central cylinder. Between the newly formed epidermis and the innermost layer of daughter cells were other daughter cells that developed into a new cortex and endodermis. Cells in the new cortex which had origins from the central cylinder were disjointed; however, cortical cells having their origin in the new root apex had developed the orderliness evident in the control root tip sections.

Recovery of Atlas 66 roots from the lethal Al treatment consisted mainly of lateral root development. Within the old meristematic cells in the root apex and central cylinder, degeneration of nuclei continued. Very little cytoplasm was present in these cells at the end of the Al treatment. The degeneration of nuclei proceeded up to the differentiated portion of the root tip, about 1000 microns. Many meristematic cell walls were collapsed during
the recovery because they lacked secondary wall development necessary for support. Lateral root development continued from those initiated both during and after Al treatment. A later section is devoted to lateral root development.

Brevor: Sublethal Treatment

Figures 7 and 8 show the same sequence of Brevor root tips collected from a sublethal (0.1 ppm) Al treatment. The slides in Figure 7 were not mordanted in iron but those in Figure 8 were. Many mitotic figures could be observed throughout the iron mordanted control root tip. The same treatment in Figure 7 was uniformly stained with fast green. After 12 hours of Al treatment, hematoxylin staining indicated that Al had entered the epidermal layer and was moving through the boundary between the root apex and rootcap. Some cortical cells had developed large vacuoles in their cytoplasm. The vacuoles were unusual because they were much larger than those found in the same relative position in the control. Mitotic activity had nearly ceased except for an occasional cell division in the central cylinder.

Twenty-four hours after Al treatment was begun, the root apex was surrounded by Al beneath the rootcap. Most epidermal cells and the outermost cortex had swollen appreciably. Although Al could be detected in and about these swollen cells, the greatest
Figure 7. Brevor root tips through 48 hours of sublethal A1 treatment - mordanted by absorbed A1.
Figure 8. Brevor root tips through 48 hours of sublethal A1 treatment - mordanted with iron ammonium sulfate.
amount of hematoxylin was to be found in the least swollen cells. Some cells about the root apex - rootcap boundary were markedly swollen while other cells were collapsed. The collapsed cells were heavily stained with hematoxylin. Mitotic activity had nearly ceased except for isolated cell divisions occurring in the meristematic cells in the central cylinder.

After 36 hours of Al treatment, most epidermal and cortical cells had become swollen but not the endodermis. These swollen cells were so enlarged that their cytoplasm occupied only a small portion of the total cell volume. Some cell divisions were occurring in the apical meristem which resulted in some enlargement of the central cylinder. All the cytoplasm in these dividing or recently divided cells appeared to be intact. There was a great deal of Al-hematoxylin adjacent to the central cylinder in Figure 8 but the meristematic cells beyond the stain appeared normal.

At the end of the 48 hour sublethal Al treatment, several more cell divisions had occurred in the apical meristem. The orientation of these divisions was periclinal to the root apex - rootcap boundary. Further swelling of both epidermal and cortical tissue had occurred. Very little hematoxylin stain was present in those swollen cells because they were void of nearly all their cytoplasm. The collapsed cells about the root apex contained a great deal of hematoxylin but
their cytoplasm was compressed into the small volume of what remained of the cell.

**Brevor: Lethal Treatment**

Figures 9 and 10 show a sequence of Brevor root tips collected during a lethal (0.4 ppm) Al treatment. The sections shown in Figure 9 were not mordanted in iron but those in Figure 10 were. Control root sections were the same shown in Figures 7 and 8 from the unmordanted and iron mordanted slides respectively. After 12 hours of Al treatment, Al had entered the epidermal layer and some cortical cells. The stain appeared darker in these cells than it did after 12 hours of sublethal treatment. Al had not completely surrounded the root apex at this time. The outermost cortical cells were becoming vacuolated.

After 24 hours of Al treatment, hematoxylin staining indicated that Al had completely penetrated the root apex - rootcap boundary and surrounded the root apex. Al had also penetrated several cortical cells but neither epidermal nor cortical cells were swelling appreciably. Some cells at the root apex - rootcap boundary were swollen and there were collapsed cells adjacent to those. Mitotic activity had essentially ceased throughout the apical meristem.

Some epidermal cells were swollen after 36 hours of Al treatment but the cortex was only slightly swollen and that was
Figure 9. Brevor root tips through 48 hours of lethal A1 treatment - mordanted by absorbed A1.
Figure 10. Brevor root tips through 48 hours of lethal A1 treatment - mordanted with iron ammonium sulfate.
confined to the outermost cells. More Al-hematoxylin had accumulated in cells adjacent to the root apex - rootcap boundary and that was restricted mostly to collapsed cells. There were several swollen cells in this region but they retained no Al-hematoxylin. The cells in the apical meristem were developing large vacuoles. If a mitotic figure could be located in these cells, it occurred in a cell which had little or no vacuolation.

At the end of the 48 hours lethal Al treatment, most of the cells throughout the apical meristem appeared to be void of most of their cytoplasm. However, examination of many of these cells by phase contrast microscopy indicated that cytoplasm was concentrated next to cell walls. In many cases, there were bridges of cytoplasm linking the nucleus with cytoplasm adhered to the cell wall. In certain cells, several such bridges existed and deformation of the nucleus resulted. The root itself was only slightly swollen and that was confined principally to the epidermis.

**Brevor : Recovery**

The recovery of Brevor root tips from both sublethal and lethal Al treatments is shown in Figure 11. Twelve hours after the end of the sublethal Al treatment, the meristematic cells in the apical meristem were actively dividing. Most of these divisions were confined to the central cylinder but some were evident in the cortical
Figure 11. Recovery of Brevor roots following sublethal and lethal A1 treatments.
tissue too. The orientation of the plane of division of most of these cells was periclinal and no divisions were occurring in cells that were collapsed or greatly swollen.

After 24 hours of recovery, a new epidermis and rootcap were evident. The meristematic region had widened appreciably by many periclinal divisions and the outermost daughter cells appeared to be differentiating into the outer root tissues. The boundary between the new root apex and the new rootcap was clearly delineated about 300 microns behind the old boundary. The old, Al-injured rootcap and collapsed cells of the old root apex were still attached to the root, but some of the old epidermis and cortical tissue had sloughed away.

Forty-eight hours after the recovery was begun in an Al free nutrient solution, the root tip had regained its structure. The new rootcap was clearly delineated from the new root apex and the new epidermis was well defined. The old Al-damaged epidermis, cortex, endodermis and rootcap had completely sloughed away at this time. Within the cortex, the cells were not organized into continuous files except for the most recently formed cells near the root apex. The many periclinal divisions in this region did not maintain any continuity along the full extent of the apical meristem.

The Brevor roots treated with lethal Al did not reinitiate apical root growth after being placed in the recovery solution. Lateral roots were initiated from the differentiated portion of the root to
about 800 microns from the root apex. Lateral root development is discussed in a later section. Most of the nuclei were still present in the Al-injured meristematic cells after 12 hours of recovery but they appeared very granular and their nucleoli were not easily distinguished. As the recovery period progressed, many nuclei were lost from Al-injured meristematic cells until most of these cells were void of any cellular constituents. As the nuclei were lost, the Al-injured meristematic cells also collapsed because they lacked any secondary wall development. Both phenomena, loss of nuclei and collapsation of cells, proceeded up the central cylinder and stopped where differentiation of vascular elements began.

Comparing Atlas 66 and Brevor

Comparing the sequences of Atlas 66 and Brevor roots in Figures 2 through 11 showed few differences between the two wheat varieties. The root anatomy of both varieties was the same but it appeared that Atlas 66 roots had a greater diameter than Brevor. An attempt was made in a "blind" experiment to relate root diameter to differential Al tolerance in wheat. In the blind experiment, ten varieties with a known Al reaction were coded and their identity was held by an impartial judge. Seedlings were grown in an Al free nutrient solution at pH 4.0 and their roots were harvested and prepared for microscopic examination. Prediction of Al reaction class was based on the diameter
of the epidermal-cortical initial cell thickness at the root apex. (This was also the diameter of the quiescent center.) The largest diameters were thought to be related to the greatest Al tolerance. However, only one out of ten varieties was correctly identified. This low degree of success did not support the involvement of root diameter in differential Al reaction among wheat varieties.

During the sublethal Al treatments, the movement of Al into Atlas 66 and Brevor roots was very much alike. Hematoxylin detected Al within epidermal cells after 12 hours of treatment. The intensity of the Al-hematoxylin was much greater in Atlas 66 than Brevor, but the amount of Al in the nutrient solution was 200 times greater for the former variety. Al continued to enter the wheat root but it followed a unique path. Al appeared to move along the root apex-rootcap boundary until the entire root apex was surrounded. From this boundary, Al penetrated the apical meristem. This is supported by the extent of damage that can be seen in the recovery sequences shown in Figures 6 and 11. Sublethal Al treatments killed cells throughout a 200 to 300 micron region in the apical meristem which paralleled the root apex-rootcap boundary. A sufficient amount of Al had failed to penetrate the endodermis or to ascend into the central cylinder to be lethal to the remaining cells. The endodermis appeared to be an effective barrier against the entry of Al into the meristematic cells about 200 to 300 microns beyond the rootcap. Meristematic cells in the central cylinder adjacent to the endodermis resumed
mitotic activity after the sublethal Al treatment. Even during the lethal treatment, the endodermis prevented injury to lateral roots and possibly stopped the entry of Al directly into the central cylinder. The consequences of Al injury, degeneration of cytoplasm and nuclei and collapsation of cells appeared to ascend up the root central cylinder suggesting that the endodermis was indeed an effective barrier to Al.

The most striking response to sublethal Al treatments in Atlas 66 and Brevor roots was the swelling induced in epidermal and cortical cells. Some swelling had occurred after the first 12 hours of Al treatment but it had not increased cell volumes appreciably yet. Further swelling continued throughout the Al exposure so that the diameter of the roots had nearly doubled. Most of this swelling was confined to the epidermis and outermost cortical cells; the endodermis and cells in the central cylinder did not swell appreciably. Additional swelling was noted about the root apex-rootcap boundary. However, there was marked collapsing of cells adjacent to swollen cells in both the root apex and rootcap.

Mitotic activity decreased abruptly after 12 hours of sublethal Al treatment. After 36 hours of Al treatment, some cell divisions were seen in the apical meristem but only within the central cylinder. The orientation of these cell divisions was generally periclinal to
the nearest root surface. These divisions continued through the next 12 hours but they did not increase root length.

After being placed in an Al-free recovery solution, the apical meristems of both Atlas 66 and Brevor roots became very active. Most of the activity was present in the central cylinder, but in Brevor, there were several divisions in the cortical tissue. The orientation of most divisions was periclinal to the nearest root surface. Cell divisions proceeded in a centripetal sequence which resulted in the inner daughter cells remaining meristematic whereas the outermost cells began to differentiate. It was through this process of cell division in the apical meristem that developed a new epidermis cortex and rootcap. These new tissues replaced those that were injured by Al and sloughed off the regrowing root.

Atlas 66 and Brevor responded to their respective lethal Al treatments very much alike. Al entered the root in the same manner as was noted during the sublethal Al treatment. But the entry of Al occurred sooner because the root apex was surrounded by hematoxylin stain in a shorter period of time. Although Al could be detected in most cells at the root apex, none could be detected in the meristematic cells within the central cylinder even after 48 hours of lethal Al treatment.

The rate of cell division in the apical meristem of Atlas 66 and Brevor was greatly reduced after 12 hours of lethal Al treatments.
Although isolated mitotic figures could be seen in meristematic cells later during the Al treatment, the orientation of the planes of division were at random. After 48 hours of Al treatment, no mitotic figures could be seen in the apical meristem. Instead, the meristematic cells developed large vacuoles in their cytoplasm. This condition appeared to start near the root apex and ascended up the central cylinder. The nuclei in both Atlas 66 and Brevor meristematic cells appeared granular and they would never reinitiate mitotic activity.

In the recovery solution, the Al injured apical meristem cells began to degenerate. Most of the cytoplasm had already degenerated or adhered to the cell wall during the Al treatment. Further degeneration of nuclei now occurred in the recovery solution and after 48 hours, they had disappeared through 800 to 1000 microns of the apical meristem. When these meristematic cells were void of their cellular constituents, they collapsed.

**Effect of Al on Cells**

The effect of Al on cell division and the constituents of the cell are discussed in this section. In the previous section, depressed mitotic activity was noted after the first 12 hours of either a sublethal or lethal Al treatment. A series of slides were prepared to study mitotic activity in relation to the length of Al exposure in Brevor
seedlings grown in one-half strength nutrient solution treated with 10 ppm Al. This level of Al is twice the amount required to inhibit Brevor roots after 48 hours of exposure (Ali, 1973) and was chosen to insure that 100 percent of the roots would be irreversibly damaged by Al. Root tips were collected before and every three hours during the 24 hour treatment. Additional collections were made every three hours for 24 hours from a group of seedlings that were recovering after 12 hours of Al exposure to study the resumption of mitotic activity. Inhibition of apical root growth in wheat roots has recently been found to be dependent on the duration of the Al exposure. In this study, all plants reinitiated root growth in a recovery solution after 6 and 12 hours of Al treatment. No Brevor seedlings reinitiated root growth after 24 hours of Al exposure; however, the 18 hour exposure had about one-third to one-half the roots reinitiate apical root growth.

Cell Division

Mitotic figures were counted in 90 micron diameter fields in the rootcap and ascending fields in the central cylinder. The diameter of a root central cylinder was somewhat greater than 100 microns. This allowed a 90 micron field to fit within the central cylinder without

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3 Personal communication with Mr. Dean Rhue, graduate student, Department of Soil Science, O.S.U., Corvallis.
including any cortical cells. The first field at the root apex contained 30 to 40 cells; however, cell size increased with ascending fields and that decreased the number of cells viewed per field. A mitotic count included any dividing cell whose nuclear component lay between late prophase and telophase without a cell plate separating the two daughter cells. Late prophase was selected to begin the mitotic count because the chromosomes were much shortened and easily observed. Occasionally adjacent sections had to be viewed to correctly identify which cells were dividing. The 3 best median sections from 8 to 10 roots were counted and averaged to obtain a mitotic count for a "normal" root section.

Figure 12 shows the sequence of Brevor root tips collected through 21 hours of Al treatment. The roots collected at 24 hours of treatment were lost during fixation. Additional collections of roots being recovered from the lost Al treatment indicated that none of those plants reinitiated apical root growth. Figure 13 shows plots of the number of mitotic figures counted in the various fields throughout the root tip. The effect of this Al treatment on root elongation was compared in Brevor at Atlas 66 in Figure 14. Ten ppm Al in one-half nutrient solution was toxic to Brevor but not Atlas 66. Decreased mitotic activity in Brevor coincided with the cessation of root elongation. Because Atlas 66 roots continued to grow, no roots tips of it were prepared for microscopic examination.
Figure 12. Brevor root tips through 21 hours of 10 ppm A1 treatment in one-half strength nutrient solution.
Figure 12. (Continued).
Figure 13. Effect of 10 ppm Al on mitotic activity in Brevor root tips.
Figure 14. Root elongation of Atlas 66 and Brevor treated with 10 ppm Al.
Mitotic figures disappeared rapidly in all fields during the first 12 hours of Al treatment. A delay was expected in their disappearance from both the second and third fields which would have reflected the upward movement of Al from the root apex-rootcap boundary. There was an indication that mitotic activity in the second field was less depressed after the first three hours but the variation between slides was large and made this observation doubtful. The major problem with mitotic counts was that they reflected the presence of Al long after it had entered the cell. Although mitotic activity may have stopped after 12 hours of exposure, another 12 hours were required before the apical growth of root tips could be completely inhibited in a recovery solution. This suggested an additional amount of Al was required after mitotic activity was stopped which would then be sufficient to kill the cells throughout the primary meristem.

When root tips were not mordanted with iron, hematoxylin staining detected Al about the root apex several hours after mitotic activity had been depressed in the apical meristem. Al may have followed the suggested path from the root apex-rootcap boundary, but the amount needed to inhibit mitosis would have been less than what was detectable with hematoxylin. Secondly, movement along this pathway could have proceeded at such a rapid rate that it would not have been reflected by delayed mitotic activity in ascending fields within the central cylinder.
Once mitosis was initiated, the process went to completion, i.e., there was a progressive disappearance of mitotic figures during the Al treatment. Had the actual process of mitosis been interrupted by Al, some mitotic figures should have been arrested in recognizable stages especially in the more severe Al treatments. If Al inhibited the mitotic cycle at a single point as Clarkson (1968) speculated, then that point would have occurred 12 hours before the completion of mitosis. Accounting backward from the completion of mitosis would place the nuclei in the S period of the mitotic cycle according to the time required for each period (Van't Hof, 1965). Clarkson (1968) had concluded that Al was inhibitory to the synthesis of DNA during the latter stages of the S period.

Reinitiation of apical root growth in Brevor seedlings after 12 hours of Al treatment is shown in Figure 15. At the end of the Al exposure, the nuclei throughout the apical meristem appeared to be in the latter stages of interphase. The nuclei were large, their chromatin was diffuse and each contained one to several prominent nucleoli. Vincent (1955) pointed out that meristematic cells will have such an appearance prior to the initiation of mitosis. In this study, these cells began to divide when they were removed from Al at the end of 12 hours. Table 3 summarizes the number of mitotic figures seen in ascending fields both during and after Al treatments.
Figure 15. Recovery of Brevor root tips from 12 hours of 10 ppm A1 treatment.
Table 3. Mitotic figures in ascending 90 micron fields within the apical meristem of Brevor roots during a 10 ppm Al treatment and after 12 hours of Al exposure. $^a$

<table>
<thead>
<tr>
<th>Hours of Al treatment</th>
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<th>Second field $^b$</th>
<th>Third field $^b$</th>
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<td>0.5</td>
<td>0.5</td>
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<td>0.3</td>
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<td>0.2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Hours of recovery after 12 hours</th>
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<th>Second field $^b$</th>
<th>Third field $^b$</th>
</tr>
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</tr>
<tr>
<td>+18</td>
<td>1.9</td>
<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

$^a$ Ten ppm Al in one-half strength nutrient solution at pH 4.0 and 25°C.

$^b$ Standard deviations given in Appendix Table 2.
Three hours after the recovery was begun, scattered mitotic figures could be seen throughout the apical meristem. There was a marked increase in their numbers over what was observed in roots at the end of the Al treatment or those remaining in the Al solution (15 hours). After six hours of recovery, the number of mitotic figures per field had further increased and was equal to what had been observed in the same respective fields in roots collected prior to the Al treatment. At this time, many cells had completed mitosis and divided into daughter cells. During the next three hours, there was a reduction in the number of mitotic figures per field but this decline had not reached the same level counted after three hours of recovery. Although mitotic activity had decreased after 9 hours of recovery, this did not indicate that the roots were dying because they continued to grow through 24 hours of recovery. Instead, the decline in mitotic figures suggested that Al may have been some delayed effect and or its effect on the mitotic cycle was only observed at this time.

Because Clarkson (1965a) could not get onion plants to reinitiate apical root growth after Al treatments, he was unable to observe when mitotic figures reappeared. He obviously had used concentrations of Al that were lethal to meristematic cells and this led him to speculate that Al was inhibitory at a specific point in the mitotic cycle as previously discussed. Wheat, on the other hand,
reinitiated apical cell divisions in an Al free solution but within three hours of removal from the stress. Instead of inhibiting the mitotic cycle at a point in the S period, it appeared more likely that Al prevented the onset of prophase during the G₂ period. Had Al arrested the mitotic cycle at one point as Clarkson (1968) suggested, a minimum of ten hours would have elapsed before mitotic figures reappeared. (Twelve hours were required for the disappearance of mitotic figures but 1.5 hours were required for the process of mitosis.)

Throughout the Al treatment, there was a marked decline in the number of mitotic figures counted in ascending fields of the central cylinder. There appeared to be a "burst" of mitotic activity after six hours of recovery although the actual mitotic counts did not surpass what had been counted in control root sections. Following this burst of mitotic activity, there was a decline in mitotic figures, especially in the first 90 micron field. These observations suggested that nuclei in the latter stages of the S period advanced unhindered into the G₂ period but that DNA synthesis in its early stages was markedly impeded.

Cytoplasm

In this study, neither binucleated nor polynucleated cells were ever observed in the thousands of sections prepared from hundreds
of root tips treated with lethal or sublethal levels of Al. Other investigators had reported that Al induced multi-nucleated cells, especially in the cortex. Figure 16 shows what might be interpreted as binucleated and trinucleated cells in the cortex. Careful examination of these cells indicated that each had but one nucleus. With phase contrast microscopy, chromatin and nucleoli could be identified in only one structure in each cell, the nucleus. The other body or bodies appeared to be masses of cytoplasm which were densely stained with hematoxylin. Al was present in these bodies because they absorbed a great deal of hematoxylin without being mordanted in iron, but these bodies were not spherical like nuclei and could be easily seen only in these specially prepared root transverse sections. In longitudinal sections, they extended throughout the cell and were not easily confused with the cell nucleus.

None of the meristematic cells within the central cylinder developed bodies of cytoplasm that could be confused with nuclei. These meristematic cells were much smaller than epidermal and cortical cells and as a consequence, had much less cytoplasm present to contribute to the formation of such a body within the cell.

Another phenomena of Al injury involved the disappearance of cytoplasm from meristematic cells throughout the root tip. This began a few hours after mitosis had been stopped in the apical meristem by a lethal level of Al. Initially, vacuoles began to develop
Figure 16. Transverse sections of Brevor root tips showing cytoplasm bodies in cortical cells.
in the meristematic cells and, as the vacuoles grew, the cytoplasm in the cell was concentrated next to the cell walls. Eventually, all the cytoplasm was adhered to the cell walls or in the corners of the cell and only the nucleus occupied the intracellular space. In many instances, cytoplasm had adhered to the nucleus and formed a bridge between it and the cytoplasm adhered to the cell wall. Occasionally, the nucleus was linked to the cytoplasm by several such bridges and that probably caused its deformation.

After 48 hours of lethal Al treatment, most of the cytoplasm had disappeared from the cell. (See Figures 6 and 11). The nucleus became a small, granular structure which also began to disappear from meristematic cells.

It was interesting to note that mitosis was resumed after the Al stress was removed if the cytoplasm remained mostly intact. This was good evidence that the inhibition of mitosis by Al was reversible. Permanent inhibition to the mitotic cycle occurred concurrently with the degeneration of cytoplasm which became evident several hours after mitosis had been stopped by Al.

### Development of Lateral Roots

Wheat lateral roots were initiated by the occurrence of several periclinal divisions of the pericycle at various locations throughout differentiated portions of the main seminal root. The endodermis
protected the developing lateral roots from the external root environ-
ment. However, once the endodermal layer was injured, usually by mechanical damage from growth, the lateral root was subject to the same environment as the main root. Figure 17 shows a lateral root initiated in Atlas 66 while the main root was subjected to a sublethal Al treatment. The endodermis had been broken and the lateral root had a great deal of Al-hematoxylin stain associated with it. This lateral root will never develop because Al has damaged many initial cells. Figure 17 shows one such root injured during the Al treatment but after 12 hours recovery. Besides the dark Al-hematoxylin stain, many cells were swollen and void of cyto-
plasm, similar to Al toxicity in the main root. When lateral roots were initiated while the plant was subjected to a lethal level of Al, they remained viable as long as the endodermis remained intact. Figure 18 shows such a protected lateral root in Brevor which would have broken the endodermis during the recovery period and would have continued to develop since the Al stress had been removed.

Fleming and Foy (1968) suggested that a lateral root was unaffected by Al while it was emerging through cortical cells. The lateral root shown in Figure 17 was injured when the endodermis was broken during the Al treatment; the cortex did not protect the emerging lateral root. This is not surprising since the cortex itself was greatly damaged by Al. Rasmussen et al. (1968) found that Al
Figure 17. Lateral root development in Atlas 66 roots exposed to a sublethal A1 treatment.
Figure 18. Lateral root development in Brevor roots exposed to a lethal A1 treatment.
did not enter the central cylinder of corn unless the epidermis was broken, usually by mechanical damage from a developing lateral root. The slides prepared in this study disagreed with the work of Rasmussen et al.; the endodermis was the barrier against the entry of Al into the central cylinder.

Figures 17 and 18 also point out another important feature of the effect of Al on wheat roots. Development of lateral roots could be inhibited by a sublethal level of Al if the endodermis was broken while the root was exposed to Al. On the other hand, a lateral root developing while the plant was being treated to a lethal level of Al was protected as long as the endodermis remained whole. This observation indicated that lateral roots may be more sensitive toward Al than main seminal roots. This has been confirmed by recent work. 4

Effect of Al on Other Varieties

Druchamp and Chinese Spring represented wheat varieties having tolerances to Al that were intermediate between Brevor and Atlas 66 (see Table 2). Slides were prepared of root tips from one collection time, that being after 24 hours of recovery from 48 hour sublethal and lethal Al treatments. Additional slides were not prepared because

4 Personal communication with Mr. Dean Rhue, graduate student, Department of Soil Science, O.S.U., Corvallis.
the response of Druchamp and Chinese were very similar to slides prepared from Brevor and Atlas 66 roots collected after the same recovery period from their respective sublethal and lethal Al treatments. Figure 19 shows recovery from both sublethal and lethal treatments in Druchamp and Chinese Spring.

Hematoxylin staining indicated that Al was concentrated in epidermal cells and surrounded the root apex through the boundary between it and the rootcap. There was the same suggestion that Al ascended into the central cylinder from behind the root apex as was noted previously in Atlas 66 and Brevor. The sublethal Al treatment induced a great deal of swelling in the epidermis and cortex that was not evident in the lethal treatment. Many cells were swollen about the boundary between the root apex and rootcap while others were collapsed. Reinitiation of apical root growth was the same in both varieties as was described previously in Atlas 66 and Brevor. The meristematic cells throughout the central cylinder began to divide actively. Many periclinal divisions occurred which increased the diameter of the meristematic cells and eventually the outermost daughter cells differentiated into a new epidermis, cortex and rootcap. As these new root tissues were being formed, the old, Al injured tissues and cells were being sloughed off the growing root tip:
Figure 19. Druchamp and Chinese Spring wheat root tips before A1 treatments and after 24 hours of recovery from sublethal and lethal A1 treatment.
Degeneration of both cytoplasm and nuclei in meristematic cells was characteristic of Al toxicity in both Druchamp and Chinese Spring. At the point where the degeneration of these cellular components ended, lateral root initiation was generally evident. The cells that were void of any constituents were collapsed. Most probably collapsation occurred because there was little secondary wall development for support in these cells at this stage of development.

**Effect of Al on the Quiescent Center**

From studies on the rate of cell division in grass root apices, Clowes (1954) found a semi-spherical group of cells at the root apex which divided very slowly if at all. This group of cells was designated the "quiescent center". Clowes (1961) discussed apical meristems and the quiescent center in some detail. DNA synthesis in the quiescent center proceeded very slowly according to tritium labelled thymidine incorporation into cell nuclei. The slow rate of cell division in this group of cells explained why little RNA and small nucleoli were found in the quiescent center. High levels of RNA and large nucleoli are evidence of rapidly dividing meristematic cells.

Clowes (1959) was able to stop DNA synthesis outside of the quiescent center in *Zea mays* with radiation. Four days after radiation, the quiescent center became smaller because margin cells started to synthesize DNA. Six to 8 days later, the quiescent center
had disappeared and only its former cells were synthesizing DNA. Clowes (1961) speculated that the quiescent center was a protected reservoir of cells which were capable of reinitiating apical root growth. While the root was growing without stress, Clowes also speculated that this group of cells may function as a site of hormone synthesis or even in the maintenance of the geometry of the root tip.

Apical root structure in wheat was very similar to some of the species Clowes (1961) discussed. Very few cell divisions were observed right at the apex of wheat roots in this study even before Al treatments were begun. Figure 20 shows the cells that Clowes would have designated a quiescent center. Also shown in Figure 20 is a root tip recovering from a sublethal Al treatment and the same cells are identified in it too. The quiescent center did not participate in the reinitiation of apical root growth. In fact, the most actively dividing cells were quite removed from the quiescent center. However, there was very little structural organization in the rapidly dividing meristematic during the first 12 hours of recovery. More orderly development proceeded after a new epidermis and rootcap had formed. Coincidental with the return of order in the root tip was the development of another quiescent center at the root apex. Exactly when a quiescent center could be identified would be best shown after.
Figure 20. The quiescent center in wheat root tips.

Control

Atlas 66

48 + 24 hours
20 ppm Al
autoradiographic analysis of the incorporation of tritium labelled thymidine into nuclear DNA.\(^5\)

Irreversible damage was sustained by the quiescent center and it did not function in the reinitiation of apical root growth. In fact, the damaged quiescent center was eventually sloughed off the growing root tip. The only function known to be attributed to the quiescent center had been as a reservoir of cells from which regrowth was initiated in irradiated roots. In this study, regrowth was initiated in the apical meristem several cells removed from the Al damaged quiescent center.

**Location of Al in Roots**

Some indication of the location of Al in wheat roots was obtained with hematoxylin. Al-hematoxylin was easily detected in the epidermis, cortex, rootcap and the boundary between the root apex and rootcap. However, no Al could be detected with hematoxylin in meristematic cells although they stopped dividing a few hours after Al treatments were begun. Failure to detect Al in meristematic cells could be explained by any of the following reasons:

1. all the Al was lost during fixation and preparation,
2. the injurious level of Al was too low to detect with hematoxylin,

\(^5\) Personal communication with Dr. F. R. Rickson, Professor of Botany, O.S.U., Corvallis.
3. inability of hematoxylin to compete with natural sites for Al in the meristematic cells, and

4. Al was never present in the meristematic cells.

Because of the extensive injury to the meristematic cells, it was reasonable to expect Al to be present in those damaged cells. Loss of Al from meristematic cells could be limited if the pH of the killing and fixing solution was adjusted to minimize the solubility of Al. Two new approaches were taken to locate Al in treated wheat roots. The first involved a fluorescent technique where Al combined with a dye and fluoresced when illuminated with ultra-violet light. Secondly, specially prepared root tips were analyzed by energy dispersive analysis of x-rays (EDAX) from the electron beam in scanning electron microscope (SEM). If either of these methods detected Al in meristematic cells, both the first and fourth reasons for the apparent absence of Al would be invalid.

Atlas 66 and Brevor seedlings were treated with 10 ppm Al in one-half strength nutrient solution while being grown in continuous light. Root tips were collected before Al treatment was begun and every 3 hours during the 24 hour treatment. For the fluorescent study, only Brevor root tips were collected. These were killed and fixed in FAA buffered at pH 4.5 because Al is not very soluble at this pH and should not be mobilized as a consequence of fixation. Atlas 66 and Brevor root tips for EDAX were killed and fixed in 10 percent Acrolein in tap water.
Fluorescent Analysis

Preparation of slides for fluorescent analysis of absorbed Al was a simple procedure. Paraplast was dissolved from the mounted sections with xylene and then the slides were transferred to absolute ethanol. After three changes of absolute ethanol, the slides were ready to be stained. A drop of buffered glycerine (pH 4.8) containing 0.5 percent Eriochrome Garnet L (2, 4, 2' - trihydroxy-azobenzene -5' - sodium sulfonate) was deposited on the specimen and a cover slip placed over it. The cover slip was held in place with adhesive tape because this was a wet mounting procedure.

Eriochrome Garnet L was also called Acid Alzarin Garnet R (AAGR) by White and Argauer (1970) who discussed the use of fluorescing dyes. The fluorescence emission, fluorescence excitation and absorption spectra for Al-AAGR were given by White et al. (1957). Al chelated with AAGR gave an intense yellow fluorescence at 580 millimicrons after being excited by ultra violet light from the 420 to 500 millimicron region. Maximum fluorescence occurred when the pH of the dye solution was buffered at 4.8. These workers reported the yellow fluorescence was specific for Al and that no other elements interfered with its determination.

Figure 21 shows Brevor root tips after 0, 9 and 21 hours of treatment with Al. There was no intense yellow fluorescence in any
Figure 21. A1 - AAGR fluorescence in Brevor root tips treated with 10 ppm A1 in one-half strength nutrient solution.
control sections. Instead, there was a dull orange auto-fluorescence from the DNA in the cell's nucleus. After 3 hours of Al exposure (not shown), an intense yellow fluorescence, characteristic of Al-AAGR chelate, appeared in some epidermal cells.

Nine hours after Al treatment was begun, Al-AAGR fluorescence had become evident in most epidermal cells but it had not surrounded the root apex at this time. Some cortical cells had been penetrated by Al but no Al-AAGR fluorescence appeared in the meristematic cells within the central cylinder which had stopped dividing at this time (see Figure 13). Further treatment resulted in a deeper penetration of Al into the cortex and its intercellular spaces. The root apex-rootcap boundary also showed a great deal of intense yellow Al-AAGR fluorescence.

Although the meristematic cells had been irreversibly damaged by Al at the end of 24 hours, no Al-AAGR fluorescence could be detected at that time. The problem of auto-fluorescence remained throughout the experiment. It was not a problem in epidermal and cortical cells because the nucleus occupied a relatively small portion of those cells. The meristematic cells, however, had the same size nucleus but it occupied nearly the entire volume of a much smaller cell. That increased the proportion of auto-fluorescence from each meristematic cell which could interfere with Al-AAGR fluorescence.
The fluorescent technique did point out what appeared to be a large accumulation of Al in epidermal and cortical cells. Additional accumulation of Al occurred in intercellular spaces and the boundary between the root apex and rootcap. The intense yellow fluorescence indicated these accumulations clearly. Hematoxylin had detected Al in the same locations throughout the root tip but it was not specific for Al. Additional information about the possibility of an accumulation of Al in meristematic cells could not be drawn because of the interference from auto-fluorescence.

**Energy Dispersive Analysis of X-rays**

Energy dispersive analysis of x-rays (EDAX) for absorbed Al was done on root tips collected from Atlas 66 and Brevor seedlings. Because of the time and material needed for preparation and analysis, EDAX was done on a Brevor control and the 12 hour, 10 ppm Al treatment on both Atlas 66 and Brevor. EDAX was done at the Materials Analysis Cost Center, Department of Mining, Metallurgical and Ceramic Engineering, University of Washington, Seattle. The specimens were mounted on a SEM stub and coated with spectographic carbon before analysis. EDAX was done over a 30 x 30 micron field in three regions of the root tip: cortical cells adjacent to epidermis, meristematic cells in the central cylinder 100 to 150 microns from the root apex and in the quiescent center.
Figure 22 shows the EDAX output for the Brevor control and glycol methacrylate embedding matrix. Neither EDAX detected the presence of Al. The major peak occurring in the Brevor control was at 2.01 KeV and that is the $K_\alpha$ energy for P. Figure 23 displays EDAX output for cortical and meristematic regions in both Al treated Atlas 66 and Brevor roots. The EDAX was stopped when the P peak reached approximately the same height and that required different amounts of time for each specimen. That did not necessarily mean that different amounts of P were present in the field but took into account the differences in the geometry of the x-ray detector. The cortical region of Brevor showed the presence of another peak at 1.47 KeV, the characteristic $K_\alpha$ energy of Al. Although there was an increased signal at 1.47 KeV in the other EDAX outputs, none exceeded the background noise as much as the Brevor cortex treated with 10 ppm Al for 12 hours.

The results of EDAX for absorbed Al in various 30 x 30 micron fields was disappointing. Identification of cellular components was difficult because of the root surface was smooth except for knife chatter marks. A 1000 fold magnification detected some micro-relief that indicated what was thought to be a nucleus within a meristematic cell of Brevor. Point projection of the electron beam on this nucleus yielded an EDAX where the Al signal went off the
Figure 22. EDAX from a 30 x 30 micron region in the apical meristem of a Brevor control root tip and the glycol methacrylate matrix.
Figure 23. EDAX from a 30 x 30 micron regions in the root tips of Atlas 66 and Brevor wheat after 12 hours treatment with 10 ppm Al in one-half strength nutrient solution.
output screen in thirty seconds while the P peak was one-half as high (data not recorded).

Further work with the EDAX system appeared to be the best means of locating absorbed Al in plant roots. It was suggested that electron transmission preparations could show cellular structure in good detail but a suitable stain would have to be found which did not interfere with the Al signal. Point analysis of various structures by EDAX would give a qualitative estimate of the elements present. That would be adequate to ascertain the location of absorbed Al in plant roots if it was not mobilized as a consequence of fixation and preparation.

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6 Personal communication with Mr. Arnold Schmidt, Supervisor, Materials Analysis Cost Center, Department of Mining, Metallurgical and Ceramic Engineering, University of Washington, Seattle.
V. GENERAL DISCUSSION

Effects of Al on Wheat Roots

Al has a profound effect on cells throughout the primary meristem of wheat roots. This is the most significant effect of Al on plants and has, for the most part, been overlooked. Only recently, the Al concentration that is required to cause the death of the root primary meristem has been used as the criteria to separate degrees of Al tolerance among several wheat varieties (Moore, 1974). This is a precise, quantitative test because it is based on a distinct phenomena, namely the primary meristem in a root tip is either living or dead.

The progression of Al toxicity was rapid; a day's exposure to Al could kill the entire primary meristem of a wheat root. Mitotic figures disappeared rapidly after the first few hours of Al treatment until virtually none were present at the end of twelve hours. While mitotic activity declined, enlargement of vacuoles began throughout the root tip. In the cortical cells the cytoplasm was displaced either by being adhered to the cell walls or into dense bodies within the cell. The smaller meristematic cells lacked a sufficient amount of cytoplasm to form similar bodies and their cytoplasm was mostly adhered to the cell walls. After the cytoplasm had been displaced, it began
to degenerate until the cells were void of that component. Nuclei persisted several hours after the cytoplasm had disappeared but they too degenerated and the cell was left void of any structures and organs.

Clarkson (1965a) noted the disappearances of mitotic figures from squashes prepared from Al treated onion root tips. Cessation of mitotic activity occurred within 5 to 7 hours after Al treatments were begun. If vacuoles developed in Clarkson's Al treated onion roots he did not note it in his results. Also, preparation of a root squash sacrifices many cells in addition to the structure of the whole root tip. That could have prevented observation of a change in the occurrences of vacuoles in relation to specific root tissue.

Inhibition of the mitotic cycle by Al did not occur at a single point in the late stages of the S period as Clarkson (1968) speculated. Instead, it appeared that the entire mitotic cycle was under an extreme stress although mitosis itself was least affected. The insensitivity of mitosis toward Al is in agreement with observations of the effects of other chemicals on mitosis (Kihlman, 1966). Some insight into a mechanism accounting for the apparent insensitivity of mitosis toward Al can be found in the work of Amoore (1963). He found that mitosis went to completion although ATP levels were reduced to one percent of their normal level which is about 2.35 μ mole per mg dry weight. Amoore noted that the duration of mitosis
doubled in roots when the ATP level fell to three percent of normal and complete arrest of mitotic figures occurred when all the ATP disappeared. But Amoore did not attribute the arrest of mitosis in roots by respiratory inhibitors to a stoppage of ATP generation. Instead, he suggested that the arrest was due to their interference with a ferrous complex he had previously found to be essential for mitosis in roots (Amoore, 1962).

Several parallels can be drawn between the results of Amoore (1963) and the observations reported in this study. For instance, mitoses would be expected to go to completion because of their very small energy requirement but the rate of mitosis could be expected to decline. This would account for the persistence of a few mitotic figures after 21 hours of lethal Al treatment (see Figure 13) although none were evident at 48 hours in other root tips. Secondly, removal of the Al stress while the cells were still viable would result in the rapid formation of mitotic figures from all nuclei slowly progressing through the G₂ period. Such a burst of activity was observed after six hours of recovery (see Table 3) and the mitotic count was equal to what had been measured in control root tip sections. However, additional mitoses would be delayed if DNA synthesis was impeded by Al, because nuclei can not divide until the chromosomes have doubled. Indeed, there was a decline in the mitotic count during the
next three hours and this was further shown after 18 hours of recovery.

There had been a suggestion that the metabolically labile fraction of DNA synthesized after an Al treatment had an unusual base composition (Sampson et al., 1965). No evidence could be found in this study to support such a conclusion. Had the DNA composition been altered, the genetic code would likewise be altered. Changes in the genetic code would be expected to cause gross abnormalities in regrowing root tips, but none were evident in this study. Similarly, the process of mitosis would have reflected any abnormalities in DNA composition. The process of mitosis is initiated only after the chromosomes have been replicated. It seems unlikely that sister chromatids would pair if the sequence of bases in the DNA had been changed. No unusual pairings of sister chromatids were ever observed during mitosis in the regrowing root tips that had been recovered after Al treatments.

Some insight into the results of Sampson et al. (1965) can be obtained from the results of this study. The 27 ppm Al treatment which caused abnormal DNA in the metabolically labile fraction of barley roots would have been lethal because barley is much more sensitive toward Al than Brevor wheat. \footnote{Personal communication with Dr. D. P. Moore, Professor of Soil Science, O.S.U., Corvallis.} In addition, the DNA
separated by these workers was labelled with $^{32}P$ for four hours but only after 48 hours of Al treatment. At this time, degeneration of the DNA in the nuclei of meristematic cells could be expected rather than synthesis. Instead of $^{32}P$ incorporation into newly synthesized DNA, it appears more likely that $^{32}P$ and P had undergone isotropic exchange. The counts of radioactivity per microgram of DNA were too low to indicate synthesis; a much higher count of radioactivity per microgram of DNA should be expected from $^{32}P$ incorporated into newly synthesized DNA.  

One of the unique aspects in the progression of Al toxicity was the particular movement of Al that could be detected with various dyes. A great deal of Al penetrated the boundary between the root apex and rootcap. Cells immediately adjacent to this boundary became heavily stained with dyes that were attracted to Al. The nuclei and cytoplasm of these cells became heavily stained. Other Al passed through the epidermis and moved through the intercellular spaces within the cortex although many cortical cells absorbed much Al as indicated by dyes. Further movement of Al into the central cylinder appeared to be prevented by the endodermis. Al did not necessarily have to cross the endodermis but rather it may have entered the central cylinder after passing through the root.

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8 Personal communication with Dr. R. S. Quatrano, Professor of Botany, O.S.U., Corvallis.
apex-rootcap boundary. From the root apex, Al could ascend through the meristematic cells of the central cylinder. No other investigators have reported such a unique path of entry for any element into a root.

The use of the dye, hematoxylin, to detect Al was applied very early by McLean and Gilbert (1927) and showed the absorption of Al by epidermal and cortical cells in corn. These workers found that Al was concentrated in the nucleus and cytoplasm as well as the intercellular spaces of cortical cells. Wright and Donahue (1953) likewise used hematoxylin to detect Al in treated barley roots. They found that the endodermis prevented the entry of Al into the central cylinder. Lately, with the introduction of the electron microprobe analyzer, there have been two studies on Al treated plant roots. Rasmussen (1968) found that Al precipitated on the epidermal root surface and did not enter the cortex or central cylinder tissue until a lateral root had penetrated the epidermis. That opened a channel whereby Al could gain entry to the plant. Waisel et al. (1970) disagreed with Rasmussen's observations because they detected Al in cortical cell vacuoles of bean and barley roots. Their roots, however, were grown in an alkaline (pH 9.3) medium. Both studies have serious limitations which restrict their findings to the broad question of Al toxicity.
Recovery of Wheat Roots from Al

Examination of the regrowing root tips showed the extent of Al damage; those cells in the primary meristem not killed by Al began to divide when the Al stress was removed. Cell divisions occurred in meristematic cells adjacent to the differentiated portion of the root and extended toward the root apex through the remaining living cells. Because the rootcap, epidermis, cortex and terminal portion of the apical meristem had been severely injured by Al, these tissues would have to be regenerated. This was accomplished by a sequence of periclinal divisions where the innermost daughter cells remained meristematic. The outermost daughter cells differentiated and thus the Al injured tissues were regenerated through a relatively simple sequence of cell division and maturation. Injured tissues and cells were eventually sloughed off and the only evidence remaining of Al damage was the disorganized files of cells created by the initial periclinal divisions throughout the primary meristem.

The nature of the regrowth of the root tip seriously questions the importance of the quiescent center. Clowes (1959) suggested that this group of cells were essential to the regrowth of injured root tips. It was clearly shown in this study that the root tip was reinitiated without the participation of the quiescent center which in fact was actually sloughed off with other Al injured tissues and cells.
Al Toxicity Phenomena

Of the many phenomena attributed to Al toxicity, most involve decreased uptake and transport of plant nutrients. This has led numerous investigators to suggest that Al toxicity in plants is a result of nutrient deficiencies. Among the various nutrient deficiencies, Ca, Mg, K and P are the most often cited as the cause. Interpretating most of these findings has been confused by either the failure to report all experimental parameters or to rigorously control them. In some instances, investigators have altered the composition of a nutrient solution without noting the effect on the plant roots. The importance of controlling the composition of the nutrient solution was demonstrated by Ali (1973). If he increased the concentration of Ca, Mg, K or Na either individually or collectively, Al toxicity in wheat could be completely overcome. Clarkson and Sanderson (1971) found that Al inhibited Ca uptake in barley within a few hours of Al exposure but adding extra Ca to the nutrient solution could overcome this toxic effect. These workers conducted their experiments over a 24 hour period with levels of Al which could be lethal to barley. During that time, Al could have killed the root primary meristem as it had in wheat during this study. Secondly, overcoming the effect of Al toxicity with added Ca could be accounted for by the observations of Ali cited previously in this discussion.
Another nutritional phenomenon involves precipitation of P by Al. McLean and Gilbert (1927) recognized that Al and P must be supplied by plants separately where they are grown in nutrient solution. Moore (1974) likewise withheld P from Al treatments with no apparent nutritional problems. Instead of an external precipitation in the nutrient solution, Wright (1943) suggested that precipitation of P by Al occurred within the plant. P was detected by EDAX in both control and Al treated roots in this study. Whether they were combined in the treated plants could not be shown. Rasmussen (1968) likewise found P and Al associated together in corn plants grown under very similar conditions. However, Waisel et al. (1970) found no such association but their bean and barley plants were grown under extremely alkaline conditions.

In his doctoral research, Kerridge (1969) concluded that the primary effect of Al was on inhibition of root elongation. Depressed nutritional status of cations was a secondary result of Al toxicity because it occurred several days later. In this study, wheat seedlings were injured by Al very much like those of Kerridge. Microscopic examination indicated that lethal Al treatments killed the entire primary meristem and this certainly could account for depressed mineral uptake. Sublethal treatments caused marked swelling of epidermal and cortical cells. Uptake of mineral through these swollen and injured cells was most probably restricted too. In
addition, nutrient absorption would be markedly reduced when root elongation ceased since maximum uptake occurs in the elongating portion of the root.

Most studies of Al toxicity can not be interpreted because the degree of injury is not known. Long term experiments measure differences in either yield or nutrient composition, but the secondary effects of Al can account for those responses. Evaluation of the primary effect of Al, injury to cells throughout the root tip - especially the primary meristem, can be done by microscopic examination of sectioned root tips. A faster means of evaluating Al injury would be to simply observe roots for reinitiation of apical root growth in a recovery solution. Moore (1974) has used this technique with good success in wheat because its basis lies in the extent of Al injury sustained by the root primary meristem.

**Varietal Al Tolerance**

Although the inheritance of Al tolerance has been demonstrated by Kerridge and Kronstad (1968), there is still much debate about the mechanism responsible for greater degrees of Al tolerance in plants. Much of this speculation has been confounded by secondary or indirect effects of Al. For example, Foy et al. (1967a) found that after 20 days of Al treatment, differential Al tolerance resulted from plant induced pH changes in the root zone. Tolerant barley plants markedly
increased the pH of an Al treated nutrient solution but it went unchanged for 20 days when sensitive plants were treated. When sensitive and tolerant plants were grown separately in control nutrient solutions, similar increases in pH were noted after 20 days. A more likely explanation for the inability of the sensitive plant to alter the pH of an Al treated nutrient solution would be the inactivity of roots resulting from the death of tissues and cells.

Another mechanism suggested to be responsible for differential Al tolerance was the root structure of various wheat varieties (Fleming and Foy, 1968). No differences in root structure could be observed until the sensitive variety, Monon, had stopped growing while the tolerant variety, Atlas 66, had continued to grow. The differences in root structure again reflected the death of tissues and cells in the more sensitive variety. Had Fleming and Foy properly increased the level of Al in the nutrient culture, they would have observed identical damage in Atlas 66 root tips and clearly, root structure would not have reflected the cause of differential Al tolerance in wheat.

The results of this thesis show that Al toxicity causes the death of root tissues and cells. This is the most definitive assessment of Al injury. To illustrate this point, the consequences of a lethal injury to Brevor and Atlas 66 root tips were identical although 100 to 200 times as much Al was required for the latter variety. Although
the toxic level of Al can be altered, the degrees of tolerance among wheat varieties remain relative. A tolerant variety will require a greater amount of Al before it sustains injury than a sensitive variety for any set of experimental conditions.

Besides the death of a cell from Al, another characteristic form of injury occurs in cells. Scarth (1923) reported adhesion of protoplasm to cell walls which developed after Al treated cells were plasmolyzed with monovalent salts. This characteristic form of plasmolysis was used by Böhm-Tüchy (1960) to study the effect of Al on several strains of Spirogyra, a green, fresh water algae. She found different degrees of Al tolerance among these strains and suggested that the plasmalemma of these algae were differentially permeable to Al. In higher plants, some evidence of the involvement of the cell plasmalemma in differential Al tolerance can be drawn from the work of Ali (1973). He found that increasing the concentration of several cations could overcome Al toxicity and suggested that Al was being kept out of meristematic cells. Either nonspecific cation competition for entry to the cell or nonspecific reduction in cell permeability could account for Ali's results. The plasmalemma is involved in the entry of Al in both instances. Differences in the cell plasmalemma could account for differential exclusion of Al from wheat cells and therefore the several degrees of Al tolerance noted in this plant (Moore, 1974). Moore further suggested that the dominant
allel may form or complete a protein in the plasmalemma which certainly could not be detected by ordinary microscopic examination. In hexaploid wheat like those used in this study, each added pair of dominant alleles may add to or further complete the protein network of the plasmalemma.

The results of this study could be interpreted to support the differential exclusion of Al at the cell plasmalemma. If a plasmalemma offers a specific resistance to Al, it can be overcome by increasing the level of Al in the nutrient solution. Therefore Atlas 66 required 100 to 200 times as much Al as Brevor before it could penetrate the plasmalemma of meristematic cells. Once inside the cell, Al was equally harmful to either the most sensitive or the most tolerant variety of wheat.
VI. SUMMARY AND CONCLUSIONS

Aluminum toxicity was studied microscopically in the root tips of four wheat varieties representing different classes of Al tolerance. All the root tips were collected from seedlings exposed to either sublethal or lethal Al treatments that were unique for each variety. The lethal treatment of Al resulted in the destruction of both cytoplasm and nuclei throughout the primary meristem of each variety. Although the root tip sustained some damage from the sublethal treatment, an adequate number of viable cells remained throughout the primary meristem to reinitiate apical root growth in seminal roots.

The slides prepared for this study give a good description of both the progression and the consequences of Al toxicity in wheat. In addition, the movement of Al into the root tip could be followed throughout the Al treatment by using dyes that either combined with Al or fluoresced when chelated with Al. There was strong evidence that Al penetrated the boundary between the root apex and rootcap and then, during a lethal treatment, ascended into and throughout the meristematic cells of the central cylinder. Al had also penetrated the epidermis and cortex but was prevented from entering the central cylinder by the endodermis which possessed an additional barrier to
the movement of Al, the casparian strip. Ascension of Al into the central cylinder from the root apex would avoid the barrier at the endodermis.

Microscopic examination of Al injured root tips revealed that the primary effect of Al was the death of cells. Death occurred rapidly, usually within the first 24 to 48 hours of exposure to Al. The mitotic cycle, however, was affected by Al almost at the onset of the Al treatment. Evidence in this study suggested that the entire mitotic cycle was impeded by Al although the process of mitosis was least affected. Some parallels could be drawn between the cessation of mitotic activity caused by Al and the effect of various respiratory inhibitors which drastically reduce the ATP level in dividing cells. If the Al stress were removed before the cytoplasm was displaced, the mitotic cycle would proceed again although some effects of Al could be detected for a period of time. Otherwise, both cytoplasm and nuclei degenerated and cells throughout the primary meristem were left void of any cellular constituents. Needless to say, these cells were unable to carry on any normal physiological functions and this could account for many of the phenomena attributed to Al toxicity which should be considered secondary effects.

The cause of varietal tolerance to Al could not be attributed to any structural feature observed through a microscopic examination of root tips from the several wheat varieties grown in this study.
Other evidence would strongly suggest that Al was excluded from meristematic cells differentially by the several varieties. Such exclusion could be overcome only by increasing the level of Al when the experimental conditions were otherwise kept constant. The only satisfactory explanation for varietal tolerance to Al at this point lies in the molecular construction of a cell's plasmalemma.
BIBLIOGRAPHY


APPENDIX
Appendix Table 1. Prediction of degrees of Al tolerance in a blind experiment.a/

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a/ Coded by Dr. R. J. Metzger, U.S.D.A., Corvallis.

* Correct
Appendix Table 2. Standard deviation among mitotic counts for the "normal" section in Brevor root tips.

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