# ULTRASTRUCTURAL RESPONSES OF WHEAT ROOT CAP CELLS TO ALUMINUM

By

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ULTRASTRUCTURAL RESPONSES OF WHEAT

ROOT CAP CELLS TO ALUMINUM

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TABLE OF CONTENTS


| Chapter | | Page |
|---|---|---|
| I. | INTRODUCTION | 1 |
| II. | LITERATURE REVIEW | 3 |
| | Effects of Aluminum on the Morphology and Physiology of Roots | 3 |
| | Ultrastructural Responses of Root Cells to Aluminum | 4 |
| | Secretion of Mucigel, a Key Process Affected by Aluminum | 5 |
| III. | METHODS AND MATERIALS | 9 |
| | Methods of Germination | 9 |
| | Determination of Growth in Primary Roots | 9 |
| | Estimation of Volume of Mucigel | 10 |
| | Electron Microscopy | 11 |
| IV. | RESULTS | 13 |
| | Inhibition of Growth in Primary Roots by Aluminum | 13 |
| | Effect of Aluminum on the Volume of Mucigel Formation | 15 |
| | Aluminum-Induced Ultrastructural Changes in the Root Cap Cells | 30 |
| V. | DISCUSSION | 54 |
| VI. | SUMMARY AND CONCLUSIONS | 58 |
| BIBLIOGRAPHY | | 60 |

LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 1. | Inhibition of Primary Root Growth by Aluminum in Three Cultivars of Wheat Differing in Sensitivity to Aluminum | 15 |
| 2. | Primary Root Tips of Wheat cv. Atlas 66 Showing the Effect of Different Concentrations of Aluminum on Mucigel Formation a) Control b) 1 μg/ml aluminum c) 4 μg/ml aluminum | 18 |
| 3. | Effect of Different Aluminum Concentrations on Mucigel Secretion in Wheat cv. Atlas 66 | 19 |
| 4. | Effect of 8 μg/ml Aluminum on Mucigel Formation in Primary Roots of cv. Atlas 66 at Different Time Intervals a) Control b) 1 hr treatment c) 2 hr treatment | 21 |
| | d) 4 hr treatment ; e) 6 hr Treatment | 23 |
| 5. | Time Course of Aluminum Inhibition of Mucigel Secretion in Wheat cv. Victory When Treated with 8 μg/ml Aluminum at Time Zero | 24 |
| 6. | Primary Root Tips of Wheat cv. Victory Treated with 1 μg/ml Aluminum a) Control b) 1 hr treatment c) 2 hr treatment | 26 |
| | d) 3 hr Treatment ; e) 4 hr Treatment | 28 |
| 7. | Time Course of Aluminum Inhibition of Mucigel Secretion in Wheat cv. Victory when Treated with 1 μg/ml Aluminum at Time Zero | 29 |
| 8. | Electron Micrograph of a Portion of Wheat Root Cap Cell Fixed in Glutaraldehyde-osmium Tetraoxide, Stained with Uranyl Acetate and Lead Citrate | 32 |
| 9. | Electron Micrograph of a Wheat Root Cap Cell Fixed in 2% Potassium Permanganate Stained with Reynold's Lead Citrate | 34 |
| 10. | Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Secretory Function of the Golgi Apparatus in the Control Treatment | 37 |
| 11. | Electron Micrograph of a Control Root Cap Cell in Wheat cv. Victory Illustrating the Cell in the State of Active Secretion | 39 |

Figure Page

12. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Effect of 1 Hour Exposure to 1 μg/ml Aluminum ..... 42

13. Electron Micrograph of a Root Cap Cell in Wheat cv. Victory Illustrating the Effect of 1 Hour Exposure to 1 μg/ml Aluminum ..... 44

14. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Effect of 2 Hour Exposure to 1 μg/ml Aluminum ..... 46

15. Electron Micrograph of a Root Cap Cell in Wheat cv. Victory Illustrating the Effect fo 1 μg/ml Aluminum ..... 48

16. Electron Micrograph of a Peripheral Root Cap Cell of cv. Victory Illustrating the Effect of 4 Hour Exposure to 1 μg/ml Aluminum ..... 50

17. Electron Micrograph of a Peripheral Root Cap Cell of Wheat cv. Victory Illustrating the Effect of 4 Hour Exposure to 1 μg/ml Aluminum..... 52

# CHAPTER I

## INTRODUCTION

Aluminum toxicity is a major problem in agriculture in many parts of the U.S. and is of increasing concern in wheat growing areas of the midwest. The use of ammonia fertilizers, combined with removal of soil cations by crops, results in soil acidification (Westerman, 1981). Under these conditions, metals such as aluminum and manganese may be absorbed to toxic levels. Aluminum thus absorbed causes overall stunting in plants, purpling of stems, curling of young leaves, and collapse of growing points. Roots affected by aluminum are stubby and brittle resulting in corraloid root systems (Foy et al., 1978).

Much of the physiological research done on plant roots has shown that aluminum interferes with cell division and cell wall formation, as well as uptake, transport and use of several elements (Ca, Mg, P, K) (Foy et al., 1978). Aluminum toxicity frequently appears as an induced calcium deficiency or reduced calcium transport problem. Reports dealing with plant response to aluminum have attached importance to the root cap in the expression of aluminum toxicity, where the earliest ultrastructural effect was observed in the Golgi apparatus activity of the peripheral cap cells. Studies conducted by Bennet et al. (1985b) on *Zea mays* showed that aluminum was inhibiting the migration of secretory vesicles which is indicative of interference of aluminum in membrane transport. Aluminum also inhibited the formation of mucigel.

Mucigel is the gelatinous material produced by the plant roots on the root surface. It consists mainly of polyuronic acids and other polysaccharides, indicating a relation between mucilage secretion and cell wall metabolism. Mucigel is the product of the

secretion process of the Golgi apparatus of outer root cap cells. Both vesicle movement and vesicle fusion at the cell surface require the coordinated function of the cell cytoskeleton and elements such as calcium for the completion of specific events in the pathway. The most important functions of mucigel (Oades, 1978) are:

(1) protection of the root from desiccation

(2) dissolving and possibly chelating certain nutrients

(3) improvement of root soil contact, thus facilitating nutrient diffusion to the root, especially in dry soils.

Most of the information on the effects of aluminum on mucigel formation and ultrastructure of root cells was obtained from studies using *Zea mays*. There is not enough information on the effects of aluminum on mucigel.

The effect of different concentrations of aluminum on the ultrastructure of root cap cells and mucigel formation in wheat could be different from that obtained in maize. So, in my project I decided to study the effects of aluminum on the ultrastructure of wheat root cap cells. I also wanted to study the effect of aluminum on the formation of mucigel in detail.

The major objectives of this study with wheat seedlings were:

(1) To determine the concentration and period of time (in hours) at which aluminum is inhibitory to root growth and mucigel formation in the root cap.

(2) To compare the effect of aluminum on root growth in three different cultivars of wheat: Atlas 66, Victory and TAM 101.

(3) To determine changes at the ultrastructural level in Golgi apparatus appearance and function in response to aluminum.

(4) To determine the effect of aluminum on the abundance of other organelles in the root cap cells.

# CHAPTER II

# LITERATURE REVIEW

Cationic aluminum in acid soils has long been known to have toxic effects on the growth of many plant species of agricultural interest (Hartwell and Pember, 1918). Since the advent of intensive nitrogen fertilization of wheat in the early 1950s, many areas have experienced a reduction in yield and in some cases crop failure, due to a marked decline in soil pH to values of 4.0-4.5 (Westerman, 1981). Under these conditions metals such as aluminum and manganese are absorbed to toxic levels. The toxic effects of aluminum on plant growth has been extensively reviewed by Foy et al. (1978).

## Effects of Aluminum on the Morphology and Physiology of Roots

Aluminum toxicity causes overall stunting of the plant, purpling of stems, leaves and leaf veins; aluminum toxicity appears as an induced calcium deficiency or reduced calcium transport problem. The major effects of aluminum are seen in the root system of plants, partly because it is the first to come in contact with the metal. Aluminum-injured roots are characteristically stubby and brittle. Root tips become thickened and turn brown. The root system is corraloid in appearance and lacks fine branching. Such roots are inefficient in absorbing nutrients and water (Foy et al., 1978).

Aluminum at toxic levels has been shown to interfere with cell division in plant roots, to fix phosphorus in less available forms in the soil and in or on plant roots, decrease root respiration, interfere with certain enzymes governing deposition of polysaccharides in cell walls, increase call wall rigidity by cross linking proteins, and interfere with the uptake,

transport and use of several elements (Ca, Mg, P, K) and water by plants (Foy et al., 1978; Rorison, 1965). Metal ions such as aluminum are known to form strong complexes and to precipitate nucleic acids (Trim, 1959).

Several investigators have studied the location of aluminum in plant roots and suggested possible physiological mechanisms of tolerance or sensitivity. Matsumoto et al. (1976) reported that in tea, an Al accumulator, old leaves contain 30,000 ppm aluminum and it was localized in epidermal cells having distinctly thickened walls. Henning (1975) found that much of the aluminum absorbed by wheat roots accumulated in the nuclei and cytoplasm of cells adjacent to this zone. He concluded from his studies that aluminum tolerance in wheat is due to exclusion of this metal at the root cell plasmalemma. Aluminum uptake is not an active process, but rather the result of passive diffusion across the plasmalemma (Rhue, 1976). Aluminum tolerance in certain cultivars of wheat and barley has been associated with the ability to resist aluminum-induced calcium deficiency or reduced calcium transport (Foy et al., 1972, Foy, 1974). Naidoo et al. (1976) suggested that aluminum was bound to esteric phosphorus in nucleic acids and to membrane lipids and that it reduced or inhibited cell division by alteration of nucleic acids. Ali (1973) reported that aluminum toxicity in wheat could be overcome by increasing the concentrations of Ca, Mg, K, or Na in the medium either individually or collectively.

## Ultrastructural Responses of Root Cap Cells to Aluminum

Al-induced ultrastructural changes were studied by Bennet and co-workers in root cells of *Zea mays*. Their studies involved finding the primary site of aluminum injury, changes induced by aluminum in the root cap and mristematic cells, and the effect of these changes on root cap function and growth (Bennet et al., 1985a, 1985b, 1987). The peripheral cap cells of the root were the first to be affected. These are the first cells of the

root apex to be exposed to aluminum. The most conspicuous activity of these cells involves the secretion of slimes and mucilages of polysaccharide or polysaccharide-protein complexes (Mollenhauer & Morre, 1966).

Progressive vacuolation of root cap cells as a result of swelling of rough endoplasmic reticulum was one of the most easily identifiable consequences of aluminum toxicity. This was followed by severe disorganization of the cytoplasmic contents within 20 hrs of exposure to < 1 μg/ml aluminum. Another effect observed by Bennet et al. (1985a) was the inhibition of migration of secretory vesicles of the Golgi apparatus, which was considered indicative of interference by aluminum in membrane transport. Complete disorganization of the Golgi apparatus was observed at 6.5 hrs exposure to 8 μg/ml aluminum. This implies that the movement of Golgi apparatus-derived vesicles to the cell surface may represent the primary target of aluminum action.

Inhibition of the Golgi apparatus function in the outer root cap cells by aluminum proceeded synchronously with diminished slime synthesis, which accords with the view (Mollenhauer & Morre, 1976) that the secretory function of the dictyosomes in the cap periphery include the packaging and export of mucilagenous materials from the cap (Bennet et al., 1987).

Decrease in amyloplast numbers with increasing aluminum concentrations coincided with diminished Golgi apparatus activity and these changes preceded reductions in mitotic activity (Bennet et al., 1987). However, nuclear structure and appearance of the nuclear membrane were unaltered even at 20 hr exposure to aluminum (Bennet et al., 1985a).

## Secretion of Mucigel as a Key Process Affected by Aluminum

The production of mucilage or slime by plant roots is a general phenomenon and contributes to the formation of the "mucigel". Jenny and Grossenbacher (1963) and Mauseth (1988) defined mucigel as the gelatinous material produced by plant roots and deposited on the root surface. Mucigel is the product of the outer root cap cells.

Synthesized in the Golgi apparatus of these cells, this material passes through the cell wall and accumulates externally as slime droplets (Rougier, 1981; Northcote and Picket-Heaps, 1966; Morre et al., 1967). The production of a carbohydrate slime secretion by the outer cells of the root cap has been studied extensively at both the cellular (Mollenhauer & Morre, 1966, 1976) and biochemical (Bowles and Northcote, 1974; Green and Northcote, 1978, 1979) level. The active secretory cells are mainly characterized by the hypertrophied form of the Golgi apparatus which is attributed to a sudden increase in carbohydrate supply within the cells, originating from the breakdown products of the stored starch grains in the amyloplasts of the peripheral cells (Juniper & Roberts, 1966).

Mucigel is mainly composed of polysaccharides (Wright & Northcote, 1974). Juniper and Roberts (1966) identified glucose, galactose, xylose, arabinose and small amounts of uronic acids in maize slime. Wright and Northcote (1974) considered mucigel to be a form of pectin modified in such a way as to provide a hydrated protective coating around the root-tip. Biochemical studies (Mollenhauer & Morre, 1966; Paull & Jones, 1975) suggest that the biosynthesis and intracellular transport of root cap slime may involve lipid and protein associated with both the membranes of the endoplasmic reticulum and the Golgi apparatus. A granulocrine process via Golgi secretory vesicles is involved in the concentration and transport of the slime precursors from their site of synthesis to the cell surface. The secretory products are released from the cell by a reverse pinocytosis. The exported material is preferentially stored between the plasmalemma and outer tangential cell wall. The final stage of the secretion process involves the passage of the secretory products through the cell wall facilitated by the progressive degradation of the cell wall to the outside where it appears as a droplet (Rougier, 1981).

Both vesicle movement processes and vesicle fusion at the cell surface require calcium for the completion of specific events in the pathway. Steer (1988b) has shown that secretion in plant cells appears to be dependent on or is triggered by a rise in the level of

internal free calcium ions from about $10^{-7}$ to $10^{-6}$M or even higher. Calcium at a critical concentration is required to activate various elements of the cytoskeleton, which in turn causes secretory vesicle movement. Effects of calcium on the cytoskeleton are mediated through calmodulin, a calcium binding protein (Klee et al., 1980). Hutton (1986) observed that inhibitors of calmodulin activity inhibit secretion.

Several functions of the mucilage for root growth and nutrient uptake by the root from the soil have been proposed (Oades, 1978). The most important functions are (1) protection of the root from desiccation (Leiser, 1968), (2) reduction of friction between the growing tip and the soil (Barley & Greacen, 1967), (3) improvement of the root-soil contact, thus facilitating nutrient diffusion to the root especially in dry soils (Nambiar, 1976), (4) to increase cation exchange and diffusion and thereby indirectly affect plant nutrition (Jenny & Grossenbacher, 1963), (5) immobilize certain toxic ions (Clarkson & Sanderson, 1969), and (6) to serve as a source of organic carbon and nitrogen in the rhizosphere (Rougier, 1981).

The roots form a polysaccharide droplet which adheres to the root tip. This provides a convenient system for the study of physiology of slime secretion. This system has been successfully utilized both for the estimation of the amount of secretory product produced by the root cap (Morre et al., 1967) and for investigations into the effects of various treatments on polysaccharide secretion (Morre et al., 1967; Jones & Morre, 1967; Mollenhauer & Morre, 1975, 1976; Paull and Jones, 1975). The rate of polysaccharide droplet formation can be directly correlated to the intensity of secretory activity of the Golgi apparatus and to the vectorial migration of the secretory vesicles from their sites of formation to the cell surface. Evidence that the movement of secretory vesicles to the cell surface is a directed phenomenon is provided in maize root. In caps treated with cytochalasin B, the secretory vesicles no longer move to the surface but accumulate at or near their sites of formation (Mollenhauer & Morre, 1976).

The primary toxic effect of aluminum is seen in the root tip meristems. However, before aluminum can migrate into the root tip meristem, it must pass through the mucigel secreted by the root tips. Since one of the functions of mucigel is to immobilize toxic ions, possibly by chelating them, the uptake of aluminum into the root tissue could be altered by mucigel. Studies conducted by Horst et al. (1982) show that root elongation is more inhibited by aluminum when the mucilage is removed from the root tips, indicating a protective function of the mucilage against aluminum injury. Mucilage has a very high and specific binding capacity of aluminum, thus reducing aluminum uptake into the root tip meristems. Clarkson and Sanderson (1969) reported an accumulation of scandium, chemically similar to aluminum, in the mucilage of onion roots.

Binding of aluminum to the mucigel was explained by exchange adsorption of aluminum on negative charges of the polyuronic acids in the mucigel (Wright and Northcote, 1974). The cation exchange capacity of the roots has been claimed to be negatively correlated to aluminum tolerance (Vose and Randall, 1962; Foy et al., 1967). The results presented by Horst et al. (1982) clearly indicate an important ecological role for mucigel excretion by root tips in the aluminum tolerance of plants growing in acid and mineral soils.

Mucigel may be an important defense substance against aluminum toxicity, yet one of the primary effects of aluminum toxicity in *Zea mays* is to inhibit production of the mucigel. Other plants need to be studied in order to determine how aluminum affects mucigel production, and where this may occur. More specifically, I was interested in answers to the following questions: (1) Does aluminum inhibit biosynthesis of mucigel from soluble precursors? (2) Does it interfere with Golgi processing of the material? (3) Does it block movement of Golgi-derived vesicles and fusion to the membranes? (4) Does it interfere with external flow of mucigel to the root cap surface? The answers to these questions would provide a better understanding of the protein that aluminum is affecting in wheat and thus this study on root cap ultrastructure was conducted.

# CHAPTER III

# METHODS AND MATERIALS

## Methods of Germination

Seeds of wheat (*Triticum aestivum*) cv. Victory, cv. Atlas 66, and Tam 101 were germinated in petri dishes, on 10-cm circles of Whatman No. 4 filter paper moistened with deionized water. In order to reduce the variation in growth rates among the seeds, the petri dishes were kept in a cold chamber at 5°C and allowed to imbibe water for 24 hours. After this, they were transferred to the growth chamber for 24 hours. The growth chamber was set to give a 16 hour light and 8 hour dark regime with a constant temperature of 26°C. The light intensity was 350 $\mu mol \cdot m^{-2}\ s^{-1}$. After this, seedlings of approximately the same growth stage were selected and placed on nylon screens with their roots immersed in 450 ml of an aerated nutrient solution of Aniol (1984) contained in plastic buckets. The seedlings were allowed to grow in the nutrient solution for 3 days. The five-day old seedlings were then transferred to fresh nutrient solution containing aluminum, supplied as $AlCl_3 \cdot 6H_2O$. The pH of the solution was adjusted to 4.5. The concentration of aluminum ions in the solution ranged from 0.1 μg/ml to 8 μg/ml. The seedlings were exposed to these concentrations of aluminum at varying treatment times. Primary roots of seedlings thus treated were used in the following studies.

## Determination of Growth in Primary Roots

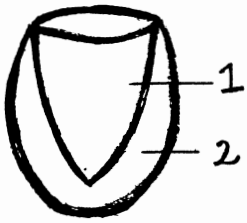
To test the effect of aluminum treatment on primary root growth, seedlings of cv. Atlas 66 (tolerant), cv. Victory (sensitive) and cv. Tam 101 (intermediate) (Dr. James D.

Ownby, Personal Commuication) were used. Seedlings were grown as described above, then exposed to five different levels of aluminum (0, 0.1, 0.5, 1.0 and 5.0 μg/ml) during a treatment time of 24 hours. Initial length of primary roots of 10 seedlings from each cultivar was recorded before exposing them to aluminum treatment. After 24 hrs, the length of the primary roots of 10 additional seedlings from each treatment was recorded. Growth of the primary roots during the treatment was estimated as the difference between mean root length at time 0 and time 24 hr. Growth curves were obtained by plotting growth of roots (in mm) vs. concentration of aluminum.

## Estimation of Volume of Mucigel

The effect of aluminum on mucigel formation in cvs. Victory and Atlas was determined. Seeds were germinated as usual until they were five days old. The five-day old seedlings of cv. Atlas were treated with 8 μg/ml aluminum during a treatment time of 0, 1, 2, 4 and 6 hours. Additional seedlings of Atlas were exposed to three different levels of aluminum (0, 1.0 and 4.0 μg/ml) for a period of 6 hours.

The seedlings of cv. Victory were exposed to 1.0 μg/ml aluminum for 0, 1, 2, 3 and 4 hours. After the treatments were completed, the primary roots of three seedlings per cultivar per treatment were harvested. The terminal 10 mm of the primary roots were excised, placed on a glass slide alongside a 6 cm ruler, and photographed using a Wild-M-8-stereomicroscope. Color transparency slides of the roots were projected onto a screen; the diameter and length of the root cap, and the mucigel drop surrounding it, were measured using a 6-cm ruler. The shape of the root tip (including the cap) approximated a cone, while the shape of the mucigel drop approximated a sphere surrounding the cone.




1 —— Cone of the root tip

2 —— Sphere of mucigel

Approximations were made since the shapes involved are not true spheres and cones, and this seemed the best way to estimate mucigel production, as there was not enough mucigel produced to measure by weight. The volume (v) of the mucigel around each root was calculated using the following formula:

$$v = 4/3\ \pi\ r_1^3 - 1/3\ \pi r_2^2 h$$

where $r_1$ = radius of the sphere of mucigel

$r_2$ = radius of the base of the root tip

h = length of the root tip from one end of the sphere to the other.

The mean of three measurements per treatment was determined for each cultivar. Using the data obtained, graphs were plotted as volume of mucigel in $mm^3$ vs. concentration of aluminum or time period of treatment.

## Electron Microscopy

Seedlings of cv. Victory were selected for the electron microscopy studies. Five-day old seedlings were treated with 1 μg/ml aluminum for 0 (control), 1, 2, and 4 hours. Initially the tissue to be studied was fixed in 2% glutaraldehyde for 2 h, washed and post-fixed in 2% $OsO_4$ for 2 h, dehydrated through an ethanol series and embedded in epoxy resin of Spurr (Spurr, 1969). Thin sections were stained with uranyl acetate and lead citrate and examined with a Joel-100 cx II Transmission Electron Microscope. It was difficult to locate membranous organelles, especially Golgi apparatus, which was important in this study, in the electron micrographs obtained by this method. After several fixation studies, a procedure developed by Mollenhauer (1959) using $KMnO_4$ was used. Root tips from each treatment were fixed in 2% aqueous, unbuffered potassium permanganate at room temperature for 15 minutes, dehydrated through an ethanol series with a terminal treatment in absolute acetone and embedded in Poly/bed 812 mixture (Luft's formula, 1961). Sections for light microscopy were cut using a Porter-Blum MT-2 Ultramicrotome and were stained with Malory's blue stain. Thin sections were post-

stained using alkaline lead citrate (Reynolds, 1963) and examined with a Joel-1000 cx II Transmission Electron Microscope. Low magnification pictures were taken to locate the secretory cells in the root cap region.

# CHAPTER IV

# RESULTS

The major observable physiological effects of aluminum in wheat roots were on the primary root growth, structure and color of root tips, and the production of mucigel. In comparison to the control, aluminum treated roots were characteristically stubby, curved and slightly brown in color. The region of the root approximately 1 to 3 mm above the tip was swollen. Aluminum treated roots showed an observable decrease in the number of root hairs. Most of the effects observed in wheat were similar to the effects described by Foy et al. (1978).

## Inhibition of Growth in Primary Roots by Aluminum

Three cultivars of wheat [cv. Atlas 66 (tolerant), cv. Victory (sensitive) and cv. TAM 101 (intermediate)] were selected for studying the effect of aluminum treatment on primary root growth. Among these cultivars, Victory and TAM 101 showed a similar response to the inhibitory action of aluminum (Fig. 1). In both these cultivars, aluminum at a low concentration of 0.1 μg/ml caused a decrease in growth of primary roots. As the concentration of aluminum was increased, there was a sharp and continuous decline in the growth of these roots. At 5 μg/ml aluminum, growth in cv. Victory was completely inhibited; whereas in cv. TAM 101 there was still some growth. This growth was, however, significantly lower than the control.

In the tolerant cv. Atlas 66 a different response to aluminum was observed. Low concentrations (0.1 μg/ml) aluminum were associated with a modest stimulation of root

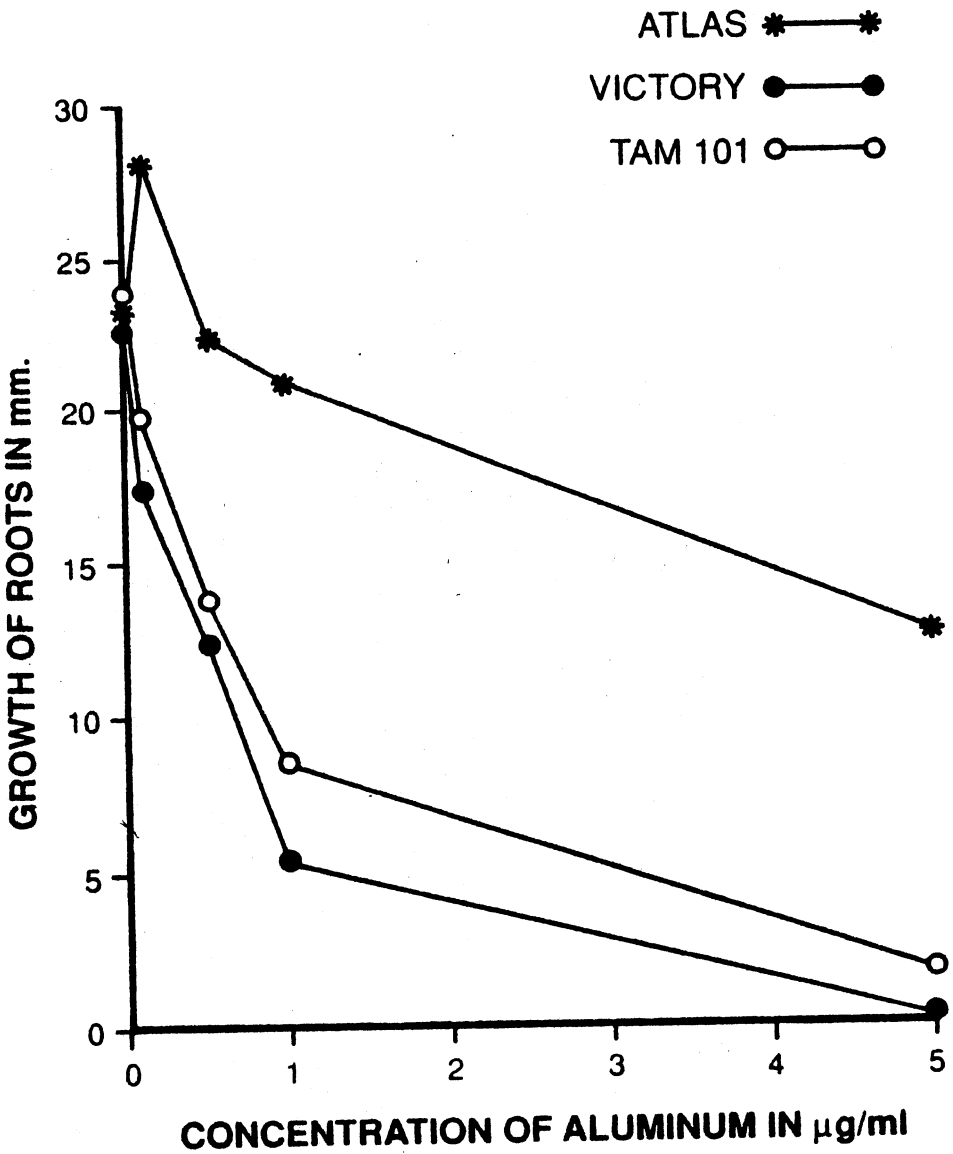



Figure 1. Inhibition of Primary Root Growth by Aluminum in Three Cultivars of Wheat Differing in Sensitivity to Aluminum. The mean root length of 10 seedlings of each cultivar was determined at time 0, then Al was added to the concentrations shown. After 24 h, the mean root length of 10 seedlings was measured.

growth. Roots exposed to 0.1 μg/ml aluminum for 24 hrs showed a 20% increase (Fig. 1). This was followed by a sharp and continuous decline of root growth when the concentration of aluminum was increased. This type of aluminum-stimulated growth response was also observed in *Zea mays* by Bennet et al. (1987). The concentration of aluminum required to inhibit growth by 50% would be about 0.6 μg/ml for Victory, 0.8 μg/ml for TAM 101 and > 5.0 μg/ml for Atlas. Thus, based on the root growth assay, Atlas 66 is about 8 to 10-times more tolerant of aluminum as Victory or TAM 101.

It can clearly be seen from the results above that aluminum even at 0.1 μg/ml concentration has an inhibitory effect on root growth in sensitive cultivars. It is also seen that the growth rate is inversely proportional to the concentration of aluminum. However, the mechanism of aluminum-stimulated growth in cv. Atlas 66 at low concentrations is presently unknown. The growth of the root requires cell division, cell elongation, and cell differentiation (O'Brien, 1982). From the results seen above, it seems possible to suggest that aluminum might inhibit growth through inhibition of a process like cell division.

## Effect of Aluminum on Mucigel Formation

The effect of aluminum on the amount of mucigel formed was studied in cvs. Victory and Atlas 66. Mucigel is the gelatinous material produced by the plant root cap and secreted onto the root surface. It is deposited in the form of a slimy droplet on the root tip. An increase or decrease in its formation can be easily observed. Any effect, therefore, that aluminum has on mucigel secretion can be observed visually, and the amount of mucigel secreted was estimated. Two experiments were designed to test this effect of aluminum.

(1) To determine the effect of different concentrations of aluminum on mucigel secretion after a treatment time of 6 hrs in cv. Atlas 66 and to determine the time course of inhibition using a high (8 μg/ml) concentration of aluminum.

(2) To determine the time course of aluminum inhibition of mucigel secretion in cv. Victory treated with 1 μg/ml aluminum.

In the first experiment, seedlings of cv. Atlas 66 were exposed to different aluminum concentrations for a period of 6 hrs to determine the aluminum concentration that was inhibitory to mucigel formation. The results can be seen in Figs. 2a-2c. The control root tip has a considerable amount of mucigel secreted. In comparison to the control, exposure to 1 μg/ml aluminum caused a large decrease in mucigel formation. Roots exposed to 4 μg/ml aluminum showed no mucigel secretion. The effect of different concentrations of aluminum on the volume of mucigel is shown in Fig. 3.

Aluminum thus has a direct effect on the amount of mucigel formed around the root tip. When seedlings of cv. Atlas 66 were exposed to 8 μg/ml aluminum, there was a decrease in the amount of mucigel formed after the first hour (Fig. 4b). Exposure to aluminum for 4 to 6 hrs resulted in complete inhibition of mucigel secretion. The volume of mucigel formed (in $mm^3$) is shown in Fig. 5. The volume of mucigel secreted was reduced from 0.14 $mm^3$ per root tip to 0.04 $mm^3$ per root tip after 1 hr.

In the case of the sensitive cv. Victory, 1 μg/ml aluminum was used to give treatment times of 0, 1, 2, 3, and 4 hrs. The response of this cultivar was somewhat different from that of cv. Atlas. Mucigel secretion began to be inhibited after 2 hr, and there was no mucigel seen by 4 hr (Fig. 6). When the volume of the mucigel formed was calculated, there was a slight, statistically insignificant increase at 1 hr, followed by a reduction to 73.5% of control at 3 hr and complete inhibition by 4 hr (Fig. 7).

It can be seen from the above results that in the sensitive cv. Victory an exposure to 1 μg/ml for 3 to 4 hrs was inhibitory to mucigel secretion. In the tolerant cv. Atlas 66, exposure to 8 μg/ml aluminum for 2 to 4 hrs and 1 μg/ml aluminum for 6 hrs resulted in complete inhibition of mucigel secretion. These results demonstrate the interference by aluminum in the secretory activity of the root cap cells, as shown by the disappearance of mucigel droplets from the root tips exposed to aluminum. The absence of mucigel on the

Figure 2. Primary Root Tips of Wheat cv. Atlas 66 Showing the Effect of Different Concentrations of Aluminum on Mucigel Formation. a) Control; b) 1 μg/ml aluminum treatment; c) 4 μg/ml aluminum treatment showing no mucigel formation.

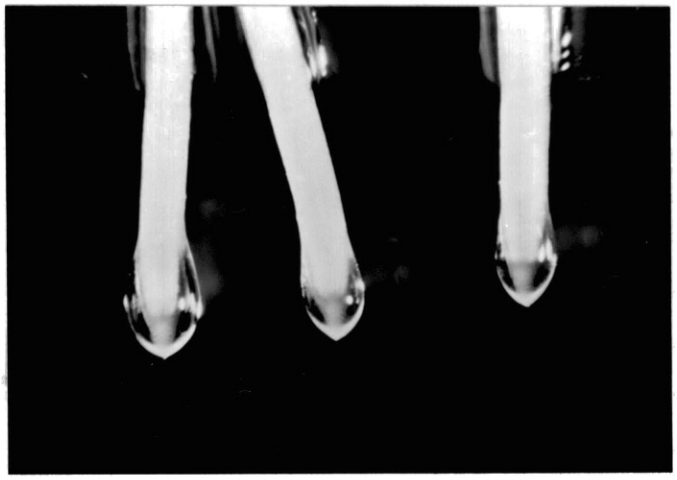
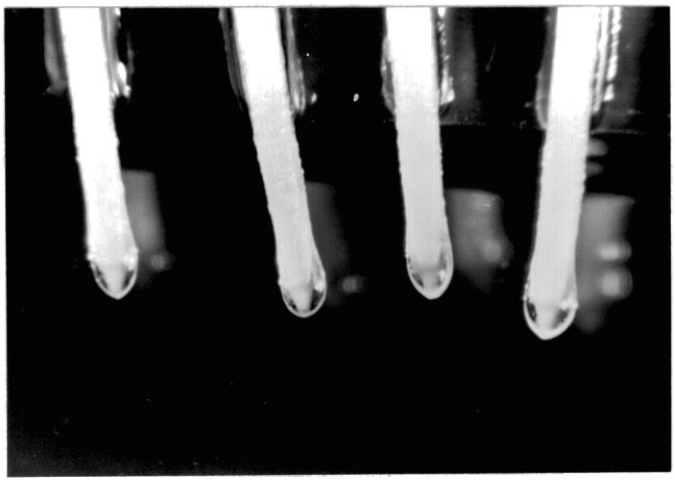


Figure 2a.

Figure 2b.



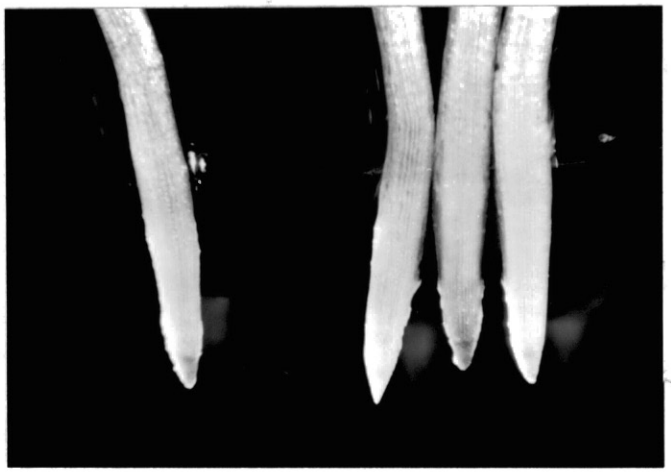


Figure 2c.

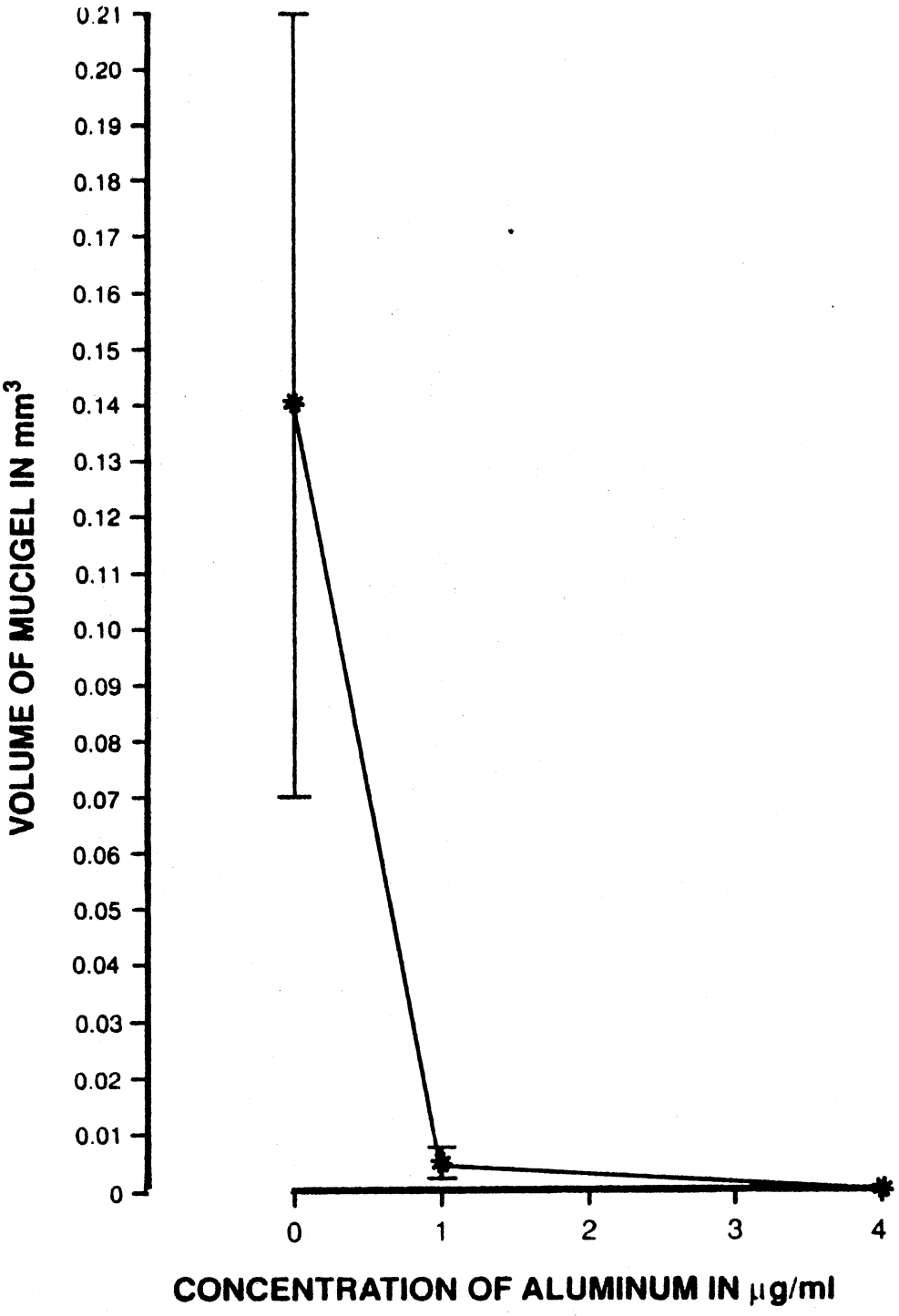



Figure 3. Effect of Different Concentrations of Aluminum on Mucigel Secretion in Wheat cv. Atlas 66. All measurements were made after 6 h of treatment. Error bars indicate the Standard Error of the Mean.

Figure 4. Effect of 8 μg/ml Aluminum on Mucigel Formation in Primary Roots of cv. Atlas 66 at Different Time Intervals. a) Control; b) 1 hour treatment; c) 2 hour treatment.

Figure 4a.

Figure 4b.

Figure 4c.

Figure 4. Primary Root Tips of Wheat cv. Atlas 66 treated with 8 μg/ml Aluminum. d) 4 hours; e) 6 hours. Both 4 and 6 hours showed no mucigel formation.

Figure 4d.

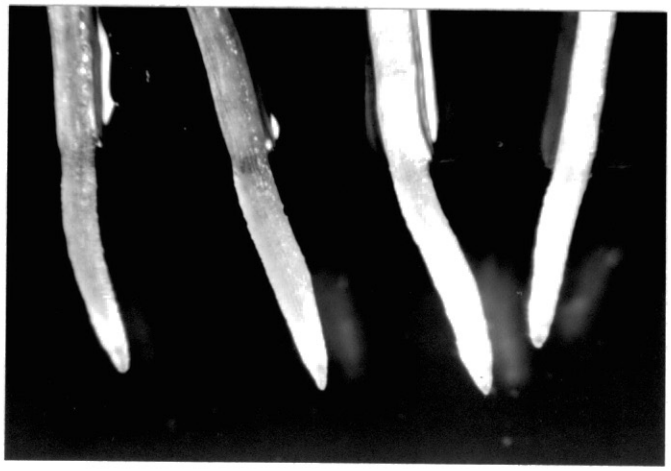


Figure 4e.

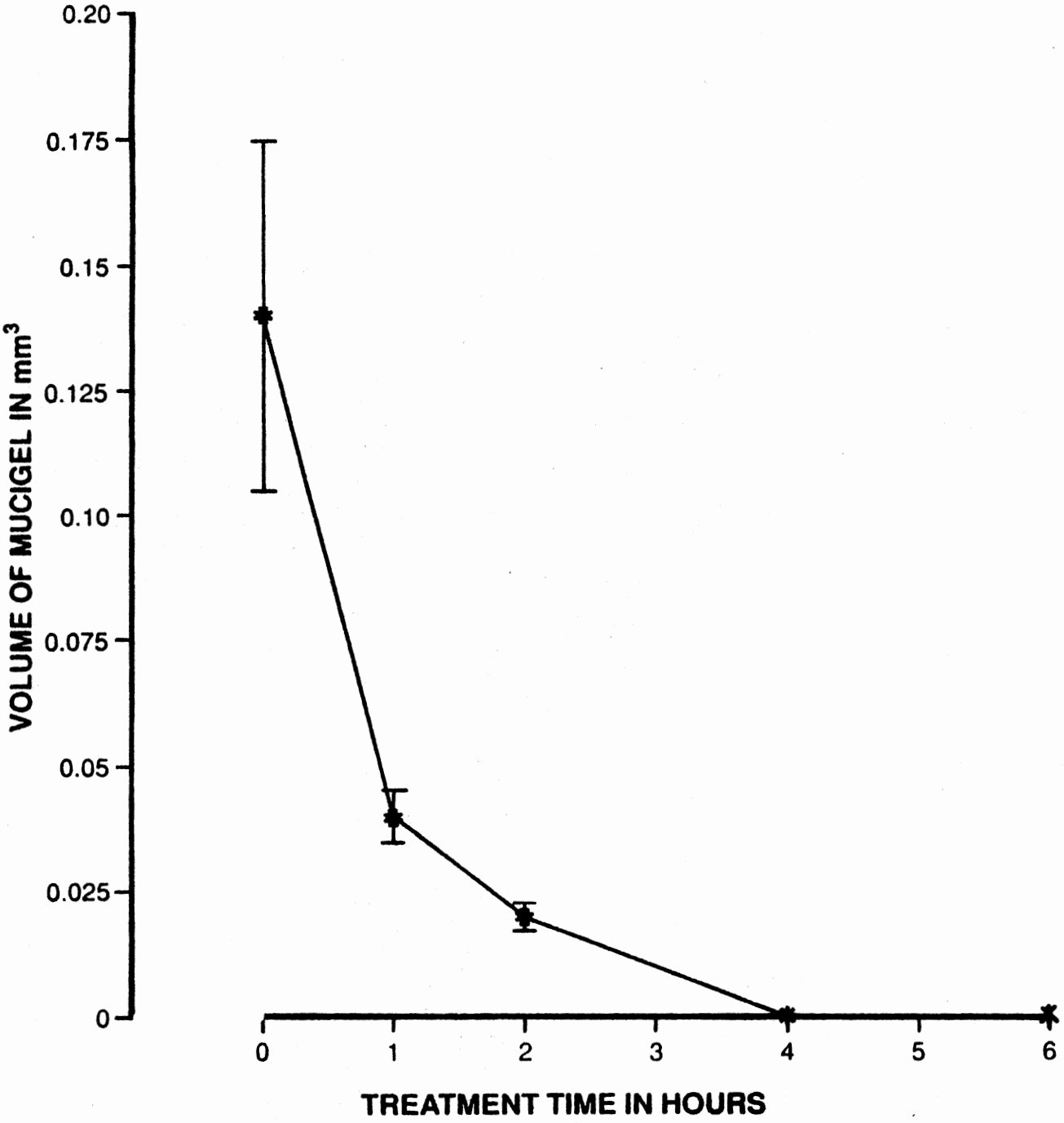



Figure 5. Time Course of Aluminum Inhibition of Mucigel Secretion in Wheat cv. Atlas 66 When Treated with 8 µg/ml Aluminum at Time Zero. Error bars indicate the Standard Error of the mean.

Figure 6. Primary Root Tips of Wheat cv. Victory Showing the Effect of 1 μg/ml Aluminum on Mucigel Formation at Different Time Intervals. a) Control; b) 1 hour treatment; c) 2 hour treatment.

Figure 6a.

Figure 6b.

Figure 6c.

Figure 6. Primary Root Tips of Wheat cv. Victory Treated with 1 μg/ml Aluminum. d) 3 hours; e) 4 hours – shows no mucigel formation.

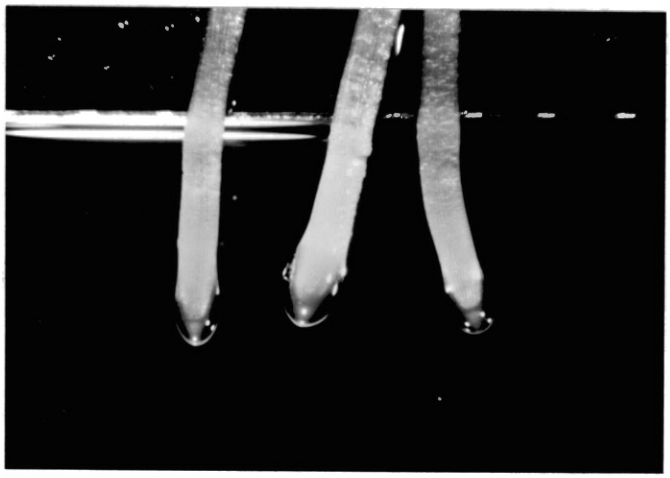


Figure 6d.

Figure 6e.

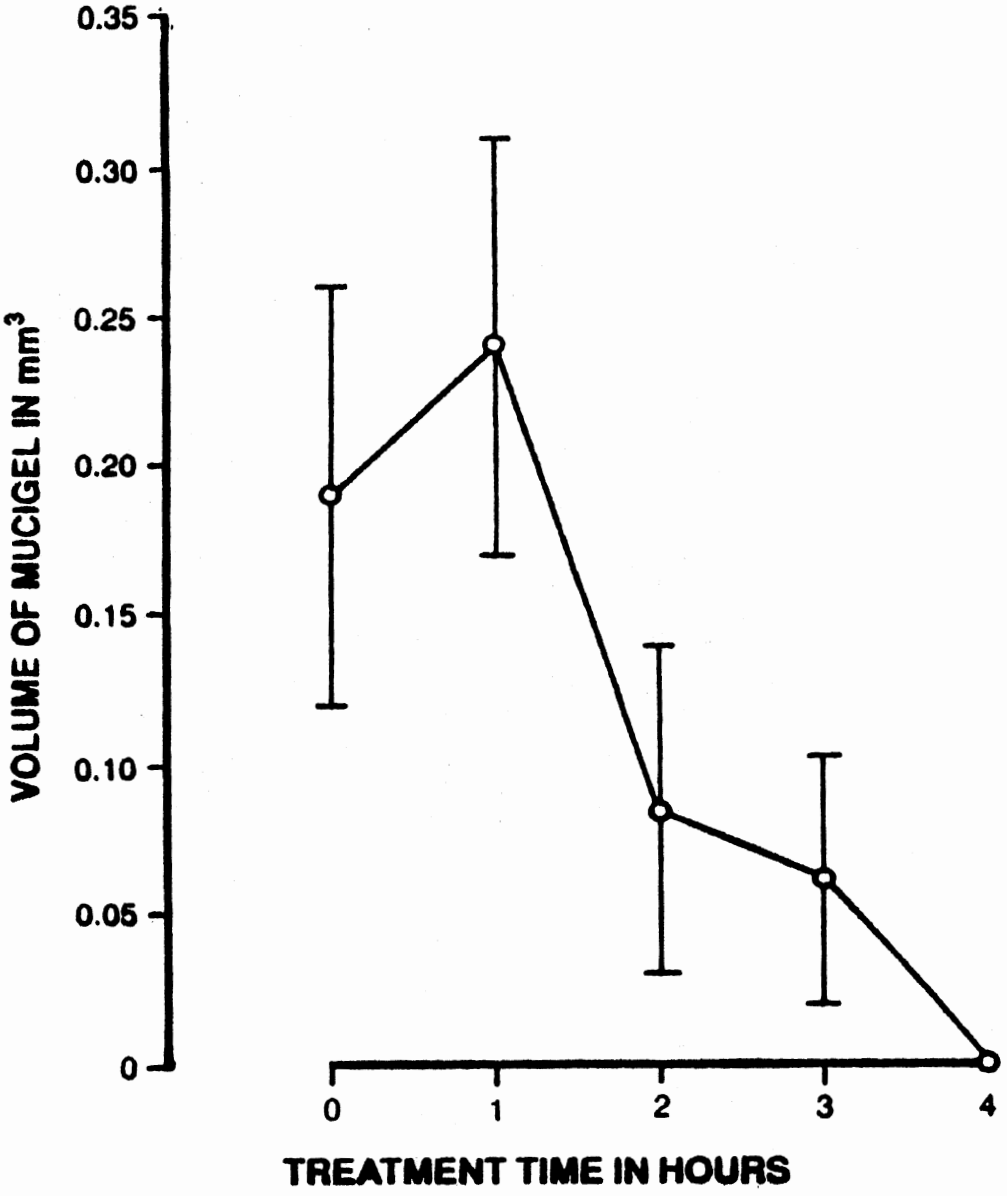



Figure 7. Time Course of Aluminum Inhibition of Mucigel Secretion in Wheat cv. Victory When Treated with 1 µg/ml Aluminum at Time Zero. Error bars indicate the Standard Error of the mean.

root tips exposed to aluminum indicates the possibility of various effects of aluminum. Since mucigel is the product of synthesis of polysaccharides in the Golgi apparatus, its absence on the root tip when exposed to aluminum may be due to a direct effect of aluminum on mucigel biosynthesis. And, because this material is released from the Golgi apparatus in vesicles which travel to, and fuse with, the plasmalemma, aluminum may be affecting the function of the Golgi apparatus. Under normal conditions, this secretory material passes through the cell wall and accumulates externally as slime droplets. Aluminum could be interfering with movement of mucigel from cell surface to the root cap surface either through changes in the cell wall or changes in the mucigel caused by aluminum.

Ultrastructural studies were, therefore, conducted in order to fully understand the above mentioned effects and to find evidence for these effects at the ultrastructural level. Specifically, evidence was sought to determine if aluminum was affecting vesicle formation, vesicle movement to the plasmalemma, or movement of the mucigel once it is released from the cell in the secretory cells of the wheat root cap.

## Aluminum-Induced Ultrastructural Changes in the Root Cap Cells

Initially, the tissue to be used for this study was fixed in glutaraldehyde, post-fixed in $OsO_4$ and stained with uranyl acetate and lead citrate. An electron micrograph of a portion of a cell from a section treated thus can be seen in Figure 8. In this electron micrograph, it is very difficult to locate membranous organelles such as the Golgi apparatus, endoplasmic reticulum, etc., which were important in this study, although ribosomes are clearly seen.

In order to be able to see the Golgi apparatus and endoplasmic reticulum membranes clearly, several procedures were tried including an old technique involving $KMnO_4$. After several fixation studies a procedure developed by Mollenhauer (1959) using $KMnO_4$ was used. The tissue was fixed in $KMnO_4$, and thin sections were stained in Reynold's lead

Figure 8. Electron Micrograph of a Portion of Wheat Root Cap Cell Fixed in Glutaraldehyde-osmium Tetraoxide, Stained with Uranyl Acetate and Lead Citrate. X 7,200.

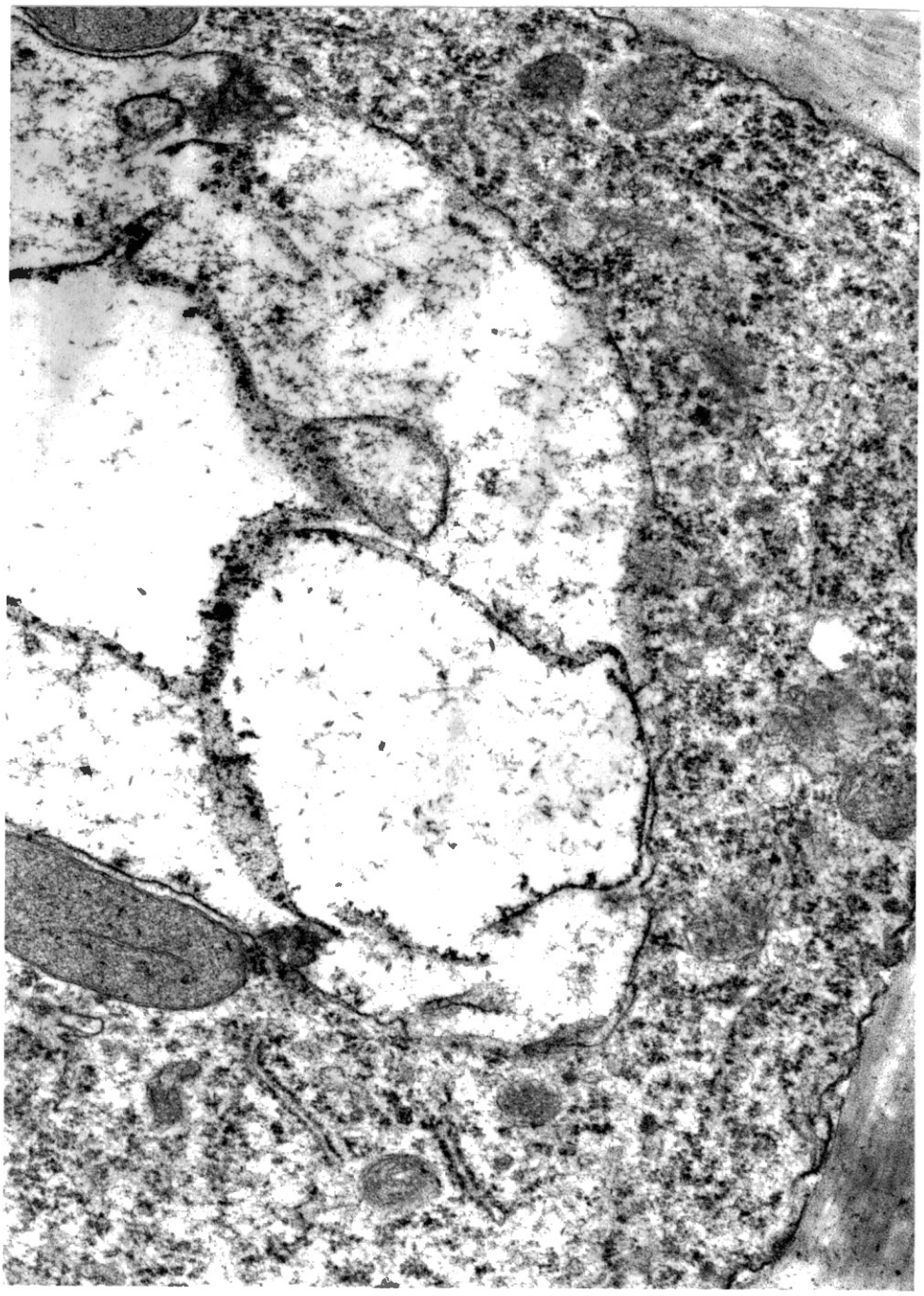


Figure 8.

Figure 9. Electron Micrograph of a Wheat Root Cap Cell Fixed in 2% Potassium Permanganate Stained with Reynold's Lead Citrate. Note the presence of numerous dictyosomes. d) endoplasmic reticulum (er); mitochondria (m) and amyloplasts (a) containing starch grains (st). X 2,900.

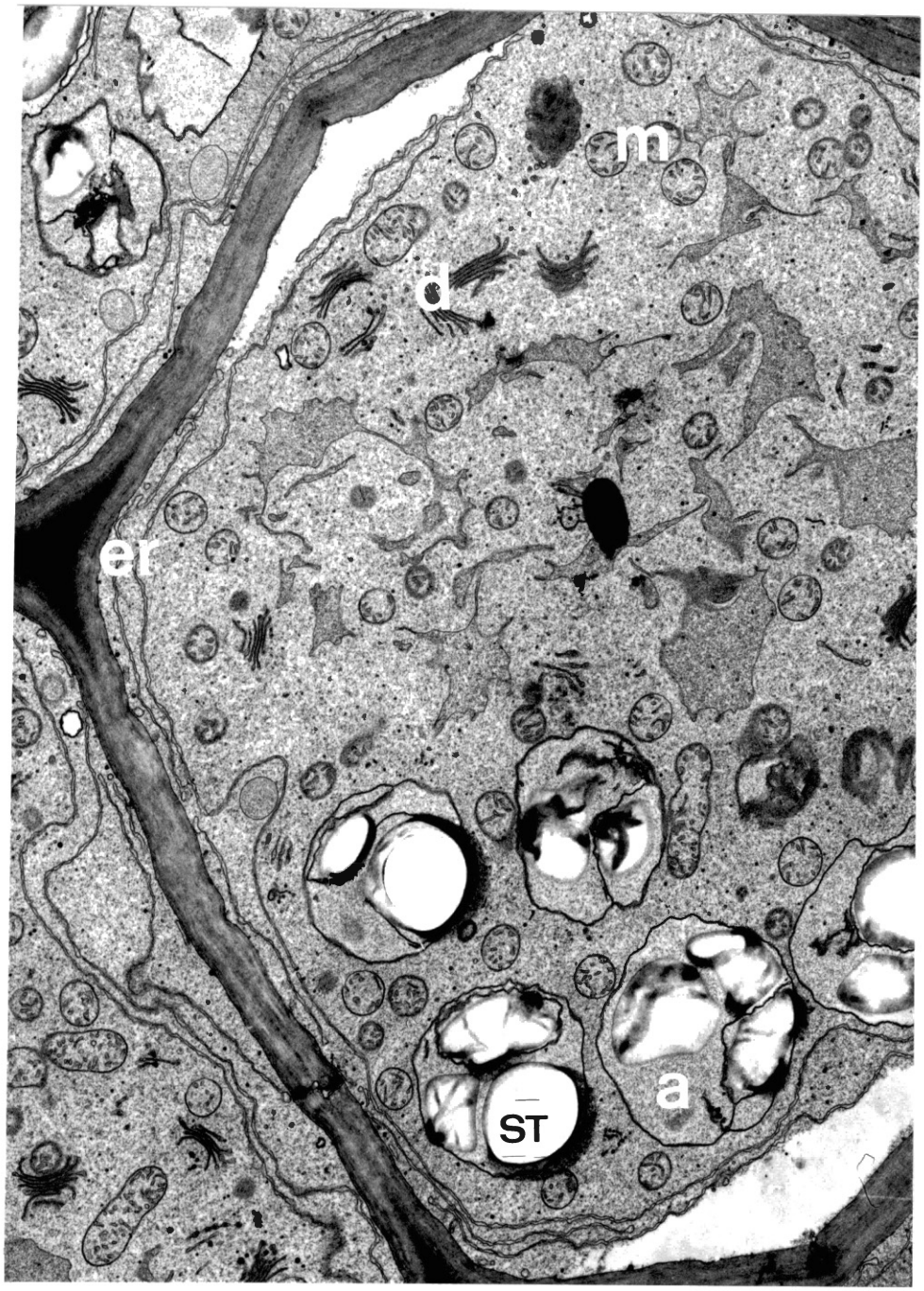



Figure 9.

citrate just before observation with the electron microscope. An electron micrograph of a wheat root cap cell from a section treated thus can be seen in Figure 9. The region of the root cap shown is the interior, where cells contain numerous Golgi bodies and much endoplasmic reticulum, but actually show little secretory activity. Although ribosomes and few protein structures are not well preserved by this technique, it proved to be more useful than the glutaraldehyde-$OsO_4$ fixation. This technique could potentially be successfully employed in morphometric analysis of the various organelles, which could be seen so clearly.

Wheat cv. Victory, which is considered sensitive to aluminum, was used in this part of the study. The effects of 1 μg/ml aluminum on the ultrastructure of secretory cells in wheat root cap at different time intervals (0, 1, 2, and 4 hrs), especially the outer layer which constitutes the secretory cells in the root cap region, were examined for aluminum-induced changes. These results are presented in Figures 10 to 17.

In the control treatments, the outer peripheral cap cells were found to be rich in dictyosomes, secretory vesicles, mitochondria and endoplasmic reticulum (Fig. 10). Amyloplasts containing starch grains are seen. The presence of amyloplasts was used to confirm that the cells being examined were part of the root cap and not part of the root meristem. Nuclei were not always seen in the plane of the section. The cell is rich in Golgi apparatus, which are hypertrophied. The hypertrophied form of the Golgi apparatus is characteristic of active secretory cells (Juniper & Roberts, 1966). Most dictyosomes show a distinct polarity with the cisternae developing sequentially across the stack from the forming face to the maturing face. Basically two types of secretory vesicles are seen, with the alteration in the shape of the secretory vesicles from elongated to round occurring during maturation. The Golgi apparatus is occasionally associated with the endoplasmic reticulum on the forming face. The secretory pattern of the Golgi apparatus is characterized by production of the secretory vesicles and their movement towards the plasmalemma. In Figure 11 the fusion of the membrane of the secretory vesicle with the

Figure 10. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Secretory Function of the Golgi Apparatus in the Control Cell. Fusion of the Golgi apparatus derived vesicles (v) with the plasmalemma (P) and release of the secretory product (sp) between the plasmalemma and cell wall (cw) is seen. X 2,900.

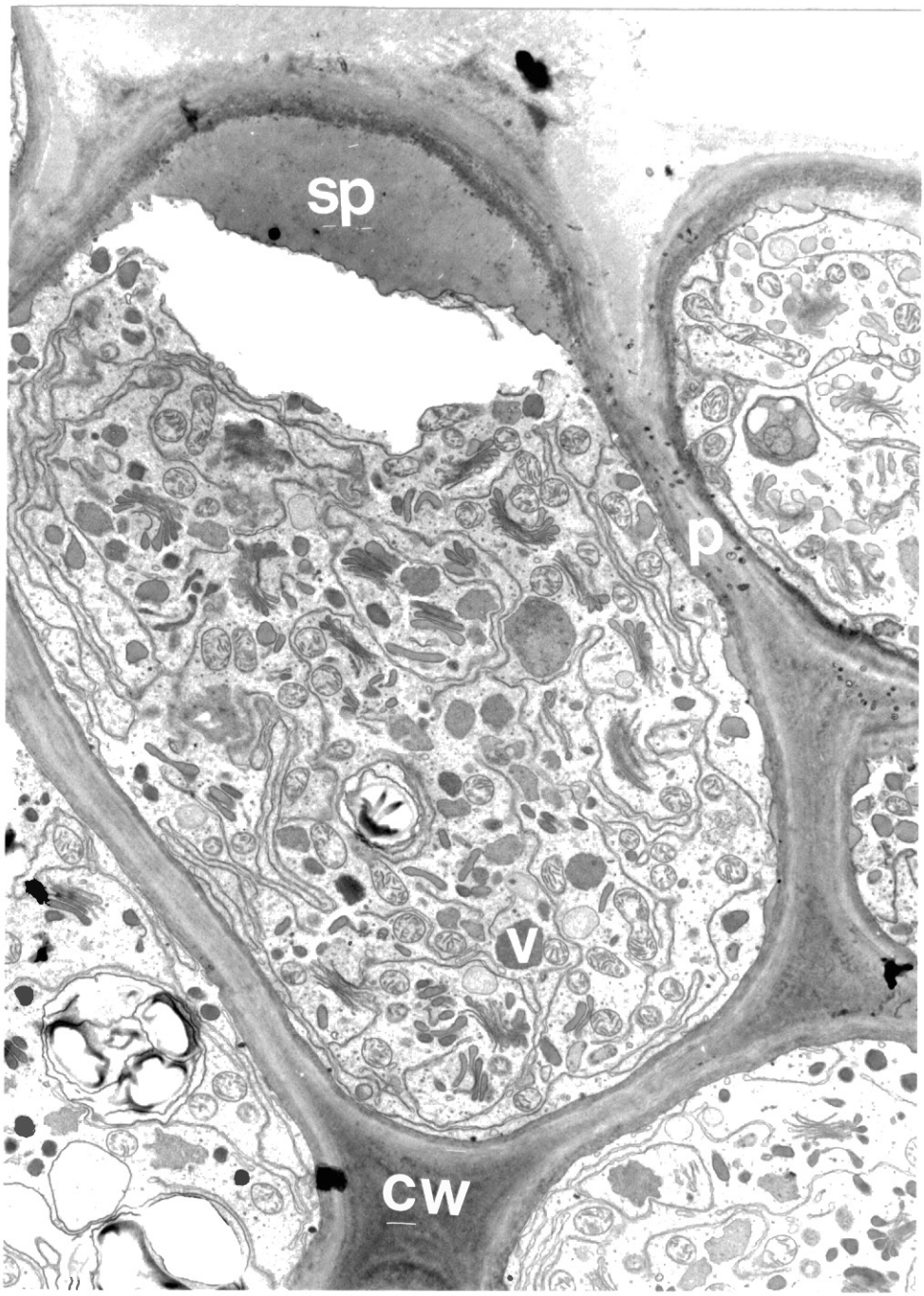



Figure 10.

Figure 11. Electron Micrograph of a Control Root Cap Cell of Wheat cv. Victory Illustrating the Cell in the State of Active Secretion. Hypertrophied dictyosomes (arrow) characteristic of active secretory cells are seen. Endoplasmic reticulum (er) is seen associated with the forming face (ff) of the dictyosomes. X 2,900.

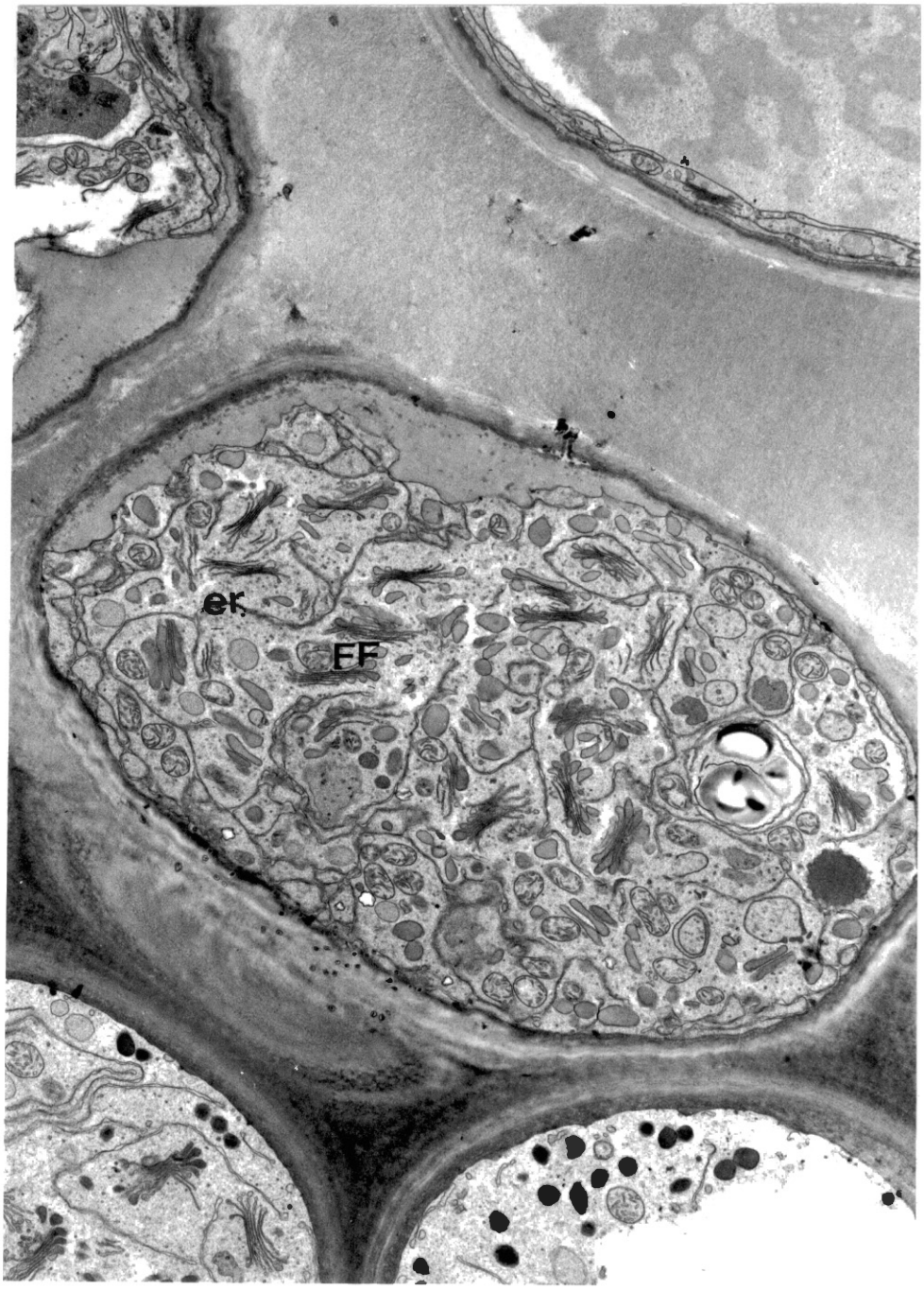



Figure 11.

plasma membrane and release of the material into the space between the plasma membrane and cell wall is clearly seen. This material must then pass through the cell wall and be deposited as a hydrated droplet on the surface of the root tip.

Figures 12 and 13 show electron micrographs of root cap cells exposed to 1 μg/ml aluminum for 1 hr. From the previous experiments on mucigel secretion, it was seen that at this time period there was not yet any reduction in the mucigel measured on the root cap surface. In the electron micrograph there is a slight increase in the number of vesicles in the cytoplasm and an increase in the amount of secretory material deposited between the plasma membrane and cell wall. Most of the organelles such as mitochondria, amyloplasts, and endoplasmic reticulum are still seen and there is little or no change in their structures. However, there is a slight disorganization of the Golgi apparatus and some of the cisternae show curling which is a symptom of stress which may be due to aluminum. Severe disorganization and curling which was observed by 2 hr exposure to 8 μg/ml aluminum in *Zea mays* by Bennet et al. (1985a) was not observed in cv. Victory, possibly because the aluminum concentration used in this study was 8-fold less. However, the appearance of the cells is in general similar to that observed by Bennet et al. (1985a).

The effects of exposure of the root cap to 1 μg/ml aluminum for 2 hrs are shown in Figures 14 and 15. Most of the cells of the outer two layers showed a marked accumulation of secretory vesicles in the cytoplasm. Visual comparison of the 2 hr treatment with the control suggested a decrease in the number and size of mitochondria. Complete disappearance of the Golgi apparatus and reduction in the endoplasmic reticulum were the primary effects of this treatment. Accumulation of the secretory vesicles within the cytoplasm is indicative of a decrease in frequency with which Golgi apparatus derived vesicles were fused with the plasmalemma and their contents released to the cell wall. Alteration in the size and shape of the secretory vesicles was observed. In some cells the transformation of cisternae of Golgi apparatus into a secretory vesicle was observed (Fig.

Figure 12. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Effect of 1 Hour Exposure to 1 μg/ml Aluminum. A slight increase in the number of secretory vesicles is observed. No alteration in the shape of mitochondria or amyloplasts is seen. Fusion of secretory vesicles with the plasmalemma is seen. X 2,900.

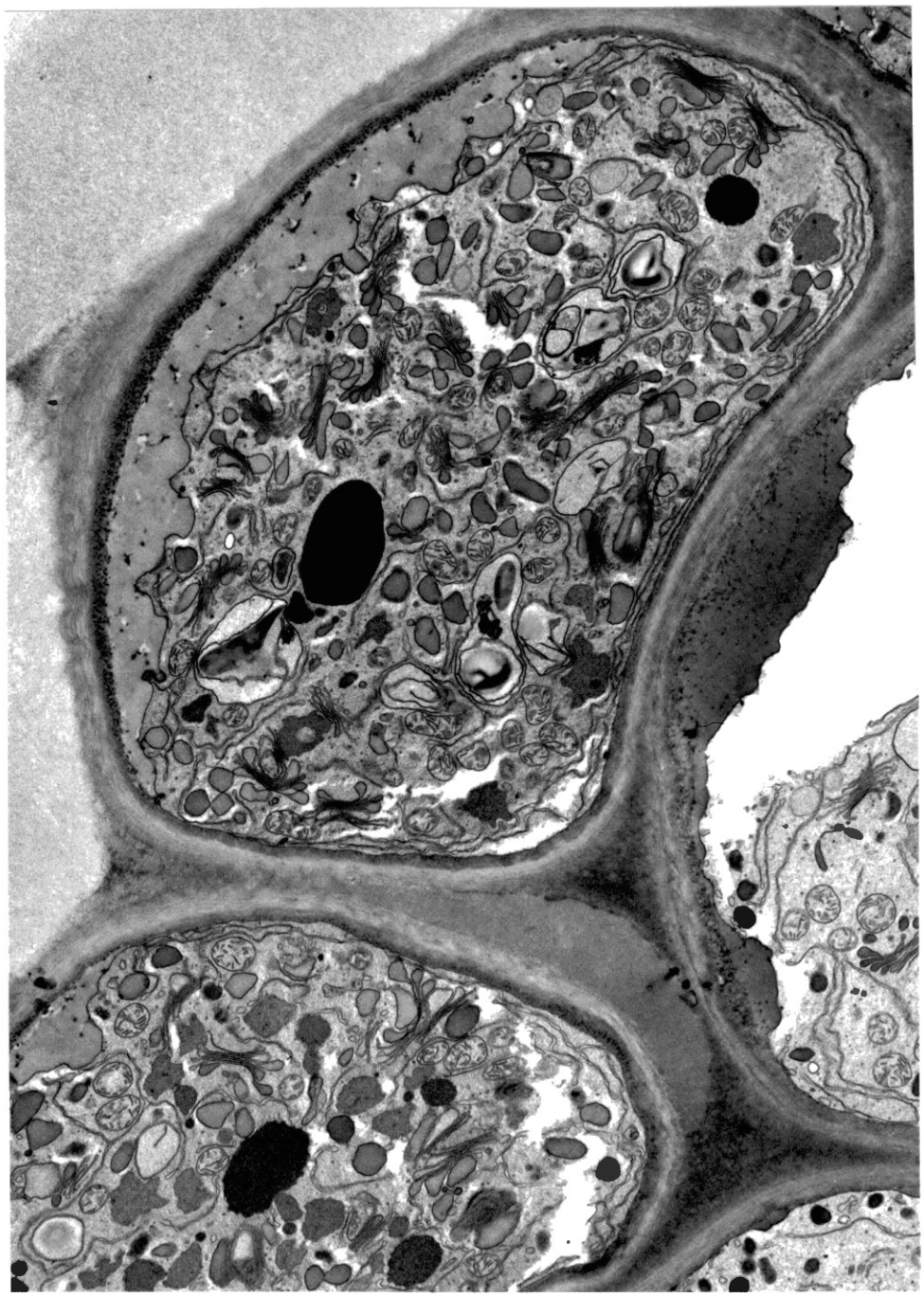


Figure 12.

Figure 13. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Effect of 1 Hour Exposure to 1 μg/ml Aluminum. Slight curling of cisternae of some of the Golgi apparatus (arrow) is seen at this treatment time. However, no change is observed in the endoplasmic reticulum, mitochondria and other organelles. X 2,900.

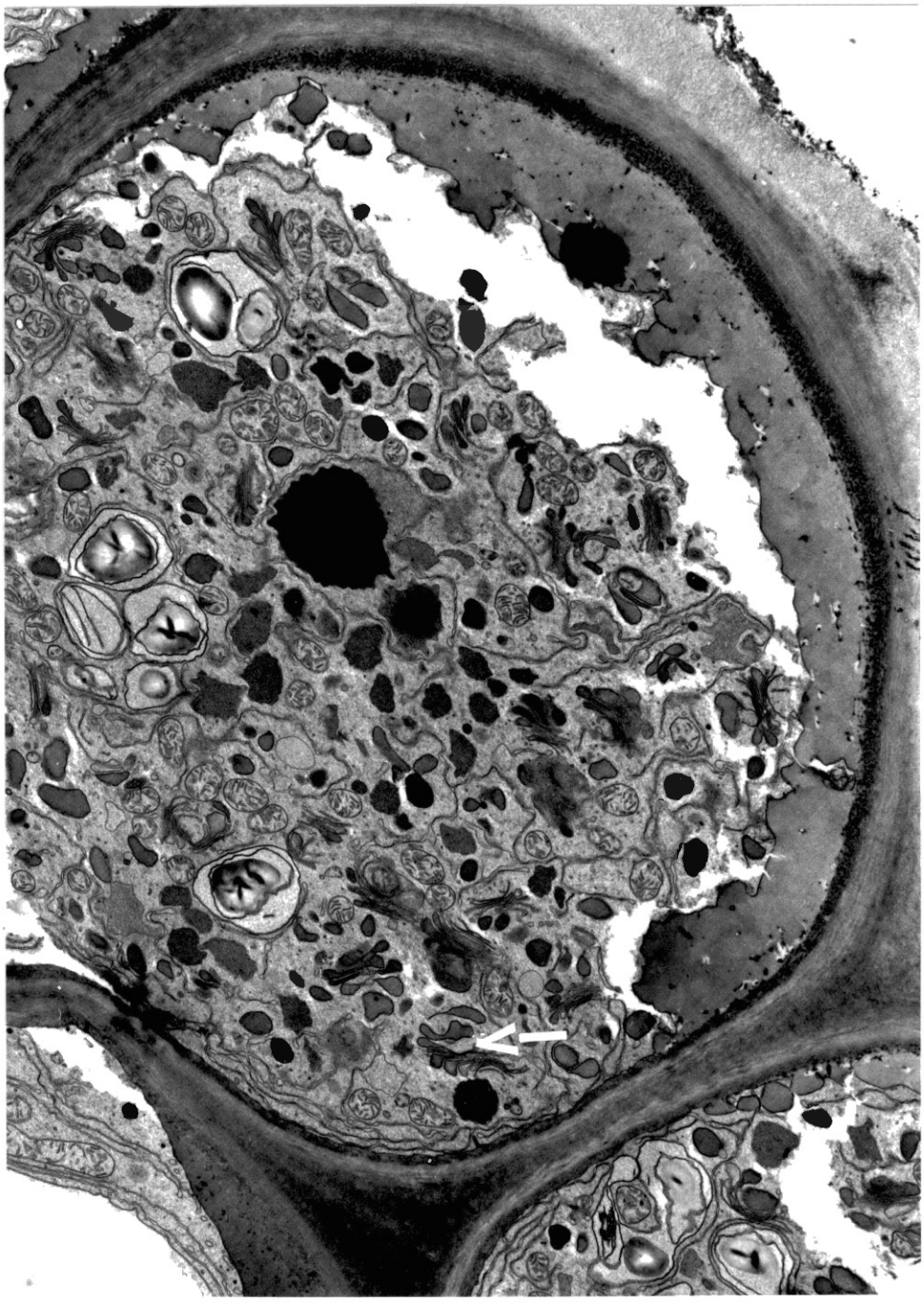


Figure 13.

Figure 14. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Effect of 2 Hour Exposure to 1 μg/ml Aluminum. A marked accumulation of secretory vesicles in the cytoplasm is seen here. Compared to the control, a decrease in the size and number of mitochondria is observed. X 2,900.

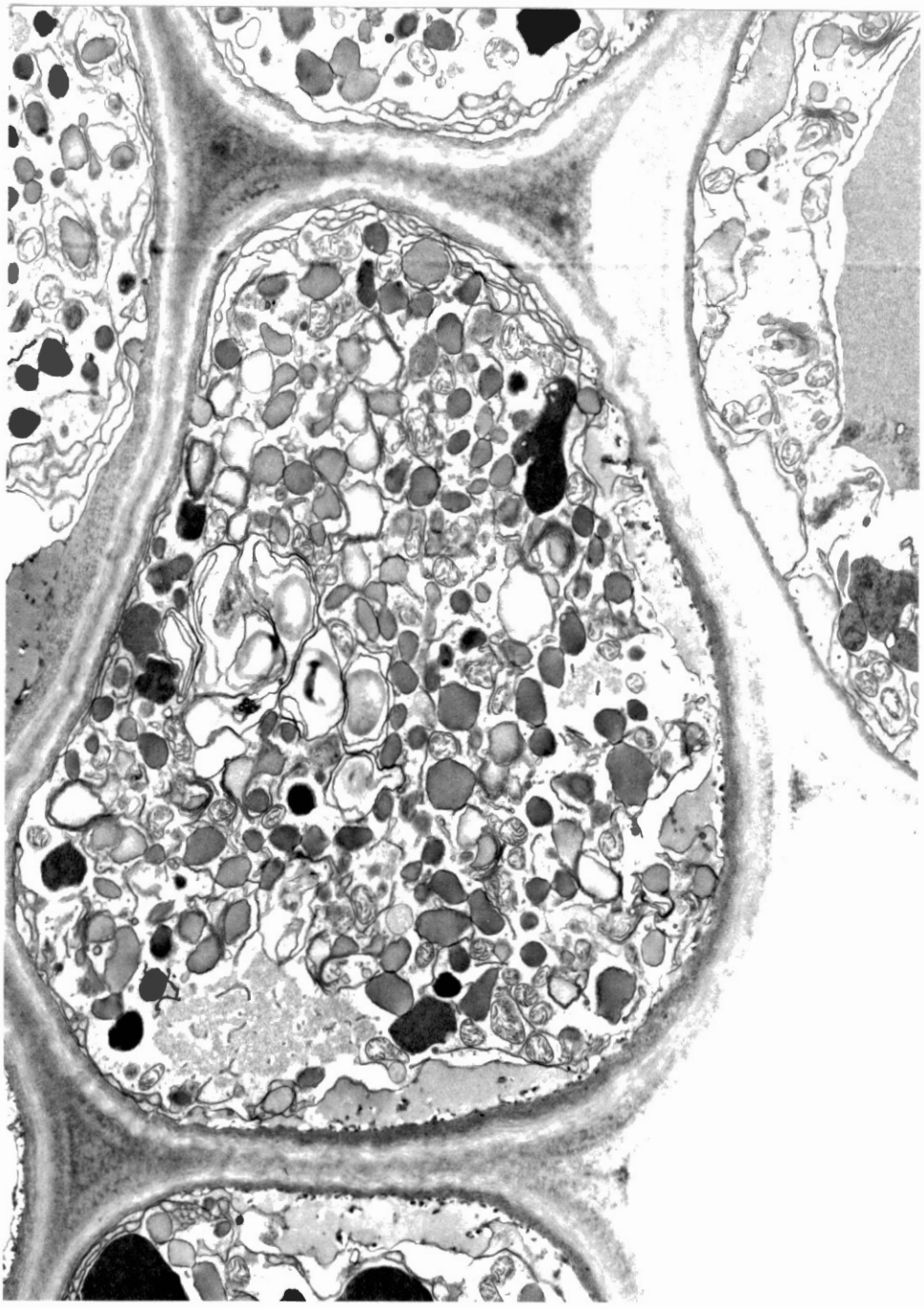


Figure 14.

Figure 15. Electron Micrograph of a Peripheral Root Cap Cell in Wheat cv. Victory Illustrating the Effect fo 1 μg/ml Aluminum. Increase in the number of secretory vesicles is seen along with the alteration in their sizes. Reduction of endoplasmic reticulum, transformation of Golgi apparatus cisternae into secretory vesicles is seen here (arrow). A decrease in the amount of secretory product (sp) is evident. X 2,900.

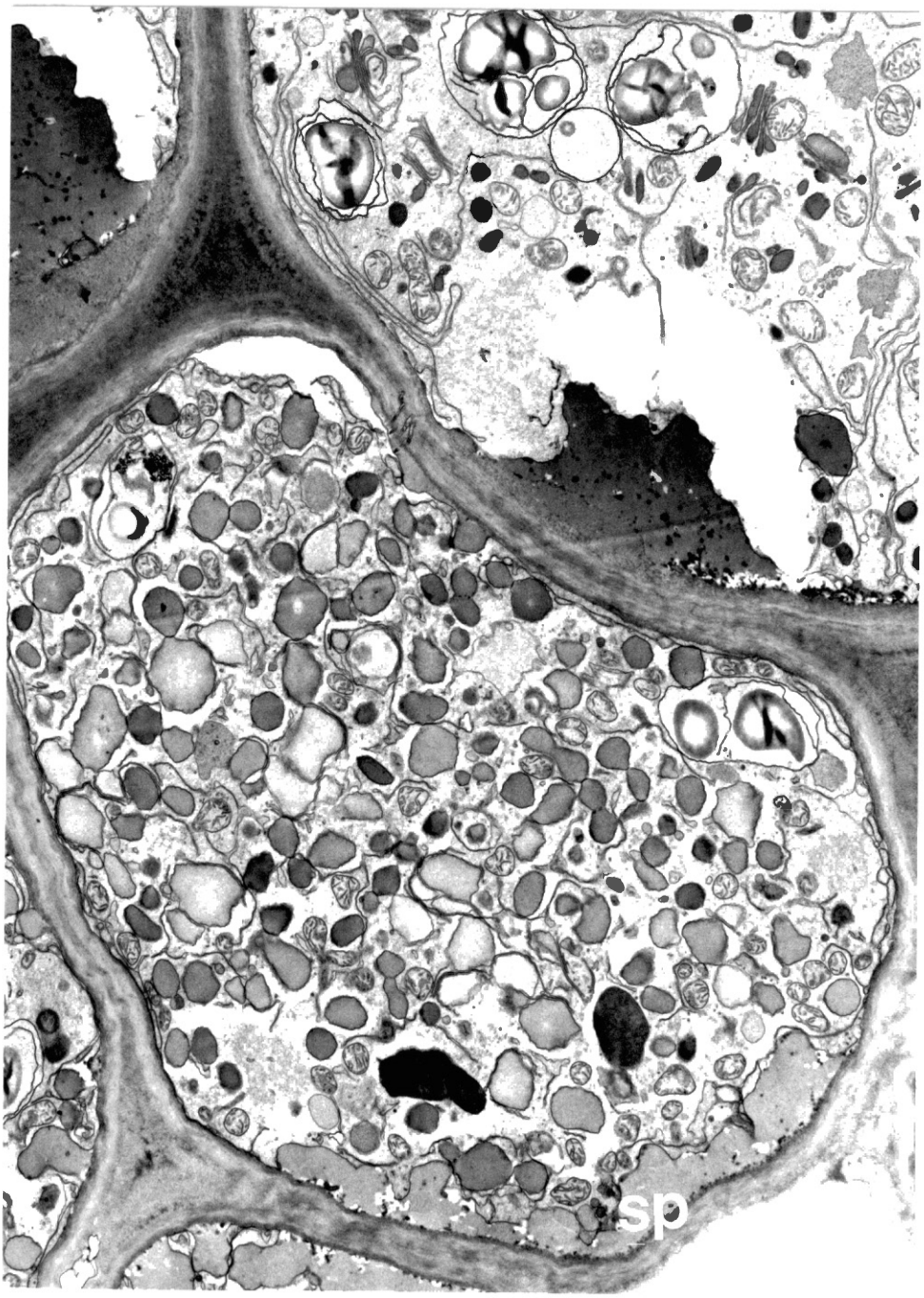



Figure 15.

Figure 16. Electron Micrograph of a Peripheral Root Cap Cell of cv. Victory Illustrating the Effect of 4 Hour Exposure to 1 μg/ml Aluminum. Secretory vesicles are still found in the cytoplasm. Very little or no endoplasmic reticulum is found. X 2,900.

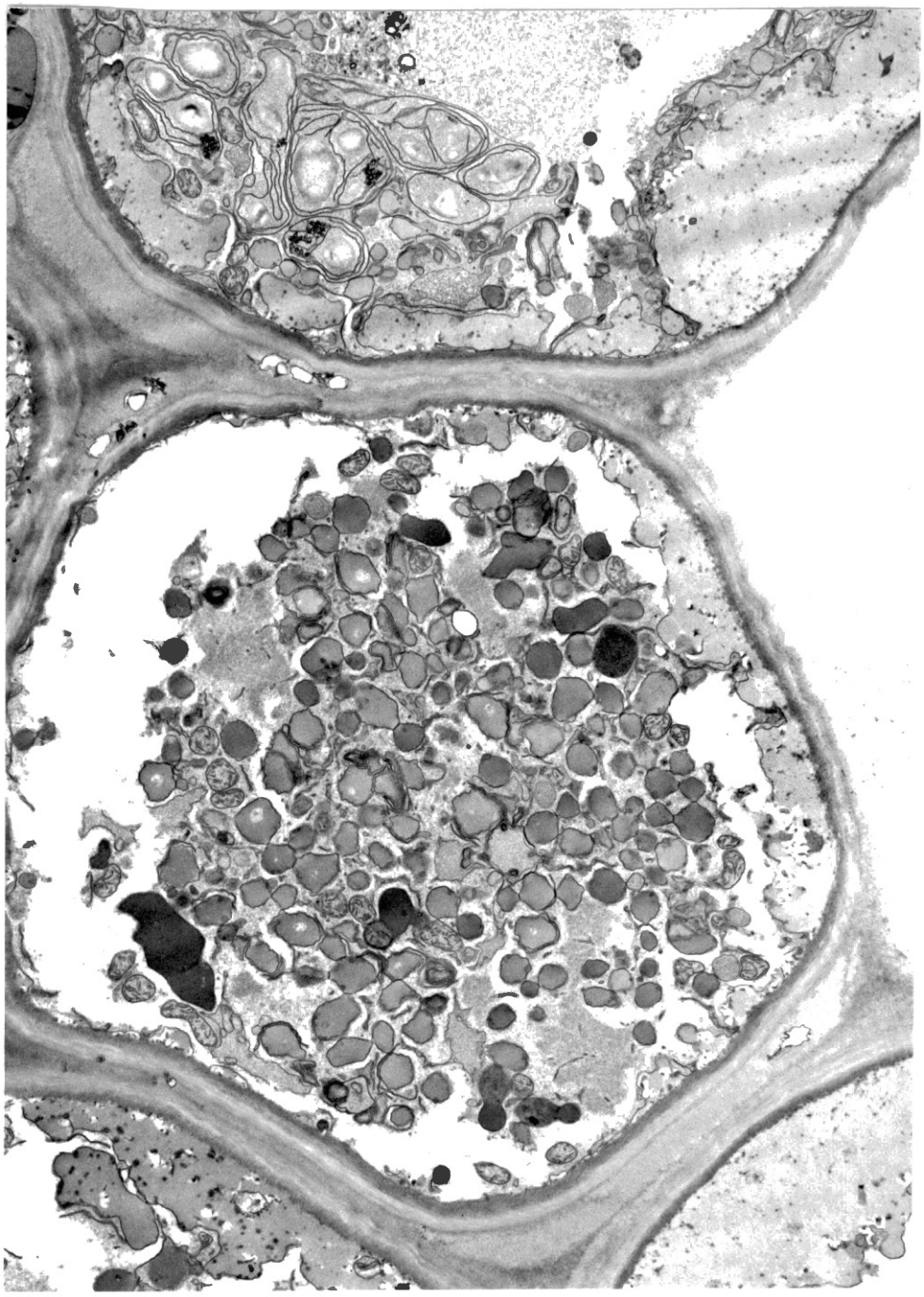


Figure 16.

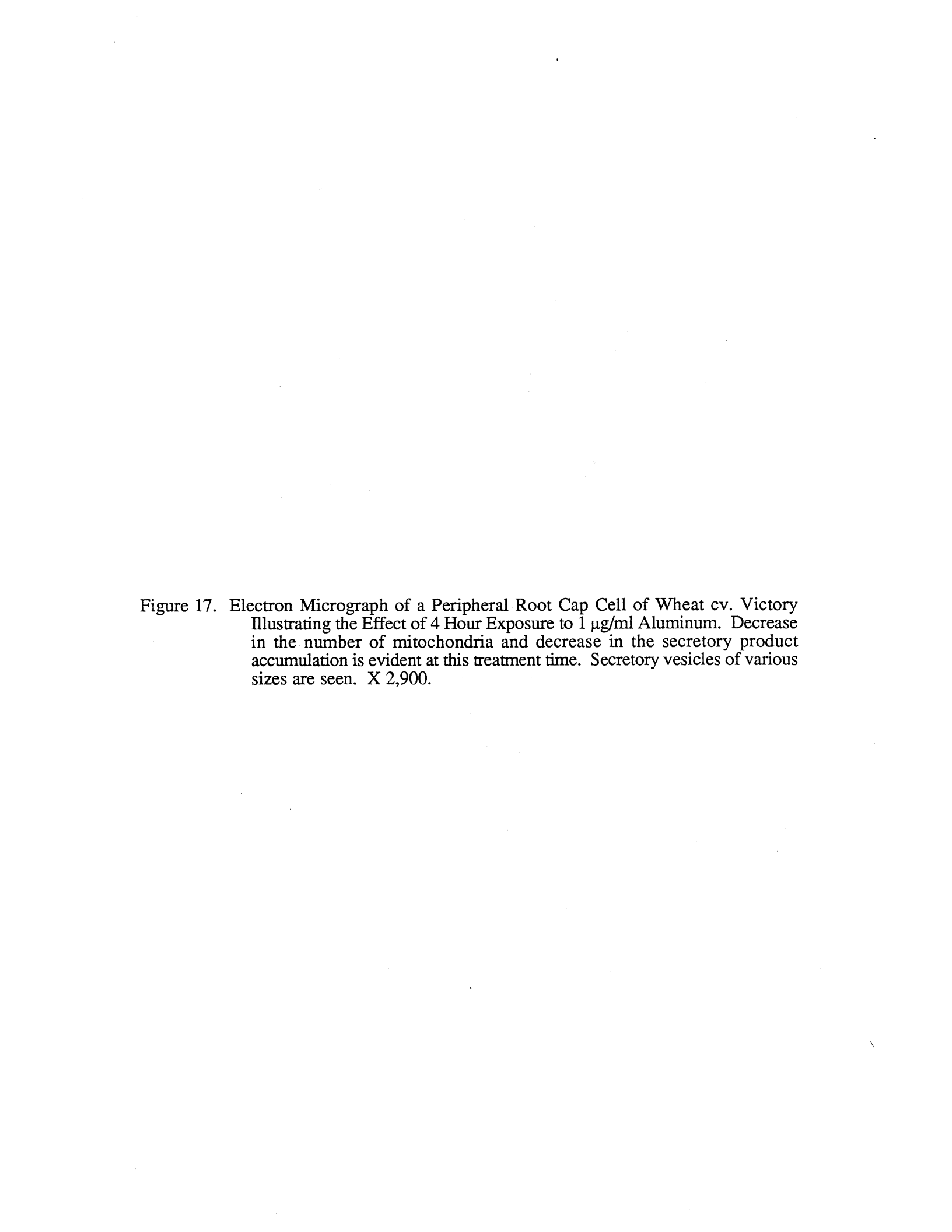


Figure 17. Electron Micrograph of a Peripheral Root Cap Cell of Wheat cv. Victory Illustrating the Effect of 4 Hour Exposure to 1 μg/ml Aluminum. Decrease in the number of mitochondria and decrease in the secretory product accumulation is evident at this treatment time. Secretory vesicles of various sizes are seen. X 2,900.

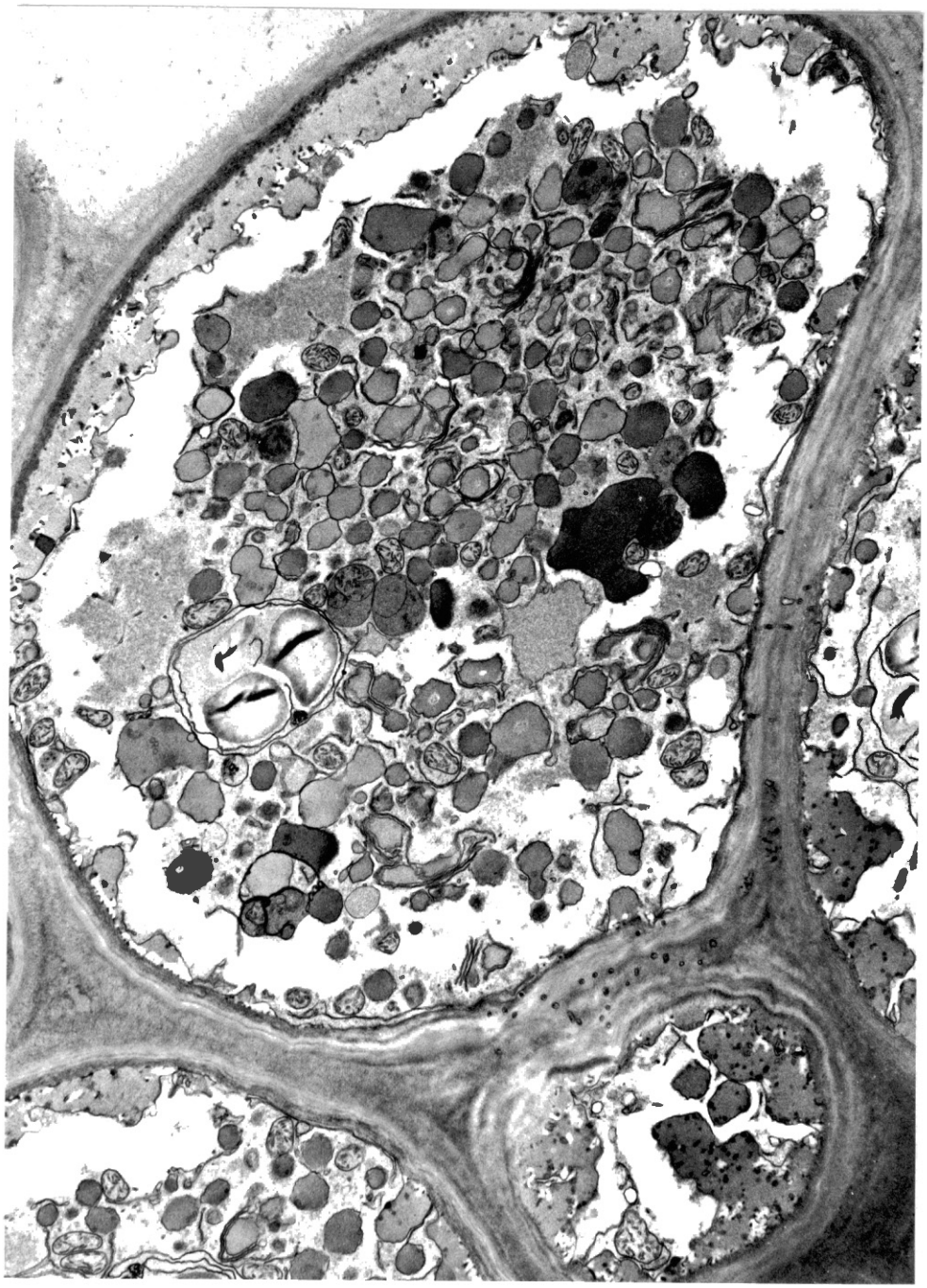


Figure 17.

14). The effects found at 4 hrs of exposure to 1 μg/ml aluminum were similar to the 2 hr exposure. Increase in the number of secretory vesicles in the cytoplasm is clearly evident in Figures 16 and 17. The electron micrographs show a reduction in the number of mitochondria, disappearance of endoplasmic reticulum and dictyosomes, the latter apparently replaced by swollen vesicles which may represent former cisternae of the Golgi apparatus. At this treatment time, there was no mucigel observed on the root tips. No effect of aluminum was observed on the nuclear structure.

# CHAPTER V

# DISCUSSION

In experiments conducted to determine the inhibitory action of aluminum on primary root growth, it was found that concentrations as low as 0.1 μg/ml were enough to inhibit growth in a sensitive cultivar of wheat, whereas in the tolerant cultivar inhibition was observed mainly at a concentration of 5 μg/ml. From the data obtained on growth rates, the concentration of aluminum required to inhibit 50% of the growth was calculated. It was found that 0.6 μg/ml in cv. Victory, 0.8 in TAM 101 and > 5 μg/ml aluminum in cv. Atlas was required to inhibit 50% of the primary root growth. One interesting aspect in these experiments was the aluminum-stimulated growth response in cv. Atlas, the mechanism of which is presently unknown. Aluminum toxicity has frequently been associated with inhibition of root growth acting through reduced mitotic activity (Bennet et al., 1985b). Aluminum at toxic levels might inhibit growth through inhibition of a process like cell division or differentiation; however, we do not know for sure what the primary target process is when aluminum inhibits root growth.

Aluminum treatment was found to inhibit mucigel formation in both the sensitive and tolerant cultivars of wheat. In the (tolerant) cv. Atlas 66 inhibition of mucigel formation was observed at 1 μg/ml aluminum whereas at the same concentration growth in primary roots was unaffected. This implies that the effect of aluminum on the primary root growth and its effect on mucigel synthesis may be two separate processes. Mucigel formation seems more sensitive to aluminum. This may be due to the fact that mucigel is formed in the peripheral cap cells, which are among the first cells to encounter aluminum ions, thus inhibition at lower concentrations. The distance from the root surface (where aluminum

ions are available) to the root cap cells is shorter than the distance between the root surface and root meristem cells, hence penetration of aluminum into the cap cells is probably greater than into root meristem cells.

The peripheral root cap cells, and the mucigel they secrete, are constantly sloughed off as the root grows, so that new root cap cells initiate secretion, forming new mucigel. There is thus a constant synthesis and turnover of mucigel. If mucigel synthesis ceases in response to aluminum, mucigel then present will slough off and disappear and not be replaced. As mentioned before, mucigel has a variety of functions, one of which is the protection of the root tip. The root tip not protected by mucigel is more susceptible to injury of any kind and presumably more susceptible to entry of aluminum into the root meristem.

Ultrastructural studies of aluminum-induced changes in mucigel secretion indicate that the primary site of aluminum action is on the structure and function of the Golgi apparatus of the outer root cap cells. The results obtained in wheat cv. Victory are in accordance with those of Bennet et al. (1985a) in *Zea mays*. Low magnification pictures of the transverse section of primary root of cv. Victory revealed that primarily the outermost layer of peripheral cells were actively involved in secretion. The layer of cells adjacent to this layer showed less secretory activity. The control root cap cells were rich in dictyosomes which were hypertrophied, and producing secretory vesicles, most of which moved towards the plasmalemma and fused with it, releasing the material inside them to the outside of the cell. The control root tips showed the droplet of mucigel on their exterior, which implies that the secreted material was continuously transported from within the cell through the cell wall to the exterior.

Evidence for the effect of aluminum on the Golgi apparatus is seen in the 1 hr treatment with 1 μg/ml aluminum, where slight disorganization and curling of the cisternae of the Golgi was observed. According to Mollenhauer and Morre (1976), these are some of the structural responses that characterize stressed Golgi apparatus.

Inhibitory action of aluminum on the movement of vesicles was observed at the 2 hr treatment time. The secretory vesicles that were formed at this period accumulated in the cytoplasm, which could be related to the decrease in the amount of mucigel formed on the root tip externally. This is indicative of the decrease in the rate of transfer of the vesicles to the plasmalemma. The relative absence of secretory vesicles from the cytoplasm of the control treatment was considered indicative of the rapidity of transfer of vesicular contents across the plasmalemma. Inhibition of vesicle transfer implies a role for aluminum in preventing the assembly of membrane material. Complete disappearance of the Golgi apparatus and endoplasmic reticulum were the primary effects of this treatment. Disappearance of the Golgi apparatus is due to the fact that the Golgi apparatus saccules are going into the formation of vesicles. Evidence for this can be seen in the electron micrographs of the 2 hr treatment where some of the cisternae of the Golgi apparatus can be seen in the process of transformation into a secretory vesicle.

At 4 hr treatment time most of these effects remained. Decrease in the accumulation of secretory material between plasmalemma and cell wall and accumulation of secretory vesicles within the cytoplasm could be related to the absence of mucigel on the root tips. From the results gathered, it is obvious that the inhibition due to aluminum sets in between the first and second hr treatment. Since most of the effects seen at 2 hrs continue to be seen even at 4 hrs, it seems possible to suggest that the secretory vesicles formed in the second hour remain in the cell and there is no further production of new secretory vesicles, which is indicative of the effect of aluminum on the secretory function of the Golgi apparatus.

By inhibiting the movement of secretory vesicles, aluminum may be interfering with the factors that facilitate this movement. It is known from the work of Steer (1988b) that calcium is required for the movement of secretory vesicles to the cell surface. There is a possibility that by interfering with the function of calcium, aluminum could inhibit the movement of secretory vesicles within the cell. Since the effects of calcium on the

cytoskeleton are mediated through calmodulin, a calcium binding protein (Klee et al., 1980), aluminum may be interfering with calmodulin activity. Hutton (1986) observed that inhibitors of calmodulin activity inhibit secretion. So it is a possibility that aluminum may be inhibiting secretion by inhibiting calmodulin activity.

Since microtubules are thought to be involved in moving vesicles through the cytoplasm to the plasmalemma, binding of aluminum to the microtubules (MacDonald et al., 1987) and "freezing" them could also be a possible mode of aluminum inhibition of secretory vesicle movement.

It has also been shown by Vierstra and Haug (1978) that aluminum may bind directly to membranes and this may interfere with ability of membranes to fuse. Thus, although this work demonstrates that Al interferes with mucigel synthesis by interfering with the secretory process at the Golgi apparatus, the exact mechanism of interference with secretion remains to be determined.

One of the functions of the Golgi apparatus in the root meristem is the formation of the cell plate during cytokinesis. In the telophase stage the middle lamella of the daughter cells is formed by the secretory vesicles of the Golgi apparatus. Any effect of aluminum on this function of the Golgi apparatus would then result in an effect on cell division and cell growth. This would seem a possible explanation for the inhibition of primary root growth in cultivars of wheat. Aluminum could be inhibiting cell division by blocking movement of vesicles to the cell plate during mitosis. Experiments related to this work were not conducted in this project. In order to determine if the effect of aluminum on cell division is due to blocking the movement of vesicles that go into the formation of cell plate, experiments could be done to measure mitotic figures in root meristem cells of the control and aluminum treated roots. If the above hypothesis is true, there would be many cells arrested in the telophase stage in aluminum treated roots, suggesting that interference with secretion and movement of Golgi apparatus-derived vesicles plays a role in both inhibition of growth and inhibition of mucigel secretion by aluminum.

# CHAPER VI

## SUMMARY AND CONCLUSIONS

The effect of different concentrations of aluminum at varying treatment times on the growth of primary roots and secretory activity in root cap cells of wheat was studied. Aluminum at toxic levels has an irreversible effect on the growth of primary roots. Complete inhibition of primary root growth in cultivar Victory was observed upon exposure to 5 μg/ml aluminum for 24 hrs. For the same treatment, root growth in TAM 101 decreased by 92% and in cultivar Atlas 66 there was a decrease by 46%.

Four hours of exposure to 8 μg/ml aluminum was inhibitory to mucigel formation in Atlas 66, whereas in cultivar Victory 4 hrs of exposure to 1 μg/ml was inhibitory. This seems to imply that mucigel secretion in cultivar Victory, like growth, is much more sensitive to aluminum than is the case for Atlas 66. Yet 6 hr exposure to 1 μg/ml aluminum was sufficient to block mucigel secretion in Atlas which implies that mucigel synthesis in cultivar Atlas 66 may be more sensitive to aluminum than growth of primary roots. Several important functions are assigned to mucigel. Basically it protects the root tip and root meristem from chemical and physical damage. By inhibiting its formation on the root tip, aluminum is causing injury to the root tip and meristem. The root tip not protected by mucigel is more susceptible to injury of any kind, and presumably more susceptible to entry of aluminum into the root meristem.

In order to study the effect of aluminum on the secretory activity, ultrastructural studies were conducted. The primary effect of aluminum was on the Golgi apparatus function. Aluminum seems to cause the swelling of the cisternae of the dictyosome into vesicles, resulting in the disappearance of the Golgi apparatus, while also inhibiting

movement of these vesicles to the plasmalemma. Secretory vesicles that were produced and accumulated within the cell at the 2 hr treatment time were also seen in the cell at the 4 hr treatment with aluminum. This implies that aluminum has an inhibitory effect on the production of secretory vesicles at the 2 hr time period, and no new vesicles are formed after this time. Four hours of aluminum treatment seemed to cause a reduction in both size and abundance of mitochondria, however, no effect was seen on nuclear structure. Most of the results observed at the ultrastructural level in wheat were similar to those observed by Bennet et al. (1985a) in *Zea mays* (1985b).

Inhibition of secretory vesicle movement by aluminum may be due to its interference with uptake and/or function of calcium in plants or a direct effect of aluminum on membrane properties, which might interfere with the fusion of membranes (i.e., fusion of vesicle membranes with the plasmalemma). Since vesicles may be transported by pathways dictated by microtubules which are part of the cytoskeleton, interference of aluminum with the cytoskeleton may also be part of the mechanism by which aluminum inhibits vesicle movement. Specific experiments should be conducted in order to determine which of the above mentioned mechanisms could be attributed to inhibitory action of aluminum on the secretory processes in root cap cells of wheat.

This study was helpful in understanding the secretory function of the Golgi apparatus, which includes the packaging and export of mucilagenous materials from the root cap and also provided some evidence for the effects of aluminum. However, it is not yet known how Al-induced changes in polysaccharide metabolism may be translated into root growth responses. Further work needs to be done on the effect of aluminum on mucigel formation in wheat cultivar Victory to see if the slight increase in the amount of mucigel produced during the first hour of exposure to aluminum is a reproducible effect. Likewise, quantifying the aluminum-induced changes would provide a better understanding of the effects of aluminum.

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