To the Graduate Council:

I am submitting herewith a dissertation written by Priyavadan A. Joshi entitled "Ultrastructural Histochemical Localization of Beta-Glycerophosphatase and Adenosine Triphosphatase Activity in Developing Cotton Fiber, Gossypium Hirsutum L. cv. Hancock." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Science.

James McD. Stewart
James M. Stewart, Major Professor

We have read this dissertation
and recommend its acceptance:

Effin T. Graham

John H. Reynolds

David L. Coffey

Accepted for the Council:

C. W. Minkel
Vice Provost and
Dean of the Graduate School

ULTRASTRUCTURAL HISTOCHEMICAL LOCALIZATION OF BETA-GLYCEROPHOSPHATASE AND ADENOSINE TRIPHOSPHATASE ACTIVITY IN DEVELOPING COTTON FIBER *GOSSYPIUM HIRSUTUM* L. cv. HANCOCK

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Priyavadan A. Joshi

June 1987

AG-VET-MED.
Thesis
876
.J683

DEDICATION

I dedicate this work to my respectful father, to my dear late mother and to my whole family; whose hard work, sacrifices, love, and educational and spiritual training helped me to achieve the knowledge and ultimately led to this stage of success in life.

ACKNOWLEDGEMENT

I wish to express my thanks to my family, friends and all those persons who have helped me during my graduate study and in the completion of this dissertation.

I am deeply thankful to my major professor, Dr. James M. Stewart, for his professional guidance, constructive criticism, and most of all, for his kind friendship during the course of this study. I am also very much thankful to the other members of my graduate committee, Dr. David L. Coffey, Dr. John H. Reynolds and Dr. Effin T. Graham, for their helpful suggestions and careful reading of the manuscript. To Dr. Graham, I am especially indebted for his personal interest, concern, and kindness toward me during the entire study.

I am thankful to the Department of Plant and Soil Science, The University of Tennessee, Knoxville and to the United States Department of Agriculture, Agricultural Research Service, for the financial support in the form of a Graduate Research Assistantship during this study. I also express my gratitude to Dr. Douglas Crater, Head of the Department of Ornamental Horticulture and Landscape Design, The University of Tennessee, Knoxville for providing research facilities, and financial support in the completion of final stage of my degree program.

I am very grateful to Dr. Hildgard Schuller, Department of Pathobiology, Veterinary Teaching Hospital, The University of Tennessee, Knoxville, for her professional suggestions and kind cooperation during the final stages of this study. My thanks also to Delores Stephenson, Department of Pathobiology, Veterinary Teaching Hospital, The University of Tennessee, Knoxville, for the technical assistance, suggestions and initial training in the operation of transmission electron microscope.

Thanks are also extended to laboratory colleagues, graduate students, secretaries and faculty members in the Department of Plant and Soil Science and also in the Department of Ornamental Horticulture and Landscape Design for their friendship, technical and typing assistance and helpful comments during this study.

My very special heartfelt thanks goes to my best friend, Norsaadah Husain, for her lovely friendship, constant inspiration and friendly cooperation during this entire study without which the completion of this manuscript would have been a much more difficult task.

The completion of this investigation would not have been possible without the financial and emotional sacrifices, love and faith shown in me throughout my academic career by my parents and my entire family. I am deeply indebted to my family for the years of training, influence,

encouragement, trust, and complete financial and moral support for the entire period of my education which have successfully led me to this stage of life. I am deeply thankful to my wife, Manju, for her courage, sacrifices, patience, endurance and complete cooperation during the entire period of the graduate program. Finally, I am proud of my lovely daughter, Dipti, for remembering me all these years as "Nana Papa."

ABSTRACT

The ultrastructural distribution of β-glycerophosphatase (β-GPase) and potassium chloride (KCl) stimulated adenosine triphosphatase (ATPase) activity was investigated in the outer integument of a linted cultivar of cotton, Gossypium hirsutum L. cv. Hancock, and a lintless (naked seed) mutant during differentiation and elongation of cotton fiber. Occurrence of β-GPase activity was investigated from one day preanthesis to three days postanthesis while ATPase activity was studied from one day preanthesis to ten days postanthesis by using a heavy metal simultaneous capture reaction technique.

No β-GPase activity was observed in the lintless line or in any controls in the linted cultivar at any stage. In the linted cultivar there was no enzyme activity before anthesis, but as fibers were initiated on the day of anthesis, activity was observed in the expanding fiber cell wall and nucleus. As the fibers started elongating, enzyme activity was particularly concentrated in the cytoplasm and cell wall where directional growth towards the micropyle occurs. By two days postanthesis, β-GPase activity was decreasing in the cell wall and nucleus but was increasing in the nucleolus. Enzyme activity in the nucleolus was highest at three days postanthesis, but

nuclear β-GPase activity was not observed then. These results indicate that β-GPase activity was associated with differentiating fiber cells, specifically with those sites where distinct anatomical, and perhaps catabolic, changes were occurring during fiber differentiation. The enzyme activity was not a precursor to, but coincided with, these changes of fiber development.

No ATPase activity other than in mitochondria was observed in the lintless mutant. In the linted cultivar no ATP-specific enzyme activity was seen in non-elongating epidermal cells, subepidermal cells of the outer integument or any controls. As fiber initials started elongating, enzyme activity gradually appeared on the tonoplasts of enlarging vacuoles. Heavier lead phosphate deposits were observed on the membrane of small vacuoles compared to the tonoplast. This activity continued at least up to ten days postanthesis. The enzyme inhibitor, N,N-dicyclohexylcarbodiimide, inhibited while KCl stimulated tonoplast ATPase activity. The gradual increase of ATPase activity on the tonoplast of expanding fibers, but not on the tonoplast of non-fiber cells, suggest the active transport of osmotically active compounds, presumably potassium (K^+) and malate, into the vacuoles of expanding fibers. Fusion of smaller vacuoles with the large central vacuole indicates that these structures contribute

additional membrane components along with their enzyme activity to the tonoplast of expanding fibers. The occurrence of ATPase activity, of endoplasmic reticulum (ER)-derived vesicular structures, and the organized pattern of deposition of these structures on the tonoplast indicate ER-originated ATPase activity. This study supports the theory of osmoregulation in cotton fiber where ATPase provides the energy for active accumulation of osmotically active compounds (K^+, malate) into the vacuoles, thereby generating and maintaining the turgor pressure required for fiber expansion.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
SITS	4 acetamido-4'-isothiocyano 2-2'-stilbene disulfonic acid
B	Bead
β-GP	Beta glycerophosphate
β-GPase	Beta glycerophosphatase
Ca^{++}	Calcium ion
CO_2	Carbon dioxide
C	Celsius
Cl^-	Chloride ion
DCCD	N,N-Dicyclohexylcarbodiimide
DIDS	4,4'-Diisothiocyano-2,2'-stilbene disulfonic acid
EM	Electron microscopic
ER	Endoplasmic reticulum
NEM	n-ethylmaleimide
$\Delta\mu_{H^+}$	electrochemical proton gradient
F	Fiber cell
Fz	Fuzz cell
G	Golgi body
GA	Gibberellic acid
H^+	Hydrogen ion
IAA	Indoleacetic acid

$Pb(NO_3)_2$	Lead nitrate
Mg^{++}	Magnesium
$Mg(NO_3)_2$	Magnesium nitrate
M	Mitochondrion
mM	Millimolar
µm	Micrometer
NF	Non-fiber
N	Nucleus
Nu	Nucelolus
NO_3^-	Nitrate ion
PM	Plasmamembrane
K^+	Potassium ion
KCl	Potassium chloride
PEP-Case	Phosphoenolpyruvate carboxylase
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
Na^+	Sodium ion
NaF	Sodium fluoride
TMT	Trimethyltin chloride
V	Vacuole
VE	Vesicle
W	Cell wall

CHAPTER I

INTRODUCTION

The cotton plant is an indeterminant woody perennial shrub of tropical origin which has been domesticated to a temperate climate as an annual. It belongs to the family Malvaceae and genus Gossypium which contains nearly 40 species (Fryxell 1978). About 90% of all cotton grown is the tetraploid species, G. hirsutum L., upland cotton. Generally, 30-40 seeds are produced in each fruit, called a boll, which is developed on a specialized flowering branch called a sympodium (Mauney 1986).

Since ancient times, cotton has been an agronomically important crop. A cotton fiber is a product of the seed and is a hairlike unicellular trichome which in botanical nomenclature is properly designated as a hair (Esau 1977). However, it is referred to as a fiber because of its usage in agriculture and the textile industry. Also, in agricultural nomenclature mature cotton fibers which are stripped from the seeds in the ginning process are called "lint." These are the raw material for spinning cotton thread and are composed of approximately 85-97% cellulose and are usually over one inch long. After lint is removed from the seed, the remaining fibers,

which are not normally removed by ginning, are termed "fuzz" (Aiyangar 1951). These structures can be distinguished by different characteristics such as length (fuzz = 2 mm, lint = 1 inch), pigmentation, strength of adherence to the seed, time of development on the cotton ovule, and spiral convolutions (Aiyangar 1951; Berlin 1986; Joshi et al. 1967).

Because of its unique characteristics, cotton fiber is a versatile subject for the investigation of many phenomena in differentiation, elongation, secondary thickening and maturation of plant cells (Aiyangar 1951; Basra and Malik 1985; Jasdanwala et al. 1977; Ramsey and Berlin 1976a,b; Stewart 1975). It presents distinct phases of growth which are marked by specific cytological and physiological events. The fiber grows along its length for about 1000-3000 times its diameter during the elongation phase without any direct intercellular connections such as plasmodesmata, pits, or middle lamellar pectic cements except at its attachment to the ovular epidermis. These basal anchorages are the only points of access for nutrient ions, sugars, hormones, and assorted metabolically active materials upon which the developing fiber cells depend.

Some of the past and present areas of research interest in cotton ovule include (1) hormonal and nutritional regulation of early embryogenesis (Jensen 1968;

Joshi et al. 1967; Mauney 1961; Schulz and Jensen 1977), (2) fiber cell differentiation and elongation (Aiyangar 1951; Ramsey and Berlin 1976a,b), (3) biogenesis of primary and secondary cell wall (Delmer et al. 1977; Huwyler et al. 1979; Meinert and Delmer 1977; Ryser 1977; Westafer and Brown 1976; Willison and Brown 1977), (4) cotton ovule culture and protoplast culture (Beasley and Ting 1973, 1974; Bhojwani et al. 1977; Finer and Smith 1982; Stewart and Hsu 1977), (5) cellulose synthesis in cotton fiber (Meinert and Delmer 1976; O'Kelley 1953), and (6) the ultrastructure of developing fiber (Itoh 1974; Ramsey and Berlin 1976a,b; Stewart 1975). However, there is hardly any information available regarding ultrastructural histochemical localization of enzymes and their possible role at the sub-cellular level during cotton fiber differentiation and elongation. Very few research efforts have been focused towards enzyme cytochemistry of cotton fiber. Even though cotton has received attention as a leading textile fiber, progress is limited by the lack of knowledge of how certain cells of the ovular epidermis are differentiated and stimulated to elongate, how nutrient ions and organic acids are transported into the elongating fiber, and how production of specific proteins (enzymes) are regulated during

fiber development. These gaps can be bridged with available histological techniques for enzyme localization at light as well as electron microscope level.

CHAPTER II

LITERATURE REVIEW

Cotton Fiber Morphology, Origin and Development

Cotton fiber has been a subject of considerable theoretical scientific as well as practical economic importance for a long time. The fundamental work by several workers (Anderson and Kerr 1938; Balls 1915; Farr 1931, 1933; Sheffield 1936) and later work by Aiyangar (1951) established the basic knowledge of cotton fiber origin and morphology. Several points of controversy existed concerning certain aspects of the origin and development of cotton fiber, but with improvements in technology and methodology it was established that cotton fibers are single-celled protrusions from the ovule epidermis (Aiyangar 1951; Ramsey and Berlin 1976a,b; Stewart 1975).

Balls (1915, 1919) published a detailed account of the development of cotton seed tissues. He reported differences in elongation of fiber cells due to distribution of fiber initiation over several days. As a result of continued mitotic division and differentiation of ovule epidermal cells up to 10-12 days after fertilization, the number of fiber cells increased up to seven-

fold (Farr 1933; Gulati 1930; Turner 1929). In addition, Sheffield (1936) found a few well-developed stomata toward the chalazal end.

Aiyangar (1951), working with 29 varieties of cotton consisting of linted and lintless, fuzzy and naked types in wild and cultivated cotton, showed that differentiation of epidermal cells occurred at 15 hours before flowering. These cells were enlarged and the nuclei were larger compared to other epidermal cells. These cells were primordial "lint cells" which appeared first at the chalazal region and then extended towards the micropyle region. In four wild types and seven lintless types studied, he observed no hair growth when primordial cells were absent. He suggested that a stimulus for lint formation occurred long before flower opening.

Baert et al. (1975) indicated that several important things including fiber initiation were related to flowering events rather than to the fertilization process. These facts were observed in unfertilized seeds and bolls which enlarged at the same rate as the fertilized ones until three days postanthesis.

Ultrastructure of Cotton Fiber Cell

Scanning electron micrographs demonstrated the sequence of initiation of fibers from the crest of the

funiculus to the later circumference to the micropylar region. The differentiating fibers became enlarged above the ovule epidermis and continued elongating tangential to the ovule in the direction of the micropyle, indicating polarity of growth (Stewart 1975).

Cytoplasmic and nuclear changes during the differentiation and elongation phases of cotton fiber have been reported in great detail (Berlin 1986; Ramsey and Berlin 1976a,b). The most significant changes were in the size of vacuoles which first decreased in size from 16 to 3 days preanthesis. By 24 hours preanthesis, these vacuoles were filled with electron dense material while the nucleus and cytoplasm appeared to be much less electron dense compared with earlier times. Phenolic compounds within vacuoles of future cells dispersed into the cytoplasm by 16 hours preanthesis. These cells became "dark cells" due to their cytoplasmic osmophilic density while adjoining epidermal cells retained phenolics in their vacuoles and appeared to be "light cells." At anthesis, elongation of fibers accompanied the formation of large central vacuoles and migration of nuclei into the centers of extending cells. At 2 days postanthesis fiber cells had numerous lipid bodies, small vacuoles, plastids, mitochondria, ribosomes, endoplasmic reticulum and dictyosomes were shown to be involved in the formation of

cell wall and plasmamembrane throughout the fiber length and not just at the fiber tip. These findings were confirmed by Westafer and Brown (1976) who suggested Golgi apparatus to be involved in the secretion and synthesis of primary well wall while endoplasmic reticulum and plasmamembrane were involved in the synthesis and secretion of secondary wall formation.

Elongation of fibers continues for 15-20 days postanthesis depending on variety and cultural condition (Anderson and Kerr 1938; Schubert et al. 1973; Westafer and Brown 1976). During the elongation phase the fiber cell makes only a thin (0.1-0.5 µm) primary cell wall which has a relatively low cellulose content (O'Kelley 1953; Huwyler et al. 1979) and constantly changing composition (Meinert and Delmer 1976). Westafer and Brown (1976) reported that secondary wall (8 µm) synthesis begins around 20 days postanthesis, but others (Meinert and Delmer 1976; Schubert et al. 1973) found that secondary deposition begins earlier. Autoradiography in elongating cotton fiber cell wall showed labeling all along the fiber length. From this study Ryser (1977) concluded that cotton fibers grew throughout their entire length but that growth was more intense at the tip region.

Hormonal Influence on Fiber Development and Ion Transport

Recent work indicated that gibberellins and auxin are important factors for fiber development (Beasley and Ting 1973, 1974; DeLanghe et al. 1978; Kosmidou 1976). Cotton fiber differentiation was shown to take place before anthesis (Ramsey and Berlin 1976a). This indicated that the differentiation phenomenon was not dependent on fertilization but was related to the hormonal influence present before fertilization. In vitro studies indicated that GA played an important stimulating role but only during a short period after anthesis; whereas, auxin was needed for the entire culture period (Beasley et al. 1974). According to Kosmidou (1976), GA had a more significant influence on fiber initiation than elongation. The presence of auxin in addition to gibberellins promoted normal initiation and elongation of fibers. Auxin alone resulted in a lower number of fiber initials than when gibberellins were present, but subsequent elongation of these initials was greater (Kosmidou 1976). Relatively short exposure (24 hours) to GA alone followed by GA plus IAA gave normal production of fibers. Comparatively longer exposure to GA from 24, 48 to 72 hours preculture resulted in decreased numbers of fibers, suggesting the

necessity of both the hormones during the early stages of fiber growth (Beasley et al. 1974).

Gibberellins and auxin induced, while abscisic acid inhibited fiber elongation (Baert et al. 1975; Beasley 1973; Beasley and Ting 1973, 1974; Bhardwaj and Sharma 1971; DeLanghe et al. 1978; Dhindsa et al. 1976; Kosmidou 1976; Singh and Singh 1975). Bhardwaj and Lad (1977) showed that seeds of a lintless mutant were deficient both in auxin and gibberellins while inhibitors in the mutant pericarp were present in higher amounts than in the linted varieties. Their results indicated that genotypic differences in fiber length were related to the levels of endogenous hormones present in the seeds. Phenolic compounds found in the dark cells during fiber differentiation (Ramsey and Berlin 1976a) were not detected in ovules of the naked seed type (Popova et al. 1979). Also, large amounts of phenolic compounds were synthesized in ovules producing more intense fiber formation and elongation. Naithani et al. (1981) reported increase of O-diphenol oxidase activity during fiber differentiation. Jasdanwala et al. (1977) showed that IAA-catabolism was low during the elongation phase, but IAA-oxidase activity increased about four times during the secondary wall thickening phase. These results emphasize the importance of auxin during fiber

elongation. Arnison (1980) proposed a hypothesis that phenolics may be acting as "auxin protectors" while peroxidase is acting as "auxin destructor."

During the early stages of fiber development, there were spectacular changes in the size of the nucleus and nucleolus, which suggested increased capacity for ribosome synthesis (Kosmidou 1976; Ramsey and Berlin 1976a,b). Nucleolar vacuolation has been reported in cotton fiber (Ramsey and Berlin 1976a,b; Vlasova 1971), as well as in other plant tissue (Barlow 1970; Galan-Cano et al. 1975; Moreno-Diaz et al. 1980; Rose et al. 1972). An increase in nucleolar material and fiber initiation was associated with the stimulus caused by pollination (Kosmidou 1976). Fuzz fiber initials (4-12 days post-anthesis) did not show any nucleolar vacuoles and had small nuclei and nucleoli (Kosmidou 1976; Vlasova 1971).

According to Cleland (1971), the rate of cell elongation is controlled by turgor pressure or wall extensibility. Expansion or elongation of cell walls in higher plants is considered to be a dual phase which requires (i) indoleacetic acid for softening and imparting stretchability to the juvenile wall and (ii) an increase in turgor pressure within the cell by accumulation of solutes in the cell sap, especially within vacuoles (Cleland 1971, 1977). Cell enlargement was

shown to be an active process that required respiration and continuous synthesis of RNA and protein. The rate of cell enlargement also was shown to be regulated in higher plants by auxin (Cleland 1971). Several plant species have been examined where auxin caused an increase in cell wall extensibility (Coartney and Morré 1980; Lockhart 1967). The rate of cell wall extension depended on the rate of biochemical wall loosening and the amount of physical extension (irreversible viscoelastic extension) which occurred as a result of each wall loosening event (Cleland 1981). This wall loosening event may result from an auxin-induced modification of the wall already present, either by (a) breakage of H-bond by the wall solution becoming acidic (Keegstra et al. 1973), or by release of calcium cross-links between pectin chains (Tagawa and Bonner 1957), (b) by non-enzymatic cleavage of wall polysaccharides at pH 4 or below (Rayle and Cleland 1970) or (c) by enzymatically mediated processes (Cleland and Rayle 1972; Fan and Maclachlan 1966). Even though the exact mechanism of auxin action is unclear, the relationship between auxin and cell elongation was demonstrated where auxin stimulated protein synthesis and RNA production by derepressing certain genes, the expression of which was necessary for the extension growth (Key 1964, 1969).

Plant cell walls are subjected to change during cell enlargement. In addition to the effects presented in the earlier section, IAA also affects the structure and viscosity of plant cell membranes (Helgerson et al. 1976; Morré and Bracker 1976) and interacts with ion transport in plant tissue. Hager et al. (1971) postulated the existence of an IAA-regulated ATP-dependent proton extrusion mechanism where IAA acts as an activator of a proton pump localized in the cell membrane. Morré and Bracker (1976) reported that auxin, through its effect on cell membrane, interacts with the ion transport mechanism. Haschke and Luttge (1975a,b; 1977a,b) showed that the counter ion of the H^+ pump and the regulation of intercellular pH during continuous H^+ extrusion was maintained by ionic charge balance during IAA-stimulated extension growth. Potassium was taken up during H^+ release and malic acid was synthesized by dark fixation of CO_2. Dymock et al. (1977) reported an in vivo study where IAA-stimulated H^+ efflux indirectly led to increased malic acid synthesis due to the alkaline pH optimum (pH 8.0) for PEP-carboxylase. Thus, enhanced acid synthesis during IAA-stimulated H^+-K^+ exchange provided both protons to be extruded and organic acid anions to balance the charge of K^+ taken up without the intracellular H^+ concentration being changed.

Presence of Stomata and Osmoregulation

It is generally assumed that stomata are not found in areas away from the external atmosphere and light. However, the presence of stomata on cotton ovules was reported by several authors in the past without any detailed description about structure or any physiological functional relationship to fiber development (Barritt 1929; Rao 1923; Sheffield 1936). Ayyangar (1948) reported interesting observations on their structural development and functional relationship in different varieties of cotton from 2 days pre-anthesis to 20 days postanthesis. Interestingly, stomata on the cotton ovule surface up to 20 days postanthesis were found to be increasing in number, living, active and capable of functioning. Stewart (1975) confirmed these observations with scanning electron microscopy and extended them to show functional stomata as early as seven days before anthesis.

In general, potassium and malate accounted for much of the osmotically active solute in vacuolar sap (Allaway 1973; Dayanandan and Kaufman 1975; Grandmann 1977; Haschke and Luttge 1975a,b; Hsiao 1976). McCall and Guthrie (1945) showed that cotton fiber contains substantial quantities of organic acids with malate being

the most prominent. This finding raised the possibility that fibers are under constant high turgor pressure. In addition, elongating fibers were shown to be the second largest sink for K^+ (Leffler 1986). Subsequent kinetic and osmoregulation studies of cotton fiber extension showed that the elongation of fiber was dependent on turgor pressure. The levels of potassium and malate in fiber cells rose and fell in relation to growth rate and reached a peak during the time when growth rate was maximum. Fiber growth was adversely affected by the absence of both atmospheric CO_2 and potassium (Dhindsa et al. 1975). Ovules did not produce fibers if cultured in a medium containing less than 0.5 mM potassium.

The occurrence of PEP-carboxylase in cotton fiber and its increased activity due to GA_3 was reported by Dhindsa (1978). Recently, interesting results concerning dark metabolism of CO_2 during fiber elongation of two cottons differing in fiber length have been presented. Potassium and malate levels rose during rapid extension growth then fell as the rate of elongation decreased. The concentration of enzymes, potassium and malate were higher in the fibers of a long staple cultivar than a short staple cultivar (Basra and Malik 1983).

Active Transport, ATPase and Extension

Potassium is the most mobile element in the plant and is present as free ions. It serves at least two roles: (a) an osmotic constituent of cell sap and (b) an activator or co-factor of many enzymes. Potassium has been shown to be effective for many different enzymes and there seems to be some correlation between the high internal potassium concentration in cells and maximal enzyme activity (Evans and Sorger 1966). Potassium plays an important role in the transport of carbohydrate from leaves to different parts of plant (Ashley and Goodson 1972). In field experiments, addition of potassium fertilizer increased both fiber length and cell wall development (Bennett et al. 1965; Bennett et al. 1967). Elongating fibers are the second largest sink for potassium (Leffler 1986), and potassium deficiency in cultured ovules caused shorter and less developed fibers (Dhindsa et al. 1975). The level of potassium and its accumulation in developing fibers was discussed earlier in this review.

Vacuoles were initially considered as compartments for intracellular excretion or, at best, as storage compartments. Later it was realized that they have an important function in cell physiology as lytic

compartments equivalent to animal lysosomes (Matile 1968). Vacuoles, being the largest membrane bound organelles in plant cells, also serve as the main organ for generating water potential deficit (turgor) and is the major storage compartment for sugars, amino acids, proteins, vitamins and organic acids (Uribe and Luttge 1984). Since the review of Matile (1978), great research interest developed around plant vacuoles, including studies on the structural and analytical characterization of vacuolar membrane (Marty 1985; Salyaev 1985); the transport mechanism across tonoplast (Uribe and Luttge 1984; Wagner and Lin 1982); isolation, properties and function of tonoplast bound ATPase activity in proton-pumps (Lin and Wagner 1985; Marin 1985; Smith et al. 1984a,b; Walker and Leigh 1981); proton pump activity and tonoplast energization (Bennett 1985; Gidrol et al. 1985; Thom and Komor 1984); and the role of tonoplast ATPase in compartmentation of solutes (Chrestin et al. 1985; Luttge and Smith 1985; Marin and Chrestin 1985).

Several functions have been attributed to the accumulation of malic acid in the vacuole of higher plants including osmotic adjustment in the cell (Cram 1976); shrinking and swelling of cells in turgor-dependent movement as in stomata (Raschke 1979); extension growth associated with the acid-growth mechanism

(Haschke and Luttge 1977a,b); and plant nutrition as in the surplus uptake of cations over anions (Torri and Laties 1966). Plants balance the charge resulting from surplus cation uptake by the synthesis of organic acids and the exchange of H^+ for the cation taken up. Plant tissue can accumulate stochiometric amounts of organic anions when cation uptake exceeds anion uptake. The level of organic acid anions may decrease if more anions are taken up than cations (Luttge and Higinbotham 1979). The relationship between acid synthesis as a result of dark fixation of CO_2 and surplus cation uptake was explained by H^+ (proton) release. With K^+-H^+ exchange at the membrane system, no organic acid anions were removed from the reaction equilibrium in the cytoplasmic phase. This could occur only when the anion (malate) accompanied the cation (K^+) into the vacuole (Torri and Laties 1966). With pulse-chase experiments Osmond and Laties (1969) observed decreased specific activity of malate when no cation uptake occurred. But with high cation uptake and low anion uptake, the radioactivity of the labeled malate remained high for a longer time, that is, the turnover of malate was decreased.

ATP is the general energy currency of the cell. Despite compartmentation, ATP is available at all the sites and locations within the cell wherever it is

needed. The enzymes concerned with active transport of potassium and other substances have been shown to be present in the membrane system (example ATPase). They hydrolyzed ATP and the energy released from this reaction could drive an active transport pump of K^+-H^+ (Luttge and Higinbotham 1979; Uribe and Luttge 1984).

The review of Hodges (1976) discussed several examples of isolation and functional role of ATPase associated with membranes which were involved in ion transport. These ATPases were activated by alkali ions, by Mg^{++} ions and to a lesser extent by Ca^{++} ions.

The role of ATPase as a proton-pump in establishing a proton-electrochemical gradient across the membrane and the coupling of solute transport with the proton electrochemical gradient has been established (Chrestin et al. 1985; Gidrol et al. 1985; Marin and Chrestin 1985). Tonoplast ATPase has been reported in all vacuole preparations, and its properties have been characterized (Leigh and Walker 1985; Marin 1985). Information on tonoplasts and their ATPase has been obtained either from mechanical isolation of vacuoles (Leigh and Branton 1976; Matile 1978) or by enzymatic isolation of protoplast (Cocking 1960; Cocking and Gregory 1963). Some of the most important characteristics of tonoplast ATPases which differentiated them from other plant membrane

ATPases were insensitivity to vanadate, stimulation by Cl^-, inhibition by NO_3^- and N,N-dicyclohexylcarbodiimide, and a slightly alkaline pH optimum in the range of pH 7 to 8. By using microelectrodes and chemical probes, Bennett and Spanswick (1983, 1984) showed that the vacuolar ATPase pumped protons into the vacuoles, thus establishing an electrochemical proton gradient, $\Delta\mu_H^+$, across the tonoplast. The vaculor ATPase indirectly regulated solute transport across the tonoplast by coupling transport with the $\Delta\mu_H^+$ established (Marin 1985). Some other examples of regulation by ATPase include cytoplasmic pH regulation in latex of Hevea brasiliensis (Chrestin et al. 1985), cytoplasmic Ca^{++} regulation (Chrestin et al. 1985; Hager and Helmle 1981; Hager and Hermsdorf 1981), sugar accumulation in sugarcane (Komor and Thom 1985), and crassulacean acid metabolism (Luttge and Smith 1985).

Different membrane bound ATPase could be distinguished with the help of inhibitors. The plasmamembrane enzyme was shown to be sensitive to vanadate (Bowman et al. 1978; Dufour et al. 1980) and the mitochondrial enzyme to azide and oligomycin (Bowman et al. 1978; Delhez et al. 1977), but the tonoplast bound activity was unaffected by these inhibitors. It was inhibited by nitrate, DIDS (4,4'-diisothiocyano-2,2'-

stilbene disulfonic acid), DCCD (N,N'-dicyclohexyl-carbodiimide), NEM (n-ethylmaleimide), SITS (4-acetamido-4'-isothiocyano-2,2'-stilbene disulfonic acid), and TMT (trimethyltin chloride (Bennett et al. 1984; Gidrol et al. 1984; Marin 1983).

Kinetic studies indicated Mg-ATP complex as the true substrate for tonoplast bound ATPase for Saccharum (Thom and Komor 1984) and Hevea (Marin 1985). Different optimum pH values such as pH 6.0 for Hippeastrum, pH 6.5 for Saccharum, pH 6.5-8.0 for Beta vulgaris, pH 6.8-7.5 for Hevea and pH 8.0 for Kalanchoe daigremontianum have been reported (Marin 1985). Wagner and Mulready (1983) demonstrated ATPase enzyme as an integral membrane protein. Difficulties in recognizing the different fluxes of ionized molecules across tonoplast have been reported. The translocators present at the tonoplast level may act as an H^+-antiport system, e.g. amino acids and Ca^{++} (Ohsumi and Anraku 1981, 1983) or H^+-symport system, e.g. malate (Luttge and Ball 1979).

Cytochemical Localization of Beta-Glycerophosphatase and ATPase Enzymes

Ultrastructural cytochemical localization of enzymes within a tissue not only establishes the structural relationship but also allows correlation of the

the possible physiological role of that tissue with general metabolism or with specific developmental phenomena. There are several reports on the application of enzyme histochemistry which has been utilized as a tool to study the process of differentiation and certain developmental phenomena (Avers 1958; Avers and Grim 1959; Cutter and Feldman 1970; Czernik and Avers 1964; Dosier and Riopel 1977; Mia and Pathak 1968; Onofeghara 1972). There are also numerous reports on the use of enzyme histochemistry to demonstrate the nature of transport processes in plant as well as animal tissue (Bentwood and Cronshaw 1978; Eleftheriou and Hall 1983; Firth 1978; Gilder and Cronshaw 1974; Hall and Butt 1969; Hall et al. 1980; Mogensen 1981; Price and Whitecross 1983; Van Steveninck 1979).

The epidermal tissue in several plant tissue species has provided a very useful site for study of tissue differentiation which can be studied with the help of histochemical work at the light and electron microscope level. Acid phosphatase has been shown to play an important role in differentiating plant tissue; however, the exact mechanism or physiological role is not fully understood (Gahan 1965; Wardrop 1968). Wilson and Cutter (1955) reported the presence of acid phosphatase in differentiating tissue of Cocos nucifera. While conducting

a histochemical survey of enzymatic distribution in differentiating cells of timothy grass roots, it was demonstrated that acid phosphatase was more active in the recently formed trichoblast cells that eventually formed root hairs (Avers 1958). Similarly, a distinct difference in the pattern of acid phosphatase distribution was shown in festucoid and panicoid grasses where high enzyme activity was noticed in trichoblasts just prior to and during root hair formation, while no activity was observed for hairless cells in the same region (Avers and Grimm 1959). Demonstration of differential phosphatase activity was also reported during trichoblast differentiation in Elodea canadensis (Dosier and Riopel 1977).

In addition to the involvement of acid phosphatase in tissue differentiation processes, there are several reports showing acid phosphatase activity in cell wall metabolism (DeJong et al. 1967a; Hall 1969; Hall and Butt 1968; Hall and Davie 1971; Halperin 1969; Maier and Maier 1972; Sexton et al. 1971). Previous studies have also reported the histochemical localization of phosphatase enzymes in the nucleus, nucleolus and at particulate sites in the cytoplasm in a wide range of plant tissue (Avers 1958; Gahan 1965; Gahan and Maple 1966; Hall 1969; Jensen 1956; Sexton and Sutcliffe 1969; Sexton et al. 1971).

Localization of β-glycerophosphatase, ATPase and other phosphatases centered around Gomori's (1952) heavy metal simultaneous capture reaction technique. Several modifications in the original technique have been suggested by different research workers on different tissues in plants (Avers 1961; Bitensky 1963; Jensen 1956). After several contradictions the validity of the technique finally was established (Barka and Anderson 1962; Hall and Butt 1969; Hall et al. 1980). The previous studies indicated that the size of experimental sample; time, temperature, concentration, osmolarity, pH, and types of fixative and buffer used; time, temperature and pH of enzyme reaction media used; use of different substrates; diffusion of reaction products; use of proper controls; and use of genetic mutants were some of the critical factors in enzyme cytochemistry (Glauert 1977; Hall 1978; Hayat 1981a,b). Within an enzymatic reaction procedure, use of proper salts, substrate and enzyme inhibitor; proper temperature and pH; freshly prepared reaction media; time of incubation; ratio of buffer to substrate; ratio of ATP/Pb^{++}; and continuity and identical conditions for the experimental treatments are critical factors to obtain valid histochemical results (Gomori 1950; Hall 1978; Hall and Butt 1969; Hall et al. 1980; Hugon 1973; Pearse 1972; Sexton and Sutcliffe 1969; Van Duijn 1973).

CHAPTER III

BETA-GLYCEROPHOSPHATASE ACTIVITY DURING FIBER DIFFERENTIATION

Introduction

Enzyme cytochemical techniques have been used as a tool to investigate differentiation. Numerous reports regarding the occurrence of acid phosphatase or β-glycerophosphatase (β-GPase) within differentiating tissue have been published for plant as well as animal tissue (Barka and Anderson 1962; Jensen 1956; Gomori 1950; Halperin 1969; Hall and Butt 1967; Love et al. 1969; Soriano and Love 1971; Waters and Butcher 1980).

Cotton fiber serves as a model system where distinct phases of growth are marked by specific cytological and physiological events. Since cotton fiber differentiation and its subsequent development show unique morphological, cytological and physiological changes, an ultrastructural study was conducted to locate β-GPase enzyme activity in a linted cultivar and a lintless mutant line. The results obtained for the occurrence of β-GPase activity during differentiation and early elongation are presented and compared with morphological events. The

results are discussed in relation to fiber cell wall metabolism and to the nucleus and nucleolus in the differentiation process.

Materials and Methods

Tissue Source and Preparation

A linted cotton, Gossypium hirsutum L. cv. Hancock, and a lintless mutant line, 9SOxHG, were grown in a glasshouse in pots containing a sand-vermiculite mixture and fertilized with a complete nutrient solution once a week. Flowers at anthesis were labeled daily for 3 days, then samples for 5 stages of fiber development were all collected on the fourth day. The sample stages were 1-day preanthesis, anthesis, 1-, 2-, and 3-days postanthesis (-1, 0, +1, +2, +3, respectively).

Ovules were excised while the flowers were attached to the plants and immediately fixed in 1.5% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.2, and kept in crushed ice (Ramsey and Berlin 1976a,b; Glauert 1977; Hall 1978). Vacuum was applied to the cold samples to facilitate rapid penetration of fixative (Glauert 1977; Hall 1978). Ovules were dissected in fixative to remove the outer integument, which is 6-7 cells thick with an average total thickness of 60-70 μm. Only the integuments were used thereafter. After 4 hours fixation, the samples

were washed overnight in 0.1 M sodium cacodylate buffer, pH 7.2, at 4°C, rinsed with 0.1 M tris-maleate buffer at pH 6.0 and advanced to the enzyme reaction step.

Enzyme Reaction Procedure

The reaction procedure was a modification of that reported by Barka and Anderson (1962) (Appendix A). Briefly, the endogenous enzyme dephosphorylates a suitable substrate to give a free phosphate group. Lead, which is included in the reaction medium as a soluble salt, captures the free phosphate, and they co-precipitate as insoluble, electron dense lead phosphate. Enzyme location is inferred by the location of the lead phosphate deposits. Incubation in complete reaction medium was conducted at 36-37°C for 1 hour. Controls included samples (1) incubated in reaction medium minus substrate; (2) pretreated with 0.1 M NaF for 5 minutes, then incubated with complete medium plus NaF; (3) without the incubation step. Tissues at each of the five stages of fiber development, with controls, were incubated in the identical, freshly prepared reaction medium, with the additions or deletions indicated.

Four compounds commonly used to detect "acid phosphatase" were individually tested as substrate in the reaction medium (Halperin 1969). The substrates, in order of decreasing reaction product, were β-GP, glucose-6-phosphate, adenosine monophosphate and p-nitrophenyl

phosphate. β-GP gave the strongest reaction; therefore, it was used as the standard substrate. Cotton tissue samples were reacted at pH 5.0, 5.5 and 6.0. Very little deposition was observed in cells incubated at pH 5.0 while tissue incubated at pH 6.0 showed intense deposition of reaction product. All subsequent reactions were conducted at pH 6.0 (Pearse 1972). Inasmuch as β-GP was the most reactive substrate tested and greater reaction occurred at pH 6.0 than 5.0, the enzyme activity is referred to as β-glycerophosphatase rather than the more general "acid phosphatase."

The cytochemical localization of enzyme activity always involves questions about specificity and artifact. In these experiments, as many variables as possible were optimized and standardized, including condition and source of ovules, vacuum infiltration of fixative and reaction medium, and time, temperature, and pH of fixation and reaction. The question of non-specific staining due to osmium tetroxide in the nucleus and nucleolus was verified by processing samples as above but without post-osmication with osmium tetroxide. Finally, controls were included to address the possibility of non-specific deposition of lead in the tissue (DeJong et al. 1967a; Gahan 1965).

Sample Preparation for EM

After incubation, all samples were rinsed with 0.1 M sym-collidine buffer at pH 7.2, then post-fixed at 4°C with 1% osmium tetroxide in the same buffer. Following an acetone series dehydration, the samples were embedded in a low viscosity plastic medium (Spurr 1969) in flat rubber molds. Since Stewart (1975) showed that fiber initials have directional growth, an effort was made to orient the outer integument such that longitudinal sections could be obtained parallel to the direction of growth. Silver-gray sections were obtained with an LKB-8800 ultramicrotome equipped with a diamond knife. Sections were retrieved on uncoated copper grids and post-stained with aqueous uranyl acetate only or with uranyl acetate followed by lead citrate (Reynolds 1963). Sections were observed and photographed with a Phillips-201 transmission electron microscope. Although conditions were controlled as closely as possible, some quality of ultrastructure was sacrificed due to the short fixation time and incubation procedure.

Results

The cytochemical localization of β-GPase activity in the linted cultivar was compared among differentiating fibers, non-fiber cells and cells of the outer integument.

The results obtained were verified by comparison with different controls and with a genetically lintless mutant. The results are presented for one day preanthesis to three day postanthesis.

Preanthesis

A portion of the epidermal layer of a -1 day ovule, after incubation in the reaction medium, is shown in Figure 1. Protrusion of the fiber initials had not yet begun, and the cells were relatively smaller compared to later stages. Nuclei were centrally located, and there were many small vacuoles. There was no indication of β-GPase activity. A region of this section is magnified in Figure 2 to show greater detail of the ultrastructure. Increased magnification revealed no fine particles of lead phosphate, although structural components of the cell could be seen external to the plasmalemma and embedded in the matrix of the cell wall. These may become sites of β-GPase activity as later results will indicate.

Anthesis

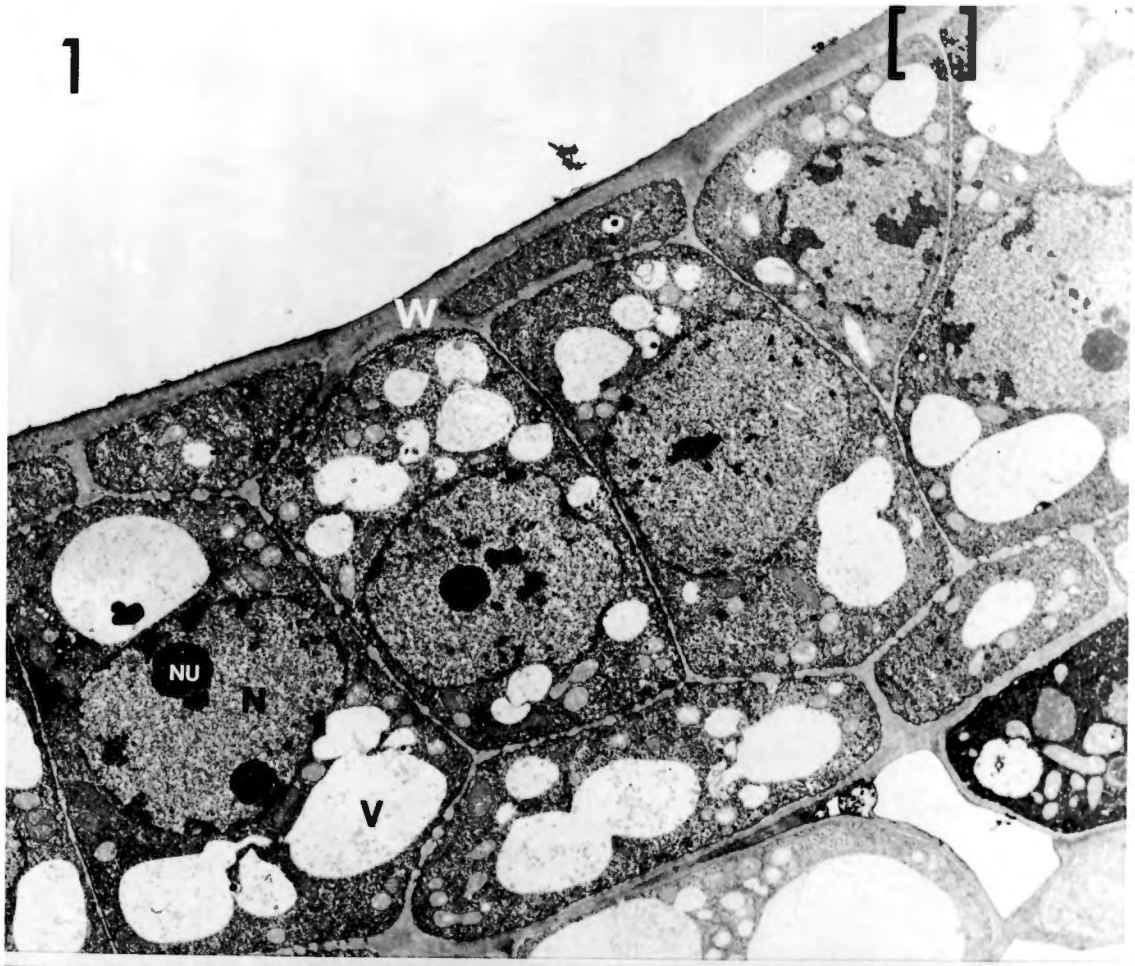
Lead phosphate deposits were first evident the day of anthesis when the fiber initial cells began to protrude above the epidermal layer. Most enzyme activity was

Figure 1.[a] One day preanthesis. Longitudinal oblique section of outer integument showing undifferentiated epidermal cells with small nucleolus and many small vacuoles. Absence of lead phosphate deposit indicates no β-GPase activity. β-GP incubated, posstained with UA and LC. x 5,900.

Figure 2. One day preanthesis. Enlargement of cell wall outlined in brackets in Figure 1. Higher magnification reveals no fine deposits of lead phosphate. Structures external to the plasmalemma (arrows) correspond to sites of enzyme activity observed in fiber initials at anthesis. x 45,000.

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[a]Legend for figures are as follows: Transmission electron micrographs of outer integument with differentiating and elongating fibers of cotton ovules, longitudinal and transverse sections with β-GPase and ATPase activity localized as electron dense lead phosphate deposits. --B beads; W cell wall; ER endoplasmic reticulum; F fiber initial; FB fat body; FZ fuzz fiber cell; G Golgi body; M mitochondrion; NF non-fiber cell; Nu nucleolus; PM plasmamembrane; V vacuole; VE vesicle; UA uranyl acetate; LC lead citrate; L.S. longitudinal section; T tonoplast; T.S. transverse section.


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Figures 1 and 2
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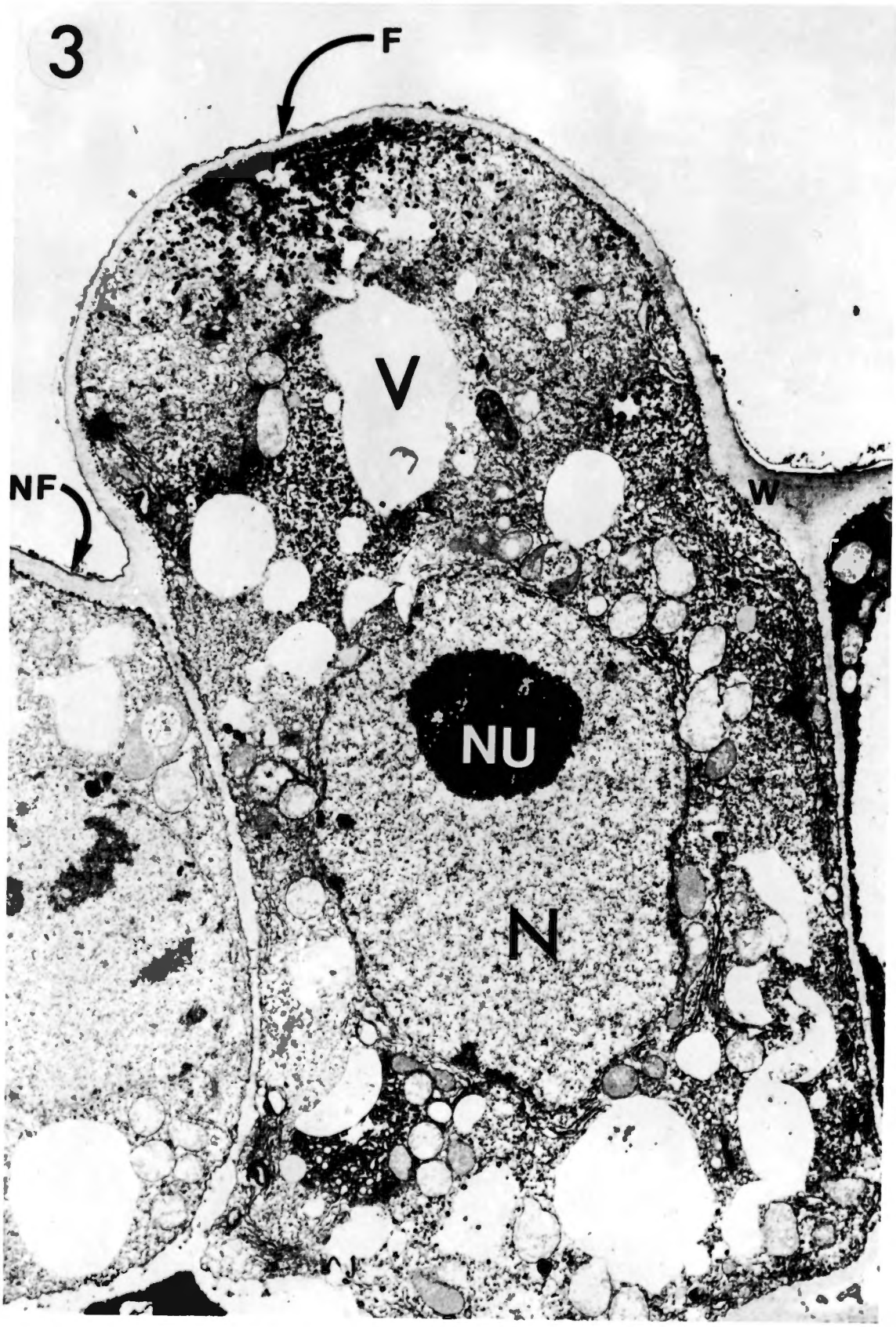
associated with the distal cytoplasm of fiber initials Figures 3, 4 and 5) and with the cell walls both of the initials (Figures 5, 6 and 7) and the newly divided epidermal cells (Figure 8). Other epidermal cells showed only minor activity.

The results at anthesis consistently showed that enzyme activity in the cytoplasm was concentrated in the expanding portion of the cell (Figures 3 and 4). Therefore, there appeared to be a relationship between β-GPase activity and directional growth of the fiber initial toward the micropylar end of the ovule (Figures 4 and 5). Deposits in the cytoplasm were associated with the endoplasmic reticulum and its related vesicles, but enzyme activity was not uniformly distributed among these (Figures 6 and 7). Occasional deposition of reaction product was observed in some vacuoles (Figures 4 and 7), but this was not consistent among fiber initials.

β-GPase activity in the cell wall of the fiber initial was predominantly in the interior wall, immediately external to the plasmalemma, but some precipitate occurred throughout the cell wall (Figure 6). The major activity corresponded to that region previously mentioned and illustrated in Figure 2.

Figure 3. Anthesis. L. S. of fiber initial with centrally located large nucleus and nucleolus, and many small vacuoles. Lead phosphate deposits predominantly in fiber initial. Note particulate deposits near expanding tip region. β-GP incubated, and poststained with UA and LC. x 10,000

NOTE: For legend see footnote page 31.


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Figure 3
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Figure 4. Anthesis. Portion of differentiating fiber initial with heavy lead phosphate deposits concentrated in expanding tip region. Note the association of lead phosphate deposits with ER and in vacuole. β-GP incubated, poststained with UA and LC. x 8,300

Figure 5. Anthesis. Longitudinal section of fiber initial with lead phosphate deposits in expanding cell wall, especially in the direction of fiber growth towards micropylar region (arrows). β-GP incubated, poststained with UA only. x 6,900

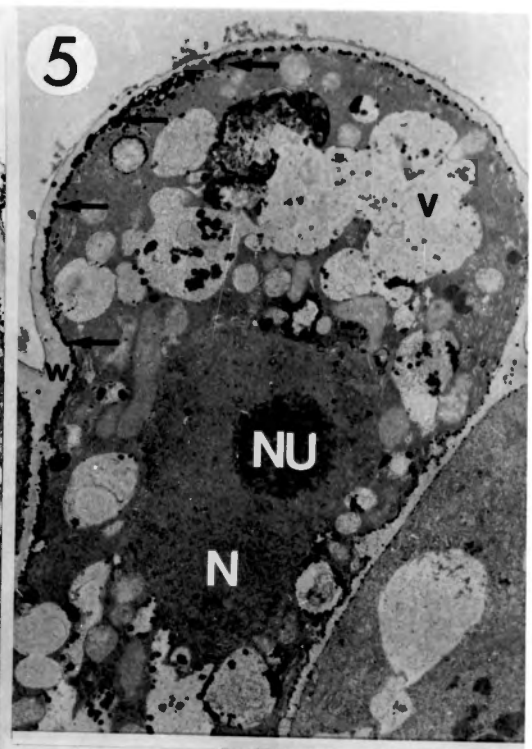
Figure 6. Anthesis. Tip of fiber initial showing lead phosphate deposits associated with ER, vesicles, and cell wall. β-GP incubated, poststained with UA only. x 58,000

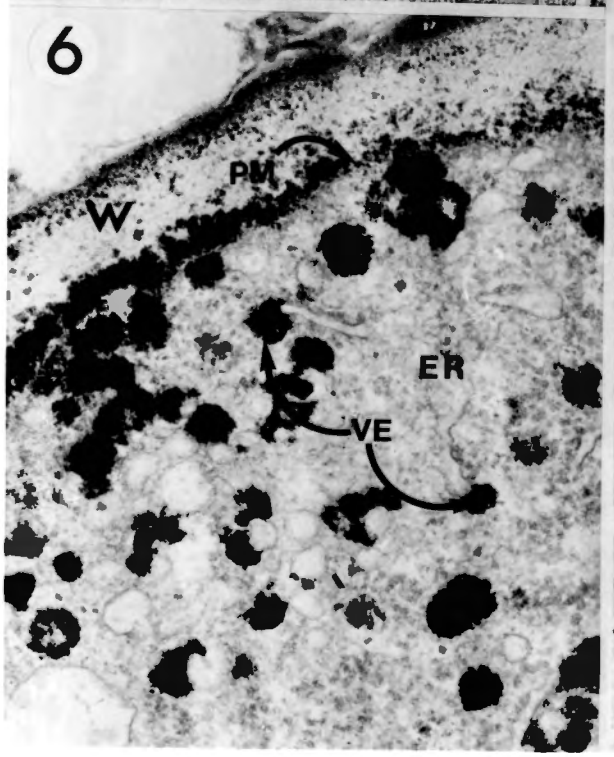
Figure 7. Anthesis. Magnified tip portion of fiber initial from Figure 4, showing occurrence of lead phosphate deposits predominantly with ER, vacuole, and vesicles. x 15,000

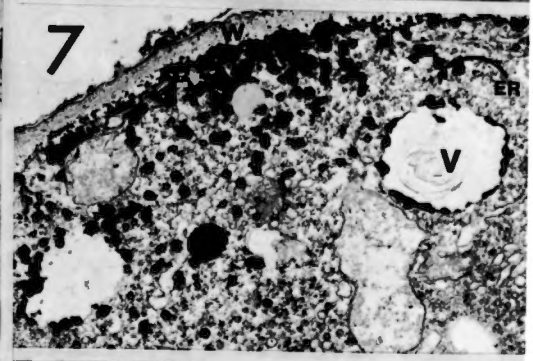
Figure 8. Anthesis. β-GPase activity in cell walls of newly divided epidermal cells. Activity in the crosswall indicated adequate penetration of fixative and reaction mixture within tissue. Sample β-GP incubated, poststained with UA only. x 14,600

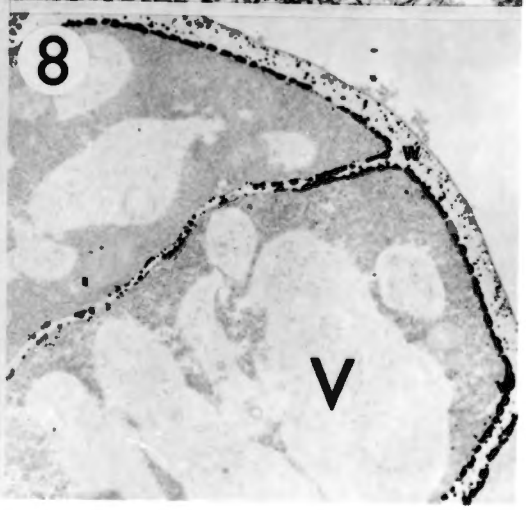
NOTE: For legend see footnote page 31.


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Figures 4, 5, 6, 7 and 8
Joshi, 1987

Fidelity of lead phosphate deposition at the sites of enzyme activity was verified by the controls. The inhibitor, NaF eliminated almost all activity (Figure 9), and there was no nonspecific deposition of lead when samples were processed without the incubation step (Figure 10), or when β-GP was excluded from the incubation medium (Figure 11).

One Day Postanthesis

By one day postanthesis, the fiber initials were expanded to their approximate final diameter, and the nuclei had enlarged and migrated into the expanding portion of the cells. The many small vacuoles began coalescing to form the large central vacuole. Coincident with these anatomical changes, there were changes in localization sites of β-GPase. Activity was generally distributed along the expanding portion of the cell (Figure 12). As in the previous stage, reaction product was heavily distributed in wall regions immediately external to the plasmalemma and more lightly distributed throughout the remaining wall (Figure 13).

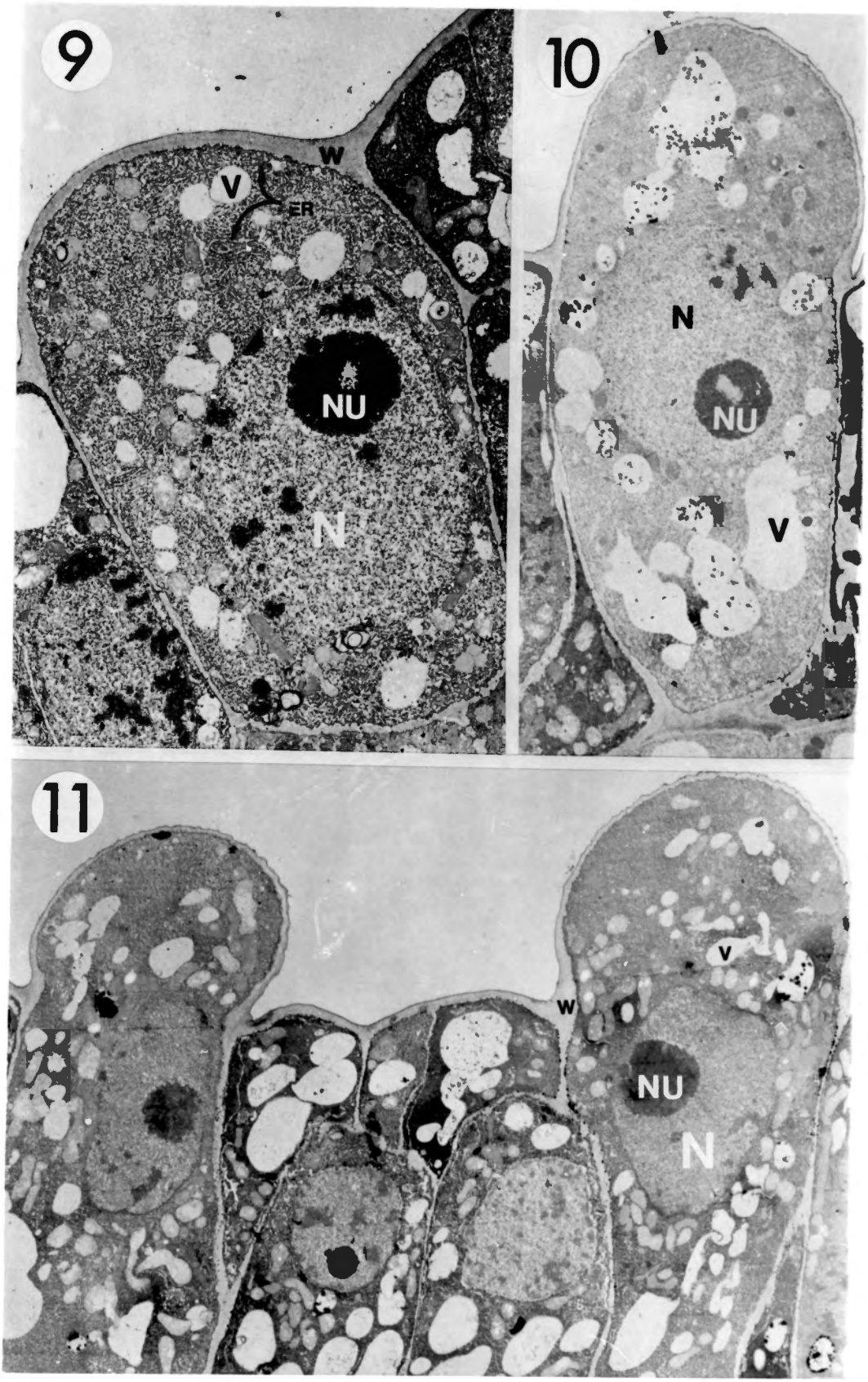
The most noticeable change in the distribution of the lead phosphate precipitate was its appearance in the nucleus and nucleolus of the fiber initials (Figure 12). Enzyme activity was scattered throughout the nucleus, but

Figure 9. Anthesis. Inhibitor control. Longitudinal section of fiber initial showing no deposits of lead phosphate in the sample, pretreated with enzyme inhibitor NaF and then incubated with enzyme inhibitor and β-GP. Poststained with UA and LC. x 4,5000

Figure 10. Anthesis. No incubation procedure control. Longitudinal section of fiber initial showing no lead phosphate deposits. Poststained with UA and LC. x 4,000

Figure 11. Anthesis. Substrate control. Longitudinal section of fiber initials shows no β-GPase activity in the sample incubated without the substrate β-GP. Poststained with UA and LC. x 4,000

NOTE: For legend see footnote on page 31.


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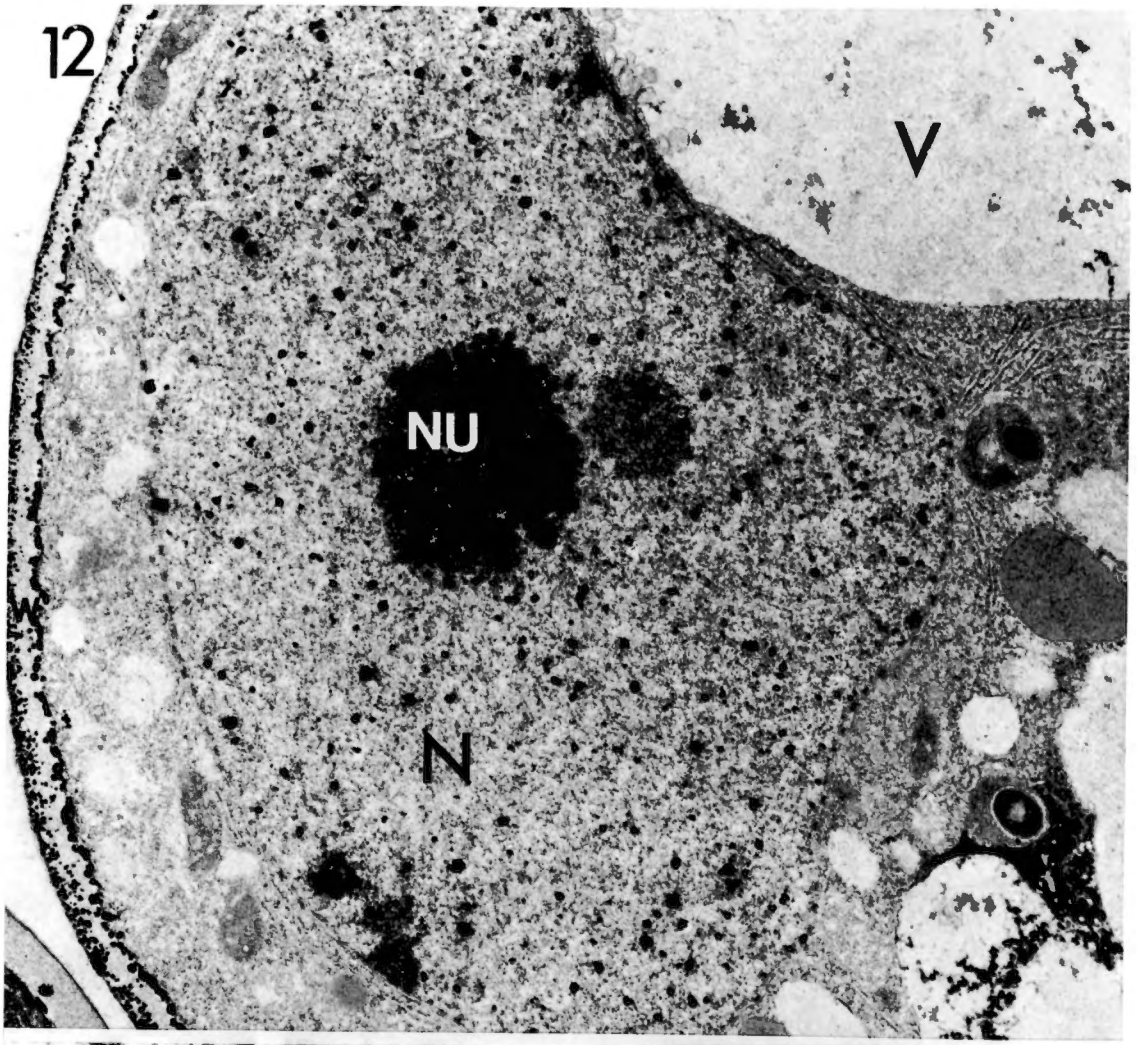
40
Figures 9, 10 and 11
Joshi, 1987

Figure 12. One day postanthesis. Portion of fiber showing β-GPase activity in the cell wall, nucleus, and nucleolus. β-GP incubated, poststained with UA and LC. x 16,000

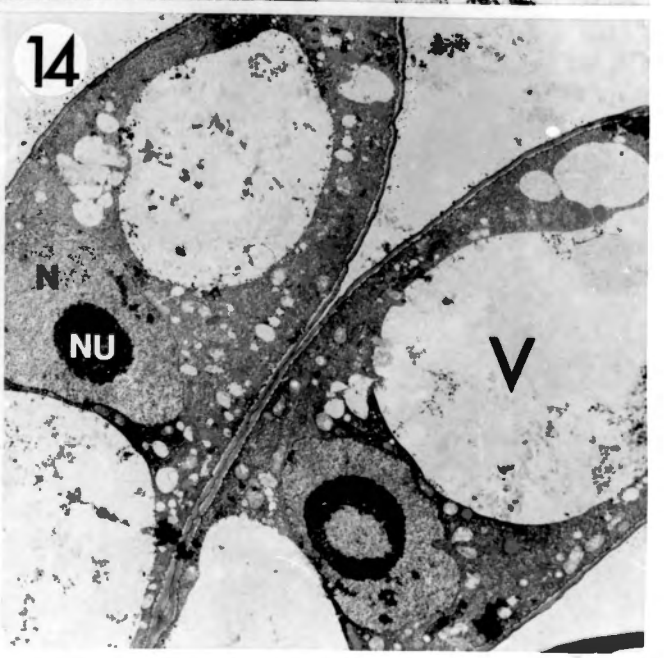
Figure 13. One day postanthesis. β-GPase activity in the cell walls of two adjacent fibers. Enzyme activity was concentrated immediately outside the plasmamembrane and in the exterior part of the cell wall (arrows). β-GP incubated, poststained with UA and LC. x 70,000

Figure 14. One day postanthesis. Enzyme inhibitor control. No lead phosphate deposits were found in cell wall, nucleus or nucleolus. NaF pretreated, NaF plus β-GP incubated, poststained with UA and LC. x 3,500

NOTE: For legend see footnote on page 31.


12
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42
Figures 12, 13 and 14
Joshi, 1987

appeared to be more concentrated in the nucleolus. The nuclei of epidermal cells that were not differentiating into fibers showed no activity. Again, NaF inhibited the β-GPase activity, so no precipitate occurred when the inhibitor was included in the reaction medium (Figure 14). The other controls confirmed that nonspecific deposition of lead was not occurring. Samples incubated with glucose-6-phosphate, adenosine monophosphate and p-nitrophenylphosphate showed comparatively decreased or no reaction product in nucleus, nucleolus or cell wall (Figures 15, 16 and 17).

Two Days Postanthesis

By two days postanthesis, the fiber initials were elongating and the central vacuole was a prominent feature of the cells. Also, there was pronounced enlargement and differentiation of the interior of the nucleolus. Associated with the nucleolar changes was an increase in β-GPase activity, as indicated by the amount of lead phosphate precipitate in that structure (Figures 18 and 19). Deposition was associated with the more electron-dense portion of the nucleolus with no activity in the differentiated interior.

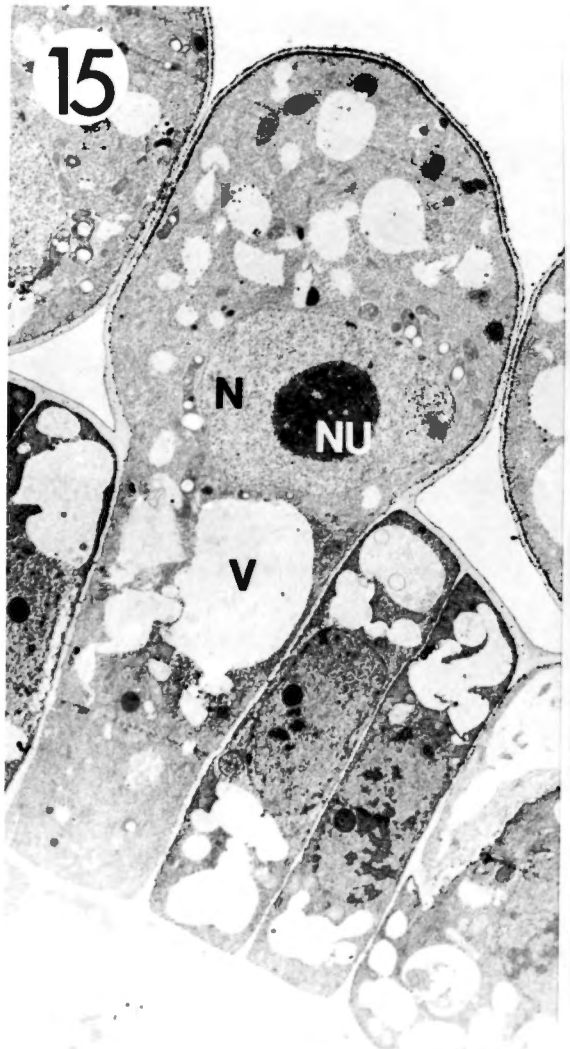
At this stage of development, β-GPase activity in the nucleus was greatly reduced compared to +1 day. Also,

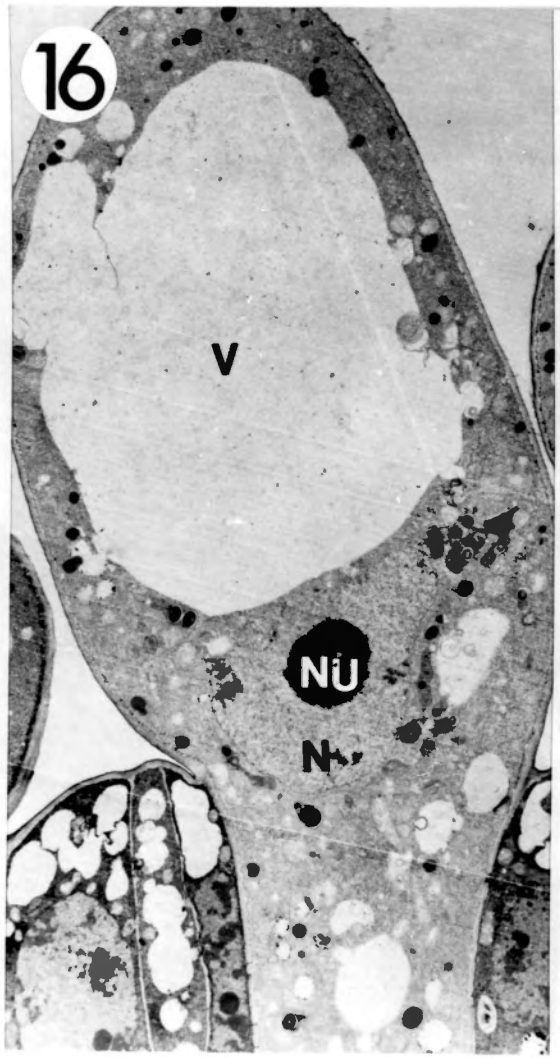
Figure 15. One day postanthesis. Outer integument incubated with glucose-6-phosphate showed some non-specific enzyme reaction product in cell wall and nucleolus. Poststained with UA and LC. x 4,000

Figure 16. One day postanthesis. Outer integument incubated with adenosine monophosphate showed some non-specific enzyme reaction product in nucleolus. Poststained with UA and LC. x 4,000

Figure 17. One day postanthesis. Outer integument incubated with p-nitrophenyl phosphate demonstrated no lead phosphate deposits in nucleus or nucleolus but some non-specific enzyme reaction product in cell wall. Post-stained with UA and LC. x 4,000

NOTE: For legend see footnote on page 31.


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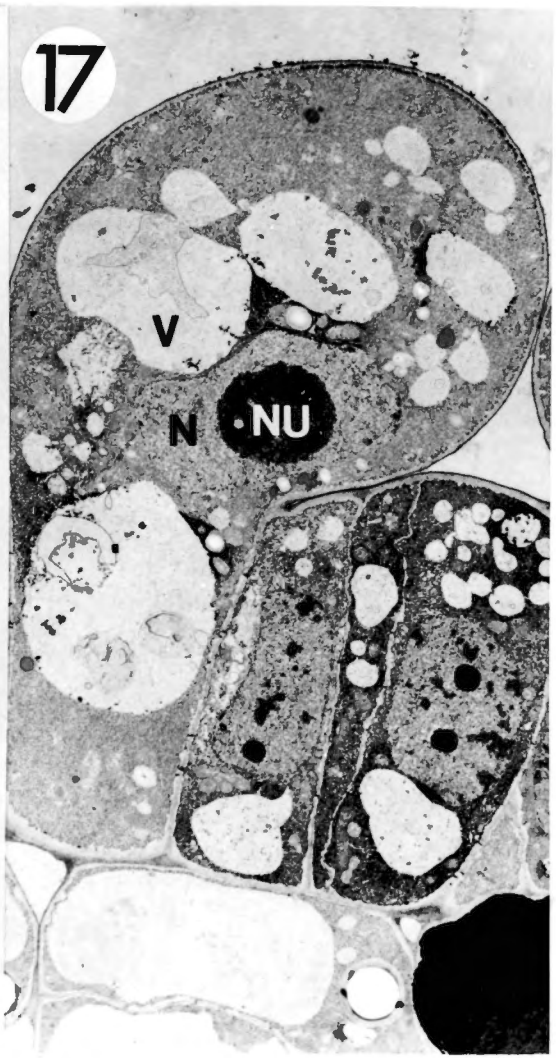

17
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Figure 18. Two days postanthesis. Two adjacent fibers showing decreased enzyme activity in cell wall but increased activity in the nucleolus. Enzyme activity was nearly absent in the nucleus. β-GP incubated, poststained with UA and LC. x 6,300

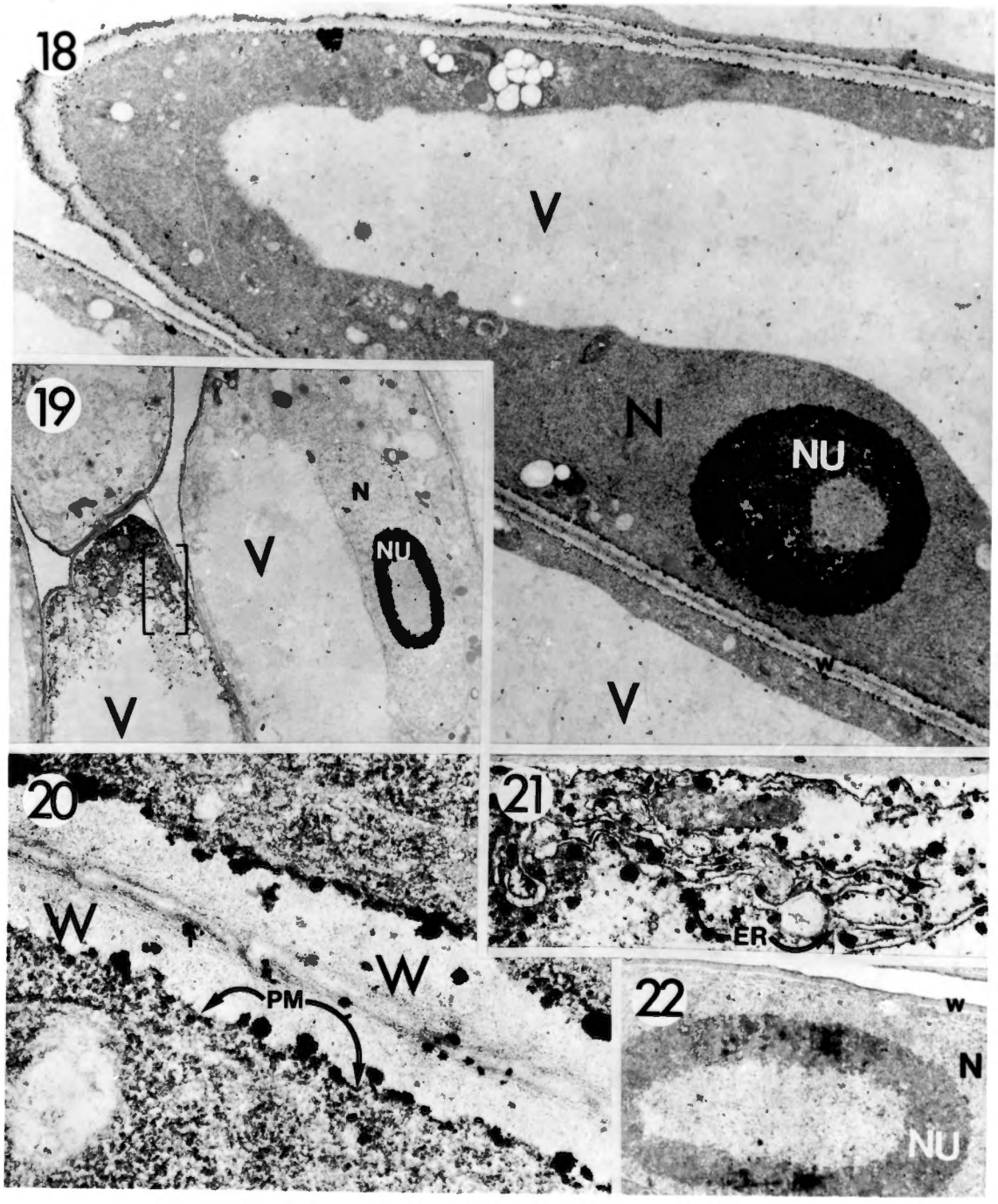
Figure 19. Two days postanthesis. Oblique section of fiber near tip region showing particulate enzyme activity. Note lead phosphate deposits in nucleolus but not in nucleus. β-GP incubated, poststained with UA and LC. x 2,000

Figure 20. Two days postanthesis fiber cell wall. Note decreased deposits in cell wall compared to Figure 13. β-GP incubated, poststained with UA and LC. x 73,600

Figure 21. Two days postanthesis. Magnified view of fiber near tip region outlined in brackets in Figure 19. β-GPase activity associated with ER. x 10,600

Figure 22. Two days postanthesis. Inhibitor control. Note absence of lead phosphate deposits in nucleus, nucleolus or cell wall. NaF pretreated, NaF plus β-GP incubated, post-stained with UA and LC. x 11,300

NOTE: For legend see footnote on page 31.


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47
Figures 18, 19, 20, 21 and 22
Joshi, 1987

activity associated with the cell wall apparently decreased (Figures 18 and 20). Near the tip of the fiber, reaction product was associated with the ER (Figures 19 and 21). Nearly-complete inhibition of the enzyme at all sites was accomplished with NaF (Figure 22).

Three Days Postanthesis

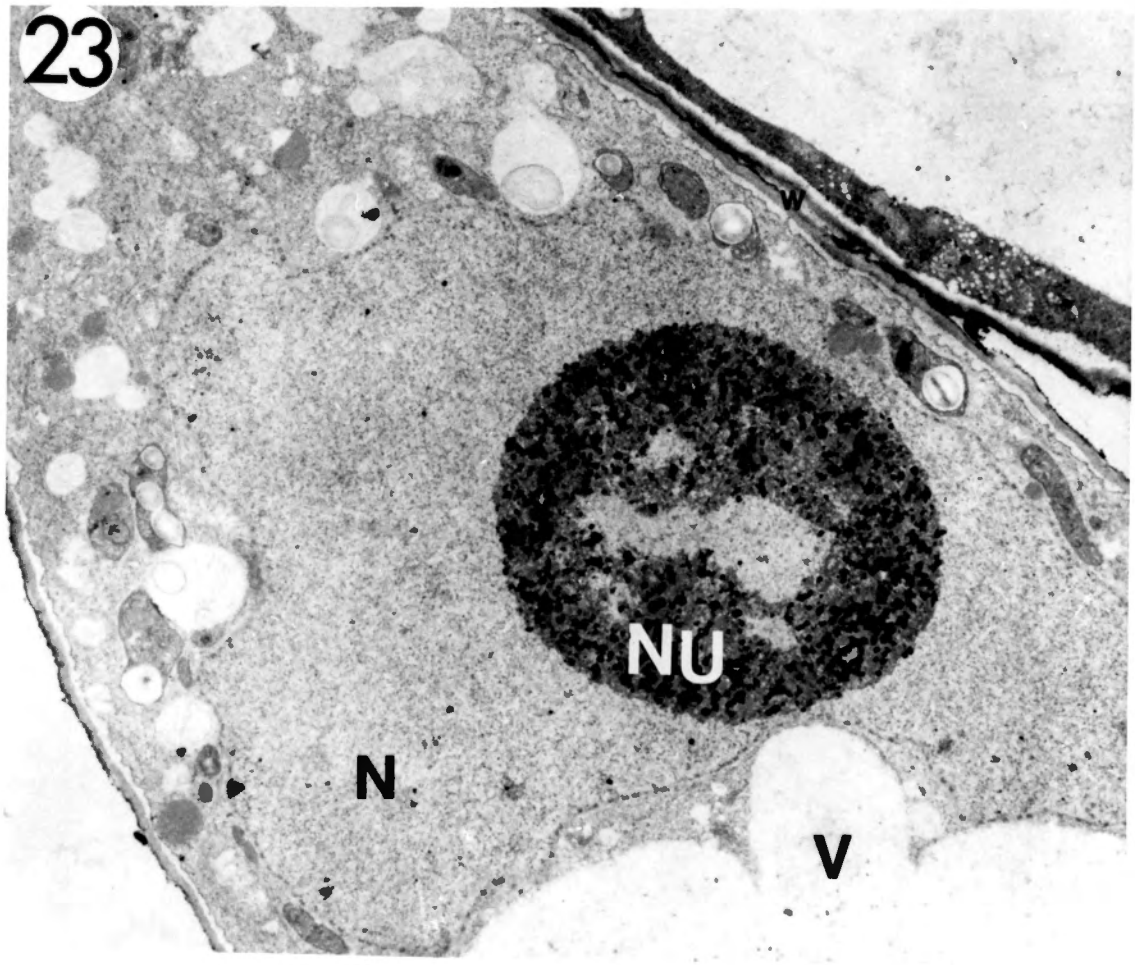
The changes occurring at the +2 day stage continued in +3 day. The nucleolus reached its maximum size and maximum activity of β-GPase (Figure 23). There was very slight activity in the nucleus, and activity in the cell wall was seen in only a few fiber cells. Non-specific staining in the nucleus and nucleolus due to osmium tetroxide was verified by processing samples without osmication after the incubation step (Figure 24). Enzyme inhibitor, NaF, completely inhibited β-GPase activity in the nucleolus (Figure 25).

Linted versus Lintless Cotton

No β-GPase activity was detected in the integument of the lintless line of cotton at any of the 5 stages examined. A representative result is presented in Figure 26. The above results indicate that enzyme activity was localized only in the epidermal layer of the linted cultivar, and then only in cells that were developing into fibers and in newly divided cells.

Figure 23. Three days postanthesis. Portion of longitudinal section of fiber cell with β-GPase enzyme activity predominantly in the nucleolus. β-GP incubated, poststained with UA and LC. x 11,000

NOTE; For legend see footnote on page 31.

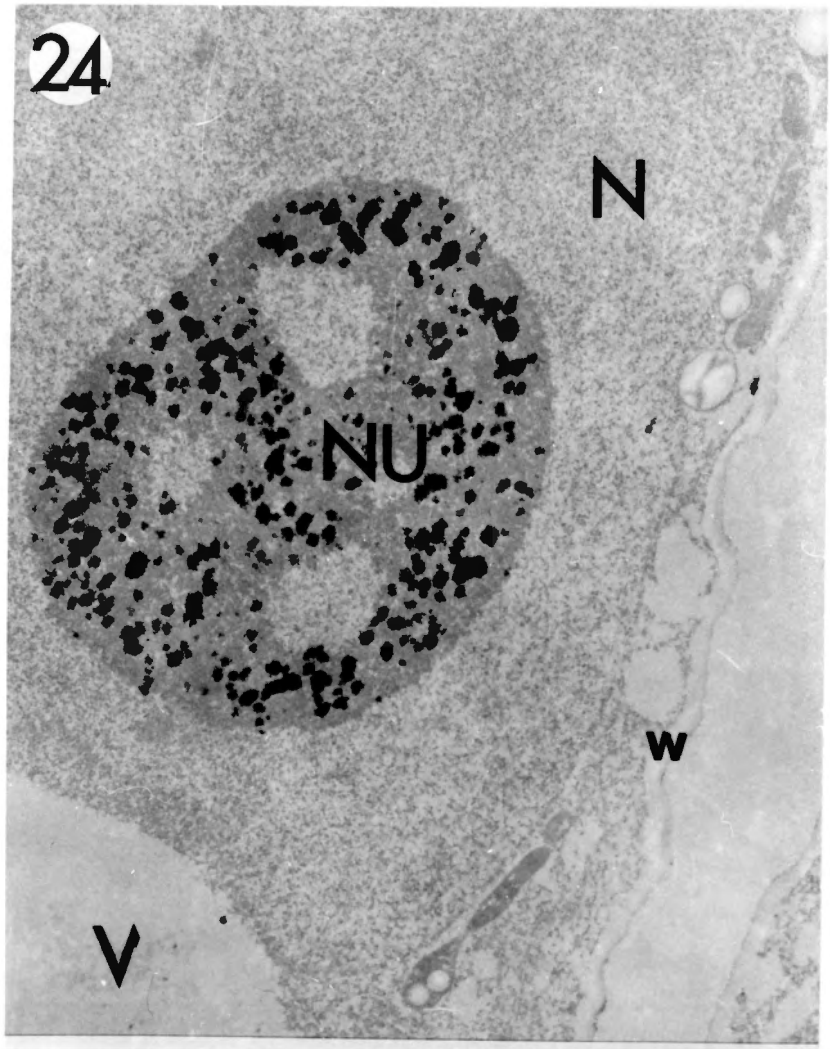

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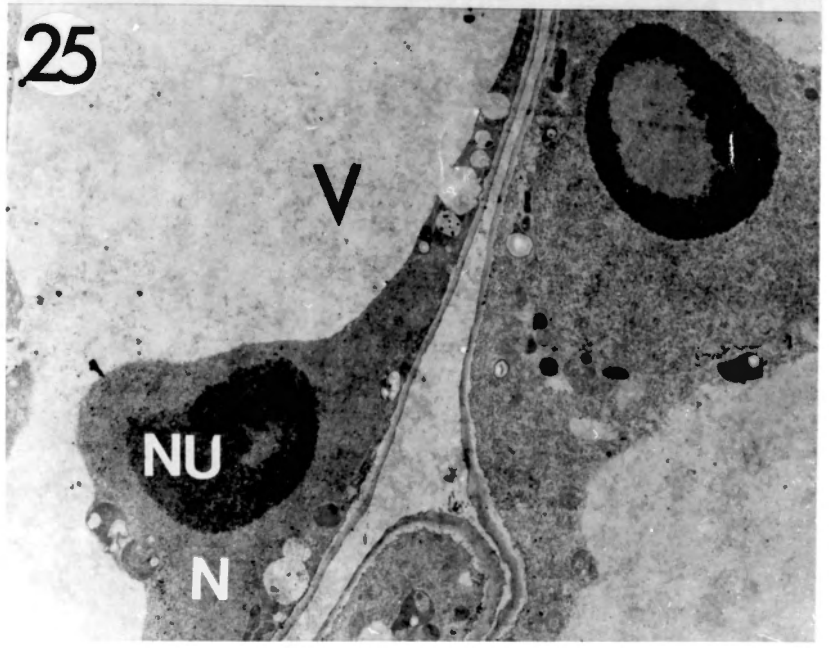
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Figure 23
Joshi, 1987

Figure 24. Three days postanthesis. Portion of fiber with lead phosphate deposits in the nucleolus. Outer integuments were processed without osmication after incubation with β-GP. Poststaining with UA only. x 22,500

Figure 25. Three days postanthesis. No enzyme activity was observed in the nucleolus in the samples pretreated with inhibitor, NaF, and then incubated with β-GP along with enzyme inhibitor. Poststained with UA and LC. x 6,000

NOTE: For legend see footnote on page 31.


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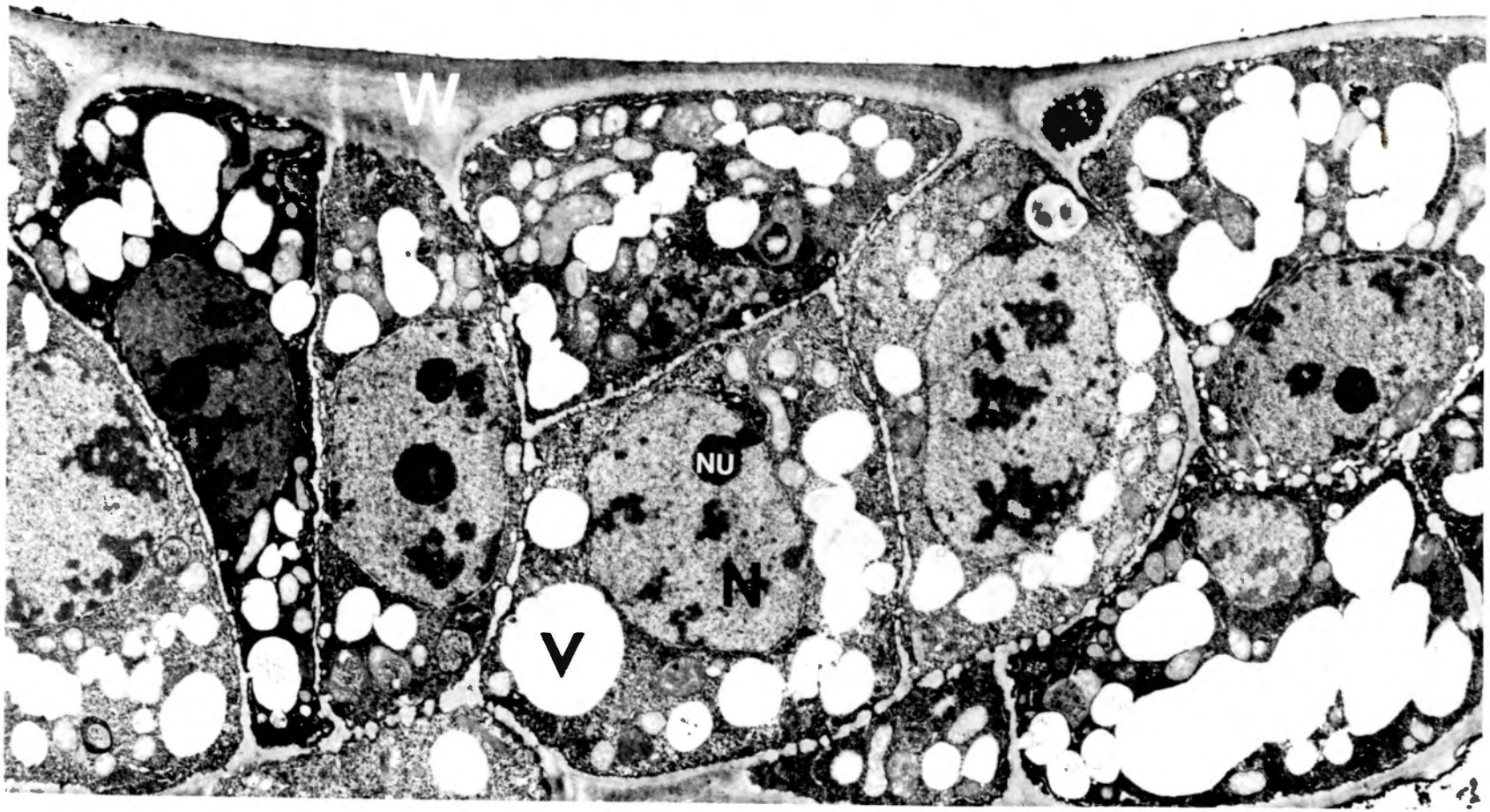

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Figures 24 and 25
Joshi, 1987

Figure 26. Lintless Hybrid line. Two days postanthesis. No β-GPase activity was detected in any cell of the outer integument. β-GP incubated, poststained with UA and LC. x 5,700

NOTE: For legend see footnote on page 31.

26



54
Figure 26
Joshi, 1987

Discussion

The fact that β-GPase activity is limited to the ovule epidermal cells that are undergoing recognizable anatomical changes associated with fiber differentiation is significant. This is reinforced by the absence of activity in the ovule epidermal layer of the fiberless mutant. The exact role of β-GPase in plant tissue is not clear, but our results, along with numerous reports by others (Avers 1958; Avers and Grimm 1959; Dosier and Riopel 1977; Forsket and Miksche 1966; Hall 1969; Mia and Pathak 1968; Sexton et al. 1971; Van den Born 1963), have shown a strong relationship between differentiation processes and β-GPase activity.

In addition to the involvement of phosphatases in the tissue differentiation process, several reports show phosphatase activity in cell walls (DeJong et al. 1967b; Hall 1969; Hall and Butt 1968; Hall and Davie 1971; Maier and Maier 1972; Sexton et al. 1971). The role of these enzymes in the cell walls has been speculated to involve intercellular transport mechanisms, phosphate reactions

and cell wall metabolism (Hall and Davie 1971; Halperin 1969; Onofeghara 1972; Sexton et al. 1971). The unique nature of the cotton fiber initial provides some limits to the possible roles for the enzyme. Since the cell wall of the fiber initial defines the limit between the cell and the external environment, transport is probably not a function of the enzyme. Likewise, the need for extensive phosphate transfer reactions external to the plasma-membrane of these cells is not evident. More likely, the β-GPase activity would be involved directly or indirectly in the cell wall metabolism. In view of its phosphorolytic activity and specific location in areas of wall expansion, especially in conjunction with the directional growth of the fiber initial, it is probably involved in cell wall loosening. Further, no activity occurred at the base of the fiber initial cell, an area where expansion does not occur, but where one can assume that transport into the cell is high.

The occurrence of β-GPase activity in limited areas of the cytoplasm is an interesting phenomenon. Figures 3 and 4, pages 35 and 37, suggest that the enzyme is transported through the cytoplasm and concentrated in the growing tip of the fiber initial. The activity is obviously associated with the ER, but our results do not conclusively show that the enzyme is confined within ER

vesicles since limiting membranes are not visible. While it is obvious that the cell wall has enzyme activity, the mechanism of how the enzymes were deposited is not clear.

The presence of β-GPase activity in the nucleus and nucleolus of the fiber initial raises questions about its function in those organelles. Since a noticeable level of activity in the nucleus occurred only during the +1 day stage, the enzyme may serve a developmental function in that organelle, or it may merely be transported to the nucleolus. Even if it is being transported, it is apparently in an active form, and is capable of reaction with suitable substrates in the nucleus. Although the range of substrates upon which the enzymes will react is not known, their transitory occurrence in the nucleus suggests dephosphorylation of nuclear protein. Such changes could influence either the quality or quantity of gene transcription, or both. This concept is similar to the Bonner (1965) model of gene suppression by histones.

Accumulation of β-GPase activity in the nucleolus is the most prominent feature of our results. Activity began accumulating the day after anthesis and continued through three days postanthesis, the last day we examined. This accumulation of activity corresponds to the enlargement of the nucleolus occurring during this period. The nucleolus

reaches its maximum size at 3 days postanthesis (Berlin 1986; Kosmidou-Dimitropoulou 1986; Ramsey and Berlin 1976a,b). Kosmidou-Dimitropoulou (1986) suggested that the dramatic change in the size and structure of the nucleolus was associated with increased capacity for ribosome synthesis. Incorporation of H-uridine into fiber initials occurred from 1 to 6 days postanthesis with the highest uptake occurring 3 days postanthesis (Berlin 1986). The coincidence of β-GPase activity and the anatomical and metabolic changes in the nucleolus during the later stages of differentiation of the fiber initial strongly imply a functional relationship among these phenomena.

The functional role of a phosphorolytic enzyme in differentiation is at first not clear. There is accumulating evidence, however, that one of the controlling mechanisms in biological systems is the phosphorylation-dephosphorylation of critical enzymes. Reviews of this area have appeared recently (Ingebritsen and Cohen 1983; Krebs and Beavo 1979). Generally, enzymes involved in synthetic pathways are activated by the phosphorolytic activity of a phospho-protein phosphatase, while those in hydrolytic functions are activated by phosphorylation achieved by a protein kinase. While we have no evidence that β-GPase functions as a phosphoprotein phosphatase,

such a theoretical role is an attractive concept in view of its frequent association with differentiation processes.

No β-GPase activity is associated with the pre-enlarging cell, but high activity is associated with expanding cell walls and with the very active nucleoli of the +1 to +3 day fiber initial. Thus, in the differentiation of the cotton fiber, β-GPase activity is apparently not involved in the critical switch that delineates a future fiber from other epidermal cells. Rather, the activity seems more closely associated with the potentiation of the cell to realize the phenomenal growth in length for which it is valued.

CHAPTER IV

ADENOSINE TRIPHOSPHATASE ACTIVITY DURING FIBER ELONGATION

Introduction

Diverse lines of evidence suggest that elongation of cotton fiber is a response to internal osmotic pressure developed by accumulation of potassium and malate in the expanding central vacuole. The question arises as to the origin and regulation of potassium and malate in the fiber cell. Why is there a differential concentration of both these elements only in fiber cells even though all the epidermal cells have a common basal area for the absorption of water or nutrients? What type of transport mechanism allows the fiber cell to accumulate more potassium (against its concentration gradient) than adjacent non-fiber cells?

Enzyme localization has proven to be an effective approach which has demonstrated the occurrence of different types of ATPase on different membrane systems in plant and animal tissue. The presence of ATPase activity on membranes has been correlated with the transport mechanism of ions and metabolites and their physiological role in different tissues (Firth 1978; Hall et al. 1980;

Mogensen 1981; Nassery and Valamanesh 1978; Price and Whitecross 1983; Sossountzov and Habricot 1985; Sossountzov et al. 1985).

In order to address questions concerning solute accumulation, a cytochemical investigation was conducted to determine if ATPase activity was present and located in such a way as to function in the accumulation of potassium and malate during fiber elongation.

Materials and Methods

Tissue Source and Preparation

A linted cotton, Gossypium hirsutum L. cv. Hancock, and a lintless mutant line 9SO x HG, were grown in a glasshouse in pots containing sand-vermiculite and fertilized with a complete nutrient solution once a week. Flowers were tagged and dated over several days from one day preanthesis to eight days postanthesis. In order to maintain uniformity in experimental design, samples for all the stages of fiber development were harvested on the tenth day. The sample stages were 1-day preanthesis, anthesis, 1, 2, 4, 6, 8, and 10 days postanthesis (-1, 0, +1, +2, +4, +6, +8, +10).

Cotton ovules were excised while the bud, flower or capsule was attached to the plant, and they were immediately fixed in 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, and kept in crushed ice

(Glauert 1977; Hall 1978; Hayat 1981). Vacuum was applied to the cold samples to facilitate rapid penetration of fixative (Glauert 1977; Hall 1978). The ovules were dissected in a pool of cold fixative to remove the outer integument which is 6-7 cells thick with an average total thickness of 60-70 µm. Only the outer integument with its associated fibers on the epidermis was used thereafter. After 4 hours of total fixation time, the samples were rinsed with 0.1 M cacodylate buffer then washed overnight with 50 mM tris-maleate buffer, pH 7.1, at 4°C to remove residual glutaraldehyde.

Enzyme Reaction Procedure

The ultrastructural enzyme cytochemical technique involved the use of heavy metal simultaneous capture reaction (Gomori 1950) and was a modification of that reported by Hall and Butt (1969) (Appendix B). Briefly, the endogenous enzyme cleaves the ATP to yield phosphate which is captured by lead to form electron dense deposits of lead phosphate at the site of enzyme activity. Final concentration of each component in the enzyme reaction medium included 50 mM tris-maleate buffer, pH 7.1; 3.6 mM $Pb(NO_3)_2$; 2 mM $Mg(NO_3)_2$; 2 mM KCl; and 2 mM ATP (K^+ or Na^+ salt). Incubation in the complete reaction medium was conducted at 31° $\pm$ 1°C for 2 hours with continuous

stirring. Controls included samples (1) incubated in the reaction medium minus substrate ATP to see the presence of any nonspecific staining due to lead in the reaction medium; (2) pretreated with enzyme inhibitor DCCD (Marin 1985), followed by incubation in complete reaction medium plus inhibitor; (3) incubated in reaction medium without KCl but with ATP as the Na^+ salt to determine the sensitivity to KCl; (4) incubated with β-GP, a common substrate for phosphatase (Joshi et al. 1985), to determine the contribution of general phosphatase, if any.

Samples for all the stages of fiber development, with controls, were fixed at the same time and incubated in the identical freshly prepared reaction medium, with the additions or deletions indicated. All the necessary precautions to ensure specificity and reduce artifacts were taken. In this experiment as many variables as possible were optimized and standardized including condition and source of ovule, vacuum infiltration of fixative and reaction medium, and time and temperature of fixation and enzyme reaction. Finally, the results obtained with the linted cultivar were compared with the lintless (naked seed) mutant. Results also were compared between expanding fiber cells and non-fiber cells of the outer integuments.

Sample Preparation for EM

After incubation, the samples were washed with 50 mM tris-maleate buffer, rinsed briefly with 0.1 M sym-collidine buffer, pH 7.2, then post-fixed with 1% osmium tetroxide for 2 hours at 4°C. Following an acetone series dehydration, the samples were embedded in a low viscosity plastic medium (Spurr 1969) in flat rubber molds and oriented so as to obtain longitudinal sections of elongating fibers. Finally, 3-4 randomly selected blocks with outer integuments from different ovules were sectioned at three different regions. Silver-gray sections were obtained with an LKB-8800 ultra-microtome equipped with a diamond knife. Sections were poststained with aqueous uranyl acetate only or with uranyl acetate followed by lead citrate (Reynolds 1963). Sections were observed and photographed with a Phillips-201 transmission electron microscope. Although conditions were controlled as closely as possible, some quality of ultrastructure was sacrificed due to the short fixation time at low temperature and the incubation procedure.

Results

Cytochemical Enzyme Localization Before Anthesis

Outer integument of the 1-day preanthesis stage showed non-elongated epidermal cells (Figure 27). Fiber initiation had not yet occurred. The cells were smaller with many vacuoles compared with later stages of ovule development. Epidermal cells showed centrally located nuclei with small nucleoli. These epidermal cells showed lead phosphate deposits only in mitochondria (Figure 28). No lead phosphate deposits were found either on the plasmamembrane or on the tonoplast (Figures 27 and 28).

Cytochemical Enzyme Localization at Anthesis

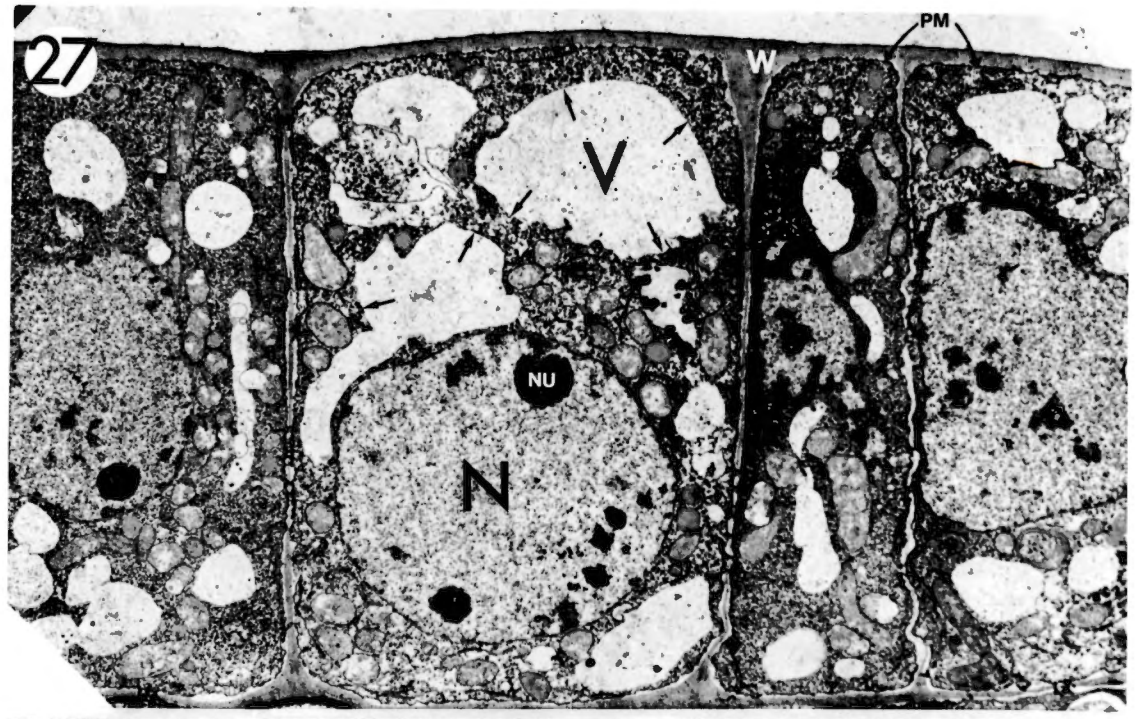
At anthesis there was no indication of ATPase activity in the cell wall, nucleus, nucleolus, or vacuoles; however, activity was obvious in mitochondria at higher magnification (Figure 29). The outer epidermal layer showed elongating fiber cells and non-fiber cells (Figure 30). In the fiber cells large nuclei and nucleoli occupying central positions were prominent. Endoplasmic reticulum (ER) and Golgi bodies were more numerous compared to non-fiber cells.

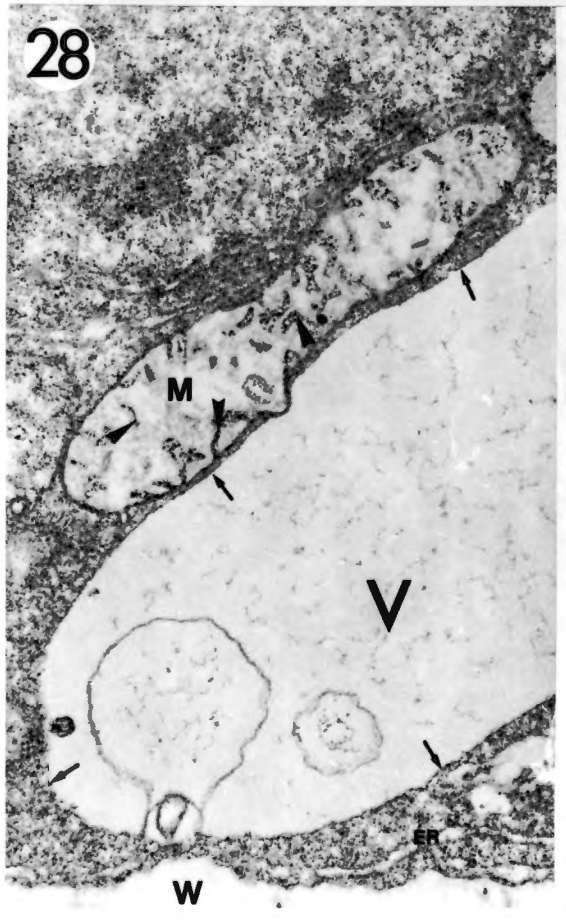
Figure 27. One day preanthesis. L. S. of undifferentiated epidermal cells. Absence of lead phosphate deposits indicates no ATPase activity on tonoplast (arrows). ATP incubated, poststained with UA and LC. x 6,000.

Figure 28. One day preanthesis. High magnification of epidermal cells shows no lead phosphate deposits on tonoplast (arrows), but deposits were present in mitochondria (arrowheads). ATP incubated, poststained with UA only. x 36,000.

Figure 29. Anthesis. No ATPase activity was found in the nucleus or on tonoplast, but enzyme activity was observed in mitochondria (arrowheads). ATP incubated, poststained with UA only. x 45,000.

NOTE: For legend see footnote on page 31.


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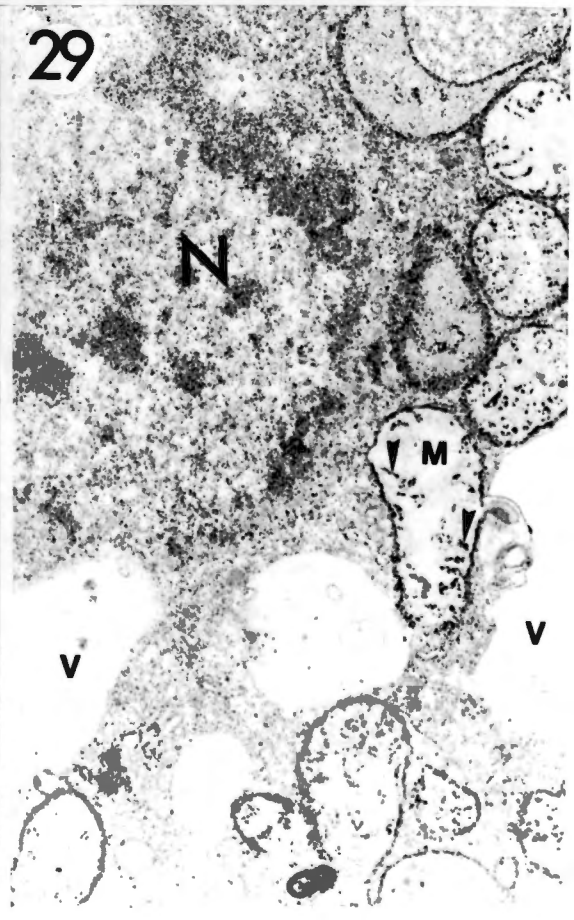

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Figure 30. Anthesis. Fibers and non-fiber cells show no lead phosphate deposits on tonoplasts. ATP incubated, poststained with UA and LC. x 7,500.

NOTE: For legend see footnote on page 31.


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Figure 30
Joshi, 1987

Cytochemical Enzyme Localization After Anthesis

By 1-day postanthesis, the fiber cells enlarged further and established expanding vacuoles that were centrally located. Many small vacuoles were coalescing to form the large central vacuole. The nucleus had migrated into the elongated portion of the cell (not shown). Numerous Golgi vesicles, lipid bodies and ER membrane and vesicles all seemed to contribute to the formation of the expanding central vacuole (Figure 31). Along with these ultrastructural changes, ATPase enzyme activity appeared on the tonoplast of the central vacuole (Figure 32) which became distinctly visible all along the fiber tonoplast (Figure 33). Tonoplasts of adjacent non-elongating, non-fiber cells and subepidermal cells of the outer integument (not shown) did not show any lead phosphate deposits.

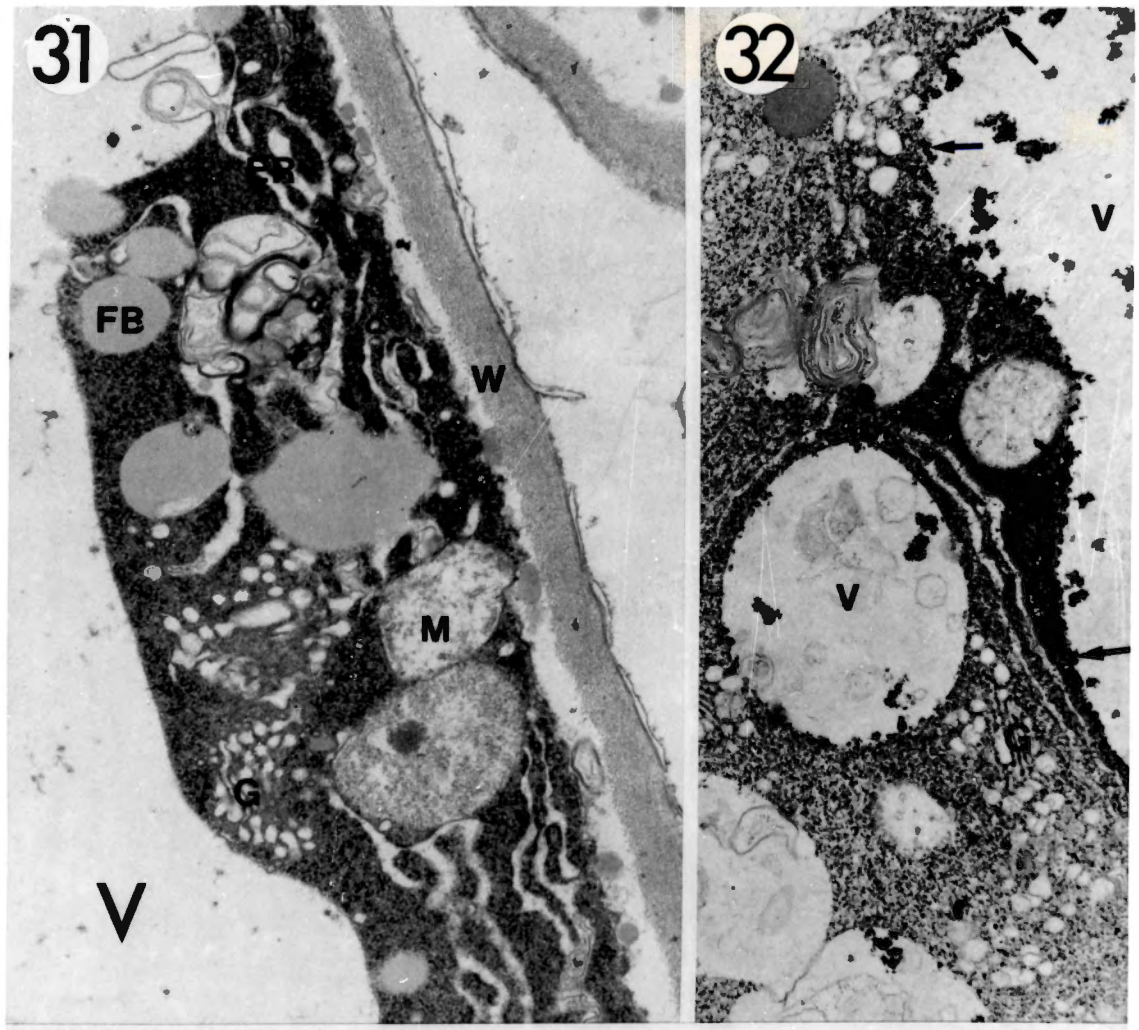
At 2-days postanthesis, the fiber cells had elongated rapidly and large expanding vacuoles were the main feature of the cell. Numerous small vacuoles distributed throughout the cytoplasm were merging into the central one (Figure 34). Lead phosphate deposits were distinctly visible, mainly on the membrane of small vacuoles and the large central one. No enzyme activity was seen on the plasmamembrane (Figure 34).

Figure 31. One-day postanthesis. Enlarged portion of L. S. of fiber. Note fusion of ER and Golgi vesicles, and ER originated fat bodies (FB) fusing with expanding tonoplast. Without incubation step, poststained with UA only. x 36,000.

Figure 32. One day postanthesis. Portion of L. S. of fiber showing occurrence of lead phosphate deposits on tonoplast. ATP incubated, poststained with UA and LC. x 20,000

Figure 33. One day postanthesis. L. S. of fiber shows ATPase activity on tonoplast (arrows). ATP incubated, poststained with UA and LC. x 12,500

NOTE: For legend see footnote on page 31.


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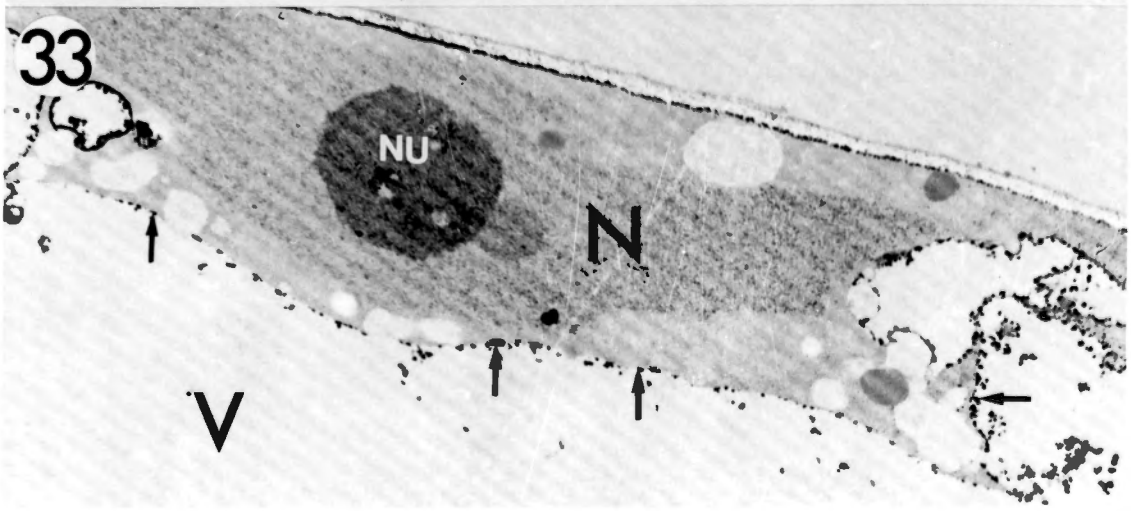
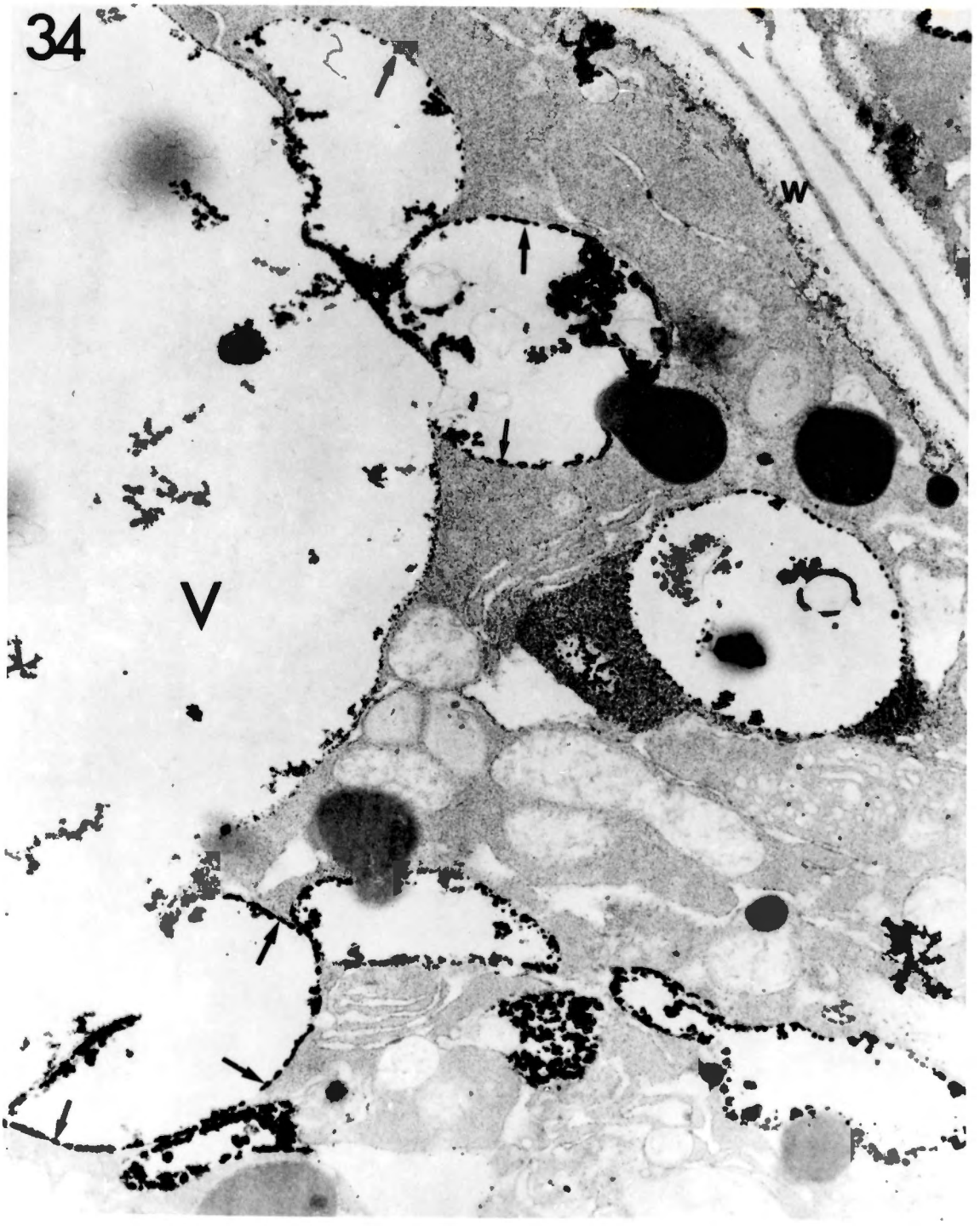

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Figure 34. Two days postanthesis. Magnified portion of L. S. of fiber shows lead phosphate deposits (arrows) on tonoplast of large and small vacuoles. ATP incubated, post-stained with UA only. x 22,000

NOTE: For legend see footnote on page 31.


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Figure 34
Joshi, 1987

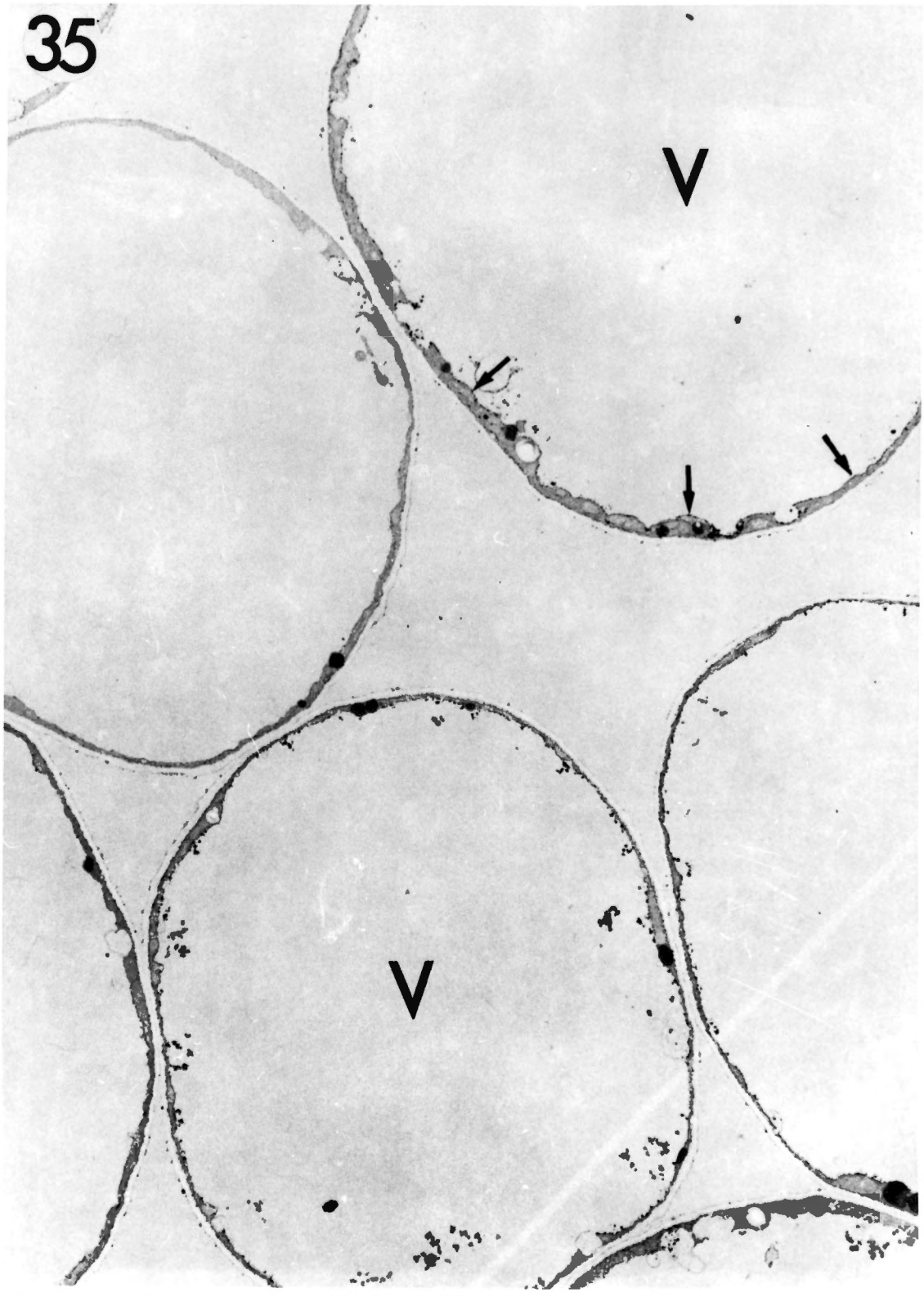
By 4- and 6-days postanthesis, fibers were too long to obtain longitudinal sections. Transverse sections of fiber showed an extremely large central vacuole with a very thin rim of cytoplasm all along the cell wall (Figure 35). At low magnification it was difficult to identify clearly the presence of lead phosphate deposits (Figure 35), but high magnification distinctly showed deposits along the tonoplast (Figures 36 and 37). These lead phosphate deposits were observed on ER in the form of discrete beads. Close association of tonoplast and ER-derived beads was observed frequently (Figure 38).

At 8-days postanthesis, the ATPase enzyme activity was predominantly present on the fiber tonoplast (arrows) and ER (Figures 39, 40, 41 and 42). ER seemed to deposit beads on the tonoplast (Figures 40 and 42) and lead phosphate deposits were consistently found as discrete beads on the tonoplast as well as on ER (Figures 40 and 42). No lead phosphate deposits were observed on the plasma-membrane of fiber cells (Figures 40 and 42) or on the tonoplast of non-fiber cells (Figure 43).

At 10-days postanthesis, lead phosphate deposits were found in a few fibers only (Figures 44 and 45). No activity was observed in fuzz fiber (Figure 44). Magnified view showed occurrence of enzyme activity mainly on tonoplast (Figure 46).

Figure 35. Four days postanthesis. T. S. of fibers with large vacuoles and thin rim of cytoplasm. Note lead phosphate deposits on tonoplast (arrows). ATP incubated, post-stained with UA and LC. x 16,000

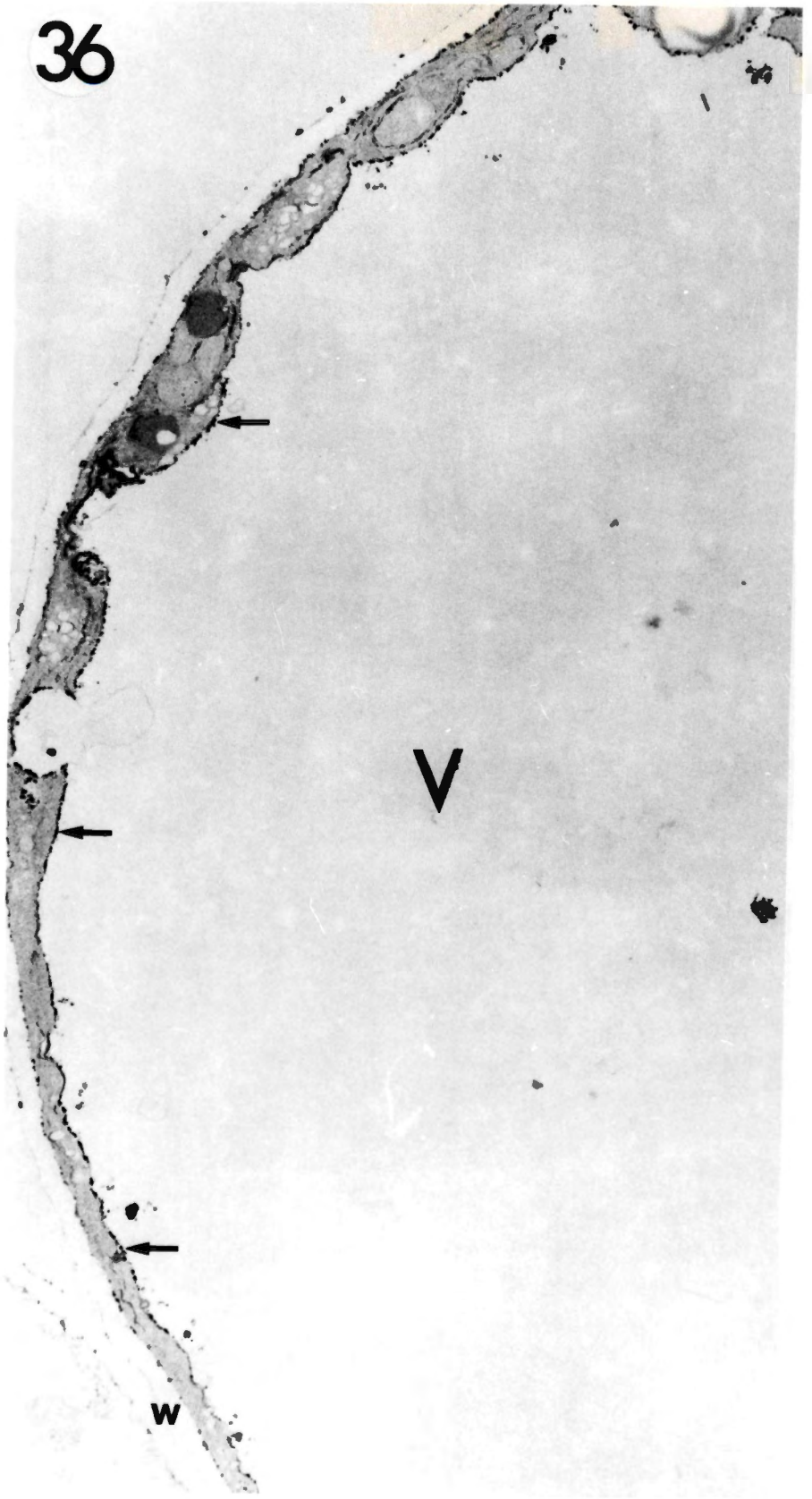
NOTE: For legend see footnote on page 31.


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Figure 35
Joshi, 1987

Figure 36. Four days postanthesis. Magnified view from Figure 35 with arrows. High magnification distinctly indicates occurrence of ATPase activity on tonoplast (arrows). x 53,300

NOTE: For legend see footnote on page 31.

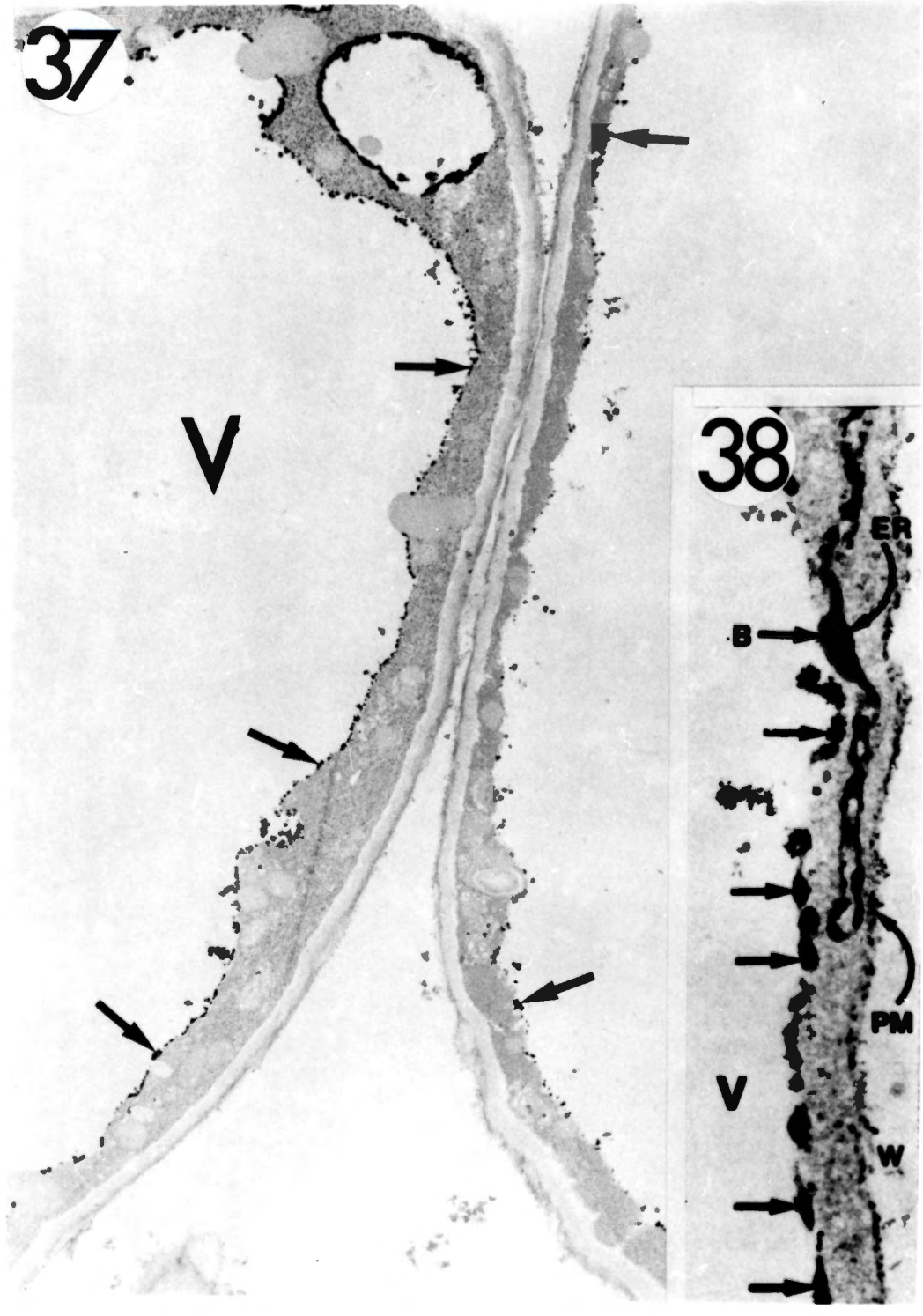

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Figure 36
Joshi, 1987

Figure 37. Six days postanthesis. T. S. of portion of two fibers showing lead phosphate deposits (arrows) on tonoplast. ATP incubated, post-stained with UA and LC. x 12,200

Figure 38. Six days postanthesis. Lead phosphate deposits on ER and tonoplast in the form of discrete beads (arrows). ATP incubated, post-stained with UA and LC. x 150,000

NOTE: For legend see footnote on page 31.

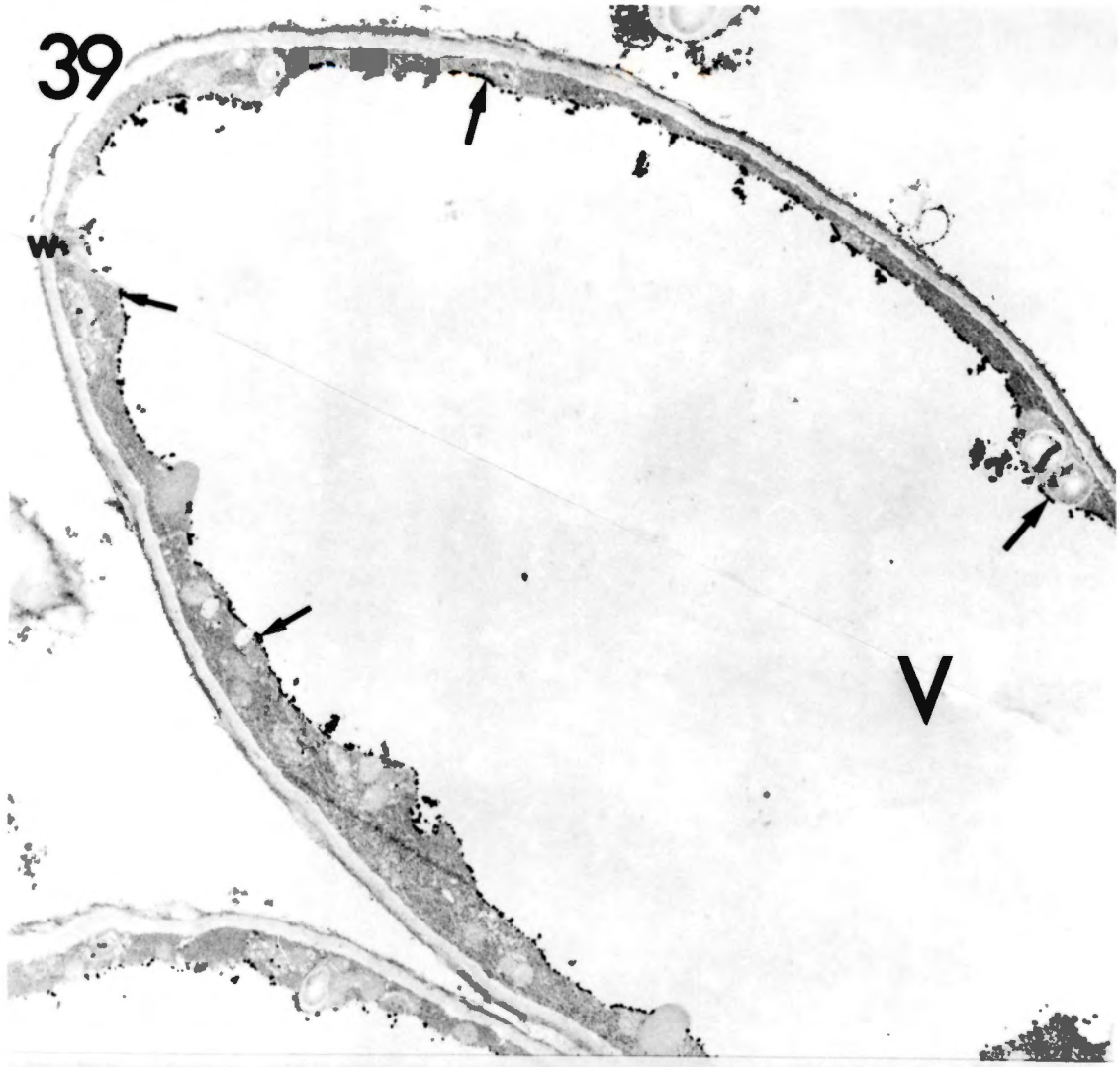

37
V
38
ER
B
PM
V
W

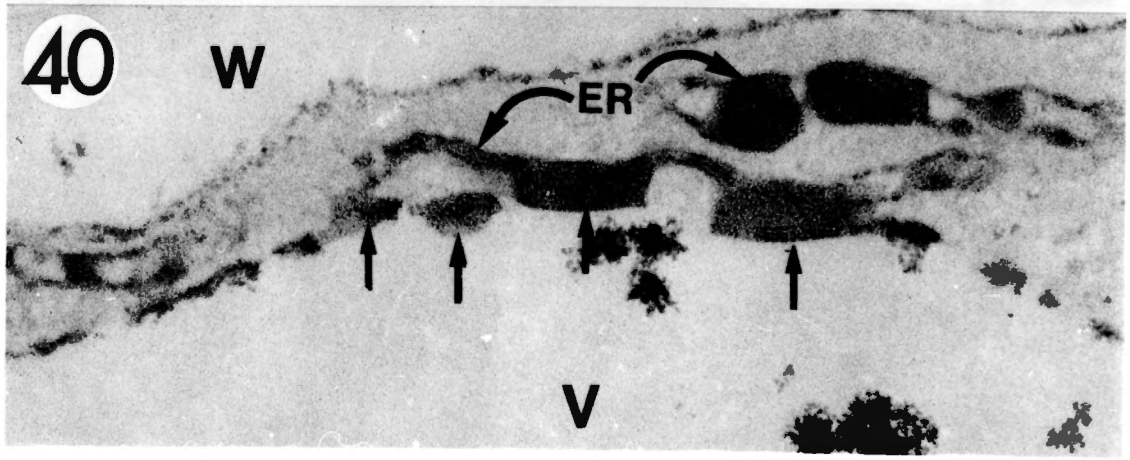
81
Figures 37 and 38
Joshi, 1987

Figure 39. Eight days postanthesis. Portion of fiber with ATPase activity on the tonoplast. ATP incubated, poststained with UA and LC. x 8,000

Figure 40. Eight days postanthesis. Very high magnification of portion of fiber indicates ATPase activity on ER and tonoplast. Note lead phosphate beads originating from ER and depositing on tonoplast (arrows). ATP incubated, poststained with UA and LC. x 162,857

NOTE: For legend see footnote on page 31.


39
W
V


40
W
ER
V

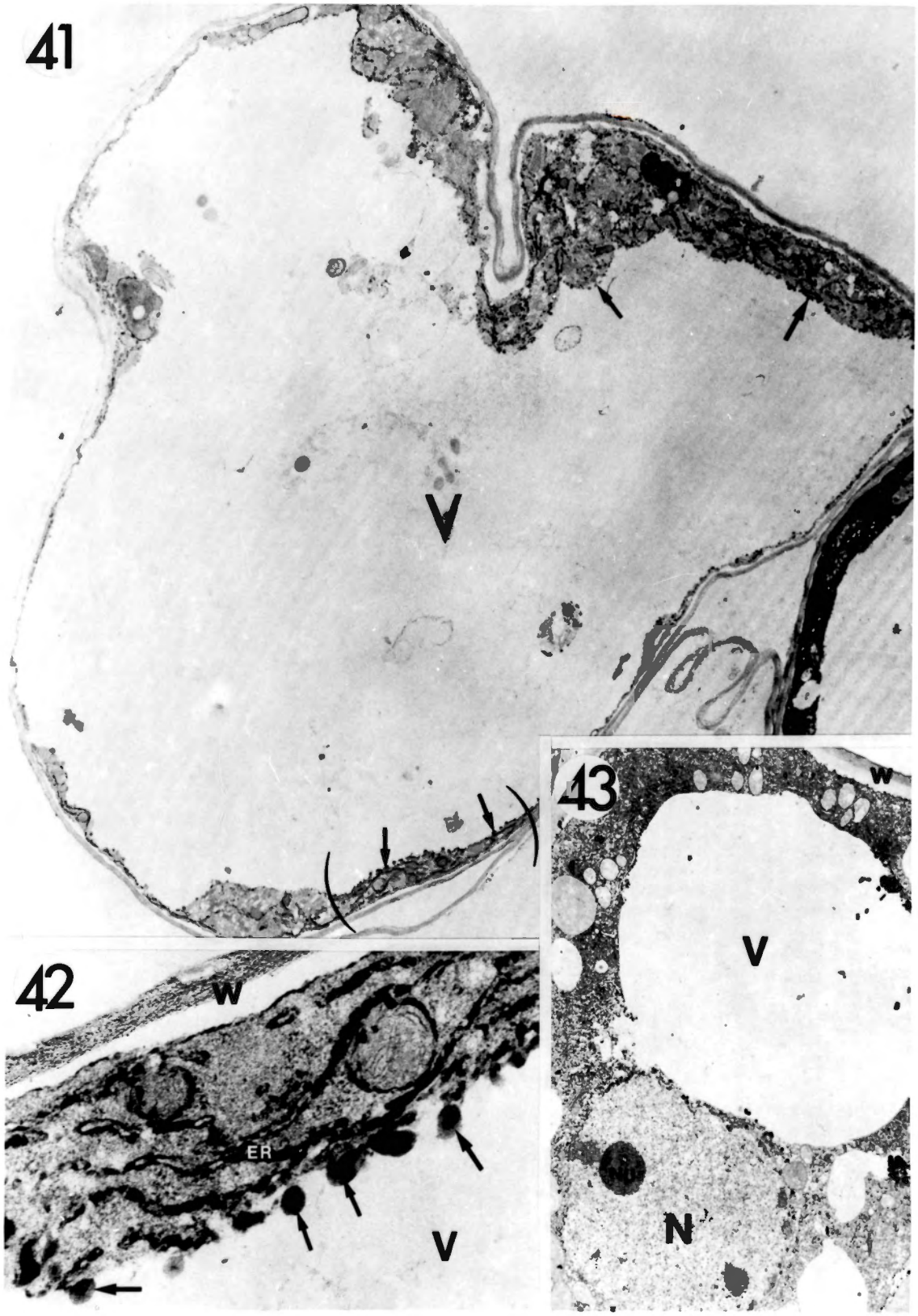
83
Figures 39 and 40
Joshi, 1987

Figure 41. Eight days postanthesis. T. S. of fiber cell shows lead phosphate deposits on ER and tonoplast (arrows). ATP incubated, poststained with UA and LC. x 5,900

Figure 42. Eight days postanthesis. Enlarged portion from Figure 41 with bracket showing ATPase activity associated with ER and tonoplast. ATP incubated, poststained with UA and LC. x 54,000

Figure 43. Eight days postanthesis. L. S. of non-fiber cell shows no lead phosphate deposits on tonoplast. ATP incubated, poststained with UA and LC. x 5,400

NOTE: For legend see footnote on page 31.


41
V
42
W
ER
V
43
W
V
N

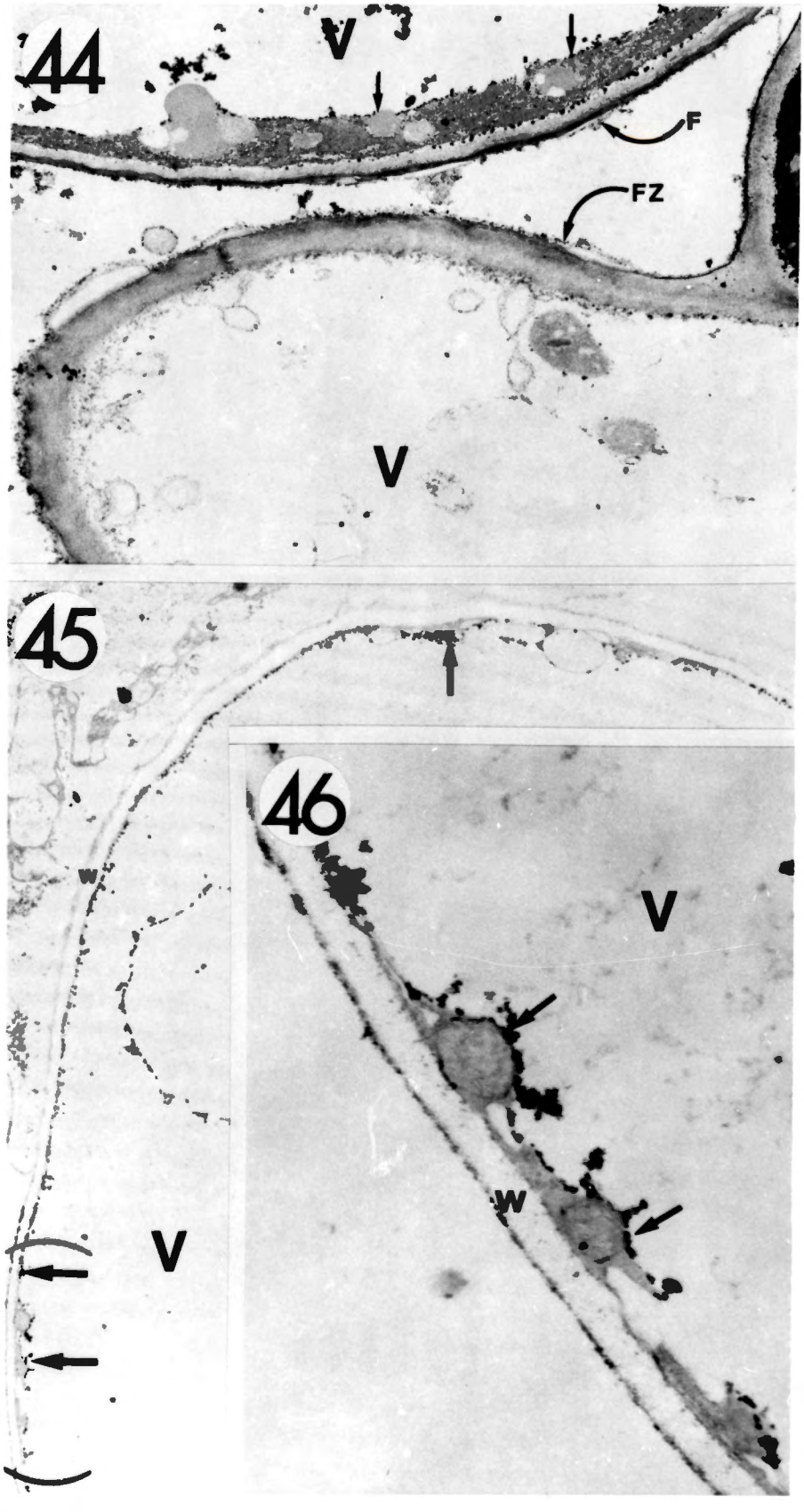
85
Figures 41, 42 and 43
Joshi, 1987

Figure 44. Ten days postanthesis. L. S. of portion of fuzz cell and fiber. Note lead phosphate deposits in fiber tonoplast (arrows) while no similar deposits were observed in fuzz tonoplast. ATP incubated, poststained with UA and LC. x 12,200

Figure 45. Ten days postanthesis. T. S. of portion of fiber cell showing large vacuole and thin rim of cytoplasm with lead phosphate deposits on tonoplast (arrows). ATP incubated, poststained with UA and LC. x 7,500

Figure 46. Ten days postanthesis. Magnified view of bracket from Figure 45 showing ATPase activity mainly on tonoplast. ATP incubated, poststained with UA and LC. x 38,000

NOTE: For legend see footnote on page 31.


44
V
F
FZ
V
45
V
46
V
W

Controls

Samples incubated with the enzyme inhibitor, DCCD, inhibited ATPase activity almost entirely (Figure 47). A magnified view showed no lead phosphate deposits on tonoplast (Figure 48). Samples incubated in a reaction medium minus the substrate ATP showed no lead phosphate deposits or any non-specific staining due to lead in the reaction medium (Figure 49). Samples incubated in a reaction medium without KCl but with ATP (sodium salt) did not show lead phosphate deposits on the tonoplast of fibers (Figure 50). Finally, samples incubated with β-GP as a substrate were devoid of lead phosphate deposits on the tonoplast of elongating fibers. Instead, there was some indication of non-specific phosphatase activity in the cell wall and nucleolus (Figure 51) as reported by Joshi et al. (1985).

Linted Versus Lintless Mutant Cotton

No lead phosphate deposits were observed on the tonoplast of epidermal cells of the outer integument of the lintless mutant. A representative result is presented in Figure 52.

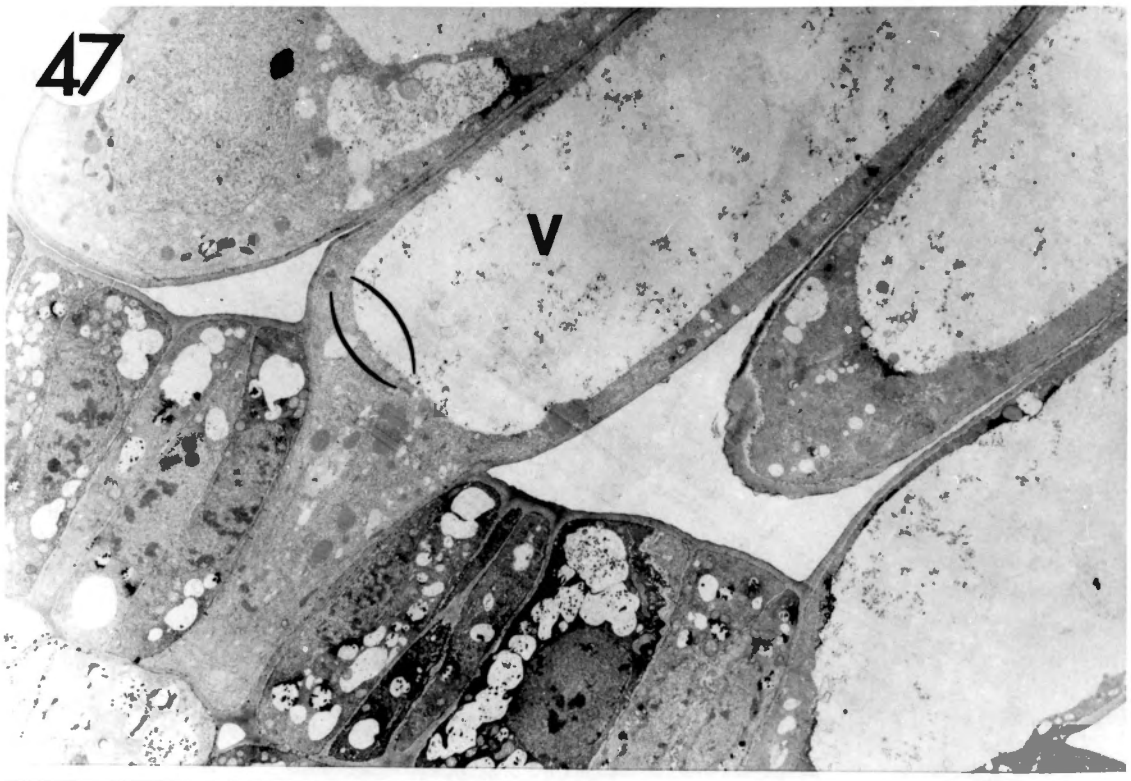
Discussion

There are numerous reports in the literature where enzyme localization has been successfully achieved

Figure 47. Enzyme inhibitor control shows no lead phosphate deposits on tonoplast of fiber. DCCD pretreated, DCCD plus ATP incubated, poststained with UA and LC. x 3,000

Figure 48. Enzyme inhibitor control. Magnified portion of tonoplast outlined in brackets in Figure 47. Note inhibition of ATPase activity on tonoplast. x 14,000

NOTE: For legend see footnote on page 31.


47
V

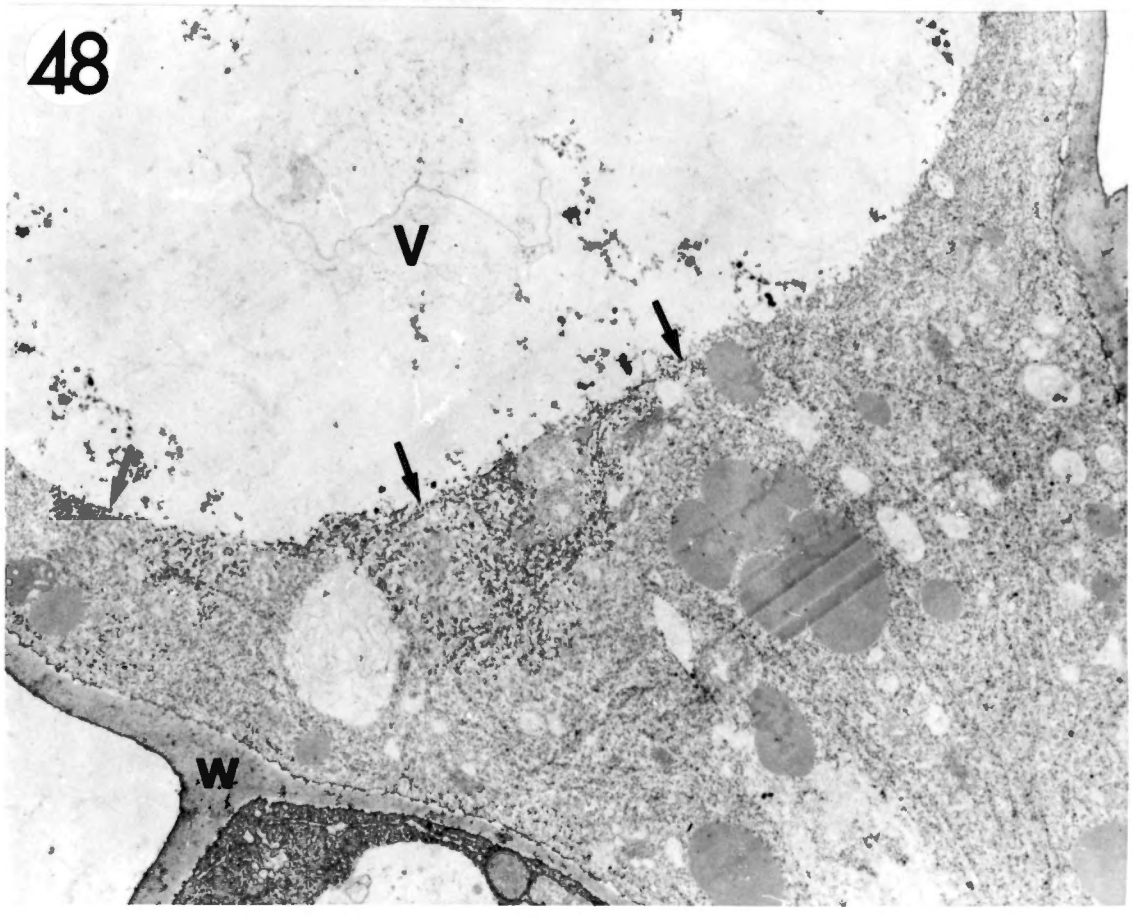
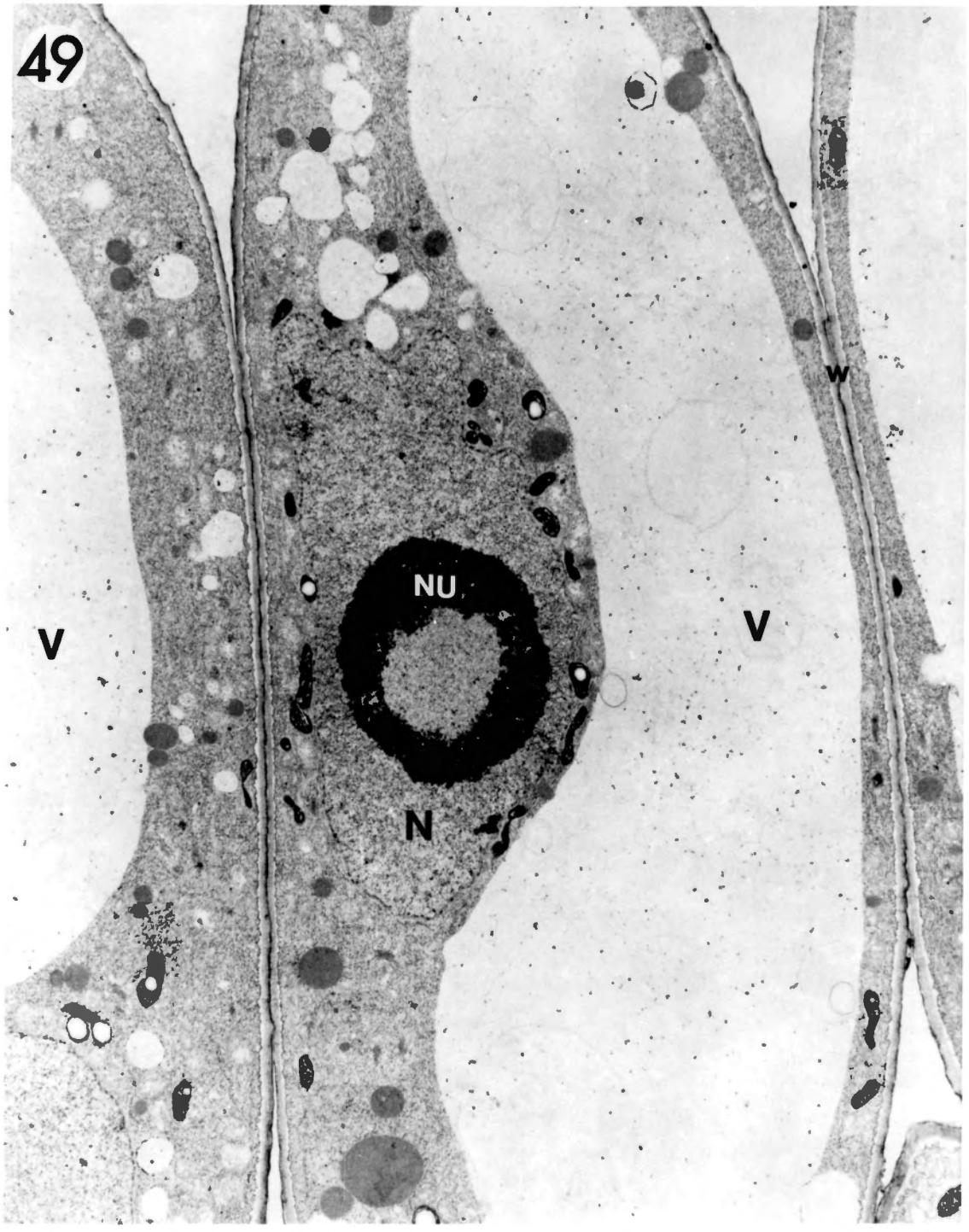

48
V
W

Figure 49. Substrate control. L. S. of three fibers shows no lead phosphate deposits on tonoplast. Incubated without ATP, poststained with UA and LC. x 7,500

NOTE: For legend see footnote on page 31.


49
NU
V
N
V
W

92
Figure 49
Joshi, 1987

Figure 50. KCl control. L. S. of two fibers near base. Note absence of lead phosphate deposits on tonoplast of large and small vacuoles. ATP (Na-salt) incubated, poststained with UA and LC. x 7,500.

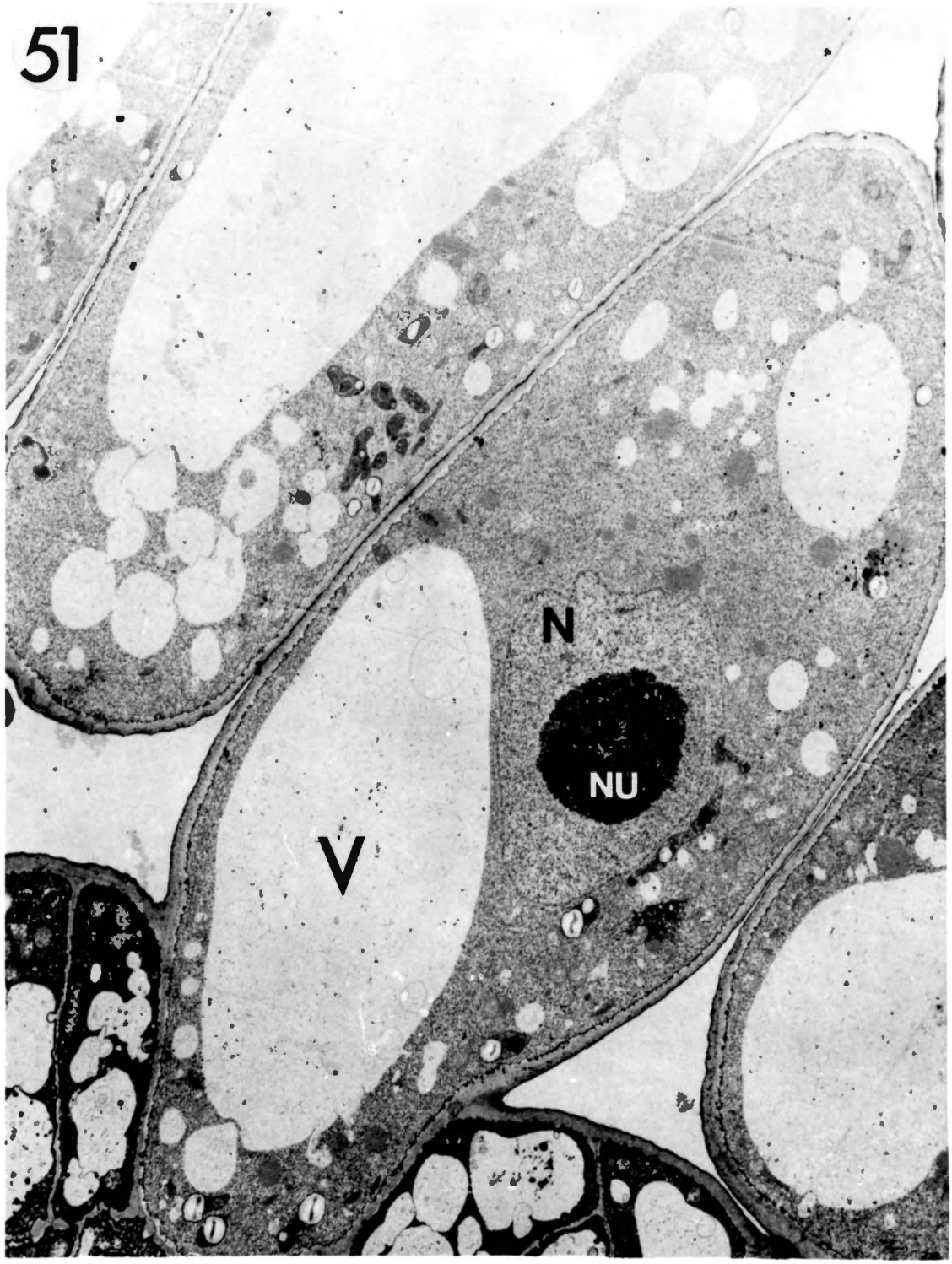
NOTE: For legend see footnote on page 31.


50
NU
V
N
W
V

94
Figure 50
Joshi, 1987

Figure 51. β-glycerophosphate substrate control. No ATPase activity was observed on tonoplast. Note non-specific phosphatase activity in cell wall and nucleolus. β-GP incubated, poststained with UA and LC. x 7,500

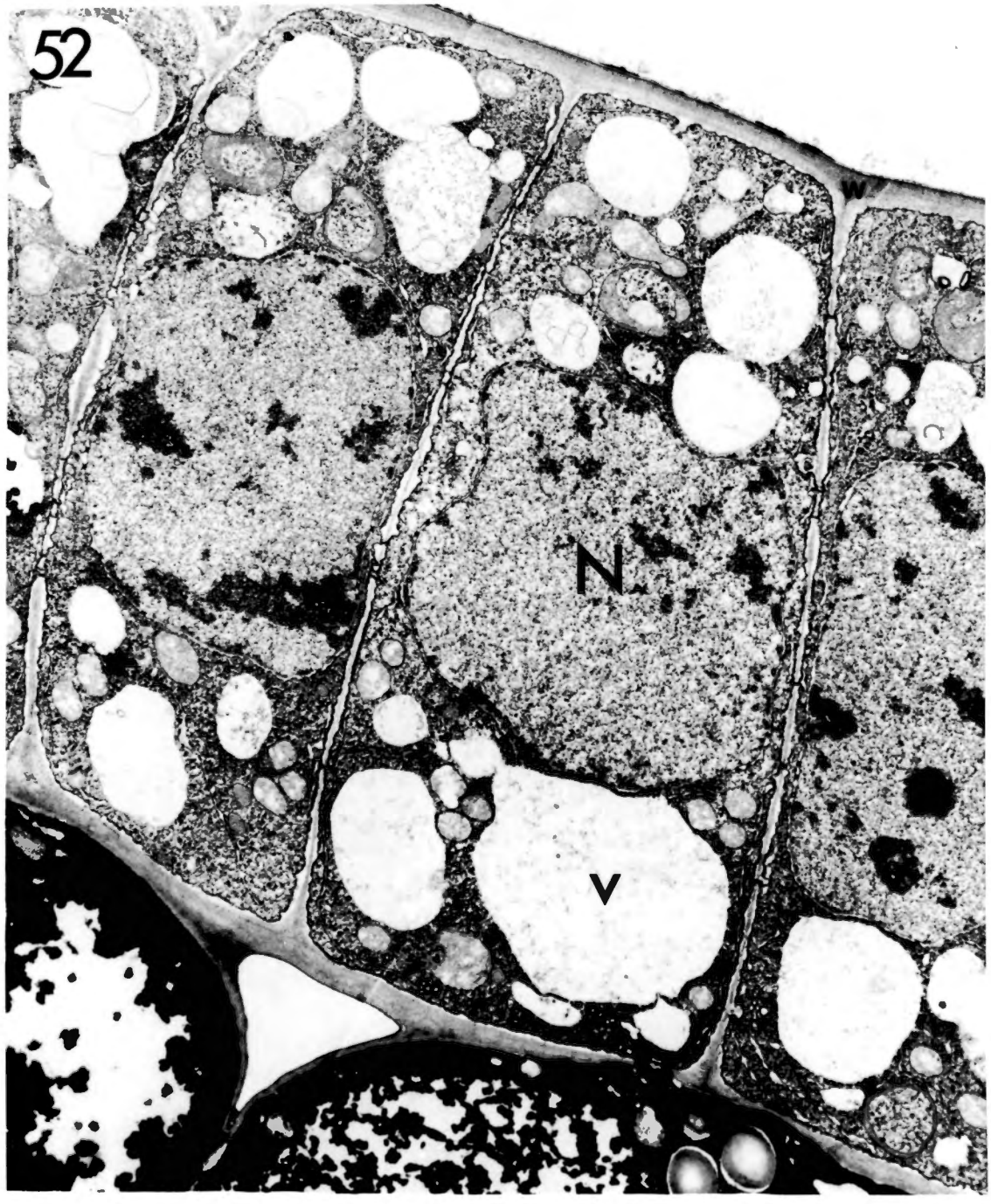
NOTE: For legend see footnote on page 31.


51
N
NU
V

96
Figure 51
Joshi, 1987

Figure 52. Lintless hybrid. No lead phosphate deposits were observed on tonoplast of non-elongating epidermal cells. ATP incubated, poststained with UA and LC. x 10,000

NOTE: For legend see footnote on page 31.


52
W
N
V

98
Figure 52
Joshi, 1987

in a number of plants (Bentwood and Cronshaw 1978; Eleftheriou and Hall 1983; Gomori 1950; Hall et al. 1980; Joshi et al. (1985). The validity of results depends upon proper controls, consideration of specific steps in enzyme cytochemistry and use of a genetic mutant line for comparison, if available (Glauert 1977; Hall 1978; Joshi et al. 1985; Winter-Sluiter et al. 1977). The present results are strengthened by the following control observations: (1) No lead phosphate deposits were observed in samples incubated in a reaction medium minus the substrate ATP and no non-specific staining due to lead in the medium was noticed. (2) The absence of deposits in the specimens incubated in a reaction medium plus the enzyme inhibitor, DCCD, agreed with the biochemical studies reported by Marin (1985). (3) No enzyme activity was observed when samples were incubated in a reaction medium without KCl but with ATP as Na^+ salt, indicating sensitivity of tonoplast ATPase to KCl as observed by Winter-Sluiter et al. (1977). (4) Incubation in a complete reaction medium with β-GP as substrate indicated no reaction product on the tonoplast but did show activity in the nucleolus and cell wall of fiber as reported by Joshi et al. (1985). (5) No enzyme activity was observed on the tonoplast of samples obtained from genetically lintless mutant cotton.

(6) Consistent tonoplast ATPase activity occurred in elongating fibers while no activity was observed in non-fiber cells or any subepidermal cells of the outer integument of the limited variety. In addition, the size of the outer integument (60-70 μm) was sufficiently small to eliminate penetration problems of the fixative and reaction media (Glauert 1977; Hall 1978). Even though all the treatments were carried out under identical conditions and all the necessary precautions were taken, we still observed that some fibers showed more intense lead phosphate deposits than others for any particular stage of fiber growth.

The results indicate the occurrence of tonoplast-ATPase activity only in those cells (fibers) which are undergoing tremendous expansion (Berlin 1986; Ramsey and Berlin 1976b) as a result of high turgor pressure, presumably created by the active transport and accumulation of ions (K^+) and solutes (malate) in these cells (Basra and Malik 1983, 1985; Dhindsa et al. 1975). No enzyme activity was observed on the tonoplast before or at anthesis, but as fiber cells started elongating enzyme activity appeared on the tonoplast of enlarging vacuoles and on small vacuoles, presumably derived either from Golgi bodies or ER (Berlin 1986). Absence of tonoplast-ATPase activity before or at anthesis in non-fiber

epidermal cells, in subepidermal cells of the outer integument, in the fiberless mutant or in the controls suggest that enzyme activity is associated with the fiber elongation or expansion process and with active transport of osmotically active substances (K^+ or malate) into the vacuoles.

Expansion or elongation of the cell wall may be a dual phase process which requires (1) IAA for stretchability of juvenile walls and (2) accumulation of solutes for maintenance of turgor pressure (Cleland 1971, 1977). According to the above theory stretching under turgor pressure (elongation phase) is primarily a physical process and is directly related to the water balance of the cell. In order to have high pressure potential in the cell, osmotically active components must be taken up or produced by the cell. In general, K^+ and malate account for the osmotically active agent in the cell vacuole (Allaway 1973; Basra and Malik 1983, 1985; Dayanandan and Kaufman 1975; Dhindsa et al. 1975; Fischer 1971; Fujino 1967; Grandman 1977; Haschke and Luttge 1975a,b; Hsiao 1976). From our results it seems appropriate to suggest that there exists an ATP-dependent proton pump which establishes an electrochemical gradient across the tonoplast membrane. This proton-electrochemical gradient may couple with ion solute transport

(Bennett and Spanswick 1983; Chrestin et al. 1985; Marin and Chrestin 1985). Tonoplast ATPase in cotton fiber may maintain the proton gradient (Wagner and Lin 1982) or may regulate intracellular pH (Chrestin et al. 1985; Raven and Smith 1979). If this is the case, then the regulation of intracellular pH may facilitate optimum pH for the enzyme (PEP-Case, pH 8.0) required for malic acid synthesis (Dymock et al. 1977; Haschke and Luttge 1975a,b).

The appearance of ATPase activity on the membranes of the central vacuole and small vesicles merging into the large one suggest that osmotically active substances might be collected and transported through small vesicles (Berlin 1986). Fusion of Golgi vesicles and ER with tonoplast seem to be major factors in the contribution of additional membrane components to the expanding vacuole. Association of lead phosphate deposits with ER in the form of beads and frequent fusion of ER-derived beads with the tonoplast suggest an organized process of ER-originating ATPase activity and then transferring it to the tonoplast of elongating fibers. Absence of enzyme activity in samples incubated in reaction medium without KCl but with ATP as the sodium salt indicate that tonoplast-ATPase activity in cotton

fiber is sensitive to KCl (Churchill and Sze 1984; Walker and Leigh 1981).

In summary, the physiology of fiber growth clearly corresponds to the theory of osmoregulation in which ATPase activity would be the source of energy for the active transport of K^+ and malate by establishing an electrochemical gradient, PEP-Case would be the source of malate, and the central vacuole would be the site of K^+ and malate accumulation (against their concentration gradient) to maintain turgor pressure in an elongating cotton fiber (Basra and Malik 1983, 1985; Dhindsa et al. 1975; Luttge and Higinbotham 1979; Uribe and Luttge 1984). The present cytochemical investigation provides strong support to the above theory by demonstrating the occurrence of ATPase activity on the tonoplast of expanding fibers.

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation consisted of an ultrastructural cytochemical localization of β-glycerophosphatase (β-GPase) activity during fiber differentiation and adenosine triphosphatase (ATPase) activity during fiber elongation in the outer integument of a linted cultivar of cotton and a lintless (naked seed) mutant. The purpose of this study was to demonstrate precisely with electron micrographs the distribution of these two enzymes during early stages of fiber development, and to correlate their possible functional role with differentiation and elongation of cotton fiber. The distribution of these two enzymes activities was achieved by using the principle of simultaneous heavy metal capture reaction technique. The results for the entire study were verified by including proper controls. Further, the results were compared among the different types of tissue in the outer integument and with a lintless mutant.

No β-GPase activity was observed in the lintless line or in any control in the linted cultivar at any stage of fiber development. In the linted cultivar there was no enzyme activity before anthesis, but as fibers were

initiated on the day of anthesis, enzyme activity was observed in the expanding fiber cell wall and nucleus. Most enzyme activity was associated with the distal cytoplasm of fiber initials and with the cell walls, both of the differentiating fibers and the newly dividing epidermal cells. Lead phosphate deposits in the cytoplasm were mainly associated with the endoplasmic reticulum (ER) and its related vesicles. Within the cell wall, β-GPase activity was mainly concentrated immediately outside the plasmamembrane. Further, as the fiber started elongating (one day postanthesis) the enzyme activity was predominantly occurring in the cell wall, nucleus and nucleolus. No enzyme activity was observed in the nucleus, nucleolus or cell walls of non-fiber cells of the epidermis at that particular stage. No lead phosphate deposits or very few deposits were observed in the samples incubated with other substrates. By two days postanthesis, β-GPase activity was decreasing in the cell wall and nucleus, but was mainly concentrated in the nucleolus only. The nucleolus reached its maximum size by three days postanthesis and along with these anatomical changes maximum β-GPase activity was observed in the nucleolus. Again, no similar enzyme activity was seen in the non-fiber epidermal cells, any sub-epidermal cell of the outer integument or in any control. The enzyme inhibitor, NaF, completely inhibited

enzyme activity. No non-specific staining due to lead in the incubation medium was observed during the entire study. No interference of osmium tetroxide during post-fixation was observed. Samples incubated without the substrate β-glycerophosphate did not show any activity at any stage of fiber development.

The results of this investigation clearly indicate that β-GPase activity was mainly occurring only in the epidermal layer of the linted cultivar. Specifically, the enzyme activity was associated with differentiating fiber cells and with newly dividing cells. Interestingly, β-GPase activity was observed only at those sites where distinct anatomical changes were occurring during fiber differentiation. Even though the specific function of β-GPase activity in plant tissue is not clear, the precise occurrence of it during these anatomical changes in differentiating fibers and not in any other cells definitely indicates some relationship with the differentiation process. The consistent presence of β-GPase activity in the cytoplasm, specifically near the tip region appears to indicate a definite relationship between enzyme activity and directional growth of the fiber initials towards the micropylar region of the cotton ovule. The occurrence of β-GPase activity in the cell wall, nucleus, and nucleolus suggests that it may be

involved in cell wall metabolism by cell wall loosening, in dephosphorylation of nuclear protein and in increased capacity for ribosome synthesis during fiber development, respectively. From the results of this study it is concluded that enzyme activity was not a precursor to, but coincided with these changes of fiber development.

The results of cytochemical localization of ATPase activity during fiber elongation clearly indicated that the enzyme activity was mainly present only in those cells which were undergoing tremendous expansion as a result of high turgor pressure, presumably created by the active transport and accumulation of ions (K^+) and solutes (malate) in the vacuole of these cells.

No ATPase activity was observed in the outer integument of the lintless hybrid line. In the linted cultivar no ATP-specific enzyme activity was seen in non-elongating epidermal cells, sub-epidermal cells of the outer integument, any control, plasmamembrane of fiber cells or in fuzz cells. Neither samples treated with enzyme inhibitor nor samples incubated without substrate showed any lead phosphate deposits or non-specific staining due to lead in the reaction medium. Also, samples incubated in a reaction medium without KCl but ATP as the Na^+ salt, did not show any sign of lead phosphate deposits. No ATPase activity was found before or at anthesis, but as

fiber initials started elongating, enzyme activity gradually appeared on the tonoplast of enlarging vacuoles. Heavier lead phosphate deposits were observed on the tonoplast of small vacuoles compared with the tonoplast of central large vacuoles. This enzyme activity continued at 10 days postanthesis.

Earlier studies have demonstrated the accumulation and need for potassium and malate in fiber cell development. The presence of ATPase activity on the tonoplast of fiber cells clearly indicates the active transport of presumably these substances into the vacuoles of elongating fiber cells. The next question arises regarding the sources of potassium and malate. Phosphoenolpyruvate carboxylase (PEP-Case) has been shown in biochemical analysis of cotton fiber. However, the precise ultrastructural site of activity and whether it may occur apart from fiber in other cells of the ovular integument are facts yet to be discovered. Further, it may be interesting to investigate the influence of CO_2, GA and IAA, since these factors play critical roles in fiber development. Considering these facts, cotton fiber will be an important model to establish the histochemical technique for PEP-Case in higher plants since such a technique for higher plants is not available yet.

Future study on the histochemical localization of potassium ions; and ATPase and PEP-Case in the outer and inner integument in addition to ultrastructural studies of the vascular structure organization within the cotton ovule, will bridge the existing lack of knowledge regarding precise sites of enzyme activity, types of transport mechanism and their physiological role during fiber development.

LIST OF REFERENCES

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Aiyangar, G. S., 1951. Origin and development of lint and fuzz in cotton. Ind. J. Agr. Sci. 214: 293-312.

Allaway, W. G., 1973. Accumulation of malate in guard cells of Vicia faba during stomatal opening. Planta 110: 63-70.

Anderson, D. B., Kerr, T., 1938. Growth and structure of cotton fiber. Ind. Eng. Chem. 30: 48-54.

Arnison, P. G., 1980. A redox model of the mechanism of action of Indole Acetic Acid (auxin) and other plant growth regulators. Spec. in Sci. and Tech. 3(1): 5-15.

Ashley, D. A., Goodson, R. D., 1972. Effect of time and K^+ status on ^{14}C-labeled photosynthate movement in cotton. Crop Sci. 12: 686-690.

Avers, C. J., 1958. Histochemical localization of enzyme activity in the root epidermis of Phleum pratense. Amer. J. Bot. 45: 609-613.

Avers, C. J., Grimm, R. B., 1959. Comparative enzyme differentiation in grass roots. I. Acid phosphatase. Amer. J. Bot. 46: 190-193.

Ayyangar, G. S., 1948. Some observations on stomata found on cotton ovules. Ind. Cott. Rev. (Bombay) 2: 187-192.

Baert, E., DeLange, E., Waterkeyn, L., 1975. In vitro culture of cotton ovules. III. Influence of growth hormones upon fiber development. La Cellule 71: 55-63.

Balls, W. L., 1915. The development and properties of raw cotton. A. and C. Black Ltd., London.

Balls, W. L., 1919. The cotton plant in Egypt. London.

Barka, T., Anderson, P. J., 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J. Histochem. Cytochem. 10: 741-753.

Barlow, P., 1970. Vacuoles in the nucleoli of Zea mays root apices and their possible significance in nucleolar physiology. Caryologia 23: 61.

Barrit, N. W., 1929. The structure of the seed coat in Gossypium and its relation to the growth and nutrition of the lint hairs. Ann. Bot. XLIII: 483-491.

Basra, A. S., Malik, C. P., 1983. Dark metabolism of CO_2 during fiber elongation of two cottons differing in fiber lengths. J. Exp. Bot. 24: 1-9.

Basra, A. S., Malik, C. P., 1985. Development of cotton fiber. Int. Rev. Cyto. 89: 65-113.

Beasley, C. A., 1973. Hormonal regulation of growth in unfertilized cotton ovules. Science 179: 1003-1005.

Beasley, C. A., Ting, I. P., 1973. The effect of plant growth substances on in vitro fiber development from fertilized cotton ovules. Amer. J. Bot. 60: 130-139.

Beasley, C. A., Ting, I. P., 1974. The effect of plant growth substances on in vitro fiber development from unfertilized cotton ovules. Amer. J. Bot. 61: 188-194.

Beasley, C. A., Ting, I. P., Linkins, A. E., Birnbaum, E. H., Delmer, D. P., 1974. Cotton ovule culture: A review of progress and a preview of potential. In: Tissue Culture and Plant Science (Street, H. E., ed.), 169-192. London: Academic Press.

Bennett, A. B., 1985. Anion-sensitive H^+-ATPase from higher plant cells: The role of chloride in stimulating proton transport. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Plants (Marin, B. P., ed.), p. 175, New York: Springer-Verlag.

Bennett, A. B., Spanswick, R. M., 1983. Optical measurements of ΔpH and $\Delta\Psi$ in corn roots membrane vesicles: Kinetic analysis of Cl^- effects on a proton-translocating ATPase. J. Membr. Biol. 71: 95-107.

Bennett, A. B., Spanswick, R. M., 1984. H^+-ATPase activity from storage tissue of Beta vulgaris: II. H^+/ATP stochiometry of an anion-sensitive H^+-ATPase. Plant Physiol. 74: 545-548.

Bennett, A. B., O'Neill, S. D., Spanswick, R. M., 1984. H^+-ATPase activity from storage tissue of Beta vulgaris: I. Identification and characterization of an anion-sensitive H^+-ATPase. Plant Physiol. 74: 538-544.

Bennett, O. L., Erie, L. J., Mackenzie, A. J., 1967. Boll, fiber and spinning properties of cotton as affected by management practices. U. S. Department of Agriculture, Technical Bulletin 1372: 109.

Bennett, O. L., Rouse, R. D., Ashley, D. A., Doss, B. D., 1965. Yield, fiber quality and potassium content of irrigated cotton plants as affected by rates of potassium. Agron. J. 57: 206-299.

Bentwood, B. J., Cronshaw, J., 1978. Cytochemical localization of adenosine triphosphatase in the phloem of Pisum sativum and its relation to the function of transfer cells. Planta 140: 111-120.

Berlin, J. D., 1986. The outer epidermis of the cotton-seed. In: Cotton Physiology, (Mauney, J. R. and Steward, J. M., eds.), Memphis, TN: Cotton Foundation, (in press).

Bhardwaj, J. S., Lad, B. L., 1977. Hormonal regulation of fiber elongation in lintless and linted genotypes of desi cotton (Gossypium arboreum L.). Ind. J. Plant Physiol. 20: 140-150.

Bhardwaj, S. J., Sharma, P. H., 1971. Influence of IAA and GA_3 on fiber properties and growth of cotton bolls. Ind. J. Agric. Sci. 41: 524-527.

Bhojwani, S. S., Power, J. B., Cocking, E. C., 1977. Isolation, culture and division of cotton callus protoplast. Plant Sci. Lett. 8: 85-89.

Bitensky, L., 1963. Modification to the Gomori acid phosphatase technique for controlled temperature frozen sections. Quart. J. Micr. Sci. 104: 193.

Bonner, J., 1965. The Molecular Biology of Development. Oxford: Clarendon Press.

Bowman, B. J., Mainzer, S. E., Allen, K. E., Slayman, C. W., 1978. Effects of inhibitors on the plasma membrane and mitochondrial adenosine triphosphatase of Neurospora crassa. Biophys. Acta 512: 13-28.

Chrestin, H., Gidrol, X., d'Auzac, J., Jacob, J. L., Marin, B. P., 1985. Cooperation of a "Davies Type" biochemical pH-stat and tonoplastic bioosmotic pH-stat in the regulation of the cystolic pH of Hevea latex. In: Biochemistry and Function of Vascular Adenosine Triphosphatase in Fungi and Plants, (Marin, B. P., ed.), p. 245. New York: Springer-Verlag.

Churchill, K. A., Sze, H., 1984. Anion-sensitive, H^+-pumping ATPase of oat roots. Plant Physiol. 76: 490-497.

Cleland, R., 1971. Cell wall extension. Ann. Rev. Plant Physiol. 22: 197-222.

Cleland, R., 1977. The control of cell enlargement. In: Integration of Activity in Higher Plants. Symp. XXXI of the Society for Experimental Biology. Cambridge University Press.

Cleland, R., 1981. Wall extensibility: Hormones and wall extension. In: Encyclopedia of Plant Physiol. New Ser. Plant Carbohydrates II. (Tanner, W., Loewus, F. A., eds.), 1313: 255-73, Berlin: Springer-Verlag.

Cleland, R., Rayle, D. L., 1972. Absence of auxin-induced stored growth in Avena coleoptile and its implications concerning the mechanism of wall extension. Planta 106: 61-71.

Coartney, J. S., Morre, D. J., 1980. Studies on the chemical basis of auxin induced cell wall loosening. Bot. Gaz. 141: 63-68.

Cocking, E. C., 1960. A method for the isolation of plant protoplasts and vacuoles. Nature 187: 962-963.

Cocking, E. C., Gregory, D. W., 1963. Organized protoplasmic units of the plant cell. I. Their occurrence, origin and structure. J. Exp. Bot. 14: 504-511.

Cram, W. J., 1976. Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Transport in plants II part A cells: Encyclopedia Plant Phys. NS Vol. 2. (Luttge, U., Pitman, M. G., eds.), 284, New York, Springer, Berlin, Heidelberg.

Cutter, E. G., Feldman, L. J., 1970. Trichoblasts in Hydrocharis. I. Origin, differentiation, dimensions and growth. Amer. J. Bot. 57: 190-201.

Czernik, C. A., Avers, C. J., 1964. Phosphatase activity and cellular differentiation in Phleum root meristem. Amer. J. Bot. 51: 424-431.

Dayanandan, P., Kaufman, P. B., 1975. Stomatal movements associated with potassium fluxes. Amer. J. Bot. 62: 221-231.

DeLanghe, E., Kosmidou-Dimitropoulou, S., Waterkeyn, L., 1978. Effects of hormones on nucleolar growth and vacuolation in elongating cotton fibers. Planta 140: 269-273.

Delhez, J., Dufour, J. P., Thines, D., Goffeau, A., 1977. Comparison of the properties of plasmamembrane-bound and mitochondria-bound ATPase in the yeast Schizosaccharomyces cerevisiae. Eur. J. Biochem. 79: 319-328.

Delmer, D. P., Heiniger, U., Kulow, C., 1977. UDP-glucose:Glucan synthetase in developing cotton fiber. I. Kinetics and physiological properties. Plant Physiol. 59: 713-718.

De Jong, D. W., Jansen, E. F., Olson, A. C., 1967a. Oxidoreductive and hydrolytic enzyme patterns in plant suspension culture cells. Exp. Cell Res. 47: 139-156.

De Jong, D. W., Olson, A. C., Jansen, E. F., 1967b. Glutaraldehyde activation of nuclear acid phosphatase in cultured plant cells. Science 155: 1672-1674.

Dhindsa, R. S., 1978. Hormonal regulation of enzymes of nonautotrophic CO_2 fixation in unfertilized cotton ovules. Z. Pflanzenphysiol. Bd. 89: 355-362.

Dhindsa, R. S., Beasley, C. A., Ting, I. P., 1975. Osmoregulation in cotton fiber: accumulation of potassium and malate during growth. Plant Physiol. 56: 394-398.

Dhindsa, R. S., Beasley, C. A., Ting, I. P., 1976. Effect of abscisic acid on in vitro growth of cotton fiber. Planta 130: 197-201.

Dosier, L. W., Riopel, J. L., 1977. Differential enzyme activity during trichoblast differentiation in Elodea canadensis. Amer. J. Bot. 64: 1049-1056.

Dufour, J. P., Goffeau, A., 1980. Molecular and kinetic properties of the purified plasma membrane ATPase of the yeast Schizosaccharomyces pombe. Eur. J. Biochem. 105: 145-154.

Dymock, I. J., Hill, B., Bown, A. W., 1977. An investigation into the influence of IAA and malate on in vivo and in vitro rates of dark carbon dioxide fixation in coleoptile tissue. Can. J. Bot. 55: 1641-1645.

Eleftheriou, E. P., Hall, J. L., 1983. The extrafloral nectaries of cotton. II. Cytochemical localization of ATPase activity and selective osmium impregnation. J. Exper. Bot. 34: 1066-1079.

Esau, K., 1977. Anatomy of Seed Plants. John Wiley and Sons, New York.

Evans, H. J., Sorger, G., 1966. Role of mineral elements with emphasis on the univalent cations. Ann. Rev. Plant Physiol. 17: 47-76.

Fan, D. F., Maclachlan, G. A., 1966. Control of cellulase activity by indoleacetic acid. Can. J. Bot. 44: 1025-1034.

Farr, W. K., 1931. Cotton fibers. I. Origin and early stages of elongation. Contributions from Boyce Thompson Institute. 3: 441-458.

Farr, W. K., 1933. Cotton fibers. III. Cell division in the epidermal layer of the ovules subsequent to fertilization. Contributions from Boyce Thompson Institute. 5: 167-172.

Finer, J. J., Smith, R. H., 1982. Isolation and culture of protoplasts from cotton (Gossypium Klotzschianum Anderss) callus culture. Plant Sci. Lett. 26: 147-151.

Firth, J. A., 1978. Cytochemical approaches to the localization of specific adenosine triphosphatase. Histochem. J. 10: 253-269.

Fischer, R. A., 1971. Role of potassium in stomatal opening in the leaf of Vicia faba. Plant Physiol. 47: 555-558.

Forsket, D. E., Miksche, J. P., 1966. A histochemical study of the seedling shoot apical meristem of Pinus lambertiana. Amer. J. Bot. 53: 694-702.

Fryxell, P., 1978. Natural History of Cotton Tribe. College Station. Texas A and M Press.

Fujino, M., 1967. Role of adenosine triphosphate and adenosine triphosphatase in stomatal movement. Sci. Bull. Fac. Educ., Nagasaki Univ. 18: 1-47.

Gahan, P. B., 1965. Histochemical evidence for the presence of lysosome-like particles in root meristem cells of Vicia faba. J. Exp. Bot. 16: 350-355.

Gahan, P. B., Maple, A. J., 1966. The behavior of lysosome-like particles during cell differentiation. J. Exp. Bot. 17: 151-155.

Galan-Cano, J., Risueno, M. C., Gimenez-Martin, G., 1975. Nucleolar ultrastructure in megaspore mother cells of Pisum sativum L. Cytobiologie 10: 475.

Gidrol, X., Marin, B., Chrestin, J., D'Auzac, J., 1984. Comparison of Hevea tonoplast adenosine-triphosphatase from freshly isolated vacuoles and lypohilized tonoplast vesicles. Z. Pflanzenphysiol. 114: 279-284.

Gidrol, X., Marin, B., Chrestin, H., D'Auzac, J., 1985. The functioning of tonoplast H^+-translocating ATPase from Hevea latex in physiological conditions. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Plants (Marin, B. P., ed.), p. 151. New York: Springer-Verlag.

Gilder, J., Cronshaw, J., 1974. A biochemical and cytochemical study of adenosine triphosphatase activity in the phloem of Nicotiana tabacum. J. Cell Biol. 60: 221-235.

Glauert, A. M., 1977. Practical Methods in Electron Microscopy. Vol. 5: Amsterdam, North-Holland Publ. Co.

Gomori, G., 1950. An improved histochemical technique for acid phosphatase. Stain Technol. 25: 81-85.

Gomori, G., 1952. Microscopic Histochemistry: Principles and Practice. Illinois: Univ. Chicago Press.

Grandmann, D., 1977. Potassium and turgor pressure in plants. J. Theor. Biol. 65: 597-599.

Gulati, A. M., 1930. A note on the differentiation of hairs from the epidermis of cotton seeds. Agric. J. India. XXV, 313-316.

Hager, A., Helmle, M., 1981. Properties of an ATP-fueled, Cl^- dependent proton pump localized in membranes of microsomal vesicles from maize coleoptile. Z. Naturfursch 36C: 997-1008.

Hager, A., Hermsdorf, P., 1981. A H^+-Ca^{++} antiporter in membranes of microsomal vesicles from maize coleoptiles, a secondary energized Ca^{++} pump. Z. Naturfursch 36C: 1009-1012.

Hager, A., Menzel, H., Krauss, A., 1971. Experiments and hypothesis concerning the primary action of auxin in elongation growth. Planta 100: 47-75.

Hall, J. L., 1969. Histochemical localization of β-glycerophosphatase activity in young root tips. Ann. Bot. 33: 399-406.

Hall, J. L., 1978. Electron Microscopy and Cytochemistry of Plant Cells. Amsterdam: Elsevier/North-Holland Biomedical Press.

Hall, J. L., Butt, V. S., 1968. Localization and kinetic properties of β-glycerophosphatase in barley roots. J. Exp. Bot. 19: 276-287.

Hall, J. L., Butt, V. S., 1969. Adenosine triphosphatase activity in cell wall preparations and excised roots of barley. J. Exp. Bot. 20: 751-762.

Hall, J. L., Davie, C. A. M., 1971. Localization of acid hydrolase activity in Zea mays L. root tips. Ann. Bot. 35: 849-855.

Hall, J. L., Browning, A. J., Harvey, D. M. R., 1980. The validity of the lead precipitation technique for the localization of ATPase activity in plant cells. Protoplasma 104: 193-200.

Hayat, M. A., 1981a. Principles and Techniques of Electron Microscopy. Biological Applications. Vol 1: Baltimore, University Park Press.

Hayat, M. A., 1981b. Fixation for Electron Microscopy. New York: Academic Press.

Halperin, W., 1969. Ultrastructural localization of acid phosphatase in cultured cells of Daucus carota. Planta 88: 91-102.

Haschke, H. P., Luttge, U., 1975a. Interactions between IAA, potassium, and malate accumulation, and growth in Avena coleoptile segments. Z. Pflanzenphysiol. 76: 450-455.

Haschke, H. P., Luttge, U., 1975b. Stochiometric correlation of malate accumulation with auxin-dependent K^+-H^+ exchange and growth in Avena coleoptile segments. Plant Physiol. 56: 696-698.

Haschke, H. P., Luttge, U., 1977a. Action of auxin on CO_2 dark fixation in Avena coleoptile segments as related to elongation growth. Plant Sci. Lett. 8: 53-58.

Haschke, H. P., Luttge, U., 1977b. Auxin action on K^+- H^+ exchange and growth. $^{14}CO_2$ fixation and malate accumulation in Avena coleoptile segments. In: Regulation of Cell Membrane Activities in Plants. (Marre, E., Ciferri, O., eds.), 243-248. Amsterdam: Elsevier/North Holland Biomedical Press.

Helgerson, S. L., Cramer, W. A., Morre, D. J., 1976. Evidence for an increase in microviscosity of plasma membrane from soybean hypocotyls induced by the plant hormone, indole-3-acetic acid. Plant Physiol. 58: 548-551.

Hodges, T. K., 1976. ATPase associated with membranes of plant cells. Encyc. Plant Physiol. 2A: 260-283.

Hsiao, T. C., 1976. Stomatal ion transport. Encyc. Plant Physiol. 2B: 195-221.

Hugon, J. S., 1973. Lead salt methods. In: Electron Microscopy and Cytochemistry (Wisse, E., Daems, W. Th., Molenaar, I., Van Duijn, P., eds.), p. 29. Amsterdam North Holland Pub. Co.

Huwyler, H. R., Franz, G., Meier, H., 1979. Changes in the composition of cotton fiber cell wall during development. Planta. 146: 635-642.

Ingebritsen, T. S., Cohen, P., 1983. Protein phosphatases: Properties and role in cellular regulation. Science 221: 331-338.

Itoh, T., 1974. Fine structure and formation of cell wall of developing cotton fiber. Wood Res. 56: 49-61.

Jasdanwala, R. T., Singh, Y. D., Chinoy, J. J., 1977. Auxin metabolism in developing cotton hairs. J. Exp. Bot. 28: 1111-1116.

Jensen, W. A., 1956. The cytochemical localization of acid phosphatase in root tip cells. Amer. J. Bot. 43: 50-54.

Jensen, W. A., 1968. Cotton embryogenesis. The zygote. Planta 79: 346-366.

Joshi, P. C., Wadhwani, A. M., Johri, B. M., 1967. Morphological and embryological studies of Gossypium L., Proc. Nat. Inst. Sci. India 33: 37-93.

Joshi, P. A., Stewart, J. M., Graham, E. T., 1985. Localization of β-glycerophosphatase activity in cotton fiber during differentiation. Protoplasma 125: 75-85.

Keegstra, K., Talmadge, K. W., Bauer, W. D., Albersheim, P., 1973: The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnection of the macromolecular components. Plant Physiol. 51: 188-197.

Key, J. L., 1964. Ribonucleic acid and protein synthesis as essential processes for cell elongation. Plant Physiol. 39: 365-370.

Key, J. L., 1969. Hormone and nucleic acid metabolism. Ann. Rev. Plant Physiol. 20: 449-475.

Komor, E., Thom, M., 1985. Transport and energization in vacuole of sugarcane. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Plants. (Marin, B. P., ed.), p. 238. New York: Springer-Verlag.

Kosmidou-Dimitropoulou, K., 1976. Physiological identification of cotton fiber technological characteristics: Hormonal action in fiber initiation and development. Doctoral thesis, State University of Ghent, Belgium.

Kosmidou-Dimitropoulou, K., 1986. Cotton fiber development-Hormonal influence. In: Cotton Physiology. (Mauney, J. R., Stewart, J. M., eds.), Memphis, TN: Cotton Foundation, (in press).

Krebs, E. G., Beavo, J. A., 1979. Phosphorylation-Dephosphorylation of enzymes. Ann. Rev. Biochem. 48: 923-959.

Leigh, R. A., Branton, D., 1976. Isolation of vacuoles from root storage tissue of Beta vulgaris L. Plant Physiol. 58: 656-662.

Leigh, R. A., Walker, R. R., 1985. Salt-stimulated ATPase and PPase activities associated with vacuoles from higher plants. In: Biochemistry and Function of Vascuolar Adenosine Triphosphatase in Fungi and Plants. (Marin, B. P., ed.), p. 45. New York: Springer-Verlag.

Leffler, H. R., 1986. Mineral compartmentation within the boll. In: Cotton Physiology. (Mauney, J. R., Stewart, J. M., eds.), Chapt. 21. Memphis, TN: Cotton Foundation, (in press).

Lin, W., Wagner, G. J., 1985. Isolation, properties, and functions of tonoplast ATPase from higher plants. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Higher Plants. (Marin, B. P., ed.), p. 67. New York: Springer-Verlag.

Lockhart, J. A., 1967. Physical nature of irreversible deformation of plant cells. Plant Physiol. 42: 1545-1552.

Love, R., Studzinski, G. P., Walsh, R. J., 1969. Nuclear, Nucleolar and cytoplasmic acid phosphatases in cultured mammalian cells. Experimental Cell Research 58: 62-72.

Luttge, U., Ball, E., 1979. Electrochemical investigation of active malic acid transport at the tonoplast into the vacuoles of the CAM plant Kalanchoe daigremontiana. J. Membrane Biol. 47: 401-422.

Luttge, U., Higinbotham, M., 1979. Transport in Plants. New York: Springer-Verlag.

Luttge, U., Smith, J. A. C., 1985. Transport of malic acid in cells of CAM plants. In: Biochemistry and Function of Vascular Adenosine Triphosphatase in Fungi and Plant Plants. (Marin, B. P., ed.), p. 227. New York: Springer-Verlag.

Maier, K., Maier, U., 1972. Localization of β-glycerophosphatase and Mg^{++} activated adenosine triphosphatase in moss haustorium, and the relation of these enzymes to the cell wall labyrinth. Protoplasma 75: 91-112.

Marin, B., 1983. Sensitivity of tonoplast-bound adenosine-triphosphatase from Hevea to inhibitors. Plant Physiol. 73: 973-977.

Marin, B., 1985. Comparative analysis of the properties of tonoplast-bound adenosine-triphosphatase from fungi and higher plants: pitfall and artifacts in the search for vacuolar ATPase and proton pump. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Plants. (Marin, B. P., ed.), p. 31. New York: Springer-Verlag.

Marin, B., Chrestin, H., 1985. Compartmentation of solutes and the role of tonoplast ATPase in Hevea latex. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Plants. (Marin, B. P., ed.), p. 212. New York: Springer-Verlag.

Marty, F., 1985. Analytical characterization of vacuolar membranes from higher plants. In: Biochemistry and Function of Vacuolar Adenosine Triphosphatase in Fungi and Plants. (Marin, B. P., ed), p. 1. New York: Springer-Verlag.

Matile, P., 1968. Lysosome of root cells in corn seedlings. Planta 79: 181-196.

Matile, P., 1978. Biochemistry and function of vacuoles. Ann. Rev. Plant Physiol. 29: 193-213.

Mauney, J. R., 1961. The culture in vitro of immature cotton embryos. Bot. Gaz. 122: 205-209.

Mauney, J. R., 1986. Vegetative growth and development of fruiting sites. In: Cotton Physiology. (Mauney, J. R., Stewart, J. M., eds.), Chapt. 2. Memphis, TN: Cotton Foundation, (in press).

McCall, F. R., Guthrie, J. D., 1945. The organic content of raw cotton fiber: Isolation of L-malic and citric acid from cotton fiber. J. Amer. Chem. Soc. 67: 2220-2221.

Meinert, M., Delmer, D. P., 1976. Biochemical changes in cell wall composition associated with in vivo and in vitro fiber development in Gossypium hirsutum. Plant Physiol. 57: Suppl. 291 A.

Meinert, M. C., Delmer, D. P., 1977. Changes in biochemical composition of the cell wall of cotton fiber during development. Plant Physiol. 59: 1088-1097.

Mia, A. J., Pathak, S. M., 1968. A histochemical study of the shoot apical meristem of Rauwolfia with reference to differentiation of sclereids. Can J. Bot. 46: 115-120.

Mogensen, H. L., 1981. Ultrastructural localization of adenosine triphosphatase in the ovules of Saintpaulia ionantha (Gesneriaceae) and its relation to synergid function and embryo sac nutrition. Amer. J. Bot. 68: 183-194.

Moreno-Diaz de la Espina, S., Medina, F. J., Risueno, M. C., 1980. Correlation of nucleolar activity and nucleolar vacuolation in plant cells. Eur. J. Cell Biol. 22: 724-729.

Morre, D. J., Bracker, C. E., 1976. Ultrastructural alteration of plant plasma membrane induced by auxin and calcium ions. Plant Physiol. 58: 544-547.

Naithani, S. C., Rama Rao, N., Krishnan, P. N., Singh, V. D., 1981. Changes in o-diphenol oxidase during fiber development in cotton. Ann. Bot. 48: 379-385.

Nassery, H., Valamanesh, A., 1978. Membrane ATPase activity of barley roots and potassium uptake by isolated stele and cortex. Plant Physiol. 42: 428-434.

Ohsumi, Y., Anraku, Y., 1981. Active transport of basic amino acids driven by a protonmotive force in vacuolar membrane vesicles of Saccharomyces cerevisiae. J. Biol. Chem. 256: 2079-2082.

Ohsumi, Y., Anraku, Y., 1983. Calcium transport driven by a protonmotive force in vacuolar membrane vesicles of Saccharomyces cerevisiae. J. Biol. Chem. 258: 5614-5617.

O'Kelley, J. C., 1953. The use of ^{14}C in locating growth regions of cell wall of elongating cotton fibers. Plant Physiol. 28: 281-286.

Onofeghara, F. A., 1972. Histochemical localization of enzymes in Tapinanthus bengwensis: Acid phosphatase. Amer. J. Bot. 59: 549-556.

Osmond, C. B., Laties, G. G., 1969. Compartmentation of malate in relation to ion absorption in beet. Plant Physiol. 44: 7-14.

Pearse, A. G. E., 1972. Histochemistry: Theoretical and Applied, 3rd ed., Vol. II, London.

Popova, P. Y., Nuritdinova, F. N., Imamaliev, A. I., Madzhitova, K. D., 1979. Differences in phenol-compound content and character of fiber formation in wild and cultivated cotton. Sel. Sk. Nauk. Im. V. I. Leniana No. 8: 22-25.

Price, G. D., Whitecross, M. I., 1983. Cytochemical localization of ATPase activity on the plasmalemma of Chara corallina. Protoplasma 116: 65-74.

Ramsey, J. C., Berlin, J. D., 1976a. Ultrastructure of early stages of cotton fiber differentiation. Bot. Gaz. 37: 11-19.

Ramsey, J. C., Berlin, J. D., 1976b. Ultrastructure aspects of early stages in cotton fiber elongation. Amer. J. Bot. 63: 868-876.

Rao, P. S., 1923. Stomata in cotton flower. J. Madras Agri. Student Union. XI: 40-42.

Raschke, K., 1979. Movements of stomata. In: Physiology of Movements. (Haupt, W., Feinleib, M. E., eds.), Encyclopedia Plant Physiol. NS Vol. 7. p. 383. New York: Springer.

Raven, J. A., Smith, F. A., 1979. Intracellular pH and its regulation. Ann. Rev. Plant Physiol. 30: 289-311.

Rayle, D. L., Cleland, R., 1970. Enhancement of wall loosening and elongation by acid solution. Plant Physiol. 46: 250-53.

Reynolds, E. S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

Rose, R. J., Setterfield, G., Fowke, L. C., 1972. Activation of nucleoli in tuber slices and the function of nucleolar vacuoles. Expt. Cell Res. 71: 1-16.

Ryser, U., 1977. Cell wall growth in elongating cotton fibers: an autoradiographic study. Cytobiologie 15: 78-84.

Salyaev, R. K., 1985. Plant vacuole membrane: Structure and properties. In: Biochemistry and Function of Vacuolar Adenosine-Triphosphase in Fungi and Plants (Marin, B. P., ed.), p. 3. New York: Springer-Verlag.

Schubert, A. M., Benedict, C. R., Berlin, J. D., Kohel, R. J., 1973. Cotton fiber development--kinetics of cell elongation and secondary wall thickening. Crop Sci. 13: 704-709.

Schulz, P., Jensen, W. A., 1977. Cotton embryogenesis: the early development of the free nuclear endosperm. Amer. J. Bot. 64: 384-394.

Sexton, R., Cronshaw, J., Hall, J. L., 1971. A study of the biochemistry and cytochemical localization of β-glycerophosphatase activity in root tips of Maize and Pea. Protoplasma 73: 417-441.

Sexton, R., Sutcliffe, J. F., 1969. The distribution of β-glycerophosphatase in young roots of Pisum sativum L. Ann. Bot. 33: 407-419.

Sheffield, F. M. L., 1936. The early development of the cotton fiber. The Empire Cotton Growing Review 13: 277-286.

Singh, O., Singh, O. S., 1975. Effect of growth regulators on early fiber development in cotton. Indian J. Exp. Biol. 13: 411-412.

Smith, J. A. C., Uribe, E. G., Ball, E., Heuer, S., Luttge, U., 1984a. Characterization of the vacuolar ATPase activity of the crassulacean-acid-metabolism plant Kalanchoe daigremontiana. Eur. J. Biochem. 141: 415-420.

Smith, J. A. C., Uribe, E. G., Ball, E., Luttge, U., 1984b. ATPase activity associated with isolated vacuoles of the crassulacean acid metabolism plant Kalanchoe daigremontiana. Planta 162: 299-304.

Soriano, R. Z., Love, R., 1971. Electron microscopic demonstration of acid phosphatase in nucleoli and nucleoplasm. Exptl. Cell Res. 65: 467-469.

Spurr, A. R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Res. 26: 31-42.

Stewart, J. M., 1975. Fiber initiation on the cotton ovule (Gossypium hirsutum). Amer. J. Bot. 62: 723-730.

Stewart, J. M., Hsu, C. L., 1977. In-ovulo embryo culture and seedling development of cotton (Gossypium hirsutum L.) Planta 137: 113-117.

Sossountzov, L., Habricot, Y., 1985. Ultracytochemical localization and characterization of membrane-bound ATPase in lateral buds from intact and decapitated plants of an aquatic fern, Marsilea drummondii A. Br. Protoplasma 127: 180-191.

Sossountzov, L., Habricot, Y., Garrec, J. P., Lamant, A., 1985. Early effect of decapitation on the Mg^{++}-K^{+} ATPase and cation content in lateral buds of the aquatic fern Marsilea drummondii A. Br. Protoplasma 127: 192-203.

Tagawa, T., Bonner, J., 1957. Mechanical properties of the Avena coleoptile as related to auxin and to ionic interactions. Plant Physiol. 32: 207-212.

Thom, M., Komor, E., 1984. Role of the ATPase of sugar-cane vacuoles in energization of the tonoplast. Eur. J. Biochem. 138: 93-99.

Torri, K., Laties, G. G., 1966. Organic acid synthesis in response to excess cation absorption in vacuolate and non-vacuolate sections of corn and barley roots. Plant Cell Physiol. 7: 395-403.

Turner, A. J., 1929. Ginning percentage and lint index of cotton in relation to the number of cotton fibers per seed. J. Text. Inst. XX: 233-273.

Uribe, E. G., Luttge, U., 1984. Solute transport and the life functions of plants. Amer. Scientist 72: 567-573.

Van den Born, W. A., 1963. Histochemical studies of enzyme distribution in the shoot tips of white spruce. Can. J. Bot. 41: 1509-1527.

Van Duijn, P., 1973. Fundamental aspects of enzyme cytochemistry. In: Electron microscopy and Cytochemistry. (Wisse, E., Daems, W. Th., Molenaar, I., Van Duijn, P., eds.), p. 3. Amsterdam: North Holland Publ. Co.

Van Steveninck, R. F. M., 1979. The verification of cytochemical test for ATPase activity in plant cells using X-ray microanalysis. Protoplasma 99: 211-220.

Vlasova, N. A., 1971. Interaction between nucleus and cytoplasm in the ontogenesis of epidermal and fibrillar cells of cotton ovules. Ontogenez. 2: 88-99.

Wagner, G. J., Lin, W., 1982. An active proton pump of intact vacuoles isolated from Tulipa petals. Biochim. Biophys. Acta 689: 261-266.

Wagner, G. J., Mulready, P., 1983. Characterization and solubilization of nucleotide-specific Mg^{++}-ATPase and Mg^{++}-pyrophosphatase of tonoplast. Biochim. Biophys. Acta 728: 267-280.

Walker, R. R., Leigh, R. A., 1981. Characterization of a salt-stimulated ATPase activity associated with vacuoles isolated from storage roots of red beet (Beta vulgaris L.) Planta 153: 140-149.

Wardrop, A. B., 1968. Occurrence of structures with lysosome-like functions in plant cells. Nature 218: 978-980.

Waters, S. E., Butcher, R. G., 1980. Studies on the Gomori acid phosphatase reaction: The preparation of the incubation medium. Histochemical J. 12: 191-200.

Westafer, J. M., Brown Jr., R. M., 1976. Electron microscopy of cotton fiber: new observation on cell wall formation. Cytobios 15: 111-138.

Willison, J. H. M., Brown, R. M., 1977. An examination of the developing cotton fiber: Wall and plasmalemma. Protoplasma 92: 21-41.

Wilson, K. S., Cutter, Jr., V. M., 1955. Localization of acid phosphatase in the embryo sac and endosperm of Cocos nucifers. Amer. J. Bot. 42: 116-119.

Winter-Sluiter, E., Lauchli, A., Kramer, D., 1977. Cytochemical localization of K^+-stimulated adenosine triphosphatase activity in xylem parenchyma cells of barley roots. Plant Physiol. 60: 923-927.

APPENDICES

APPENDIX A

INCUBATION MEDIUM FOR BETA-GLYCEROPHOSPHATASE

Stock Solution	Volume	Final Concentration
0.2 M Tris-maleate buffer, pH 6.0	10 ml	40 mM
0.1 M Na-β-glycerophosphate	4 ml	8 mM
0.02 M Lead nitrate	6 ml	2.4 mM
Distilled water	30 ml	

APPENDIX B

INCUBATION MEDIUM FOR ADENOSINE TRIPHOSPHATASE

Components	Final Concentration
Tris-maleate buffer, pH 7.0	50.0 mM
Lead nitrate	3.6 mM
Potassium chloride	2.0 mM
Magnesium nitrate	2.0 mM
ATP- K^+ or Na^+ salt	2.0 mM

VITA

The author, Priyavadan Amritlal Joshi, was born in Mokalsar, India on August 1, 1946. After passing the S. S. C. Examination from M. M. Pupil's Own High School (Bombay), he entered M. M. D. College, The University of Bombay. He received the Bachelor of Science degree in botany and chemistry (both as a major field) in 1970, and the Master of Science degree in botany (cytogenetics) in 1973 from The University of Bombay.

Upon graduation, he was employed as a laboratory teaching instructor by the Department of Botany, Bhavan's College, The University of Bombay until he decided to continue for further studies in the U. S. A.

He entered The University of Tennessee, Knoxville, in the Fall of 1975 and was graduated with a Master of Science degree in Plant and Soil Science in December 1978. His elder brother, Dr. L. A. Joshi, provided the complete financial support for his first degree in the U. S. A. Later on, he was awarded a graduate research assistantship at The University of Tennessee, Knoxville, and he began study for a Doctor of Philosophy degree in the Department of Plant and Soil Science in the Winter of 1980.

He worked as a Laboratory Research Technician from October 1984 to October 1985 in the Department of

Ornamental Horticulture and Landscape Design. Then he continued working as a laboratory technologist in the Department of Pathobiology. He received his Ph. D. in June 1987.

He is married to the former Manju N. Trivedi of Arvi. They have a lovely daughter, Dipti.