ABSTRACT

THE ULTRASTRUCTURE AND MICROANATOMY
OF THE ROOT TIP OF PISUM SATIVUM

by Gordon C. Spink

A descriptive study of the first millimeter of the seedling root tip of Pisum sativum var. Alaska was undertaken. Although the pea root has no distinct areas, it was sub-divided into seven regions for analysis: quiescent center, cortex, central cylinder, epidermis, columella, tip-cells, and epidermis-root-cap-complex.

The tissue was fixed, dehydrated and embedded according to standard electron microscopy techniques. Thin sections (400 to 1,000 Angstroms) were observed in the electron microscope and thick sections (½ to 10 microns) were observed in the phase microscope. Electron microscope sections were related to the complete root picture. Autoradiography of paraffin sections of tritiated thymidine treated cells were made to observe the quiescent center, as described by Clowes (1961).

THE ULTRASTRUCTURE AND MICROANATOMY OF THE ROOT TIP OF *PISUM SATIVUM*

By

Gordon C. Spink

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1966

Gen 493
10-15-66

ACKNOWLEDGEMENTS

I wish to sincerely thank Dr. G. B. Wilson for his guidance and help; Mrs. June P. Mack for her technical assistance and cooperation; Dr. Lee V. Leak, formerly of Michigan State University and now at Massachusetts General Hospital, Boston, for his help and advice with electron microscopy; and my wife, Jane, for her understanding.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	iv
LIST OF TABLES	vi
INTRODUCTION	1
DEFINITIONS	3
LITERATURE REVIEW	6
MATERIALS AND METHODS	9
OBSERVATIONS	13
DISCUSSION	35
SUMMARY	46
FIGURES	47
BIBLIOGRAPHY	88

LIST OF FIGURES

Figure	Page
1.--Phase microscope montage photograph of the first millimeter of root tip	48
2.--Quiescent center	50
3.--Quiescent center	52
4.--Quiescent center	54
5.--Cross-sectional montage of cortex and central cylinder	56
6.--Cortex cells	58
7.--Central cylinder cells	60
8.--Cross-sectional montage of central cylinder cells.	62
9.--Epidermal cells.	64
10.--Epidermal cells.	66
11.--Cross-sectional montage of epidermal cells	68
12.--Columella cells.	70
13.--Columella cells.	72
14.--Cross-sectional montage at 400 microns from tip.	74
15.--Tip-cells.	76

List of Figures--continued.

Figure	Page
16.--Epidermal-root-cap-complex	78
17.--Golgi bodies	80
18.--Lipoidal material.	82
19.--Rough-endoplasmic reticulum and microtubules . .	84
20.--Autoradiograph showing quiescent center.	86

LIST OF TABLES

Table		Page
1.	QUIESCENT CENTER DATA SHEET	28
2.	NUMBER OF ORGANELLES PER SQUARE MICRON OF CYTOPLASM	29
3.	CORTEX DATA SHEET	30
4.	CENTRAL CYLINDER DATA SHEET	31
5.	EPIDERMIS DATA SHEET.	32
6.	COLUMELLA DATA SHEET.	33

THE ULTRASTRUCTURE AND MICROANATOMY OF THE ROOT TIP OF PISUM SATIVUM

INTRODUCTION

The root apical meristem, being a convenient tissue for experimental study, is often used in cytological, morphological and physiological investigations. The meristematic area contains a large number of mitotically active undifferentiated cells which contain few if any vacuoles and lack secondary cell walls. The cells are easy to obtain and relatively easy to handle and can be treated simply with chemicals and metabolic inhibitors (Wolff, 1964).

The root apical meristem of Pisum sativum var. Alaska has been used for many years in the Cytology Laboratory of the Biology Research Center, Michigan State University, for the study of mitosis, the mitotic cycle, and as a tissue for testing cytological effects of pesticides and herbicides. A fairly clear picture has been obtained concerning cell kinetics and chromosome structure of the meristematic area of the root apex in the light microscope.

THE ULTRASTRUCTURE AND MICROANATOMY OF THE ROOT TIP OF *PISUM SATIVUM*

INTRODUCTION

The root apical meristem, being a convenient tissue for experimental study, is often used in cytological, morphological and physiological investigations. The meristematic area contains a large number of mitotically active undifferentiated cells which contain few if any vacuoles and lack secondary cell walls. The cells are easy to obtain and relatively easy to handle and can be treated simply with chemicals and metabolic inhibitors (Wolff, 1964).

The root apical meristem of *Pisum sativum* var. Alaska has been used for many years in the Cytology Laboratory of the Biology Research Center, Michigan State University, for the study of mitosis, the mitotic cycle, and as a tissue for testing cytological effects of pesticides and herbicides. A fairly clear picture has been obtained concerning cell kinetics and chromosome structure of the meristematic area of the root apex in the light microscope.

This investigation was undertaken to extend knowledge obtained at the light microscope level to the electron microscope level and to study the microanatomy and ultrastructure of cells of the first millimeter of the primary root of Pisum seedling.

DEFINITIONS

ACROPETAL.....Proceeding from the base (the region of the shoot-root connection at the ground level) toward the apex.

BASIPETAL.....Proceeding from the apex toward the base.

LIPOIDAL MATERIAL...Material is considered lipoidal if it reacts to certain fixatives as reported in the classical electron microscope literature, and no attempt is made to specify the type.

MERISTEM.....'A meristem is, by definition, a group of plant cells in a state of active division and growth. These cells are not homogeneous since a meristem may be subdivided into regions that differ anatomically or in cellular activity, which indicates that differentiation has already progressed to a considerable extent in these embryo-like cells." (Miksche, 1964)

MICROANATOMY.....The anatomical details visible in the electron microscope.

MICROTUBULES.....Tubular structures found primarily at the periphery of the cell, having an outside diameter of 230-270 Angstroms.

NORMAL ROOT.....A root grown under the standard conditions, not chemically treated or caused in any manner to be different; it is used as the control for comparison.

QUIESCENT CENTER.....Located at the junction of the columella cells and the central cylinder region; it is the zone that should contain the initial cells according to the classical histogen theory; it is described as an area in which cells seldom or never divide, roughly hemispherical in shape which may change shape and size with time, and is surrounded by actively dividing cells, the initials. (Clowes, 1961)

ROOT APICAL MERISTEM..Used in a broad sense, it is various lengths of root proximal to the apex. (Esau, 1965)

ROOTCAP.....Is commonly regarded as a structure that protects the root meristem and assists the root in the penetration of the soil during its growth. (Esau, 1965) In Pisum this is more a descriptive term than an anatomical term.

ULTRASTRUCTURE.....Is the resolution and portrayal of cytological units in the electron microscope. (Frey-Wyssling, 1965)

PROTOMERISTEM.....The least determined part of the meristem and includes the initials and their most recent derivatives. (Esau, 1965)

LITERATURE REVIEW

The anatomy of the root using the optical microscope has been adequately covered by Stocking (1956) and Esau (1965). Esau (1965) has updated her earlier work with the limited use of electron microscope observations and photomicrographs.

Whaley, Mollenhauer, and Leech (1960), in a review article, described the components and ultrastructure of the apical root meristematic cells of various plants. While different types of cells from various plants were discussed, Whaley *et al*. used their own photographs of the meristematic cells of rootcap of *Zea mays* for illustration. Leech, Mollenhauer, and Whaley (1963) in a later related work, dealt with certain changes in the cellular characteristics which become apparent in the development of particular cell types basipetal to the initial area. They followed this development in part for 10 centimeters from the initial area. Clowes and Juniper (1964) studied the fine structure of the quiescent center and its neighboring tissue in *Zea* and

attempted to compare the undifferentiated, non-meristematic cells of the quiescent center with those of meristematic, non-differentiated cells of the meristem and those of the differentiated, non-meristematic cells of the cap. Clowes (1961, 1964) has studied the root apical meristem with particular interest in the quiescent center, and published photographs of tritiated thymidine treated Pisum roots showing this quiescent center. No data, however, as to the age or length of roots or conditions of growth were given.

While limited investigations are being pursued on the cellular anatomy of meristems, much information has accumulated on cellular organelles of plant material. Frey-Wyssling and Mühlethaler (1965) have thoroughly discussed ultrastructural plant cytology and the relationship to molecular biology. Porter and Machado (1960) studied the Allium root tip extensively with particular interest in the endoplasmic reticulum. Golgi bodies and their possible functions have been investigated by Whaley's group at the University of Texas (Whaley et al. 1960; Mollenhauer, Whaley, and Leech, 1961; Mollenhauer, 1965). Most recently, Ledbetter and Porter (1963, 1964) have reported a new component of plant cells, microtubules. Exclusive of microtubules, the cellular

components of plant cells have been thoroughly reviewed in The Cell, vol. II (Brachet, 1961) and The Cell, vol. VI (Brachet, 1964).

The previous anatomical studies on Pisum sativum have been the investigations with the optical microscope of Popham (1955), Reeve (1948), and Neumann (1939) in which they attempted to classify the anatomical boundaries and levels of tissue differentiation. There is still disagreement on the interpretation of their results and also on the development of the anatomical organization. Torrey (1955) has done a thorough descriptive study of the Pisum root tip with comparison of the intact to the excised tip. Although particularly interested in the development of vascular patterns, Torrey's study of the pea root anatomy was quite extensive.

MATERIALS AND METHODS

The observations in this study were made on the first millimeter of the root tip of Pisum sativum, var. Alaska. Ferry Morse and Vaughn Seed Companies, whose seeds were used, guaranteed that the peas possessed relative genetic homogeneity, were disease-free and had not been treated with herbicides or insecticides.

The peas were soaked for 6 hours in glass distilled water (pH 5.5 - 5.7) at 22.5° C; rolled in paper toweling and placed vertically in beakers containing water. Wax paper was placed around the rolled toweling to prevent drying of the peas by evaporation.

After 36 hours of germination at 22.5° C, the peas, the roots of which were 1-2 centimeters in length, were suspended on waxed, one-quarter inch wire mesh over dishes which contained 350 milliliters of full strength modified Hoaglund nutrient (balanced salt) solution. To allow for acclimatization, the peas remained in the nutrient for 4 hours. The nutrient solution, pH 5.5 - 5.7, was constantly aerated by

filtered air. The Hoaglund solution consists of the following in grams per liter:

Calcium nitrate $Ca(NO_3)_2$	7.6
Ammonium nitrate NH_4NO_3	10.32
Magnesium sulfate $MgSO_4 \cdot 7\ H_2O$	14.4
Potassium monobasic phosphate KH_2PO_4	10.68
Potassium dibasic phosphate K_2HPO_4	.56

Seedlings were grown in the aerated nutrient solution in a constant temperature water bath at 22.5° C in a 22.5° C constant temperature room. Samples were taken at various times from 0 hour to 120 hours.

Root tips 1 to 2 millimeters in length were harvested with a clean sharp razor blade and placed immediately into the fixative. Various fixatives were used: 5% unbuffered potassium permanganate ($KMnO_4$), 2.5% $KMnO_4$ buffered at pH 7.5 with veronal acetate, 2.5% $KMnO_4$ buffered at pH 7.2 with Sorenson's buffer, 6.25% glutaraldehyde and post-fixed in 2.5% $KMnO_4$ buffered to pH 7.2 with Sorenson's buffer, and 6.25% glutaraldehyde and post-fixed in 1% osmic acid (OsO_4) buffered in Sorensen's at pH 7.2. Duration of fixation, all at room temperature, was 1½ to 2 hours and that of post-fixation 1 hour. The material was dehydrated in one hour

in a series of alcohols; then embedded in Epon 812 according to Luft (1961).

Sections were obtained with glass and diamond du Pont knives on a Leitz ultramicrotome and a Porter-Blum ultramicrotome MT-2. For the best tissue orientation, flat embedded root tips were placed in a vise-type holder on the MT-2. Thick sections of ½ to 10 microns were viewed unstained and photographed on a Wild phase contrast M-20 microscope on 35 millimeter film using various filters. Naked 400 mesh and parlodion-carboned grids of 100 and 200 mesh were used. Some sections were stained with Reynold's lead citrate (1963), uranyl acetate (Pease, 1964), or double stained with a combination of both (Pease, 1964). Grids were observed on a Philips EM-100B and photographed on 35 millimeter Kodak Fine Grain Positive film.

Seedlings needed for the autoradiographic studies were suspended over dishes containing tritiated thymidine (New England Nuclear) at a concentration of 2 microcuries per milliliter of nutrient solution. Samples were taken from 0 to 120 hours and fixed in Pienaar's fixative (1955), which consists of a 6:3:2 mixture of absolute methanol, chloroform, and propionic acid; placed in a vacuum for 10

in a series of alcohols; then embedded in Epon 812 according to Luft (1961).

Sections were obtained with glass and diamond du Pont knives on a Leitz ultramicrotome and a Porter-Blum ultramicrotome MT-2. For the best tissue orientation, flat embedded root tips were placed in a vise-type holder on the MT-2. Thick sections of ½ to 10 microns were viewed unstained and photographed on a Wild phase contrast M-20 microscope on 35 millimeter film using various filters. Naked 400 mesh and parlodion-carboned grids of 100 and 200 mesh were used. Some sections were stained with Reynold's lead citrate (1963), uranyl acetate (Pease, 1964), or double stained with a combination of both (Pease, 1964). Grids were observed on a Philips EM-100B and photographed on 35 millimeter Kodak Fine Grain Positive film.

Seedlings needed for the autoradiographic studies were suspended over dishes containing tritiated thymidine (New England Nuclear) at a concentration of 2 microcuries per milliliter of nutrient solution. Samples were taken from 0 to 120 hours and fixed in Pienaar's fixative (1955), which consists of a 6:3:2 mixture of absolute methanol, chloroform, and propionic acid; placed in a vacuum for 10

minutes; and stored in the refrigerator until embedded in paraffin. Paraffin blocks were sectioned 8 microns thick on an A-O Spencer microtome and sections were mounted on albumin coated glass slides. Paraffin was removed by xylol and slides containing the radioactive material were then hydrated to water at which time they were coated with Kodak liquid emulsion NTB-3 according to Prescott (1964). At varying times from one to three weeks, slides were developed to determine the progress of exposure (Prescott, 1964). After development, slides were then stained in leucobasic fuchsin Schiff reagent (Lillie, 1951) and mounted permanently in diaphane mounting medium.

OBSERVATIONS

For convenience in describing the first millimeter of the root apical meristem of Pisum sativum, the meristem was subdivided into seven areas (Figure 1). It should be remembered, however, that in the pea root tip there are no discrete areas with well defined limits, and that various types of transitional cells are found between these seven areas. The seven areas to be discussed are as follows: quiescent center, cortex, central cylinder, epidermis, columella, tip cells, and epidermis-root-cap-complex.

In the following descriptions of a typical cell of the quiescent center, cortex, and central cylinder, it is assumed when average measurements or quantities are given, they are for a thin section through the center of the cell in a median longitudinal section of the pea root. These values should therefore be used only for comparison. The criterion used was that the nucleus and cytoplasm be cut equally in two. Because of the random placement of the nucleus, vacuoles and specific areas in the cytoplasm, the

OBSERVATIONS

For convenience in describing the first millimeter of the root apical meristem of Pisum sativum, the meristem was subdivided into seven areas (Figure 1). It should be remembered, however, that in the pea root tip there are no discrete areas with well defined limits, and that various types of transitional cells are found between these seven areas. The seven areas to be discussed are as follows: quiescent center, cortex, central cylinder, epidermis, columella, tip cells, and epidermis-root-cap-complex.

In the following descriptions of a typical cell of the quiescent center, cortex, and central cylinder, it is assumed when average measurements or quantities are given, they are for a thin section through the center of the cell in a median longitudinal section of the pea root. These values should therefore be used only for comparison. The criterion used was that the nucleus and cytoplasm be cut equally in two. Because of the random placement of the nucleus, vacuoles and specific areas in the cytoplasm, the

cells of the epidermis, columella, tip-cells, and epidermis-root-cap-complex lacked bilateral symmetry when cut longitudinally or transversely. It was, therefore, impossible to arrive at average numbers of organelles, as was done for the other described areas.

Table 2 shows a comparison of the number of organelles per square micron of cytoplasm for the quiescent center, cortex, and central cylinder. Data were calculated from a thin section of material through the center of the cell in a median longitudinal section. Of the 7 regions, only the quiescent center, cortex, and central cylinder could be compared in this manner for these 3 exhibit the necessary bilateral symmetry.

The following information was obtained from observations of numerous sections of pea root seedlings. Data were obtained directly from photographs and microscopic observation (light, phase, and electron).

Quiescent Center

Cells in the quiescent center (Figures 2, 3, and 4) were found to be irregularly shaped though more or less

spherical, packed tightly in a random manner, and approximately 12 microns in diameter. (See Table 1 and 2 for more detailed information.) Autoradiographic studies of paraffin sections concurred with those of Clowes (1961), and demonstrated that this area had a very low tritiated thymidine uptake during the range of time of this study. Therefore it can be concluded that the cells in this area were not dividing, or at least, as a group, were dividing very slowly during the period of exposure to the radioactive isotope. The nucleus, centrally located, filled the majority of the cell volume. The nuclear envelope showed typical pores, areas of larger discontinuities, and very few connections with endoplasmic reticulum (ER). Cytoplasmic free ribosomes and rough-ER were evident in osmium tetroxide (OsO_4) fixed material. Short pieces of ER were found randomly displaced, while longer pieces were usually found around the periphery of the cell. Small lipoidal droplets, usually separated, were scattered throughout the cells. Proplastids, about 8 per thin section, were equally divided between spherical and cylindrical shaped bodies, of which about ¼ exhibited a bright refractive substance, probably stored starch. Few Golgi bodies (an average of 3 per thin sectioned cell) with

associated small vesicles were seen in this area. Most of the mitochondria were spherical types, less than 1 micron in diameter, and were found in lower number than in most other areas. Although vacuoles were not prominent in this area, five different small types were located. Plasmodesmata connected adjacent cells.

Cortex

The cells of the cortex tissue, when cut transversely (Figure 5), appeared oval (12x20 microns) with very conspicuous intercellular spaces at the junctions of surrounding cells. The nucleus was centrally located while the vacuoles were found at the ends of the oval. In a median longitudinal section the cells were square (12x12 microns) to rectangular with the longer axis cross-wise to the long axis of the root (Figure 6). Since the longer axis of the oval shape was arranged along the circumference, in median longitudinal sections the smaller diameter of the cell was evident. (See Tables 2 and 3 for more detailed information.) The 3 most characteristic features of cells in this region were: 1) squarish shape of cells in longitudinal sections, 2) large

number of organelles, and 3) very conspicuous intercellular spaces. Another characteristic feature of cells in the cortex was the great abundance of microtubules. These were found only in glutaraldehyde-OsO_4 fixed material. They were also found, however, to a lesser extent in the epidermis-root-cap-complex. Microtubules were more evident only when the cell periphery was cut obliquely. This was due to their complex (not yet fully understood) distribution pattern. The nucleus occupied about 1/3 of the cell volume and characteristically showed many pores. There appeared to be some question concerning the number and types of vacuoles. In $KMnO_4$-fixed material, however, there appeared to be no membrane-limited vacuoles having grainy contents. This is further pursued in the discussion. The cells in this region contain what Whaley et al. (1960) have termed prevacuoles and appeared as dark homogeneous areas, less than 1 cubic micron in volume, and were characteristically star-shaped. These also appeared to be in association with or part of the ER. All lengths, up to 6 microns, of ER were found randomly dispersed throughout the cell. In cross-section many plasmodesmata between all cells were evident, while in longitudinal section, because of the section being parallel to the intercellular

spaces, connections appeared only between cell walls perpendicular to the long axis of the root. Lipoidal droplets, usually single, were found scattered in the cells. Proplastids were evenly divided between spherical shapes with a diameter of about 2 microns and cylindrical, dumbell, and irregularly shaped types. Cylindrical proplastids were of the typical size, as found previously, 3x1 microns, while many irregular and dumbell shaped types, not seen in the quiescent center, were evident. Almost twice as many spherical and cylindrical mitochondria were found as any other type per thin sectioned cell. Very few dumbell shaped mitochondria were evident. Golgi bodies were more numerous as were their vesicles.

Central Cylinder

As one proceeds basipetally from the quiescent center to 1 millimeter from the tip, distinct but gradual changes were evident. The cells elongated into regularly stacked cylinders (Figures 5, 7, and 8) with the long axis parallel to that of the root. It appeared that this region of the tissue was in transition, and a stable cell type was not

noted. Great variations in vacuole types and cytoplasm were noted. Cell organelles, however, remained fairly constant for the region. (See Tables 2 and 4 for more detailed information.) It is very possible that this variation of the cells is due to early differentiation into pre-phloem and/or pre-xylem. Dense quantities of free cytoplasmic ribosomes and rough-ER were evident. The ribosome content appeared to vary between cells in this region. Again as in the quiescent center, ER was randomly dispersed but the longer pieces were more apt to be located around the cell periphery. More of the ER was 1 to 6 microns in length, than 1 micron or less. Plasmodesmata were found in all cells. Single lipoidal droplets, ¼ micron in diameter, were randomly scattered, while clumps were very rare. Because of the increased cell size, the nucleus filled about 25% of the cell, and exhibited the usual pores, holes, and ER-connections. Vacuoles could best be described as in a transition from the membrane-limited textured interior type found in the quiescent center, to the highly sculptured or clear area vacuoles found at 1000 microns from the tip. Very few dumbbell shaped proplastids were seen, while 3 times more spherical types than cylindrical ones were

present. Nearly all of the mitochondria were 3/4 to 1 micron diameter spheres, while a very few long dumbells were found. Typical appearing Golgi bodies were evident, each with approximately 5 vesicles, ¼ micron in diameter.

Epidermis

The epidermal cells (Figures 9, 10, and 11) to be described were located at 1000 microns from the tip and were oval cylinders with their long axis the same as that of the root. (See Table 5 for more detailed information.) Approximately 90% of the cell was composed of a vacuole, which may or may not be limited with a membrane, whose contents may be grainy and/or fibrous. As a result of the large vacuolar area, the dense cytoplasm and nucleus were located at the periphery. Depending on the manner in which the cell was sectioned, either dense cytoplasm or vacuolar area or some of both were found. Few free ribosomes were evident in the cytoplasm and, as a result, rough-ER was clearly seen. Long single strands of weaving ER (up to 10 microns) along with many short pieces were found at the periphery of the cytoplasm. Up to 10 loosely stacked individual

strands of ER were also found localized in this area. Numerous Golgi bodies with many vesicles were seen, and these vesicles appeared to be engaged in the process of depositing their contents outside the plasma membrane. These vesicles appeared to move toward the plasma membrane, become fused to it, then burst open giving the plasma membrane a slightly scalloped appearance. Lipoidal droplets were absent as were plasmodesmata. Proplastids were both spherical and cylindrical and filled with many starch grains. Mitochondria were equally divided between spherical and cylindrical bodies. Epidermal cells were separated from each other and from the interior cell layer by a prominent cell wall, 2 to 5 microns thick. A granular substance extended about 4 microns into the mucilaginous material that covered the root tip.

Columella

The cells located in the columella exhibited very distinctive characteristics (Figures 12, 13, and 14). (To my knowledge these columella cells have not been previously described in the literature using electron microscope

techniques.) The cylindrical cells formed regular columns between the quiescent center and the tip-cells. (See Table 6 for more detailed information.) The diameter averaged 15 microns and the long axis averaged 18 microns. The plasma membrane was slightly scalloped probably due to the vesicles from Golgi membranes. Again as in the epidermal cells, these do not show bilateral symmetry when cut transversely or longitudinally. The irregularly shaped nucleus, containing pores, was located toward the basipetal end of the cell. Large irregularly shaped spherical proplastids containing large refractive starch grains were located in the acropetal end of the cell (Esau, 1965). Numerous mitochondria both spherical and cylindrical shaped were found. Lipoidal droplets ½ micron in diameter were seen scattered in the cytoplasm in some cells, while in others they were concentrated at the periphery of the cell. Long single strands of ER, about 10 microns in length, along with shorter pieces, were usually seen near the periphery. Unique to this region were the specific areas of great amounts of ER, usually not longer than 4 microns, in close association with vesicles. These vesicles, less than ¼ micron in diameter appeared to be the result of ER-budding. Many Golgi bodies with up to 6 cisternae

and numerous small vesicles were found adjacent to, but not in this region. Also vesicles ½ to 1 micron were found scattered in the cytoplasm. These vesicles may be larger free Golgi vesicles. Plasmodesmata were evident between cells having cell walls of less than 1 micron. Some membrane-limited vacuoles were seen, but more prominent were the clear areas located in the cytoplasm. (The clear areas were considered vacuoles for they appeared as such in the light microscope.) The membrane-bounded vacuoles were about 4 microns in diameter and exhibited a typical fibrous-textured interior. Some vacuole membranes contained many pores or small holes. Also observed were vacuoles, single or in clusters, with very whorled membranes. In some cells, membrane-bounded areas of less dense cytoplasm containing scattered organelles were in association with these irregularly whorled vacuoles.

Tip-Cells

Tip-cells (Figure 15) can best be described in terms of differences from columella cells. There are four obvious changes; 1) the cytoplasm is denser, 2) clear areas are no

longer found in the cytoplasm, 3) there is a great increase in the clear membrane bounded vesicles ½ micron in diameter, and 4) these vesicles appear to be moving from the interior of the cytoplasm to the plasma membrane and discharging their contents outside of the plasma membrane, thus giving the typical highly scalloped cell shapes found throughout this area. This scalloping rapidly progresses acropetally from the slightly scalloped columella cells to the deeply scalloped tip-cells. Highly scalloped cells were evident 3 to 4 cells basipetal to the tip. As the scalloping progresses, the cytoplasm is equally reduced, and that which is left is more dense. The substance deposited by the vesicles, between the cell wall and the plasma membrane, was of different texture than the still evident original cell wall (middle lamella). Even with the most scalloped cells, it was possible to see the original shape of the cell and this corresponded to the size and shape of the columella cells.

Epidermis-Root-Cap-Complex

The epidermis-root-cap-complex (Figure 16) is that part of the root tip apex that is basipetal to cells we can

designate as typical epidermal cells (with smooth plasma membranes) and extending inward until columella cells or cortical cells are reached and down to the apex to the typical tip-cells. Thus in 3 dimensions, it is a cone with a hole at the vertex. The epidermal-root-cap cells can best be described by comparing them to the typical epidermal type, columella cells, and tip-cells. Cytoplasmic free ribosomes and rough-ER similar to that of columella cells were seen. In longitudinal section they usually appeared lenticular and circular in cross-section. Their long axis approximately paralleled that of the tip and the size was approximately 30 by 12 microns. They have large membrane bounded vacuoles, whose contents are of granular texture, and may also have small whorled membrane inclusions in or near the large vacuoles or isolated. The vacuoles may also be of the sculptured type. Some microtubules were apparent in the glutaraldehyde-OsO_4 fixed material. A wide variance in organelle contents and distribution was noted; however, the large number of Golgi bodies and vesicles that were present in the columella and tip-cells were not found. Also the distribution of nuclei and plastids was not found to be as regular. The plasma membrane is slightly scalloped

but never to the extent of that of the tip-cells. The most characteristic features are their lenticular shape in longitudinal section and prominent large membrane bounded vacuoles. The closer they are to the columella and tip-cells, the more they resemble them; the closer they are to epidermal cells, the more they look like them. Their lenticular shape is a practical shape for it lends itself to packing in the area, as the tip curves toward the point.

In the light of the above observations, it is evident that for any cell classification or identification, it is necessary to consider both the gross cell morphology and the ultrastructure of the protoplasm. With the electron microscope it is impossible to classify a cell by studying only the organelles or only the gross shape of the cell. However when both are coordinated, it is possible to classify and identify a cell, even with the artificial divisions that must be used.

These artificial divisions become very evident when one attempts to draw in boundaries on a photograph of a median longitudinal section of the root apex. It is all a series of gradations. To go from any one point to another in this area, it is necessary to proceed through a gradual

change of cell types. To go from the outer epidermis to the central cylinder, one must traverse the following cell types: outer-epidermis, inner-epidermis, epidermal-like cortex, cells looking more like cortex, cortex, central-cylinder-looking cortex cells, and finally central cylinder cells. And one may also go through variations by starting in central cylinder cells and proceeding to the tip-cells. This even gradation into all areas may be due to the lack of an apical initial cell (or cells), which would form definite layers and regions (Clowes, 1961). In a photograph of the pea seedling root, at all stages of development, even from early embryogenesis (Reeve, 1948), it is impossible to draw definite boundaries for the apical meristem. It is possible, however, to draw in definite regions and layers only a short distance more basipetal than 1 millimeter from the tip.

TABLE 1

QUIESCENT CENTER DATA SHEET

CELL ARRANGEMENT.....irregularly packed
CELL SHAPE.....irregularly shaped spheres
CELL DIMENSIONS.....12x12x12 microns
CELL VOLUME.....about 900 cubic microns
RIBOSOMES.....yes**

ENDOPLASMIC RETICULUM (ER)
- Rough.....yes**
- Av.# of pieces***
  - 1 micron or less.....11 (3-17)*
  - 1-7 microns..... 7 (2-20)
- Distribution pattern.....long pieces usually around cell periphery

NUCLEUS
- Location.....centrally located
- % of cell filled.....more than 50%
- Nuclear envelope.....pores and larger holes
- Nuclear envelope-ER connections.....few

LIPOIDAL
- Single--av. #.....14 (4-21)
  - --av. diameter..... $\frac{1}{4}$ micron
- Clumps--av. #..... 3 (0-4)
  - --av. size..... $\frac{1}{4}$x1 micron

PROPLASTIDS
- Spherical--av. #.....4 (1-8)
  - --av. diameter.....1 micron
- Cylindrical--av. #.....3 (0-6)
  - --av. size.....3x1 micron
- Dumbell--av. #.....1 (0-5)
  - --av. size.....$2\frac{1}{2}$x1 micron
- Reflective material.....$\frac{1}{4}$ of all proplastids

GOLGI
- Av. #..... 3 (0-6)
- Av. size..... 3/4 micron long
- Vesicles..... 3 per Golgi, 1/10 micron diameter

MITOCHONDRIA
- Spherical--av. #.....15 (4-28)
  - --av. diameter.....3/4 micron
- Cylindrical--av. #..... 2 (0-6)
  - --av. size.....2x1 micron
- Dumbbell--av. #.....less than 1
  - --av. size.....2x1 micron

PLASMODESMATA.....15 connecting all adjacent cells

Table 1--Quiescent Center Data Sheet (continued)

VACUOLES

Type.....	Membrane-limited	
Shape....	spherical	cylindrical
Contents.	grainy	grainy
Av. #....	2 (0-6)	2 (0-5)
Av. size.	1 micron diameter	3x1 micron ($\frac{1}{4}$x1 to 3x5)

COMMENTS: other types rarely found, 1) membrane-limited with clear contents, 2) star-shaped opened-ER, 3) swollen-ER, 4) clear areas in cytoplasm which may or may not have scattered organelles.

NOTE: Av. # is given per thin sectioned cell, median longitudinal section.

*Range
**OsO_4 fixation
***$KMnO_4$ fixation

TABLE 2

NUMBER OF ORGANELLES PER SQUARE MICRON OF CYTOPLASM

Organelles	Quiescent Center (63 sq. microns)*	Cortex (105 sq. microns)*	Central Cylinder (231 sq. microns)*
ER--1 micron or less	.17 (.05-.27)	.21 (.03-.53)	.03 (.01-.07)
1-7 microns	.11 (.03-.32)	.25 (.11-.44)	.06 (.04- .1)
LIPOIDAL			
Single	.22 (.06-.33)	.16 (.07-.31)	.02 (0-.04)
Clumps	.05 (0-.06)	.00	.00
PROPLASTIDS			
Spherical	.06 (.02-.13)	.07 (0-.16)	.03 (.01-.04)
Cylindrical	.05 (0-.1)	.05 (.02-.12)	.01 (0-.02)
Dumbbell	.02 (0-.08)	.01	.00
GOLGI	.05 (0-.1)	.06 (.01-.1)	.02 (0-.04)
MITOCHONDRIA			
Spherical	.24 (.06-.44)	.26 (.04-.47)	.09 (.07-.1)
Cylindrical	.03 (0-.1)	.06 (.02-.13)	.01 (.00-.02)
Dumbbell	.00	.00	.00

*The average square microns of cytoplasm per cell, taken from electron micrographs of sections of material 600-1000 Angstroms thick.

TABLE 3

CORTEX DATA SHEET

CELL SIZE, SHAPE, AND ARRANGEMENT (cross section)...oval, 12x20 microns, with large intercellular spaces between cells; long axis of oval-shaped cell is arranged along the circumference of the root.

CELL SIZE, SHAPE, AND ARRANGEMENT (median longitudinal section)...square to rectangular, longer axis perpendicular to long axis of root; av. size 12x12 microns; due to arrangement of oval-shaped cell, we see short axis of oval in median longitudinal section.

CELL VOLUME.....about 2410 cubic microns

RIBOSOMES.....cytoplasm very dense due to heavy concentration**

MICROTUBULES.....yes**

ENDOPLASMIC RETICULUM (ER)

- Rough.....yes**
- Av. # of pieces***
  - 1 micron or less.....22 (3-56)*
  - 1-7 microns.....26 (12-46)
- Distribution pattern.....none

NUCLEUS

- Location.....central
- % of cell filled.....30%
- Nuclear envelope (NE).....many pores
- NE-ER connections.....none

LIPOIDAL

- Single--av. #.....17 (7-33)
  - --av. diameter.....$\frac{1}{2}$ micron
- Clumps--av. #.....very few
  - --av. size.....$\frac{1}{2}$x1 micron

PROPLASTIDS

- Spherical--av. #.....7 (0-17)
  - --av. diameter.....2 micron
- Cylindrical--av. #.....5 (2-13)
  - --av. size.....3x1 (1x2 to 1x6 to 2x5)
- Dumbbell--av. #.....1
- (& Irregular)- size range.....(1x7 to 2x3 to 1x3)

GOLGI

- Av. #.....6 (1-10)
- Av. size.....2 micron in length
- Vesicles.....Av. 8 per Golgi, $\frac{1}{4}$ micron diameter

MITOCHONDRIA

- Spherical--av. #.....27 (4-50)
  - --av. diameter.....1 micron or less
- Cylindrical--av. #.....6 (2-14)
  - --av. size.....2x1 ($\frac{1}{2}$x1$\frac{1}{2}$ to 1x8)
- Dumbbell--av. #.....very few

PLASMODESMATA

25 connections to all cells but not across intercellular spaces

Table 3--Cortex Data Sheet (continued)

VACUOLES
- Type.....prevacuolar areas
- Shape.....irregular star-shaped, maybe in association with ER
- Contents...dark, homogeneous
- Av. #.....either none or 6 to 10
- Av. size...less than 1 cubic micron
- COMMENTS: No membrane limited vacuoles in $KMnO_4$; some in OsO_4

NOTES: Av. # is given per thin sectioned cell, median longitudinal section.

*Range
**OsO_4 fixation
***$KMnO_4$ fixation

TABLE 4

CENTRAL CYLINDER DATA SHEET

CELL SHAPE AND ARRANGEMENT...regularly stacked cylinders, long axis parallel to root long axis
CELL DIMENSIONS.....11x30 microns
CELL VOLUME.....about 2850 cubic microns
RIBOSOMES.....dense cytoplasm due to free ribosomes**
ENDOPLASMIC RETICULUM (ER)
- Rough.....yes**
- Av. # of pieces***
  - 1 micron or less..... 8 (2-20)*
  - 1-7 microns.....15 (10-23)
- Distribution pattern.....long pieces usually at cell periphery

NUCLEUS
- Location.....centrally located
- % of cell filled.....25%
- Nuclear envelope (NE)....pores and larger holes
- NE-ER connections.....yes

LIPOIDAL
- Single--av. #.....4 (0-9)
  - --av. diameter.....$\frac{1}{4}$ micron
- Clumps--av. #.....very rarely found
  - --av. size.....$\frac{1}{2}$ micron

PROPLASTIDS
- Spherical--av. #.....7 (2-10)
  - --av. diameter..1$\frac{1}{2}$ micron
- Cylindrical--av. #.....2 (0-5)
  - --av. size....1x3 microns
- Dumbbell--av. #.....less than 1
  - --av. size.....3x1$\frac{1}{2}$ micron

Table 4--Central Cylinder Data Sheet (continued)

GOLGI
- Av. #.....4 (0-10)
- Av. size.....1 micron
- Vesicles.....5 per Golgi, $\frac{1}{4}$ micron diameter

MITOCHONDRIA
- Spherical--Av.#.....22 (16-28)
  - --Av. diameter.....3/4 to 1 micron
- Cylindrical--Av. #.....3 (1-5)
  - --Av. size.....3x1 micron
- Dumbbell--Av. #.....less than 1
  - --Av. size.....2x$\frac{1}{2}$ micron (spherical ends 1 micron in diameter, connecting rod $\frac{1}{2}$ micron in width, total length may be 5 microns)

PLASMODESMATA.....30 connecting all adjacent cells

VACUOLES
- Spherical, cylindrical, and dumbbell shapes with and without distinct limiting membranes.
- Opened ER connected to spherical vacuoles
- Sculptured vacuoles
- ER in association with sculptured vacuoles
- COMMENTS: Appear to be in transition from membrane-limited textured interior type of quiescent center to the sculptured and clear areas evident at 1000 microns from tip which appear to be the typical vacuoles for the region.

NOTE: Av. # is given per thin sectioned cell, median longitudinal section.

*Range
**OsO_4 fixation
***$KMnO_4$ fixation

TABLE 5

EPIDERMIS DATA SHEET

CELL SHAPE AND ARRANGEMENT...oval cylinders with long axis parallel to that of the root
CELL DIMENSIONS (cross-section).....oval 15x10 microns
CELL DIMENSIONS (median longitudinal section)....15x50 microns
CELL VOLUME.....about 5660 cubic microns
RIBOSOMES.....few**
ENDOPLASMIC RETICULUM (ER)
- Rough.....very evident due to few free ribosomes **
- Distribution pattern.....long single strands (up to 10 microns) of weaving ER along with many short pieces found at periphery of cell. Up to 10 loosely stacked strands of ER also found at periphery.

Table 5--Epidermis Data Sheet (continued)

NUCLEUS
- Location.....compressed between vacuole and cell membrane at periphery of cell.
- % of cell filled.....very small

LIPOIDAL.....none evident

PROPLASTIDS
- Spherical--#.....about ½ of all present
  - --av. diameter..2 microns
- Cylindrical--#.....about ½ of all present
  - --av. size....3x1½ micron
- Dumbbell--#.....none
- Reflective material.....filled with many large bodies

GOLGI
- Av. #.....numerous
- Av. size.....1½ micron
- Vesicles.....many, ¼ micron in diameter

MITOCHONDRIA
- Spherical--#.....about ½ of all present
  - --av. diameter..½ micron
- Cylindrical--#.....about ½ of all present
  - --av. size....½x1 micron
- Dumbbell--av. #.....none

PLASMODESMATA.....none

VACUOLES
- Type.....may or may not be limited with a membrane
- Shape.....irregular
- Contents.....grainy and/or fibrous
- Av. #.....usually only 1
- Av. size.....fills 90% of cell

NOTE: Av. # is given per thin sectioned cell, median longitudinal section.

**OsO_4 fixation

TABLE 6

COLUMELLA DATA SHEET

CELL SHAPE AND ARRANGEMENT...regular cylindrical columns (in cross section, oval; in a median longitudinal section, squarish)

CELL DIMENSIONS.....cylinder diameter 15 microns; long axis 18 microns

CELL VOLUME.....about 3180 cubic microns

RIBOSOMES.....few**

Table 6--Columella Data Sheet (continued)

ENDOPLASMIC RETICULUM (ER)
Rough.....very evident due to fewer free ribosomes**
Distribution pattern.....(1) Great numbers of ER (not longer than 4 microns) in close association with vesicles, found in definite regions. (2) Long single strands of ER, up to 10 microns, with shorter pieces were usually found near the periphery.

NUCLEUS
Location.....toward basipetal end of cell, shape very irregular
% of cell filled.....small
Nuclear envelope (NE).....numerous pores but no large holes
NE-ER connections.....none

LIPOIDAL
Single--#.....scattered pieces, some localized at periphery
--av. diameter.....½ micron

PROPLASTIDS
Distribution pattern.....irregularly shaped spherical proplastids located near acropetal end of cell
Size.....up to 6 microns
Reflective material.....many large starch bodies

GOLGI
Av. #.....many, up to 6 cisternae
Av. size.....2 microns in length
Vesicles.....numerous small vesicles

MITOCHONDRIA
Spherical--av. #.....numerous
--av. diameter.....1 micron
Cylindrical--av. #.....numerous
--av. size.....2x1 micron (½x2 to 5x1)

PLASMODESMATA.....between cells having cell walls less than 1 micron thick

VACUOLES
Type.....membrane limited vacuoles (membrane may have many pores or small holes)
Shape.....may be highly irregular or whorled
Contents.....fibrous textured, some may have less dense cytoplasm with scattered organelles
Av. #.....few
Av. size.....4 microns diameter
COMMENTS: More prominent were cytoplasmic clear areas with no membrane limited boundary.

CYTOPLASM.....large clear areas, no ribosomes evident
.....regions containing both short pieces of ER and many vesicles

VESICLES
½ to 1 micron in diameter, found scattered in cytoplasm
¼ micron diameter in definite regions with short pieces of ER

NOTE: Av. # is given per thin sectioned wall, median longitudinal section.

**OsO_4 fixation

Table 6--Columella Data Sheet (continued)

ENDOPLASMIC RETICULUM (ER)
Rough.....very evident due to fewer free ribosomes**
Distribution pattern.....(1) Great numbers of ER (not longer than 4 microns) in close association with vesicles, found in definite regions. (2) Long single strands of ER, up to 10 microns, with shorter pieces were usually found near the periphery.

NUCLEUS
Location.....toward basipetal end of cell, shape very irregular
% of cell filled.....small
Nuclear envelope (NE).....numerous pores but no large holes
NE-ER connections.....none

LIPOIDAL
Single--#.....scattered pieces, some localized at periphery
--av. diameter.....½ micron

PROPLASTIDS
Distribution pattern.....irregularly shaped spherical proplastids located near acropetal end of cell
Size.....up to 6 microns
Reflective material.....many large starch bodies

GOLGI
Av. #.....many, up to 6 cisternae
Av. size.....2 microns in length
Vesicles.....numerous small vesicles

MITOCHONDRIA
Spherical--av. #.....numerous
--av. diameter.....1 micron
Cylindrical--av. #.....numerous
--av. size.....2x1 micron (½x2 to 5x1)

PLASMODESMATA.....between cells having cell walls less than 1 micron thick

VACUOLES
Type.....membrane limited vacuoles (membrane may have many pores or small holes)
Shape.....may be highly irregular or whorled
Contents.....fibrous textured, some may have less dense cytoplasm with scattered organelles
Av. #.....few
Av. size.....4 microns diameter
COMMENTS: More prominent were cytoplasmic clear areas with no membrane limited boundary.

CYTOPLASM.....large clear areas, no ribosomes evident
.....regions containing both short pieces of ER and many vesicles

VESICLES
½ to 1 micron in diameter, found scattered in cytoplasm
¼ micron diameter in definite regions with short pieces of ER

NOTE: Av. # is given per thin sectioned wall, median longitudinal section.

**OsO_4 fixation

DISCUSSION

The cellular organization of the root tip of Pisum has been well established with the use of the light microscope (Popham, 1955). The foregoing electron microscope observations substantiate the descriptions based on light microscope studies and provide further information on the organization as well as the ultrastructure and microanatomy.

The interpretation of the pattern of organization, termed the open or Pisum type, has been modified only slightly since first reported by Janczewski (1874). This open type is characterized by the lack of distinct organizational patterns and the apparent continuation of the cell column from the tip into the central cylinder cells. This characteristic pattern is also clearly apparent at the electron microscope level. As has been reported earlier, there are no distinct cell or organelle changes detectable by electron microscopic observation. From any one point to any other point, one must go through a transition of cellular and organelle changes. Despite this, however, it is still possible in a general way to subdivide the meristematic segment into seven areas.

This open or Pisum type of organization is only one of many forms. Another type, which has a closed or very distinct pattern, is found in Zea. Because of this and the ease of identification of areas, it is often used for study. Whaley, Mollenhauer, and Leech, Cell Research Institute, University of Texas, have made extensive electron microscope studies using Zea and, up to the present, their publications are the most complete in this field. Thus, our finding should be compared to theirs.

Since the work of Whaley et al. was the first and still is the most complete published investigation on the root meristem using the electron microscope, it is often used as the standard for plant cells. Unfortunately, it is often forgotten that their results and micrographs are based only on Zea and thus the impression, either implied or stated, is given in the general literature that this may be typical for plants. Until further comparative studies are made this is an unjustified assumption. Pisum and Vicia are certainly different from Zea and many other variations may yet be found.

It is unfortunate also that the earlier work of Whaley is most often quoted. This is a general review of

the meristematic region, more concerned with organelles than cell types, and does not consider the characteristics of discrete regions. The published micrographs certainly do not indicate the diversity of cell types. The micrograph used as the typical cell of this region is termed a "transverse section through a meristematic rootcap cell" (Whaley et al., 1960) and in later works which use this as a reference, it is implied and inferred that this is a typical meristematic root tip cell. Because of the great diversity in the apical root meristem found in our work on Pisum, I do not believe one can show a typical meristematic root tip cell. Fortunately, a more complete study of cell types found in Zea was later published (Leech, Mollenhauer, and Whaley, 1963) and showed some of the diversity of cells found within 10 centimeters of the tip. However they still did not show all the cell types found or the cellular relationships. If one needs to show a typical meristematic root tip cell, the definition of meristem should be made very clear and very rigid.

While no criticism of the work of Whaley, Mollenhauer and Leech is implied, the generalizations from the work, either implied or stated, are a little disturbing as this

indicates a uniformity which does not exist. Because the organization in root meristems is so diverse and the fact that Zea has such a distinctive type, I do not believe that it should be implied or considered as typical of plants. Zea has a well organized and clearly evident meristem with a distinct rootcap. It also has a distinct quiescent center that may be easily outlined on a photograph of a median longitudinal section. Other plants have equally distinct, but differently organized meristems. On the other hand, plants like Vicia, Pisum, and others, have no distinct regional boundaries (Clowes, 1961).

Nevertheless, even with the great difference in organization between Zea and Pisum, electron micrographs do show similarities in cytoplasmic organelles (Whaley et al., 1960) and in most cell types (Leech et al., 1963). No specific organelle comparison is possible with Zea, since the data were analyzed in a different manner. Whaley et al. (1960) reported generally on the organelles of the whole root tip and Clowes and Juniper (1964) are concerned only with organelle content in the root axis. Although electron micrographs of Zea and Pisum are generally comparable, they do differ in specific regions. The micrograph

that Whaley et al. (1960) used as the representative of the meristem, the "transverse section through a meristematic rootcap cell," would certainly not be a representative cell for Pisum. The nearest equivalent cell type in Pisum is that of the quiescent center, not of the rootcap.

Apparently, no cells are found in Zea which are comparable to the columella and tip-cells of Pisum. No mention in the literature was found of these cell types being studied by electron microscopy. The regions of short pieces of ER and small vesicles may be unique to the columella cells. The tip-cells appear to be extreme cases of the result of action of active Golgi vesicles. The cytoplasm is much denser as if the clear-watery-material is being collected in the Golgi vesicles and then deposited outside the plasma membrane.

The work on Pisum fully supports that of Mollenhauer, Whaley, and Leech on Golgi bodies. In agreement with them (Figure 17), we found that larger (more active appearing) Golgi bodies with large flattened vesicles were located in the outer cells (epidermis, epidermis-root-cap-complex, and tip-cells) and the smaller Golgi with smaller and fewer vesicles were located in the interior of the root (Whaley,

Kephart, and Mollenhauer, 1959; Mollenhauer, 1965). Also, the observations reported here fully support Mollenhauer's and Whaley's theories of Golgi vesicle involvement with cell wall deposition. Zea cells were shown with slightly scalloped edges though never to the extreme of that of the columella and tip-cells seen in Pisum. Also Zea cells revert to smooth plasma membranes, while those of Pisum do not (Mollenhauer, et al., 1961). Nevertheless, the mode of action appears to be the same.

A feature of Pisum that was not found discussed in the literature is the occurrence of a high concentration of lipoidal material in the quiescent center region (Figure 18). After OsO_4 fixation the material appears as a very dark, smooth, homogeneous material, well-contained with no apparent unit membrane. After $KMnO_4$ fixation, since $KMnO_4$ dissolves away some of the lipoidal material, the material may appear as an "empty-body" with a heavy, dark, thick layer at the periphery (Figure 18). This heavier concentration of lipoidal material is not only evident in the electron microscope, but also very evident in the phase microscope because of its highly refractive property.

As has been noted, different fixatives and fixation procedures may give different results. Variation of the concentration, duration, temperature, and pH of the basic fixative may each give different results. Depending on the fixative used, different buffers even at the same pH may give different results. On the choice of fixatives, there are 2 schools of thought: 1) Whaley and his group believe permanganate to be the best; 2) K. R. Porter, Harvard University, and his followers stress the importance of glutaraldehyde fixation followed by post-fixation in OsO_4.

For membranes and ER, $KMnO_4$ gives very dark, sharp images, almost like a line drawing. The ground substance of the cytoplasm is very smooth and structureless (Mollenhauer, 1959). Glutaraldehyde fixation with Sorenson's buffer (Ledbetter and Porter, 1963) followed by a post-fixation of OsO_4 or $KMnO_4$ apparently gives the most accurate picture, but certainly in some cases not the sharp images of $KMnO_4$ alone. After glutaraldehyde fixation and a post-fixation, ribosomes, rough-ER and microtubules are clearly seen (Figure 19), but in some cases due to the dense ribosomes, smooth-ER may be completely hidden and organelles barely visible. It is felt that glutaraldehyde

fixation is superior, since everything seen in $KMnO_4$ is present, plus other definite components (ribosomes, rough-ER, microtubules, plus perhaps many other things). Morphologically, the basic picture obtained with $KMnO_4$ and glutaraldehyde fixation followed by post-fixation of OsO_4 or $KMnO_4$ are the same. The fixative choice depends upon what is to be studied and emphasized in one's micrographs. Ledbetter has done extensive work on buffers for glutaraldehyde and we concur with his choice of Sorenson's buffer at pH 7.2. Quite obviously fixatives need much more study in terms of the artifacts they produce.

Buffers are also of particular importance. Different buffers or pH's cause great variations in the occurrence and type of vacuoles found. The differences may range from swollen ER to a great increase in small 1 micron diameter spheres to a few large vacuoles that nearly fill the cell. Much care must be taken to compare regions only when they have had the same treatment. This was painfully learned by the author.

As previously mentioned, the vacuoles evident in the cortex region do not present a constant picture. In this region, it appears that different types of vacuoles

may be seen after different fixations, but the results may not always be consistent. Perhaps under different physiological conditions, different fixations create different results. Also, and perhaps this is the most logical explanation, it may be only a technical problem that is caused if the section is not an exact median longitudinal section or if the column of cortex cells in the root itself is twisted or at an angle. As mentioned above, the cells in cross-section are oval with the nucleus in the center and vacuoles usually found at either end of the oval. Thus, if the short axis of the oval is not exactly cut, the appearance would be variable. This technical problem may be evident only in the cortex cells due to their particular arrangement. It certainly will not give a different picture if central cylinder cells are cut slightly off-center.

The work of Clowes (1959, 1961, 1964) on the quiescent center was also compared to our work. He demonstrated the existence of this region in many types of plants, e.g. Zea, Pisum, and others. Our autoradiographic photographs of paraffin sections fully reproduced his results (Figure 20). Clowes and Juniper (1964) published an excellent electron micrograph montage of the quiescent center and

the surrounding cells in Zea on which they were able to draw in the distinct boundaries of this region. Again as in the case of the Zea work by Whaley et al., incorrect generalizations have been made. It is sometimes assumed by persons not completely familiar with the work of Clowes or with patterns of organization in roots, that a distinct quiescent center can be drawn on photographs of all plants. Although this region can not be clearly seen structurally in Pisum, it is equally evident in Pisum and Zea after autoradiographic procedures.

In conclusion, it is clear that the first millimeter of the Pisum root is a complex tissue containing a great variety of cell types. It is in fact a highly specialized region, the function of which is the production of new cells at a controlled rate. It also is in this region that the vascular pattern is determined (Torrey, 1955).

Using this work as a base-line, comparative studies of either induced or random changes may now be pursued. At present, the induced changes caused by treatment with 5-amino uracil (an analog of thymine) are being studied in our laboratory. Also a complete series of electron microscope autoradiographic experiements are planned to further

study the quiescent center. Considerable knowledge could also be gained by observing antimitotic and herbicidal action on the meristematic region. Until the present study of the normal root tip was completed, no real comparative studies could be attempted since there was no base-line for a standard.

SUMMARY

1. A descriptive study of the ultrastructure and microanatomy of the first millimeter of the root tip of Pisum has been presented.

2. Since the pea root tip has no distinct boundaries, it was necessary to sub-divide the root tip into 7 regions: quiescent center, cortex, central cylinder, epidermis, columella, tip-cells, and epidermis-root-cap-complex.

3. Each area was investigated and data collected.

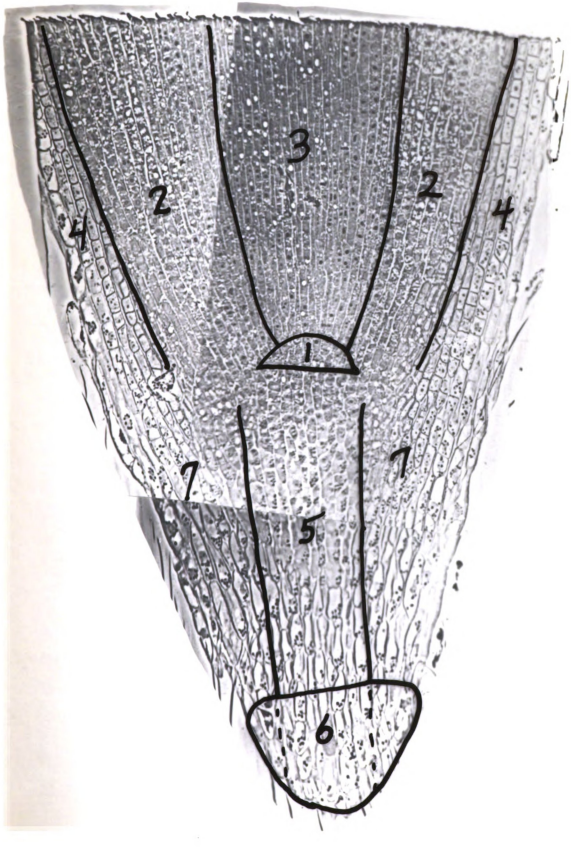
4. The areas studied in the electron microscope were shown in relationship to a phase contrast photograph montage of a median longitudinal section of a root tip.

5. Comparison of the ultrastructure and microanatomy was made between the work on Pisum of this study and the published work on Zea.

FIGURES

Figure 1.--Phase microscope montage photograph of the first millimeter of root tip.

A phase microscope montage of the first millimeter of the pea root in a median longitudinal section. Specimen is a ½ micron thick section of material fixed by usual electron microscope techniques and embedded in Epon and sectioned by a diamond knife on an ultramicrotome. Overlay shows the 7 regions: 1) quiescent center; 2) cortex; 3) central cylinder; 4) epidermis; 5) columella; 6) tip-cells; 7) epidermis-root-cap-complex. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 450.


1
2
2
3
4
4
5
6
7
7

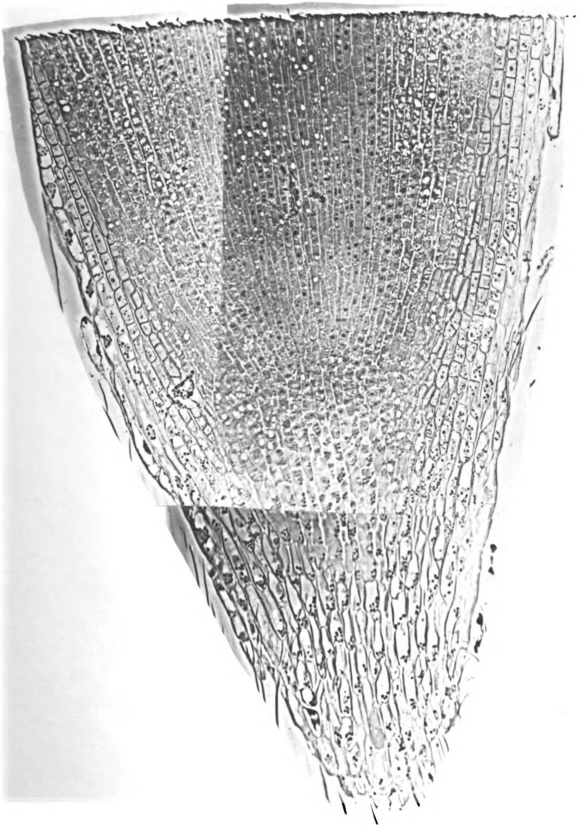
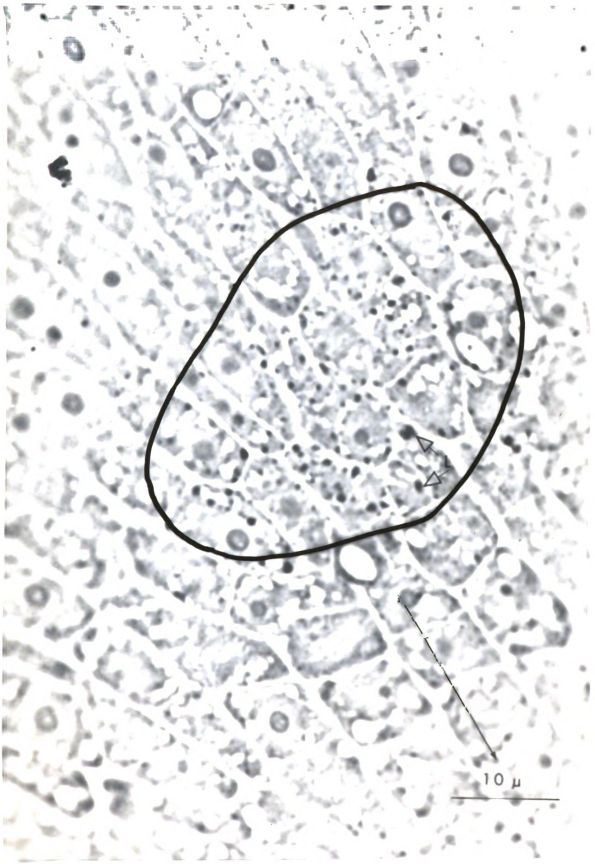


Figure 2.--Quiescent center.

An enlargement of Figure 1, showing q
(circled on overlay). Note the concentration
oidal material (L). Arrow, pointing to root
root axis. Glutaraldehyde-OsO_4 fixation. Ap
2,800.


10 μ

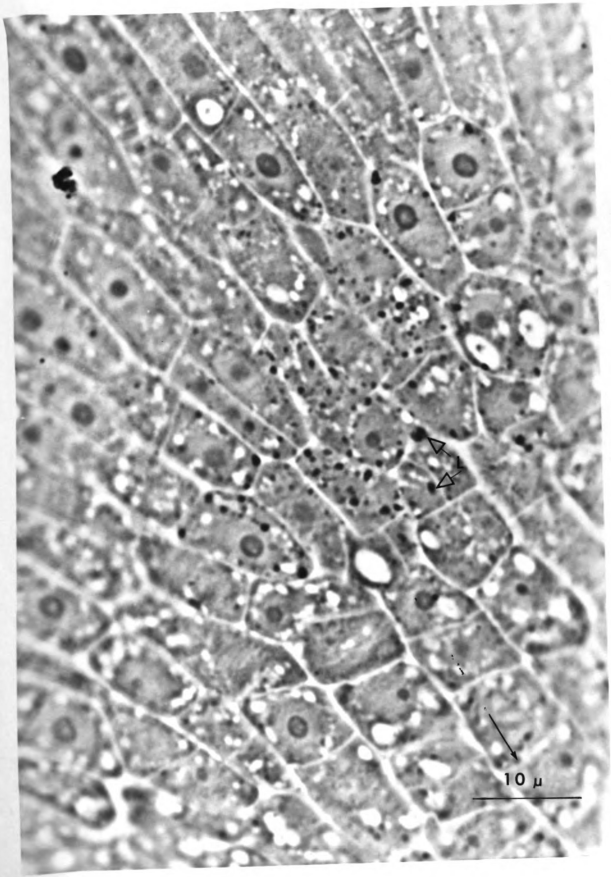

10 µ

Figure 3.--Quiescent center.

An electron micrograph of cells in the quiescent center following Glutaraldehyde-$KMnO_4$ fixation. Note the nucleolus (NU), nuclear envelope pores (P), chromatin material (C), mitochondria (different shapes) (M), proplastids (different shapes) (PP), plasmodesmata (PD), cell wall (W), lipoidal material (L), endoplasmic reticulum (ER), and Golgi (G). Arrow, pointing to root apex, indicates root axis. Median longitudinal section. Approximately X 6,000.

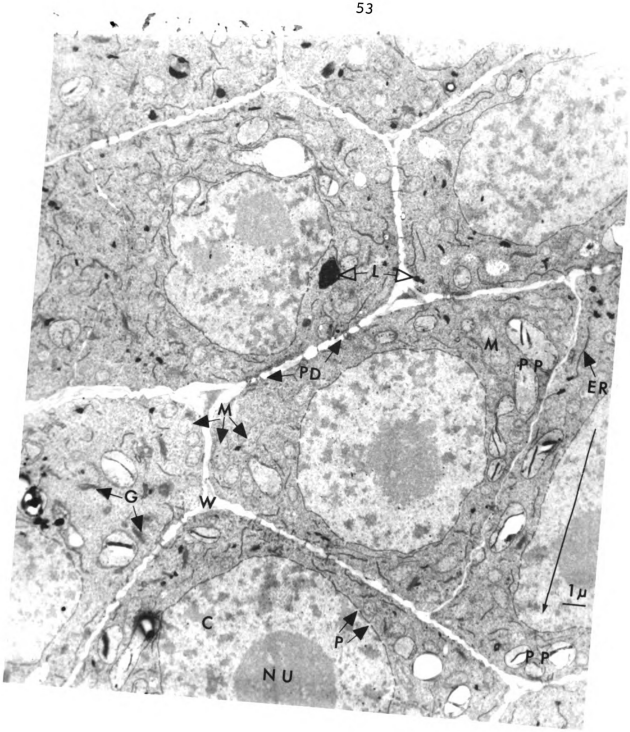

L
M
PP
ER
PD
M
G
W
1μ
C
P
PP
NU

Figure 4.--Quiescent center.

Cells of the quiescent center after
Note the differences from Figure 3. New cel
formed between daughter cells (X). Nuclear
not yet completed after division. Arrow, po
apex, indicates root axis. Median longitudi
Two and one-half percent $KMnO_4$, pH 7.5 with
tate. Approximately X 6,000.

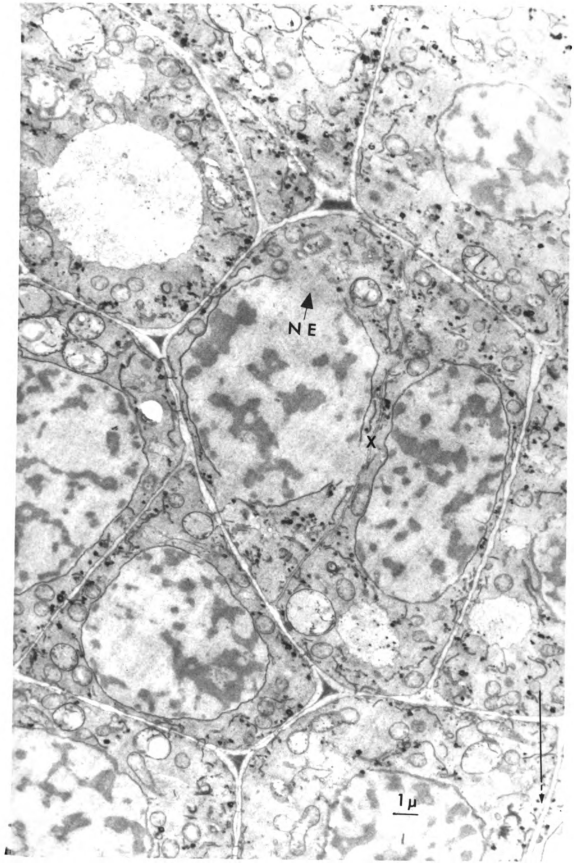
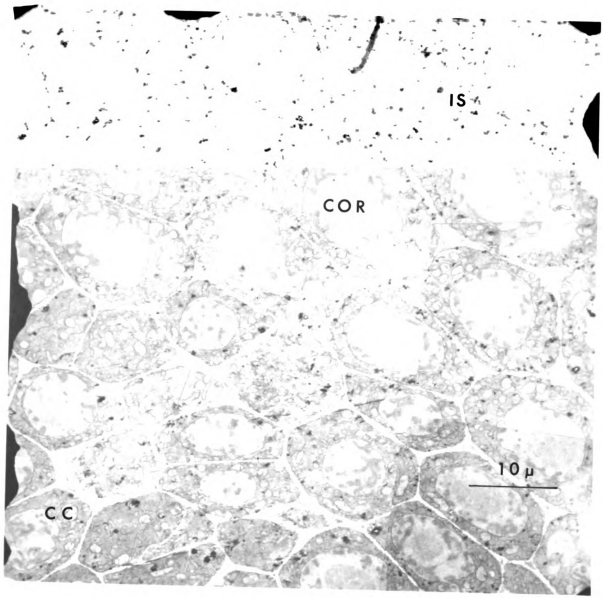

NE
X
1 μ

Figure 5.--Cross-sectional montage of cortex and central cylinder.

A cross-sectional montage of a portion of root at 1000 microns from the tip, showing cortex (COR) and central cylinder cells (CC). Note the intercellular spaces (IS). Fixation in 2.5% $KMnO_4$, pH 7.5 with veronal acetate buffer. Approximately X 2,300.


IS
COR
10 µ
CC

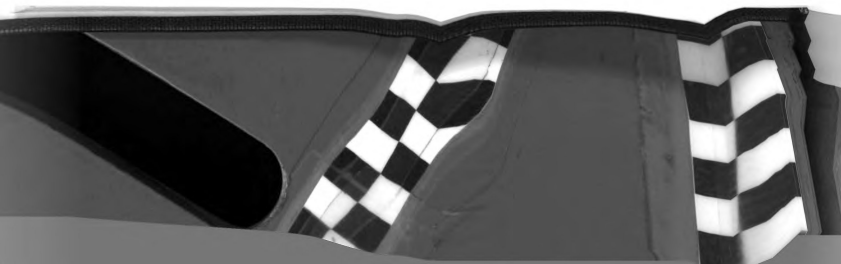


Figure 6.--Cortex cells.

A section showing cortex cells. Note the large intercellular spaces (IS). Arrow, pointing to root apex indicates root axis. Median longitudinal section. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.

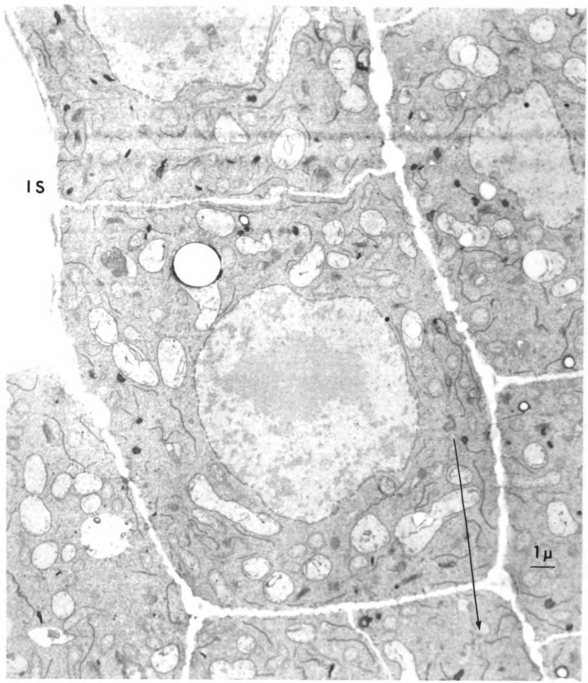

IS
1μ

Figure 7.--Central cylinder cells.

Portions of central cylinder cells. Note the double nucleolus (NU), in a polyploid cell, and the pore (P) in the nuclear envelopes which are evident because o the oblique cut of the nuclei. Arrow, pointing to the root apex, indicates root axis. Glutaraldehyde-$KMnO_4$ fixation. Median longitudinal section. Approximately X 6,000.

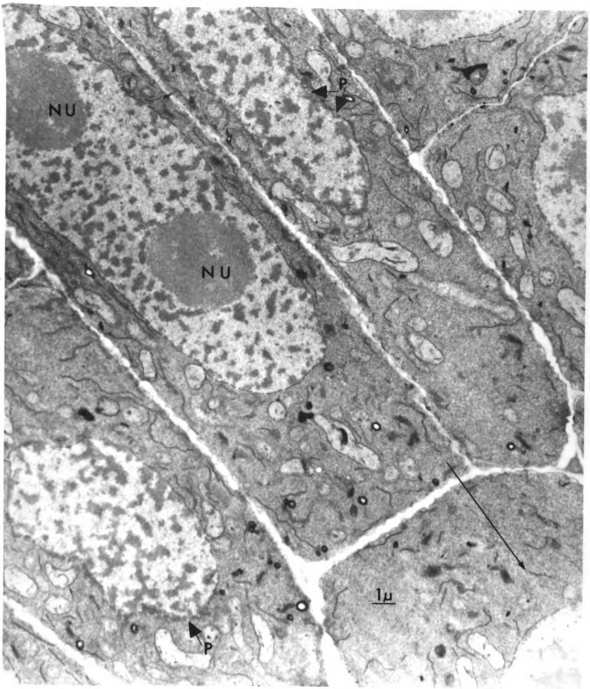
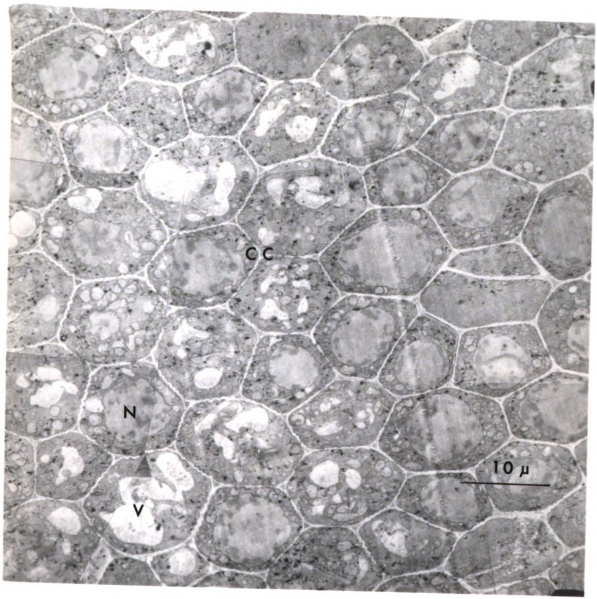
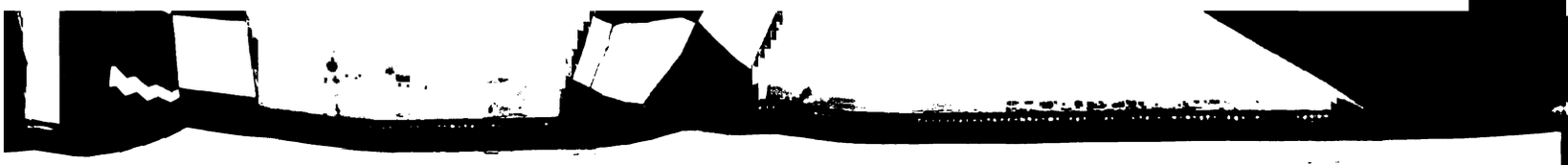

NU
NU
P
P
1μ

Figure 8.--Cross-sectional montage of central cylin
cells.

A cross-sectional montage of a portion of root
1000 microns from the tip, showing central cylinder cel
(CC). Note the sculptured vacuoles (V) and nuclei (N).
Fixation in 2.5% $KMnO_4$, pH 7.5 with veronal acetate buf
Approximately X 2,300.


CC
N
V
10 μ



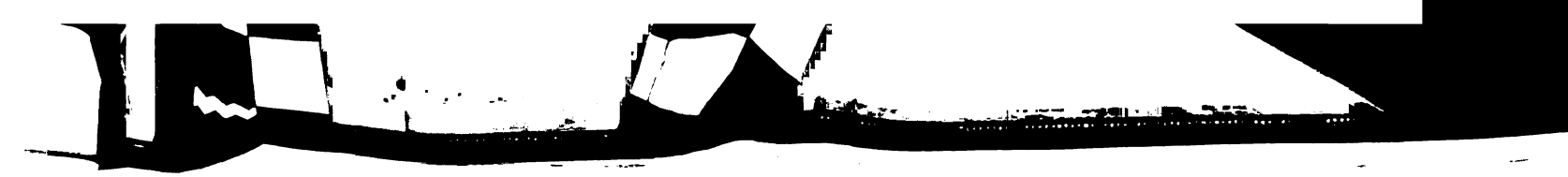


Figure 9.--Epidermal cells.

Portions of epidermal cells. Note the endoplasmic reticulum (ER) localized at periphery, clear cytoplasmic areas (X), heavy concentration of starch in proplastids (PP), Golgi (G) and Golgi vesicles (GV), and highly scalloped plasma membrane (PM). Arrows at bottom of photograph indicate portions of 2 cells located at the periphery of the root. Median longitudinal section. Arrow, pointing to root apex, indicates the root axis. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.

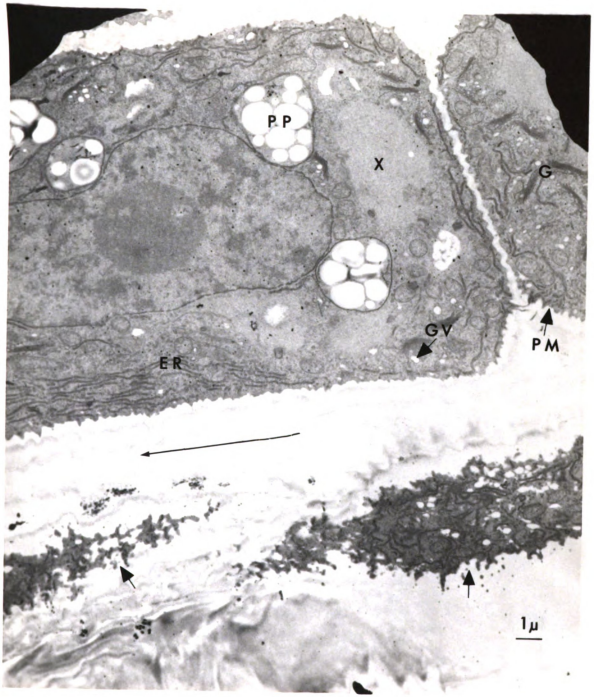
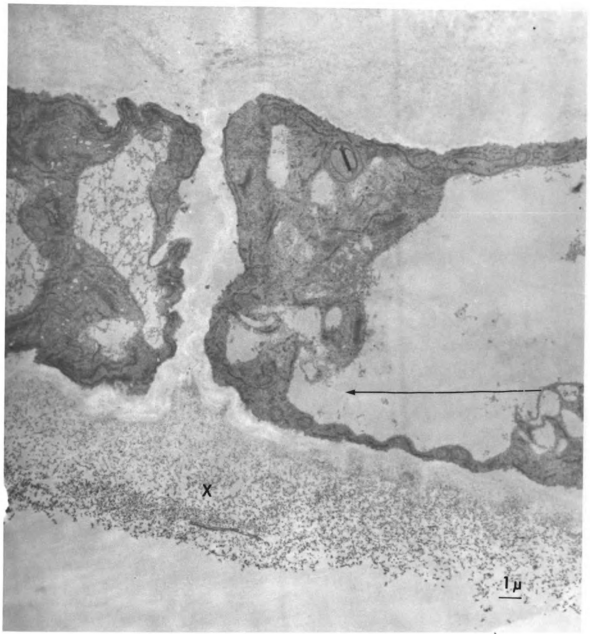

PP
X
G
GV
PM
ER
1μ

Figure 10.--Epidermal cells.

Portions of 2 epidermal cells showing the central large vacuoles and cytoplasm at the periphery of the cells. Note the grainy mucilaginous covering of the root (X). Median longitudinal section. Arrow, pointing to root apex, indicates root axis. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.



tral
cells.
apex,
Ap-

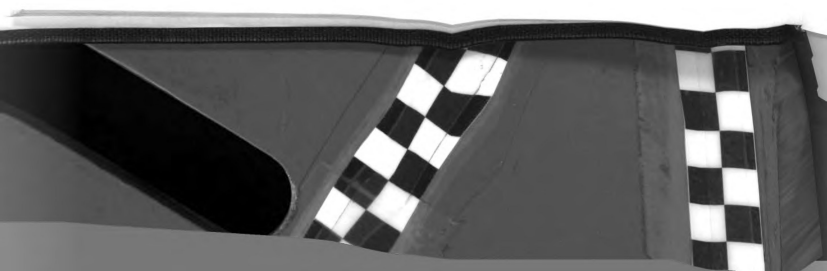


Figure 11.--Cross-sectional montage of epidermal cells.

A cross-sectional montage of a portion of root at 1000 microns showing epidermal (EPI) and sub-epidermal cells. Note the smooth plasma membrane (PM) of the outer epidermal cells. Fixation in 2.5% $KMnO_4$, pH 7.5 with veronal acetate buffer. Approximately X 2,300.

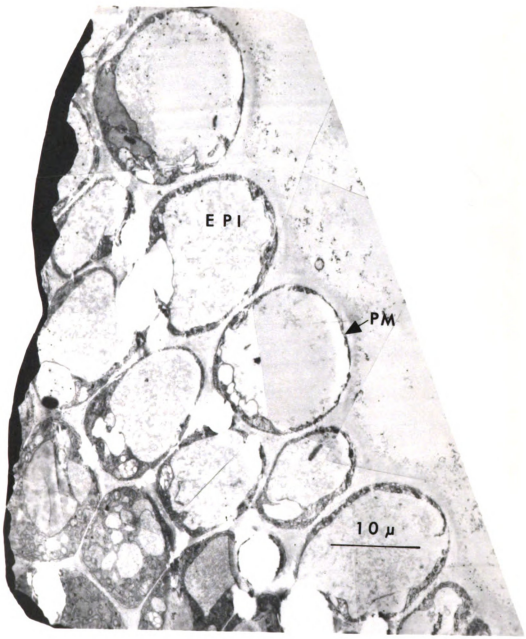

EPI
PM
10 μ

Fig. 12.--Columella cells.

Photograph showing cells of the columella. Note the endoplasmic reticulum (ER) - vesicle (V) region and placement of the nucleus (N) and proplastids (PP). Median longitudinal section. Arrow, pointing to root apex, indicates root axis. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.

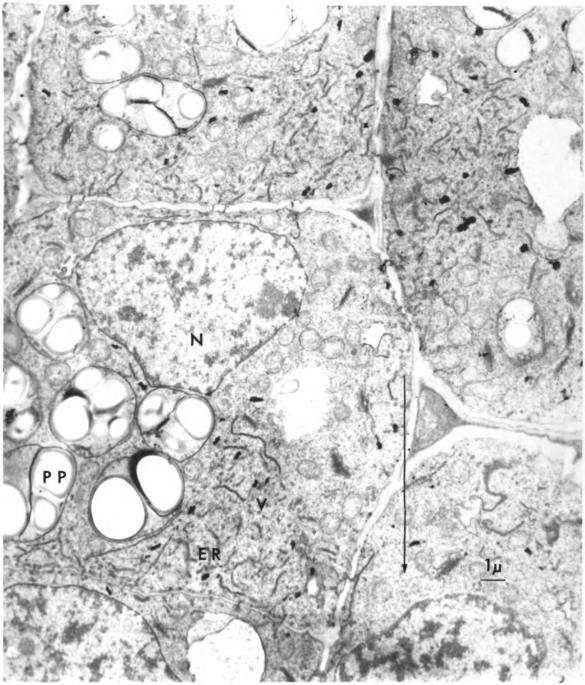

N
PP
V
ER
1μ

Figure 13.--Columella cells.

Portion of a columella cell located closer to the root apex than those in Figure 12. Note the placement of the nucleus (N) and proplastids (PP), endoplasmic reticulum (ER) - vesicle (V) region, Golgi (G), and cytoplasmic clear area with scattered organelles (X). Median longitudinal section. Arrow, pointing to root apex, indicates root axis. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.

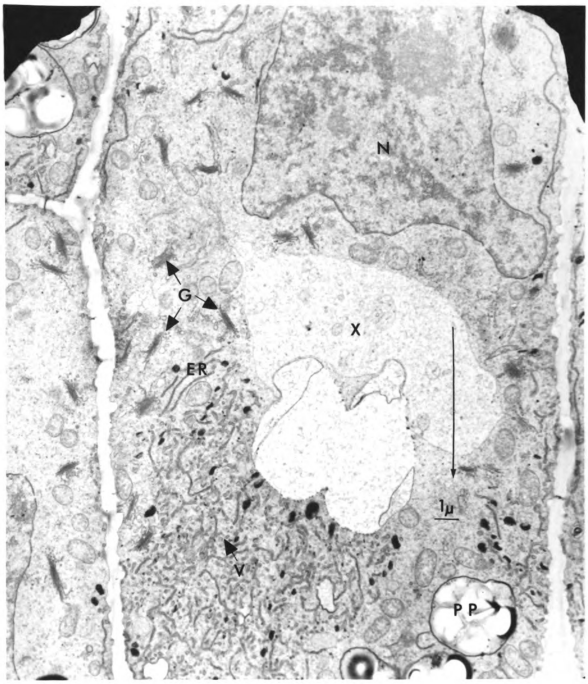

N
G
X
ER
1μ
V
P P

Figure 14.--Cross-sectional montage at 400 microns from tip.

A cross-sectional montage of a portion of root at 400 microns from the tip. Note the highly scalloped outer cells (X) and the gradations to the columella cells (COL) containing lipoidal material (L) and endoplasmic reticulum (ER) localized at the periphery in some cells. Fixation in 2.5% $KMnO_4$, pH 7.5 with veronal acetate buffer. Approximately X 1,170.

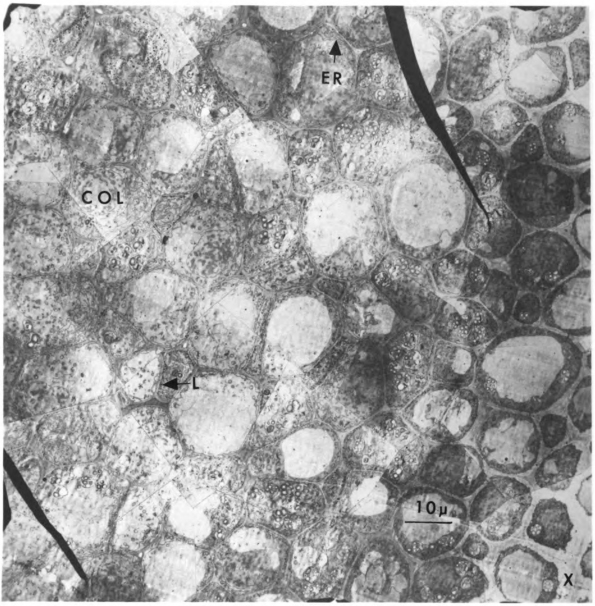

ER
COL
L
10μ
X

Figure 15. Typical

Histograms of ...

extinction of ...

deposited ...

shape and ...

magnification ...

Figure 15 A ...

the to ...

MnO_2 ...

Figure 15.--Tip-cells.

Portions of highly scalloped tip-cells. Note great numbers of Golgi bodies (G) and Golgi vesicles (GV), deposited Golgi vesicle contents (X), and former cell shape indicated by original cell wall (W). For higher magnification, and details of the Golgi bodies, see Figure 17 A. Median longitudinal section. Arrow, pointing to root apex, indicates root axis. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.

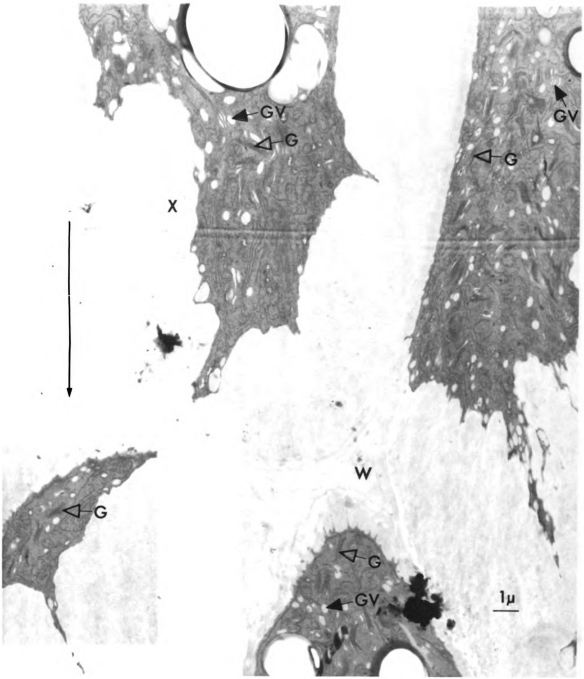
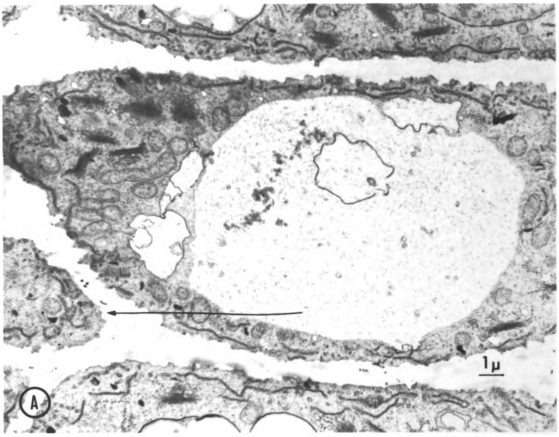

GV
G
X
GV
G
G
W
G
GV
1μ

Figure 16.--Epidermal-root-cap-complex.

Portions of cells showing the variation of types found in the epidermis-root-cap-complex. The cell in photograph A is located near the columella cells and the cell in photograph B is near the epidermis and tip-cells. Both exhibit the lenticular shape. Arrow, pointing to root apex, indicates root axis. Median longitudinal section. Glutaraldehyde-$KMnO_4$ fixation. Approximately X 6,000.


1μ
A

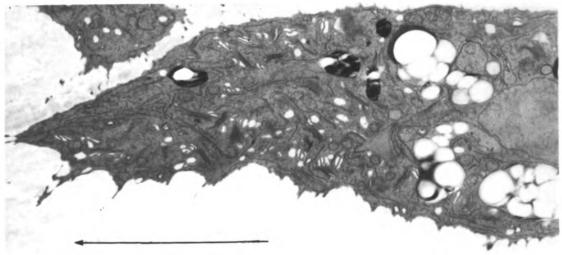
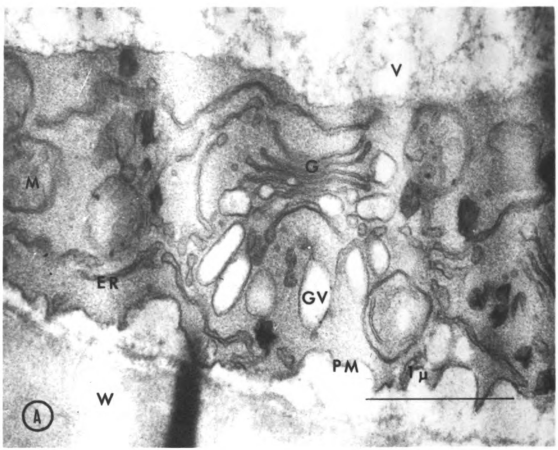

1μ
B

Figure 17.--Golgi bodies.

This plate shows two types of Golgi bodies found in the pea. Photograph A has a Golgi body (G) with larger vesicles (GV) found in cells near the periphery of the root. Photograph B shows a Golgi body (G) with smaller vesicles (GV) found in cells in the interior of the root. Note the endoplasmic reticulum (ER), plasma membrane (PM) being more scalloped in A, mitochondria (M), vacuoles (V), and cell wall (W). Fixation 2.5% $KMnO_4$, pH 7.5 with veronal acetate. Approximately X 38,500.


V
G
M
ER
GV
PM
1 μ
W
A

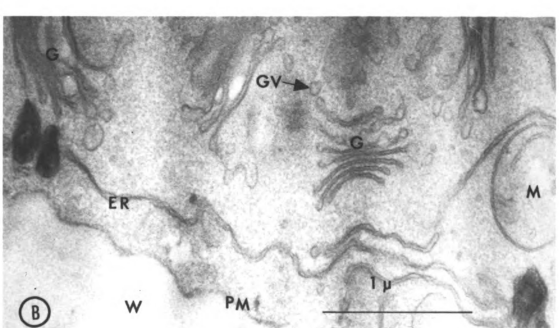

G
GV
G
M
ER
1 μ
B
W
PM

Figure 18.--Lipoidal material.

This plate shows lipoidal material (L). A. Lipoidal droplet (L) after Glutaraldehyde-OsO_4 fixation. Approximately X 42,300. B. Lipoidal droplet (L) after $KMnO_4$ fixation. Approximately X 42,300. C. Showing high concentration of lipoidal material (L) found in the quiescent center. Glutaraldehyde-OsO_4 fixation. Approximately X 5,000.

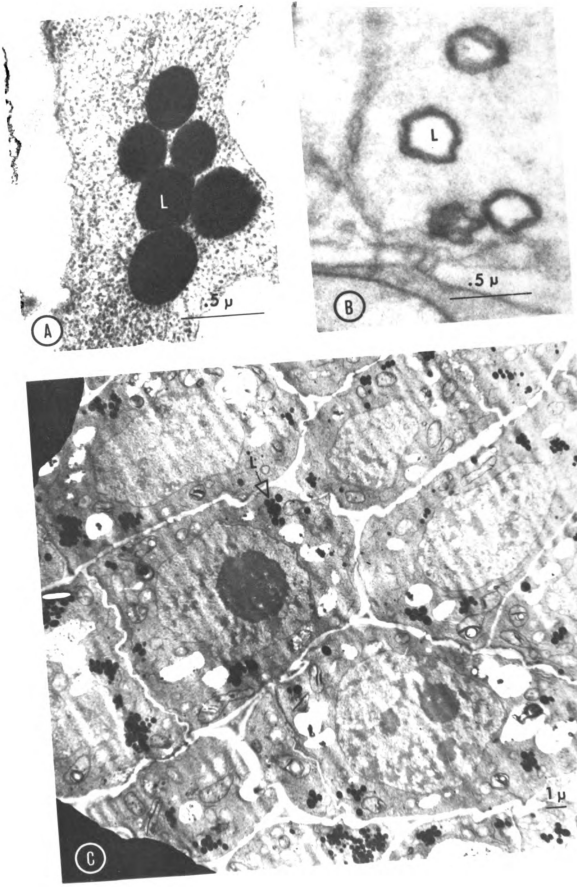
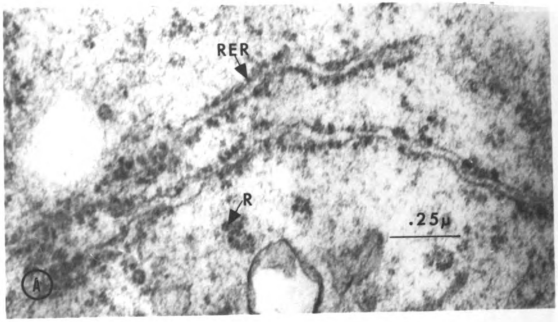
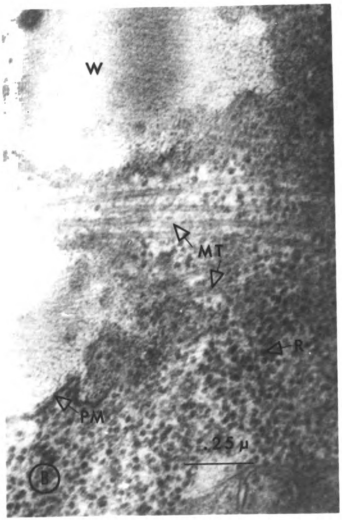

L
.5 μ
A
L
.5 μ
B
L
1 μ
C

Figure 19.--Rough-endoplasmic reticulum and microtubules.

This plate shows rough-endoplasmic reticulum and microtubules. A. Shows rough-endoplasmic reticulum (RER) and free ribosomes (R). Glutaraldehyde-OsO_4 fixation. Approximately X 72,000. B. Shows microtubules (MT) at the periphery of the cell. Note plasma membrane (PM), cell wall (W), and dense free cytoplasmic ribosomes (R). Glutaraldehyde-OsO_4 fixation. Approximately X 72,000. C. Cross-sections of 3 microtubules (MT) at the periphery of a cell. Glutaraldehyde-OsO_4 fixation. Approximately X 72,000.

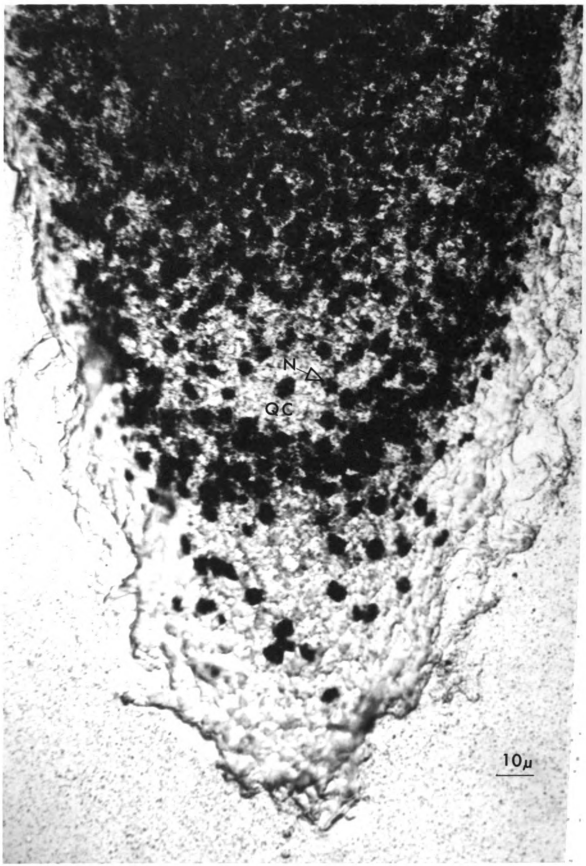

RER
R
.25μ
A


W
MT
R
PM
.25μ
B


MT
.25μ
C

Figure 20.--Autoradiograph showing quiescent center.

An autoradiographic paraffin section of root tip grown for 18 hours in nutrient solution and tritiated thymidine. Note the quiescent center (QC) with low uptake of tritiated thymidine into the nuclei (N). Approximately X 700.


N
QC
10μ

BIBLIOGRAPHY

Brachet, J. and A. E. Mirsky (ed.). 1961. The cell, II. Academic Press, New York. 916 p.

Brachet, J. and A. E. Mirsky (ed.). 1964. The cell, VI. Academic Press, New York. 564 p.

Clowes, F. A. L. 1959. Apical meristems of roots, Cambridge Philosophical Society, Biol. Rev. 34:501-529.

Clowes, F. A. L. 1961. Apical meristems. Blackwell, Oxford. 217 p.

Clowes, F. A. L. 1964. The quiescent center in meristems and its behavior after irradiation, p. 46-58. In Meristems and differentiation. Brookhaven Symp. Biol. 16, Office of Technical Services, Dept. of Commerce, Washington.

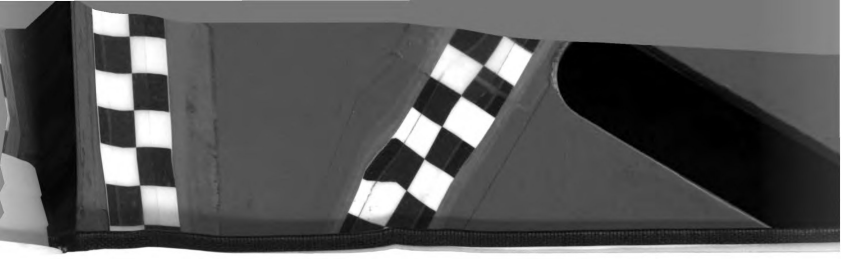
Clowes, F. A. L. and B. E. Juniper. 1964. The fine structure of the quiescent centre and neighbouring tissues in root meristems. J. Exp. Bot. 15:622-630.

Esau, K. 1965. Plant anatomy. 2nd ed. John Wiley & Sons, New York. 767 p.

Frey-Wyssling, A. and K. Mühlethaler. 1965. Ultrastructural plant cytology. Elsevier Pub. Co., New York. 377 p.

Janczewski, E. 1874. Recherches sur l'accroissement terminal des racines, dans les Phanérogames. Ann. Sci. Nat. Bot. Sér. V. 20:162-201.

Ledbetter, M. C. and Porter, K. R. 1963. A "mictrotubule" in plant cell fine structure. J. C. B. 19:239-250.



Ledbetter, M. C. and Porter, K. R. 1964. Morphology of microtubules of plant cells. Science 144:872-874.

Leech, J. H., H. H. Mollenhauer, and W. G. Whaley. 1963. Ultrastructural changes in the root apex, p. 74-84. In G. E. Fogg (ed.), Cell differentiation, Symp. Soc. Exp. Biol. XVII, Cambridge University Press.

Lillie, R. D. 1951. Simplification of the manufacture of Schiff reagent for use in histological procedures. Stain Tech. 26:163-165.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414.

Miksche, J. P., chmn. 1964. Meristems and differentiation. Brookhaven Symp. Biol. 16, Office of Technical Services, Dept. of Commerce, Washington. 240 p.

Mollenhauer, H. 1959. Permanganate fixation of plant cells. J. Biophys. Biochem. Cytol. 6:431-436.

Mollenhauer, H. H. 1965. Transition forms of Golgi apparatus secretion vesicles. J. Ultra. Res. 12:439-446.

Mollenhauer, H. H., W. G. Whaley, and J. H. Leech. 1961. A function of the Golgi apparatus in outer rootcap cells. J. Ultra. Res. 5:193-200.

Neumann, O. 1939. Über die bildung der wurzelhaube bei Juglans, Mimosa and Lupinus. Planta 30:1-20.

Pease, D. C. 1964. Histological techniques for electron microscopy. Academic Press, New York. 381 p.

Pienaar, R. de V. 1955. Combinations and variations of techniques for improved chromosome studies in the Gramineae. J. S. African Bot. 21:1-8.

Popham, R. A. 1955. Zonation of primary and lateral root apices of Pisum sativum. Am. J. Bot. 42:267-273.

Porter, K. R. and R. D. Machado. 1960. Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip. J. Biophys. Biochem. Cytol. 7:167-180.

Prescott, D. M. 1964. Autoradiography with liquid emulsion, p. 365-370. In D. M. Prescott (ed.), Methods in cell physiology. Academic Press, New York.

Reeve, R. M. 1948. Late embryogeny and histogenesis in Pisum. Am. J. Bot. 35:591-601.

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

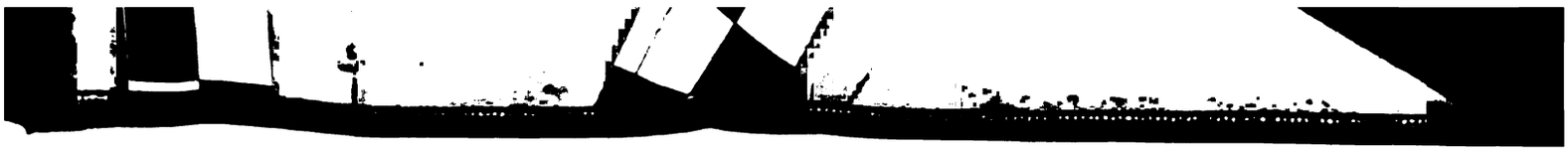
Stocking, C. R. 1956. Histology and development of the root, p. 173-187. In W. Ruhland (ed.), Encyclopedia of plant physiology, III. Springer-Verlag, Berlin.

Torrey, J. G. 1955. On the determination of vascular patterns during tissue differentiation in excised pea roots. Am. J. Bot. 42:183-198.

Whaley, W. G., J. E. Kephart, and H. H. Mollenhauer. 1959. Developmental changes in the Golgi-apparatus of maize root cells. Am. J. Bot. 46:743-751.

Whaley, W. G., H. H. Mollenhauer, and J. H. Leech. 1960. The ultrastructure of the meristematic cell. Am. J. Bot. 47:401-449.

Wolff, S. 1964. Handling of root tips, p. 215-227. In D. M. Prescott (ed.), Methods in cell physiology. Academic Press, New York.



MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 03177 5624