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AUTORADIOGRAPHIC EXAMINATION OF THE INCORPORATION
OF TRITIATED SUGARS IN CELLS OF THE ROOT CAP
AND EPIDERMIS OF CORN (ZEA MAYS) AND
ASSOCIATED MUCILAGINOUS SECRETIONS

BY

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A thesis submitted to the Faculty of Graduate
Studies and Research in partial fulfilment
of the requirements for the degree of
Master of Science

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ABSTRACT

Young corn root tips incubated in solutions containing three radioactive sugars - glucose-6-³H, glucose-1-³H and fucose-6-³H were shown to exhibit different labelling patterns in the cells of the root cap and associated slime and cells of the epidermis and associated mucilage with both continuous and pulse chase experiments. The origin of the mucilage overlaying the epidermis was shown to be the epidermal cells by adding to known differences between the root cap slime and the epidermal mucilage and by pulse chase grain counting data. Cell walls were found to be well labelled by all three precursors, especially new cell plates. Three patterns of cell plate formation were well elucidated by the autoradiographic procedure. Young root hair tips were also labelled.

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IN LOVING MEMORY OF MY LATE
FATHER - EURSAL JAMES KAINB

TABLE OF CONTENTS

Abstract.	ii
Acknowledgements.	iii
Dedication.	iv
Table of Contents	v
1. Introduction.	1
2. Materials and Methods	17
2:1 Plant Material.	17
2:2 Radioactive Chemicals	17
2:3 Incubation with Radioactive Precursors.	19
2:4 Fixation, Infiltration and Sectioning	21
2:5 Staining Procedures	23
2:6 Autoradiography	25
2:7 Microscopy and Photomicrography	27
2:8 Grain Counting Procedure.	28
2:9 Plasmolysis Experiment.	30
2:10 Controls and Checks	30
3. Results	32
3:1 Introduction to the Results	32
3:2 Continuous Labelling.	39
3:3 Pulse Chase Labelling	53
3:4 Examination of the Epidermis - Specific Topics.	77
3:5 Controls.	86
4. Discussion.	87
5. Conclusions	114
Appendices.	116
References Cited.	158

The most beautiful thing we can experience is the mysterious. It is the source of all true art and science To know that what is impenetrable to us really exists, manifesting itself as the highest wisdom and the most radiant beauty which our own dull faculties can comprehend only in their most primitive forms - this knowledge, this feeling, is at the centre of true religiousness.

Albert Einstein

1. INTRODUCTION

Roots release into the soil many different substances which were termed exudates by Hale et al. (1967). More recently Rovira et al. (1979) redefined released compounds by two terms, exudates and secretions. (Exudates are defined as low molecular weight compounds whose release is not metabolically mediated whereas secretions can be low or high molecular weight compounds released by metabolic processes. These compounds include a non-diffusible mucilaginous material (Rovira and McDougall, 1967) which has been referred to in the literature through the years as mucigel, mucilaginous material, gelatinous material, gelatinous sheath, mucilaginous sheath, gel-like sac material, slime, mucilage, root jelly, etc. all implying that this substance has a viscous nature (Rovira, 1969). Being non-diffusible, this mucilage remains in the root/soil interface zone (the rhizosphere) through which passes nutrients for the plant, the diffusible exudates and secretions of the plant and water. The elaboration of the nature and possible functions of these mucilaginous substances secreted from the root surface is becoming more essential for the understanding of plant nutrition and root/soil interactions.

There is a long history of noticed mucilage on the root surface. It was first described on root hairs (Schwarz, 1883 from Leiser, 1968; Roberts, 1916; Howe, 1921 and later corroborated by several workers

i.e. Frey-Wyssling and Muhlethaler, 1948; Dawes and Bowler, 1959; Head, 1964 and Werker and Kislev, 1978 for many root types) and also on roots in general (Scott et al. 1958; Rovira, 1962; Jenny and Grossenbacher, 1963; Dart and Mercer, 1964; Leiser, 1968; Brams, 1969).

Several functions have been suggested for this mucilaginous substance on the root;

1. It provides a zone of close contact between the root and soil particles (termed the rhizosphere) (Old observations - Schwarz, 1883 from Leiser, 1968; Price, 1912; Roberts, 1916; Light microscopy - Lund, 1965; Blevins et al. 1968; Leiser, 1968; Soileau et al. 1964; Oades, 1978; Transmission electron microscopy - Jenny and Grossenbacher, 1963; Greaves and Darbyshire, 1972; Blevins et al. 1970; Foster and Rovira, 1976; Scanning electron microscopy - Campbell and Rovira, 1973; Rovira and Campbell, 1974).

2. It may be beneficial to cation exchange (Jenny and Grossenbacher, 1963; Crooke et al. 1960a, 1960b; Jenny, 1966).

3. It may be important in the maintenance and genesis of soil structure proximal to the root (Soileau et al. 1964; Finch et al. 1971; Swincer et al. 1972; Breisch and Reisinger, 1975; Guchert et al. 1975).

4. It may affect the relative proportions of soil micro-organisms and the numbers and virulence of viable organisms (including pathogens) surrounding the roots, therefore having an influence on root disease.

The presence of micro-organisms on the root surface is a well documented fact (Dart and Mercer, 1964; Brams, 1969; Samstevich, 1965; Breisch and Reisinger, 1975; Campbell and Rovira, 1973; Greaves and Darbyshire, 1972; Barber and Martin, 1976; Foster and Rovira, 1976;

Oades, 1978).

5. It may exert some selectivity on ion uptake by the plant root and may also be a reserve of ions (Brams, 1969; Oades, 1978).

6. It may also protect the root from mechanical damage and from desiccation (Leiser, 1968; Price, 1912; Samstevich, 1965; Oades, 1978).

7. It may be a source of food for soil organisms (Pareek and Gaur, 1964; Oades, 1978).

Mucilage being persistently present seems to be important to the root, especially for root/soil interactions. It would therefore be useful to discover its source within the root and to determine its nature.

In the preceeding references the word mucilage has been used to describe many different types of secretions and has therefore become a misused term lacking a clear meaning. Oades (1978) and Rovira et al. (1979) have attempted to clear up this matter and it is from Oades work that I have extracted one of the terms for defining the secreted material of the roots.

On corn roots two different types of mucilaginous material can be distinguished. These will be referred to as root cap slime (from historical precedence) and epidermal cell mucilage or for short epidermal mucilage (after Oades, 1978). Figure 1 indicates the positions of these two types of mucilaginous secretions.

Harris and Northcote (1970) were the first to recognize a difference between the root cap slime and the epidermal mucilage (although Price noticed a difference in desert grasses in 1912).

4.
Figure 1:

A diagram of a Zea mays L. root tip showing the various extracellular secretions of the root and the structure of the epidermal cells where:

- A is the root cap slime
- B is the epidermal cell wall (outer periclinal)
- C is the inner layer of the mucilage
- D is the outer layer of the mucilage

These classifications will be used in Table 1 for the staining reactions. The regions marked in the root cap (Regions 1 and 2) are indicated to provide easier understanding of the portion of the root cap being discussed in the Results and Discussion.

Figure 1a:

An epidermal cell (stained with PAS) with its associated mucilage. Outer mucilage (om), inner mucilage (im), the thick outer wall (periclinal) of the epidermal cell (cw) and the epidermal cell (EC). x2000.

In their examination of the epidermis Bell and McCully (1970) first noted a histochemical difference between the root cap slime and the epidermal mucilage. These two secretions have been since thoroughly histochemically and physically distinguished (Miki, 1977; Clarke *et al.* 1979; Miki *et al.* 1980). These results are summarized in Table 1. Two histochemically different regions are present in the mucilage and are referred to as outer and inner mucilage (Figure 1).

The root cap slime has been extensively analyzed (Table 2). The results of the analyses and also the staining characteristics indicate that the slime is carbohydrate in nature. It is said to consist of units made of a cellulosic central polymer surrounded by a pectin-like material and to contain 1-4 linkages. There is however limited agreement on the chemical composition of the slime (Table 2).

The epidermal mucilage, on the other hand, has not been analyzed, but it is also known to be polysaccharide in nature from its staining properties (Table 1 especially the PAS positivity).

The origin of the slime has been suggested from time to time. Haberlandt (1914) believed that it was derived from the middle lamella of the older root cap cells. Juniper and Roberts (1966) believed that it came from degraded cell walls. It is now known to be a product of the peripheral root cap cells. This is a well documented fact (Whaley *et al.* 1959a and 1959b; Mollenhauer *et al.* 1961; Northcote and Pickett-Heaps, 1966; Mollenhauer and Morrè, 1966; Pickett-Heaps, 1967a, 1967b; Roberts and Butt, 1968b, 1970; Kirby and Roberts, 1971; Rougier, 1971; Jones and Morrè, 1973; Juniper and Pask, 1973;

Table 1: Histochemistry of the Root Cap Slime, the Epidermal Cell
Mucilage and Walls.

	A Slime	B Epidermal Cell Walls	C Inner Mucilage. Layer	D Outer Mucilage Layer	**
Toluidine Blue O	pale pink	pale pink	pale green	pale pink	
Alcian Blue	light blue	light blue	-	blue	
Auto- fluorescence	++	+++	+++	+++	
PAS *	pink +	pink +++	pink ++	pink +++	
Calcofluor	blue +	bright blue +++	blue ++	bright blue +++	

after Clarke et al. 1979

+, ++ and +++ are subjective judgements of relative intensity.

- = no stain.

* means periodic acid Schiff reaction.

** categories from Figure 1.

Table 2: Analyses of Neutral Sugars of Zea mays L. Root Cap
Slime.

	1	2	3	4	5	6
Fucose	38.58	35	32	8	16.7	-
Galactose	30.41	23	21	39	37.7	35
Arabinose	14.51	26	15	7	6.7	-
Xylose	7.45	16	4	5	12.8	5
Glucose	5.68	-	22	22	16.7	37
Ribose	0.00	-	-	-	-	11
Rhamnose	-	-	-	1	-	-

1. Percentage of neutral sugars from ethanol wash. Paull and Jones (1975).
2. Percentage from nodal roots: Floyd and Ohlrogge (1970).
3. Expressed as percentage. Galacturonic and glucuronic acid are also present. Harris and Northcote (1970).
4. mg per 100 mg of slime. Galacturonic acid present - 12%. Jones and Morrè (1973).
5. Expressed as percentage. Slime from little roots developed by callus of coleoptile tissue. Wright and Northcote (1974).
6. Expressed as percentage. Galacturonic acid are also present. Jones and Morrè (1967).

Dauwalder and Whaley, 1974; Paull and Jones, 1976; Chrispeels, 1976).

The epidermal mucilage has been greatly neglected by microscopists, but has been noted by some (Scott, 1966, 1970; Old and Nicholson, 1975; Whaley *et al.* 1959a; Price, 1912, etc.). It has been misidentified as late as 1978 by Shen-Miller *et al.* as a cuticle and even more recently (1979) by Smith and O'Brien as the wall. The origin of the epidermal mucilage is still in dispute. Older theories include that of Scott *et al.* (1958) which suggested that the liquid precursors of the mucilage exuded from the plasmadesmata of the epidermal cell walls and Esau (1965) stated that it originates through the gelatinization of the root cap and the protoderm. Other workers say that it in part originates from the root cap slime and is simply drawn up over the surface of the epidermal cells (Foster in Oades, 1978). Others postulate that the epidermal cells themselves are the source of the mucilage (Price, 1912; Pickett-Heaps, 1967b; Dauwalder *et al.* 1969). In any case, the origin of the epidermal cell mucilage is still in doubt. Is it secreted by the root cap cells and drawn up over the root during growth, is it totally secreted by the epidermal cells or is it a composite from both?

Several factors point to the possibility that the epidermal mucilage is secreted by the epidermis and is not simply an extension of the root cap slime.

1. The root cap slime and the epidermal mucilage are histochemically different (Table 1) and are also biochemically distinguishable due to the fucose content of the slime. The detection of fucose (6-deoxy-L-galactose) is significant because it is a common constituent

of plant polysaccharides but is rarely found in such high concentration as in the root cap slime (Table 2), (Nevins et al. 1967). Fucose is more prevalent in the cap of Zea mays than elsewhere in the root and it occurs in only trace amounts in the rest of the seedling (Wright and Northcote, 1976; Wright, 1975). Because it is present in such large quantities it is considered as a specific marker for slime (Barlow, 1975). It is not a major component of root cap slime from wheat or other species analyzed (such as sycamore and peas - Wright, 1975), but is common in all corn cultivars so far examined. Fucose is therefore an end product of a differentiation process more or less specific to the maize outer root cap cells. In earlier analyses (as Number 6 of Table 2), fucose was not detected. It has now been determined that fucose was misidentified as ribose. Since this report ribose has not been detected (1 to 5 of Table 2). The slime and the mucilage are also physically different. The slime is easily removed whereas the mucilage is not; the slime is very easily dehydrated and rehydrated whereas the mucilage is not (Jones and Morrè, 1967, 1973).

2. The secretory character of the epidermal cells;

(a) The epidermal cells have enlarged Golgi cisternae and vesicles compared to other root proper cells as do the outer root cap cells for the root cap (Leech et al. 1963, Lopez-Fando et al. 1976). Enlarging of the Golgi has been linked to a secretory character and occurs in other known secretory cells (Whaley et al. 1959a, 1959b; Mollenhauer et al. 1961; Mollenhauer and Whaley, 1963; Rougier, 1971; Barlow and Sargent, 1978; Coulomb and Coulon, 1971;

Perner, 1957). The Golgi apparatus has long been known to be important in secretion (old work - Bowen, 1929a, 1929b; animal cells, especially mucopolysaccharides and carbohydrate proteins - Beams and Kessel, 1968; Bennett and Leblond, 1970; Bennett, 1970; Bekesi and Winzler, 1967; Neutra and Leblond, 1966a, 1966b; Strugess et al. 1973; Droz, 1966, 1967; Rambourg et al. 1969; Outrecht et al. 1969; Warshawsky et al. 1963; Dorfman, 1963; plants - first suggested by Drawert and Mix, 1962 and confirmed for plant slimes and other secretions - Mollenhauer and Morrè, 1966; Northcote and Pickett Heaps, 1966; Schnepf, 1961, 1963ab, 1964a, 1964b, 1965; Schnepf and Busch, 1976; McCully, 1966; cell walls and algal scales - Northcote, 1971; Fowke and Pickett-Heaps, 1972; Mollenhauer and Morrè, 1966; Bisalputra et al. 1966; Sievers, 1963; Brown et al. 1969, 1970; Franke and Brown, 1971; Brown, 1968; Schnepf and Deichgraber, 1969).

(b) Staining Density:

Bell and McCully (1970) found in lateral roots that the forming epidermis is very intensely stained. According to Clowes (1959) an indirect measurement of meristematic activity can be made from the density of the staining of the cytoplasm by basic dyes (such as toluidine blue). Strong staining with such dyes is characteristic of cells which are secretory. Another characteristic of secretory cells is a high metabolic activity. Clowes (1959) noted that when he applied L-leucine to the first 3 mm of the root apex of Zea mays that the highest rate of incorporation per volume occurred in the cap initials, the meristematic epidermis and the adjacent outer cortex. These same cells also have been shown to have high

β - glucosidase activity (Ashford and McCully, 1970).

(c) Ribosomal Content:

The ribosomal content of epidermal cells is more like that of a pancreatic acinar cell (a very active secretory cell) than that of the typical plant cell (Karas and McCully, 1973).

(d) The epidermis also has the typical columnar shape of known secretory cells.

Until recently, very little work was done to determine the structure of the epidermal cells. Most cytological work was done to discover aspects of root hair development and structure (Roberts, 1916; Snow, 1905; Scott et al. 1958; Cormack, 1935, 1937, 1949, 1962). In 1970 Bell and McCully while examining the development of lateral roots in corn noted the structure of the epidermal cells of the meristematic region of these roots. The younger root epidermis has been examined (Lewis and Crotty, 1976) however, the epidermis has only recently been examined for its developmental sequence (Clarke et al. 1979). Clarke et al. (1979) examined the entire developmental sequence from the periclinally flattened hexagonal shaped cells in the quiescent centre to the root hair initiation zone (Clarke and McCully, 1979). They distinguished and examined four developmental stages: (1) the young epidermal cells within the root cap which are flattened and "irregularly polygonal" in shape. These cells are not examined in this work because the major thrust of the epidermal examination was the mucilage origin towards the exterior of the root. (2) the columnar epidermal zone cells which are prominently rectangular in shape, with the short

dimension in the anticlinal direction. Further basipetal, (3) the transition zone epidermal cells are more cuboidal in shape with the periclinal dimension becoming longer. (4) the cells elongate in the periclinal dimension to become rectangular (or tabular) with the long dimension in the longitudinal plane in the elongation zone.

Three of these regions are examined in this work and are described in Section 3:1.

Earlier autoradiographic studies concerned the meristematic or columnar shaped cells but not the older cells. There is a general lack of observation that needs to be filled.

The technique which is used in my work to (1) distinguish the origin of the epidermal mucilage and (2) compare the developmental regions of the epidermis is autoradiography.

Pickett-Heaps (1967) says that the process of autoradiography has "great significance to cytologists, since by observing the sites of incorporation of labelled metabolites within the cells, the possibility exists of relating ultrastructure to function and to sites of some biochemical processes."

Autoradiography has been defined as the use of photographic emulsions to study the occurrence and distribution of radioactive substances in a specimen which contains radioactive material (Fischer and Werner, 1971; Hendee, 1973).

In autoradiography, for the best possible accuracy, sensitivity and resolution, the emulsion should be a thin layer and lie in close proximity to the specimen (DeRobertis et al. 1975). Other factors important in resolution are the grain size of the emulsion, the

section thickness and the radioactive moiety used.

The technique involves first labelling the specimen with a precursor which contains a radioactive label. Labelled compounds are those containing one or more radioactive atoms of the same element in the compound. The theory is that this replacement causes no other changes (other than the property of radioactivity) in the compound and therefore the compound will behave chemically and biologically like the unlabelled compound, except that it will eventually "betray its location by radioactivity" (DeRobertis et al. 1975). The fate of the precursor and its metabolites can therefore be followed. The radioactive moiety used for the best resolution is tritium (^3H), because it emits a low energy β particle with a short range (Caro, 1964; Michaux-Ferriere, 1976). It is also an efficient grain producer and compounds of high specific activity can be obtained (Mercer and Birbeck, 1972).

The technique is well known and is adequately described in the literature (Caro, 1964; Glauert, 1965; DeRobertis et al. 1975; Caro and van Tubergen, 1962; Masse, 1976; Hendee, 1973). Precautions must be taken during processing to avoid leaching and migration of radioactive compounds. It has been noted that radioactivity is lost at each processing stage (fixation, dehydration, infiltration) (Heinrich and Kuschki, 1978).

The production of consistently thin, uniform sections is an important factor in resolution. Caution must also be taken not to use a stain that may react with the emulsion (Hendee, 1973) for example toluidine blue causes chemical fogging of the emulsion (Mercer and Birbeck, 1972). PAS is routinely used as it causes no problems if

the extra Schiff's reagent is thoroughly removed.

The emulsions used are called nuclear track emulsions. They are mixtures of silver halide crystals and gelatin as all photographic emulsions, but have a higher concentration of the silver halide and a greater fineness and uniformity of grain conformation than other photographic emulsions (DeRobertis et al. 1975; Masse, 1976; Hendee, 1973).

There are two ways to label tissue: (a) continuous labelling which means that the tissue is labelled until it is fixed and (b) pulse chase in which the specimen is labelled with the radioactive (hot) precursor for a certain time period which is called the pulse followed by a chase which involves the incubation with a higher concentration of the same unlabelled compound (cold, usually a solution of 100 times the concentration). Competition occurs between the cold and hot compounds and in theory stops the uptake of the radioactive compound. Both of these techniques were used in this work.

One of the most important parts of using autoradiography is to pick precursors that will label the tissues of concern. In this work, three sugars were chosen because the main thrust was to discover the origin of the epidermal mucilage. The three sugars were glucose-1-³H, glucose-6-³H and fucose-6-³H.

Glucose-6-³H and glucose-1-³H were chosen since glucose is a common sugar of roots and would therefore be expected to enter the epidermal cell mucilage and the root cap slime. The two glucoses were applied to compare the fates of the two different labelling positions. When glucose is metabolised to uronic acids and amino

acid precursors, the sixth position carbon is lost, therefore glucose-6-³H would lose its radioactive character whereas glucose-1-³H would not. Glucose-6-³H, therefore, is called a "cleaner" label because the radioactivity does not enter as many non-target molecules (proteins, etc.) and is said to have a higher specificity for labelling hexoses.

Fucose was chosen because of its supposed specificity for the root cap slime and to see if it labelled other structures in the root. Fucose is not usually catabolized, unlike the glucoses, but is used directly in the synthesis of carbohydrates, glycoproteins, etc. via a nucleotide form (Paull and Jones, 1975).

In order to be visible by autoradiography, these sugars must be metabolized into compounds of high molecular weight. Free glucoses and fucoses and ones in low molecular weight compounds would be washed away during fixation and subsequent processing (Neutra and Leblond, 1966b; Northcote and Pickett-Heaps, 1966).

Since the two secretions of the root seem to be important for the existence of the root in the soil, it seems important that the origins of these two be understood. Using autoradiography, some insight into the origins of these two secretions may be found.

The purpose of this research is therefore (1) to establish whether the mucilage is indeed a product of the epidermal cells and not the root cap cells by (a) adding to the recognized differences between the mucilage and the root cap slime, a known product of the root cap and (b) using pulse chase results and grain counting procedures to follow the movement of the mucilage from the cells,

(2) to compare the localization of the three sugars after incorporation, (3) to examine the epidermis in detail in the following aspects (a) comparison of the three developmental regions for labelling patterns, (b) cell wall labelling and (c) root hair labelling, (4) to examine autoradiographic labelling patterns for the root cap and the root cap slime.

2. MATERIALS AND METHODS

2:1 Plant Material:

Corn seeds (Zea mays L. variety Seneca Chief) were surface sterilized in a 6% sodium hypochlorite solution (commercial Javex) for four minutes. The seeds were washed three times in sterile distilled water and allowed to imbibe under sterile conditions in distilled water at 25°C for three to six hours, then transferred to sterile Petri dishes (15 cm) containing approximately 50 ml of 0.9% agar (Oxoid agar number 3- Oxoid Limited, England). The edges of the Petri dishes were sealed with Parafilm (American Can Company, Greenwich CT) to retain moisture and were incubated at 25°C in the dark until the primary roots were three to five centimetres long (usually three to four days).

2:2 Radioactive Chemicals:

Glucose, D- [1-³H] (N) (specific activity 18 Ci/mmol), glucose, D- [6-³H] (N) (specific activity 33.99 Ci/mmol) and fucose, L- [6-³H] (specific activity 13.12 Ci/mmol) were obtained from New England Nuclear (Boston, Mass.). (N denotes nominally labelled. For an explanation see Appendix I.) The chemical names will be abbreviated to the following three forms respectively: glucose-1-³H, glucose-6-³H and fucose-6-³H. Structural representations of each molecule indicating the position of the radioactive tritium (³H) are presented in Diagram 1. The stock chemicals were received as ethanol:water solutions (9:1).

Diagram 1:

(a) Glucose, D $[6-^3\text{H}(\text{N})]$. (b) Glucose, D $[1-^3\text{H}(\text{N})]$ (c) Fucose, L $[6-^3\text{H}]$.

The small numbers represent the carbon number.

* represents the position of the tritium (^3H) moiety.

The purity of these radioactive stocks was periodically checked as described in Appendix III.

2:3 Incubation with the Radioactive Precursors:

Radioactive stocks were diluted with distilled water to a concentration of 100 μ Ci/ml. Each root tip was bathed in 100 μ l of solution. A rectangular plastic box with a tight fitting lid was used as an incubation chamber (Figure 4). Two box length pieces of Parafilm were affixed to the bottom of the chamber with masking tape. Small wells were formed in the Parafilm with light pressure (by a blunt instrument). One hundred microlitres (0.1 millilitres) of the solution (or chase solution or distilled water) was pipetted into the wells by automatic pipette (Oxford, Fisher Scientific) (with disposable tips). For the incubation, the root tip (4-8 mm) of an intact seedling was inserted into a droplet of liquid (Figure 2). High internal humidity was maintained by lining the sides of the chamber with moist chromatography paper and by placing water soaked paper on the bottom (on top of the Parafilm between the seedlings).

The temperature of the incubation chamber was maintained at 25°C by floating it in a temperature controlled water bath. Incubation with sugar solutions showed no difference compared to water grown material (Appendix II) in growth patterns, so water was usually used for bathing the controls (30-60 minutes).

After the incubation period (continuous or pulse chase) the root was washed with a stream of distilled water from a wash bottle for 15-30 seconds to remove the excess radioactivity before the

Figure 2:

A view of a corn seedling from the side showing how much of the root tip is submerged into the droplet of liquid for feeding. x 1.5.

Figure 3:

A diagram of the glass dipping jar. The measurements are in millimetres.

Figure 4:

A diagram of the incubation chamber. The measurements are in centimetres.

excision of the root tip for fixation or bathing in the chase solution whichever was the case.

For pulse chase, the pulse time for all three sugars was 60 minutes. Between the pulse and the chase the roots were thoroughly rinsed in a stream of distilled water as mentioned above and then moved to a well containing the chase solution - a sugar solution 100 times the concentration of the radioactive sugar (glucose-6-³H-0.053g D-glucose/100 ml = 3mM, glucose-1-³H-.1g D-glucose/100 ml = 6mM and fucose-6-³H-.13g L-fucose/100 ml = 8mM). After the chase, the roots were again washed before fixation.

The specific times for continuous and pulse chase labelling for all three labels are presented in Table 3.

2:4 Fixation, Infiltration and Sectioning:

Radioactively incubated and control root tips were excised (approximately 3-6 mm) in cold (approximately 4°C) 3% glutaraldehyde (JBEM Services, Pointe Claire, Quebec) in 0.025 M potassium phosphate buffer pH 6.8 and fixed overnight (8 to 16 hours) at 4°C (Feder and O'Brien, 1968). The fixative was replaced by distilled water and the vials were put into a boiling water bath for five minutes (procedure of Dr. M. Smith, personal comm.). The water inside the vials did not boil but became very hot. The roots inside the vials migrated back and forth from the top of the vial to the bottom of the vial during this procedure. This procedure has no effect on labelling patterns or on the intensity of labelling using glucose-6-³H (Kaine, 1977), but results in better infiltrated tissue, so it was routinely used.

Table 3: Summary of Procedures Performed.

		Continuous			Pulse Chase		
		G1H	G6H	F6H	G1H	G6H	F6H
Incubation Times	15	x	x	-	-	-	-
(Minutes)	30	x	x	x	-	-	-
(Pulse)	60	x	x	x	x	x	x
	120	x	x	x	-	-	-
	180	-	x	-	-	-	-
Wash		x	x	x	x	x	x
Chase Times	0	-	-	-	x	x	x
(Minutes)	10	-	-	-	-	x	-
	15	-	-	-	x	x	x
	30	-	-	-	x	x	x
	60	-	-	-	x	x	x
	90	-	-	-	x	x	x
Wash		-	-	-	x	x	x
Fixation		x	x	x	x	x	x
Boiling		x	x	x	x	x	x
Dehydration		x	x	x	x	x	x
Infiltration & Embedding		x	x	x	x	x	x
Sectioning		x	x	x	x	x	x
Staining (PAS)		x	x	x	x	x	x
Dipping		x	x	x	x	x	x
Exposure		x	x	x	x	x	x
Development		x	x	x	x	x	x

G1H = glucose-1-H; G6H = glucose-6-H; F6H = fucose-6-H;

x = part of procedure; - = not part of procedure.

The roots were dehydrated in a methyl cellosolve (2 methoxy-ethanol), ethanol, n-propanol, n-butanol series two changes of each lasting 15 to 60 minutes at 0°C and infiltrated in glycol methacrylate monomer mixture for two weeks at room temperature after dehydration (Feder and O'Brien, 1968). Polymerization in fresh monomer mixture was performed at 60°C for 24 to 48 hours (Feder and O'Brien, 1968).

After trimming by file, 2 μ m thick longitudinal sections were cut using glass knives. The sections were mounted in drops of sterile distilled water on glass slides (Gold Seal - Clay Adams, Parsippany, NJ). The sections were dried down for 12 to 18 hours on a hotplate at 37°C.

2:5 Staining Procedures:

2:5:1 Periodic Acid Schiff's (PAS) Reaction (modified from Feder and O'Brien, 1968):

This stain was used routinely to show up the roots for autoradiography. It was performed before the slide was emulsion coated. The general procedure of Feder and O'Brien (1968) was followed using one of the aldehyde blocking agents they suggested - saturated 2,4 dinitrophenol (DNPH) in 15% acetic acid for 10 minutes. The slides were then washed in running water for 30 minutes and dried down on a hotplate. After this the procedure of Feder and O'Brien (1968) was followed.

2:5:2 Aniline Blue (after Smith and McCully, 1978b):

Aniline blue (#1523 Polysciences, PA) staining was also performed before emulsion coating. Unstained sections were mounted in 0.05% aniline blue in 0.67 M potassium phosphate buffer at pH 8.6

under a coverslip and observed with epifluorescence optics.. The beamsplitter used was F1, the barrier filter 44 and the excitor filter BG12 (Zeiss, West Germany). For the experiment to correlate aniline blue staining with autoradiography, the aniline blue stained slides were washed overnight in running water, dried down and stained by the PAS reaction before the emulsion coating.

2:5:3 Toluidine Blue O (modified from Feder and O'Brien, 1968 - CI#52040 Fisher Sci.):

Sections embedded in methacrylate were stained in 0.05% toluidine blue in benzoate buffer pH 4.4 for one to two minutes. The stain was then rinsed off and the section was dried before mounting in immersion oil. This stain could be applied after autoradiography as it stains through the emulsion. It could not be used before coating with the emulsion because it causes chemical fogging of the emulsion (Mercer and Birbeck, 1972). Before mounting in immersion oil the sections are breathed on and the condensation allowed to clear before mounting.

The following stains (2:5:4 to 2:5:7) were performed on uncoated slides and also in one case on hand sections.

2:5:4 Alcian Blue 8GS (lot#71123, JBEM Services, Pointe Claire, P.Q.):

The sections were stained with 0.3% alcian blue (aqueous) for one to four minutes. The stain was then washed off and the slide was air dried before mounting in immersion oil.

2:5:5 Methylene Blue (1% in 1% Sodium borate - Edward Gurr Ltd., England):

Stain was performed for one to two minutes and then the sections were washed in tap water and allowed to dry before mounting in immersion oil.

2:5:6 Calcofluor White M2R New (Am. Cyanamid Co., Montreal)

(Hughes and McCully, 1975):

Sections were mounted in 0.01% calcofluor (aqueous) (diluted from a 0.1% stock solution) (aqueous) and observed with epifluorescence optics. The filter combination used was BG12 as an excitor filter and barrier filter 44 with an F1 beamsplitter.

2:5:7 Autofluorescence:

Sections were equilibrated and mounted in 0.067 M phosphate buffer pH 8.6. The sections were examined with epifluorescence optics using excitor filter UG1 and barrier filter 50 with an F1 beamsplitter. The technique was also used for handsections for the removal of mucilage experiments (Appendix VI). Handsections were cut, equilibrated and mounted in the 0.067 M buffer pH 8.6 for examination with the fluorescence microscope.

2:6 Autoradiography:

2:6:1 Preparations for Autoradiography:

The sections were first stained (in the majority of experiments) by the PAS reaction before dipping.

Small black plastic slide boxes (25 slide capacity - Fisher Scientific) were cleaned. A small amount of Drierite (W.A. Hammond-Drierite Com., Xenia, Ohio) was wrapped in gauze and was separated from the experimental slide section by a divider slide three spaces from one end of the box. The place next to the divider

slide was left vacant in the experimental slide section.

The emulsion (Kodak NTB-2 Nuclear Track) was packaged in a plastic bottle and to melt the emulsion the bottle was placed in a water bath at 43°C for 30 minutes. The emulsion was then poured into a warmed dipping jar diluted with an equal amount of warmed (43°C) distilled water and mixed by decanting between two dipping jars. The diluted emulsion was allowed to stand until no bubbles clung to clean slides dipped into it (four to six minutes). The safelight used was a Kodak Wratten number 1. (using a 15 watt bulb).

2:6:2 Dipping:

Slides were dipped individually with the section side away from the experimenter. The slide was held vertically over the emulsion, immersed until the tip touched the bottom of the dipping jar, left for two seconds and withdrawn slowly and steadily (over a five second period). To dry, the slides were leaned vertically against a wall at a 45° angle on paper towels for 10 to 20 minutes. The lower end of the slide was then blotted on paper towels and the slide was deposited in the slide box with the tissue side facing the Drierite. The box was then sealed with black electric tape and wrapped in aluminum foil.

An examination of the dipping technique was performed to check for any irregularities in the emulsion thickness (see Appendix IV).

2:6:3 Exposure and Processing:

The slides were stored at 3°C for two weeks (in one

case for one week).

Development was under proper safelighting (Kodak Wratten #1). Slides were transferred to a glass slide rack which could be moved between glass tanks of solutions. The procedure for the development was as follows: (adapted from Rogers, 1967).

All solutions were equilibrated to 17°C.

- (i) Develop in Kodak Dektol diluted one part to two parts distilled water for two minutes.
- (ii) Rinse in distilled water (30 seconds).
- (iii) Fix in 30% sodium thiosulphate (aqueous) for eight minutes.
- (iv) Wash in running water and air dry.

2:7 Microscopy and Photomicrography:

Dried slides were mounted in immersion oil (Carl Zeiss - Oberkochen, W. Germany) under a coverslip. When staining with toluidine blue as mentioned previously, the sections were breathed on and the condensation just allowed to clear before mounting in the oil. Sections were viewed directly in aniline blue and calcofluor solutions. All slides were examined with a Carl Zeiss microscope equipped with epifluorescence and bright field optics. Photographs of fluorescence were recorded on Kodak Panatomic-X 35 mm film and the autoradiographs on 4 x 5 inch Ektapan sheet film. Filters were used to take photomicrographs of the autoradiography to modulate the background PAS stain (deep amber (Cinemoid 33) and primary red (Cinemoid 6) - the Strand Electrical and Eng. Com., England). The large format (4 x 5 inch) negatives were taken using a Leitz camera.

with a bellows attachment. The 35 mm camera was a Nikon camera back. For the picture of the root positioned in the droplet of liquid a Minolta SRT201 camera with a Vivitar macro focusing auto zoom lens was used.

2:8 Grain Counting Procedures:

Slides representing the various chase times were prepared and mounted in immersion oil. Measurements were made using a Carl Zeiss microscope on bright field using a 40X objective with an 8X ocular. An ocular scale was placed in one of the eyepieces of the microscope. Calibration with a stage micrometer showed each division on the ocular scale equalled 1.25 μ m. The scale was aligned with an anticlinal wall of an epidermal cell (Diagram 2). A block (area) of wall and mucilage was counted with the dimension along the root being 10 ocular divisions and the other dimensions as per Diagram 2. The mucilage thickness was also measured. To compensate for root growth (Appendix II) for chase times of 10, 15, 30 and 60 minutes only the columnar zone cells were counted whereas for 90 minute chase both the columnar and the transition zone cells were counted.

At the same time measurements of cell length were also made of each for the developmental zones.

2:9 Plasmolysis Experiment:

Seedlings were grown (as per Section 2:1) and fed glucose-1- 3 H for times of 30 and 60 minutes. The roots were then washed and half of the sample immediately fixed. The other half was placed in a beaker containing 2M mannitol (30°C) for five minutes. (A preliminary

Diagram 2: Counting Procedure for Pulse Chase Data.

Areas Counted (Hatched Areas)

Outer	$(3 \times 1.25)(10 \times 1.25)$ $= 46.875 \mu\text{m}^2$	$(1 \times 1.25)(10 \times 1.25)$ $= 15.625 \mu\text{m}^2$	$\left. \begin{array}{l} \\ \end{array} \right\} (1 \times 1.25)(10 \times 1.25)$ $= 15.625 \mu\text{m}^2$
Inner	$(7 \times 1.25)(10 \times 1.25)$ $= 109.375 \mu\text{m}^2$	$(2 \times 1.25)(10 \times 1.25)$ $= 31.25 \mu\text{m}^2$	
Wall	$(1.5 \times 1.25)(10 \times 1.25)$ $= 23.44 \mu\text{m}^2$	$(2 \times 1.25)(10 \times 1.25)$ $= 31.25 \mu\text{m}^2$	$(2 \times 1.25)(10 \times 1.25)$ $= 31.25 \mu\text{m}^2$

The numbers on the diagrams refer to the dimensions on the ocular micrometer.

test to determine the best sugar to use, the optimal time of plasmolysis and whether the normal glutaraldehyde fixative would reverse plasmolysis is described in Appendix IX.) The roots were rapidly rinsed in phosphate buffer and fixed and embedded as described in Sections 2:4 to 2:6.

2:10 Controls and Checks:

The following experiment was performed to determine if prestaining the sections by the PAS reactions can cause some variation in labelling patterns or in the amount of label. The roots were incubated for 60 minutes with glucose-1-³H and processed in the routine fashion. Some slides were stained and adjacent slides were not stained. All were subsequently dipped in a similar fashion and developed together at the same time. Similar regions were examined (Figures 168-171). The exposure time was two weeks. The amount and positioning of the label was essentially identical in the stained and unstained sections. The grains were easier to see without the background stain, but the necessity of the stain is apparent since there was a lack of accurate structural and positional information in the unstained sections.

This procedure (PAS staining) must be carried out before coating with emulsion, since it is a rather vigorous and complex procedure which would probably damage the emulsion both chemically and physically (due to continuous dehydration, rehydration and washing).

A control was run to determine whether the aniline blue reacted with the emulsion in some way to produce an artifacts. Slides were stained with aniline blue, viewed, washed overnight and then emulsion

coated. The slides were stained by the PAS reaction then coated with emulsion. Adjacent slides were stained by the PAS reaction then emulsion coated. No apparent differences in labelling were found, therefore it was concluded that prestaining with aniline blue caused no artifacts and was an acceptable procedure.

In order to check for background grains which might originate from the emulsion itself, a clean slide was dipped in the emulsion and developed as the experimental slides. At least one of such slides was included in each box of slides.

3. RESULTS

3:1 Introduction to the Results:

Autoradiographic labelling patterns are compared for the root cap and the various developmental regions of the epidermal cell layer.

Definitions and areas of the root to be examined are outlined in Diagram 3. The areas are:

Area A: Root cap and associated slime.

Three developmental regions of the epidermis:

Area B: Columnar epidermal cells and associated mucilage.

Area C: Transition zone of the epidermis and associated mucilage.

Area D: Elongation or root hair zone of the epidermis and associated mucilage.

For the subjective portions of these observations (continuous and pulse chase) the terms more, less and related terms are based on subjective visual evaluations of the density of the silver grains over an approximately equal area.

The root cap (Area A) also has a developmental sequence (Figure 1). Just under the junction of the root proper and the cap are located the cuboidal meristematic root cap cells. As the root cap cells mature they are pushed towards the periphery of the root cap and become less cuboidal. In the central cap, the cells are still cuboidal with prominent starch grains. Further out, the

Diagram 3: Diagram of Roots Indicating Areas of Interest and Definitions.

cells just adjacent to the outermost layers show different labelling patterns (Region 2). The mature cells at the tip of the root cap are spherical to oval in shape, whereas those at the flanks are bent-sausage in shape (Region 1).

The root cap slime has two distinct layers - both are PAS positive but to different staining densities. The less PAS positive slime occurs outside the root cap proper between the sloughed cells whereas the more PAS positive slime occurs between the cells of the outer layer and between the plasmalemma and the cell wall (Miki *et al.* 1980). When slime is referred to in the text, it refers to the outer slime and references to the inner slime refer to that located in the root cap proper.

The three developmental areas of the epidermis are characterised by (a) a dense band of label along the outer periclinal edges of the epidermal cells and (b) by the occurrence of the mucilage.

The dense band of label is a common feature of the incorporation patterns of all three radioactive sugars. The band is first seen low down in the epidermis at the most acropetal portion of the columnar zone (Figures 20 and 25). The dense band of label is continuous up the root except over cells forming new anticlinal walls. Using comparative histochemistry and high resolution optical microscopy (Figures 5 to 7), the dense band locus seems to be that of the thick outer periclinal wall. This dense band is continuous if colchicine is applied (Figure 8). If the roots are plasmolysed after incubation with the radioactive sugars, even though the epidermal cells do not plasmolyse as easily as other cells and usually plasmolyse at the

Dense Band Location:

Figure 5:

Epidermal cells and mucilage stained with alcian blue. The arrowhead points at the outer periclinal wall of the epidermal cells. x3000.

Figure 6:

Epidermal cells and mucilage stained with methylene blue. The arrowhead points at the outer periclinal wall of the epidermal cells. x3000.

Figure 7:

Epidermal cells stained with the PAS reaction followed by autoradiography. The majority of the dense band of label appears to be in the outer periclinal wall of the epidermis. This root was incubated for 60 minutes with glucose-1-³H. The arrowheads point at the outer periclinal wall. x3000.

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base of the cells, there are enough cells that plasmolysed at the top of the cells (nearest the thick outer periclinal wall) to verify that this dense band of label is indeed present in this outer periclinal wall (not in the cytoplasm or between the plasmalemma and the wall) (Figures 9 to 11).

The epidermal mucilage occurs outside the thick outer periclinal wall and closely abuts the surface of the epidermal cells (Figure 1). Initially, the mucilage forms the boundary between the root proper and the root cap in a thin layer, but at the flanks of the root it gradually becomes a thick layer (12-25 μm). Along the surface of the maturing epidermis, this mucilage layer slowly thins from the end of the columnar zone (Area B) to the elongation zone (Area D) where it is about 1.25 μm in thickness and in some cases is not present at all. Clarke *et al.* (1979) state that they could no longer distinguish the "extracellular layer" at the position of the first root hairs. In this work, however, the mucilage layer though thin is most often observed (Figures 151, 159, 160). Regions C and D of the mucilage as shown in Figure 1, are the histochemically distinguishable ones and do not refer to the regions counted for pulse chase data.

One of the characteristics of secretory cells already outlined in the introduction was the density of basophilic staining. With toluidine blue, the epidermis stains the most densely basophilic of all the cell types of the root tissue (Figure 12). The epidermis also accumulates more of the labelled material than any other cell type of the root proper (Figure 13).

Plasmolysis Experiments:

Figure 8:

The side of a root showing the dense band is present over the entire epidermal length. (No plasmolysis) x220.

Figure 9:

Plasmolysis of epidermal cells following a 60 minute incubation period with glucose-1-³H. Plasmolysis has occurred towards the outer periclinal wall and the label remains in the wall area. (Arrowheads point at the plasmolysed areas.) x1700.

Figure 10:

Plasmolysis of the epidermis following a similar procedure as for Figure 9. Again note that the label remained in the wall over those areas where the cytoplasm has shrunk back (arrowheads). x1700.

Figure 11:

Plasmolysis following similar conditions as Figures 9 and 10. Note that also here most of the label is associated with the wall. x1700.

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38.

Figure 12:

A longitudinal section through the epidermis stained with toluidine blue O. Note that the epidermis stains darker than the rest of the cell layers in the root proper. x470.

Figure 13:

A section through a root which was incubated for 60 minutes with glucose-1-³H. Note that the epidermis has accumulated a great deal more label than the rest of the cell layers in the root proper. x230.

2

3:2 Continuous Labelling:

With increased times of labelling, there is an overall increase in the amount of silver grains located over the root tissue. A common feature is that the outer root cap is always the most heavily labelled cell layer of the root cap and the epidermis is the most heavily labelled cell type of the root proper. These observations apply to all three sugar precursors.

The results of the continuous labelling are summarized in Tables 4 to 6. Table 4 is for glucose-6-³H, Table 5 for glucose-1-³H and Table 6 for fucose-6-³H. The tables are a summary of several different roots and experiments whereas the micrographs are individual examples of the labelling patterns. A comparison of the three sugars for the four areas of the root presented in Diagram 3 is given below. Regions 1 and 2 of the root cap are shown in Figure 1.

3:2:1 Root Cap Cells (Area A):

The glucoses have similar incorporation patterns. After 15 minutes of incubation with glucose-6-³H, there is a small amount of label in the cytoplasm and walls of the outer cap cells. There is also a very small amount of label in the surrounding slime (Figures 14 and 15). At 30 minutes, the labelling pattern is similar and there is an increase in the total label (Figure 17). Using glucose-1-³H on the other hand, at 30 minutes incubation, the label is accumulated just inside the walls of the peripheral cap cells (Region 1) but not over the walls, unlike the glucose-6-³H labelling (Figure 30). At longer times (60, 90 and 120 minutes), glucose-6-³H label takes on the form of the 30 minute time of the glucose-1-³H (i.e., just beneath

Glucose-6-³H Continuous - 15 and 30 Minutes:

Figures 14 to 18 show sections of root tips that have been incubated for the times indicated.

Figure 14:

15 minutes. Root cap. Most of the label is located in the peripheral root cap cells (Region 1). x230.

Figure 15:

15 minutes. In the columnar epidermis there is very little label at 15 minutes. The label is mainly in the cytoplasm. There is a small amount of label in the walls, but very little in the mucilage. x440.

Figure 16:

15 minutes. For the transition epidermal cells the label is located primarily in the cytoplasm. There is some label in the wall, but very little in the mucilage. x265.

Figure 17:

30 minutes. Root cap cells on the flanks showing cytoplasmic labelling, no labelling in the starch grains and quite a bit of wall label, x480.

Figure 18:

30 minutes. There is heavy cytoplasmic labelling in the peripheral root cap cells (tip towards the upper right hand corner). The young columnar cells also have a high intensity of labelling and a definite increase in wall label over the time of 15 minutes. There are also a number of root cap cells embedded in the slime that contain no label. Perhaps these are dead cells. x265.

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15

16

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18

Table 4: Summary of Glucose-6-³H Continuous Labelling Results.

		Labelling Time in Minutes				
		15	30	60	90	120
Outer Root Cap						
(A)	cell walls	-	*	**	***	***
	cytoplasm	*	**	***	**	**
	slime	*	*	**	**	***
	starch grains	-	-	*	*	*
	figure numbers	14 15	17	19	24	29
Columnar Epidermal Cells						
(B)	cytoplasm	*	*	***	***	***
	mucilage 2	*	**	**	***	*** 1
	dense band 2	-	*	***	****	****
	figure numbers	15 16	18	22 23 19 20	25 26	27 28
Transition Epidermal Cells						
(C)	cytoplasm	*	*	***	***	***
	mucilage 2	*	**	**	***	***
	dense band 2	-	*	***	****	****
	figure numbers			21		
Elongation Epidermal Cells						
(D)	cytoplasm	*	*	*	*	*
	mucilage 2	-	-	*	*	*
	dense band 2	-	*	***	****	****

Ratings: - = no label
 * = very little label
 ** = moderate amount of label
 *** = heavily labelled
 **** = very heavily labelled

Notes: 1 in the inner mucilage near the outer boundary of the cells.
 2 a common feature is that over cells with new anticlinal walls, there is a discontinuity of label in the mucilage and the outer periclinal wall.

Glucose-6-³H Continuous Label - 60 Minutes:

Figure 19:

In the peripheral root cap (1-2 layers - Region 1) the label is dense in the cytoplasm and also in the walls of the cells. In more interior cells, the label is generally cytoplasmic for 2-3 layers and mainly located in the starch grains. x230.

Figure 20:

An enlargement of the cap flank region of a section similar to that of Figure 19 showing heavy labelling in the cap cells. The dense band of label is beginning to accumulate over the epidermal cells and the new cell wall is heavily labelled. x510.

Figure 21:

The transition zone of the epidermis showing heavy cytoplasmic labelling, the dense band of label in the outer periclinal wall and some label in the mucilage but not so much as in some regions of the columnar zone epidermis mucilage. x230.

Figure 22:

Columnar region cells (root cap in the lower right hand corner) showing the dense band of label, the heavy mucilage label and discontinuity in the dense band of label and the mucilage label above cells with a new anticlinal wall. x230.

Figure 23:

Columnar cell cytoplasm and new anticlinal cell walls (top left hand corner) are heavily labelled, the dense band is quite apparent over the cells except those with new anticlinal walls. The mucilage is quite heavily labelled. x230.

43.

Glucose-6-³H Continuous - 90 and 120 Minutes:

Figure 24:

90 minutes. Peripheral cap cells are labelled heavily in the walls and in the starch grains. There is little label in the inner cytoplasm but more in the walls and the outer cytoplasm. x260.

Figure 25:

90 minutes. The label is quite heavy in the peripheral cap cells, especially in the cell walls. The slime is also labelled. x260.

Figure 26:

90 minutes. There is label in the cytoplasm, the dense band locus and the mucilage of these columnar epidermal cells similar to the 60 minute pattern. x260.

Figure 27:

120 minutes. The columnar zone (Area B) near the flanks of the root cap (root cap in the upper right hand corner). Note the dense band of label in the outer periclinal wall and the mucilage which is most heavily labelled closer to the cells than further out in the mucilage. x230.

Figure 28:

120 minutes. The mucilage over these columnar epidermal cells is very heavily labelled as is the outer periclinal wall (dense band) except over new anticlinal walls. x230.

Figure 29:

120 minutes. There is high slime label and heavy wall label of the root cap cells and some label in the starch grains. x230.

Table 5: Summary of Glucose-1-³H Continuous Labelling Results.

		Labelling Time in Minutes. ✓		
		30	60	90
Outer Root Cap				
(A)	cytoplasm	**	***	***
	cell walls	-	*	**
	slime	*	*	**
	starch grains	-	-	*
	figure numbers	30	35 36	42 44
Columnar Epidermal Cells				
(B)	cytoplasm	**	**	**
	mucilage 2	*	**	***
	dense band 2	**	***	***
	other walls	*	**	**
	figure numbers	31 32	37	41
Transition Epidermal Cells				
(C)	cytoplasm	**	**	**
	mucilage 2	**	***	***
	dense band 2	***	****	****
	other walls	**	**	**
	figure numbers	33	38	43
Elongation Epidermal Cells				
(D)	cytoplasm	*	*	*
	mucilage	*	*	*
	dense band	***	***	***
	other walls 1	***	***	***
	figure numbers	34		

Ratings: The same as for Table 4.

Notes: 1 all four walls (visible) are heavily labelled.

2 a common feature of these structures is that over cells with new anticlinal cell wall, there is a discontinuity of the label.

Glucose-1-³H Continuous - 30 Minutes:

Figure 30:

The root cap label is found in the peripheral cap cells (Region 1) just beneath the cell wall, unlike the glucose-6-³H label which is cytoplasmic at 30 minutes. The label is not in the walls. There is very little label in the slime. x265.

Figure 31:

Columnar epidermal cells (Area B) are lightly labelled in the cytoplasm, the outer periclinal wall and the other walls. There is a discontinuity in the dense band of label over the cells forming a new anticlinal wall. x480.

Figure 32:

Columnar epidermal zone (Area B). The dense band of label is present and the mucilage is lightly labelled. The cells are quite heavily labelled in the cytoplasm. x260.

Figure 33:

Transition epidermal zone cells (Area C). The cells are less heavily labelled in the cytoplasm and the dense band seems more heavily labelled than the columnar zone. x260.

Figure 34:

Elongation zone epidermis (Area D). The amount of wall label is almost the same all the way up the root in the outer periclinal walls and continues into this zone. The label is sometimes heavy in all visible walls as in this section in the elongation zone. There is very little label in the mucilage. x260.

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34

Glucose-1-³H Continuous - 60 Minutes:

Figure 35:

The peripheral cap is labelled in the cytoplasm quite heavily. There is some label in the cell walls of these cells and a small amount in the slime. Note the unlabelled cells in the slime which are probably dead. x230.

Figure 36:

On the flanks of the root. There is the same general labelling pattern in the root cap as noted for Figure 35. Underlying the root cap cells, the mucilage is somewhat labelled as is the outer periclinal wall and the cytoplasm of the epidermal cells. x260.

Figure 37:

Columnar region of the epidermis. Note the dense band of label in the outer periclinal wall and the label in the mucilage which lies close to the epidermis. x350.

Figure 38:

Transition zone of the epidermis (Area C). The dense band of label is present and heavy and the mucilage and new cell walls are very heavily labelled above the cells with the new cell walls there is some mucilage label; an example of recovery of the discontinuity. x350.

Figure 39:

The columnar region with the dense band and label in the mucilage. Note also the large amount of label from the slime which is totally unrelated to the mucilage (arrowhead). x280.

Figure 40:

A magnification of the mucilage and the tail of slime label from Figure 39. (Post autoradiography stained with toluidine blue in addition to regular PAS staining.) x1260.

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Glucose-1-³H Continuous - 90 Minutes:

Figures 41 to 43:

Epidermal cell labelling. Mucilage and slime overtop epidermal cells. In these roots the slime was dragged up the root. The cell walls of the epidermis are heavily labelled as is the mucilage. The slime, which can be distinguished from the mucilage by PAS staining intensity is much less labelled than the mucilage (small arrowhead - slime; large arrowhead - mucilage). x245.

Figure 44:

The root cap with the peripheral cells labelled mainly in the walls and also in the starch grains with some asymmetry of the label towards the slime. x245.

Table 6: Summary of Fucose-6-³H Continuous Labelling Results.

		Labelling Time in Minutes			
		60	90	120	180
Outer Root Cap					
(A)					
	cytoplasm	**	***	**	**
	cell walls	*	**	**	**
	slime	*	*	**	***
	starch grains	-	-	-	-
	asymmetric labelling 1	***	***	***	***
	figure numbers	45 48	49	52	
Columnar Epidermal Cells					
(B)					
	cytoplasm	**	**	**	**
	mucilage 2	*	**	***	***
	dense band 2	**	**	**	**
	other walls	*	*	*	*
	figure numbers	46	50 51	53 54	
Transition Epidermal Cells					
(C)					
	cytoplasm	**	**	**	**
	mucilage 2	*	**	**	**
	dense band 2	**	**	**	**
	other walls	**	**	**	**
Elongation Epidermal Cells					
(D)					
	cytoplasm	*	*	*	*
	mucilage	*	*	*	*
	dense band	**	**	**	**
	other walls	**	**	**	**
	figure numbers			55 56	

Ratings: The same as for Table 4.

- Notes:
- 1 the asymmetric distribution takes the form of accumulations of label in the outer cap cells parallel to the perimeter of the root cap.
 - 2 in these areas, there is a discontinuity of label accumulation over cells that are forming new anticlinal cell walls.

49.

Fucose-6-³H Continuous - 60 Minutes:

Figure 45:

The side of a root showing the pattern illustrated in Figure 47 in the peripheral root cap cells. This label appears to be inside the walls of the cells. The epidermis has a dense band present in the outer periclinal wall and there is a small amount of label in the mucilage. x295.

Figure 46:

Columnar epidermal cells with the dense band of label and lightly labelled mucilage. x295.

Figure 47:

A diagram of the way the asymmetric distribution of the label occurs in the root cap.

Figure 48:

The tip of the root cap containing the asymmetric distribution of the label. x295.

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• Fucose-6-³H Continuous - 90 and 120 Minutes:

Figure 49:

90 minutes. The root apex of the root proper showing the peripheral root cap cells with the typical fucose pattern of labelling of Figure 47 and the new epidermal mucilage being labelled low down in the flanks of the root cap cells. x320.

Figures 50-51:

90 minutes. Columnar epidermal cells. The dense band of label in the outer periclinal wall which is much less than for the glucoses at the same time is discontinuous over cells with new anticlinal walls as is the mucilage label. x329.

Figure 52:

120 minutes. There is little labelling in the root cap cells except on the side towards the outside and the starch grains are not labelled. The slime is labelled quite heavily. x280.

Figures 53-54:

120 minutes. Columnar epidermal cells. The concentration of label in the dense band in the outer periclinal wall and the mucilage is quite high. There is a discontinuity in the dense band label and in the mucilage label over cells with new anticlinal walls (arrowhead). x480.

• Figures 55-56:

120 minutes. Elongation zone epidermis. There is a dense band of label in the outer periclinal wall but little mucilage label. x280.

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or in the walls of the outer root cap cells (Figure 19). Glucose-1-³H retains its labelling pattern at the longer times. The adjacent inner cell layers (Region 2) have general label in the cytoplasm but not over the walls. This is the same for both glucoses at 60 and 90 minutes except in some cases, there is an asymmetric distribution of the label towards the outside of the root or facing a slime deposition area if loose cells are examined for glucose-1-³H (Figure 44). At longer times, the slime is heavily labelled with both sugars. Glucose-6-³H seems to be incorporated by the cells into the slime more readily than is glucose-1-³H.

Fucose on the other hand labels the outer side of the cells and the slime located there (the darker staining slime distinguished by Miki *et al.* 1980) on the peripheral three to five layers of cells (Region 1) for all times. This pattern is illustrated in Figure 47. The label is primarily inside the wall, but there is also some in the walls. Fucose also quite strongly labels the slime at the longer incubation times (two and three hours). In general, fucose seems to require longer incubation periods than the two glucoses to label structures with a comparative amount of label. This is also noted for the epidermis.

Glucose-1-³H and glucose-6-³H label the starch grains of the outer cap cells (Region 1) at the longer labelling periods (90 and 120 minutes for glucose-6-³H and 90 for glucose-1-³H). In the central root cap cells (interior to Region 2) at all times, the starch grains are labelled and the cells are lightly labelled in the cytoplasm. Fucose, however, does not under any circumstance label

the starch grains of any root cap cells.

3:2:2 Columnar Epidermal Cells (Area B)

The two glucoses label in a similar way. At lower incubation times, only the cell cytoplasm is labelled and with longer incubation times the label is also found in the walls and the mucilage. In general, glucose-6-³H seems to label the walls and mucilage rather than the cytoplasm whereas glucose-1-³H seems to label primary or new cell walls more heavily than does glucose-6-³H. In both cases, the dense band of label in the outer periclinal wall is present and even the very young epidermis covered by the heavily labelled flanks of the root cap has the dense band of label (Figures 19 and 20). Glucose-6-³H also seems to label the mucilage better and at shorter incubation times than glucose-1-³H.

Fucose-6-³H, on the other hand, requires longer incubation times to label walls and the dense band locus (outer periclinal wall). It does not label the outer periclinal wall as densely as the glucoses. It does label primary wall and the mucilage quite well. In all cases with increasing labelling times there is an increase in the amount of label in the mucilage with all three sugars. Over cells with new anticlinal walls, there is a discontinuity of the label in the outer periclinal wall and the mucilage also for all three sugars (Figures 22, 23, 31, 46).

A specific difference in the labelling of the slime and the mucilage was noted as in Figures 41 to 43, where the slime and the mucilage are side by side. Not only is the PAS staining difference apparent, but also the discrepancy in the amount of

label present (much more in the mucilage). Also in Figures 39 and 40 there is a heavy "tail" of label in the slime which is unrelated to the mucilage label.

The label in the mucilage is often accumulated close to the outer periclinal wall of the epidermis (e.g. Figure 27).

3:2:3 Transition Epidermal Cells (Area C):

All three sugars are incorporated by this zone. The dense band of label in the outer periclinal wall is found with all three radioactive sugars, more pronounced than in the columnar zone in many cases, most notably with fucose. There is more label in the mucilage with the glucoses and less with fucose than the columnar zone mucilage.

The cells are quite heavily labelled in the cytoplasm and the other cell walls are also labelled but none as heavily as the thick outer wall.

3:2:4 Elongation Epidermal Cells (Area D):

The cell walls are well labelled, especially the thick outer periclinal wall with all three sugars. The very thin mucilage is sparingly labelled with all three precursors. Root hairs are labelled in the PAS positive deposit at the tips. In some cases, all four visible walls are heavily labelled (Figures 16, 34) but in most cases the other three walls are less labelled than the outer wall.

3:3 Pulse Chase Labelling:

Two experimental approaches have been used for the material labelled by the pulse chase method; (a) a subjective approach done

on several samples, the results of which are summarized in Table 7 for glucose-6-³H, Table 8 for glucose-1-³H and Table 9 for fucose-6-³H. The figures are individual representatives of the various labelling patterns whereas the tables cover several samples and (b) silver grain counts on the epidermal cells and associated mucilage only.

3:3:1 Subjective Observations:

In general, it is found that all three radioactive sugars are absorbed by the root and are metabolised by the cells and subsequently are secreted to become a constituent of the two secretions being examined: the root cap slime and the epidermal cell mucilage. As the chase time increases there is a distinguishable change in the location and amount of label within the various components involved.

The tables are arranged for the root cap, so that when bearding (the asymmetric distribution of label towards the outside of the cells) is present, the label is rated under bearding and when bearding is not present, the label accumulated is rated under the heading 'general'. The characteristics of the three labelling precursors will be compared for the root cap, the epidermis and the mucilage.

3:3:1:1 Comparisons of Three Areas of the Root:

(a) Root Cap:

For glucose-6-³H, at the 60 minute pulse time, the cytoplasm and walls of the cells in the peripheral cap are labelled uniformly. For glucose-1-³H, the label is in the cytoplasm around the perimeter of these same cells (Region 1) (compare Figures

Table 7: Summary of Glucose-6-³H Pulse Chase Results.

Pulse + Chase Time	60	60+10	60+15	60+30	60+60	60+90
ROOT CAP						
Region 1						
(a) General label						
cytoplasm	**	*	*	*	**	*
walls	**	2	2	**	**	**
slime	*	2	2	**	**	**
starch grains	**	-	***	***	***	***
(b) Bearding						
wall	-	***	***	-	-	3
interior 5	-	-	-	3	3	3
slime	-	**	***	3	3	3
Region 2						
(a) General label						
cytoplasm	**	*	*	*	-	-
walls	**	2	2	*	-	-
starch grains	*	*	***	-	-	-
(b) Bearding						
walls	-	**	***	-	-	-
interior	-	-	**	-	-	-
Interior Cells						
cytoplasm	**	*	*	-	-	-
walls	**	**	**	-	-	-
starch grains	**	***	**	-	-	-
interior 4	-	***	-	-	-	-
Figure Numbers	58	60 61	64 64a	67	70	73 73a 74
EPIDERMIS & MUCILAGE						
inner mucilage 1	*	***	***	***	***	***
outer mucilage 1	-	*	**	**	**	**
outer peri. wall 1	**	**	**	**	**	**
cytoplasm	**	**	**	*	*	*
Figure Numbers	58 59	62 63	65 66	68 69	71 72	75 76

Ratings: - = no label or very little
 * = small amount of label.
 ** = moderate amount of label
 *** = heavy label

Notes: 1 as indicated in Figure 1.
 2 ignore when rated in bearding section.
 3 bearding present but only at the flanks.
 4 rated when bearding present in this cell layer.
 5 inside of the wall.

Glucose-6-³H Pulse Chase Results:

Figure 57: 60 minute pulse + no chase (base time)

A view of the side of a root showing the flanks of the root cap and young epidermal cells. The root cap label is cytoplasmic as is the label in the epidermal cells. The dense band of label is present in the outer periclinal wall of the epidermal cells but there is not much mucilage label. x245.

Figure 58: 60 minute pulse + no chase (base time)

Columnar epidermal cells showing cytoplasmic labelling the dense band of label and some label in the mucilage. x480.

Figure 59: 60 minute pulse + no chase (base time)

Transition zone epidermal cells showing heavy labelling in all walls, more in the outer periclinal than the others. The mucilage is also lightly labelled. x230.

Figure 60: 60 minute pulse + 10 minute chase

The tip of the root cap with asymmetric distribution of the label towards the exterior of the root (similar to the way fucose labels these cells) in the outer root cap (Regions 1 and 2). x250.

Figure 61: 60 minute pulse + 10 minute chase

An enlargement of a tip similar to Figure 60. The label appears to be inside of the wall (arrowhead) for the more interior cells, whereas for the more external cells the label seems to be in the wall and outside of the cells. x510

Figure 62: 60 minute pulse + 10 minute chase

Columnar region epidermal cells with the dense band of label and the mucilage label only part way out into the mucilage. There almost seems to be a band or wave of label moving outwards. x290.

Figure 63: 60 minute pulse + 10 minute chase

Columnar region of the epidermis showing clearly the wave of label moving out into the mucilage. x570.

Figure 64: 60 minute pulse + 15 minute chase

The side of a root cap showing the same pattern of labelling as the 10 minute chase time. The starch grains are also labelled in the outer cells in some cases. x 240.

57.

Glucose-6-³H Pulse Chase Results (Continued):

Figure 64a: 60 minute pulse + 15 minute chase

An enlargement of the root cap tip showing the same asymmetric distribution pattern as for the 10 minute chase. The starch grains are labelled. x540.

Figure 65: 60 minute pulse + 15 minute chase

Columnar epidermal cells with the same wave of mucilage label beneath the scattered root cap cells and a discontinuity in the dense band of label and the mucilage label over cells with new anticlinal walls (arrowhead). There seems to be a reduction in cytoplasmic label. x240.

Figure 66: 60 minute pulse + 15 minute chase:

Columnar epidermal cells with a dense band of label in the outer periclinal wall and some mucilage label. x240.

Glucose-6-³H Pulse Chase Results (Continued):

Figure 67: 60 minute pulse + 30 minute chase

Root cap (side of) showing a reduction of label, a loss of the asymmetric distribution pattern in some parts of the cap, general cell wall labelling and starch grain labelling. x240.

Figure 68: 60 minute pulse + 30 minute chase

Epidermal cell with the label most intense in the mucilage close to the cell boundaries. The dense band of label and the mucilage label are discontinuous over cells with new anticlinal cell walls even after a chase period (arrowhead). x240.

Figure 69: 60 minute pulse + 30 minute chase

Columnar epidermal cells (Area B). There is an increase in mucilage label but a decrease in the cytoplasmic label. x240.

Figure 70: 60 minute pulse + 60 minute chase

The side of the root cap showing a cleaned out appearance. The label is in the cytoplasm and the starch grains. Some cells on the flanks (arrowheads) show asymmetric distribution of label towards the outside. x240.

Figure 71: 60 minute pulse + 60 minute chase

Younger epidermal cells with very little label in the mucilage or in the cells. x245.

Figure 72: 60 minute pulse + 60 minute chase

Older columnar epidermal cells (than Figure 71) with plenty of label in the mucilage. This would be the more appropriate section to examine due to root growth. x245.

Figure 73: 60 minute pulse + 90 minute chase

The side of the root cap showing bearding in some of the cells on the flanks. There is a general reduction of label in all areas of the root cap. x230.

Figure 73a: 60 minute pulse + 90 minute chase

The tip of the root cap showing the much reduced label and the general wall and starch grain labelling. x230.

Glucose-6-³H Pulse Chase Results (Continued):

Figure 74: 60 minute pulse + 90 minute chase

Further down the root cap from the flanks towards the tip. There is a general wall and cytoplasmic labelling (including starch grain labelling) in the peripheral cap. Starch grains are also labelled in the interior cap. x230.

Figure 75: 60 minute pulse + 90 minute chase

Epidermal cells with mucilage label both in the inner and outer regions of the mucilage. The dense band of label in the outer periclinal wall is still present. x230.

Figure 76: 60 minute pulse + 90 minute chase

Epidermal cells still with the dense band of label and the mucilage label. x230.

Table 8: Summary of Glucose-1-³H Pulse Chase Labelling Results.

Pulse + Chase Time	60	60+15	60+30	60+60	60+90
ROOT CAP					
Region 1					
(a) General label					
cytoplasm	**	**	*	*	**
walls	**	**	1	1	***
slime	**	**	1	1	**
starch grains	-	-	+	**	**
(b) Bearding					
wall			***	**	
interior of cell 2	-	-	-	-	-
slime	-	-	**	**	-
Region 2					
(a) General label and					
Bearding(b)					
cytoplasm	*		*	*	-
walls	*(-b)		***b	**b	-(-b)
starch grains	**		***	**	*
interior 2	-b		**b	-b	-b
Figure Numbers	77	79	82 83	86 87	91
EPIDERMIS & MUCILAGE					
inner mucilage	**	**	**	***	****
outer mucilage	*	*	*	*	**
outer periclinal wall	**	**	**	**	**
cytoplasm	**	**	*	*	*
Figure Numbers	78	80 81	84 85	88 to 90	92 to 95

Ratings: The same as Table 8, except that, **** means very heavily labelled.

Notes: 1 ignore, is rated in the bearding section.
2 this means inside of the wall.

Glucose-1-³H Pulse Chase Results:

Figure 77: 60 minute pulse + no chase (base time),

The root cap is labelled in the outer cytoplasm and in the wall. The epidermal cells (columnar) have a dense band of label in the outer periclinal wall with some label in the mucilage. x320.

Figure 78: 60 minute pulse + no chase (base time)

For the transition epidermis (Area C) the dense band of label is present, but there is more mucilage label than for the columnar cells. x300.

Figure 79: 60 minute pulse + 15 minute chase

In the root cap, the pattern of labelling is basically the same as that of the unchased material with only a slight tendency towards asymmetry of label distribution in a few cells. x280.

Figure 80: 60 minute pulse + 15 minute chase

Columnar epidermal cells (Area B) show an increase in the label in the mucilage close to the outer cell boundaries. x280.

Figure 81: 60 minute pulse + 15 minute chase

Epidermal cells, showing mucilage label close to the cell boundaries. x280.

Figure 82: 60 minute pulse + 30 minute chase

Side of a root cap, some cells of which exhibit the bearding phenomenon (asymmetry of label). Some cells have starch grains labelled. Note also the label in the more PAS positive slime. x250.

Figure 83: 60 minute pulse + 30 minute chase

The tip of the root cap showing asymmetry of label towards the exterior of the root. For the more interior of the bearded cells (Region 4 - 2nd layer), the label is inside of the cell wall, for the more external cells (Region 4 - 1st layer) the label seems to be in the walls or outside of the walls. Note the label in the more PAS positive slime. x250.

Figure 84: 60 minute pulse + 30 minute chase

Epidermal cells with the dense band of label in the outer periclinal wall and quite heavy mucilage labelling. x250.

Glucose-1-³H Pulse Chase Results (Continued):

Figure 85: 60 minute pulse + 30 minute chase

Epidermal cells with the dense band of label and mucilage labelling. A discontinuity in the dense band of label is also present over cells with new anticlinal cell walls. x250.

Figure 86: 60 minute pulse + 60 minute chase

A composite of a root cap tip. The bearding phenomenon is still present, but the amount of label is noticeably reduced in general. The more PAS positive slime is also labelled. x250.

Figure 87: 60 minute pulse + 60 minute chase

The side of a cap halfway between the flanks and the tip. The asymmetry of labelling is still present here as well. x250.

Figures 88 to 90: 60 minute pulse + 60 minute chase

Three columnar epidermis areas showing the accumulation of mucilage label close to the outer surface of the epidermis in a definite band. x250.

Figure 91: 60 minute pulse + 90 minute chase

The outer root cap with some asymmetric distribution remaining. There is also a lot of label in the more PAS positive slime. x280.

Figure 92: 60 minute pulse + 90 minute chase

Columnar area of epidermis. The mucilage label is beginning to be also in the outer portion of the mucilage, and is still increasing in the inner portion of the mucilage. Note also that even after 90 minutes of chase - the discontinuity of labelling over cells with new anticlinal cell walls in the dense band of label and in the mucilage is still present. x280.

Figure 93: 60 minute pulse + 90 minute chase

A similar view of columnar region of epidermis as Figure 92. x280.

Figure 94: 60 minute pulse + 90 minute chase

Almost transition zone (Area C), with label in whole of the mucilage and also in outer periclinal wall in a dense band. x280.

Glucose-1-³H Pulse Chase Results (Continued):

Figure 95: 90 minute pulse + 90 minute chase

There is label in the total mucilage and in the outer periclinal wall in a dense band of these transition zone (Area C) epidermal cells. x280.

Table 9: Summary of Fucose-6-³H Pulse Chase Labelling Results.

Pulse + Chase Time	60	60+15	60+30	60+60	60+90
ROOT CAP 1					
Region 1					
cytoplasm	*	*	*	*	*
walls	**	**	**	**	**
slime	*	**	**	***	***
starch grains	-	-	-	-	-
Region 2					
cytoplasm	*	*	*	*	*
walls	*	*	**	**	*
starch grains	-	-	-	-	-
interior 2	**	**	**	**	**
Inner 3					
cytoplasm	*	*	*	**	**
walls	-	**	-	-	-
starch grains	-	-	-	-	-
interior 2	-	-	-	-	-
Figure Numbers	96	100	105	111	115
	97	101	to 107	112	116
EPIDERMIS & MUCILAGE					
inner mucilage	*	*	*	**	***
outer mucilage	*	*	*	*	**
outer peri. wall	***	***	***	**	**
cytoplasm	**	**	**	**	**
Figure Numbers	98	102	108	113	117
	99	to 104	to 110	114	to 119

Ratings: As per Table 8.

- Notes:
- 1 the pattern always appears asymmetric and is always located on the outer side of the cells.
 - 2 interior refers to being inside of the walls of the cells.
 - 3 inner refers to the root cap cells inside of Region 2.

Fucose-6-³H Pulse Chase Results:

Figure 96: 60 minute pulse + 0 minute chase (base time)

The side of the root demonstrating the same bearding phenomenon as Figure 47. x295.

Figure 97: 60 minute pulse + 0 minute chase (base time)

Root tip with the label accumulated inside of the cells towards the outside of the root surface. x295.

Figure 98: 60 minute pulse + 0 minute chase (base time)

Columnar epidermis with a dense band of label in outer periclinal wall but with little label in the mucilage. x295.

Figure 99: 60 minute pulse + 0 minute chase (base time)

Columnar epidermal cells. The label is found in the outer periclinal wall as a dense band with very little mucilage label. x295.

Figures 100-101: 60 minute pulse + 15 minute chase

Two examples of the root cap. The asymmetric distribution of label towards the outside of the cells and parallel to the root surface is still apparent. x295.

Figures 102-104: 60 minute pulse + 15 minute chase

Views of the columnar epidermis, showing that the dense band of label is still present in the outer periclinal walls and that there was an increase of label in the mucilage. x295.

Figures 105-108: 60 minute pulse + 30 minute chase

Views of the root cap showing a similar asymmetric distribution to earlier times

Figure 105:

Side of the root cap. x295.

Figure 106:

Some cells seem to already have some material present within them as a PAS positive accumulation in the cells and the labelled material bounds these areas (arrowheads). x295.

Fucose-6-³H Pulse Chase Results (Continued):

Figure 107:

Tip of the root cap.

Figures 108-110: 60 minute pulse + 30 minute chase

Three views of the epidermal layer with the dense band of label present and with label present in the mucilage near the cells. x295.

Figure 111: 60 minute pulse + 60 minute chase

The side of the root cap, with the bearding phenomenon still present in the cells. The label seems to be moving outwards in the cells. x295.

Figure 112: 60 minute pulse + 60 minute chase

The tip of the root cap with the asymmetric distribution of the silver grain label. x295.

Figure 113: 60 minute pulse + 60 minute chase

The columnar region of the epidermis near the root cap with the dense band of label present and mucilage well labelled. The outer mucilage however is not as well labelled. x295.

Figure 114: 60 minute pulse + 60 minute chase

The columnar zone (Area B), farther out from the root cap with the dense band of label and mucilage label present. x295.

Figures 115-116: 60 minute pulse + 90 minute chase

Views of the root cap with the asymmetry pattern still apparent. x295.

Figures 117-119: 60 minute pulse + 90 minute chase

Three views of the epidermis. There appears to be a reduction in the amount of label located in the outer periclinal wall (dense band locus) and perhaps more label in the mucilage. The mucilage is not labelled in the outer part. x295.

57 and 77). By ten minutes chase, for glucose-6-³H in the peripheral cell (Region 1) the label is asymmetrically distributed with most label in the external periclinal wall to the exterior of the root cap (similar to the pattern in Figure 47 for fucose labelling). The label is within and outside the walls for the most exterior cells, within the walls for more interior cell layers of Region 1 and inside of the walls for Region 2 (Figures 60 and 61). For 15 minutes chase (Figure 64) bearding is still present, but the cells with label inside the walls are now in Region 1, otherwise a similar pattern occurs. For glucose-1-³H, on the other hand, no bearding pattern has occurred by 15 minutes chase (Figure 79), but by 30 minutes is present similar to the pattern for glucose-6-³H at 15 minutes (Figures 82 and 83). For glucose-6-³H from 30 to 90 minute chases, the bearding pattern disappears from the outer root cap and there is general wall and cytoplasm label, except at the flanks where bearding still occurs (Figures 67, 70, 74). There is also a reduction in the amount of label. For glucose-1-³H bearding is still present at 60 and 90 minutes of chase, but somewhat reduced (Figures 86 and 91).

The starch grains in the Region 1 cells are labelled from 30 minutes chase to the 90 minutes chase for glucose-6-³H and glucose-1-³H. Region 2 cell starch grains are labelled at 15 minutes for both glucoses as well.

The darkly staining slime (characterised by Miki et al. 1980) is labelled by both of these precursors and also by fucose-6-³H.

At all times for fucose-6-³H, the bearding orientation is present, even at the base time (no chase) of 60 minutes. The pattern remains the same for all the chase times with a slight increase in total accumulation.

Glucose-6-³H seems to label slime more specifically and also more quickly than glucose-1-³H.

(b) Epidermis:

For all three sugars and all chase times, the epidermis is lightly labelled in the cytoplasm and in the walls excluding the outer periclinal wall. The amount of label slowly decreases in these structures with time. The outer periclinal wall is densely labelled at all chase times for the three sugars, except that for the 60 and 90 minutes chase for fucose-6-³H it seems somewhat reduced. Over cells with new anticlinal walls, the discontinuity in dense band is present for all three sugars (Figures 65, 68, 92, 99).

(c) Mucilage:

For all three sugars at the pulse time, there is a small amount of label in the mucilage. With increasing chase times, there is an increase in the amount of label in the mucilage localized in the region (with a definite abrupt boundary in many cases - Figures 62, 63, 89, 90, 92, 113, 114, etc.) near the outer cells edges. This does not coincide with the histochemically distinguishable region of Figure 1. At longer times, there is some label in the outer portion of the mucilage, but not a great deal (Figures 92 and 118).

See Table 10 for a brief comparison of the three labels.

3:3:2 Grain Count Data:

The following corrections were taken into account in order to arrive at the accumulated count data presented below:

1. For exposure times of seven days, the grain counts are doubled to correspond to the regular exposure time of two weeks.
2. For the 90 minute chase time, counts for the mucilage and wall were made in the columnar and the transition zones in order to cover any ambiguity that may be caused by root growth. (See Appendix II.)
3. For the transition zone counts mentioned above, in order to compensate for the change in mucilage area being counted between the columnar and transition zone, the grain counts are multiplied by three. This correction was based on how thick the mucilage was in comparison to the columnar zone and is also used as a correction for cell wall expansion. The mucilage thickness in the columnar zone is on the average $10 + 9 + 7 = 26$ (divisions on the ocular scale) and for the transition zone the value is three (divisions on the ocular scale). Therefore, the factor to multiply the values from the transition zone is $26/3 = 8.67$ or approximately three.
4. If the background counts are high, a sample background for a similar area as is counted for the grain count is subtracted from the values before they are averaged into the data.

Table 10: Summary of the Pulse Chase Experiments Observations.

	Glucose-6- ³ H		Glucose-1- ³ H		Fucose-6- ³ H	
	Epidermis	Root Cap	Epidermis	Root Cap	Epidermis	Root Cap
60 *	dense bd. in OPW, not much in M	outer RCC - cyto. (total)	dense bd. in OPW, very little in M	outer RCC in cyto. & some in walls	same as G-6-H	in outer edges of outer RCC no SG label in slime
60+10 *	dense bd. more in M	bearded cells outer RC walls & cyto. & SG label starts	no data		no data	
60+15 *	increase in M, close to EC, dense bd. - OPW	bearding still present- reduced SG label	increase in M, inner part dense bd. in OPW	bearding starting	same as G-6-H	bearded look, no SG label
60+30 *	dense bd. in OPW, increase in M near to cells	reduced label in outer RC some beard SG label	more into inner M, dense bd. in OPW	bearding still present SG label starts	same as G-6-H	same as 60+15
60+60 *	dense bd. in OPW still, M near cells	much reduced label, some beard SG label	dense bd. same OPW inner M increase	reduced label, bearding still present	same as G-6-H but less in dense band	same as 60+30
60+90 *	M still labelled dense bd. in OPW also	similar to 60+60	similar to 60+60 except more in outer M	reduced RC label similar to 60+60	reduced dense bd. label, M label same as 60+60	same as 60+60

Abbreviations used:

RC = root cap
 RCC = root cap cells
 OPW = outer periclinal wall
 M = mucilage
 bd. = band

cyto. = cytoplasm

* e.g. 60+90 where the first no.
 stands for the pulse time and
 the second no. stands for the
 chase time.

In all cases where notation such as 60 + 15 is used, the first number represents the pulse time and the second number represents the chase time. The wall data refer to the outer periclinal wall at all times.

(a) Glucose-1-³H Counts:

Counts of silver grains for incubation with glucose-1-³H are presented in Table 11. Raw data from which means and variances are calculated are presented in Appendix VII.

For the wall (again this refers to the outer periclinal wall), there seems to be a change in the number of silver grains with an increase of chase time, whereas the counts over the inner and outer mucilage (as defined by Diagram 2) increase as time progresses. These are inferences drawn from looking at the data.

Analysis of variance followed by a multiple range test (if required) of the data were performed and the results are reported in Table 11. Important points are noted below.

1. For the wall, silver grain counts seem to be intuitively the same except for the 60 + 15 category. With the exclusion of the 60 + 15 result, no significant difference was found after the analysis of variance was performed using both the columnar and transition results for 90 minutes. The amount of wall label is shown not to change over the chase period.

2. In the case of the inner layer of the mucilage there is a trend of increasing counts that is shown to be statistically significant. There is a definite difference from the beginning of the

2

Table 11: Means and Statistical Results of Grain Counts in Various Parts of the Mucilage and Wall of Epidermal Cells with Glucose-1-³H as Precursor.

Chase Time	Wall			Inner Mucilage			Outer Mucilage		
	$\bar{x}$	N	SE	$\bar{x}$	N	SE	$\bar{x}$	N	SE
0	21.5	20	1.60	8.5	20	0.87	1.95	20	0.39
15	11.2	25	0.87	16.9	25	1.55	7.0	25	1.06
30	21.6	19	1.68	32.4	19	2.35	13.4	19	1.67
60	19.7	19	1.14	38.4	19	10.78	11.4	19	1.93
90T	26.3	24	1.30	59.5	23	6.71	20.2	23	3.15
90C	19.79	24	1.37	29.75	24	2.81	8.58	24	1.05

$\bar{x}$ = mean

N = number of samples

SE = standard error

C = counts from the columnar zone

T = counts from the transition zone epidermis

Significant Analyses:

Transition

Wall

60+15+60=60+30=60+60=60+90
without 60+15, there is no significant difference

Inner Mucilage

60=60+15+60+30=60+60+60+90

Outer Mucilage

60 60+15 60+30 60+60 60+90

Columnar

60+15+60=60+30=60+60=60+90
without 60+15, there is no significant difference

60+60+15+60+60+60+30=60+90

60 60+15 60+30 60+60 60+90

Multiple Linear Regression and Correlation:

	Transition			Columnar		
	Y-intercept	Slope	Correlation	Y-intercept	Slope	Correlation
Wall	16.67	0.09	0.57	17.85	0.02	0.19
Inner Mucilage	10.13	0.54	0.98	15.52	0.25	0.74
Outer Mucilage	4.03	0.17	0.91	6.13	0.06	0.49

chase to the end of the chase indicating an increase of label accumulation into this region of the mucilage that is significant.

3. In the case of the outer mucilage the numerical values indicate that there is an increase in the label in this portion of the mucilage and statistical analysis indicates that this increase is real. There is good correlation in all cases especially for the mucilages.

(b) Glucose-6-³H Counts:

Values of the glucose-6-³H counts are presented in Table 12. The raw data from which this table of results is calculated are presented in Appendix VII. For the wall and the outer mucilage, there seems to be little change in the values with increasing chase times. For the inner mucilage, however, there seems to be a tendency toward an increase of grains located over it as the chase time increases. Analysis of variance and Newman-Keuls multiple range tests were performed and the significant differences are presented on Table 12. Important points are noted below.

1. For the wall (using the transition results for 60 + 90), it is important to note that there is basically no difference in values except that the 60 + 90 value is statistically different from all other values. Using the columnar results, the results are confusing and are therefore difficult to interpret.

2. In the case of the inner layer of the mucilage, again the results are very difficult to interpret and the only concrete thing that can be concluded is that 60 + 90 is different from all other values, which is evidence that the mucilage label

Table 12: Grain Count Data and Statistical Significance for
Glucose-6-³H in the Outer Wall and the Mucilage of
the Epidermal Cells.

Chase Time	Wall			Inner Mucilage			Outer Mucilage		
	$\bar{x}$	N	SE	$\bar{x}$	N	SE	$\bar{x}$	N	SE
0	24.6	29	2.79	9.86	30	1.38	7.69	29	1.12
10	25.3	24	2.45	28.1	24	3.08	11.38	24	1.94
15	22.92	25	1.47	21.2	25	2.75	11.44	25	1.89
30	11.48	25	0.91	11.88	25	1.07	5.0	25	0.93
60	17.75	12	3.76	14.25	12	3.67	9.36	11	5.34
90T	33.7	18	2.97	46.72	18	7.04	29.2	18	6.54
90C	17.29	26	1.68	14.97	29	2.22	9.14	28	1.47

$\bar{x}$ = mean

C = counts from the columnar zone epidermis

SE = standard error

T = counts from the transition zone
epidermis

N = number of samples

Statistical Analyses Results:

Transition
Wall
60 60+10 60+15 60+30 60+60 60+90
Inner Mucilage
60 60+10 60+15 60+30 60+60 60+90
Outer Mucilage
60 60+10 60+15 60+30 60+60 60+90

Columnar
60 60+10 60+15 60+30 60+60 60+90
60 60+10 60+15 60+30 60+60 60+90
no significant difference is found

Multiple Linear Regression and Correlation:

	Transition			Columnar		
	Y-intercept	Slope	Correlation	Y-intercept	Slope	Correlation
Wall	20.31	0.07	0.31	21.16	-0.04	-0.29
Inner Mucilage	14.63	0.23	0.58	16.73	0	-0.00
Outer Mucilage	5.58	0.23	0.86	9.26	-0.01	-0.09

increases over the chase time when the transition results are used.

For the columnar results a relatively inconclusive result is found.

3. For the outer layer of the mucilage in one case (transition results used for 60 + 90) only the 60 + 90 is different from all the other times. For the columnar value used for 60 + 90, no statistical difference is found. These results may indicate that there is no real difference or that insufficient time has elapsed to record any real difference. There is very poor correlation in all cases.

(c) Fucose-6-³H Grain Counts:

The fucose results are presented in Table 13.

The raw data from which these results are taken are presented in Appendix VII. The wall grain count values tend to reduce as the chase progresses, whereas the inner mucilage counts seem to increase with increasing chase times. The values for the outer mucilage seem to show no significant increases or decreases. Analysis of variance followed by a multiple range test if required was performed and the significant differences are reported in Table 13. The important points are summarized below.

1. For the wall counts (using the columnar region for 60 + 90) after the analysis of variance, there is found to be a significant difference. After the completion of the Newman-Keuls test however, there is found to be no significant difference. The analysis of variance is more sensitive to differences than is the Newman-Keuls test and that is the cause of this apparent discrepancy - the difference is not therefore that significant. If the transition zone is used, the tendency towards a decrease is shown to be somewhat

Table 13: Grain Count Data and Statistical Results for Fucose-6-³H Label in the Outer Wall and the Mucilage of the Epidermal Cells.

Chase Time	Wall $\bar{x}$	N	SE	Inner Mucilage $\bar{x}$	N	SE	Outer Mucilage $\bar{x}$	N	SE
0	12.4	15	1.17	10.6	15	1.59	4.4	15	1.02
15	11.6	10	1.12	10.7	10	1.65	2.9	10	0.55
30	10.7	30	0.65	12.23	30	1.11	3.07	30	0.45
60	10.8	20	1.06	10.8	20	1.93	2.85	20	0.85
90T	9.17	30	0.61	18.7	30	3.56	4.1	30	1.06
90C	9.47	30	0.47	15.47	30	1.20	2.73	30	0.44

$\bar{x}$ = mean

C = counts from the columnar zone epidermis

SE = standard error

T = counts from transition zone epidermis

N = number of samples

Statistical Differences:

Transition
Wall
60 60+15 60+30 60+60 60+90

Columnar
no significant difference with multiple range test, but a significant difference registered with analysis of variance.

Inner Mucilage
all values to same except
60+30=60+90

60 60+15 60+30 60+60 60+90

Outer Mucilage
no significant difference

no significant difference

Multiple Linear Regression and Correlation:

	Transition			Columnar		
	Y-intercept	Slope	Correlation	Y-intercept	Slope	Correlation
Wall	12.16	-0.03	-0.94	12.10	-0.03	-0.94
Inner Mucilage	9.62	-0.08	0.80	10.47	0.04	0.75
Outer Mucilage	3.46	0	0	3.71	-0.01	-0.70

statistically upheld, although not too conclusively.

2. For the inner mucilage, the columnar results suggest that there is an increase which is real, whereas for the transition value the increase does not seem to be statistically upheld. There is, however, a tendency towards an increase.

3. For the outer mucilage, no statistical difference is found using either the columnar or transition results for 60 + 90. This could be a result of not a long enough time of chase to register anything as moving out to the outer mucilage. There is good correlation for all, especially when columnar results are used and also for wall and inner mucilage with transition zone results.

3:4 Examination of the Epidermis - Specific Topics:

3:4:1 Comparison to the Three Regions of the Epidermis by Cell Length:

The average cell lengths (periclinal dimension) determined in this study are 10-15 μ m in the columnar region (Area B), 15-22 μ m in the transition zone (Area C) and 25 μ m for the smaller cells in the elongation zone and up to 75 μ m for the long cells.

3:4:2 New Anticlinal Cell Wall and Root Hair Formation:

The patterning of cell plate formation in the anticlinal walls is found to be quite dramatically demonstrated by means of the autoradiographic technique. The three types of division which occur are (see Diagram 4), (a) classical form of cell plate formation where the cell plate begins in the centre of the cell and grows out to meet the mother cell walls, (5) cell plate formation beginning from the inner periclinal wall and growing outwards and (c) cell plate formation beginning from the outer periclinal wall and growing inwards.

Diagram 4: Patterns of Cell Wall Formation.

Cell Plate Patterns:

Figure 120:

A cell plate forming in the classical mode (from the centre and growing outwards towards the mother cell walls), this cell plate began to form before the autoradiographic feeding (* - unlabelled portion) and was completed (labelled portion) within the time period. The precursor was glucose-6-³H for 60 minutes. This is from Kaine (1977). x1760.

Figure 121:

A cell plate also forming by the classical method, except the beginnings of this wall are labelled, whereas it has not been completed within the time period of the labelling. (Glucose-6-³H pulse chase 60+60.) x1200.

Figure 122:

Another cell plate forming as Figure 121; (glucose-6-³H pulse chase 60+60) with the label occurring in the middle of the cell. x400.

Figure 123:

A cell plate that is forming from the outer periclinal wall inward, the part with the arrowhead was formed before feeding with the precursor (glucose-6-³H - 30 minutes) and the rest of the wall was finished during the labelling time. This is extracted from Kaine (1977). x1400.

Figure 124:

Another cell wall just beginning to form from the outer periclinal wall and growing inwards. (Fucose-6-³H for 90 minutes.) (see arrowhead) x500.

Figure 125:

Two small cell walls (arrowheads) forming from the inner periclinal wall with glucose-6-³H for 30 minutes. This is extracted from Kaine (1977). x1200.

Figure 126:

Another cell wall forming from the inner periclinal wall. This is the more common form of new cell wall development found in these experiments. The label for this photomicrograph was fucose-6-³H for 90 minutes. x800.

Cell Plate Patterns* (Continued):

Figure 127:

Another cell plate forming from the inner periclinal wall, this time labelled with glucose-1-³H for 30 minutes. x1500.

Figure 128:

These internally originating cell walls (arrowheads) were labelled by fucose-6-³H for 90 minutes. x480.

Figure 129:

This internally originating cell (arrowhead) was labelled with fucose-6-³H for two hours. x460.

Figure 130:

These two cell walls forming from the inner periclinal wall were labelled with glucose-1-³H for 30 minutes. x600.

Note that over all of these cells, there is a discontinuity in the dense band of label and in mucilage label.

120

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130

Most of the new cell plates occur in the columnar zone epidermal cells (Area B), but there are also quite a few in the transition zone epidermal cells (Area C).

The new cell plates are highly labelled and as such can be easily distinguished from nearby older cell walls. The classical cell plate (a) forming from the centre of the cell and growing outwards towards the mother cell walls) is labelled with all three radioactive sugars. In some cases, part of the plate (central portion) has formed before the incubation with the sugars and the silver grains are located over the outer parts of the cell plate (Figure 120) which formed during the incubation period and in other cases the plate has begun to form during the incubation period and the silver grains are located over the central portion of the plate (Figures 121 and 122).

Cell plates forming from the outer periclinal wall (Figures 123 and 124) and growing inwards and from the inner periclinal wall outwards (Figures 125 to 130) are also labelled by all three radioactive sugars. There seems to be more of the type which grow from the inner periclinal wall than from the outer periclinal wall.

The cells which are forming the cell plates are longer along the longitudinal axis of the root than nearby cells that have already divided (Figures 126 and 127). Cells also have the peculiar association of being in groups of four (which can be recognized by the scalloping pattern on the outer edges of the cells) and so often two adjacent cells will divide to form such a group of four (Figure

Correlations Between Autoradiography and Aniline Blue Induced Fluorescence:

Figures 131-133:

Three views of the same section to show correlation between autoradiographic label in the new cell walls and aniline blue induced fluorescence. The aniline blue staining was done before the autoradiography. The precursor used was glucose-1-³H for 30 minutes. The magnification is x400.

Figure 131: PAS stain + autoradiography.

Figure 132: Aniline blue induced fluorescence.

Figure 133: PAS + autoradiography + toluidine blue staining.
Note that in the cells with the new cell walls, there is a large number of small vacuoles close to the new cell wall.

Dense Band of Label Discontinuities:

Figures 134-141 are extracted from the continuous labelling, Figures 142-146 from pulse chase results and Figures 147-150 are examples of recovery of this discontinuity - all show the same pattern of lack of label in the outer periclinal wall and in the mucilage.

Figure 134: Glucose-6-³H - 60 minutes (x240)

Figure 135: Glucose-6-³H - 60 minutes (x120)

Figure 136: Glucose-1-³H - 30 minutes (x275)

Figure 137: Glucose-1-³H - 60 minutes (x150)

Figure 138: Glucose-1-³H - 30 minutes (x520)

Figure 139: Glucose-6-³H - 60 minutes (x420)

Figure 140: Glucose-1-³H - 60 minutes (x150)

Figure 141: Fucose-6-³H - 120 minutes (x330)

Figure 142: Glucose-6-³H - PC-60+10 (x150)

Figure 143: Glucose-1-³H - PC-60+90 (x275)

Figure 144: Glucose-1-³H - PC-60+90 (x340)

Figure 145: Glucose-6-³H - PC-60+90 (x210)

Figure 146: Fucose-6-³H - PC-60+15 (x150)

Figure 147: Glucose-1-³H - PC-60+60 (x125)

Figure 148: Glucose-6-³H - PC-60+15 (x220)

Figure 149: Glucose-6-³H - PC-60+30 (x180)

Figure 150: Glucose-1-³H - 60 minutes (x180)

128). This grouping into fours is more obvious in some cases than others (Figure 128).

For new cell walls correlations can be made between aniline blue induced fluorescence and labelling patterns (Figures 131 to 133). Walls that fluoresce with aniline blue are also heavily labelled.

It is also noted in general that cells that are forming a new cell wall are less labelled by all three sugars in the cytoplasm than are nearby non-dividing cells. The cells also seem to have a large accumulation of small vacuoles on both sides of the new cell wall (Figure 133).

As has been previously noted, over cells forming new anticlinal walls, the dense band of label in the outer periclinal wall and the mucilage label directly above these cells is absent or reduced compared to nearby non-dividing cells. This labelling pattern has been observed with all three sugars as precursor, both with the continuous and pulse chase experiments (Figures 134 to 150).

In most roots observed, there are a number of new cell walls along the length of the epidermis but no new cell walls are observed in tissue bathed in colchicine prior to incubation with the radioactive precursors. The dense band is continuous (Figure 11).

Over cells that have finished making a new anticlinal wall, the outer periclinal wall and the mucilage begin to become labelled (Figures 147 to 150).

In the elongation zone (Area D), root hairs develop. Root hairs are found to have developed in some cases and to have

Root Hair Development:

Figure 151:

A young root hair labelled with fucose-6-³H for 120 minutes. Note the accumulation of label at the tip of the hair. x1400.

Figure 151a:

Another young root hair also labelled for two hours with fucose-6-³H, note again a small accumulation at the tip. x150.

Figure 151b:

Another young root hair exhibiting similar labelling characteristics labelled with glucose-6-³H for 15 minutes. x150.

Figure 152:

The tips of older root hairs are also massively labelled by fucose-6-³H for three hours. x1200.

Figure 153-155:

Consecutive sections through an area of root (labelled with fucose-6-³H for 90 minutes) showing very young root hair primordia. The numbers indicate the primordia, so that they can be followed from section to section. x150.

Figures 156-157:

The root tips of older root hairs again with the large accumulation of label at the tips. The roots were incubated with fucose-6-³H for two hours. x600.

Figure 158:

The young root primordia (arrowhead) is aniline blue positive as are the tips of older root hairs. This was done on an unstained section by mounting in aniline-blue. x600.

Figures 159-160:

The same area of the root, bearing two young root hair primordia, Figure 159, with PAS stain and autoradiography and Figure 160, by phase-contrast microscopy. From this pair it can be seen that the label occurs in an indentation of the wall in the mucilage. x900.

Root Hair Development (Continued):

Figure 161:

A copy of a plate from Lerch (1960) - (figure from) showing that where he found callose is the same as where it is found in the root hairs for this experiment including the small root hair primordia.

a particular labelling pattern of their own. As indicated previously, the elongation zone epidermal cells have a dense band of label located in the outer periclinal walls of the cells.

Young root hair tips are labelled with the glucoses and fucose (Figures 151, 151a and 151b). The tips of longer root hairs are also labelled (Figures 152, 156 and 157). Root hair tips have a strong aniline-blue-induced fluorescence. The root hairs are always located at the acropetal ends of the cells and are present on both the long and the short cells found in this region.

Small pockets of label are found in near adjacent cells (towards the tip) from the cells with the youngest root hairs at the acropetal ends of the cells (Figures 153 to 155). The location of the pocket is not cell size dependent. These pockets which are located outside of the outer periclinal wall (in a small indentation of the wall and shaped like a half moon) as indicated by phase-contrast microscopy can be traced from section to section (Figures 159 and 160). They are between 6 to 8 μm in length, have aniline-blue-induced fluorescence (Figure 158) and stain similarly to mucilage with other stains (PAS, Toluidine blue).

3:5 Controls:

In the course of each experiment the controls (sections of roots that were not fed with a radioactive precursor, but emulsion coated and developed as per normal procedure) were examined. In all cases they were clean of background and had similar morphological and histochemical properties to the experimental tissue.

4. DISCUSSION

Science is always wrong. It never solves a problem without creating ten more. George Bernard Shaw

The technique of autoradiography is useful for localization studies of various compounds for biological specimens. There are, however, several problems associated with the interpretation of autoradiographs.

One problem which could occur is that the precursor used is not taken up by the specimen. However, the root tip of corn is known to be capable of absorbing and utilizing sugars from an exogenous source and indeed did absorb the exogenously supplied radioactive glucoses and fuçose provided in these experiments.

During the performance of the technique several artifacts such as high background resulting from faulty handling or contamination, fogging of the emulsion from incorrect use of stain can occur. However, if proper technique is utilized these problems are minimized.

In examination of the prepared specimens, there can be some doubt arising as to the positioning of the silver grains over structures. This is a greater problem with electron microscope autoradiography and is negligible in the present work.

There is always some doubt as to the specificity of

transference of the label with a precursor to the expected product, i.e., glucose is not only metabolised into polysaccharides but also is metabolised into proteins. There are also the additional problems of differential rates of polymer synthesis related to kinetics of the various biochemical reactions and also different rates of synthesis of similar compounds in different regions of a tissue and differential dispersion rates of labelled compounds in different regions. Also the dilution of precursors within cell pools could affect the rate of utilization of precursors and also the size of a pool in a particular region could affect the rates of migration or secretion of labelled polymers.

The final problem is the possibility of different penetration rates of precursors and also of differential uptake of precursors into cells. This problem has been confronted in studies of the two outer cell groups examined in this work - the root cap and the epidermis. The outer root cap accumulates the most label of all cell layers of the root cap with the three sugars used. This observation is common in the literature (Juniper and Roberts, 1966 (glucose- ^3H with corn); Pickett-Heaps, 1966 (glucose-1- ^3H and 6- ^3H with wheat); Dauwalder and Whaley, 1973, 1974 (glucose- ^3H and galactose- ^3H with corn); Barlow, 1974 (^3H glucose with corn); Barlow and Sargent, 1978 (^3H glucose with regenerating corn root cap from the quiescent centre). The epidermis is also more heavily labelled with all three sugars than other cell types of the root proper at all times, including very long time (Figure 6). This higher concentration in these outer cell types could be interpreted as a

function of unequal penetration. Of course, for accurate interpretations of metabolism, it is important that entry is made to the entire area of the tissue to be studied. In a study by Roberts and Butt (1968a), although uptake of glucose was linear with time for corn roots, autoradiography of the roots revealed that the rate of incorporation was different for individual tissues. At short incubation times all radioactivity was associated with the epidermis, its thickened wall and the outer cortex with no radioactivity in the inner tissues. Also the flanks of the root cap and other marginal root cap cells were heavily labelled. At longer times, the isotope was incorporated consecutively into the inner region of the cortex and then the pith, but the epidermis and outer cortex increased greatly (Roberts and Butt, 1968a - Figure 2). They state that it cannot be definitely determined whether the differences in incorporation rates were due to a higher metabolic rate and/or synthetic rate of the outer tissues or a more rapid equilibration of the labelled compound into the precursor pools of the outer tissue or due to penetration differences and/or greater availability to the outer tissues.

However, in tracer studies with foliage supplied with $^{14}\text{CO}_2$, radioactive exudates from the roots are rapidly found (Rabideau and Mericle, 1952; McDougall and Rovira, 1970; McDougall and Rovira, 1965; McDougall, 1970; Barber and Martin, 1976). Cormack and Lemay (1963) noted that exogenously supplied glucose entered the cortex of Sinapsis alba fairly rapidly. Also the fact that at longer times, the outer root cap and epidermis are always heavily labelled indicates that this is not a penetration phenomenon, but a function of a higher

metabolic rate. This interpretation has also been proposed by other workers (Pickett-Heaps, 1967b; Juniper and Roberts, 1966; Barlow, 1975). It could also be due to differences in cell pool size.

The root cap and the epidermis have been found to be distinct from the rest of the cell types of the root in other ways. The epidermal layer stains more intensely than the other cell types of the root proper with toluidine blue especially the columnar region (Figure 12). The outer root cap cells contain highly hypertrophied Golgi vesicles (Whaley *et al.* 1959a; 1959b; Mollenhauer *et al.* 1961; Juniper and Roberts, 1966; Northcote and Pickett-Heaps, 1966a, 1966b; Rougier, 1971). Similar morphology of some Golgi vesicles has been seen in epidermal cells of corn roots (Leech *et al.* 1964; Lopez-Fando *et al.* 1976). A positive correlation between hypertrophied Golgi and high rates of incorporation of radioactive sugars has been made (Juniper and Roberts, 1966; Bowles and Northcote, 1972; Northcote and Pickett-Heaps, 1966) for several cell types that are known to be secretory. This indicates the possible secretory character of the epidermis.

Several other observations point to a secretory character of the epidermis. For example, the high ribosome count (Karas and McCully, 1973) and the columnar shape of the cells. Regardless of the evidence, a possible secretory function for the epidermis has been neglected and the origin of the overlying mucilage is still in doubt even though Price as long ago as 1912 describes epidermal cells as "mucilage secreting glands" and emphasized that this activity is indicated by their dense protoplasmic contents

and by the presence of large and conspicuous nuclei near the base of the cells. In spite of this there are still experimenters who believe that the mucilage is a product of the root cap (Foster in Oades, 1978).

Evidence provided in this work suggests the mucilage-secretory nature of the epidermis. In addition the incorporation patterns of the three precursors for the epidermis and the root cap will be discussed and a comparison of the incorporation of the three sugars will be made.

1. The Root Cap and Its Slime:

For continuous labelling, the incorporation patterns for the glucoses is similar to those observed by several other workers. That is most of the label from the glucose isotopes is accumulated in the peripheral root cap cells (Region 1) (Figures 10, 19, 24, 30, 35). Many workers have found that the peripheral root cap cells are the most highly labelled cells of the cap (Dauwalder and Whaley, 1974; Northcote and Pickett-Heaps, 1966; Juniper and Roberts, 1966; Roberts and Butt, 1970).

For both glucoses in the present work, at shorter times, there is little slime label, but at longer times, the amount of label increases greatly in the slime. The glucose-6-³H precursor seems to label slime more readily than does the glucose-1-³H precursor. Pickett-Heaps (1967) also noted that for wheat roots, glucose-6-³H gives more overall labelling than does glucose-1-³H.

The fucose precursor on the other hand has a very interesting pattern for continuous labelling. The label is always

located in the outer peripheral cells toward the exterior portion of the cell cytoplasm and parallel to the outer perimeter of the root cap (Figures 45, 48, 52). This observation has been noted previously by Kirby and Roberts (1971), Paull and Jones (1975b, 1976), Paull et al. (1975), except that they state that the characteristic pattern does not occur in the outermost layer of cells (rather they have a diffuse distribution in the walls and outside the cells). In the present study, while this is sometimes the case, the mentioned pattern does manifest itself in the outermost cells as well.

With pulse chase techniques, totally different observations are made. The difference between pulse chase and continuous data can be explained by the saturation of the cell pools. In continuous labelling, the pools are continually being filled up but no high influx of sugar is given whereas in the pulse chase, the cells are filled up during the pulse and then the high concentration of glucose in the chase causes the labelled material to move differently. Therefore later times in the continuous labelling could have similarities to lower chase times as they do in this work. This would be because at shorter pulse chase times and longer continuous labelling times the same amount of sugars would be present therefore resulting in similar labelling patterns.

In the outer root cap cells, the bearding pattern is found for both glucoses at lower times of the chase. The labelling patterns are similar, but the label is not so intense with glucose-1-³H as it is with glucose-6-³H. The bearding phenomenon has been noted before

and has been considered as partial evidence of directionality of secretions (Morrè et al. 1967; Mollenhauer and Morrè, 1966, 1976; Juniper and Pask, 1973).

Barlow and Sargent (1978) found that this directionality occurs even in a root cap which is being regenerated from the corn root quiescent centre. Not only did the exposed quiescent centre cells begin to produce slime, but the typical hypertrophied dictyosomes were present. The slime accumulates asymmetrically towards the outer edges of the cells. After some time the accumulation fills the entire perimeter of the cells outside the cytoplasm with increasing amounts of produced slime. The factors that control directionality are not as yet understood, but Barlow and Sargent (1978) state that directionality may be a consequence of position, but also say that the properties peculiar to such a position that would cause a change in the dictyosomes and the asymmetric accumulation of slime are unknown, but could be related to the availability of oxygen and the substrate for slime synthesis. At longer times, the amount of bearding decreases; indicating that the pulse chase is at least partially effective. At these longer times, when the lower portion of the outer root cap has lost its bearding, the flank cells are often still bearded. Contrary to the theory of root cap development, that states that these cells are not very active (Barlow, 1975), they remain active even later than the lower peripheral cells.

For glucose-6-³H the asymmetric distribution of the label toward the outer part of the cell in the outer root cap cells

developed quickly (by 10 minutes) (Figures 60 and 61). This quick change of label position points to a rather rapid rate of slime production. This is comparable to other synthetic rates of mucopolysaccharides, glycoproteins and other plant slimes from the literature both for animals and plants. It is possible that this quick synthetic rate of the slime may be affected by two phenomena, periodicity and enhancement of secretion by exogenous sugars. If by chance the roots were caught just prior to a secreting phase, a quick turnover would have occurred. The roots are believed to be in phase since the roots are believed to be synchronized by dipping in water (Jones and Morrè, 1977, 1973). Thus periodicity may or may not be a factor depending on the time period after dipping. The secretion of slime has been shown to have a periodicity of approximately three hours. There is also a periodicity of the production of dictyosome vesicles which is suggested as the cause of the periodic secretion of the slime (Jones and Morrè, 1973). Secretion has been shown to be enhanced by supplying mono- and di-saccharides to the growth medium of the corn root. Sucrose at 0.04 M doubles the secretion (Jones and Morrè, 1977). In further examinations of this phenomenon, it was found that the exogenous sugars enhanced secretion most effectively from 0.01 M to 0.1 M for glucose, sucrose, fructose, maltose, lactose, ribose, xylose, fucose and mannitol (between 1.5 to 2 fold increase). Mannose at 50 mM gives a phenomenal increase of 5 to 10 fold, where galactose, arabinose and galacturonic acid are inhibitory. Sucrose at 40 mM stimulates the production of fucose, glucan and galactan polysaccharide

synthesis, whereas fucose at 40 mM stimulates fucose polysaccharide synthesis, only slightly stimulates galactan polysaccharide synthesis and does not stimulate glucan polysaccharide synthesis (Paull et al. 1975). This phenomenon of stimulation of secretion by exogenous sugars has been reported by Schnepf (1961) for the secretion of the trapping slime of the gland cells of carnivorous plants. With the pulse chase used in the present work, the concentrations of the chase solutions were just under 0.01 M, within a range which might cause increased secretion. Both the periodicity and enhancement by exogenous sugars could be factors in the rate at which the radioactive material is channelled from the cells into the slime.

The labelling of starch grains in Region 1 first occurs at 60 minute pulse + 10 minute chase for glucose-6-³H and 60 minute pulse + 30 minute chase for glucose-1-³H. This does not occur in continuous labelling until 90 minutes (for both precursors). This shows the similarity between the long continuous labelling times and the shorter chase periods as previously discussed. This has been noted by Northcote and Pickett-Heaps (1966) for wheat roots and they tendered the following explanation. During the pulse, most of the soluble glucose was channelled into the Golgi apparatus and immediately into the slime material and no label was available for starch synthesis. Once, however, the chase glucose became available, synthesis of starch began from the pool of labelled glucose, so the labelled sugars could therefore accumulate in the storage polysaccharides. This could depend on the metabolic activity of the cells and also on the availability of the externally supplied glucose (Pickett-Heaps, 1967c).

The closely related inner root cap cells (Region 2) on the other hand have label accumulated on the outer side of the cells (inside the walls for both glucoses) but also have their starch grains labelled from the very beginning of the chase and are not as involved in slime secretion as the outer cells (Region 1). Pickett-Heaps (1966) also noted the starch grain labelling in these cells for wheat. He says that these inner cells have continuous starch grain label because they are not competing for the pool of labelled glucose to the same extent as those in the outer cap. Juniper and Roberts (1966) note that the amyloplasts accumulate starch as the meristematic activity ceases and the starch grains become more prominent in the expanding cells of the cap but are lost again as the cells approach the periphery of the cap. In the present work contrary to that of Pickett-Heaps label can occur in starch grains of the outer cells for the two glucoses. A correlation between starch degradation and slime secretion has been suggested (Juniper and Roberts, 1966; Paull and Jones, 1976; Juniper and Pask, 1973) but the regeneration study of Barlow and Sargent (1978) on root caps shows that starch degradation and slime synthesis are not linked although they seem to coincide in the normal cap.

For the fucose precursor under the pulse chase condition the outer root cap cells (Region 1) accumulate label in the outer edges in the walls of the cells, tangential to the outer surface of the cap similar in pattern to the continuous labelling. More internal cell layers have the label inside the walls.

Recent studies done with lectins (Rougier et al. 1979) which

bind specifically to L-fucose have shown that these lectins have a strong positive reaction with slime areas, the surface of peripheral root cap cells and sloughed cells embedded in the slime. (This has recently been corroborated, Vermeer, 1981, in press) This, of course, corresponds to where the radioactive sugars label the cells and slime. Other lectins specific for glucose and galactose have weaker reactions at the outer root cap (Rougier *et al.* 1979). Fucose also never labels starch grains, either after continuous or pulse chase experiments. This is understandable since very little modification and metabolism of fucose exogenously supplied is believed to occur and in order for it to label starch grains it would have to be metabolised.

In a few cases it was observed that an accumulation of secretion occurs between the plasmalemma and the cell walls of the outer cap cells (Figures 35, 106, 111). This has often been noted in the literature (Jones *et al.* 1966; Morrè *et al.* 1967; Rougier, 1971; Juniper and Pask, 1973; Branton and Moor, 1964; Barlow and Sargent, 1978). The occurrence of this phenomenon is believed to be related to water stress (Jones *et al.* 1966; Jones and Morrè, 1966) and can be removed by dipping the roots in water (Morrè *et al.* 1967). This does not occur very often in the present work since the roots are in a solution at all times. Other cell types also show the same internal accumulation of products (Schnepf and Busch, 1976 for glandular hairs; Schnepf, 1965 for oil appendices; and Schwab *et al.* 1969 for Venus fly traps).

2. The Epidermis:

(a) Cells:

In general, with increasing time for continuous labelling, the amount of label in the cytoplasm and walls of the cells increases, whereas with pulse chase the amount of cytoplasmic label is inversely related to increasing time. This indicates a partially effective pulse chase.

The outer periclinal wall of the epidermis is quite thick and is also massively labelled by all three precursors with continuous and pulse chase labelling (referred to as the dense band). The high level of activity in this wall has been previously observed (Pickett-Heaps, 1967b, 1967c - ^3H glucose in wheat; Dauwalder *et al.* 1969 - ^3H glucose on corn; Dauwalder and Whaley, 1974 - ^3H galactose on corn). In general, the other three visible cell walls are not as heavily labelled except that sometimes in the elongation zone epidermal cells all four visible walls are heavily labelled, whereas three of them (that is except the outer periclinal wall) are normally lightly labelled. The reason for this is unknown. Whaley and Mollenhauer (1963) and Mollenhauer and Mollenhauer (1978) found that the Golgi vesicles are incorporated almost exclusively into the outer periclinal wall of the epidermis and very few are associated with the other walls. The outer wall is obviously a most important aspect of these cells since it is so intensely labelled and the amount of label does not get reduced by chasing with cold sugars. An understanding of the exact function of the wall would probably show why this wall is so heavily labelled. Roberts and Butt (1969) noted that incorporation of galactose into alkali insoluble polysaccharide (cellulose) was at

a maximum at 1.5 mm and fell as far back as 3 mm from the tip and then remained constant through the elongation zone and non-growing cells. They state that this is largely associated with the thick outer wall.

Another feature in the labelling of the thick outer wall is that there is a discontinuity in this dense band of label over cells with new anticlinal wall or over cells that are dividing (in telophase) (Figures 134 to 150). This has been previously observed (Pickett-Heaps, 1967b; Dauwalder et al. 1969; Dauwalder and Whaley, 1974) and was found to be present for all three sugars under the two experimental conditions - continuous labelling and pulse chase in the present work.

The change of position of the labelling incorporation may indicate the presence of a vectorial mechanism. Although directionality mechanisms are not completely understood, it would be interesting to examine the cause of this labelling change. It may indicate that similar organelles perform two functions of wall and mucilage production and when a new cell plate is being formed no label is added to the outer surface. Mollenhauer and Mollenhauer (1978) examined the distribution of vesicles at different times in the mitotic phase and found a change in the location of vesicle fusions paralleling the change of labelling patterns found in the present work. For example, the number of vesicles fusions per μm of wall section during interphase with the outer periclinal wall was .45 fusions as opposed to .01 vesicles for the anticlinal (they call them transverse) and inner walls, but during cytokinesis the

numbers are the following: .03 for the transverse, .18 for the outer wall and 2.54 for the cell plate. It is obvious that during cell division that the wall of importance is the new cell wall. These results parallel the observations of the massive label in the new cell wall and that during interphase the outer periclinal wall is the most heavily labelled, but is not labelled during cytokinesis.

(b) Mucilage:

It has been noted that the mucilage layer is quite thick at the flanks of the roots (12-22 μm) and gradually thins until it is 1.5 μm or less or not present at all in the root hair zone. Price (1912) also noted that the mucilage thinned. Clarke *et al.* (1979) stated that there was no mucilage at the elongation zone contrary to the present work but recent work from the same lab has shown a secretion to be present on these older portions of the root, although not necessarily the same type of mucilage as is present on the younger portions of the root (Vermeer, 1980 - per. comm.).

Mucilage thickness varies with the plant species, environmental and microbial conditions and in the case of microscopical measurements, with the methods of preparation, but the present results are of the same order of magnitude as those obtained by others. Brams (1969) determined a mucilage layer on citrus roots to be 2 μm . Greaves and Darbyshire (1972) found mucilage on wheat roots to be .5 μm of all parts of axenic roots and to be .5 to .8 μm on non-axenic roots. Foster (1977) showed that mucilage on roots of grasses from irrigated pastures were 20 to 50 μm thick. O'Brien (1972) stated that the

mucilage layers on oats, peas, corn and willow were about 19 μ m in thickness. Mucilage layers have been observed for aquatic plants of up to 1 mm in thickness (Oades, 1978). Measurements of these mucilages is tedious since they are prone to swelling and shrinking upon wetting and drying. The relative thickness of the mucilage indicates that it gradually thins, therefore thickness also varies with distance from the root tip. The evidence points to the epidermis as the originator of the epidermal mucilage.

There are two approaches for associating the origin of the mucilage with the epidermis rather than the root cap: (a) first to add to the recognized differences between the root cap slime and the mucilage and (b) by direct autoradiographic observations of the movement of label into the mucilage under pulse conditions.

- (a) Further differences between the mucilage and the slime as indicated by autoradiographic labelling:

A quick summary of the differences already found include (a) histochemical (Table 1), (b) physical - slime is easily removed from the root whereas mucilage is difficult to remove (Appendix VI), and (c) biochemical - fucose content of slime is much higher than the epidermal mucilage (Harris and Northcote, 1970; Bowles and Northcote, 1972, 1974).

New Observations:

- (i) Pulse chase data shows a difference in labelling rates between the slime and the mucilage. The glucoses are metabolized and extruded into the slime much quicker than into the mucilage. Even after a ten minute chase (Figures 60 and 61)

the bearding pattern for the slime production is highly pronounced (especially for glucose-6-³H). This high rate is comparable to other synthetic rates from the literature both from plants and animals (30-60 minutes from injection of radioactive material to secretion for animal glycoproteins and mucopolysaccharides - Neutra and Leblond, 1966a, 1966b; 30-45 minutes for root cap slime - Bowles and Northcote, 1974; vesicles in Drosophyllum can traverse the cytoplasm in 2.5 minutes - Mollenhauer and Morrè, 1966; the scales of Pleurochrysis are synthesized and discharged in under two minutes - Brown et al. 1969). The difference in rate of secretion between the mucilage and the slime could possibly be related to the metabolic rates of the cells making the two secretions and also may be an indication of a degree of complexity of the two secretions.

(ii) Specific observations of differences in label amount between the root cap slime and the mucilage have been observed for the continuous labelling (see Figures 39 to 43, 54).

(b) Evidence from Autoradiographic Labelling of the
Epidermal Mucilage Alone:

(i) Continuous Labelling:

At short incubation times, there is little label present in the epidermal mucilage but at longer times there is a noticeable increase (compare Figure 16 with 23 and Figure 31 with 41). This does not in itself prove that this label comes from the epidermal cells, but since in most cases the slime is not associated with the mucilage, it seems that the origin of this label is probably the epidermis. There is of course a reduction of the amount of label

in the mucilage as one moves basipetally up the root, but this may be due simply to the majority of the mucilage being made by the younger cells and being drawn up over the cell surfaces. The same would also be true if the root cap were partial originator of the mucilage, but often the accumulation is towards the inner portion of the mucilage, whereas if the slime were the contributor one would assume it would add to the outer edge of the mucilage. Another point is that over cells that are forming new anticlinal walls, there is a discontinuity in the dense band of label in the outer periclinal wall and also in the mucilage label. If the root cap were responsible for the laying down of the mucilage there is no physiological basis why or how this cell wall formation would affect root cap secretion or the accumulation of a root cap secretion at a specific point removed from the root cap.

(ii) Pulse Chase and Grain Count Data:

For the pulse chase condition, results show that after the pulse time of 60 minutes there is some label found in the mucilage, but as the chase time continues, there is an increase in the mucilage label for all three sugars most notably in a layer near the periphery of the epidermal cells. Counts of silver grains are considered to be proportional to exposure times therefore grain counts are indicative of the radioactive content of the structure being examined (Leblond, 1976). The same observation as to the position of the label is borne out by the grain count data (as with the photographic observations) that is, there are higher counts in the inner part of the mucilage than in the outer part of the mucilage.

The mucilage label seems to move out as a wave with an abrupt boundary. The reason for this is unknown but may point to the possibility that the outer mucilage is of a different character than the inner mucilage. Indeed the very outer portion of the mucilage is histochemically distinguishable from the rest of the mucilage. This remains to be clarified. In this outer mucilage, there may be a cellulose component or some other more complex molecule which takes a longer period to make than can be made with the experimental timing and therefore the label seems to have this abrupt boundary. However, for longer times with the glucose the label begins to move into this outer zone. Since very often the outer mucilage pulse chase counts show no significant difference over the chase times, another explanation of this lack of label in the outer mucilage is that there is not a long enough time period to allow the label to reach this far out. The fact that most of the label is located close to the outer periclinal cell boundaries seems to indicate that the labelled material originates from the epidermal cells and not from the root cap. If the labelled material originated from the root cap, one would expect that the area of greatest labelling would be in the outer portion of the mucilage, but the opposite is the case.

Mucilage label does not appear to be coming from the dense band of label in the outer periclinal wall therefore is not coming from degraded wall material (grain count data). For the glucoses, the wall has a constant grain count from the beginning of the chase to the end.

Other evidence that the walls are not a

source of the labelled material has been found. Plasmolysis of cells of sycamore culture inhibits secretion. If the secretion were from the wall, plasmolysis would not affect it. It has also been noted that metabolic inhibitors also stop secretion, but secretion from the cell walls would be passive and would not be inhibited (Brett, 1978). With increasing chase times, the amount of fucose label in the mucilage slightly increase but in the thick outer wall it decreases. If in the glucoses case at least, the label is not coming from the wall, then this means the mucilage precursors are made and secreted by the epidermal cells and this labelled material makes its way through this outer epidermal wall. It was noted in the results that the glucose-6-³H grain count data were a bit erratic (matching in most cases the photographic evidence). This cannot truly be explained, except that variations in working with living material can occur.

(iii) Recovery From Dense Band and Mucilage

Discontinuity:

In some cases, a recovery from the discontinuity in the mucilage label over cells with new anticlinal walls was observed (Figures 147 to 150 and Figure 13 of Dauwalder and Whaley, 1974). The recovery label in the mucilage always occurs close to the outer boundary (inner mucilage) which suggests that the mucilage is formed by the epidermal cells. Since, if the root cap were responsible for this label, as already mentioned, this discontinuity would be incomprehensible - it is not understood why a root cap secretion would respond to an epidermal cell phenomenon, but

secondarily during this recovery, the label would not be expected to occur first near the outer periclinal boundary of the cells but at the outer edge of the mucilage. Also in most cases, these cells are spatially well separated from the root cap.

3. Anticlinal Walls and Root Hairs:

Cell walls are generally well labelled by the radioactive sugars. Incorporation into walls is continuous as shown by supplying labelled material for a long period since it continues to accumulate (Northcote and Pickett-Heaps, 1966).

New cell walls formed in the epidermis are always labelled more heavily than nearby older cell walls. This has been previously noted (Pickett-Heaps, 1967b for wheat with ^3H glucose).

The classical interpretation of cell plate formation was that the cell plate originates in early telophase as a free-floating lamella in the middle of the equatorial plane of the mitotic apparatus and then grows peripherally until it reaches and fuses with the walls of the mother cell (Essau, 1965; Fowke and Pickett-Heaps, 1978; Whaley *et al.* 1966; Pickett-Heaps and Northcote, 1966; Frey-Wyssling *et al.* 1964). Golgi vesicles have been postulated as the precursors of the plate (Whaley and Moellenhauer, 1963).

In corn roots, the classical type of cell wall formation has been found for epidermal cells (Figures 120 to 122). However, Clarke *et al.* (1979) found for the radial walls of the epidermis that cell walls could begin to form from either periclinal mother cell wall and grow towards the other periclinal wall. All three types of anticlinal walls were demonstrated by autoradiography with

all three sugars (Figures 123 to 150). Initiation from the inner periclinal wall seems to be more common than from the outer periclinal wall for these corn cultivar roots but the reason for this is not known. Ingrowth of the cell plate from one side of the cell has been described for Marchantia species (Fowke and Pickett-Heaps, 1978; Volker, 1978). Northcote (1977) describes this type of division for sycamore cells and non-classical cell wall formation has been induced by caffeine in Vicia faba roots (Roper and Roper, 1978).

New cell walls have aniline-blue-induced fluorescence (Figure 132) and a good correlation of aniline-blue-induced fluorescence for new cell walls with autoradiographic label patterns has been made. This has not been previously observed. Aniline-blue-induced fluorescence has been used often as an indication of new cell walls (Waterkeyn, 1967; Smith and McCully, 1978a, 1978b; Fulcher et al. 1976; Sutherland and McCully, 1976; Brett, 1978; Jones and Payne, 1978) labelled.

The substance which is induced to fluorescence is called callose (Eschrich, 1956). Callose is reputed to be a β 1,3 glucan but the evidence is scanty (Aspinall and Kessler, 1954; Eschrich and Currier, 1964; Faulkner et al. 1973; Currier, 1957; Sutherland and McCully, 1976). Aspinall and Kessler (1953) have the only undisputed analysis of callose identifying it as β 1,3 glucan from the grape vine (Vitis viniferis). Recent evidence (Vithanage et al. 1980) contrary to this states that callose contains glucan compounds with both 1,4 and 1,3 linkages (Secale cereale).

PAS does not interfere with aniline-blue-induced fluorescence. This is evidence in support of the 1,3 linked nature since three substituted glucosyl residues are resistant to periodate oxidation (Smith and McCully, 1977, 1978b). Therefore since callose is theoretically a glucan (Eschrich, 1954), it seems reasonable that a correlation can be made between the labelled wall and fluorescence with aniline blue. Fucose also labels the new cell walls, although it is not necessarily a part of callose.

Root hairs were found to form on the acropetal ends of the cells of the elongation epidermal cells (Area D). This developmental pattern has been observed for several plants (Snow, 1905; Cornsack, 1935; Sinnott and Bloch, 1935; Troughton, 1962). Root hair positioning is believed to be correlated with a weakness in the cell wall (Roberts, 1916).

Root hairs were found on both the long and short cells of this region. This has been noted for roots that lack well-defined trichoblast cells (Snow, 1905).

The root hairs discovered in this work were labelled (by all three precursors) at the tips of the root hairs (Figures 151, 152, 156, 157). The root hair tips also have aniline-blue-induced fluorescence. This has been observed before (Ridgway, 1913 - many species including Zea mays; Smith and McCully, 1977 - tomato and stated that from unpublished results all root hairs examined had fluorescent tips; Howe, 1921; Currier, 1957). Fluorescence is generally limited to young root hairs and may not always be observed there. Older hairs and non-callosoed areas of young root.

In order to see if there is any statistical difference in the length changes between the three different bathing solutions the growth changes for the second segment were used and compared at times of one and two hours. For the one hour time, after the analysis of variance, the F test value is larger than the table value ($\alpha = 0.05$) which means that there is a significant difference present. Upon completion of the test, changes in growth for fucose are statistically different from those of water, but at a less significant level of significance ($\alpha = 0.025$) the F value (table) is greater therefore there is no significant difference. In the case of the two hour results no significant difference was found ($\alpha = 0.05$), so water was used as a control bath.

The fact that the root grew so much within the time period examined, which also falls into the time period of labelling, shows that for the pulse chases and in fact for the basic continuous labelling, caution must be taken about statements of amounts of label in any particular region of the root over time.

(b) Measurements of Fixed, Dehydrated and Embedded Roots (mm):

The values for the fixed, dehydrated and embedded roots are: 5, 4.5, 4, 4, 4, 4.5, 4, 4, 5, 4.5, 4, 4.5, 4. The mean is 4.3. Since the fresh length was 5mm, this represents a percentage reduction of 14% in length.

(c) Measurements of the Region of Columnar Epidermal Cells of Sectioned Roots:

The values in mm are the following: .8, .86, .88, 1.0, .6, .9, .9, .92, .6, 1.0, .92, .6, 1.0, .92, 1.1, 1.0, .8, .92, .84, 1.0, 1.02, 1.14, 1.1, 1.06,

There is also a similarity of these indentations to the so-called "wall appositions" which are formed in response to wounds caused by fungi to plant cells (Aist, 1976; Kunogh et al. 1979; Aist and Israel, 1977a, 1977b). These papillae as they are also called are often hemispherical as are the indentations in the wall for the root hair primordia and are also aniline blue positive, indicating the presence of callose (Aist, 1976) a common constituent of plant wound plugs (Currier, 1957; Currier and Strugger, 1956; Faulkner et al. 1973; Ramming and Henrichs, 1973). The size (5-15 μ m) is also similar to the indentations of the root hairs. The possibility that there was fungal infestations of the roots used in the experiments is remote, although not entirely impossible. It is interesting that a root-hair initial so closely resembles the site of a response to fungal attack.

4. Comparison of the Three Sugars:

In general the glucoses labelled in a similar fashion except that the radioactivity appeared more readily into new cell walls and primary cell wall regions with glucose-1-³H as precursor. This has been attributed to the formation of labelled uronic acids from this position of radioactivity and not with the sixth position. Primary walls of the epidermis contain a great deal of uronic acids and pentoses (Roberts and Butt, 1968; Roberts, 1967). Roberts and Butt (1969) state that cellulose synthesis is low in the meristem as indicated by low accumulation of ¹⁴C-6-glucose. They state that the maximum accumulation occurs at 3 mm basipetally from the cap junction.

For the root cap however, glucose-6-³H labels the slime and outer root cap more readily than glucose-1-³H. Both of these results are no doubt due to the fact that a larger percentage of the label from glucose-6-³H goes directly into hexoses, whereas in the case of glucose-1-³H some of the label can be diverted to uronic acids which are not such a prominent component of slime as the hexoses. Uronic acids derived from glucose-6-³H would be non-radioactive and therefore radioactivity remaining would be slime specific (Harris and Northcote, 1970; Katz *et al.* 1965; Pickett-Heaps, 1967c; Fowler and Rees, 1970). This also explains why glucose-1-³H labels the new cell walls better, due to the high concentration of uronic acids.

Fucose was incorporated differently from the two glucoses. It is interesting to note that in the outer root cap cells the accumulation of fucose is asymmetric even without a chase, whereas for the glucoses, the asymmetry is an effect of the chase procedure. Fucose does not as already noted label starch grains. Fucose is known for its specificity for glycoproteins of animal tissue and as a component of serum proteins, blood group substances and bacteria polysaccharides (Ishihara *et al.* 1964). In bacteria, fucose is biosynthesised by this pathway; GPP mannose $\longrightarrow$ GDP-4 keto-6 deoxy-D mannose $\longrightarrow$ GDP L-fucose (Jones and Morrè, 1973). Fucose can be added to a glycoprotein in animal tissue by this reaction sequence: L-fucose $\longrightarrow$ fucose-1-P $\longrightarrow$ GDP-fucose Glycoproteins. A similar pathway may be in operation for plants. A mechanism for forming GDP-fucose has been isolated in corn

roots (Kirby and Roberts, 1971). Recent work has discovered fucosyl transferases in the outer root cap cells in the dictyosomes and smooth endoplasmic reticulum (James and Jones, 1979a, 1979b). Roberts (1968) states that the direct route from fucose may function only in the presence of exogenously supplied fucose and de novo synthesis probably occurs by the bacterial method from mannose. Fucose has also been found to be (in most glycoproteins) a terminal sugar (Spiro, 1970; Bekesi and Winzler, 1976; Roberts, 1968; Ishihara et al. 1964; Ishihara and Heath, 1968; Melchers, 1971; Parkhouse and Melchers, 1971; Dorfman, 1963). Fucose has been recently shown to be terminal in cell wall formation as well (Morris and Northcote, 1977; Keegstra et al. 1973; Aspinall et al. 1969).

There are several parallels between glycoproteins and plant secretions. Recent work has been done to see if the presence of fucose in the slime is indicative of the presence of glycoproteins in the slime with some positive results (Green and Northcote, 1979; Pont Lezica et al. 1978).

Fucose is not extensively metabolised to Kreb's cycle or other glycolytic intermediates (Bekesi and Winsler, 1967) but usually is used directly as fucose (Bennet and Leblond, 1970; Haddad et al. 1971). It has recently been implicated in the receptors of toxins and hormones and is reputed to protect glycoproteins against proteolytic attack, to regulate the lifetime of the glycoproteins and to mask certain cellular antigens (Bishayee and Dorai, 1980).

5. CONCLUSIONS

The outer cell layers (outer root cap cells and the epidermis) have been shown to be different in many aspects from the other cell types of the root not the least of which is the high accumulation of radioactive material.

The epidermal mucilage has been shown to originate from the epidermis and not the root cap by (a) adding to the known differences between the slime and the mucilage and (b) by pulse chase and grain counting on the mucilage. The following additional proofs were provided by this work - the location of the label in the mucilage is close to the outer boundary of the cells for both continuous and pulse chase experiments and also from the recovery from a discontinuity of dense band in label in the outer periclinal wall and the mucilage. Further observations into the mechanisms of the mucilage information would be advantageous to a more complete understanding of the mucilage's importance.

The different forms of new cell wall (anticlinal) formation (cytokinesis) was clearly elucidated by autoradiographic techniques.

Further experimentation should include an examination of the various developmental stages of epidermis, further elucidation of the difference between the slime and the mucilage, and an examination of the new cell plates and root hair primordia by electron microscopy. An additional experiment that should be performed is to remove the

mucilage maintaining the integrity of the underlying epidermis and examining the return of the mucilage by microscopy and autoradiography.

APPENDIX I: Explanation of the (N) Designation in the Chemical

Names of the Radioactive Precursors:

This designation is one given by the company (New England Nuclear) and is used when the method of preparation requires (usually a significant amount of) the label to be in a specific site or sites but no further information is available as to the extent of labelling (if any) at other positions.

^3H compounds designated as nominal (N) are generally prepared by a reduction process during which ^3H may exchange in positions other than those labelled by the reduction. Thus 7- Bromocholesterol reduced to a ^3H labelled cholesterol has been demonstrated to contain 86% of the label at position 7 and the remainder distributed over position 3, 4 and 6. It is however designated as 7- ^3H labelled cholesterol. If it is found that greater than 95% of the ^3H is at any specific site, the N designation is dropped (as in the case of the fucose label used), and it is considered specifically labelled, but for the glucoses the label is not specifically confined to the designated positions in amounts above 95%.

APPENDIX II: Root Growth Experiment and Other Related Experiments:

During the course of experimentation it became obvious that in order to understand the autoradiographic patterns, the rate of growth of the corn roots would have to be determined.

Specimens used were grown under similar conditions as experimental autoradiographic specimens. The roots were then marked with thin lines made by a blue Sharpie pen every 2mm from the tip of the solid root cap (that is, excluding the diffuse mucilaginous portion) up the root for approximately 2 to 3 cm as shown in Diagram 5.

Diagram 5:

The roots were then placed in the same chamber as was used in the labelling experiments with similar arrangements for maintaining a humid atmosphere and the use of the wells for holding the liquid in which the tips were bathed. Three different bathing solutions were used: (a) glucose at a concentration similar to the radioactive glucoses (an average of the two therefore using .001g/100ml), (b) fucose at the same concentration as the autoradiographic experiments (.00126g/100 ml) and (c) distilled water.

Measurements (mm) were made of the marked segments at various time intervals.

Other measurements that were necessary for the understanding of the root growth experiment were also performed: (a) measurement of the length of the columnar zone using an ocular micrometer that was calibrated with a stage micrometer, (b) measurements of encapsulated roots cut to a known length of 5 mm before fixation were measured after processing and embedding in order to calculate a shrinkage factor.

(a) Root Growth Data:

During the times over which the roots were observed the roots elongate, especially the first and second segments (originally 2mm in length each - see Diagram 5). The results are recorded in Tables 14 to 16 and in Figures 162 to 164.

The change in length per segment in mm was examined for the three different bathing solutions. In order to determine whether the changes in length are statistically significant a multiple range test was performed (Newman-Keuls test, Zar 1974). This test involves firstly an analysis of variance and then a multiple range test. An example of this test is given in Appendix VIII.

In general the acropetal 3 to 4 segments showed measurable changes and these were statistically tested. The results show that the root does grow within the time periods of labelling used and that the growth of the first two segments and especially segment 2 is such that the difference in length is statistically different from changes of length of the rest of the segments.

119.

Root Growth Data:

Figures 162-164:

Note that for all three treatments that the second segment in particular grows a great deal but so does the first. The root in the middle in each case is the control root (that is it was marked just before taking the pictures).

Figure 162: Bathed in fucose.

Figure 163: Bathed in glucose.

Figure 164: Bathed in water.

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Table 14: Length Changes (mm per segment) of Roots Grown in Water.

Root Area *	Time in Minutes														
	30			60			120			180			240		
	$\bar{x}$	N	SE	$\bar{x}$	N	SE	$\bar{x}$	N	SE	$\bar{x}$	N	SE	$\bar{x}$	N	SE
1	^a .13	30	.04	^a .18	51	.04	^a .33	51	.05	^a .36	16	.10	^a .36	16	.12
2	^a .36	30	.07	^a .42	51	.06	^b .68	51	.06	^b .78	16	.11	^b .94	16	.12
3	^b .05	30	.03	^b .09	51	.03	^c .17	51	.04	^c .11	16	.07	^c .11	16	.07
4	0	30	0	0	51	0	0	51	0	^c .03	16	.04	^c .06	16	.04
5-10	0	30	0	0	51	0	0	51	0	0	16	0	0	16	0

* See Diagram 5.

 $\bar{x}$ Mean

N, Number of Samples

SE Standard Error

Means with different letters are statistically different.

Table 15: Length Changes (mm per segment) of Roots Grown in
Glucose.

Root Area *	Time in Minutes					
	60			90		
	$\bar{x}$	N	SE	$\bar{x}$	N	SE
1	^a .425	20	.09	^a .59	20	.08
2	^a .48	20	.07	^a .78	20	.08
3	^{ba} .25	20	.08	^b .345	20	.08
4	^b .075	20	.05	^c .1	20	.06
5-9	0	20	0	0	20	0

* Refer to Diagram 5.

$\bar{x}$ Mean

N Number of Samples

SE Standard Error

Means with different letters are statistically different.

Table 16: Length Changes (mm per segment) of Roots Grown in
Fucose.

Root Area *	Time in Minutes					
	60			120		
	$\bar{x}$	N	SE	$\bar{x}$	N	SE
1	^a .205	20	.06	^a .395	20	.10
2	^b .67	20	.07	^b .89	20	.05
3	^a .325	20	.08	^c .7	20	.24
4	^a .225	20	.20	^d .08	20	.05
5-11	0	20	0	0	20	0

* Refer to Diagram 5.

$\bar{x}$ Mean

N Number of Samples

SE Standard Error

Means with different letters are statistically different.

In order to see if there is any statistical difference in the length changes between the three different bathing solutions the growth changes for the second segment were used and compared at times of one and two hours. For the one hour time, after the analysis of variance, the F test value is larger than the table value ($\alpha = 0.05$) which means that there is a significant difference present. Upon completion of the test, changes in growth for fucose are statistically different from those of water, but at a less significant level of significance ($\alpha = 0.025$) the F value (table) is greater therefore there is no significant difference. In the case of the two hour results no significant difference was found ($\alpha = 0.05$), so water was used as a control bath.

The fact that the root grew so much within the time period examined, which also falls into the time period of labelling, shows that for the pulse chases and in fact for the basic continuous labelling, caution must be taken about statements of amounts of label in any particular region of the root over time.

(b) Measurements of Fixed, Dehydrated and Embedded Roots (mm):

The values for the fixed, dehydrated and embedded roots are: 5, 4.5, 4, 4, 4, 4.5, 4, 4, 5, 4.5, 4, 4.5, 4. The mean is 4.3. Since the fresh length was 5mm, this represents a percentage reduction of 14% in length.

(c) Measurements of the Region of Columnar Epidermal Cells of Sectioned Roots:

The values in mm are the following: .8, .86, .88, 1.0, .6, .9, .9, .92, .6, 1.0, .92, .6, 1.0, .92, 1.1, 1.0, .8, .92, .84, 1.0, 1.02, 1.14, 1.1, 1.06,

.92, 1.12, 1.1, .7. The mean is .928mm. With the 14% correction to compensate for shrinkage during processing (Section b above) this length would be 1.06mm or approximately 1.0mm.

(d) Calculations Based on Root Growth Experiment to Determine How to Perform Grain Counting for the Pulse Chase Data:

The root cap is approximately 1mm in length and it maintains a constant length of 1mm for the time period concerned (Table 17). Therefore the remaining 1mm of the first segment is responsible for the growth in this region, thus since the area designated B of columnar epidermal regions is approximately 1mm in length, segment 1 of the root growth experiment contains the root cap and the columnar epidermal regions.

After 30 minutes, region 1 has grown .13mm. That would displace a cell at the very base of Area B into the lower one quarter of the same area, similarly a cell in the middle of Area B would be displaced one eighth of the distance up from the middle and one near the top would be displaced into the transition zone (Area C). Since the counting was done at the middle and bottom of the zone, no correction is necessary for this time.

After one hour, segment 1 has grown on an average .23mm. This would take a cell at the base of the zone one quarter of the way up and one in the middle three quarters of the way up. Again the problem is not severe and no correction is performed.

After two hours the root segment 1 has grown .45mm, which means that a possible correction may be needed. A cell at the bottom of the zone would be displaced one half the way up and one in the middle

Table 17: Lengths for Root Segments 1, 2 and 3 Bathed in Water,
Glucose and Fucose (Change in Length in mm).

	Water			Glucose			Fucose			Average		
	1	2	3	1	2	3	1	2	3	1	2	3
30 min.	.13	.36	.05	-	-	-	-	-	-	.13	.36	.05
60 min.	.18	.42	.09	.3	.48	.25	.205	.67	.33	.23	.52	.22
2 hours	.37	.68	.19	.59	.73	.35	.395	.89	.70	.45	.77	.41
3 hours	.36	.78	.11	-	-	-	-	-	-	.36	.78	.11
4 hours	.36	.94	.11	-	-	-	-	-	-	.36	.94	.11

would be displaced to the top of the zone. It would therefore be best if some compensation was made, so in order to cover this both the columnar and the transition zones were counted for the 90 minute chase data, but the transition zone would probably be more statistically sound.

If such corrections are not undertaken, then the entire body of the research is in doubt. Much of the older research was done without taking into account the fact that the researcher was dealing with a dynamic system and yet samples were taken over time in a static position when in reality the cells that were properly labelled are somewhere else in the tissue. With this problem in mind, the data in this case was corrected at the later chase times.

Recent work has shown that the old studies of cell cycles on growing root systems were lacking in clarity of approach, were too simplistic and made too many assumptions (Clowes, 1971; Lopez-Saez *et al.* 1975 for example). Other work has taken into account the dynamic properties of the root and many have made attempts to understand it (Goodwin and Avers, 1956; Goodwin and Stepka, 1945; Brumfield, 1942). Green (1976) and Gander (1980) point out all the previous errors and offer new methods of analysis. The most basic error was that most authors totally separate cell division from cell elongation as two processes which never occur together (Steeves and Sussex, 1972 for example). Green changes the names of these to "partitioning" and "surface extension" respectively and indicated that growth is not accomplished first by a zone of division followed by a zone of elongation but by mutually balancing combinations of the two

(Green, 1976, Figure 1). This of course is recognized in the corn root in the columnar region of the epidermis where a cell must elongate before it divides. He points out the problem with measuring rates of growth by studying the behaviour of pairs of points on a surface. He states that if the marks are widely spaced and the time is long, they may span changing rates and that the entire area could be displaced basipetally up the roots.

In autoradiography, especially for the roots being considered (the first of the areas in the growth experiment) the upper mark is displaced upward causing the length of the interval to change. Therefore, it is important that this be taken into account to insure that the same group of cells which was initially labelled are counted for each of the chase times.

APPENDIX III: Verification of the Purity of the Radioactive Stock:

In order to be certain that the precursors presented to the experimental organisms were the expected compound and were pure; each shipment of radioactive stock was checked using the following procedure when received and prior to each major experiment.

Thin-layer chromatography was chosen as the method to verify the purity of the radioactive stocks.

A sugar mixture containing 5 mg each of glucose, galactose, xylose, mannose, rhamnose and arabinose in 1 ml of distilled water was used to test activation procedures (methanol prewash, heating, heating plus methanol prewash); solvent systems and application volumes for the best possible separation, spot size and shape. This mixture was later used on each chromatogram as a check of the solvent system efficiency.

The application volume which gave the best results was 2.5 μ l. The best activation procedure/solvent system combination was a prewash in methanol prior to spotting and a solvent system of pyridine/ethyl acetate/water = 1/2/2.

Procedure:

(i) Materials:

Eastman Chromatography sheets, Number 13179 Silica Gel
(without fluorescent indicator) size 20 x 20 cm

Kodak no-Screen NS-5T X-ray film

pyridine

ethyl acetate

(ii) Spotting of the Chromatogram:

Volumetric capillary tubes were used to spot the chromatogram on the origin which was ruled with a pencil at 2 cm from the bottom of the sheet. The volume spotted for the mixture and the pure cold sugar standards of fucose and glucose (5mg/ml) was 2.5 μ l whereas for the radioactive precursors (fucose-6- 3 H, glucose-6- 3 H and glucose-1- 3 H) 1 μ l was used. The spots were thoroughly dried prior to immersion in the chromatography tank.

(iii) Solvent Preparation and Running of the Chromatogram:

The solvent was prepared just prior to use. 40 ml of pyridine, 80 ml of ethyl acetate and 80 ml of distilled water were mixed in a separatory funnel in a fumehood. The funnel was shaken and the layers allowed to separate. After separation, the lower layer was discarded and the upper layer (approximately 100 ml) was saturated with 1 ml of pyridine and 2 ml of ethyl acetate. This mixture was then poured into the chromatography tank. The chromatogram was leaned against a glass plate in the chromatography tank, the cover was seated and weighted. The run time was approximately four to eight hours.

(iv) Exposure and Development:

After the chromatogram was run and dried, a portion of it was removed and sprayed with benzidine reagent and developed at 100°C for three to four minutes in order to check separation. The benzidine reagent is prepared as follows: 0.5 g of benzidine is dissolved first in a mixture of glacial acetic acid/TCA (10 ml of glacial acetic acid + 10 ml of 40% tricarboxylic acid) before the addition of absolute

ethanol (80 ml). This reagent colours hexose sugars brown and pentose sugars pink/purple. If the separation was satisfactory, the remainder of the chromatogram was stapled in the dark to a piece of X-ray film and placed in a light tight envelope in a drawer at 20°C for three to four weeks.

After the exposure time, the X-ray film was developed as follows: (a) removed from the chromatogram in total darkness, (b) Kodak D-11 developer for four to five minutes (20°C)(in dark), (c) water rinse for 30 seconds, (d) Kodak fixer for 10 to 20 minutes (in dark), (e) water rinse, (f) photoflo and (g) air dry.

The chromatogram was then sprayed by means of a spray gun in a fume hood with the benzidine reagent prepared as above and developed at 100°C for three to four minutes.

(v) Results:

The X-ray (Figure 165) shows small distinct spots which indicates that the compounds have not disintegrated into other compounds. From Table 18 the migration distances (Rf values) of all sugars are listed. A sample chromatogram and X-ray were photographed using a Minolta SRT201 equipped with a Vivitar MacroZoom lens on a light box (Figures 165 to 167). For the superimposed picture, the alignment was done using the staple holes.

The various chromatograms and X-ray pairs showed all the stocks to be pure at all times. The purity of the stocks is of great importance and without such a verification, experimentation could be in doubt.

Table 18: Rf Values for the Various Sugars on the Chromatogram
for the Verification of Purity.

Category	cm 1	Rf	cm 2	Rf	cm 3	Rf	Rf Average
Mix 5 (galactose)	3.5	.201	4.0	.280	3.3	.204	.23
Mix 4 (glucose)	4.45	.256	4.9	.243	4.45	.276	.26
Mix 3 (mannose)	5.32	.306	5.8	.406	5.7	.353	.36
Mix 2 (xylose & arabinose)	7.1	.408	7.5	.524	7.5	.464	.46
Mix 1 (rhamnose)	10.1	.580	10.4	.727	10.1	.625	.64
Glucose (alone)	4.6	.264	5.15	.360	5.75	.356	.33
Fucose (alone)	7.4	.425	8.0	.560	9.0	.557	.51
Glucose-1- ³ H	4.7	.270	5.4	.378	-	-	.32
Glucose-6- ³ H	4.7	.270	5.2	.364	-	-	.32
Fucose-6- ³ H	7.4	.425	8.0	.560	-	-	.49
Solvent Line	17.4	-	14.3	-	16.15	-	-

Figure 165: Verification of the Purity of Stock
Radioactive Chemicals.

The x-ray that was placed against the chromatogram after it was run. Note that the three dots are circular with no tailing.

165

Fuc-
6-³H

Glu- Glu-
1-³H 6-³H

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Figure 166: Verification of the Purity of the Stock
Radioactive Precursors.

The chromatogram after it has been developed.
The mix (which was used as a test of the way the
chromatogram ran) has the following components:
1. rhamnose, 2. xylose, 3. arabinose, 4. glucose and
5. galactose.

166

6H Mix G-1H G-6H Glu Fuc

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Figure 167: Verification of the Purity of the Stock
Radioactive Precursors.

The chromatogram and x-ray superimposed showing
that the Rf values of the radioactive sugars and cold
samples are the same.

167

R-6H

Mix

Fuc

Mix

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APPENDIX IV: Check of Dipping Technique:

The purpose of this experiment was to determine what type of irregularities, if any, may have occurred in emulsion thickness during the dipping procedure.

Outdated emulsion was removed from the dark and exposed to light. Five different types of dipping tests were made as follows: (1) undiluted emulsion (exposed). Since, however, the emulsion is normally used as a 1:1 dilution with water, it was decided that a combination of $\frac{1}{2}$ emulsion and $\frac{1}{2}$ something else would be better. So the following were tried: (2) emulsion:water:drawing ink = 2:1:1. This was found to be a little dark in colour, but was still usable. Another mixture was made with less ink (3) emulsion:water = 1:1. A few drops of ink were added to the water before it was mixed with the emulsion. Two other facets of the problem were also examined: (4) slides with sections in them were dipped in emulsion type mixture number 3 and (5) slides with PAS stained sections on them were dipped in emulsion mixture number 5.

The slides were dipped and dried as the experimental slides were and as described in Section 2:6:2.

Figures 172 a, b and c show three different sets of dippings. They show that there is a minimum amount of irregularities with slides without section. With the addition of sections there is an increase in the irregularities, usually due to drainage problems after dipping. The irregularities are not, however, significant enough to anticipate any problems with the results that may be related to emulsion thickness

irregularities or to the general dipping techniques. The idea of using ink was gleaned from Sanchez-Ramos et al. (1978) who used it to test to see if ganglia in their preparations were coated by emulsion. By this technique they found no carbon particles over the ganglia and therefore no emulsion was present, an important thing to discover and to avoid as an artifact.

APPENDIX V: Removal of Emulsion Attempts and Post-Autoradiographic
Aniline Blue Staining Attempts:

When it was realized that aniline blue would be used as a stain to attempt to correlate the new cell wall fluorescence with the autoradiographic labelling of new cell walls attempts were made to stain with aniline blue after autoradiography.

Most of the slides which were of interest were also mounted in oil. The oil was solubilized with xylene. The slide was dried with a hair dryer, and then rinsed in running tap water for 10 to 20 minutes. Aniline blue was then applied. No fluorescence was found and therefore a further attempt was made on the same slide by soaking it overnight in aniline blue with another negative result. Next, it was assumed that perhaps the emulsion was the problem and attempts were made to remove the emulsion. There was ~~some~~ reason for the stain not to penetrate through the tissue. The major constituent of the emulsion is gelatin (Rogers, 1967). Gelatin is soluble in hot water and acetic acid (Merck, 1968). A slide was heated from 60 to 100°C and checked at 10°C intervals with no positive results. Boiling the slide also had negative results. The same result occurred with 1% acetic acid with the same method of heating and testing. An old section which had been processed through autoradiography but not mounted in oil was stained with aniline blue and there was fluorescence in the pits in a few minutes. Therefore the emulsion itself is not the cause. However, there may be a reaction between the emulsion and the oil that occurs with time that prevents the stain from penetrating. A slide which

had been mounted in oil for a long time (Toluidine Blue O stained) was deoiled and then stained and a positive result was found. Therefore the oil itself is not the cause.

Still in an attempt to remove the emulsion, Froland's solution of KI and I_2 in water incubated for one hour, rinsed for 30 minutes in 30% sodium thiosulphate (pentahydrated) and rinsed in water was tried next. It removed the silver grains and allowed some aniline blue in, but not in the proper places. Also slides were soaked in hyponeutralizer undiluted for an hour to try to remove the emulsion with a negative staining reaction.

An alkaline digestion method of Revel and Hay (1961) for the removal of emulsion was tried. It involved soaking of the slide for 20 minutes in 0.05 N sodium hydroxide. This worked to a certain degree.

It is probably a result of some interaction of the stain used (PAS) and the developing which stops aniline blue from staining through the emulsion.

It was later found that prestaining with aniline blue before PAS reaction and dipping gave the best results.

APPENDIX VI: Mucilage Removal Attempts:

At one point in this research, when ideas were being considered to prove by any conceivable means that the epidermal cells were the originators of the mucilage; attempts were made to remove the mucilage. This was done with the idea in mind of watching the mucilage regenerate and determining a time chart of regeneration. With this knowledge, a time of radioactive labelling would have been chosen in order to just label the cells before regeneration of the mucilage and to watch the mucilage gradually regenerate and accumulate label as time went on.

The procedure to remove the mucilage therefore had two prerequisites: (1) to remove the mucilage, but not the epidermal cells and (2) to leave the root in a viable condition.

After each test was made transverse hand sections were made of the roots with an Ingram Blue Blade razor blade prewashed in 1:1 methanol acetone to remove oil from the blade. Tests were made of the best way to monitor the presence of the mucilage - PAS reaction, toluidine blue, alcian blue and autofluorescence. It was discovered that autofluorescence was the best and was therefore used routinely.

No positive results were obtained. The following are the various methods which were tried:

(a) Scraping - with a thin Ingram blade. This was not very effective and often the epidermis was damaged so this was not considered a proper method.

(b) Celite - dry rubbing inside a Kimwipe. Celite is a dry powder, an abrasive often used in virus studies to abrade a leaf to allow the

entrance of the virus being introduced to the plant. It did not however remove the mucilage.

(c) Sonification:

(i) in a beaker containing approximately 100 ml of water. The roots were submerged totally in the water. Times of 5, 10, 11, 15, 30, 60 and 90 minutes were used, all without conclusive results. Some of this is documented in Figure 173 a to f. After sonification the roots were always translucent for half the root length regardless of their length and with longer times became difficult to hand section.

If the roots which had been sonicated were placed in a Petri dish in the dark for approximately 12 to 18 hours, the translucence had disappeared but the roots had a little crook near the tip.

(ii) in .2mM CaCl_2 (100 ml) for 5, 10 and 15 minutes (Barber and Gunn, 1974).

(iii) with sulphuric acid 5% + 5 minutes sonification 7.5% + 5 and 10 minutes of sonification. There may have been some reduction, but the treatment makes the tissue very soft so this may be undesirable from the viability context.

(iv) in celite - 5.0 g/150 ml water for 15, 30 and 60 minutes.

(d) from Brams (1969):

(i) 1 minute in 1 N NH_4Cl_2 .

(ii) 1 minute in 1 N NH_4Cl_2 followed by 30 minutes in 1N HCl.

(e) Detergents:

(i) Liqinox - 10 ml of concentrated soap + 90 ml of water and sonified for 15 and 30 minutes.

(ii) Extran 300 - 100% - turned the root a bright green even after a quick dip and very soft after 5 minutes. 75% also produces the green colouration and 30% for 5 minutes also still leaves the mucilage intact.

(f) after Paull and Jones (1975) - 2 M KOH was used for fractionation and general extraction but it turned the roots very green upon dipping.

(g) Macerase enzyme was used at 3% at room temperature in water at a pH of approximately 5.8 for times of 5 minutes, 5 minutes + 10 minutes sonification and 15, 60, 90, 120 and 240 minutes + 15 minutes of sonification. Some of this is documented in Figure 173 g to j. Macerase has been often used to produce protoplasts and for general maceration of plant tissue (Bateman, 1968; Jensen et al. 1971; Kanai and Edwards, 1973; Cataldo and Berlyn, 1974; Edwards and Black, 1971; Krause et al. 1978; Mbaki et al. 1978; McClendon and Somers, 1960; Otsuki and Takebe, 1969; Rehfeld and Jensen, 1973; Sato, 1968; Shepard and Totten, 1975; Walker and Izawa, 1979; Takebe et al. 1968).

It appears that there needs to be new ideas to attempt this project. Perhaps the use of a cellulase in conjunction with the macerase could help since there are suggestions that the outer layer of mucilage may contain cellulose. Also perhaps macerase could be used together with an esterase since the existence of phenolic compounds has been discovered in the mucilage (Smith and O'Brien, 1979).

Figures 168-169: PAS Control:

Photomicrographs of similar areas of the root cap. Figure 168 stained with PAS before autoradiography and Figure 169 unstained. There is little difference in label amounts or patterns. (Glucose-1-³H - 60 minutes.) x230.

Figures 170-171: PAS Control:

Similar regions of the epidermis. Figure 170 stained with PAS prior to autoradiography and Figure 171 unstained. There is no noticeable difference in labelling. x230.

Figures 172 a-c: Dipping Control:

Three duplicate dipping control experiments, with the following categories from left to right: undiluted exposed emulsion on a clean glass slide, emulsion: india ink = 1:1 on a clean glass slide; emulsion: water = 1:1 with a few drops of india ink on a clean glass slide; the 1:1 emulsion: water-ink mixture on a slide with sections on it and finally, the same mixture on a slide with PAS stained sections on it. There are some irregularities in emulsion thickness, but they are usually not serious enough to cause any problems with interpretation of the results.

Figures 173 a-j: Mucilage Removal Attempts:

The magnification is 90x and all pictures were taken of handsections using autofluorescence.

- (a)(b) controls.
- (c)(d) sonification for 5 minutes in water.
- (e) sonification for 10 minutes in water.
- (f) sonification for 15 minutes in water.
- (g) macerase for 30 minutes.
- (h)(i)(j) macerase for 60 minutes.

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APPENDIX VII: Raw Data for Grain Counting.

See Tables 11 to 13 and refer to Diagram 4 in the Materials and Methods for positions of counting.

Table 19: Raw Data for Glucose-1-³H Counts:

	Wall	Inner Mucilage	Outer Mucilage
60	30,24,33,30,36, 25,27,18,18,20, 14,15,12,14,14, 22,20,26,20,12.	8, 9, 7, 7,14, 7, 6,10,14,14, 5, 5, 8,10,14, 0, 6, 6, 6,14.	3, 5, 1, 0, 0, 2, 0, 1, 0, 0, 5, 4, 4, 1, 3, 0, 2, 2, 2, 4.
60+15	17, 7,14,13,13, 7, 7, 8,17,14, 4,15, 8, 8,12, 6,13, 8,12,15, 12,11,16, 4,20.	20,16,25,16,25, 15,19,23,17,27, 0, 8, 9,12,16, 19, 8, 5,25,15, 12,19,36,15,20.	8, 8, 6, 5,22, 17,14,10, 4,12, 2, 6, 8, 6, 0, 5, 0, 2, 3,12, 3, 5, 2, 5,10.
60+30	25,13,25,20,13, 16,26,10,24,14, 20,20,20,15,24, 25,40,30,30.	39,45,27,28,34, 20,22,28,24,12, 30,33,36,44,36, 45,27,55,30.	8,12,10, 4,13, 10, 8, 8, 0,18, 15,13,20,25,26, 12, 8,25,20.
60+60	18,30,18,25,24, 20,18,24,17,15, 14,26,24,24,12, 25,40,30,30.	39,45,27,28,34, 48,53,25,50,40, 22,24,56,38,15, 48,25,23,48.	23, 0,20,11,10, 24, 4,14, 6,11, 0, 8,14, 6,11, 27, 8, 0,14.
60+90	24,20,25,20,14, 19,28,20,20,20, 30,40,24,26,24, 30,30,30,30,30, 30,32,26,40.	12,20, 3,24,24, 10,50,28,14,12, 7,34,20,27,32, 26,28,20,19,14, 18,12,18.	8, 7, 1,12,14, 3,14, 9, 9,15, 3, 3, 0,11,10, 0, 8, 4, 8,12, 12,10, 2.
60+90 Col.	10,12,20,25,14, 15,12, 8,18,22, 25,21,25,28,28, 20,35,20,27,25, 11,17,17,20.	11,22,24, 6, 9, 19,13,26,27,22, 47,50,42,32,47, 55,27,37,40,22, 28,28,30,50.	0, 5,14, 3, 3, 7, 3,12,18,11, 8,10,20,10, 7, 5, 6, 9, 8, 9, 7, 6, 9, 5.

Trans. = counted in the Transition Zone

Col. = counted in the Columnar Zone

Table 20: Raw Data for Glucose-6-³H Counts:

	Wall	Inner Mucilage	Outer Mucilage
60	5, 6, 40, 40, 50, 50, 10, 12, 8, 30, 50, 20, 50, 40, 13, 23, 16, 6, 12, 40, 30, 15, 18, 18, 40, 18, 18, 21, 15.	4, 2, 6, 3, 18, 6, 3, 3, 5, 11, 2, 6, 0, 12, 32, 20, 16, 7, 8, 16, 17, 19, 0, 4, 13, 15, 18, 16, 10, 4.	10, 1, 8, 12, 12, 2, 1, 5, 2, 16, 12, 0, 14, 24, 6, 14, 9, 20, 6, 10, 3, 6, 4, 2, 2, 6, 8, 8, 0.
60+10	24, 29, 50, 60, 30, 18, 16, 18, 18, 14, 36, 42, 22, 28, 30, 11, 20, 23, 30, 12, 16, 20, 15, 25.	20, 29, 43, 52, 43, 34, 16, 32, 21, 33, 62, 52, 30, 32, 38, 14, 27, 11, 18, 10, 15, 18, 8, 27.	24, 2, 10, 23, 18, 12, 14, 12, 13, 14, 10, 42, 16, 18, 2, 6, 15, 0, 6, 4, 2, 2, 2, 6.
60+15	18, 26, 22, 30, 32, 28, 19, 20, 25, 21, 28, 26, 32, 34, 42, 11, 21, 15, 12, 16, 15, 20, 20, 20, 20.	37, 38, 39, 20, 20, 18, 36, 21, 24, 18, 10, 40, 24, 28, 60, 12, 14, 13, 10, 7, 7, 10, 4, 8, 12.	17, 19, 15, 17, 10, 12, 15, 20, 7, 14, 4, 22, 38, 4, 30, 7, 0, 6, 1, 0, 10, 4, 3, 6, 5.
60+30	9, 7, 8, 9, 12, 4, 8, 3, 7, 10, 14, 12, 20, 18, 18, 13, 12, 12, 16, 9, 18, 18, 18, 12, 10.	27, 14, 9, 4, 11, 6, 10, 9, 8, 10, 15, 16, 22, 10, 20, 12, 13, 14, 14, 11, 13, 8, 12, 9, 10.	1, 5, 4, 5, 1, 3, 2, 0, 0, 3, 8, 1, 21, 2, 8, 6, 3, 7, 5, 5, 12, 10, 1, 3, 9.
60+60	25, 50, 30, 13, 20, 14, 24, 12, 11, 10, 8, 6.	21, 20, 50, 3, 14, 13, 10, 12, 13, 6, 7, 2.	48, 42, 2, 0, 0, 2, 2, 4, 0, 0, 3.
60+90 Trans.	30, 25, 40, 50, 50, 50, 30, 30, 30, 45, 45, 45, 45, 45, 3, 24, 24, 24.	20, 15, 29, 6, 11, 21, 29, 0, 30, 29, 8, 5, 17, 0, 22, 17, 12, 12.	3, 1, 7, 2, 3, 21, 12, 8, 23, 35, 11, 7, 15, 0, 15, 2, 3, 7.
60+90 Col.	17, 14, 14, 19, 18, 20, 20, 30, 30, 19, 15, 15, 25, 13, 18, 35, 15, 20, 9, 5, 5, 0, 3, 30, 20, 20.	6, 9, 15, 19, 15, 6, 1, 14, 16, 15, 40, 2, 3, 0, 2, 18, 33, 28, 29, 12, 50, 10, 18, 19.	2, 4, 10, 11, 7, 6, 4, 8, 9, 3, 0, 0, 0, 0, 3, 10, 20, 12, 18, 10, 35, 22, 14.

Trans. = counted over the Transition Zone Epidermis

Col. = counted over the Columnar Zone Epidermis

Table 21: Raw Data for Fucose-6-³H Counts:

	Wall	Inner Mucilage	Outer Mucilage
60	6, 8, 10, 16, 12, 10, 11, 5, 6, 12, 17, 16, 20, 16, 18.	13, 14, 12, 8, 26, 5, 5, 5, 6, 10, 2, 15, 15, 7, 16.	8, 5, 10, 4, 10, 4, 7, 6, 0, 10, 0, 2, 0, 0, 10.
60+15	15, 19, 15, 7, 10, 17, 13, 12, 12, 16.	4, 5, 6, 18, 16, 8, 18, 12, 10, 10.	2, 3, 4, 1, 2, 7, 3, 3, 3, 1.
60+30	18, 14, 20, 16, 14, 14, 15, 12, 14, 10, 8, 15, 12, 6, 10, 11, 11, 11, 10, 14, 12, 10, 15, 7, 11, 3, 10, 9, 15, 10.	12, 12, 22, 14, 24, 4, 5, 1, 10, 14, 11, 7, 6, 4, 14, 22, 16, 20, 10, 15, 14, 8, 16, 25, 13, 8, 6, 9, 12, 13.	1, 4, 5, 3, 4, 5, 4, 0, 2, 1, 1, 3, 0, 3, 1, 10, 0, 3, 2, 6, 4, 3, 6, 3, 6, 0, 8, 1, 1, 2.
60+60	13, 20, 12, 20, 20, 6, 12, 14, 10, 8, 8, 8, 5, 8, 10, 6, 5, 9, 10, 12.	23, 24, 30, 20, 22, 6, 2, 9, 4, 14, 13, 4, 4, 0, 10, 9, 3, 5, 5, 9.	9, 5, 10, 6, 12, 1, 0, 0, 5, 0, 1, 1, 0, 0, 0, 0, 0, 2, 1, 4.
60+90 Trans.	14, 9, 9, 12, 12, 5, 14, 4, 9, 11, 10, 8, 8, 9, 10, 6, 9, 9, 9, 12, 7, 10, 8, 8, 7, 15, 12, 12, 8, 8.	27, 18, 32, 22, 22, 3, 11, 8, 18, 8, 24, 11, 18, 19, 15, 8, 8, 20, 12, 17, 12, 6, 18, 9, 14, 18, 18, 20, 16, 12.	0, 0, 5, 3, 4, 1, 3, 2, 0, 3, 5, 3, 7, 6, 4, 0, 1, 0, 4, 0, 6, 0, 0, 0, 5, 4, 3, 8, 1, 4.
60+90 Col.	14, 12, 9, 9, 11, 13, 11, 10, 13, 11, 12, 6, 8, 12, 12, 10, 6, 5, 10, 9, 6, 5, 6, 8, 2, 10, 8, 1, 12, 14.	6, 3, 3, 6, 3, 27, 36, 36, 0, 0, 0, 12, 27, 66, 42, 24, 12, 13, 21, 33, 0, 0, 0, 6, 0, 60, 51, 15, 36, 33.	0, 0, 0, 0, 0, 6, 3, 3, 15, 18, 0, 0, 9, 3, 0, 0, 0, 0, 0, 9, 0, 0, 0, 0, 12, 9, 6, 15, 15.

Trans. = counted over the Transition Zone Epidermis

Col. = counted over the Columnar Zone Epidermis

APPENDIX VIII: Statistical Tests for Grain Count Data and Root Growth Experiment (Newman-Keuls Multiple Range Test):

The same statistical test was done for the root growth data and the grain count data. The test first involves a single factor analysis of variance to test the null hypothesis $H_0: \mu_1 = \mu_2 = \dots = \mu_l$ where l is the number of samples. The assumptions are that the samples are normally distributed, but flexible enough to accommodate considerable differences in variances and deviations from normality. After the null hypothesis is tested and if it is rejected, this means that there is a difference between the samples, but does not necessarily mean that all of the samples are different from one another. If the hypothesis is accepted, this means that no statistically significant difference exists between the samples. In the case of the rejection of the hypothesis, the Newman-Keuls Multiple Range Test is then applied. The sample sizes do not have to be the same since the test contains provisions for different sample sizes. Examples of the test are given on the following pages.

An example of the Grain Count Data Test:

Inner layer of the mucilage for G-1-³H

$$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$$

H_A : that the grain counts in this layer at different chase times are not all equal.

$$\alpha = 0.05$$

	K				
	60	60+15	60+30	60+60	60+90
n_i	20	25	19	19	23 N=106
$\sum_{j=1}^{n_i} x_{ij}$	170	422	615	730	1368
$\bar{x}_i$	8.5	16.9	32.4	38.4	59.5
$\left(\sum_{j=1}^{n_i} x_{ij} \right)^2$	1445	7123.36	19906.6	28047.4	81366.26
$\sum n_i$					

$$\sum_{i=1}^k \frac{\left(\sum_{j=1}^{n_i} x_{ij} \right)^2}{n_i} = 137898.57$$

$$\sum_{i,j} x_{ij} = 3305$$

$$\text{Total degrees of freedom} = N-1=106-1=105$$

i, j

$$\text{Group degrees of freedom} = K-1=5-1=4$$

$$\sum_{i,j} x_{ij}^2 = 167961$$

$$\text{Error degrees of freedom} = N-K=106-5=101$$

$$C = \frac{\left(\sum_{i,j} x_{ij} \right)^2}{N} = \frac{(3305)^2}{106} = 103047.41$$

$$\text{Total sum of squares} = \sum_{i,j} x_{ij}^2 - C = 167961 - 103047.41 = 64913.594$$

$$\text{Groups sum of squares} = \sum_{i=1}^k \frac{\left(\sum_{j=1}^{n_i} x_{ij} \right)^2}{n_i} - C = 137898.57 - 103047.41 = 34851.164$$

Error sum of squares = Total SS - Groups SS = 64913.594 - 34851.164
= 30062.43.

Summary of Analysis of Variance: (set up according to the following table (Zar, 1974).

Source of Variation	Sum of Squares (SS)	Degrees of Freedom	Mean Square (MS)
Total $[X_{ij} - \bar{X}]$	$\sum_{i=1}^k \sum_{j=1}^{n_i} X_{ij}^2 - C$	$N-1$	
Groups (i.e., between groups) $[\bar{X}_i - \bar{X}]$	$\sum_{i=1}^k \left(\frac{\sum_{j=1}^{n_i} X_{ij}}{n_i} \right)^2 - C$	$k-1$	$\frac{\text{Groups SS}}{\text{Groups DF}}$
Error (i.e., within groups) $[X_{ij} - \bar{X}_i]$	Total SS - Groups SS	Total DF - Groups DF (or $N-k$)	$\frac{\text{Error SS}}{\text{Error DF}}$

Now for the same table except with the numerical values:

Source of Variation	SS	DF	MS
Total	64913.594	105	-
Group	34851.164	4	8712.79
Error	30062.43	101	297.65

$$F = \frac{GMS}{EMS} = \frac{8712.79}{297.65} = 29.27$$

Table value is $F(1)(k-1)(N-k)$

Since $F_{0.05}(1, 4, 101)$ (use 100 for table) is equal to 2.46 (table D11 Zar, 1974)

H_0 is rejected

$p < 0.0005$

Since a significant F resulted from the analysis of variance, the Newman-Keuls multiple range test is applied:

Ranks of sample means (i)	1	2	3	4	5
Ranked sample means ($\bar{x}_i$)	8.5	16.9	32.4	38.4	59.5
Size of samples (n_i)	20	25	19	19	23

1 is 60; 2 is 60+15; 3 is 60+30; 4 is 60+60; and 5 is 60+90.

A table is then set up in order to compare the five conditions in pairs.

Comparison (B vs A)	Diff- erence ($\bar{x}_B - \bar{x}_A$)	SE	q	p	q 0.05, 100, p	Conclusion
5 vs 1	51.0	3.73	13.67	5	3.917	R Ho: $\mu_5 = \mu_1$
5 vs 2	42.6	3.52	12.10	4	3.685	R Ho: $\mu_5 = \mu_2$
5 vs 3	27.1	3.78	7.17	3	3.356	R Ho: $\mu_5 = \mu_3$
5 vs 4	21.1	3.78	5.58	2	2.800	R Ho: $\mu_5 = \mu_4$
4 vs 1	29.9	3.91	7.65	4	3.685	R Ho: $\mu_4 = \mu_1$
4 vs 2	21.5	3.71	5.80	3	3.356	R Ho: $\mu_4 = \mu_2$
4 vs 3	6	3.96	1.52	2	2.800	A Ho: $\mu_4 = \mu_3$
3 vs 1	23.9	3.91	6.11	3	3.356	R Ho: $\mu_3 = \mu_1$
3 vs 2	15.5	3.71	4.18	2	2.800	A Ho: $\mu_3 = \mu_2$
2 vs 1	8.4	3.66	2.30	2	2.800	A $\mu_2 = \mu_1$

$SE = \sqrt{\frac{S^2}{n}}$ when n_i is the same. $S^2 = \text{error MS}$

$SE = \sqrt{\frac{S^2}{2} \left(\frac{1}{n_A} + \frac{1}{n_B} \right)}$ when n_i 's are different,
where $S^2 = \text{error MS}$

$$q = \frac{\bar{X}_B - \bar{X}_A}{SE}$$

p = range of means between two samples

i.e., 5 vs 1 $p = 5$

q table value = q, Error DF, p.

SE's

$$5 \text{ vs } 1 = \sqrt{\frac{297.65}{2} \left(\frac{1}{23} + \frac{1}{20} \right)} = 3.73 \quad 4 \text{ vs } 1 = \sqrt{\frac{297.65}{2} \left(\frac{1}{19} + \frac{1}{20} \right)} = 3.91$$

$$5 \text{ vs } 2 = \sqrt{\frac{297.65}{2} \left(\frac{1}{23} + \frac{1}{25} \right)} = 3.52 \quad 4 \text{ vs } 2 = \sqrt{\frac{297.65}{2} \left(\frac{1}{19} + \frac{1}{25} \right)} = 3.71$$

$$5 \text{ vs } 3 \text{ and } 4 = \sqrt{\frac{297.65}{2} \left(\frac{1}{23} + \frac{1}{19} \right)} = 3.78 \quad 4 \text{ vs } 3 = \sqrt{\frac{297.65}{19}} = 3.96$$

$$2 \text{ vs } 1 = \sqrt{\frac{297.65}{2} \left(\frac{1}{25} + \frac{1}{20} \right)} = 3.66$$

If the calculated q is equal to or greater than the critical value, q, v, p from Table D12 of Zar, then $H_0: \mu_B = \mu_A$ is rejected.

In this case the overall conclusion was that

$$\mu_1 = \mu_2 \neq \mu_3 = \mu_4 \neq \mu_5$$

or in another form, 60-

60 + 15

60 + 30

60 + 60

60 + 90

where — means are statistically the same

Here is an Example for the Root Growth Experiment:

Fucose - 1 hour

$$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4$$

H_A : that they are not all the same changes in length.

$$\alpha = 0.05$$

	Area 1	Area 2	Area 3	Area 4	
n_i	20	20	20	20	$N = \sum_{i=1}^k n_i = 80$
$\sum_{j=1}^n x_{ij}$	4.1	13.4	6.5	4.5	$\sum_{i=1}^k \sum_{j=1}^n x_{ij} = 28.5$
$\left(\frac{\sum_{j=1}^n x_{ij}}{n_i} \right)^2$	.205	.67	.325	.225	$\sum_{i=1}^k \left(\frac{\sum_{j=1}^n x_{ij}}{n_i} \right)^2 = 12.94$
$\sum_{j=1}^n x_{ij}$	.84	8.98	2.11	1.01	

$$\sum_{i,j} x_{ij} = 28.5$$

$$\sum_{i,j} x_{ij}^2 = 18.25$$

$$C = \frac{\left(\sum_{i,j} x_{ij} \right)^2}{N} = \frac{(28.5)^2}{80} = 10.15$$

$$\text{Total Sum of Squares} = \sum_{i,j} x_{ij}^2 - C = 18.25 - 10.15 = 8.1$$

$$\text{Group Sum of Squares} = \frac{\sum_{i=1}^k \left(\sum_{j=1}^n x_{ij} \right)^2}{n_i} - C = 12.94 - 10.15 = 2.79$$

$$\text{Error Sum of Squares} = \text{Total SS} - \text{Group SS} = 8.1 - 2.79 = 5.31$$

Summary of Analysis of Variance:

Source of Variation	SS	DF	MS
Total	8.1	79	-
Groups	2.79	3	.93
Error	5.31	76	.070

$$F = \frac{\text{Group MS}}{\text{Error MS}} = \frac{.93}{.070} = 13.29$$

Table value .05 (1), 3, 76 (use 80) = 2.72

The calculated F is larger than the table value therefore there is a significant difference.

The Newman-Keuls test is therefore applied:

Ranks	1	2	3	4
	.205	.225	.325	.67
	(Area 1)	(Area 4)	(Area 3)	(Area 2)

$$SE = \sqrt{\frac{s^2}{n}} = \sqrt{\frac{.07}{20}} = .059$$

Comparison	Difference	SE	q	p	q table	Decision
4 vs. 1	.465	.059	7.88	4	3.737	reject
4 vs. 2	.445	.059	7.54	3	3.399	reject
4 vs. 3	.345	.059	5.85	2	2.829	reject
3 vs. 1	.1	.059	1.69	3	3.399	accept
3 vs. 2	.12	.059	2.03	2	2.829	accept
2 vs. 1	.02	.059	.34	2	2.829	accept

Therefore the overall conclusion is $\mu_1 = \mu_2 = \mu_3 \neq \mu_4$ or
root area 2 $\neq$ root area 1 = area 3 = area 4

This test can deal with samples of different sizes but this was not required in this case.

APPENDIX IX: Preliminary Plasmolysis Experiments with Hand

Sections and Plasmolysis Patterns:

When it was decided that an extra experiment should be performed to confirm the position of the dense band of label the technique of plasmolysis was chosen.

In order to test to see (a) whether the epidermal cells would indeed plasmolyse and (b) whether the normal glutaraldehyde fixative would disturb the plasmolysis (i.e. reverse it) and (c) which technique of plasmolysis, i.e., concentration of sugar and time of application, would be the most appropriate; transverse hand sections of three to four day old corn seedlings were cut. Two sugar solutions were tested, (1) 2M sucrose and (2) 2M mannitol. Both were applied for five minutes and it was found that mannitol was the better of the two.

2M mannitol was then applied to some hand sections which were mounted on a glass slide under a cover slip. Several areas of plasmolysis were located under the microscope. 3% glutaraldehyde in 0.025M phosphate buffer was drawn through under the cover slip by placing a paper tissue on the opposite side of the cover slip to the pipette applying the fixative. This was repeated several times to ensure exposure of the areas being examined. These areas were checked periodically for a two hour period. There was no change in the plasmolysed cells due to the introduction of the glutaraldehyde.

It was therefore decided that a plasmolysis period of five minutes in 2M mannitol would be sufficient and that the normal means of fixation were acceptable.

After processing, it was discovered as noted in the results that the dense band of label was in the outer periclinal wall, but along with this there were also interesting patterns of plasmolysis. In the root proper, in any particular file of tissues, generally the cells in the file plasmolysed in a similar fashion (Figure 176). The epidermis as seen in the plasmolysis experiment, can sometimes plasmolyse at the outer edge of the cells but more often it occurs at the base of the cells between two adjacent cells at the same corner (Figures 177, 179). The root cap plasmolysed differently for each region; the outermost cells (in the slime) plasmolysed all the way around so that the cytoplasm was in the middle of the cells; the outer cells of the root cap proper generally plasmolysed at the outer wall (the same position as label would go if they were chased or with the fucose label) (Figures 178, 180 to 182); more internal cells of the root cap did not plasmolyse on any particular face of the cells.

Sachs (1972) says that patterns of plasmolysis for many plants is repeatable and considers them indicative of the strength of plasmadesmata, certainly this would be true for the root cap as these cell walls often give way to allow an accumulation of slime between the wall and the plasmalemma. For the cortex Sachs (1972) found that the orientation of the plasmolysed cytoplasm was perpendicular to the major direction of growth and parallel to the major plane of cell division. Strugger (1934) stated that plasmolysed cytoplasm sticks preferentially to growing cell walls. In the corn root most of the cytoplasm does seem to be oriented in this fashion for the cortex and also the young xylem vessels.

APPENDIX X: Protease Experiments:

In order to do a qualitative check as to the protein present and what labelling characteristics may be due to protein, roots were incubated with glucose-6-³H for times of 15 minutes, 30 minutes and 60 minutes followed by two hours in a protease solution at 25°C. The solution used was a 10 mg protease/ml solution in 0.01M glycine buffer pH 7.5 containing 5 mM CaCl₂. The protease is a bacteria purified protease (non-specific) from Streptomyces griseus repurified type VI (Sigma, St. Louis). Proteases are known to break the peptide bond and since the proteins are broken down into peptides of a low molecular weight, they would be washed away during the rest of the processing. In general, the label is reduced over the cytoplasm, but label remains in such areas as the label in the root cap cells (Figures 187, 190 to 192) and in the mucilage (Figures 187, 189a) and in the dense band of label in the outer periclinal wall (Figures 190, 191). The protease bathing acts as a light chase, not as effective as an actual chase (with a concentrated sugar solution), but still causing some asymmetry in the outer root cap cells (Figures 190, 192). Roberts and Butt (1968a) analysed metabolic products at various stages of incubation with radioactive glucose. After 30 minutes the radioactivity was still entirely in glucose but after four hours there were many amino acids labelled. The tissue seems to lack clarity in form from tissue not incubated with protease as well but since all three times have essentially the same concentration of silver grains over the cytoplasm the protease may have reduced the amount of label from protein at the longer times.

Plasmolysis Patterns and Protease Experiment:

Figure 174:

Plasmolysis patterns of the central portion of corn root. Note that the portion where the connections between the wall and the plasmalemma is the weakest is almost always on the same side of any file of cells, usually on the basipetal ends of the cells. x190.

Figure 175:

The side of a corn root after plasmolysis. Note that the most common plasmolysis pattern of the epidermis is to release at the lower periclinal wall at the boundary between two cells (arrowheads). x190.

Figure 176:

The root cap, showing that where plasmolysis occurs is the same position as where the label accumulates in the cells - towards the outer side of the root. Some cells even contain a pink staining substance (with PAS) (symbol), the pattern of plasmolysis mirrors the position of bearding and fucose label (Figure 47). x190.

Figure 177:

An enlargement of the epidermis, showing the plasmolysis pattern as notch shape at the base of the cells. x800.

Figure 178:

An enlargement of the area marked with on Figure 178. These are the cells containing the pink material; strands of pink staining material extends from the wall to the plasmalemma. x700.

Figure 179:

An enlargement of the area marked with on Figure 178. x700.

Figure 180:

Another view of root cap plasmolysis. x400.

Plasmolysis Patterns and Protease Experiment (Continued):

Figures 181-186 Protease Experiment:

This experiment shows that the label which is present in most of the other experiments is generally not protein label, except for inside of the cells. Because the protease was applied after the precursor incubation, the labelling patterns resemble that of the pulse chase results, i.e. the bearding phenomenon in the root cap of Figures 186 and 188. The label used was glucose-6-³H.

Figure 181:

15 minutes with the glucose followed by 120 minutes in protease. Note that there is less cytoplasmic label than usual, but root cap label is untouched. x250.

Figure 182:

15 minutes glucose + 120 minutes protease; note again that the label in the cytoplasm is reduced but not the root cap wall label or slime label. x250.

Figure 183:

30 minutes glucose + 120 minutes protease. The epidermis, again with cleaner cytoplasm - as compared to glucose - label continuous and pulse chase labelling; note that from the inset, the mucilage label is not removed by protease. x250.

Figure 184:

60 minutes glucose + 120 minutes protease. Note that the bearding phenomenon material is not removed by protease action. x250.

Figure 185:

Epidermis after 60 minutes glucose + 120 minutes protease. The dense band and mucilage label is not removed by protease. x250.

Figure 186:

60 minutes glucose + 120 minutes protease. The root cap tip, and the wall label are not protease sensitive. x200.

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