HELEN P.V. LANGLEY.

THESIS: "Studies on the Absorption of Salts by Plant Roots"

A copy of the thesis has now been sent to the External Examiner who is Dr L.W. Poel, University of Birmingham.

Your Sub-Committee is

PROFESSOR WALTON (Convener)

PROFESSOR HAWTHORN (R.C.S.T.)

DR BOND.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

STUDIES ON THE ABSORPTION OF SALTS BY PLANT ROOTS.

Salt absorption by plants is generally considered to be a two stage process, one phase of which is dependent on the metabolism of the cell The experimental evidence on the subject is limited and the proposed mechan of absorption are based mainly on theoretical considerations.

The purpose of this thesis , therefore, is to study the uptake o salts using radioactive tracer techniques and to identify the centre of each phase of the absorption process in plant roots. A technique of autoradio has been developed for use with water soluble isotopes, giving results at th cellular level with thin sections of root material. Four ions, calcium, rubidium, sulphate and iodide, were used in these experiments.

It has been shown by this technique that there are two phases in the process of salt absorption by excised plant roots and the existence of a 'active' phase has been established. However, contrary to an assumption made in the method of isotopic exchange, it has been shown that the material reta after exchange is not all actively absorbed.

The passive phase of absorption is associated with the epidermis the hypodermis and the endodermis. The ions taken up by the epidermis were found to be readily exchangeable. The nature of the localisation in the hypodermis is assumed to be a binding of the ion which has a low degree of exchangeability. It is proposed that the endodermis acts as a barrier to diffusion in that part of the root beyond the region of calcium accumulation

The active phase of absorption is primarily associated with the parenchyma of the cortex in the apical region of the root, this region bein

termed the region of calcium accumulation. Cations appeared to be localised in the protoxylem initial cells and in the central cells of the root cap.

Cation absorption occurs in the epidermis, hypodermis, endodermi cortical parenchyma of the apical region of the root and in the protoxylem initial cells. Anion absorption does not appear to occur in the epidermis, protoxylem initials or in the central cells of the root cap.

On the autoradiographic evidence, it is proposed that vacuolar accumulation of cations occurs in the cortical parenchyma in the region of calcium accumulation. Anion accumulation does not occur in the vacuoles bu the case of sulphate ions, it is localised in the region of the nucleus of the cell. It is proposed that anions are absorbed into their respective metabolic cycles in the cytoplasm of the cell. The results obtained with sulphate ions suggest that the site of protein metabolism in the cell, is restricted to the region of the nucleus of the cell.

The autoradiographs show that there is an intense localisation o non-exchangeable ions in the cortex in a region of the root in which the endodermis does not retain ions. It is possible that these accumulated ion form an osmotic pump which promotes the flow of ions by diffusion from the external solution directly into the stele in this region of the root. The region of maximum salt absorption, therefore, is also the region of maximum translocation.

Helen P.V. Langley

September 1961

STUDIES ON

THE ABSORPTION OF SALTS BY PLANT ROOTS.

by

Helen Pauline Valentine Langley .

A thesis submitted in accordance with the requirements of the Faculty of Science of the University of Glasgow for the Degree of Doctor of Philosophy.

September 1961.

ACKNOWLEDGEMENTS.

The work described in this thesis was initiated on a suggestion by Professor John Hawthorn,B.Sc.,Ph.D.,A.R.C.S.T.,F.R.I.C. and the author gratefully acknowledges his continuing support and assistance.

The author is greatly indebted to Dr. Ronald B. Duckworth, B.Sc. for his constant guidance and encouragement in this work.

Thanks are also due to Mr. Curtis, Curator of the Botanic Gardens, Glasgow for the supply of roots of Dendrobium sp. and to Dr. J.C. Haig of the National Vegetable Research Station, Wellesbourne, Warwich from whom a standard variety of onions was obtained.

The author is indebted to the Muirhead Trust for the award of scholarship during the three years of practical work.

TABLE OF CONTENTS.

PREFACE 1

INTRODUCTION

I General. 2

II Review of the Literature 2

1. Passive Absorption 2
2. Active Absorption 5
3. Suggested Mechanisms of Active Absorption. 10
4. Translocation. 12

III Summary of the Present State of Knowledge. 14

EXPERIMENTAL PART I : TECHNIQUE

1. Formulation of the Conditions of Treatment 17
2. Development of a Method of Fixation 23
3. Development of the Technique of Autoradiography. 31
4. Presentation of Results. 39

EXPERIMENTAL PART II : AUTORADIOGRAPHY

I Studies with a Divalent Cation 41

1. Uptake and Exchange of Calcium Ions in Living Onion Roots. . .41
2. Uptake and Exchange of Calcium Ions in Dead Onion Roots. . . .46
3. Uptake and Exchange of Calcium Ions in Roots of Dendrobium sp. and Amaryllis sp. 49
4. Uptake and Exchange of Calcium Ions in the Presence of Strontium 53
5. Attempts to Identify the Centres of Calcium Uptake 57
6. Discussion 60

II Studies with a Monovalent Cation 67

1. Uptake and Exchange of Rubidium Ions in Onion Roots. 67
2. Discussion 70

III Studies with Anions. 72

1. Uptake and Exchange of Sulphate Ions in Onion Roots. 72
2. Uptake and Exchange of Iodide Ions in Onion Roots. 75
3. Discussion 76

DISCUSSION 78

CONCLUSIONS 87

REFERENCES 89

Appendix I Standard Experimental Procedure i
Appendix II Details of the Isotopes Used and the Exposure Times Required iv

PREFACE.

The work described here is part of a general programme of research into the occurrence of radioactive fallout in plants.

Salt absorption by plants is generally considered to be a two stage process, one phase of which is dependent on the metabolism of the cell. The experimental evidence on the subject is limited and the proposed mechanisms of absorption are based mainly on theoretical considerations.

The purpose of this thesis, therefore, is to study the uptake of salts using radioactive tracer techniques and to identify the centre of each phase of the absorption process. The technique of autoradiography was considered to present the most practical approach to the problem. The major part of the work is therefore concerned with the development of a suitable technique for use with water soluble isotopes and with the establishment of this method by repeated observations on root material.

Four ions, calcium, rubidium, sulphate and iodide were used in investigations on plant roots and the results obtained at the cellular level of autoradiography are discussed in relation to proposed hypotheses of salt absorption.

INTRODUCTION.

GENERAL.

The absorption of salts into plant roots has been a subject for discussion for many years but with the introduction of new techniques, principally using isotopic tracers, a certain amount of experimental evidence has been obtained. These data, however, only apply to particular points in the process of absorption and the major part of what remains purely hypothetical.

In this introduction it is intended to review the literature on salt absorption and to summarise the mechanisms of absorption proposed previously, thus forming a basis for further discussion.

REVIEW OF THE LITERATURE.

It is now established (1-7) that there are two phases in the absorption process, one passive and one 'active' in the sense that it is associated with the expenditure of energy by the cell.

1.PASSIVE ABSORPTION.

Diffusion:-

Diffusion is the first step in the process of absorption and it has been established that ion uptake by this means is a rapid and reversible process (9-11).

It has been suggested by different workers that the region of

the cell accessible to diffusion includes the cell wall and part of the cytoplasm (12) or the cell wall and all of the cytoplasm (13-17) or the total water space (9,18,19). Hope and Stevens (20) have demonstrated the existence of a Donnan equilibrium with the external solution due to the presence of immobile anions or cation exchange spots. These spots are considered to be located in the cell wall (21-23) and are therefore included in estimations of the space accessible to diffusion. Hence the term Apparent Free Space (A.F.S.) has arisen which includes both the space accessible to diffusion and the Donnan Free Space (D.F.S.).

The mathematics of the measurement of Apparent Free Space have been examined by Briggs and Robertson (18) and values have been reported ranging from 8-34% of the volume of the tissue depending on the material used and the particular ion under investigation (13,24-26). Levitt (24) criticises these estimations on the grounds that the bathing solution is not completely removed from the material during blotting. It has also been shown that the concentration of the solution immediately around the specimen during treatment is greater than that in the bulk of the solution (27). This surface film effect would increase the amount of salt left on a specimen and add to the value of the Apparent Free Space. Estimates of Apparent Free Space are higher in excised roots than in roots of intact seedlings (28 cf. 29) which would suggest that the Apparent Free Space of intact roots does not extend into the stele.

The barrier to diffusion has not been positively identified but some recent findings uphold the classical view of a functional plasmalemma(30-35). MacRobbie and Dainty(36) by inserting micro-electrodes into different regions of a cell, have shown that the cytoplasm is bordered by barriers to diffusion both at the inner and outer surfaces. In relation to the movement of sodium, the latter appears to be more resistant to diffusion(37). For calcium, the opposite has been found using bean plants(38).

Cation Exchange:-

The occurrence of cation exchange is well attested in storage tissue and in the roots of higher plants(12,39-41). It is greater in less active tissue (e.g. storage tissue compared with meristematic tissue) (3) and greater at low concentrations(42-44).

Epstein and Leggett (45) have shown that the strontium^{89} absorbed into barley roots in the first relatively rapid, passive uptake is readily replaced by other cations and by hydrogen ions when the roots are placed in weak acid. It has been reported that a cation can be replaced by one of greater valency (45-48) and that two cations of similar valencies compete for the same site.

Cation exchange has been demonstrated in ether killed roots (49,50) but Helmy and Elgaby (48) have also shown that the groups responsible for cation exchange are easily destroyed by killing the roots with heat. Poisons may diminish the number of sites available in the

tissue (51) but in contrast, 2.4 dichlorophenoxyacetic acid ,by providing additional 'sites' can increase the cation exchange capacity (52).

Several authors have presented theoretical discussions (53-55) on the exchange of ions on single molecules which are capable of binding both anions and cations but since at normal external pH levels, acid dissociation predominates, adsorptive ion exchange is largely cationic(20).

The nature of the exchange sites is uncertain but they are believed to be located in the cell wall (21-23,30-32) and the more recent papers attribute ion exchange to unmethylated carboxyl groups of the cell wall constituents (30,31). Despite the diversity in terms used to describe these sites, there is general agreement as to their meaning, viz.,fixed or at least constrained negative valencies to which cations are attracted.

2. ACTIVE ABSORPTION.

Relation to Metabolism:-

Hoagland (56,57) has demonstrated that salt accumulation is suppressed under conditions unfavourable to respiration and an increment of respiration has been observed to accompany the absorption of salts by a cell (58,59).

By the use of respiratory inhibitors, it has been found that the absorption of both cations and anions is suppressed by substances

which inhibit glycolysis and the Krebs cycle (60-64). Lundegårdh (59,65-67), however, bases his scheme of active absorption of anions on the specific stimulation of a cyanide-sensitive system, the latter being distinct from the ground respiration which is insensitive to cyanide. The occurrence of the cyanide-sensitive respiration and the light reversible inhibition of ion absorption by carbon monoxide (64,68) has led Lundegårdh (59,65-67) and others (68-76) to consider the cytochrome system.

Middleton (77) has found that dinitrophenol, glucose and potassium chloride all stimulate respiration in the same way and has concluded that the stimulation of a cyanide-sensitive system is a general consequence of stimulating the respiration, however caused, rather than a direct specific stimulation of a terminal oxidase; cytochrome oxidase in the hypothesis proposed by Lundegårdh. Middleton has suggested that the stimulated respiration may be cyanide-sensitive because the cyanide-insensitive system is of limited capacity. If the total rate of respiration exceeds that of which the latter is capable, any excess would be carried by the cyanide-sensitive system. This hypothesis would explain the partially cyanide-sensitive respiration found in beet discs by Robertson (68,78).

It is not known whether both anions and cations can cause this 'salt' respiration (60-64) or whether only anions are responsible(59,65-67). Sutcliffe and others (77,79-83) have suggested that the presence of potassium alone can cause salt respiration.

Absorption of cations in excess of anions has been demonstrated.

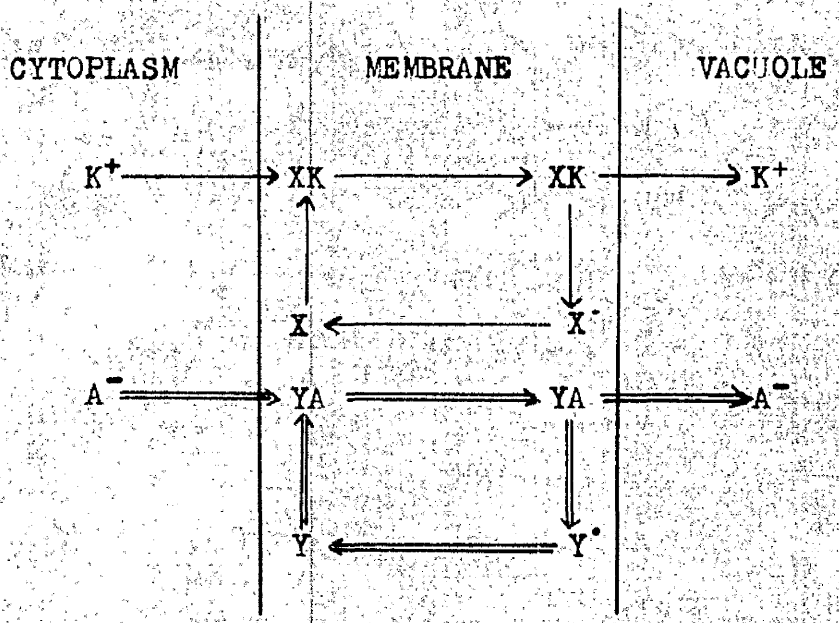


Figure 1. A Carrier Mechanism after Sutcliffe (7).

This excess cation uptake is believed to be balanced by the production of organic acid anions and by the replacement of previously absorbed cations (28,83,84). At low pH levels, carbon dioxide is believed to take the place of bicarbonate (83). Since these reactions do not occur under conditions of rapid uptake when both ions of a salt are equally absorbed, it would appear that this exchange is a secondary effect related to the maintenance of electrical neutrality.

Isotopic Exchange:-

The later experimental evidence supporting the theory of active absorption has been derived using isotopic exchange techniques. In this method the material is treated with a labelled ion for such a time as to involve all phases of absorption. Subsequent treatment with the same but unlabelled ion replaces all the exchangeable material with the inactive ion, leaving the actively accumulated material in the tissue, presumably behind a highly impermeable barrier (45,86).

This work has led to the suggestion that absorption involves the operation of carriers (86-88). It is assumed (2,3,5,8,89-93) that the ion forms a complex with the carrier molecule, passes through a barrier (presumably the tonoplast) between an outer phase (cytoplasm) and an inner phase (vacuolar sap) with the release of the ions in the inner space, as summarised in Figure 1.

TABLE I

Substrate ion	Competing ion	Non-competing ion	Reference
Rb^+	K^+ Cs^+	Na^+ Li^+	86
Li^+	Ca^{++}	—	92
Br^-	Cl^- I^-	NO_3^-	87
Sr^{++}	Ca^{++} Ba^{++}	Mg^{++}	45
SO_4^{--}	SeO_4^{--}	$H_2PO_4^-$ NO_3^-	26
HPO_4^{--}	OH^-	—	94
$H_2PO_4^-$	OH^-	—	94

Kinetics of Active Absorption:-

Epstein and Hagen (86) have compared ion absorption involving carriers with enzyme catalysis. In both cases, the agent (ion or enzyme) combines with a substance (carrier or substrate) to form an intermediary complex which subsequently breaks down, transport or catalysis thus having taken place.

On plotting the rate of active absorption of an ion obtained by isotopic exchange, as a function of its concentration according to the Lineweaver-Burke method, a characteristic enzyme saturation curve is obtained (45,86). The analyses were developed further using the Michaelis-Menten theory to determine whether cations were competing with one another for the same carrier molecule. On plotting the reciprocals of absorption rate and concentration, straight line graphs were obtained. Competitive interference was reflected in these graphs by an increase in the ratio of slope to intercept. Non-competitive interference, on the other hand, caused no increase in either factor. Table 1 summarises these interactions (45).

It would appear that ions of similar chemical behaviour act as metabolic analogues and compete for the same carrier. At high concentrations some non-competing ions have been shown to be competitive (3) but the effects are not all antagonistic. Synergistic effects have been observed by Viets (46) and Overstreet and Handley (47) who find that absorption of monovalent cations is promoted by divalent and polyvalent cations. Increased absorption of sulphate (26), potassium (95),

rubidium and phosphate ions (42) has been observed in the presence of calcium ions.

The nature of the carriers is unknown but it has been proposed that more than one carrier might be involved in the absorption of a single ion species (96-99). Fried and Noggle (98) suggest that one carrier might dominate at low concentrations and another at high concentrations. Laties (100) has presented evidence that carriers are generated by the cell prior to the absorption process.

The evidence on selectivity of the carrier action and non-selectivity of the exchange process would suggest that the entities involved in each case are not identical. Assuming the entities to be the same, carrier formation would be expected to take place at the site of exchange absorption which is generally understood to be the cell wall. The carrier process would then entail the movement of the carrier complex across a possible barrier in the plasmalemma, through the cytoplasm and across the tonoplast. The reduced carrier would then be required to return to the cell wall. The suggestion that mitochondria act as carriers introduces similar problems to the system of identical exchange and carrier molecules.

It is uncertain whether an initial exchange precedes active accumulation. Russell (101) and others (90,102-104) consider this intermediate stage to be necessary but Hylmo (105) has suggested that the immediate substrate for active absorption is the solution in the free space of the tissue which tends to equilibrium with the external solution.

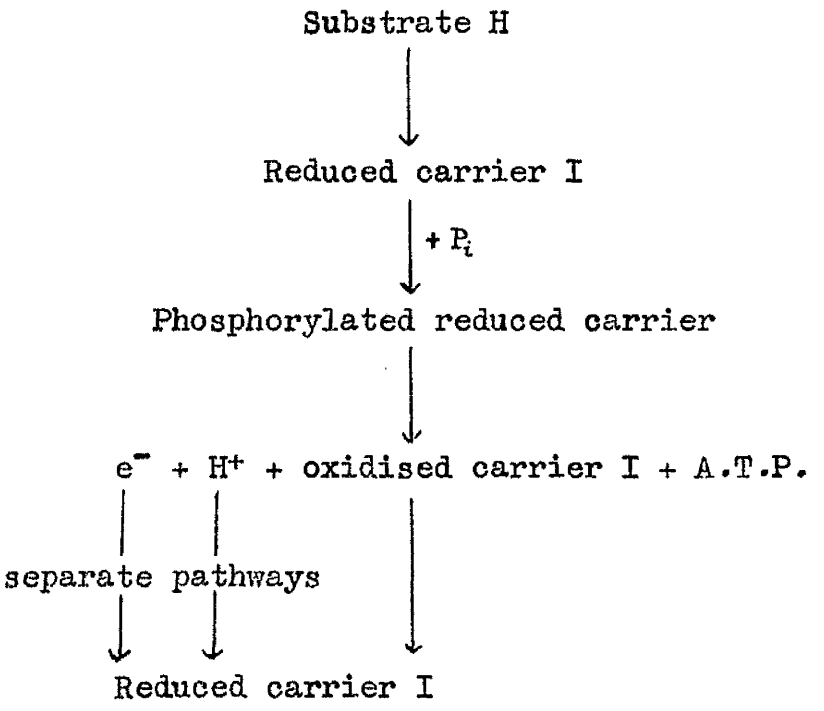


Figure 2. Phosphorylation Mechanism after Robertson (8).

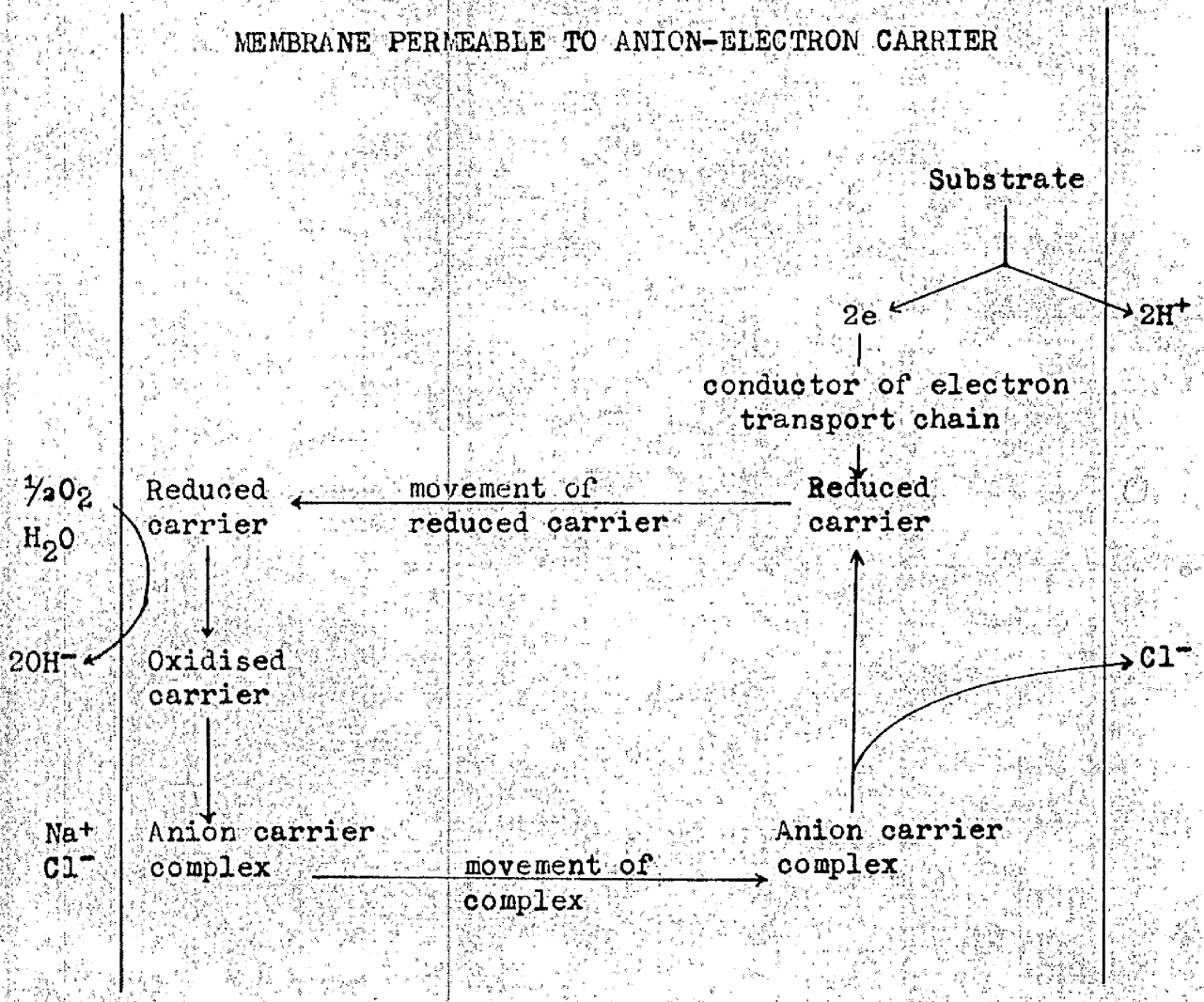


Figure 3. Absorption of Salts after Robertson (8).

5. SUGGESTED MECHANISMS OF ACTIVE ABSORPTION.

Various theories have been proposed as to the nature of the carriers , their mode of action and the source of energy for absorption. No single hypothesis attempts to explain all three points but the following are the most comprehensive.

Phosphorylation Mechanisms:-

Robertson (8) postulates that the first act of secretion or active absorption,depends on the separation of positive and negative charges (H^+ and e^-). An electron is believed to move through an electron carrier system in a membrane and lead to the formation of a hydroxyl ion at the other side of the membrane. Evidence that an anion carrier might also be involved, suggested that a substance which carries an electron in one direction might be able to carry an anion in the opposite direction. It is essential to this hypothesis that a complex between the anion and the oxidised redox substance is formed,but the complex must occur in the lipoid membrane so that the anion ceases to exist as a negative charge in water and becomes part of a molecule in a membrane of low permeability to the free ion. Cations may then move along the electrochemical potential set up by the active absorption of the anion. A diagrammatic representation of this hypothesis is shown in Figure 2 with the proposed mechanism of energy production by phosphorylation in Figure 3.

Several methods have been based on the utilization of phosphate

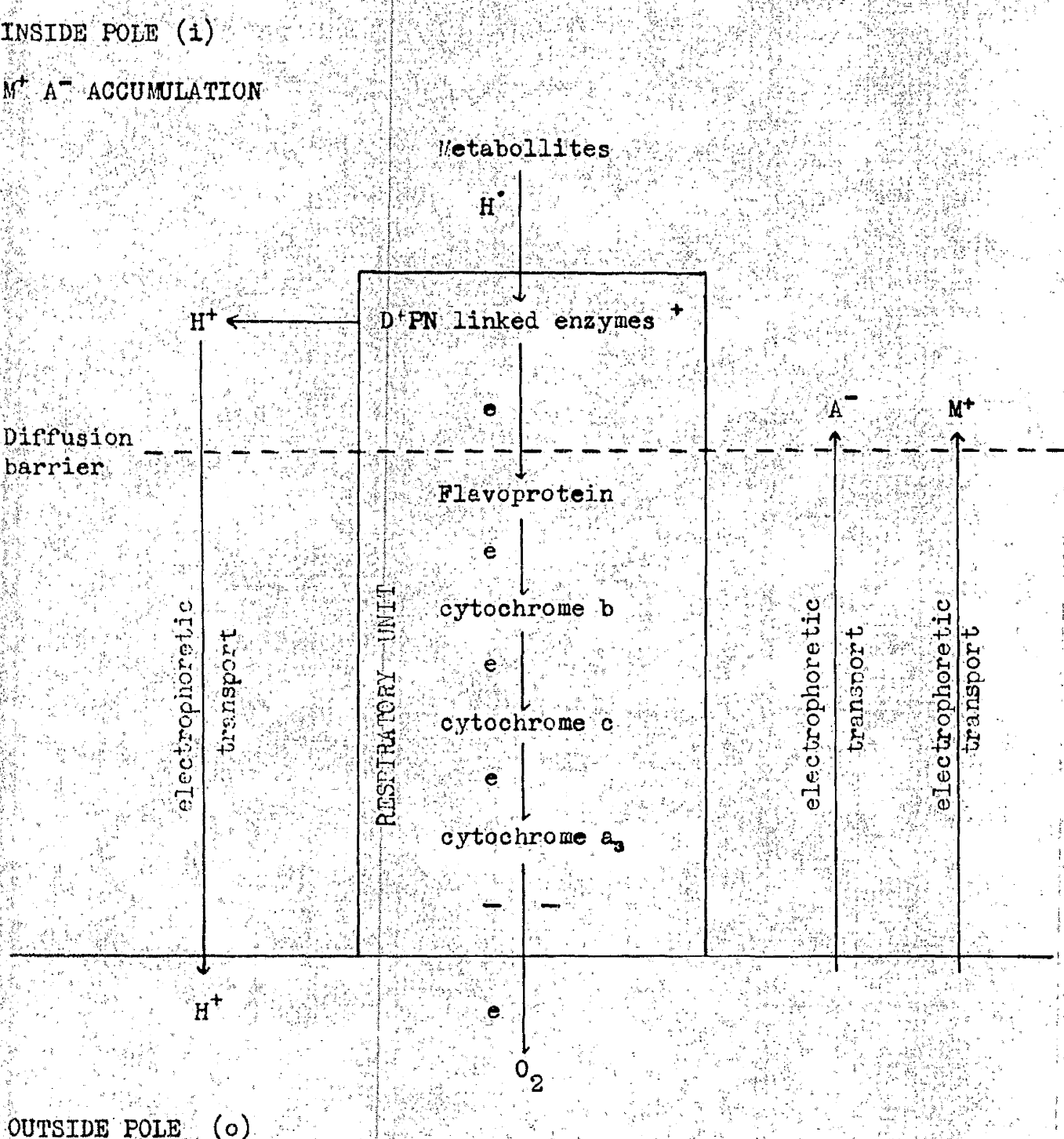


Figure 4. Anion Absorption after Lundegardh (111).

energy but differing in the nature of the carrier. Ribonucleic acid (106), phosphatidic acid (107) and lecithins (108) have been suggested as possible carriers and salt uptake has been linked with protein synthesis (109), the proposed carriers being amphoteric nitrogen compounds (110).

Redox Systems:-

The Lundegårdh hypothesis (111) is similar to that of Robertson in that only anions are considered to be actively accumulated. These are again exchanged for electrons but whereas Robertson does not specify the electron transport system involved, Lundegårdh proposes the cytochrome pathway as detailed in Figure 4. Both of these authors and others (3,112,113) consider that accumulation of ions takes place in the mitochondria. The hypothesis laid down by Lundegårdh has received much criticism and he has modified it to apply to the halogens only.

Conway (114) has proposed a mechanism for the transport of cations which resembles that of Lundegårdh for anions. He has suggested that a cation becomes bound to a reduced respiratory intermediate which becomes oxidised on the other side of the membrane by transferring an electron to another oxidised substance which he does not specify. The oxidised carrier returns across the membrane where it is reduced and becomes capable of accepting another ion.

Alternatives to the Carrier Hypothesis:-

1. A recent paper (115) describing invaginations in the cell

walls of plants gives support to Bennett's theory (116) that ions become bound to the outer surface of the membrane and are transferred to isolated vesicles within the membrane by invagination and pinching off. The membrane surrounding the vesicle is then decomposed and the ions are released in the free state.

2. Hutton (117) has suggested a mechanism in animal cells which may be applicable to plant cells. It involves reversible hydration and dehydration of the membrane and permits a unified explanation of the electrical behaviour of cells and of differential permeability.

3. Saltman has proposed that anions and some cations (zinc and copper) are absorbed directly into metabolic cycles which would imply that the link with respiration is indirect (118,119).

4. TRANSLOCATION.

It is generally accepted that the movement of salts across the cortex is passive. The cortex is considered to act as a single cell in passing ions across successive layers of protoplasm — a symplast (69,104) Plasmodesmata are known to permeate plant cell walls giving a continuous cytoplasm across the cortex (120,121).

It has been suggested that the non-metabolic flow of salts may be caused by the concentration gradient (2) or the increasingly anaerobic conditions in the region enclosed by the endodermis(117). However this region is considered to be adequately aerated by the intercellular spaces (124).

Ions are known to be able to move against a high concentration gradient (122) into the stele which would necessitate the expenditure of energy to transport ions into the xylem. The conclusions, therefore, are that the endodermis acts as a high resistance barrier and that its action is comparable to the tonoplast of a single cell (28,123). Hylmo agrees with this suggestion and proposes that salts also enter the stele passively by breaks in the endodermis and are actively accumulated by the protoxylem (105).

The ions for transport into the stele are considered to be derived from those freely moving in the symplast (1-7). The vacuole is regarded as a cul de sac (6,7,124-126) but Epstein (3) considers that ions may be released from the vacuoles for transport to the shoot in cases of starvation. Lundegårdh (2) has proposed that the salts present in the vacuolar sap act as an osmotic pump which promotes the flow of ions into the stele.

Upward movement of salts is generally understood to occur in the xylem (18,38,127) but several authors consider that they are transported in the phloem (128-130). Lateral transport is assumed to occur from the xylem to the cells along the path of transport by exchange and active accumulation. Downward movement from leaves of normally immobile calcium has been demonstrated in the xylem in the presence of diethyl ether (38).

SUMMARY OF THE PRESENT STATE OF KNOWLEDGE.

It is established that the passive phase of absorption includes cation exchange, adsorption and diffusion resulting in a Donnan equilibrium with the external solution. These are essentially physical processes and will proceed at low temperatures or in an inert gas. The regions of the cell accessible to diffusion are not certain and values of the Apparent Free Space have been found to be dependent on the plant material and the ion under investigation. The tonoplast is considered to form a barrier to diffusion while the plasmalemma may or may not constitute a barrier. The nature of the cation exchange spots is not known but they are considered to be located in the cell wall.

The dependence of active uptake on the metabolism of the cell is accepted but the source of energy for absorption and the link with respiration are not clear. It has been proposed that the latter may be direct as in the Lundegårdh hypothesis or indirect as indicated by Middleton. Anions alone or the presence of anions and cations together may be required to cause salt respiration.

The carrier mechanism is generally regarded as presenting an explanation for the spatial transport of ions across a membrane. Several compounds have been suggested as carriers but none are based on experimental evidence.

It is unlikely that the carriers in active absorption are identical to the entities involved in cation exchange. The latter process

TABLE II (after Epstein (45))

ACTIVE UPTAKE	PASSIVE UPTAKE
1. Linear with time; no equilibrium reached in experiment.	1. Non-linear with time ; equilibrium reached in 30 minutes.
2. Ions are essentially non-exchangeable.	2. Ions are readily exchangeable.
3. Selective with respect to various ions or groups of ions.	3. Non-selective.
4. Requires energy expenditure (under anaerobic conditions active absorption is negligible; exchange adsorption is not diminished.	4. Does not require energy expenditure on the part of the tissue.

is not generally considered to be a necessary step preceding active accumulation. The substrate for active absorption is taken to be the freely diffusible material in the Apparent Free Space.

Table II summarises the data on the two phases of absorption .

Translocation is believed to occur passively by diffusion and mass flow across a symplast. It is possible that the salt accumulated in the vacuoles forms an osmotic pump to promote the flow of salts into the xylem. The endodermis is considered to form a barrier and it is concluded that there is an active process involved in transporting ions across the endodermis to the xylem for transport to the shoot.

It is evident from the survey of the literature on salt uptake that, although the existence of a two stage absorption process is generally accepted, very little is known of the active phase. The mechanism involving the use of carriers is hypothetical as are most of the proposed mechanisms. From investigations using the technique of isotopic exchange, Epstein and others have built up a pattern of the interrelationships of different ions on salt uptake. The remaining experimental evidence is not great and is concerned with isolated details. The field for experimental investigation is therefore extensive.

It was felt that an attempt should be made to establish the site of cation exchange and of the active process in plant roots and in individual cells. The technique of autoradiography was considered to

afford the most practical approach to the problem. The first experiments, therefore, are concerned with formulating the conditions of treatment of the root material. The use of water soluble isotopes has necessitated the development of a method of fixation which would prevent any displacement of the salt in the material during the preparation of the autoradiograph.

Autoradiographs obtained with a monovalent anion and cation and with a divalent anion and cation are described and are discussed in relation to the hypotheses of uptake detailed in the introduction.

EXPERIMENTAL.

PART 1. TECHNIQUE.

FORMULATION OF THE CONDITIONS OF TREATMENT.

The technique of isotopic exchange has been used to obtain quantitative estimations of the absorption of several anions and of potassium and strontium in barley, pea and bean roots. In the present investigation, the selection of the root material was determined by the size of the cells, the ease with which the material could be handled and sectioned and by the availability of a large number of roots of uniform size and stage of growth at any given time. Allium cepa, Narcissus sp. and Amaryllis hippeastrum were found to give suitable roots when grown in constantly aerated tap water. Roots of Dendrobium sp. were of interest later since they have a many layered epidermis and a clearly defined hypodermis. These were obtained from the Botanic Gardens, Glasgow. Excised roots were used in all experiments and were considered to absorb salts in the same way as roots of intact plants except that the vessels of the stele were open at one end to the bathing solution , thus removing the effect of transpiration. The roots were assumed to be viable since plasmolysis of the cells could be demonstrated.

Because of the initial connection of this work with fall-out studies, attention was directed to strontium. However, calcium, the metabolic analogue of strontium, was chosen since it is more suitable for autoradiography as will be shown in a later section. Ca^{45} as a solution

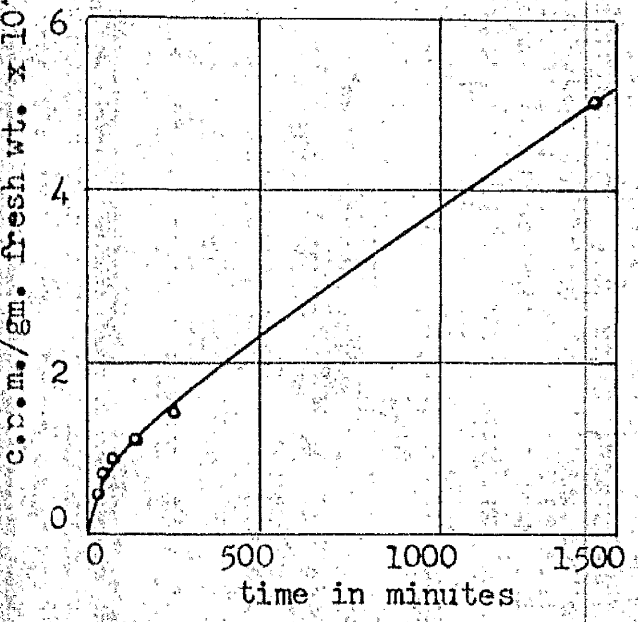


Figure 5. Uptake from a solution containing 0·005meqCa/l.

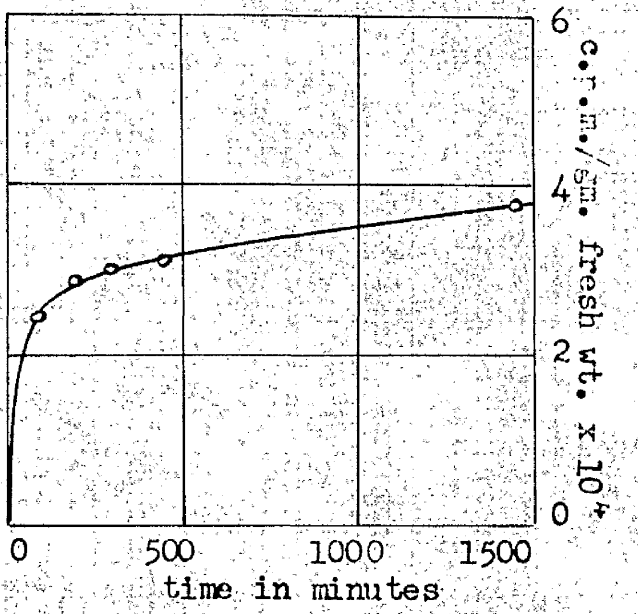


Figure 6. Uptake from a solution containing 0·3 meqCa/l.

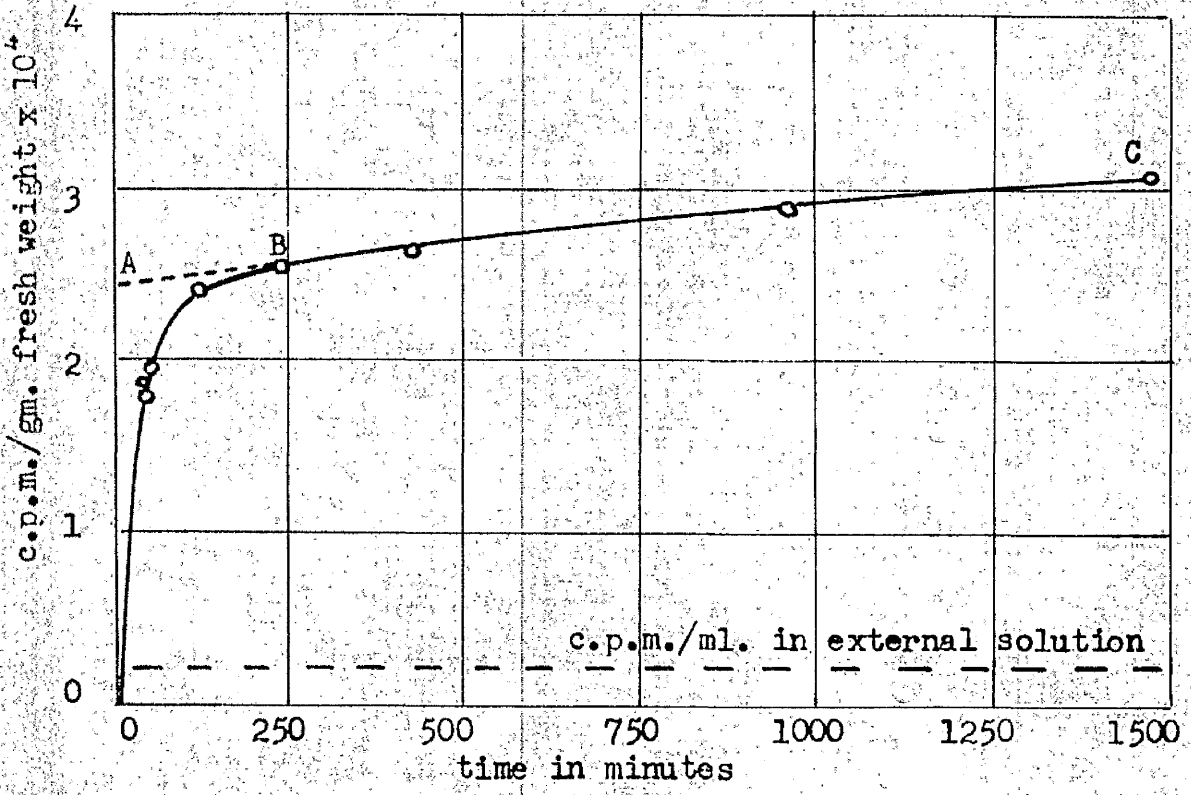


Figure 7. Uptake from a solution containing 0·5 meq. Ca/litre.

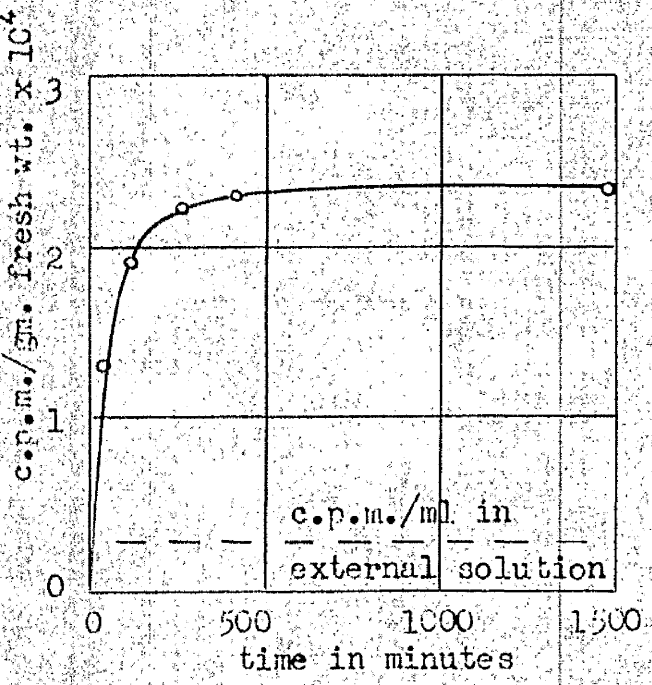


Figure 8. Uptake from a solution containing 1·0meqCa/l.

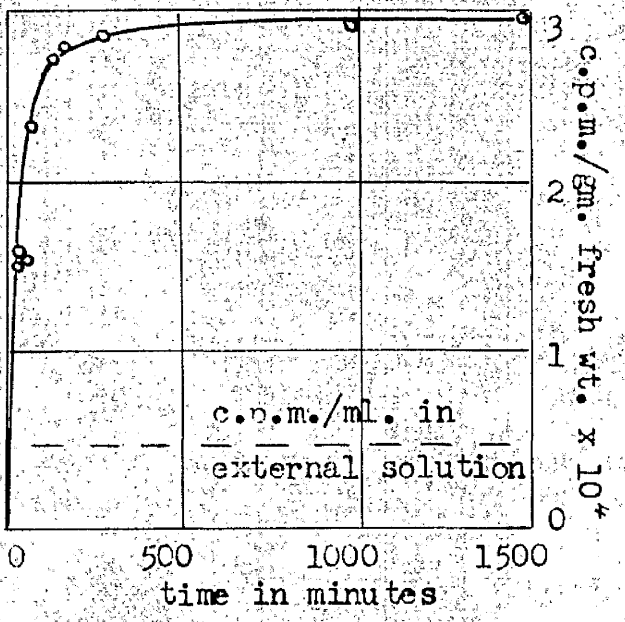


Figure 9. Uptake from a solution containing 2·0meqCa/l.

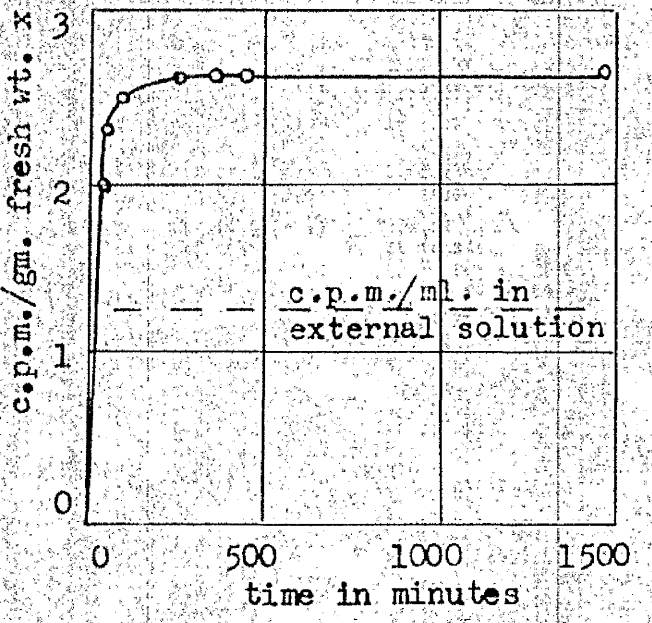


Figure 10. Uptake from a solution containing 5·0meqCa/l.

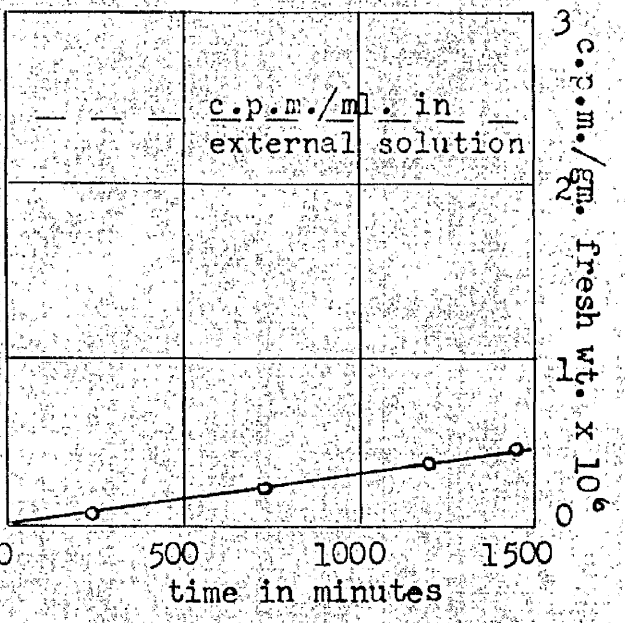


Figure 11. Uptake from a solution containing 35·0meqCa/l.

of calcium chloride was obtained from the Radiochemical Centre, Amersham.

UPTAKE OF CALCIUM

1. Effect of Concentration:-

To determine the optimum concentration for the absorption of calcium, onion roots were excised 1 inch long and were treated at 30°C with labelled calcium chloride solutions having concentrations ranging from 35- 0·005meq.Ca/l. Roots were removed from the tubes at regular time intervals and rapidly rinsed three times in deionized water. The material was blotted and weighed before ashing at 700°C for 2 hours. The ash was dissolved in dilute hydrochloric acid and made up to 25mls. 1 ml. of this solution was dried evenly on a watch glass (1 inch) and counted with a Geiger Müller end window counter. The weight of calcium chloride on the watch glass was less than 0·01gm. and self absorption was negligible. The counts were converted to c.p.m./gm. fresh weight of root material and these values were plotted against time for each concentration of calcium in the external solution (Figures 5-11).

The ratio of the activity in the root (c.p.m./gm. fresh weight) to the activity in the external solution (c.p.m./ml.) at a given time will be referred to as the concentration factor. In Figure 12 the results of the above experiments are summarised where the value of the concentration factor at 400 minutes for each concentration used is plotted against the concentration of calcium in the external solution.

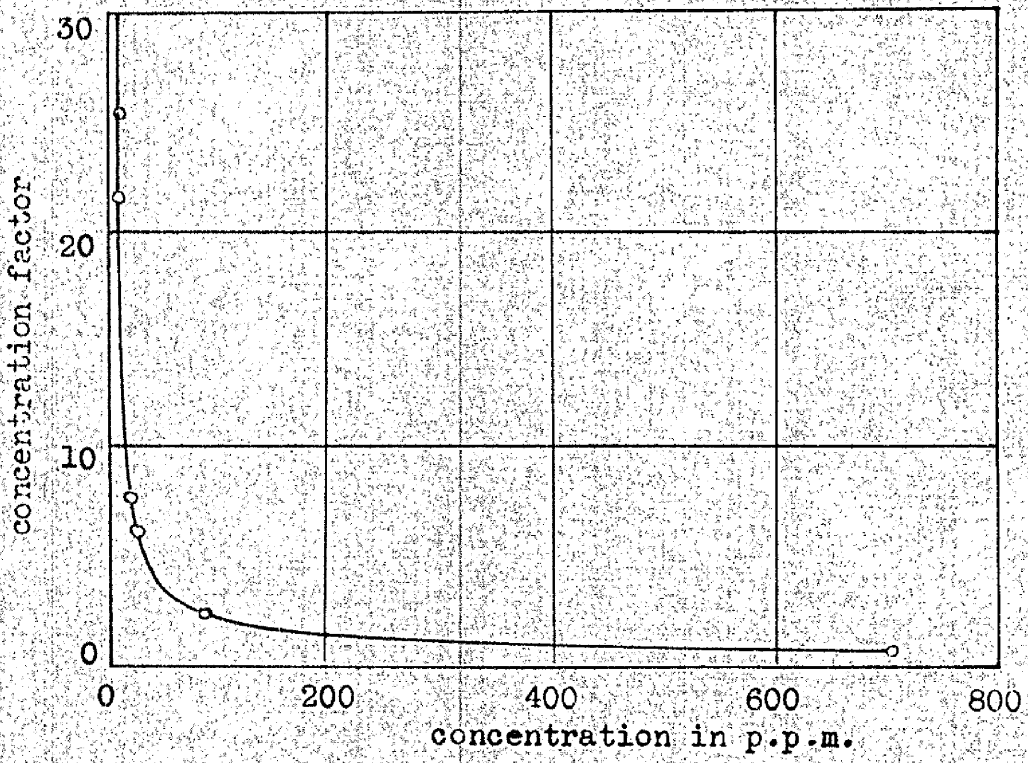


Figure 12. Relation between concentration factor and concentration of calcium in uptake solution.

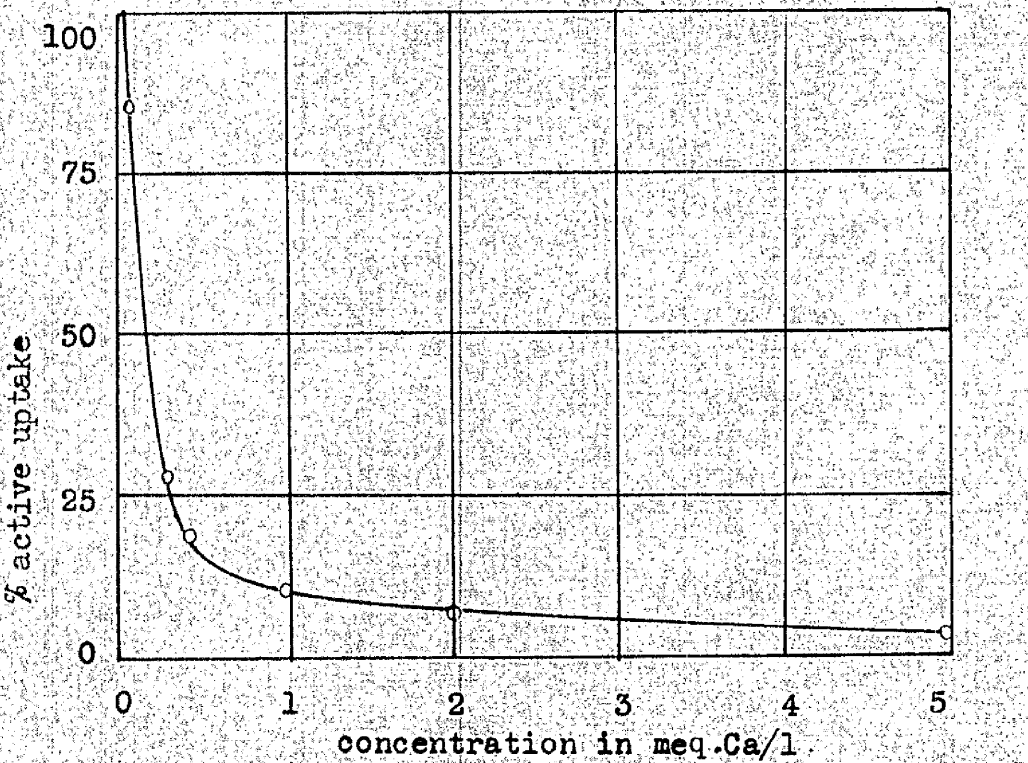


Figure 13. Relation between percentage active uptake and concentration of solution used.

TABLE III

Concentration of calcium in external solution (meq/l)	Absolute activity absorbed (c.p.m.)
35	$7{\cdot}0 \times 10^6$
5	$9{\cdot}0 \times 10^6$
2	$10{\cdot}0 \times 10^6$
1	$10{\cdot}0 \times 10^6$
0·5	$10{\cdot}5 \times 10^6$
0·3	$9{\cdot}0 \times 10^6$

It would appear from this curve that the amount of calcium taken up by the roots decreases as the concentration of calcium in the external solution is increased.

If 1 meq. of calcium is taken to contain a certain amount of activity and the relative amounts of activity absorbed at each concentration are calculated using the concentration factor, then an estimate of the absolute activity absorbed is obtained. It was found by this calculation that absorption was maximal between 0·5 and 2·0 meq. Ca/litre. (Table III).

2. Active Absorption:-

In the original uptake curve (e.g. Figure 7) the part of the curve above the shoulder (BC) is taken to represent the period of active absorption (45,86). By extrapolation of this line to zero time, (BA) an estimate of the active uptake is obtained from the difference between the count at 1440 minutes (C) and the count at zero time (A). This calculation was made at each concentration and converted to percentage active uptake of the total uptake at 1440 minutes. This percentage was related to concentration as shown in Figure 13.

A calculation of the activity absorbed by active processes as absolute activity showed that the latter increased with decreasing concentration of calcium in the external solution.

3. Effect of pH level:-

Calcium chloride solutions of 0·5 meq.Ca/l. were prepared with

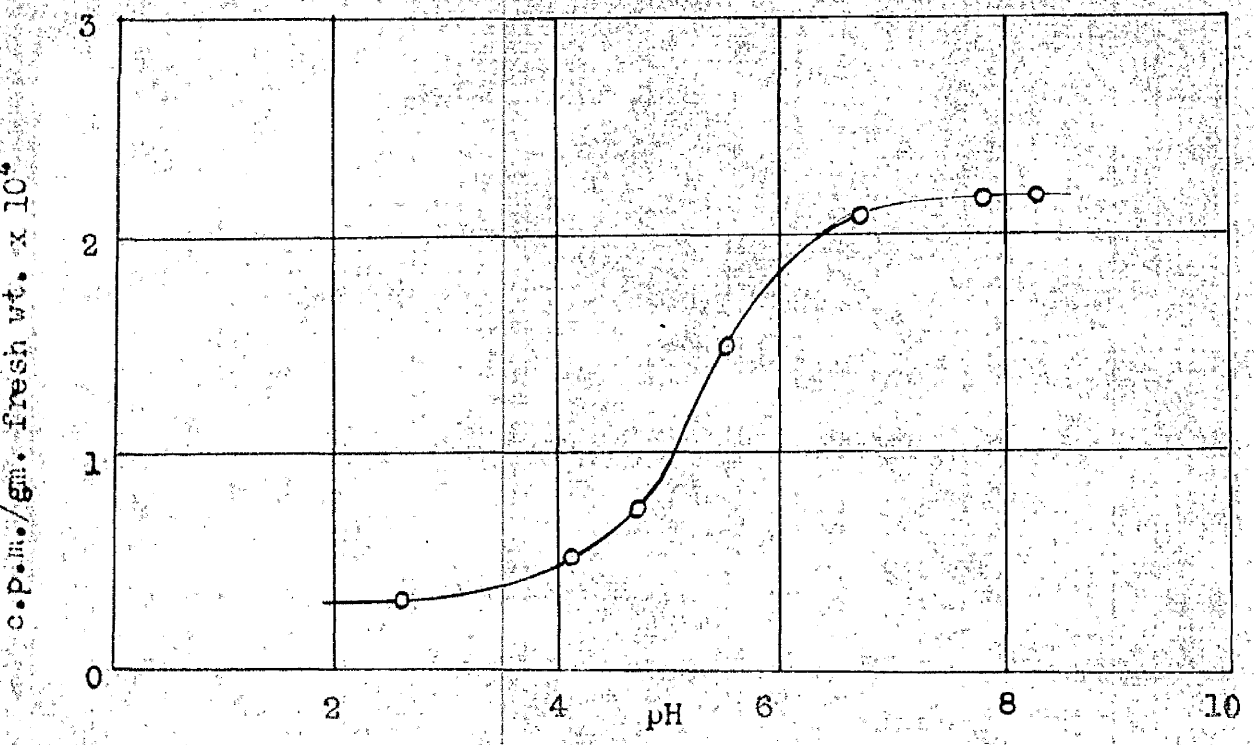


Figure 14. The effect of different pH levels on the absorption of calcium into onion roots

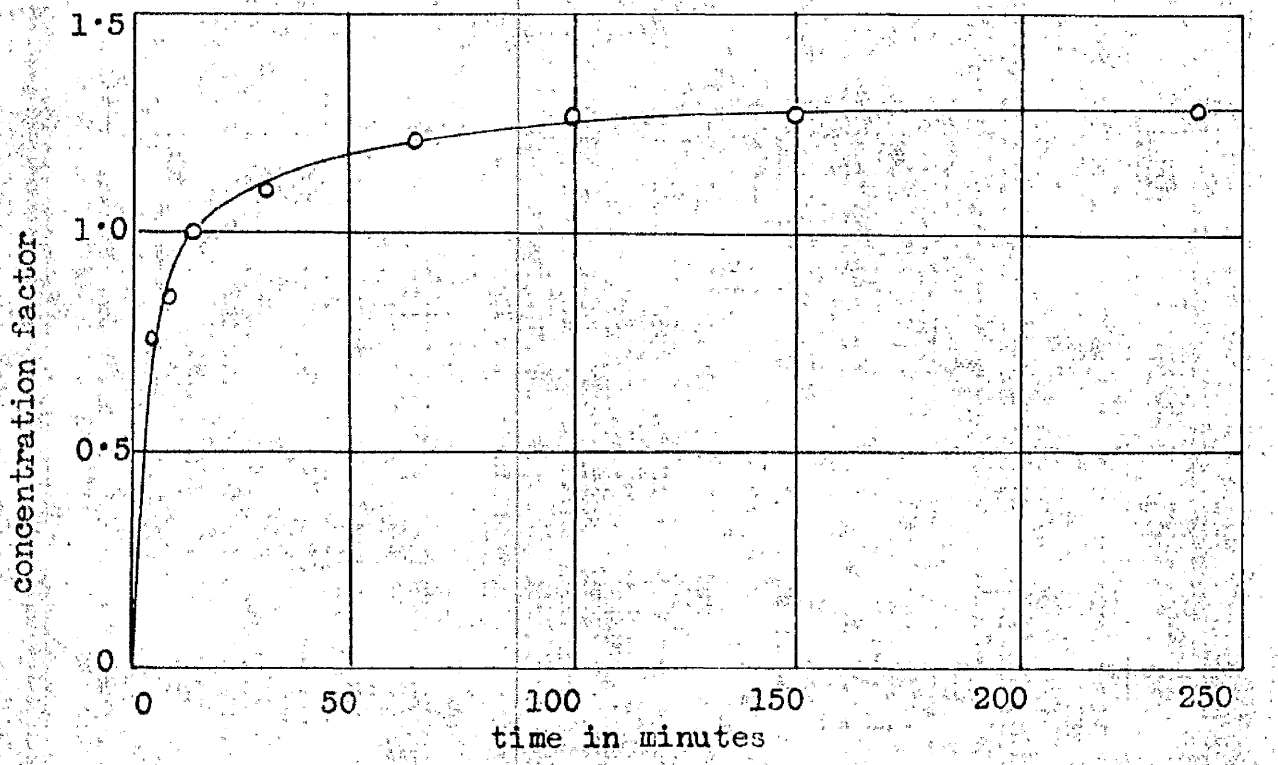


Figure 15. Absorption of Ca^{45} by onion roots from a Hoagland solution.

hydrochloric acid and ammonium hydroxide such that a range of pH values was obtained between pH 2.8 and 9·4, as measured on a Cambridge pH meter. Figure 14 represents the uptake of calcium by onion roots (c.p.m./gm. fresh wt.) at various pH levels. The results agree with Olsen's findings (131) that at pH levels above 5·5, cation uptake is increased while below this level, the uptake is diminished. It would appear that at pH 5·5 anions and cations are equally available for absorption.

4. Uptake from a Full Nutrient Solution:-

The absorption of calcium from a full nutrient solution was followed by repeating the uptake procedure for a single salt but using a Hoagland solution containing Ca^{45} (Figure 15). The presence of other cations did not appear to alter the absorption characteristics but the concentration factor was less than that obtained from a single salt treatment, using a solution of the same calcium concentration.

EXCHANGE OF CALCIUM.

1. Exchange Curve:-

Onion roots were treated with a labelled calcium chloride solution of 0·5 meq.Ca/l. for 240 minutes at 30°C, rinsed three times in deionized water, blotted and transferred to an unlabelled solution of the same concentration of calcium. Samples were taken off after

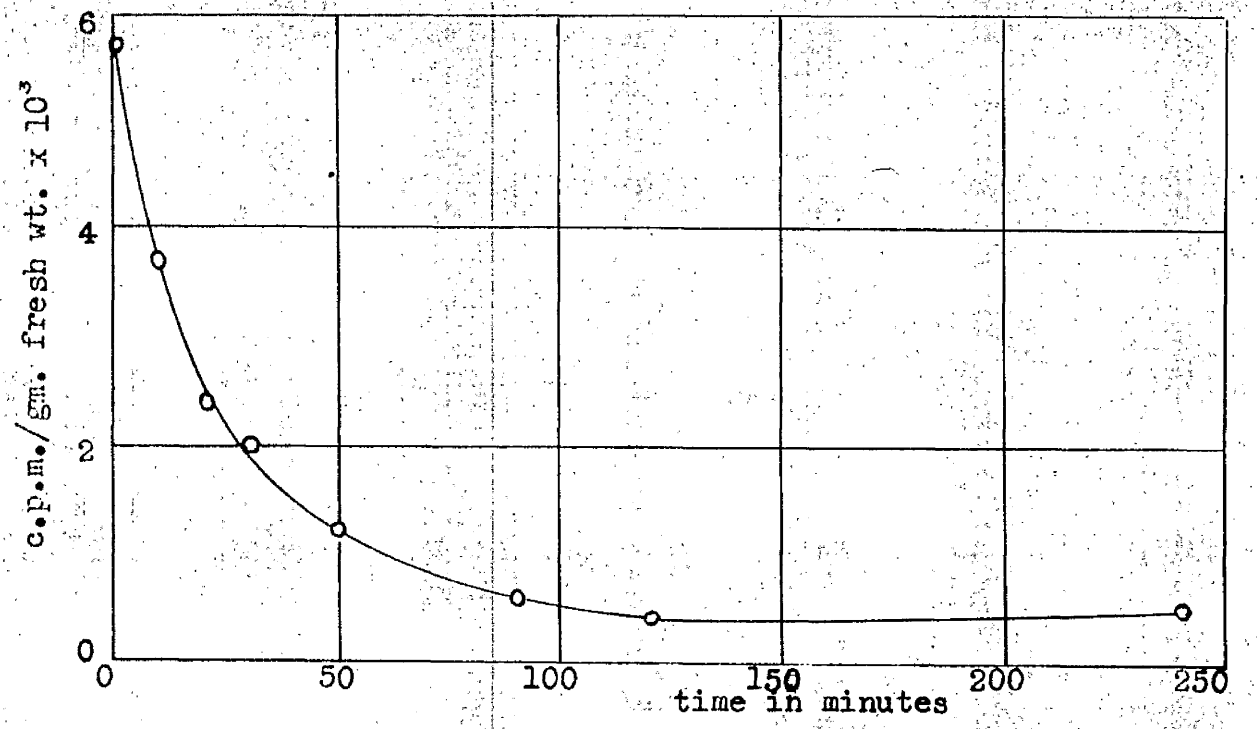


Figure 16. Exchange curve for calcium using onion roots (0·5 meq/l.)

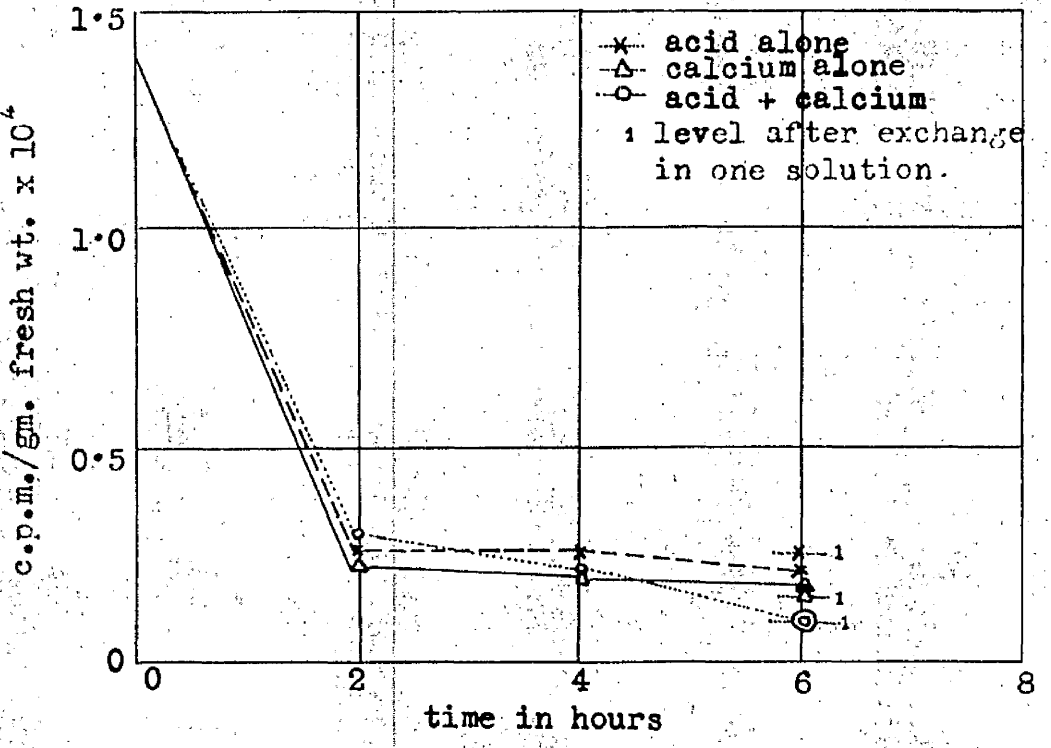


Figure 17. The effect of renewed solutions and pH on the exchange of calcium in onion roots.

several time intervals and counted as before. The exchange curve of c.p.m./gm. fresh weight against time is shown in Figure 16. It is evident from this curve that exchange did not go to completion under the conditions of the experiment. The activity (c.p.m./gm.) left in the material was 1·5 times the count per ml. of the labelled treatment solution and approximately 40 times the count per ml. of the exchange solution after use. The calcium remaining in the roots after exchange is considered to be actively absorbed.

2. Exchange in Several Solutions and at Low pH levels.

To determine whether exchange was complete under the conditions outlined above, exchange was carried out in three successive solutions containing 0·5 meq.Ca/l. The effect of a low pH on the exchange of calcium was also investigated by exchanging in a calcium chloride solution prepared with hydrochloric acid to give a pH of 2·8 . The results are presented in Figure 17. It would appear that there is no difference in the extent of exchange when three successive exchange solutions are used. At the lower pH level, exchange appeared to continue after 6 hours and might have gone to completion with increased exchange times. This would suggest a loosening of some binding of calcium which does not occur at the normal experimental pH of 5·5 .

3. Exchange in a Full Nutrient Solution:-

An exchange curve was obtained by repeating the above procedure

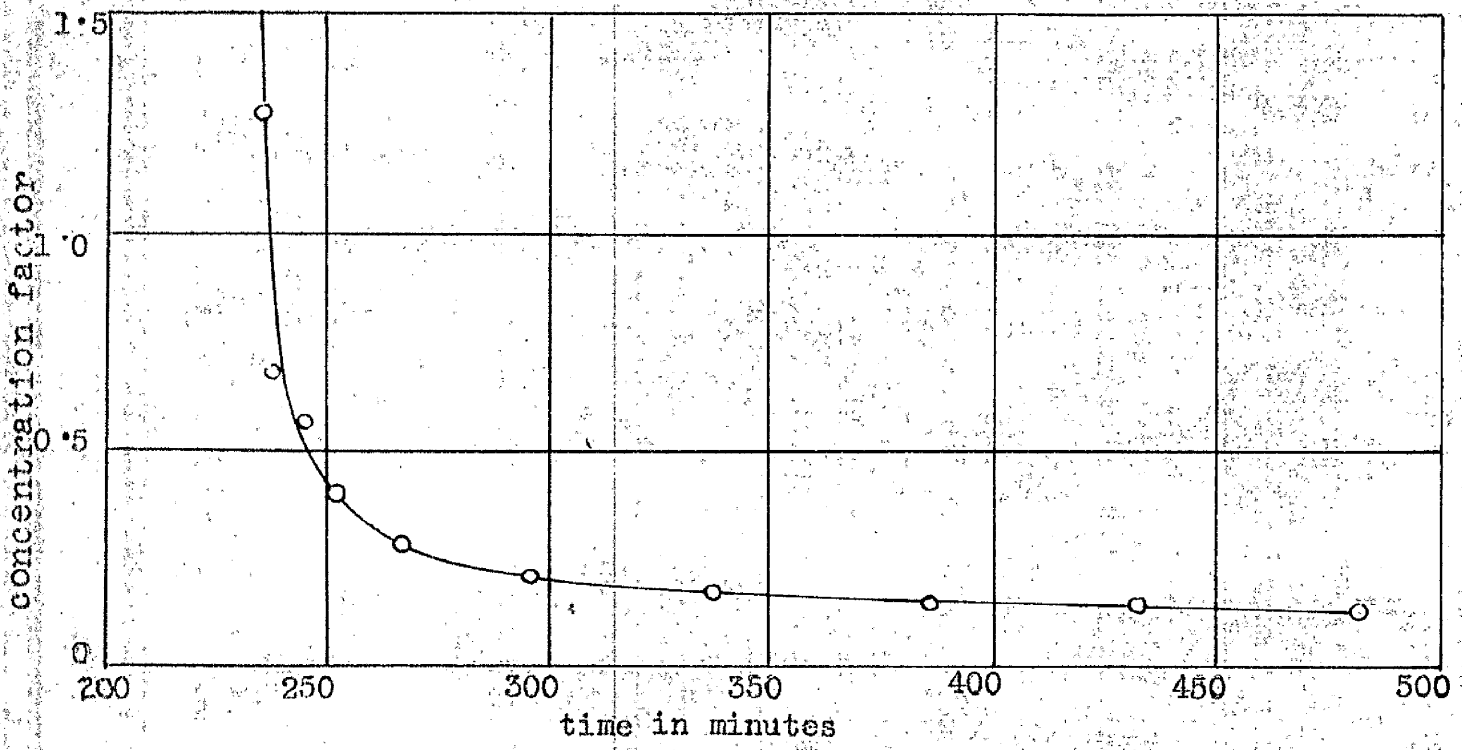


Figure 18. Exchange of Ca^{45} in Onion Roots in Hoagland Nutrient Solution.

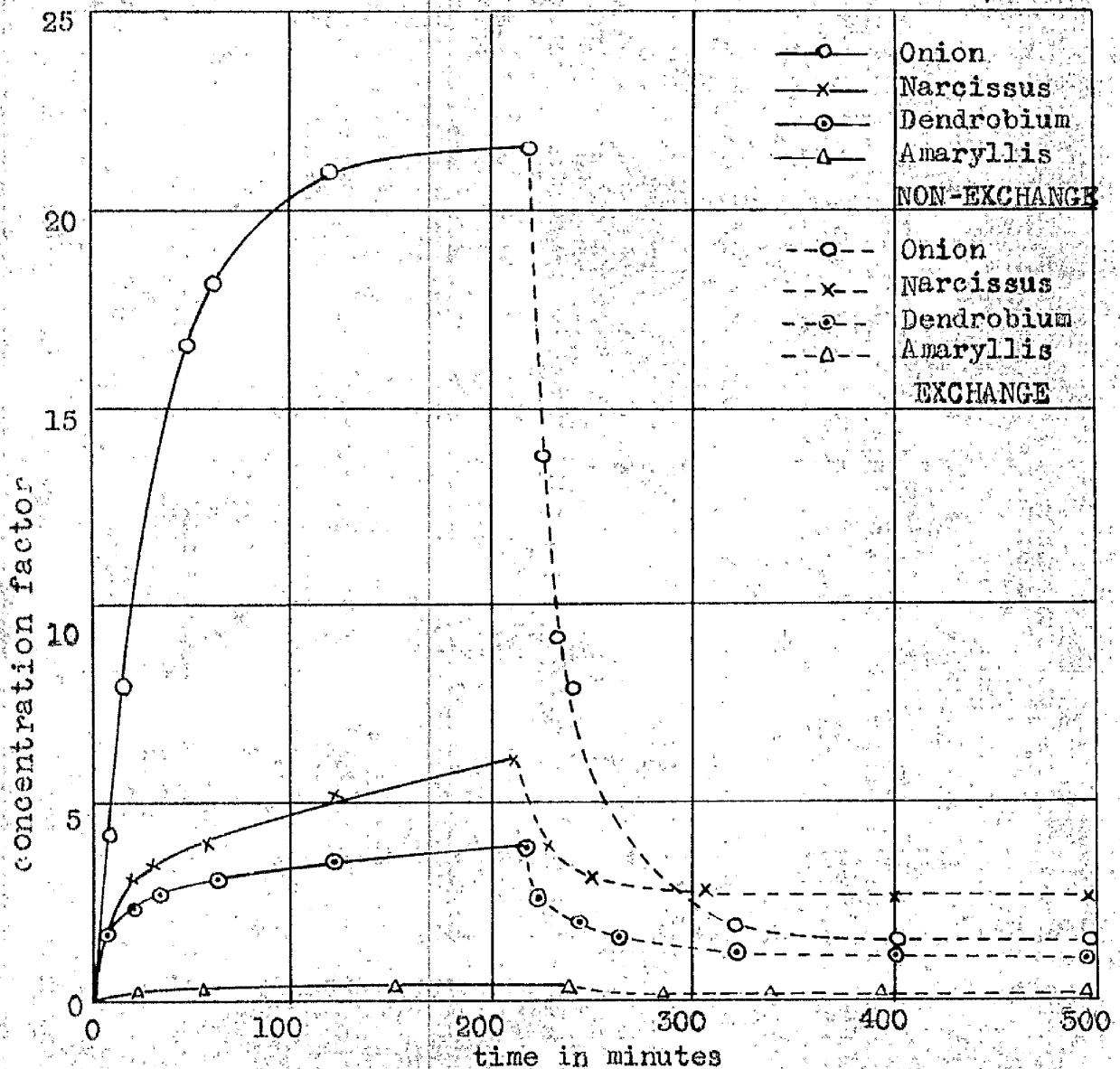


Figure 19. Comparison of Absorption by Four Root Materials.

using a full nutrient solution for exchange (Figure 18). The exchange characteristics were similar to those obtained by exchange in a single salt solution. The final count per gm. of root material was found to be greater than the count per ml. of the uptake solution from which it is assumed that the calcium retained by the root was actively accumulated.

ROOT MATERIALS.

The uptake and exchange characteristics of roots of Narcissus sp., Dendrobium sp. and Amaryllis hippeastrum were obtained by repeating the procedure followed with onion roots. The calcium concentration used was 0·5 meq.Ca/l. and the results for the four different materials are compared in Figure 19. where the concentration factor is plotted against time. Amaryllis hippeastrum appears to absorb calcium very slowly and under the conditions of the experiment, the count per gm. of roots did not equal the count per ml. of the external solution. From the differences obtained in the concentration with these roots, it would appear that the absorption of calcium by excised roots varies greatly from genus to genus though the physiological state of the plants may have a considerable effect.

It was decided as a standard procedure to treat onion roots at 30°C with Ca^{45} as a solution of calcium chloride containing 0·5 meq.Ca/l. at a pH of 5·5 for more than 200 minutes to include the active phase of absorption. Exchange would then be carried out in one solution of calcium chloride of the same concentration, pH and temperature for the same time.

DEVELOPMENT OF A METHOD OF FIXATION.

The successful application of the autoradiographic method at the cellular level requires that the method of fixation shall prevent the displacement of labelled ions or molecules from the locations which they occupy at the end of the experimental treatment. The difficulties of achieving this are maximal when, as in the present investigation, the material being studied is largely in the form of freely soluble, readily diffusible ions. While it was realised therefore, that the classical methods of liquid fixation were likely to be of little use in this case, tests were carried out to determine whether some significant fraction of the absorbed tracer was present in a form not readily removable by the liquid treatments.

LIQUID FIXATIVES.

1. Roots treated as described in the previous section were fixed in Carnoy le Brun fluid for 15minutes, washed in alcohol and taken through xylol to paraffin. Counts on the solutions after use showed that considerable leaching of Ca^{45} had in fact occurred, mainly in the fixative but also in the alcohol washes. Autoradiographs of the apical region of the root showed many large reduced grains after very short exposure times. These appeared in the same focal plane as the autoradiographic film and were not due to precipitation of the stain. It was considered

that an artefact had arisen due to some constituent of the fixative. Ca^{45} was located only above the outer layers of cells of the root and there was no visible localisation in the cortex or endodermis of exchanged or non-exchanged roots as was found with the freeze dried material. It was assumed therefore, that most of the Ca^{45} had been leached from the tissue.

2. Scott Russell (132) has detailed a method of precipitation 'in situ' of water soluble P^{32} with a basic lead acetate solution in alcohol at -70°C . A parallel precipitation was carried out on roots labelled with Ca^{45}. Alcohol saturated with oxalic acid at -70°C was used but on counting the solution after use, it was found that practically all the calcium had been leached from the tissue.

FROZEN SECTIONING.

Frozen sectioning was attempted on a horizontal sledge microtome with the blade cooled by carbon dioxide. Sections were immediately brushed onto slides cooled on a dish of drikold and transferred to the darkroom. Sections were pressed against slides previously prepared with autoradiographic film and exposed in a bin of drikold. The practicability of keeping the sections frozen and the unavoidable formation of a layer of ice crystals above the frozen section resulted in a certain amount of leaching and loss of resolution. In addition the

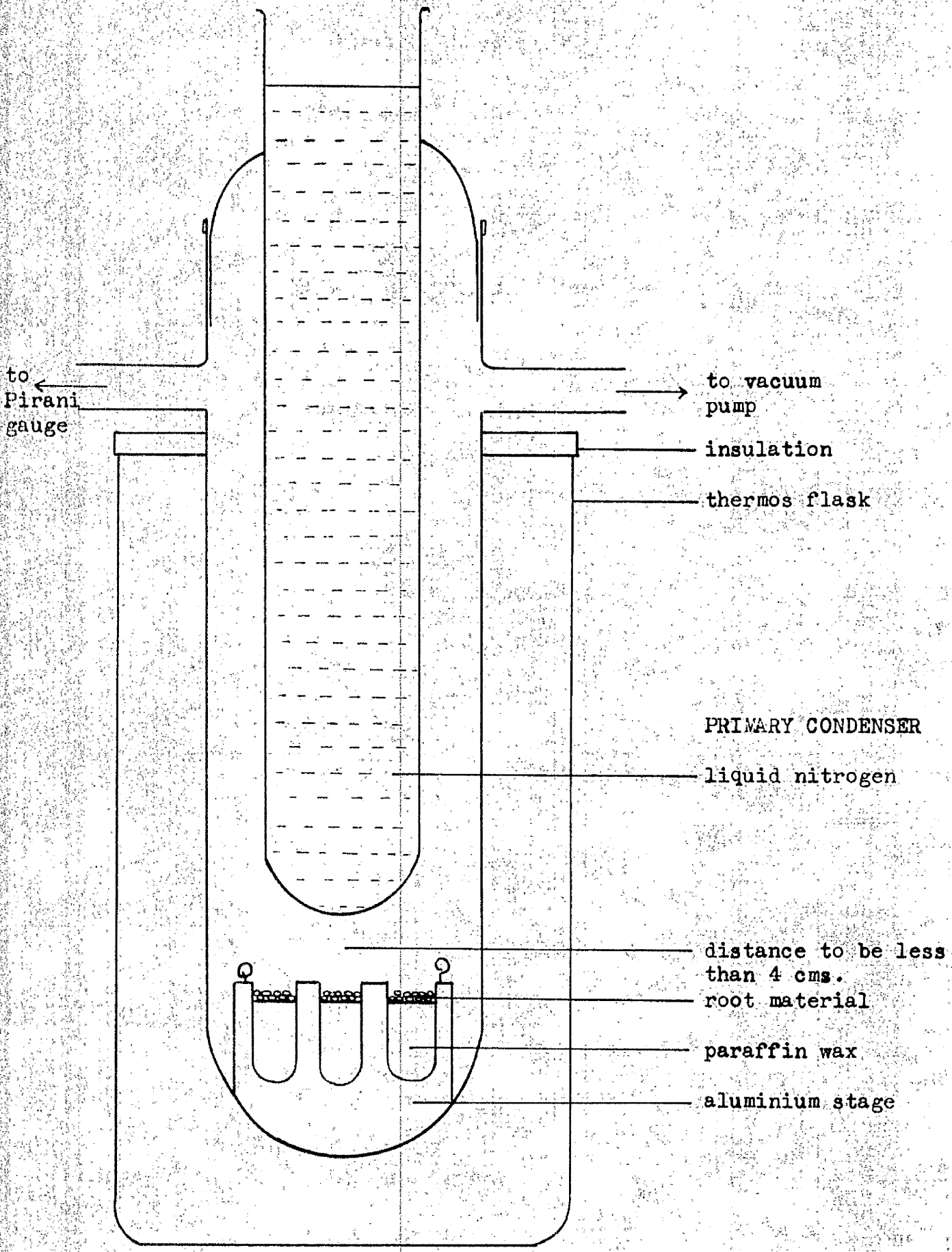


Figure 20. Freeze-drying Apparatus.

displacement of the sections during development prevented an accurate association of the autoradiographic image with any tissue. Realignment was not successful and the method was not further investigated.

FREEZE DRYING.

The primary concern is to prevent movement of water soluble ions in the material during freezing and drying. A temperature of -40°C is considered to be necessary to prevent enzymatic decomposition of the tissue (133,134) and it has been proposed that a specimen temperature of -55°C, or the eutectic point of the salt systems in the tissue, should be maintained during drying to prevent movement of inorganic ions. Gersh and Stephenson (135), however, consider that data on the eutectics of the various salts have no bearing on the problem.

1. Apparatus:-

This temperature requirement has determined the design of the freeze drying apparatus to be used. Glick and Malmstrom (136) have devised an apparatus for drying at very low temperatures but which consists of a single unit involving complicated glass blowing. The important features of their apparatus have been incorporated into the final design used in the present work. The apparatus is shown in Figure 20.

The drying chamber consists of an outer glass tube of 2·5 inches diameter with a ground glass connection to an inner glass tube or liquid nitrogen probe. During the primary drying the water drawn from the material

is condensed on the surface of the probe and is later removed when the drying under liquid nitrogen is complete,to a phosphorus pentoxide trap placed above the pump. The system is evacuated by means of a Speedivac Rotary pump No. 2SC50. (with air ballast) which is capable of a vacuum of below 0·001 m.m. mercury when liquid nitrogen is present in the system. Vacuum measurements are made on a Pirani gauge connected directly to the dehydration chamber. Other designs of apparatus connect the gauge between the pump and the drying chamber but it was considered that a more accurate value of the vacuum in the chamber itself would be obtained by placing the gauge after the chamber in the apparatus.

2. Drying Rate:-

To obtain the maximum drying rate of the material , several features of design and operation were considered. It was necessary that the material to be dried should be held at a distance of less than the mean free path of water molecules from the condenser surface. Under the expected drying vacuum, the calculated distance was 4 cms. An aluminium stage 2·5 inches in height and drilled to a depth of 0·75 inch was designed to carry the roots at the required distance from the probe.

The roots (1 inch in length) as used in the uptake experiments, although of small diameter, required excessive drying times. Therefore half inch roots were treated and after freezing were split into three using a cooled scalpel, forceps and platform (-70°C) to give pieces approximately 4 mm. in length and 1·5 mm. in diameter. These were readily

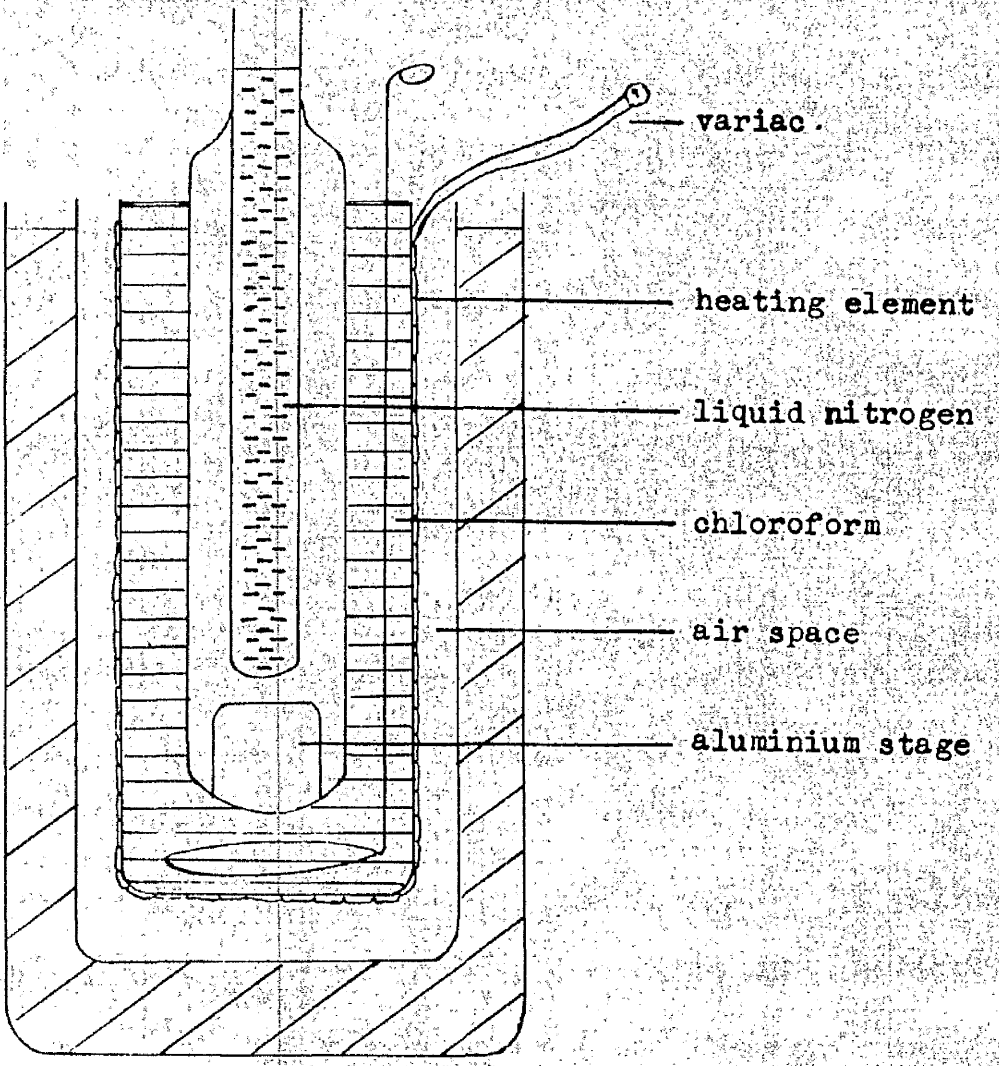


Figure 21. System for Maintaining the Freeze-drying Apparatus at a Steady Temperature

dried and embedded.

Any changes in the temperature of the material during drying was followed by means of a thermocouple. A copper/ nickel chrome thermocouple was calibrated against an iso-pentane thermometer (10°-200°C). The wires in the apparatus were covered with polythene tubing to prevent temperature effects due to the liquid nitrogen probe. Since it was not possible to attach the thermocouple to the aluminium stage, an 8 mm. cube of 5% agar was frozen in liquid nitrogen on the thermocouple tip and immediately introduced into the apparatus. The temperature rose to -49°C and this was maintained during drying. A steady specimen temperature of -55°C was obtained by holding the material in liquid nitrogen in the apparatus and then applying the vacuum .

Andrew and Hale (137) recommend the use of a thermistor system to control the temperature of the material during drying but the use of a cryostat in the apparatus as shown in Figure 21. was considered to be as effective. The cryostat employed was chloroform in this case, which solidifies at -63·5°C but constant stirring of the cryostat was required to maintain an even temperature along the length of the tube. The drying rate using this system was approximately the same as that in which no external coolant was used. The use of an acetone/drikold mixture immediately around the drying chamber slightly decreased the drying rate.

A drying rate curve was obtained for the above system but with no external coolant. Samples of onion root material were taken off after

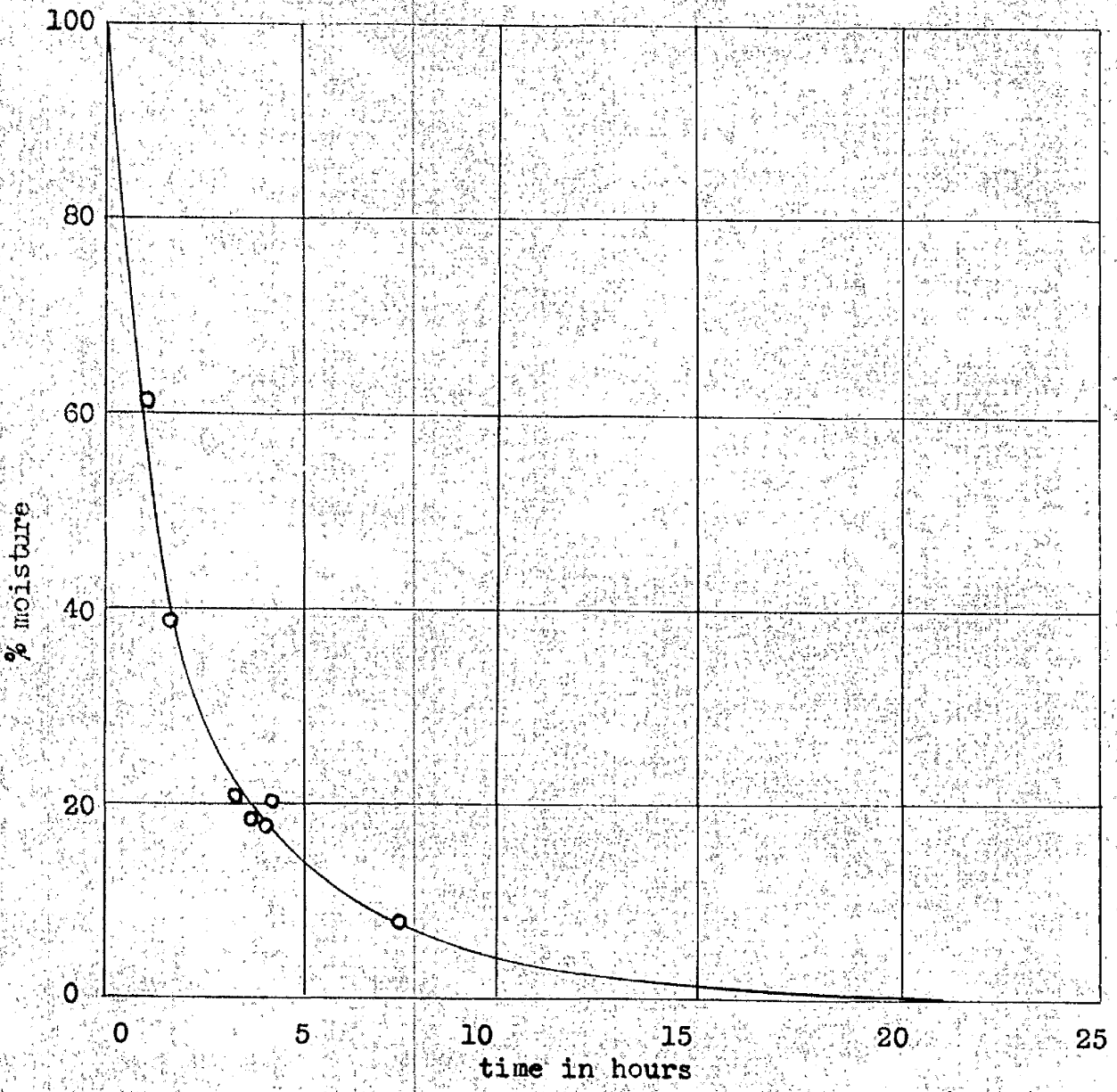


Figure 22. Drying rate curve for onion roots.

different intervals of time, weighed and dried in an air oven to constant weight (Figure 22). When the drying time exceeded 10 hours the apparatus was held overnight in acetone/drikold and drying continued the following day. The period in acetone/drikold did not apparently cause any appreciable drying of the material. From the drying curve it can be seen that approximately 90% of the moisture was removed after 24 hours in liquid nitrogen and it was found that drying with phosphorus pentoxide after this period was similar to prolonged drying in liquid nitrogen. The moisture content after 24 hours was lower than the corresponding monolayer condition (B.E.T. theory) (138) and would not form a continuous phase permitting ionic diffusion. The rise to room temperature would not therefore be expected to allow movement of calcium. Only small amounts of material could be dried at one time which resulted in very low weights of freeze dried material. The limitation of the balance increased the percentage error in determinations of residual moisture in material of less than 10% moisture.

The drying curve, however, does give an indication of the time required for drying under the adopted system. The conditions selected involved drying for not less than 30 hours in liquid nitrogen followed by not less than 100 hours with phosphorus pentoxide as the secondary condenser. Freezing was carried out in iso-pentane cooled with liquid nitrogen to a temperature of -160°C and the material held in liquid nitrogen in the grooves of the stage until the application of the vacuum such that a steady specimen temperature of -55°C was maintained during drying.

3. Tests to Detect Displacement of Calcium Ions:-

Freezing in iso-pentane cooled with liquid nitrogen is very rapid and it was not anticipated that displacement of inorganic ions would occur. However, the following tests were carried out to confirm this assumption.

5% agar gels were prepared with (a) water (b) a calcium solution of 0·5 meq.Ca/l. (c) a labelled calcium solution of 0·5 meq.Ca/l. and an activity of $3·5 \times 10^3$ c.p.m./8 mm. cube. 8 mm. cubes of each gel were joined together on a fuse wire and immediately frozen in iso-pentane cooled with liquid nitrogen. Counts were made of each gel after freezing for several time intervals up to 30 minutes. The maximum count in the unlabelled gels was 6 c.p.m. which could be attributed to contact of the pieces rather than to diffusion of Ca^{45}. Less than 15 seconds was required to freeze a 1 cm. cube of agar gel to a temperature of -160°C.

A similar examination of the adopted freeze drying procedure was made by drying three cubes of gel prepared as above, under the normal drying conditions. Counts were obtained of the same order as those in the tests on freezing. From autoradiographs on x-ray film of the dried gels, it was apparent that no displacement of calcium had occurred. The characteristic pattern of calcium absorption was found to be exactly reproduced in each of eight separate freeze drying runs which would also support the contention that the procedure developed in this instance does not permit displacement of inorganic ions.

4. Embedding:-

It was found to be an advantage to embed the dried material in the apparatus without breaking the vacuum. Paraffin wax (M.Pt. 54°C) was therefore deaerated in the cavities of the stage before drying of the root material. Embedding was rapid (15 minutes) but was usually continued for 3 hours, after which the solid paraffin cylinders, with the root material embedded, were transferred to a 55°C incubator for 12 hours. In this way almost all the roots were completely infiltrated with wax.

DEVELOPMENT OF THE TECHNIQUE OF AUTORADIOGRAPHY.

The technique of autoradiography originated from studies by Becquerel on fluorescence but the results were not recognised as being due to radioactivity until uranium compounds were found to produce the same effect on photographic plates. Several new techniques have been developed in recent years with the introduction of more sensitive emulsions and the ready availability of a wide range of isotopes. In the present investigation, the stripping film technique originated by Pelc (139) was selected for use since it is capable of the highest resolution with thin sections of material.

1. RESOLUTION.

Resolution is an arbitrary term which is used to indicate the relative merits of an autoradiograph. In the autoradiographic image the reduced grains are often grouped together to form aggregates. Quantitative measurements of resolution have been based on the diameter and density of these aggregates or on the distance between separate aggregates. However, from a practical point of view, in the work with sectioned material, the concept of resolution has come to mean the precision of locating a source of activity in a specimen, that is to say, the more precisely one can associate the autoradiographic image with a histological structure or single cell, the higher is the resolution.

There are many factors which influence resolution, such as exposure time, isotope and section thickness so that it becomes difficult to standardise this property. Quantitative measurements on autoradiographs are only feasible where the conditions of preparation of the autoradiographs have been exactly reproduced.

2. SECTIONING.

Sections were cut on a horizontal sledge microtome from paraffin blocks. It was desirable that the sections should be less than one cell thick such that the image obtained could be accurately related to a single cell without interference from activity in cells or parts of cells underneath. In work with water soluble isotopes, floating-out of the sections to eliminate crinkling is not possible and therefore the thinnest sections available which remained flat after sectioning were 10μ thick. These were pressed onto cleaned slides and the paraffin removed with xylol to prevent ballooning when the slides were developed.

3. IMPERMEABLE FILMS.

To obtain maximum resolution it is necessary to apply the autoradiographic film directly above the sections since the greater the distance of the film from the source of activity, the greater is the

area of the emulsion over which the rays from the source cause grain reduction. In the stripping film technique, the film is applied wet and therefore an impermeable film must be included above the section to prevent leaching of the isotope. To maintain optimal resolution it was necessary that the film applied should be less than 1μ thick. At this thickness dry films could not be handled and the selection of the film material therefore depended on the use of a solvent which would not cause leaching. A 1·5% wt./vol. solution of Saran (1000 c.p.s. vinylidine chloride) in methyl ethyl ketone was found to be suitable. The surface of the Saran coating was strongly hydrophobic when dry and it was found that the autoradiographic film was readily displaced during development. To prevent this, an intermediate film of celloidin was used. Good contact between the autoradiographic film and the slide was obtained by dipping the Saran coated slide into a 1% wt./vol. solution of celloidin in a 50/50 vol./vol. mixture of absolute alcohol and ether, 30 seconds before the application of the autoradiographic film. It was assumed that leaching did not occour during coating with celloidin since the alcohol/ether solvent was not able to penetrate the Saran layer. The average distance from the section to the autoradiographic film was 1·1μ. The additional property of Saran in being able to withstand strong alkali, has decreased the damage to the sections normally caused by the developing solutions.

At first, sections were simply pressed onto slides coated with gelatin or celloidin but it was found that these layers adsorbed the stains used after development and were not readily destained by water

or alcohol. When a 0·5% solution of Saran was used as the primary adhesive, the sections were readily mounted and when the impermeable coating was applied, the result was an even layer of Saran enclosing the sections and this completely prevented leaching. It was found that the Saran layer adsorbed the stains to only a very limited extent.

4.STRIPPING FILM TECHNIQUE.

The advantages of the stripping film technique over other methods is that a very thin and uniform sensitive layer of silver halide can be applied to a specimen. Comparative studies of the various stripping emulsions have not been made but Boyd (140) gives estimates of the relative sensitivities as a guide in selecting a film. Kodak A.R. 10 emulsions are less sensitive but give a higher resolution than the Kodak A.R.50 emulsions which give a quick response to the activity in the specimen but have only a low resolution. The A.R.10 plates were used for investigations at the cellular level.

The stripping layer was divided into 6,8 or 12 depending on the area of the slide to be covered,and the pieces stripped slowly and evenly to prevent flashing due to electrostatic discharges. They were turned over before placing on the surface of a water trough such that the emulsion would face the sections when lifted. The gelatin expanded fully in 3 minutes in water which spread the emulsion into an even finer layer. The film was lifted by dipping the prepared slide under

the film which then wrapped tightly around the slide. The films were dried at room temperature and exposed at 4°C . Exposure of the films at a reduced temperature cut down the background on the slides.

5. EXPOSURE TIME.

The time of exposure which is necessary depends on the energy of the β emission and the half life of the isotope under investigation, the activity in the sections and the sensitivity of the film used. The sensitivity of the film is determined by the degree of resolution required and the selection of the isotope by the nature of the investigation undertaken. Calcium was selected in preference to strontium because of the low maximum energy of the β radiation of Ca^{45} which makes possible a higher resolution and reduces the spreading of the image. The activity in a section may be evenly distributed or localised in distinct areas. In the latter case a shorter exposure can be expected due to the concentration of activity. In a 10μ section the amount of isotope is very low and therefore the isotope applied must be of a high specific activity.

Estimates of exposure times can be obtained by calculation (141) but these are usually underestimates and the simplest method is to expose a series of slides of similar activity for different periods of time to determine the optimum exposure time for the particular isotope and material investigated.

6. DEVELOPMENT.

Kodak films were developed in D.19b developer for not more than 10 minutes since there is an increase in grain size with increasing development time, thus reducing resolution. The temperature of the developer and fixing solutions should be accurately controlled at 20 ± 0·5°C since uneven temperatures may lead to distortion of the image. The strongly alkaline developing solutions may affect the sections but in the present investigations the Saran layer almost entirely prevents this occurring.

7. STAINING.

Staining was found to be necessary and several methods were investigated.

(a) Vital Staining :-

Onions were grown for three days in water and transferred to 0·001% solutions of the following stains with varying effects:-

Janus Green: The stain was precipitated on the surface of the root, only the cell walls of the epidermis being lightly stained.

Malachite Green: All the tissues were stained but with insufficient differentiation.

Methylene Blue: The staining effect was satisfactory but this stain has been found to inhibit phosphorus metabolism and it was not investigated further.

Trypan Blue: The nuclei in the cells of the apical region of the root were clearly stained but the cell walls did not take up this stain.

Congo Red: Only the epidermal cell walls were stained. The stain was precipitated on the surface of the root.

Vital New Red: The stain was taken up by the epidermal cells only.

Crystal Violet: The nuclei were more deeply stained than the cell walls and a satisfactory degree of differentiation was obtained. A lower concentration of stain could have been used.

A simpler method of vital staining was devised where excised roots were treated with calcium solutions also containing a low concentration of the stain. Crystal violet and neutral red were used but as with the previous method, destaining of the sections occurred during the processing of the slides. This method, however, was used to give clear differentiation of non-exchanged and exchanged material by using crystal violet in the exchange solution and neutral red in the uptake solution. In this way, two different types of material could be freeze dried in the same run and be easily differentiated when embedded in paraffin.

(b) Staining after Development of the Autoradiograph:

The selection of a stain for use after development of the autoradiograph was restricted by the ease with which the celloidin layer and the gelatin of the emulsion take up the stain and the ease with which they can be destained. Normal botanical stains such as haematoxylin were found to stain the gelatin intensely and to destain only slightly in alcohol. Several stains, previously used in autoradiographic studies of animal tissues (142) were investigated as follows:-

Celestine Blue: The cell walls were only lightly stained. Little differentiation was found.

Toluidine Blue: The various cell parts were not adequately differentiated.

Crystal Violet: The gelatin layer of the emulsion became deeply stained.

Pyronine Methyl Green: This stain gave green nuclei and pale purple cell walls but was not found to be consistent in its staining action. Destaining had to be rapid to prevent complete removal of the stain from the section.

Neutral Red and Carbol Fuchsin: The definition obtained was not as clear as if a green counter stain had been used but sufficient clarity was obtained. This stain was selected for standard use.

The adopted procedure for the preparation of autoradiographs from plant material is given in Appendix I. Details of the isotopes used in the present investigation and the approximate exposure times required for 10μ sections are presented in Appendix II.

PRESENTATION OF RESULTS.

The largely visual nature of the evidence and, in particular, the fact that the autoradiographic image is not in the same focal plane as the section, create unusual problems with regard to the presentation of results.

The general distribution of activity in the root sections is demonstrated by means of scale diagrams showing the distribution of the main tissues and cell layers and the principal areas of blackening of the emulsion. The dots on these diagrams are not intended to represent reduced grains of an autoradiographic emulsion but to indicate the relative intensity of the darkening of the autoradiographic image. These diagrams are accompanied by high power drawings giving the precise relationship between the silver grains of the image and the fine structure of the tissue. Only the cell detail necessary to demonstrate this relationship has been included. The vacuoles are shown by a single line denoting the vacuolar membrane and the nuclei are shaded in. All diagrams and drawings have been made using a camera lucida.

Photomicrography has also been used and in this case, it has usually been necessary to prepare two plates for each field of interest, one focussed in the plane of the section and the other in the plane of the autoradiographic image. The result obtained with black and white photographs was not satisfactory since the black grains of the autoradiographic image did not contrast sufficiently with the cell structure.

Colour photography was more successful but the resulting photographs still are not comparable to the direct examination of the slides through the microscope. In this instance, one photograph focussed in a plane intermediate between that of the section and that of the autoradiographic image was found to be as effective as two photographs taken, one at each focal plane. Some definition was lost on printing and enlarging from the transparency but the overall effect is similar to a stained preparation.

The autoradiographs showing distinct aggregates of reduced grains were found to be most suitable for photography. Some prints of the distribution of calcium 45 in non-exchanged and exchanged onion roots have been prepared.

EXPERIMENTAL.

PART 2. AUTORADIOGRAPHY

STUDIES

WITH A DIVALENT CATION.

The autoradiographic technique using x-ray films has been applied in studies on salt uptake with whole plants. However, the distribution of activity obtained by this method can be related to the different plant parts only, leaves, veins, roots etc., since the technique does not permit a higher resolution. The most successful use of detailed autoradiography with plant material has been in cytological studies using P^{32}, S^{35} and As^{8} (149-151). No work with other isotopes has been reported on plant tissues at the cellular level of autoradiography.

The major part of the work reported in this thesis was carried out using calcium. Rubidium was used in a smaller number of experiments.

1. UPTAKE AND EXCHANGE OF CALCIUM IN LIVING ONION ROOTS.

Onion roots for autoradiography were treated with a solution of calcium chloride containing 0·5 meq. Ca/l. and having an activity of 5·5 μc/ml. Treatment with the isotope was continued for 16 hours to allow sufficient active uptake to take place, as shown by the uptake curve (Figure 7.). Some roots were treated with the above solution for

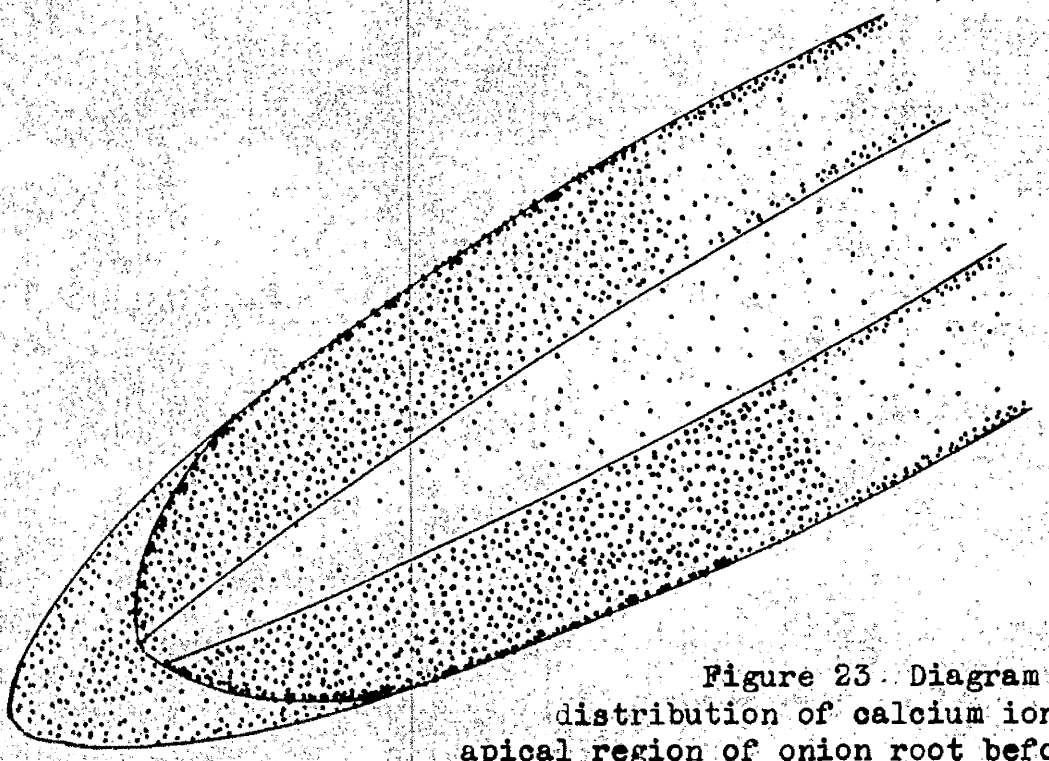


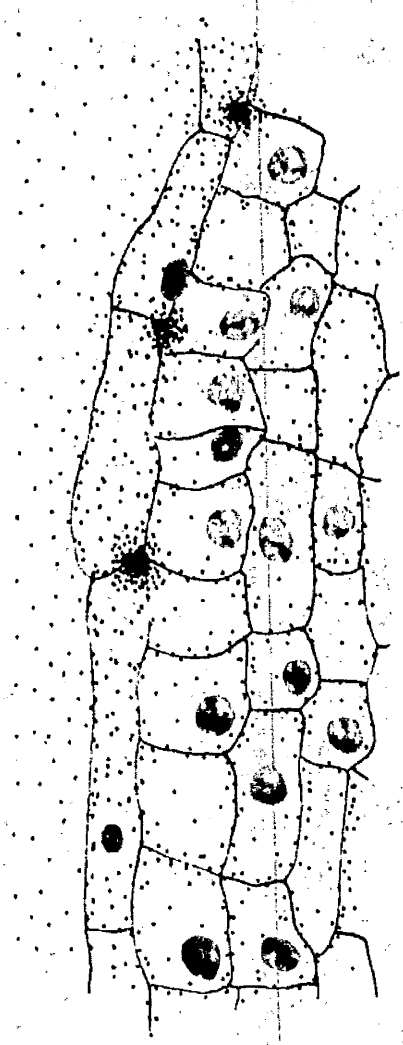
Figure 23. Diagram of the general distribution of calcium ions in the apical region of onion root before exchange

7 hours and subsequently exchanged for 16 hours in an inactive calcium chloride solution of the same concentration. Both types of material were freeze dried and prepared for autoradiography by the procedure outlined in Appendix 1. The material treated with the radioactive solution only is referred to as non-exchanged and that treated also with inactive calcium, as exchanged.

Autoradiographs of sections of roots from eight similar freeze drying runs were set up so that adequate reproduction of results could be demonstrated. Median sections, 10-40 μ thick, were used and an average of 150 slides were exposed from each run. The characteristic features of the autoradiographs were found to be readily reproducible.

General Distribution of Ca^{45} in Non-exchanged Roots:-

The distribution of Ca^{45} in non-exchanged onion roots is illustrated in Figure 25. There was a relatively high general darkening of the autoradiographic film above the whole section but some areas of calcium localisation were evident. A certain amount of calcium was present in the outermost layer of cells and central cells of the rootcap. The epidermis demonstrated a high calcium uptake, particularly in the first 3 mm. length of root back from the tip. Beyond this region the calcium was more closely associated with the hypodermis. There was a general darkening of the film above the cortex in the region 1-4 mm. back from the tip. Beyond this region, the number of reduced grains



? mod Cap

Figure 24. Longitudinal section of the apical region of onion root showing the localisation of calcium in the epidermis before exchange.

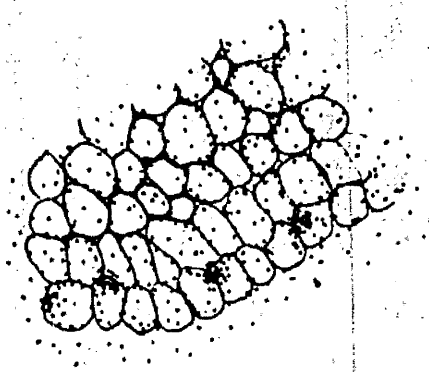


Figure 24a. Transverse section of the apical region of onion root showing the localisation of calcium in the epidermis before exchange

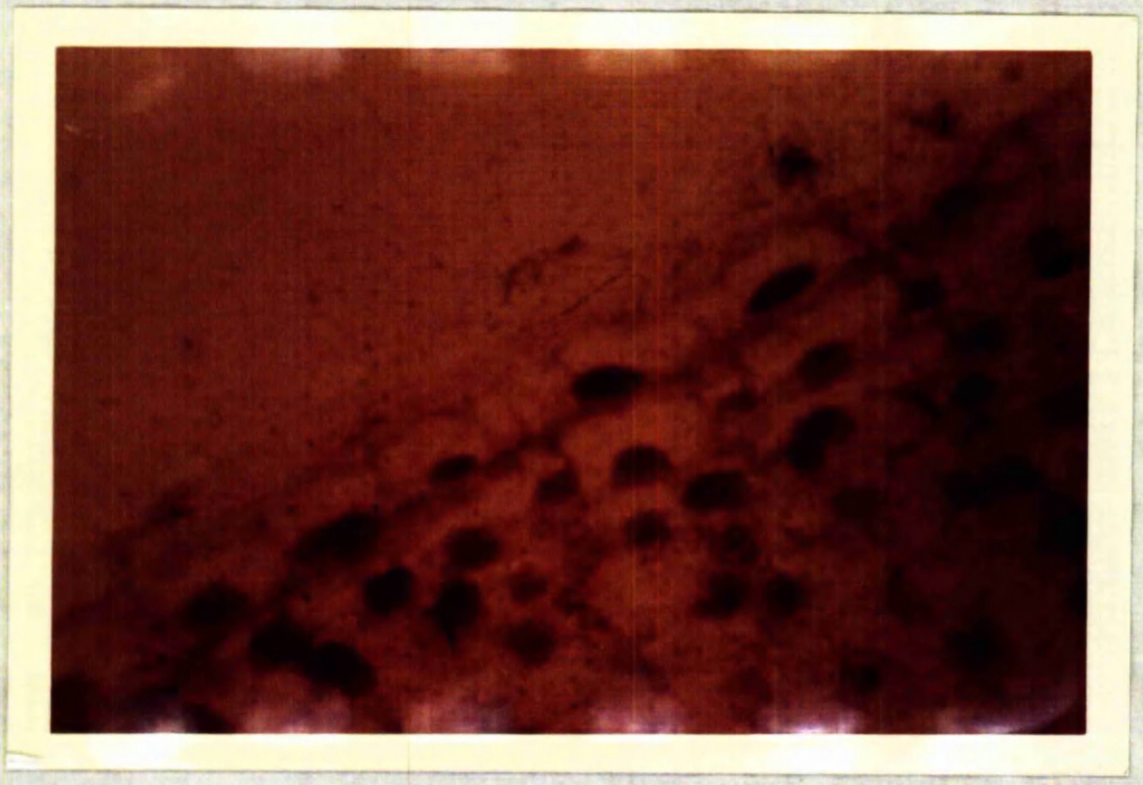


Figure 24b. Photograph of a longitudinal section of the apical region of onion root showing the localisation of calcium in the epidermis before exchange.

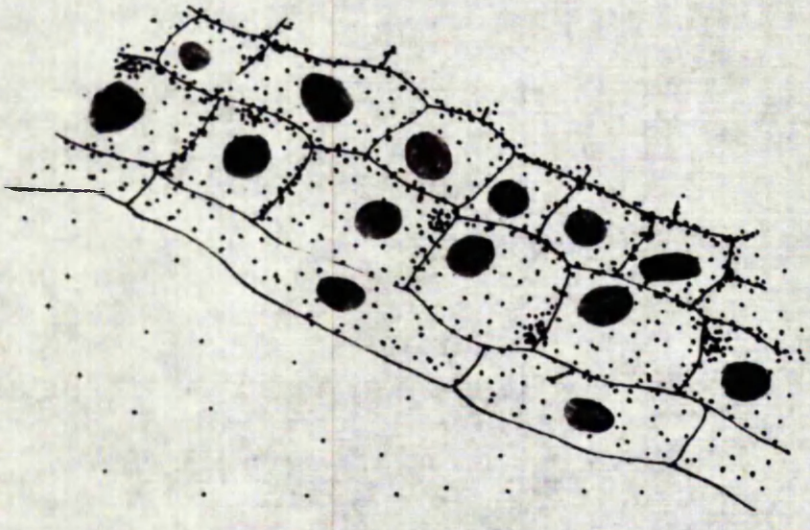


Figure 25. Longitudinal section of the apical region of onion root showing the localisation of calcium in the hypodermis before exchange.

was much lower. However, the endodermis, in this latter region, did show a definite localisation of calcium.

Distribution at the Cellular Level:-

Root Cap: The Ca^{45} localised in the outermost layer of cells formed aggregates of grains in the autoradiograph which were situated above the inner wall of the cell, that is to say, the cell wall not in contact with the bathing solution. The aggregates occurred mainly above the cells of the tip and no more than one aggregate was found above each cell. The uptake in the central cells appeared to be of the same order in the cell wall and the cytoplasm.

Epidermis: This tissue also absorbed calcium, the autoradiograph appearing as aggregates of grains. Again these were positioned above the cell wall, one per cell, and were found above this layer of cells in the meristematic region as well as the more mature tissues. Aggregates of grains were not found above those cells more than 3-4 mm. back from the tip. Figure 24 illustrates the localisation of Ca^{45} in this tissue.

Hypodermis: The activity associated with this tissue was confined to the cells more than 3 mm. back from the root tip. A few aggregates of grains were observed but the number of reduced grains above the cells was high. (Figure 25.)

Cortical Region: There was a general darkening of the film above the cortical parenchyma of the root tip, involving the meristematic

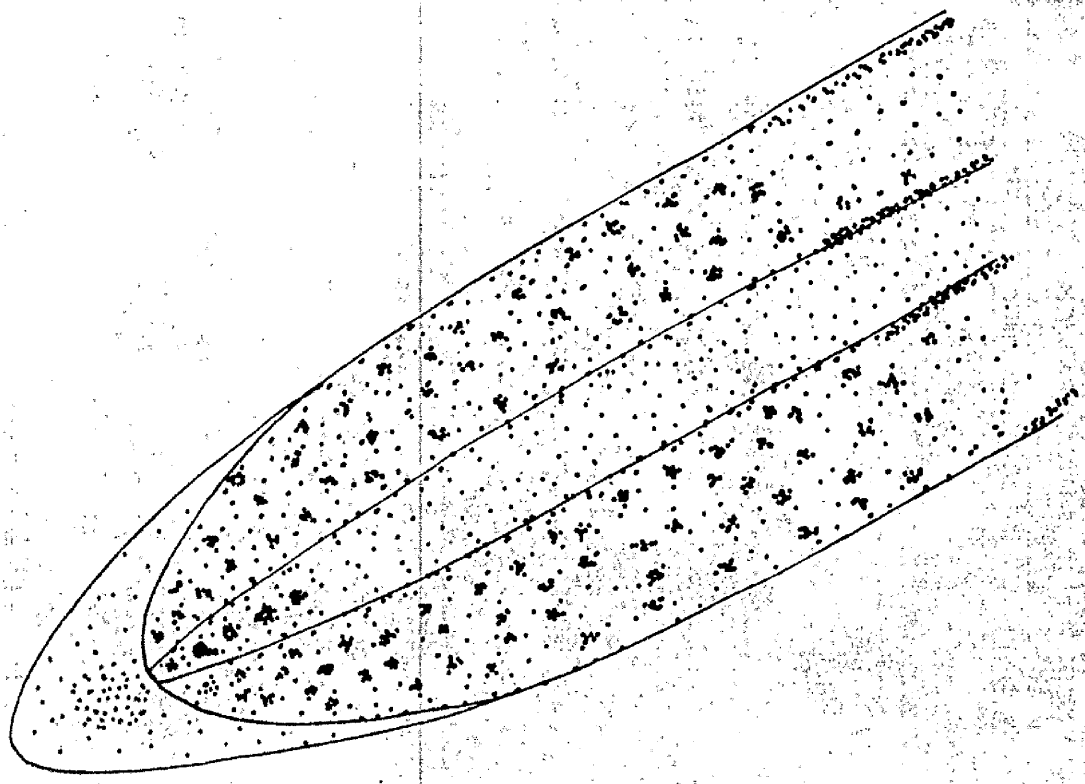


Figure 26. Diagram of the general distribution of calcium ions in the apical region of onion root after exchange.

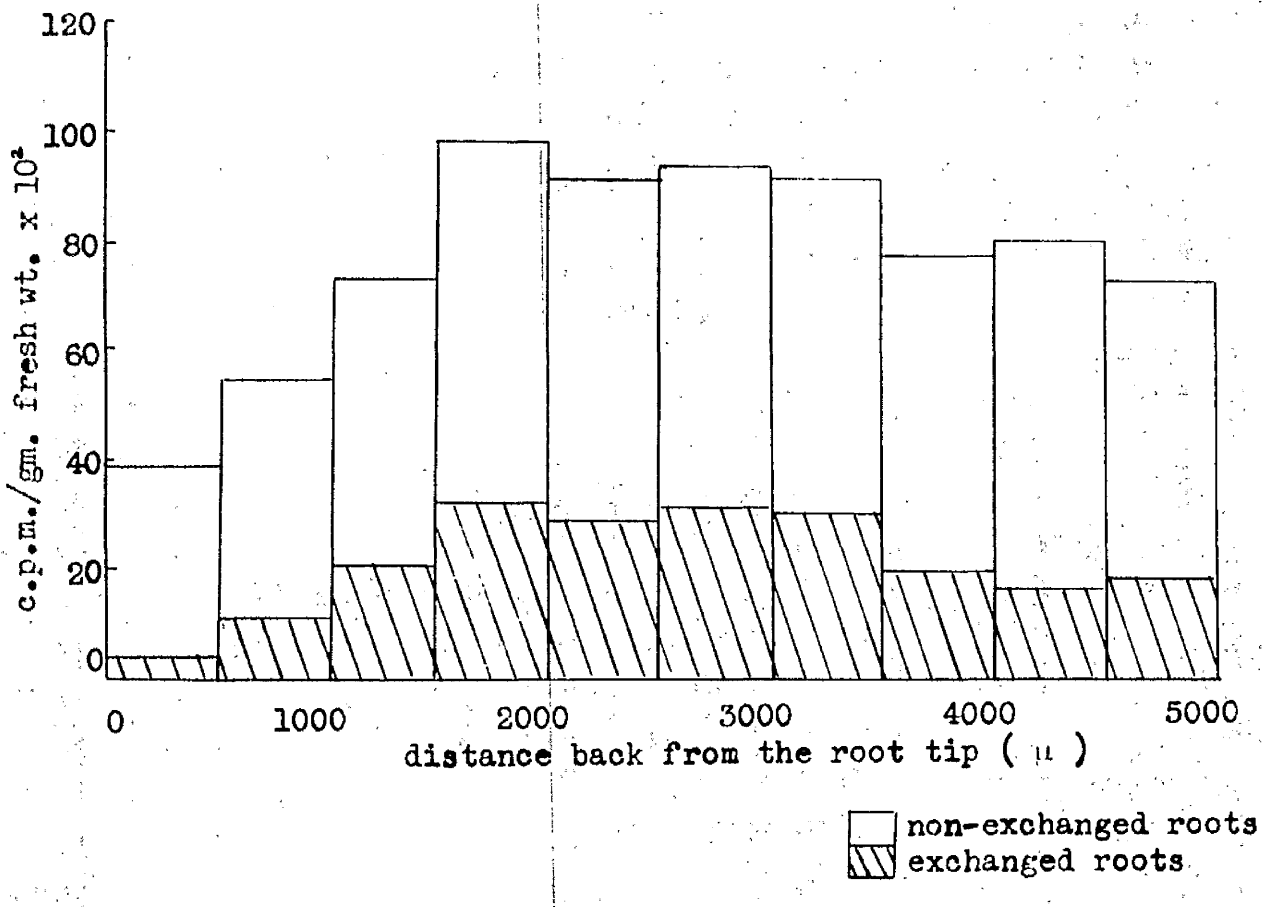


Figure 27. Distribution of Ca^{45} in non-exchanged and exchanged onion roots.

cells and the mature cells of the cortex up to a distance of 3-4mm. back from the root tip. No aggregates of grains were found.

Endodermis: The localisation of Ca^{45} was apparent as a general darkening of the autoradiograph above the cells more than 3 mm. back from the tip, no aggregates of grains being found.

General Distribution of Ca^{45} in Exchanged Roots:-

The autoradiographs of exchanged material have shown that calcium was retained predominantly in the cortical parenchyma, as well as the endodermis, hypodermis and the central region of the root cap. (Figure 26.). The retained calcium is localised over a definite length of cortical tissue stretching from the tip to a point approximately 3·5 mm. back from the tip. Counts on serial sections, cut transversely, showed that there was a zone of especially high activity per unit area of section in both non-exchanged and exchanged material, between 1·0 mm. and 3·5 mm. back from the tip (Figure 27.). For ease in describing this region it will be termed 'the region of calcium accumulation'.

Distribution at the Cellular Level:-

Root Cap: The activity in the outermost layer was removed by exchange but a few aggregates of grains were apparent in the central cells of the root cap. These aggregates were of a relatively small diameter and appeared to be situated within the cell wall but a more

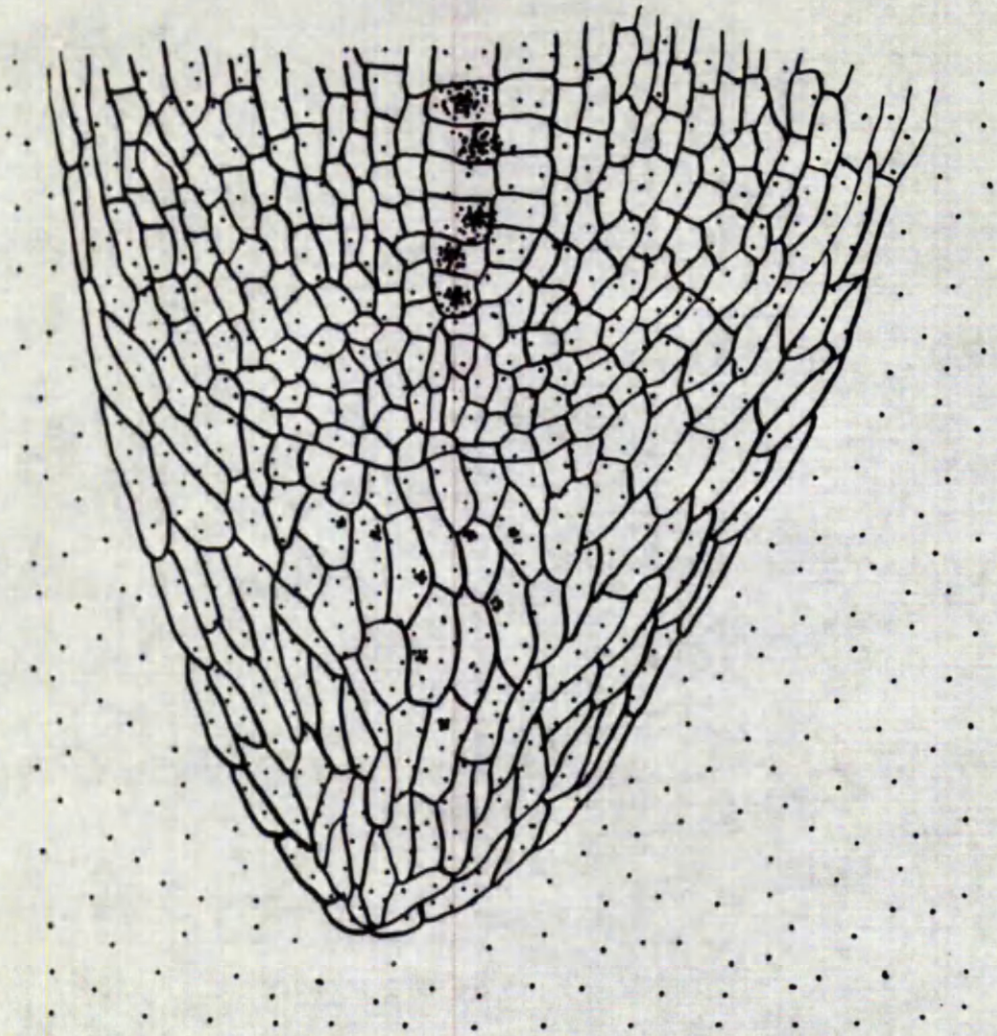


Figure 28. Tangential section of the apical region of onion root showing the calcium retained in the inner cells of the root cap and in the protoxylem initials after exchange.

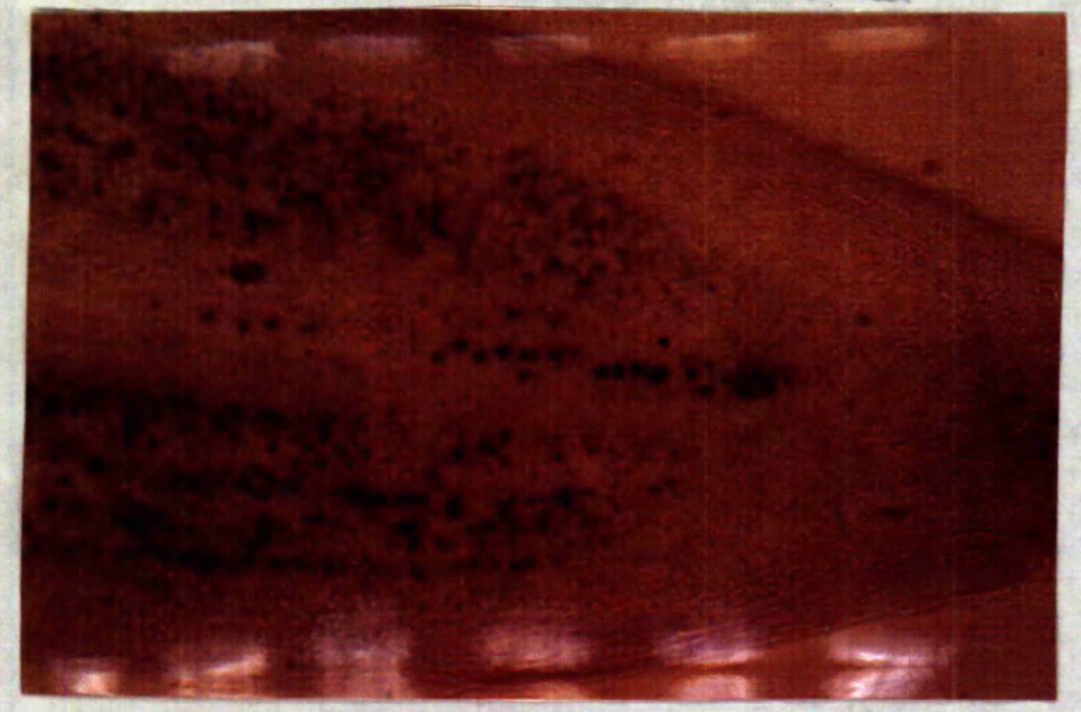


Figure 28a. Photograph of the above region.

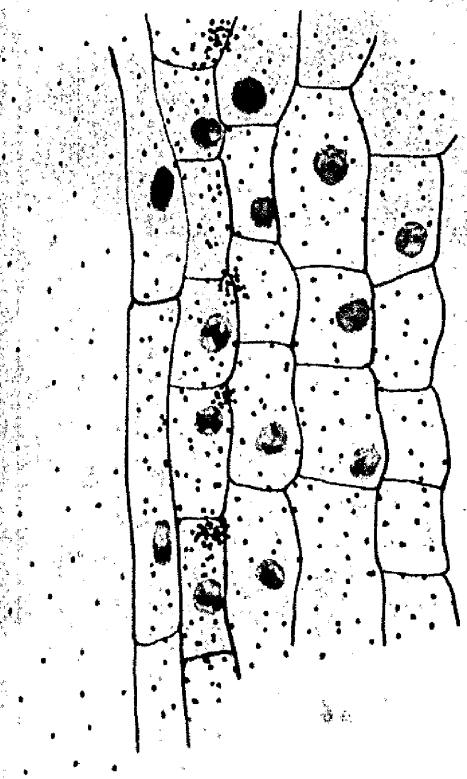


Figure 29. Longitudinal section of the apical region of onion root showing the calcium retained in the hypodermis after exchange.

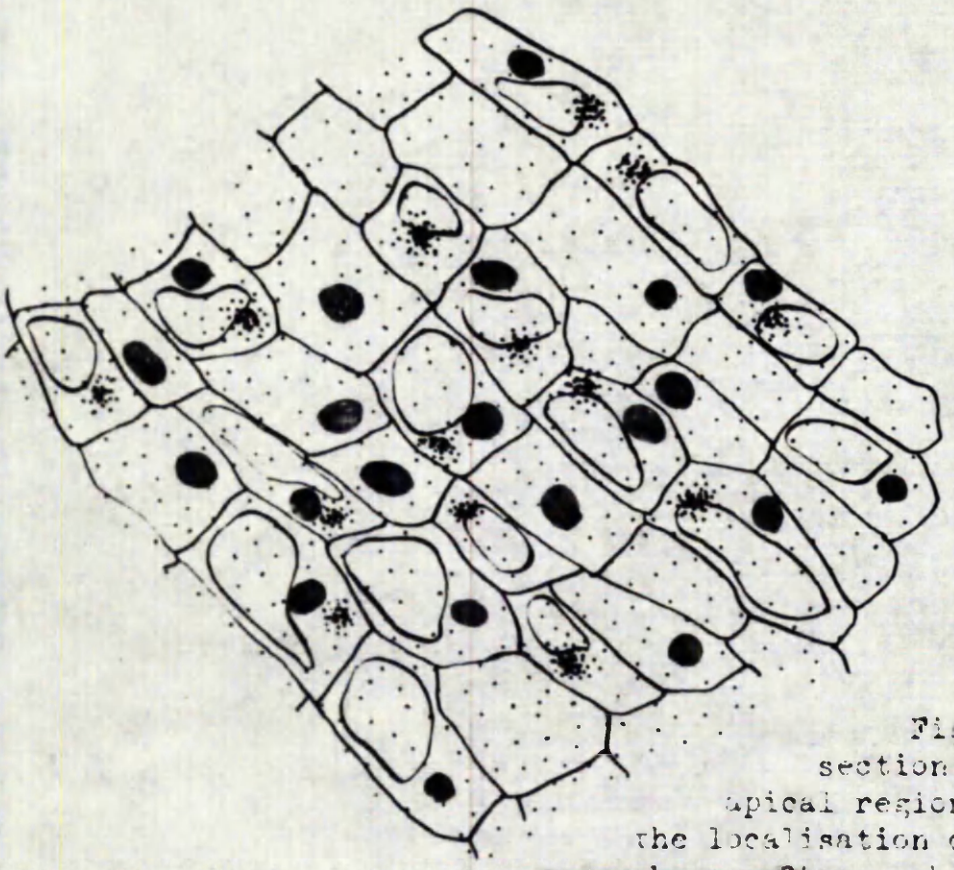


Figure 30. Longitudinal section of the cortex in the apical region of onion root showing the localisation of calcium in the parenchyma after exchange in inactive calcium.

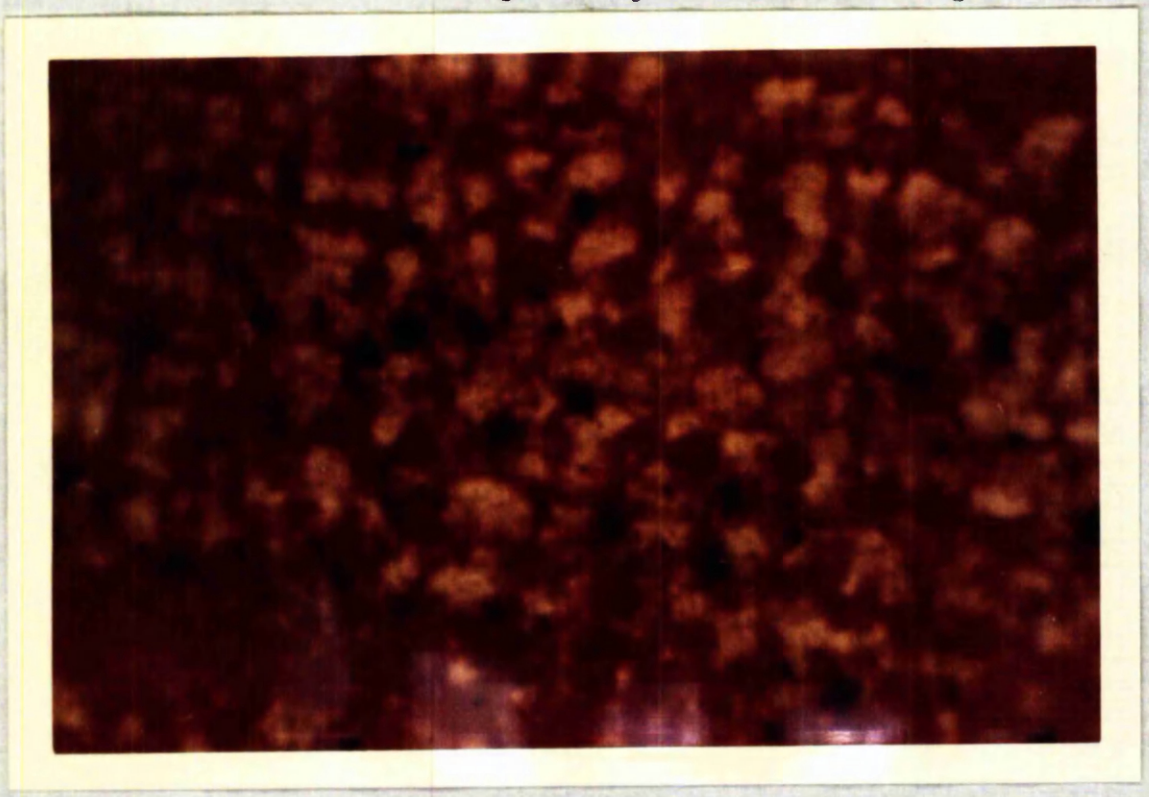


Figure 30 a Photograph to show the distribution of Ca^{45} in the cortical parenchyma after exchange in inactive calcium.

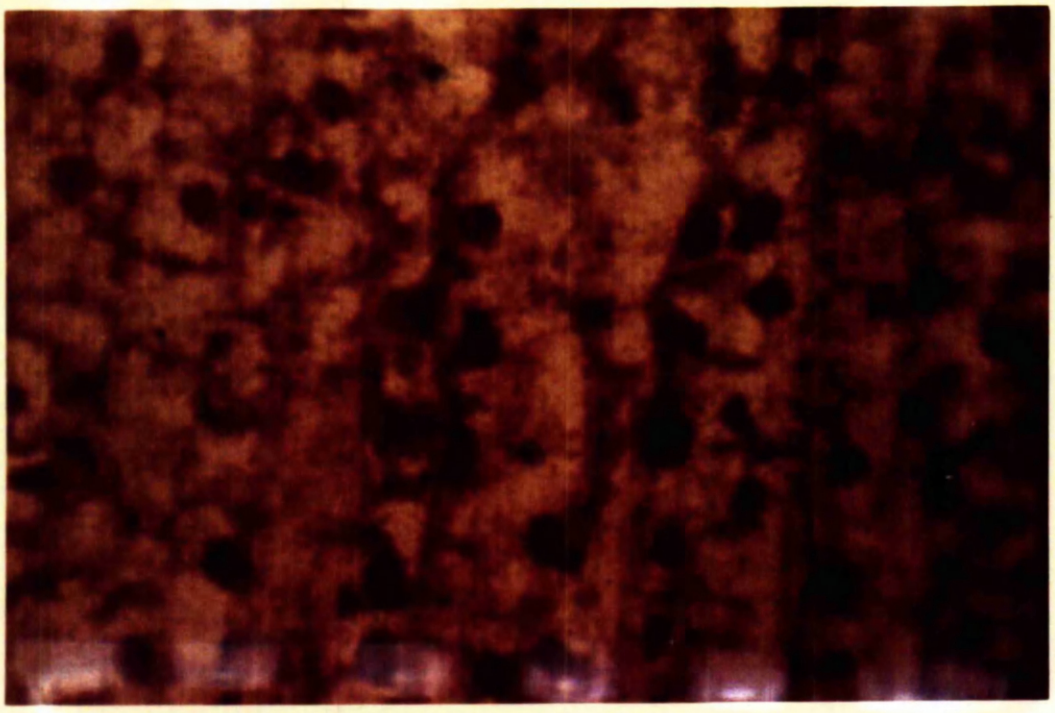


Figure 30b. Photograph to show the distribution of Ca^{45} in the cortical parenchyma after exchange.

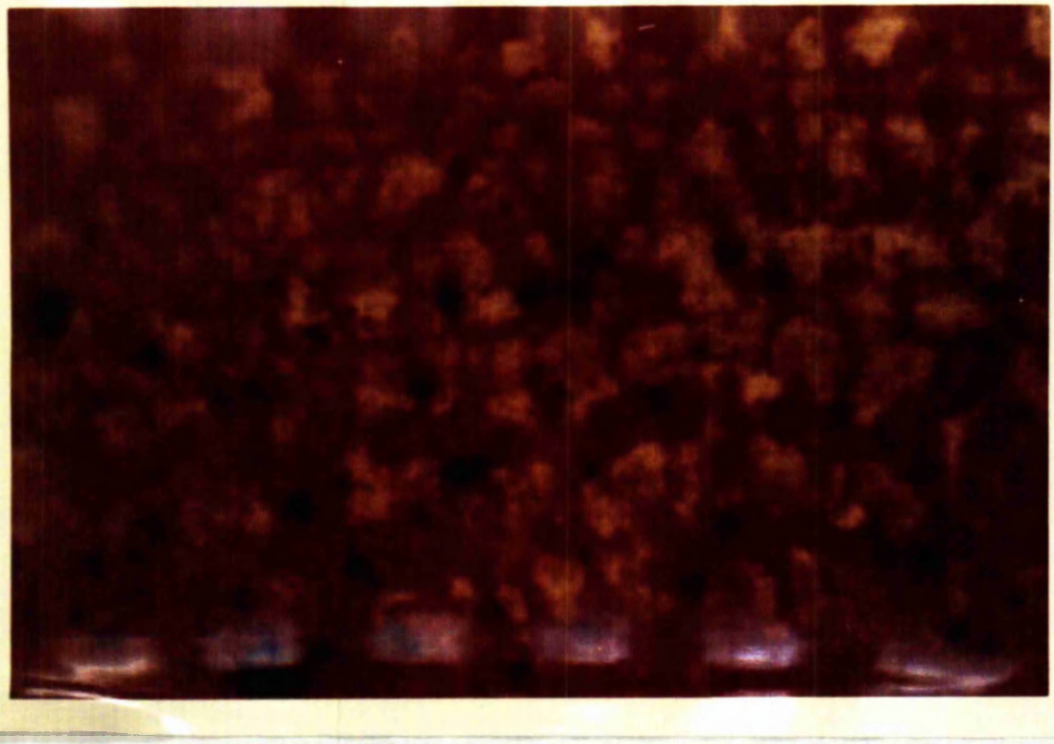


Figure 30c. Photograph to show the distribution of Ca^{45} in the cortical parenchyma after exchange.

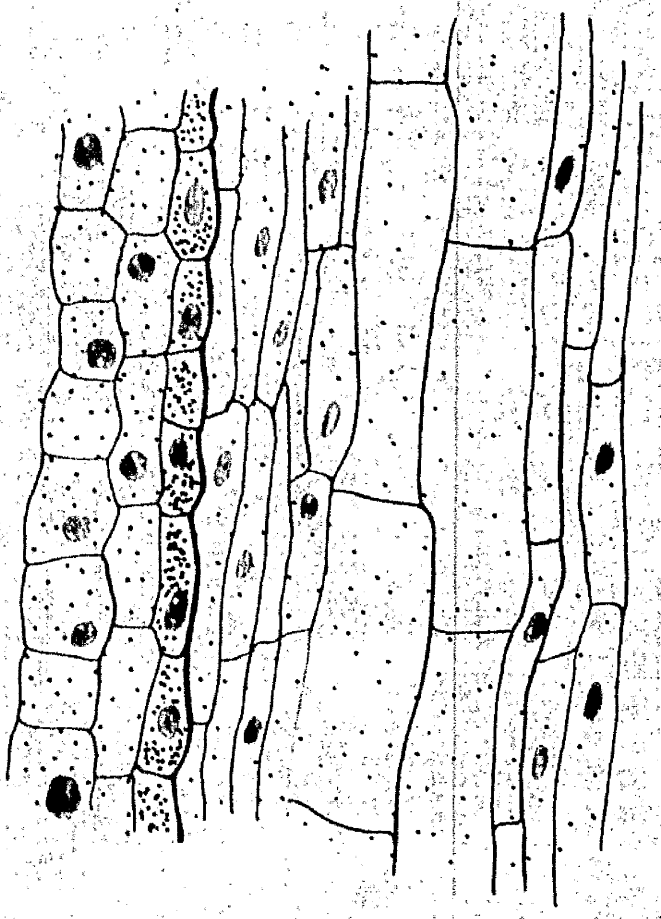


Figure 31. Longitudinal section of the apical region of onion root showing the calcium retained in the endodermis after exchange.

accurate association of the autoradiographic image with a particular cell component was not possible. (Figure 28.)

Epidermis: The calcium absorbed from the uptake solution was completely removed.

Hypodermis: The localisation of calcium was more distinct on exchange. Aggregates of grains were found mainly above the inner tangential walls of the cells of this tissue beyond the region of calcium accumulation. (Figure 29.)

Cortical Region: The autoradiographs showed calcium to be retained in the region of calcium accumulation. In the more deeply stained preparations, the aggregates of grains appeared to be over the edge of the cytoplasm of the cell near to the vacuole. The number of cells demonstrating this localisation was greater in the inner cortex and the region nearer the tip. The aggregates of grains were circular and very dense. (Figure 30.)

Endodermis: The localisation was more distinct on exchange. The autoradiographic image consisted of a high number of reduced grains distributed generally over the cell cytoplasm as illustrated in Figure 31.

Protoxylem Initials: After exchange, aggregates of grains of greater density and diameter than those above the cortex, were found above the protoxylem initials as shown in Figure 28. They were positioned within the cell wall but the spread of the aggregates prevented their being associated with a particular cell component.

The distributions are summarised in Table IV.

TABLE IV

Distribution of Ca^{45} in Non-exchanged and Exchanged Onion Roots.

TISSUE	NON-EXCHANGED	EXCHANGED
Root Cap	Ca^{45} evenly distributed over cell wall and cytoplasm. Aggregates of grains in film above cell wall in outermost layer of cells.	Aggregates of grains in film above cells in centre of root cap: those above outermost layer of cells not present in exchanged material.
Epidermis	Aggregates of grains in film above cell walls in apical region of root. High number of reduced grains above length of tissue.	Aggregates of grains not found above exchanged material. Low number of reduced grains above cells.
Hypodermis	Some aggregates of grains found in film. Highest uptake in cells beyond region of Ca accumulation.	Aggregates of grains in film above those cells beyond the region of Ca accumulation.
Cortical Region	General uptake, mainly in cell walls. Region of high Ca^{45} uptake from 1-3·5 mm. back from the root tip.	Circular aggregates of grains in film, greatest in number above inner cortex and cells at tip of root. Region1-3·5 mm. back from tip. Localisation within cell wall.
Endodermis	General uptake in cells beyond region of Ca accumulation.	Localisation of Ca^{45} within cell wall, does not appear as aggregates of grains in film.
Protoxylem Initials	No localisation.	Aggregates of grains in film. Localisation within cell wall.

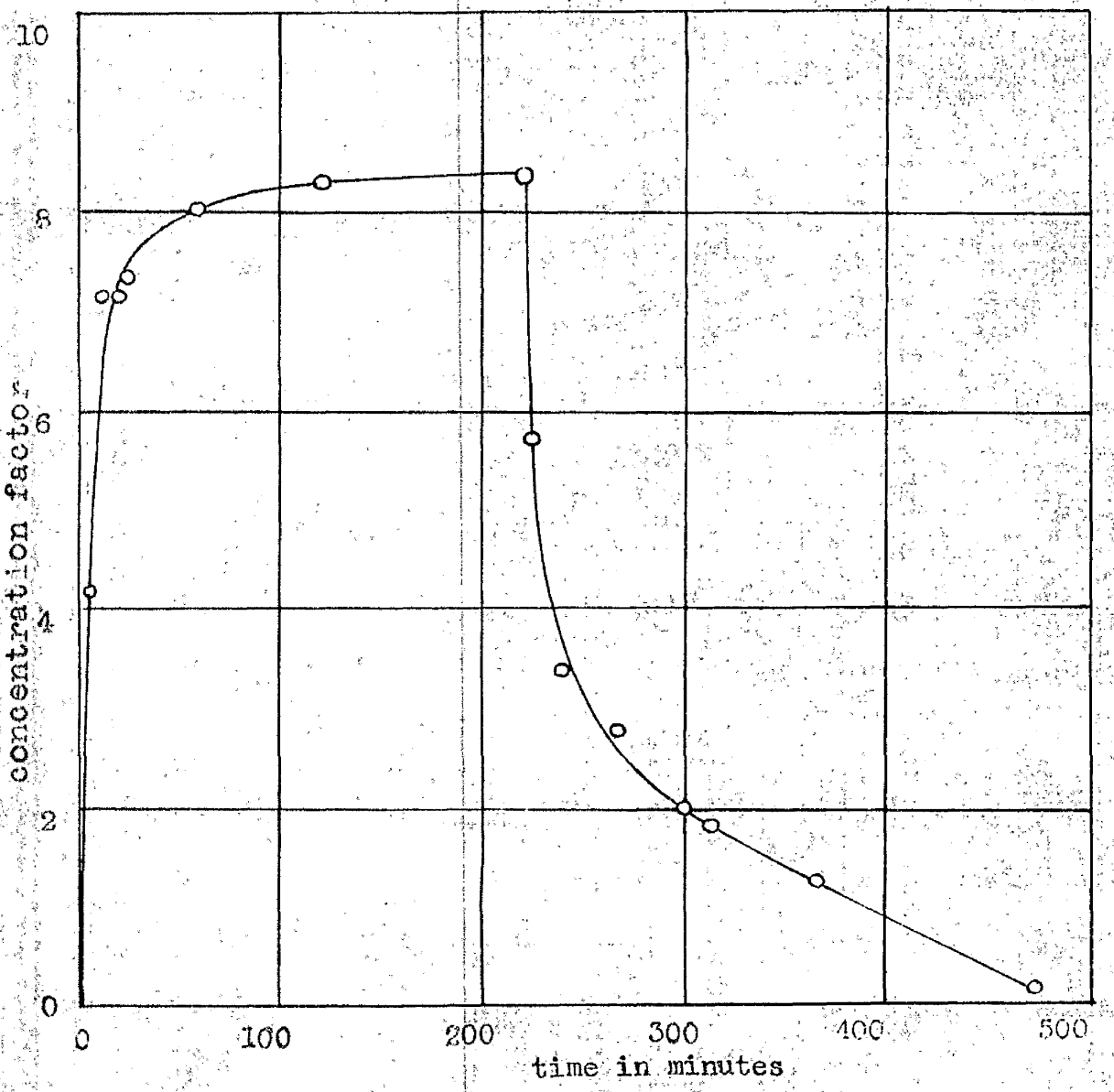


Figure 32. Uptake and exchange curve for calcium ions with roots killed by boiling.

2. UPTAKE AND EXCHANGE OF CALCIUM IN DEAD ONION ROOTS.

The retention of calcium after several hours of exchange was not considered to be sufficient evidence to assume that this retained calcium was, in fact, actively accumulated. Also it does not preclude the possibility that all the calcium taken up by the root has been passively absorbed and is bound to different cell constituents with varying degrees of exchangeability. In order to determine the sites of passive and active processes in the root, a comparison has been made in the following experiment between the uptake of calcium in living and dead onion roots.

Onion roots were killed (a) by boiling in water for 15 minutes and (b) by placing in liquid ether for 30 minutes. Uptake and exchange curves were constructed using solutions containing 0·5 meq.Ca/l. as shown in Figure 32. for boiled roots. In each instance, the rapid initial uptake was followed by a slower phase to a level which remained constant after 150 minutes. A concentration factor of 8·4 was obtained in comparison with a factor of 21·4 at 400 minutes for living roots. The exchange in inactive calcium was relatively rapid and tended to go to completion with boiled roots but, in the case of ether-killed material, the exchange curve levelled off as was observed with living roots. This would suggest that the roots treated with ether were not killed but merely anaesthetized and that they had regained their ability to retain calcium.

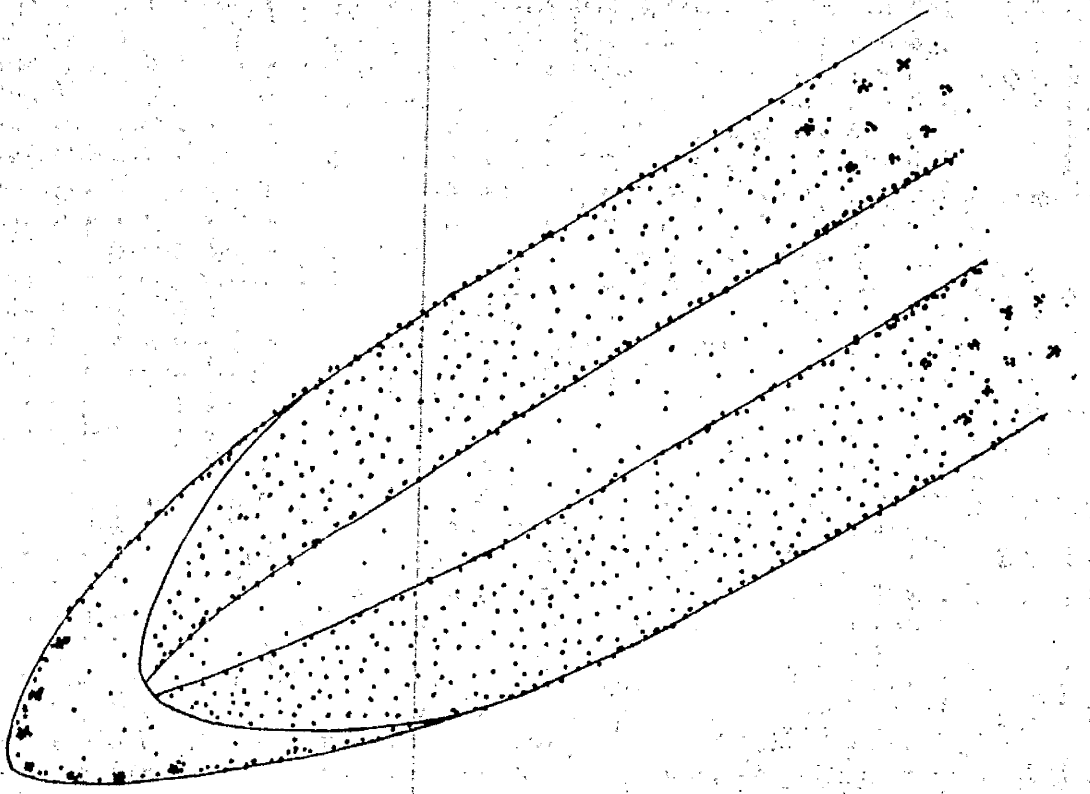


Figure 33. Diagram of the general distribution of calcium ions in the apical region of dead onion root before exchange.

Roots were held in boiling water for 15 minutes and subsequently treated according to the procedure for living roots. Autoradiographs of non-exchanged and exchanged material were set up. The roots became soft on heating and when finally rinsed, the tissues tended to separate.

General Distribution of Ca^{45} in Non-exchanged Dead Onion Roots:-

Figure 33. illustrates the general distribution in non-exchanged boiled roots. The number of reduced grains above the entire section was high but calcium was localised in the root cap, epidermis and endodermis. Also there was a certain amount of calcium distributed generally in the cortical region up to 3·5 mm. back from the tip. Beyond this area, calcium appeared to be localised in individual cells.

Distribution at the Cellular Level:-

Root Cap: Aggregates of grains were evident above the outermost layer of cells, being positioned above the cell walls. No localisation was found in the central cells.

Epidermis: Aggregates of grains were found above the cell walls of the epidermis in the region of calcium accumulation as shown in Figure 34.

Hypodermis: Beyond the region of calcium accumulation there was a high general darkening of the film above this tissue. No aggregates of grains were found.

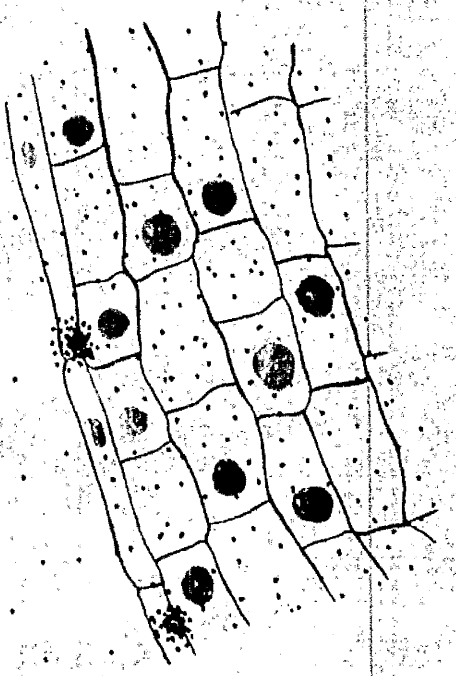


Figure 34 Longitudinal section of the apical region of onion root showing the localisation of calcium in the epidermis after killing of the roots and treatment with isotope.

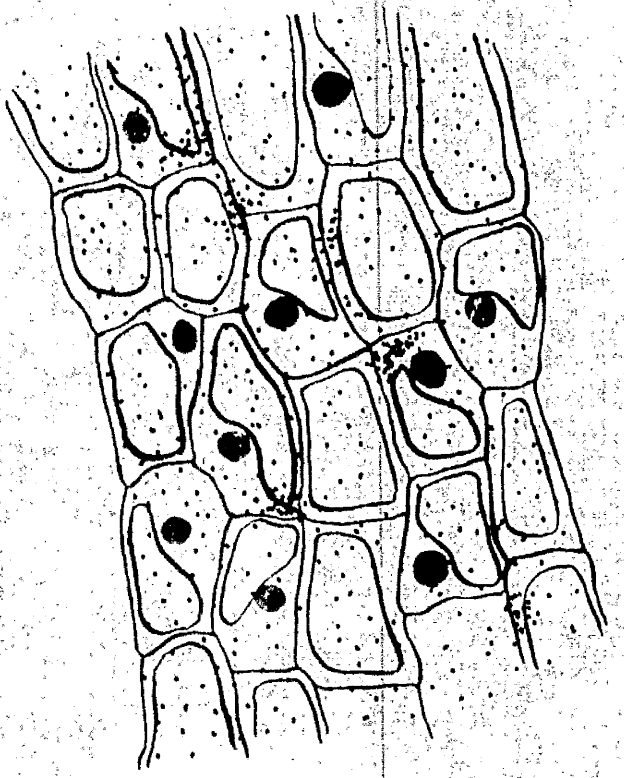


Figure 35. Longitudinal section of the apical region of dead onion root showing the distribution of calcium in the cortex beyond the region of calcium accumulation before exchange.

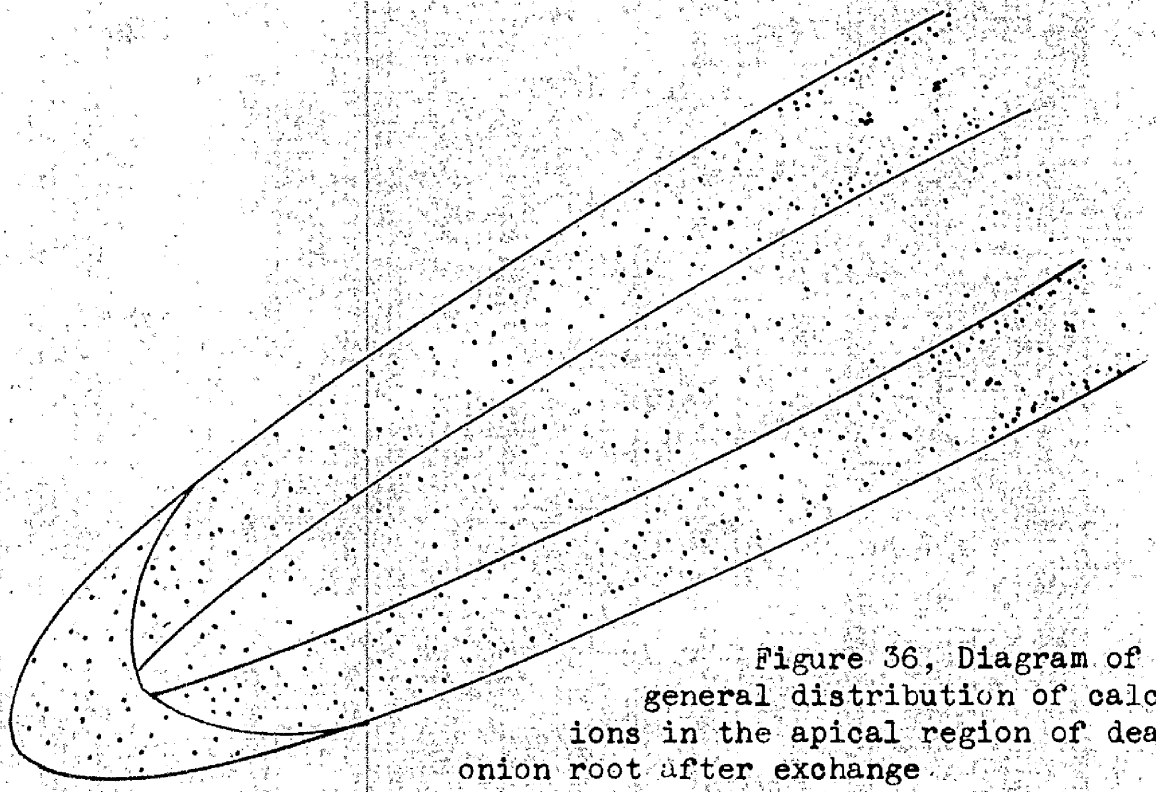


Figure 36, Diagram of the general distribution of calcium ions in the apical region of dead onion root after exchange.

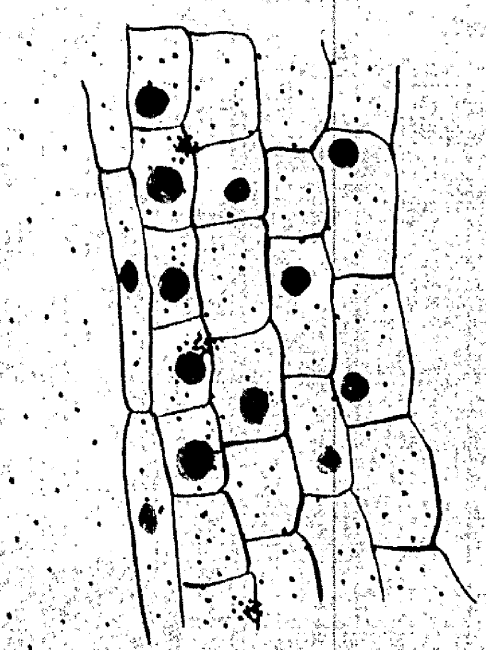


Figure 37. Longitudinal section of the apical region of onion root showing the localisation of calcium in the hypodermis in dead roots after exchange

Cortical Region: A very high number of reduced grains was found over this region, distributed evenly over the cell walls and the cytoplasm. The extent of this region was similar to the region of calcium accumulation in living roots, though it was less well defined. Reduced grains were collected into rough aggregates which were positioned above the cell walls of the parenchymatous cells of the cortex in the part of the root beyond the region of calcium accumulation. These aggregates were distinct from the ones observed in living roots after exchange as shown in Figures 35. and 30.

Endodermis: The uptake of calcium in the endodermis was shown by a general darkening of the film above the cytoplasm of the cells, no aggregates of grains being present.

General Distribution of Ca^{45} in Exchanged Dead Onion Roots:-

The distribution of the calcium retained by the roots after exchange is shown in Figure 36. The activity in the root cap and epidermis was removed completely while the major part of the calcium in the cortex was exchanged. The calcium absorbed by the endodermis and the hypodermis was more distinct on exchange, in the region beyond the region of calcium accumulation. Only a slight uptake was found in the cortex over 3·5mm. back from the root tip. No localisation was found in the proto-xylem initials of non-exchanged or exchanged dead roots.

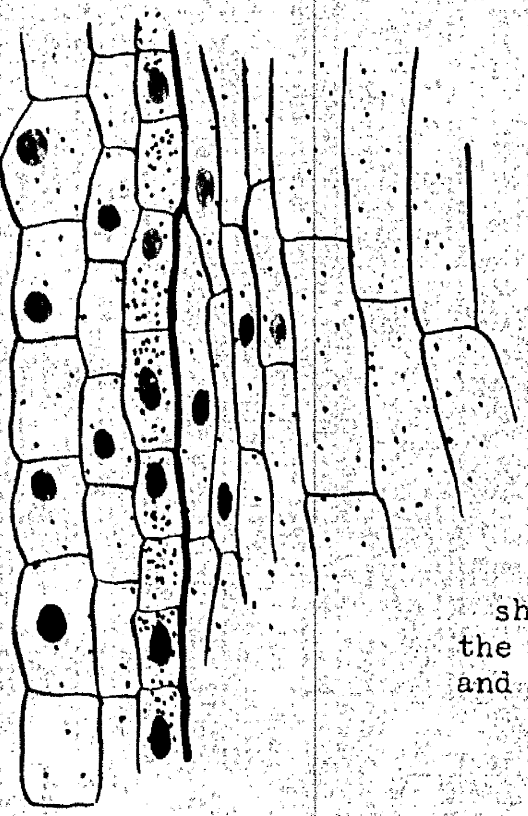


Figure 38. Longitudinal section of the apical region of onion root showing the retention of calcium in the endodermis after killing the roots and exchanging under normal conditions.

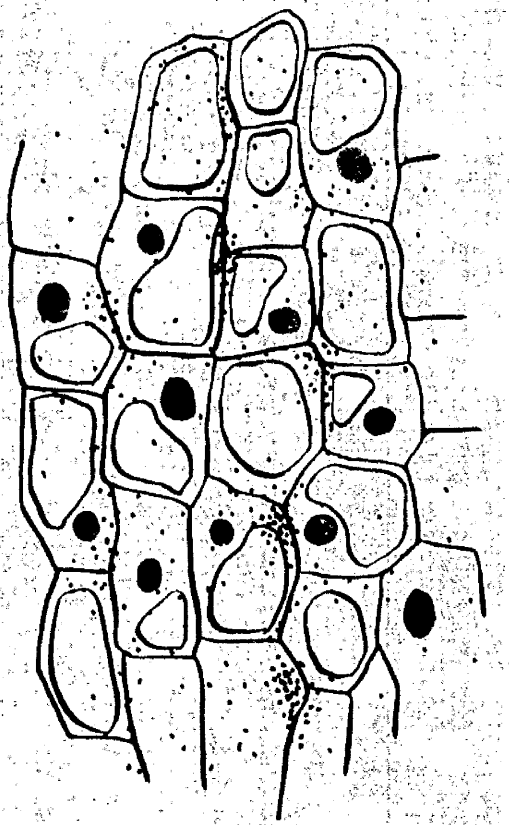


Figure 39 Longitudinal section of the apical region of dead onion root showing the calcium held in the cells of the cortex beyond the region of calcium accumulation, after exchange.

TABLE V

Comparison of the Distribution of Ca^{45} in Living and Dead Onion Roots

TISSUE	NON-EXCHANGED		EXCHANGED	
	Living	Dead	Living	Dead
Root Cap	General and aggregates of grains in film	As in living roots.	Aggregates above cells in centre of root cap.	No localisation.
Epidermis	Aggregates above cell walls in region of Ca accumulation.	As in living roots.	No localisation.	No localisation.
Hypodermis	Highest uptake in cells beyond 3·5mm.	As in living roots.	Aggregates above cells beyond region of Ca accumulation.	As in living roots.
Cortical Region	General uptake, most in cell walls. Region 1-3·5mm	General uptake in walls and cytoplasm. Region same.	Numerous aggregates Localisation within cell walls.	Slight general uptake.
Upper Region of Root	No localisation.	Rough aggregates above cell walls.	No localisation.	Small amount Ca^{45} retained.
Endodermis	General uptake in cells beyond 3·5mm.	As in living roots.	Localisation within cell walls.	As in living roots.
Protoxylem Initials	No localisation.	No localisation.	Localisation within cell walls. Aggregates present in film.	No localisation.

Distribution at the Cellular Level:-

Hypodermis: Circular aggregates of grains were found above the walls of those cells beyond the region of calcium accumulation (Figure 37.).

Endodermis: In the tissue beyond the region of calcium accumulation the endodermal uptake appeared as a general darkening of the film which was similar to the distribution in living roots (Figure 38.).

Cortical Region: Traces of calcium were left after exchange in the region of the root more than 3·5 mm. back from the tip (Figure39.).

The features of the distribution of calcium found in living and dead onion roots before and after exchange are summarised in Table V. The significance of particular points of distribution in relation to the mechanisms involved in uptake will be considered at the end of the section. The following sub-sections describe the results of other experiments designed to extend the scope of the investigation on calcium uptake and to yield additional information bearing on particular features of the distribution of calcium described above.

3. (a) UPTAKE AND EXCHANGE OF CALCIUM IN ROOTS OF DENDROBIUM SP..

It was felt that the epidermal/hypodermal uptake in onion roots was not sufficiently well defined and that a root having a clearly differentiated epidermis and hypodermis should be investigated. An uptake and exchange curve for roots of Dendrobium sp. was plotted as shown in Figure 19. The shape of the curve was similar to that obtained using onion roots but the concentration factor of 3·7 was considerably lower than that

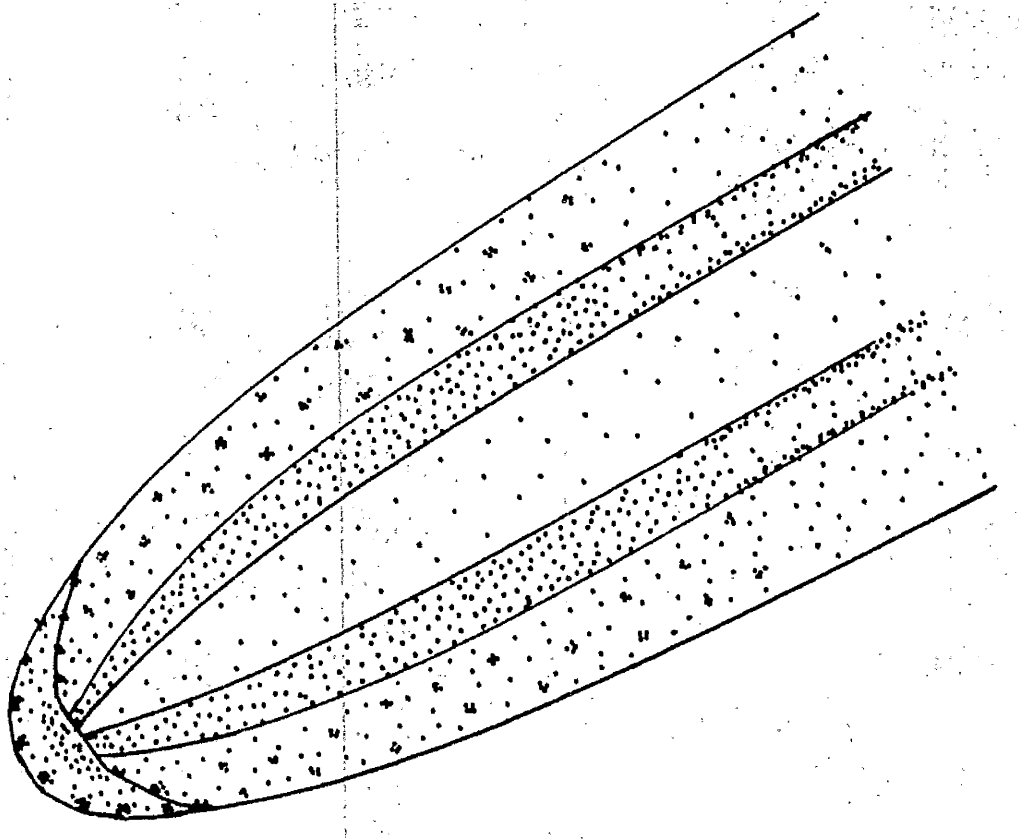


Figure 40 . Diagram of the general distribution of calcium ions in the apical region of the root of Dendrobium sp. before exchange.

obtained for onion roots. Roots of Dendrobium sp. were treated with a labelled solution of calcium chloride and exchanged in an inactive solution according to the procedure detailed in Appendix 1.

Since the roots are of a much greater diameter than onion roots, smaller pieces, of shorter length than diameter, were freeze dried in order to prevent unduly long drying times.

General Distribution of Ca^{45} in Non-exchanged Roots:-

Figure 40. illustrates the overall distribution of calcium in non-exchanged roots and it appears to be generally similar to that obtained with onion roots. There was a region of high calcium uptake stretching from the root tip to a point approximately 5 mm. back from the tip. The localisation in the outermost layer of cells and inner cells of the root cap was repeated. The increased endodermal and hypodermal uptake in the tissues beyond the region of calcium accumulation was similar to the uptake in onion roots. The higher epidermal uptake occurred as before in the region of calcium accumulation and was evident in each layer of this tissue.

Distribution at the Cellular Level:-

Root Cap: Several large aggregates of grains were observed above the outermost layer of cells, predominantly in those cells at the tip of the root. They appeared to be situated above the cell walls. The localisation in the inner cells of the root cap was not as well defined

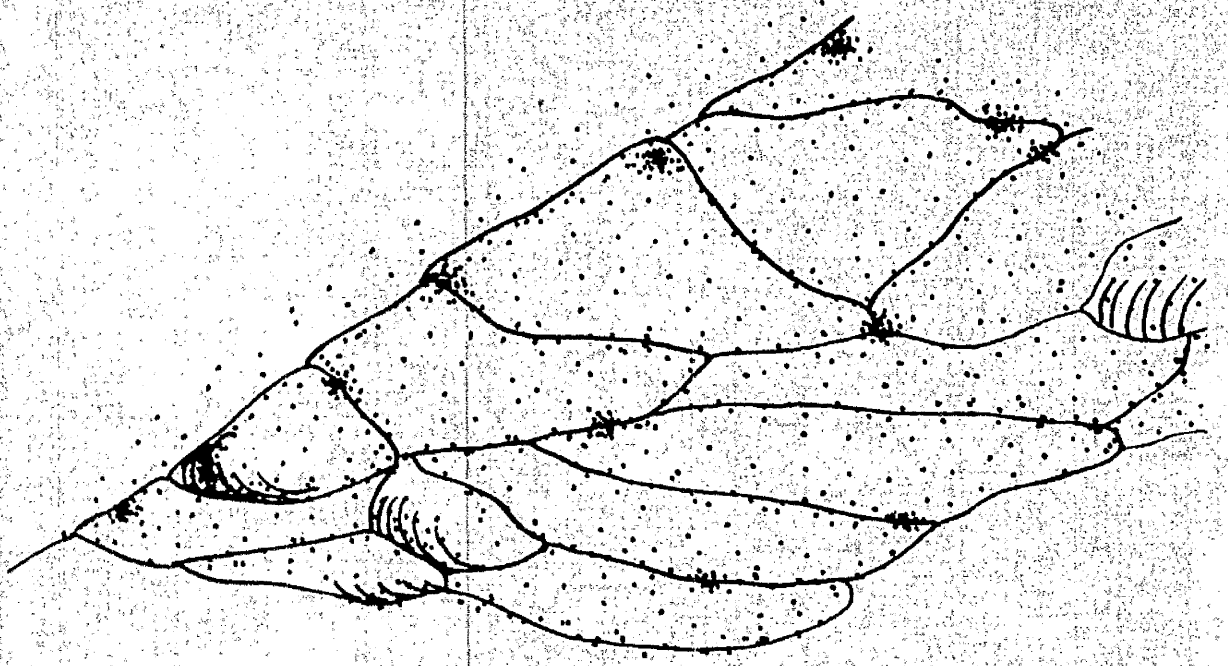


Figure 41. Longitudinal section of the apical region of a root of Dendrobium sp. showing calcium localised in the epidermis before exchange.

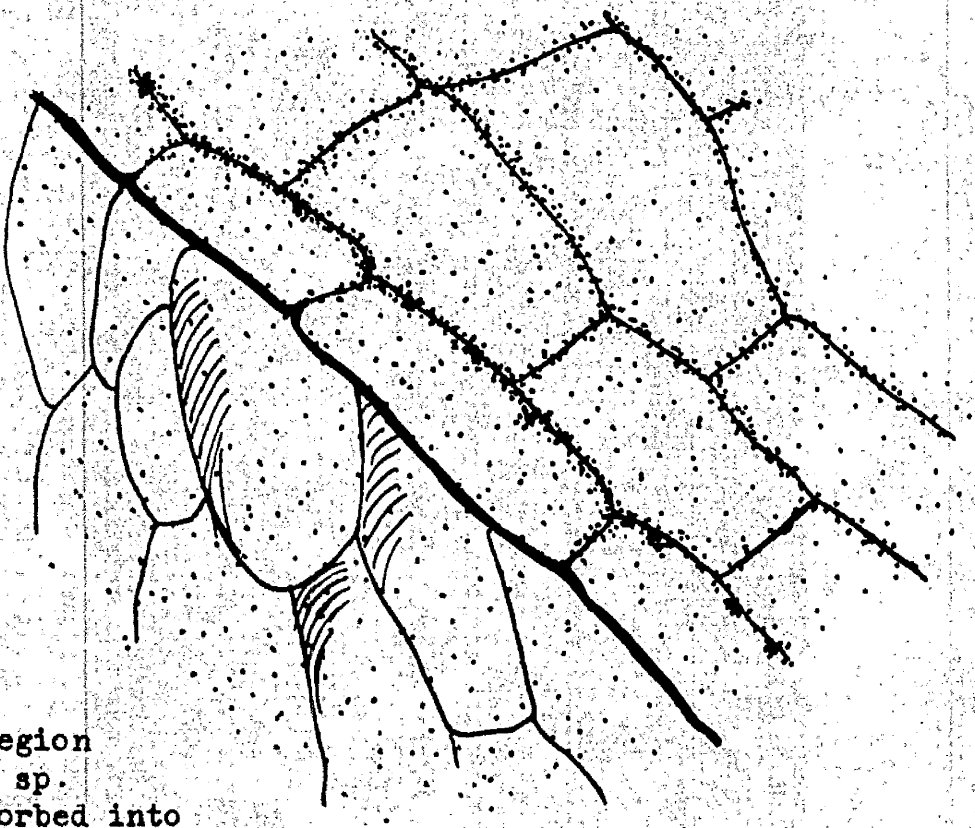


Figure 42. Longitudinal section of the apical region of a root of Dendrobium sp. showing the calcium absorbed into the hypodermis and parenchyma of the the cortex before exchange.

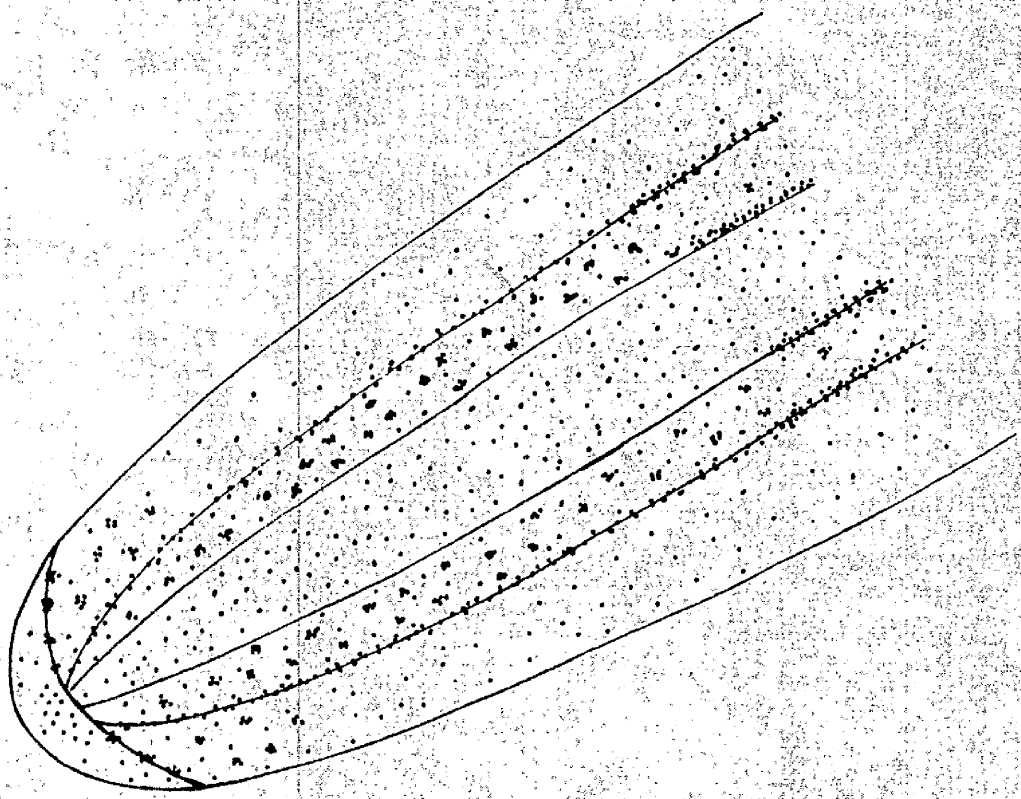


Figure 43. Diagram of the general distribution of calcium ions in the apical region of the root of Dendrobium sp. after exchange.

TABLE VI

Distribution of Ca^{45} in Roots of Dendrobium sp. before and after Exchange.

TISSUE	NON-EXCHANGED	EXCHANGED
Root Cap	Aggregates of grains in film above outermost layer	No localisation.
Epidermis	Several aggregates per cell; most in cells near tip; localisation in region of Ca accumulation	Most of Ca^{45} exchanged; some aggregates in cells at tip.
Hypodermis	Uptake greatest in cells beyond region of Ca accumulation. A few aggregates present in film.	Several small aggregates of grains above each cell: above inner radial wall.
Cortical Region	Mainly above cell walls: no aggregates visible: region of uptake as in onion roots.	A few aggregates in film above this tissue: Ca^{45} localised within cell wall.
Endodermis	General uptake within cell walls : in cells beyond region of Ca accumulation.	Several aggregates above outer radial cell wall of each cell beyond region of Ca accumulation.
Protoxylem Initials	No localisation.	No localisation.

as that in onion roots and it appeared as a general darkening of the film above this area.

Epidermis: In many instances, more than one aggregate of grains was found above the cell walls of a single cell. Each layer of cells exhibited calcium localisation, the outermost layer showing the greatest number of aggregates of grains (Figure 41.).

Hypodermis: The darkening of the autoradiographic film due to calcium present in the cell walls was sufficient to mask any localised isotope. A few rough aggregates of grains were observed above the inner tangential wall of the cells beyond the region of calcium accumulation as shown in Figure 42.

Cortical Region: There was a distinct darkening of the film above the cell walls of the parenchyma of the cortex, particularly in the region of calcium accumulation but no aggregates of grains were found (Figure 41.).

Endodermis: The endodermis demonstrated an increased absorption of calcium in the same longitudinal region of the root in which the hypodermal uptake occurred (Figure 42.).

General Distribution of Ca^{45} in Exchanged Roots:-

The major part of the calcium absorbed into the epidermis appeared to be exchangeable but the isotope taken up by the hypodermis and endodermis was retained. The root cap and cortical parenchyma showed a limited retention of calcium (Figure 43.).(Table VI).

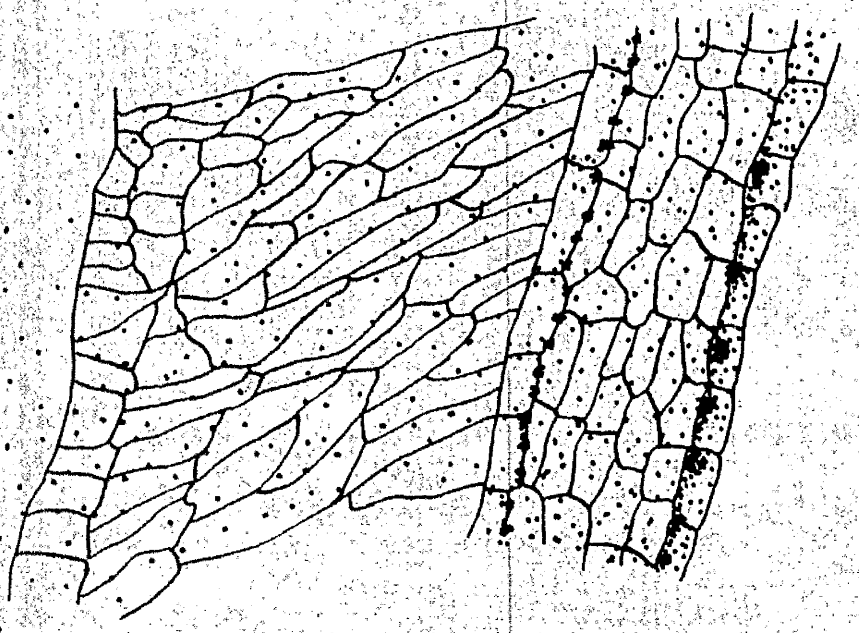


Figure 44. Longitudinal section of the apical region of a root of Dendrobium sp. showing localisations of calcium in the hypodermis and endodermis after exchange.

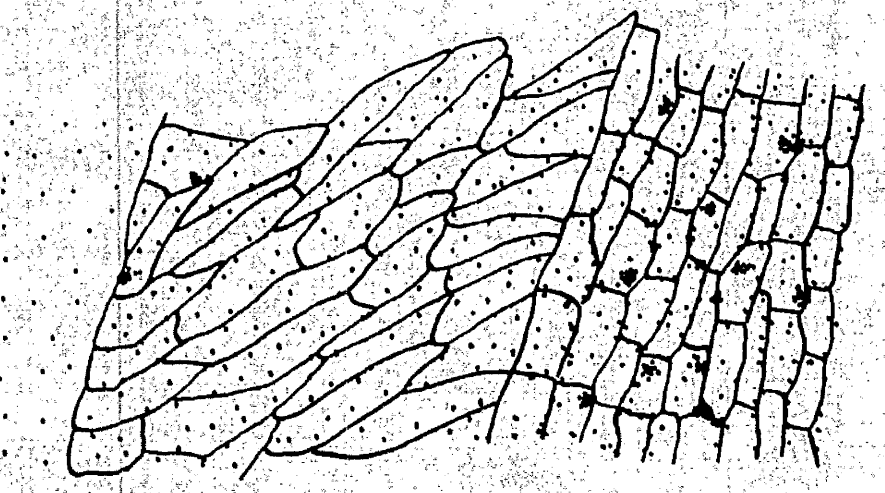


Figure45. Longitudinal section of the apical region of a root of Dendrobium sp. showing a localisation of calcium in the parenchyma of the cortex and the epidermis after exchange.

Distribution at the Cellular Level:-

Root Cap: Most of the calcium was exchanged, the remainder being held in the central cells. No definite aggregates of grains were found.

Epidermis: Some calcium remained in the meristematic cells. Aggregates of grains were found above this area but to a lesser extent than in non-exchanged roots. In the more mature tissues, a few aggregates of grains were found in the outermost layer only.

Hypodermis: Several aggregates of grains were visible above the inner tangential wall of each cell in the region beyond the region of calcium accumulation. These were roughly circular in shape and occurred above the cell wall.(Figure 44.)

Cortical Region: Small aggregates of grains were found to be present above the parenchymatous cells of the cortex. Normally more than one aggregate occurred above each cell, some above the vacuole and some more closely associated with the cell wall (Figure 45.). The region of uptake was similar to that in onion roots.

Endodermis: Figure 44. also illustrates the localisation in the endodermis. In the roots of Dendrobium sp., the autoradiographs showed roughly circular aggregates of grains associated with the outer tangential walls of the cells, often more than one per cell. The greatest uptake occurred in the cells beyond the region of calcium accumulation.

Protoxylem Initials: No aggregates of grains were found above this tissue, only a general darkening of the autoradiographic film.

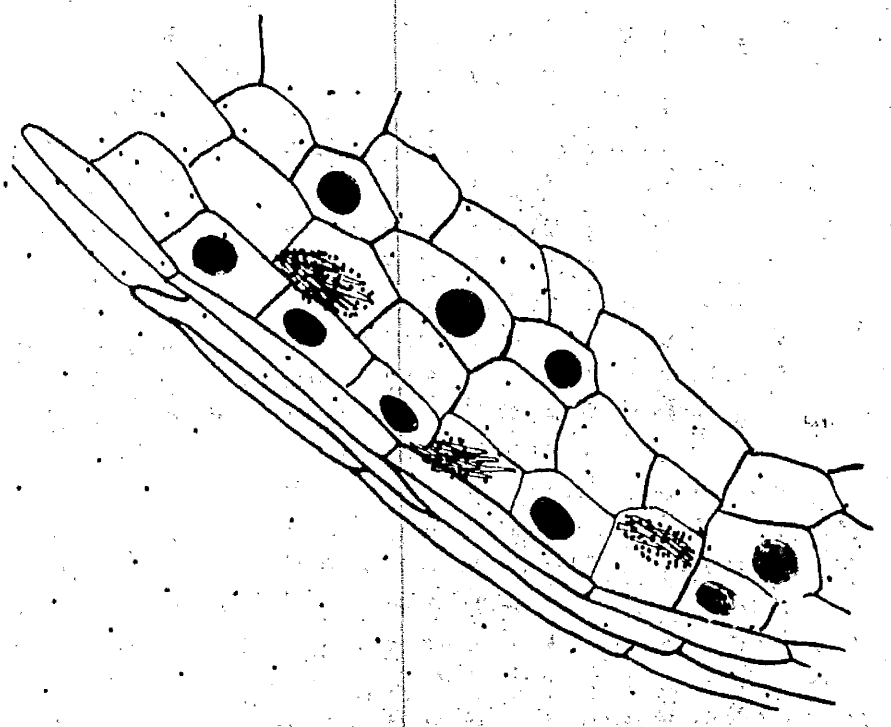


Figure 46. Longitudinal section of the apical region of a root of Amaryllis sp. showing the presence of Ca^{45} in calcium oxalate crystals.

3. (b) UPTAKE AND EXCHANGE OF CALCIUM IN ROOTS OF AMARYLLIS SP..

In the preliminary experiments, several different roots were investigated, of which the roots of Amaryllis sp. were the least efficient in absorbing calcium. The concentration factor, in this case, did not exceed one. A few autoradiographs were set up of exchanged and non-exchanged material. The distribution of calcium in the epidermal and hypodermal tissues in the apical region of the root is of interest since calcium oxalate crystals were present in these tissues. There was a definite localisation of reduced grains in the autoradiographic film above the needle-shaped crystals (Figure 46.). Due to the low amount of calcium absorbed, the only area of calcium uptake in the other tissues of the root, was in the mature cells of the endodermis. After exchange a certain amount of calcium was still present in the clusters of crystals suggesting a very slow exchange.

4. UPTAKE AND EXCHANGE OF CALCIUM IN THE PRESENCE OF STRONTIUM:

(a) The Effect on Calcium Uptake:-

Cation exchange is assumed to be unselective but the active phase has been shown to be selective (3,5-7). The following experiment was designed to determine the selectivity of the absorption processes occurring in the different tissues.

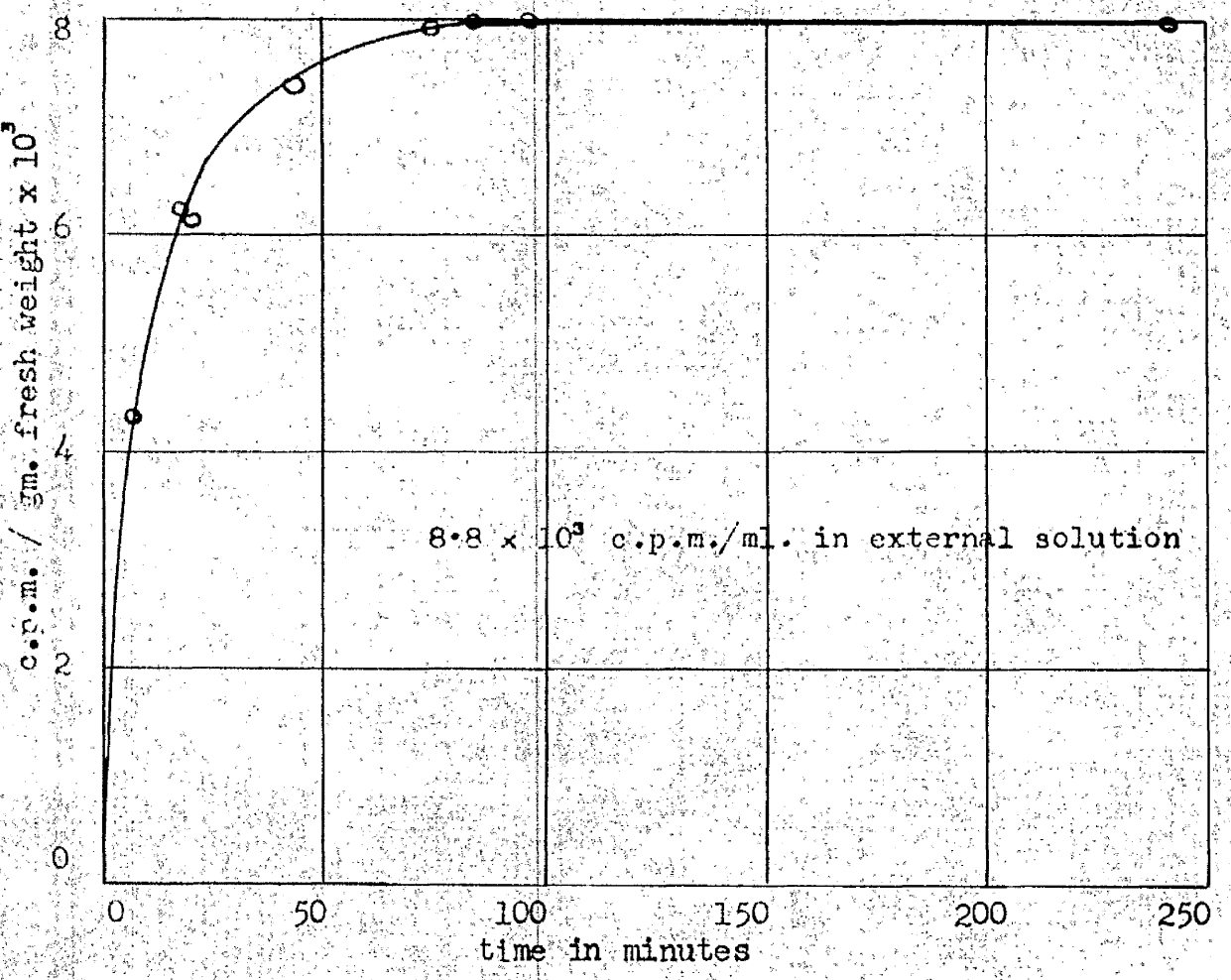


Figure 47. Uptake of calcium from an equimolar solution of calcium chloride and strontium chloride.

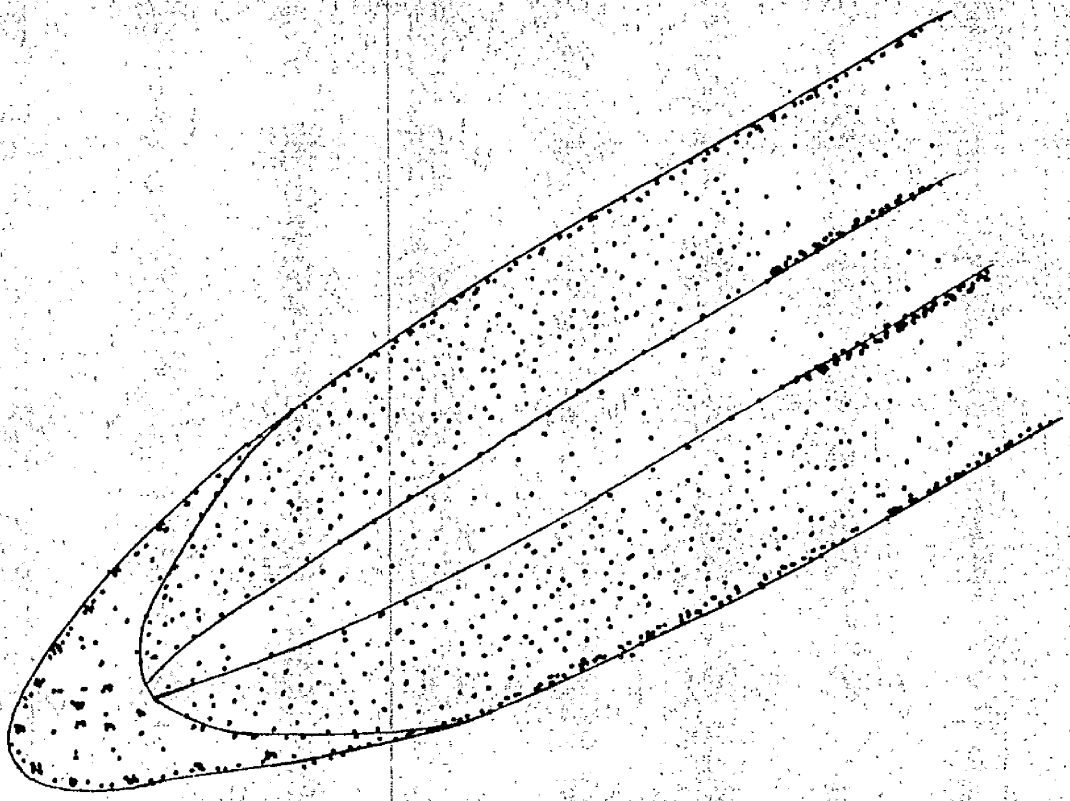


Figure 48. Diagram of the general distribution of calcium ions in the apical region of onion roots after treatment with a two salt solution.

An uptake and exchange curve for calcium in the presence of an equimolar concentration of strontium is given in Figure 47. The total cation concentration was maintained at 0·5 meq.Cation /l. It can be seen that the curve rapidly levels off after an initial rapid uptake, and the concentration factor in this instance , did not exceed one.

Autoradiographs were set up of material treated with the two salt mixture and a few after exchange in inactive calcium.

General Distribution of Ca^{45}:-

Figure 48. represents the distribution of Ca^{45} in non-exchanged material. A localisation of calcium was found in the epidermis of the apical region of the root and, in the region beyond 3·5 mm· back from the tip, the uptake appeared to be associated with the hypodermis. Also, in this latter region, the endodermis showed an increased absorption of Ca^{45}. The parenchyma of the cortex showed a slight concentration up to a distance of approximately 3·5 mm back from the root tip, (Table VII).

Distribution at the Cellular Level:-

Root Cap: The root cap showed a slight general uptake in the central region. Aggregates of grains were found above the cell walls of the outermost layer of cells. The localisation was not found after exchange.

Epidermis: The uptake pattern in the epidermis was similar to that obtained after treatment with a single salt solution. Aggregates

TABLE VII.

Comparison of the Distribution of Ca^{45} in Onion Roots Treated with Single and Two Salt Solutions.

TISSUE	UPTAKE IN $CaCl_2$	UPTAKE IN $CaCl_2$ + $SrCl_2$
Root Cap	Ca^{45} evenly distributed over cell wall and cytoplasm. Aggregates of grains in film above cell wall in outermost layer of cells.	Aggregates of grains as in roots treated with $CaCl_2$.
Epidermis	Aggregates of grains in film above cell walls in apical region of root. High number of reduced grains above length of tissue.	Similar distribution showing aggregates of grains above this tissue.
Hypodermis	Some aggregates of grains found in film. Highest uptake in cells beyond region of Ca accumulation.	Similar distribution to that in roots treated with $CaCl_2$.
Cortical Region	General uptake, mainly in cell walls. Region of high Ca^{45} uptake from 1-3·5 mm. back from root tip.	No aggregates of grains were found. Region of uptake 1-3·5 mm.
Endodermis	General uptake in cells beyond region of Ca accumulation.	Similar distribution of Ca^{45}.
Protoxylem Initials	No localisation.	No localisation.

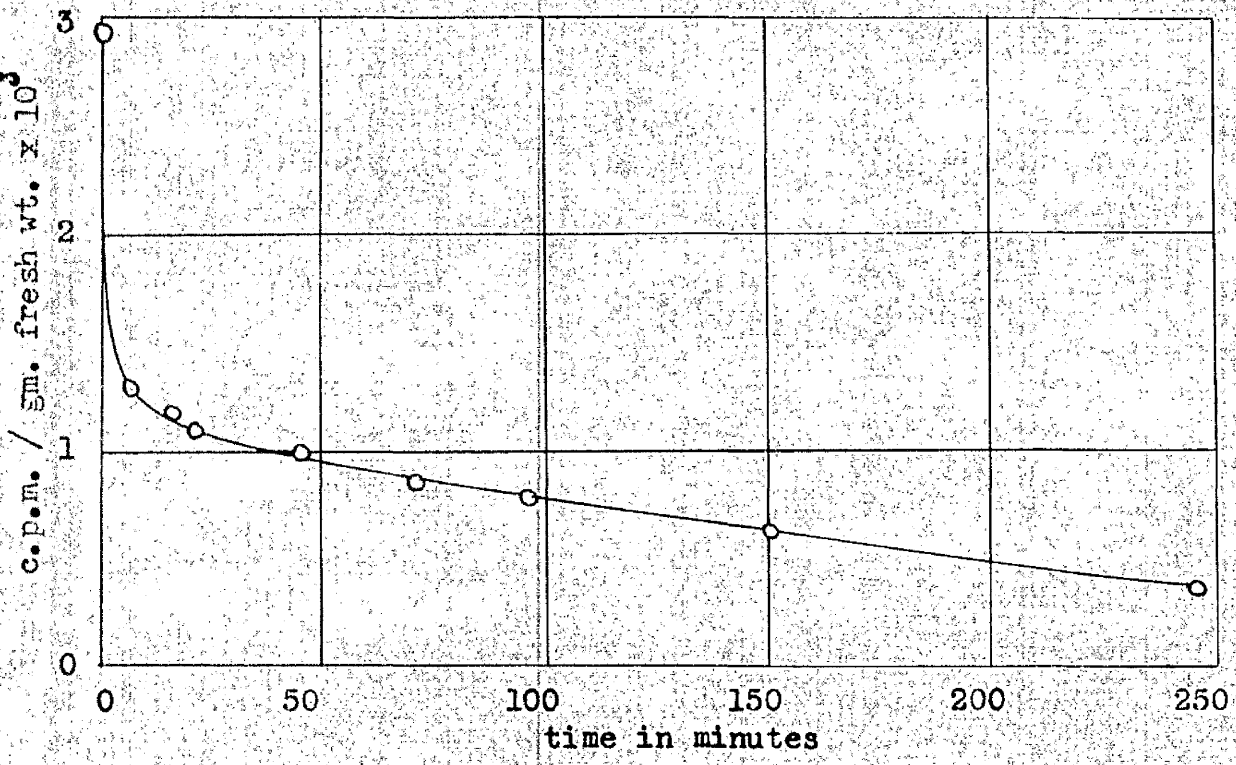


Figure 49. The effect of exchange in strontium chloride solution on the retention of calcium by onion roots.

of grains were found above the cell walls of this tissue up to a distance of approximately 3·5 mm back from the root tip. No calcium remained after exchange.

Hypodermis: A few aggregates of grains were found above the cell walls of the hypodermis in the region beyond the region of calcium accumulation. The calcium was retained after exchange.

Cortical Region: The cell walls of the parenchyma showed a slight localisation of calcium which was removed by exchange.

Endodermis: There was a slight darkening of the film above the cytoplasm of the cells, no aggregates of grains being present. After exchange a certain amount of calcium was retained.

Protoxylem Initials: No localisation was observed. Table VII summarises the distribution of calcium in onion roots after treatment with a single salt and a two salt solution.

(b) Exchange of Calcium with Strontium:-

The effect of strontium on the exchange characteristics of onion roots is shown in Figure 49. Exchange was carried out in a solution of strontium chloride containing 0·5 meq.Sr/l. after treatment with an active solution of calcium chloride having a similar concentration of the cation. It appears from the curve that with extended treatment, exchange of calcium by strontium may have gone to completion.

Autoradiographs of exchanged roots were set up by the procedure detailed in Appendix 1.

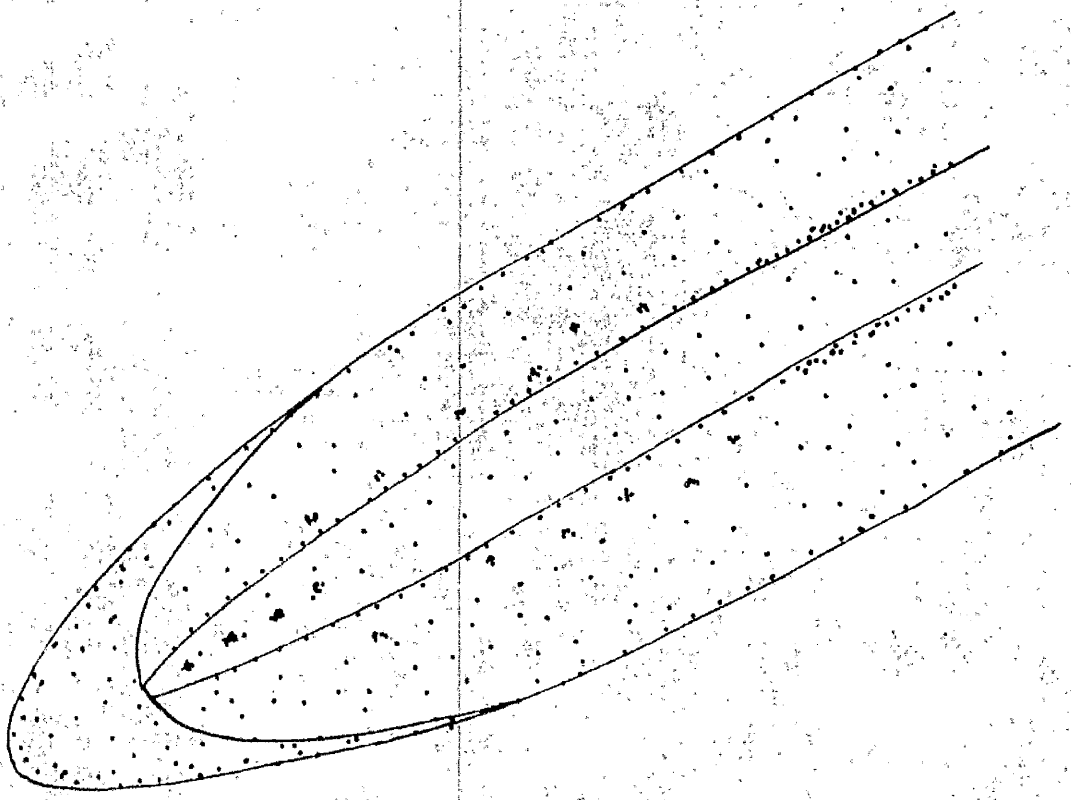


Figure 50. Diagram of the general distribution of calcium ions in the apical region of onion roots after uptake from a solution of calcium chloride and subsequent exchange in strontium chloride.

TABLE VIII

Comparison of the Distributions Obtained on Exchange in Calcium Chloride and Strontium Chloride after Uptake in Labelled Calcium Chloride.

TISSUE	EXCHANGE IN CALCIUM	EXCHANGE IN STRONTIUM
Root Cap	Aggregates of grains in film above central cells.	No localisation.
Epidermis	No localisation.	No localisation.
Hypodermis	Aggregates in film above cell walls in those cells beyond the region of Ca accumulation.	No localisation.
Cortical Region	Numerous circular aggregates of grains in film above apical region of root. Localisation within cell walls.	Ca^{45} almost entirely removed. The isotope in the region of the endodermis was last to exchange.
Protoxylem Initials	Localisation of Ca^{45} within cell walls.	Aggregates of grains present in film.

General Distribution of Ca^{45}:-

Figure 50. illustrates the distribution of Ca^{45} after 17 hours exchange in strontium chloride. The epidermal calcium and that of the outer layers of the root cap have been removed which is similar to roots exchanged in calcium chloride. The calcium left after exchange was present in the hypodermis, endodermis and inner cortex, (Table VIII).

Distribution at the Cellular Level:-

Root Cap: No activity was retained in the central cells of the root cap.

Hypodermis: A few aggregates of grains were visible after exchange. These were confined to those cells beyond the region of calcium accumulation. They were positioned above the cell wall as shown in Figure 29. for roots exchanged in the usual way.

Cortical Region: The calcium retained in the cortex was limited to the inner cortex, again localised within the cell wall, as in Figure 30.

Endodermis: The autoradiographs showed the same distribution of calcium in the endodermis as that obtained with exchange in calcium chloride.(Figure 31.)

Protoxylem Initials: Aggregates of grains were present above these cells, as in Figure 28.

5.ATTEMPTS TO IDENTIFY THE CENTRES OF CALCIUM UPTAKE.

An indication of the nature of the sites involved in passive binding mechanisms is given by the autoradiographs. The localisations in the epidermis and hypodermis are particularly associated with the cell wall and it is possible that ion exchange occurs in the epidermis on the pectic components of the cell wall. The possibility of an active binding mechanism to constituents of the cell cytoplasm cannot be disregarded in the case of the endodermal and cortical localisations. An attempt has been made in the following experiments to extract the labelled calcium compounds from sectioned material and excised roots by the use of solvents and enzymes.

Since the freeze drying technique was time-consuming, the effect of normal chemical fixatives on the distribution of absorbed and retained calcium was first determined.

(a) Chemical Fixatives:-

Roots were treated under the adopted conditions with calcium chloride and exchanged in the following two fixatives, firstly with no added calcium and secondly with calcium added to give a concentration of 0·5 meq.Ca/l.

Fleming's Strong Fixative: 5·3% acetic acid
0·42% osmic acid
0·79% chromium trioxide
water to 100 mls.

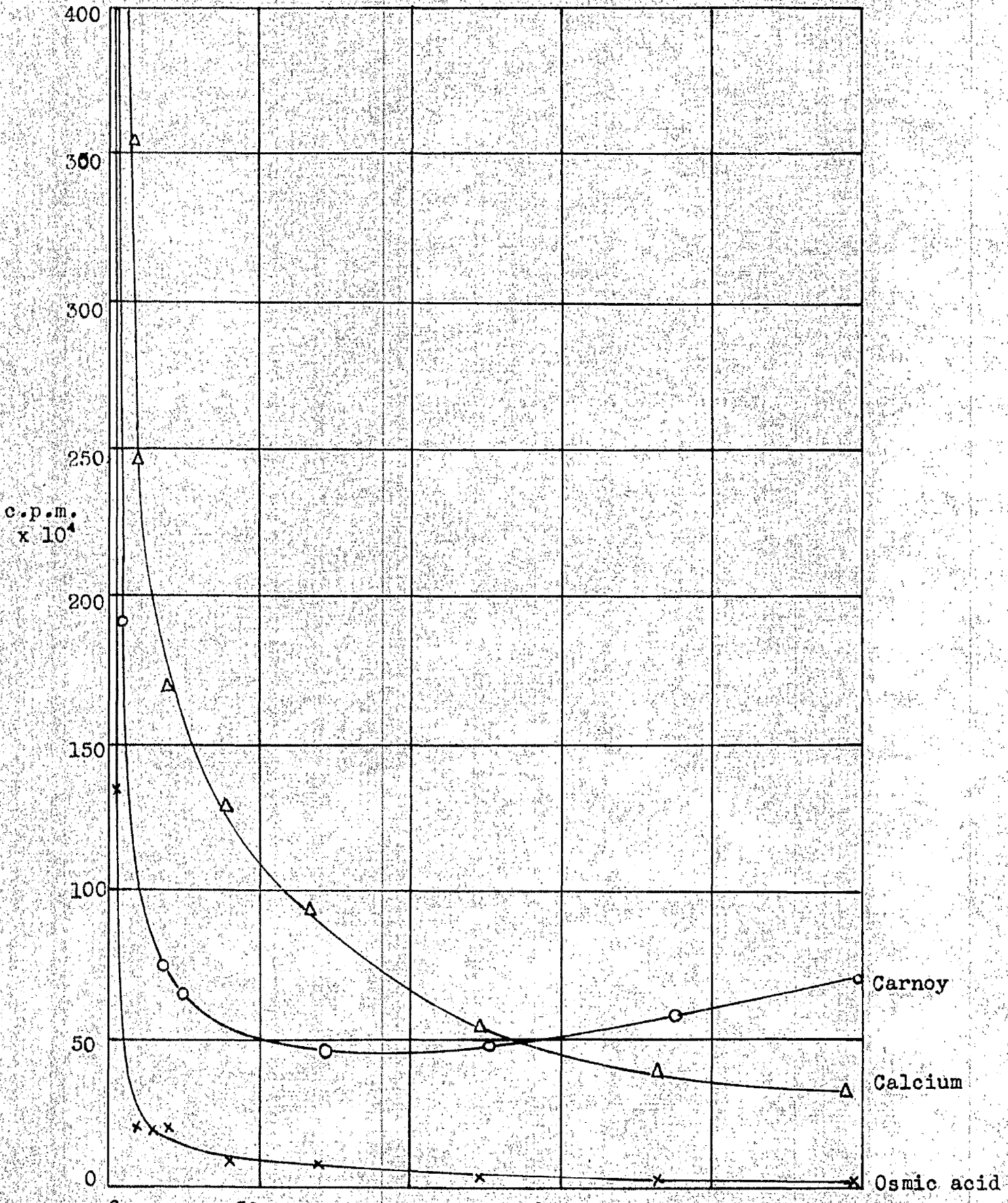


Figure 51. Exchange of Ca^{45} from onion roots in fixative solutions.

Carnoy le Brun Fixative: 10 ccs. 100% ethyl alcohol
10 ccs. glacial acetic acid
10 ccs. chloroform
mercuric chloride to saturation.

The exchange curves are presented in Figure 51. for the experiment with added calcium. The curves obtained with no added calcium were of a similar nature.

There was a marked increase in the activity in the roots after 100 minutes in the Carnoy le Brun fixative which might be attributed to the formation of additional binding sites on the surface of the root.

The final count in the roots after exchange in the fixative containing osmium tetroxide or osmic acid, was markedly lower than that in the roots exchanged in calcium alone. The only difference in the action of the two fixatives, due to their chemical components, appears to be in their ability to split carbohydrates. The chromium trioxide present in Fleming's fixative is reported to oxidise carbohydrates to aldehydes (144). Aldehydes were found to be present in this fixative after use.

The results suggest, therefore, that the chromium trioxide component acts either on the constituents of the cell wall or by affecting the permeability of the tonoplast, thereby allowing a free exchange of calcium.

Autoradiographs of exchanged and non-exchanged onion roots, prepared after fixation in Carnoy le Brun fixative for 2 minutes and embedding in paraffin, did not show a localisation of Ca^{45} in any tissue.

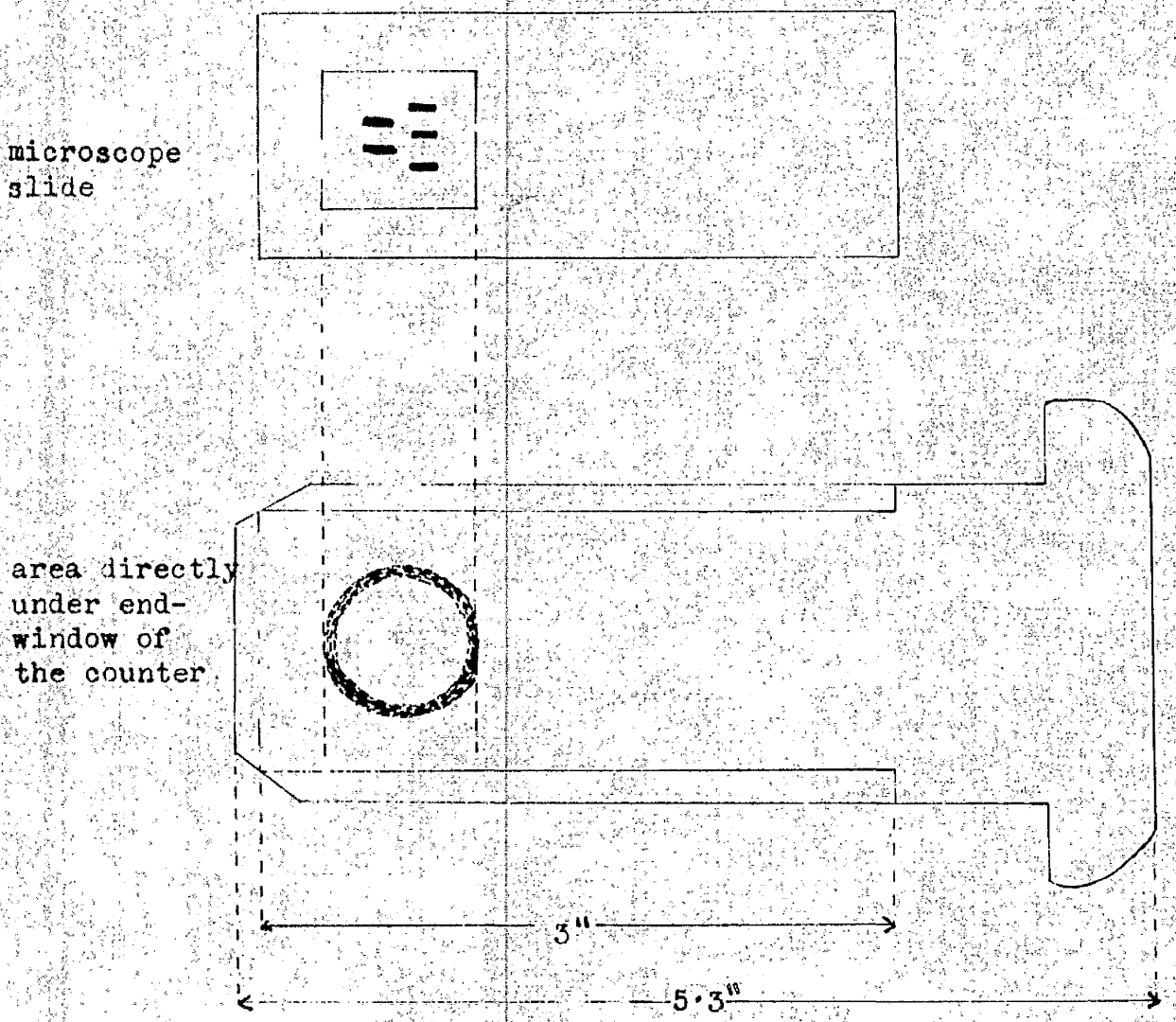


Figure 52 Planchet for counting sectioned material

TABLE IX

Solvents used in the extraction of Ca^{45} from onion roots.

SOLVENT	TREATMENT	MATERIAL REMOVED	REFERENCE
Perchloric acid	10% acid at 4°C, 12 hrs.	RNA	(146)
Perchloric acid	5% acid at 60°C, 30mins.	RNA + DNA	(146)
Hydrochloric acid	1 N , at 37°C, 3 hrs.	RNA + DNA	(147)
Trichloracetic acid	4% acid at 90°C, 15mins.	RNA + DNA	(147)
Trichloracetic acid	10% acid at 4°C, 18 hrs.	RNA + DNA	(147)
Alcohol/ether	Hot , 30 mins.	Lipid phosphorus	(147)
Sodium hydroxide	Dilute , 15 mins.	Phosphoprotein	(147)
Pyridine	17°C, 30 mins.	Lipids	(147)
Pyridine	60°C, 4 hrs.	Lipids	(147)
Ether	Hot, 3 changes.	Lecithins	(147)
Chloroform/alcohol	Hot, 3 changes.	Lipids	(147)

(b) Action of Solvents:-

Exchanged onion root material was prepared and sectioned according to the procedure outlined in Appendix 1. A special planchet was prepared from Perspex so that a microscope slide could be carried on it. Sections were placed on slides so that when introduced into the lead castle of a Geiger counter, the sections were directly underneath the end window, as shown in Figure 52. In this way, sections could be counted before and after treatment. Five 10μ sections were placed on each slide and these were treated with the solvents listed in Table IX.

It was found that aqueous solvents caused an immediate and complete loss of activity from the sections while fat solvents removed only slight amounts. In the latter instance, the distribution of calcium remaining in the root was similar to the distribution in exchanged living roots.

(c) Action of Enzymes on Excised Roots:-

The unrestricted removal of activity from sectioned material by aqueous solvents led to an examination of the action of enzymes on excised roots. The five enzymes selected for use were cellulase, pectinase, pepsin, ribonuclease and alkaline phosphatase. Onion roots were treated (a) for 3 hours in contact with the enzyme followed by normal uptake and exchange (pre-treatment) and (b) with Ca^{45} followed by exchange in calcium chloride and subsequently treated for 3 hours

TABLE X

Effect of Treatment with Enzymes on the Retention of Ca^{45} by Onion Roots after Exchange.

ENZYME	PRE-TREATMENT	POST-TREATMENT
Pectinase	The distribution of Ca^{45} in each tissue was similar to that in untreated roots except in the hypodermis where no localisation was found.	Distribution similar to pre-treated roots.
Cellulase	Similar distribution to that obtained with untreated roots.	Hypodermal localisation was not found.
Pepsin	Similar distribution to untreated roots but the endodermal uptake was less marked.	Distribution similar to pre-treated roots.
Ribonuclease	Similar distribution to normal exchanged roots but the size of the aggregates of grains above the cortical parenchyma was increased.	Similar distribution to untreated roots.
Alkaline Phosphatase	Similar distribution to untreated roots.	Similar distribution to untreated roots.

with the enzyme (post-treatment).

The root cells were found to undergo normal plasmolysis after treatment with the enzyme solutions and it is assumed that the latter did not have an adverse effect on the root metabolism. The counts on the roots were too alike to show any effect of the enzyme treatment. Autoradiographs were prepared and the distribution of Ca^{45} in the main tissues is detailed in Table X. Pectinase and cellulase appear to have the greatest effect. The action of pepsin and ribonuclease is less definite.

DISCUSSION.

1. Validity of the Autoradiographic Results:-

It was shown in the preliminary experiments on the development of the technique that displacement of Ca^{45} during freezing and drying is unlikely under the adopted conditions. Movement of the autoradiographic film during the preparation of the permanent mount is possible but any displacement of the aggregates of grains in relation to their characteristic position above the cell was not sufficient to be noticeable. The ready reproducibility of the results also confirms that the autoradiographs obtained, are a true representation of the distribution of the calcium absorbed and retained by the roots following the different treatments.

2. Vacuolar Accumulation of Calcium:-

In Table V, the pattern of uptake and exchange of Ca^{45} in living roots is compared with that in dead material. In the living roots, calcium was retained in the cortical parenchyma, hypodermis, endodermis, cells of the root cap and the protoxylem initial cells. In similar dead material, however, the localisations in the parenchyma of the cortex, root cap and protoxylem initials were absent and it is assumed, therefore, that the uptake into these tissues occurred by an 'active' process. Since the accumulation in the root cap is small in comparison to that of the cortex, and the uptake into the protoxylem initial cells is probably connected with translocation, the discussion of possible vacuolar accumulation will be limited to the localisation observed in the cortical parenchyma.

The formation of dense circular aggregates of grains suggests a small regularly shaped or 'point' source of activity. The number of atoms involved in the formation of each aggregate is in the order of 4×10^{10} A.M.U. or $2{\cdot}5 \times 10^{12}$ atoms of calcium when calculated from the activity present in a 10μ section. It is not easy with such a small weight of material (approximately 4×10^{-11} gm.), to anticipate how the soluble solids of the vacuolar sap would dry during freeze drying and how the dry material would behave when prepared for sectioning. By analogy with large scale models, it is possible that the material dries to a powder which might be expected to fall to the floor of the vacuole. It is most likely that the material would form an arc at the edge of the

vacuole, giving rise to an elongated aggregate of grains in an autoradiograph. The weight of the dry material will be very low and a redistribution of the powder might occur during subsequent treatment. The manner in which this redistribution would occur is purely speculative. The material might be carried on the surface of the embedding medium to the opposite side of the vacuole or be evenly distributed in the medium. In either case, it is unlikely that a small source of activity would be formed. Adhesion of the atoms to the sides of the vacuole may occur but an arc of material would still be present.

In the autoradiographs of stained sections, the aggregates are more closely associated with the edge of the cytoplasm than the vacuole. However, a section thickness of 10 μ is not ideal for the identification of an autoradiographic image with a particular cell component since, in many cases, part of the cell beneath the one being investigated, may influence the result. The question then arises as to whether there is any possible centre of accumulation in the cytoplasm consistent with such a small source. Calcium uptake has been associated with the mitochondria and microsomes by several authors (70,113,143), but there is no evidence to suppose that they or any other cell constituent are localised into a particular area in the cell.

The proximity of the aggregates of grains to the vacuole suggests that the tonoplast itself may be the site of calcium accumulation. However, it is expected that any accumulatory mechanism would operate over the entire surface of the membrane. In terms of ion pumps, as used

by MacRobbie and Dainty, it is possible that a calcium pump might be associated with the tonoplast but the autoradiographic results would require the restriction of such a mechanism to a particular point in the membrane, which seems improbable.

The autoradiographs of exchanged living roots show that the meristematic cells are particularly active in accumulating calcium. In the event of vacuolar accumulation, the mature cells would be expected to accumulate calcium to a greater extent than meristematic cells. The occurrence of a definite region of calcium accumulation also suggests that there are several factors influencing the accumulation of salts by plant roots which have yet to be assessed.

3. Calcium Uptake by Roots of Dendrobium sp.:-

The investigations on the roots of Dendrobium sp. indicate that the general pattern of calcium uptake and consequently the basic mechanisms of uptake are common to the roots of different plant species. Some slight differences are apparent in the extent to which calcium is localised in the various tissues in the two species studied but this is to be expected in view of the structural and physiological differences between the two types of roots. In the case of onion roots, a nutrient solution constantly surrounds the roots but the roots of Dendrobium sp. must absorb salts very rapidly from intermittent bathing solutions, the spongy epidermis being equipped to retain moisture and dissolved nutrients

after the bathing solution is removed. Therefore it is to be expected with these roots that the physical processes of absorption, such as ion exchange and adsorption, will be of particular importance giving a rapid uptake. The epidermis was found to demonstrate a high proportion of calcium held by ion exchange in the cell walls, being freely exchangeable. The relatively large amount retained in the meristematic region of this tissue, after exchange, suggests that adsorption to components of the cell wall also occurs with the formation of complexes having a low degree of exchangeability of the mineral ion.

In the roots of Dendrobium sp., the calcium held in the epidermis, hypodermis and endodermis which is assumed to be passively absorbed, will together represent a relatively large proportion of the total calcium retained after exchange. As a result, the amount of actively absorbed material appears to be comparatively low. This fact is borne out by the autoradiographs of exchanged material which show a correspondingly low number of aggregates of grains above the parenchyma of the cortex.

4. Selectivity:-

Further evidence to associate the active phase of absorption with the parenchyma of the cortex is available from the results of the experiments concerned with selectivity. In these experiments, the total cation concentration in the treatment and exchange solutions was maintained at 0·5 meq./l. and the calcium activity, although reduced to 2·5 μc/ml. was still sufficient to give the characteristic autoradiograph

No difference was found between the autoradiographs of material treated with a single salt solution and those of roots treated with a mixture of calcium and strontium ions. On exchange, however, it was found that no calcium had been accumulated from the two salt treatment into the cortical parenchyma and protoxylem initials. It is assumed, therefore, that the processes of absorption into these tissues are selective and hence are active processes.

The effects of exchange in a solution of strontium chloride after the normal uptake treatment are outlined in Table IX. A slow exchange was found to occur under these conditions from the parenchyma of the cortex, the activity in the inner cortex being the last to exchange. It is not certain whether that taken up by the protoxylem initials is merely slow to exchange or is not available for exchange. The endodermal and hypodermal calcium was also slow to exchange but that in the epidermis was readily removed. It is assumed, therefore, that under conditions favouring exchange, the actively absorbed calcium can be removed. At first this result appears contrary to the proposed mechanism of vacuolar accumulation. However, it has been reported (6), that by depriving a root of a particular ion, the ions in the vacuolar sap are released for translocation within the plant.

5. The Action of Solvents and Enzymes on Accumulated Calcium:-

Since relatively few autoradiographs were exposed in the studies on the extraction of the binding sites, it is considered that no definite

conclusions can be drawn from the results. At first, the rapid loss of calcium from sectioned material when exposed to aqueous solvents appears contrary to the continued retention of calcium observed after 17 hours exchange. In the event of vacuolar accumulation, however, the material in the now open vacuoles of the section would be expected to diffuse readily in water. The results, therefore, support the hypothesis of vacuolar accumulation.

The observations made on the effect of pectinase and cellulase on excised roots lend support to the possibility that pectic compounds or cellulose are involved in the process of ion exchange. The action of pepsin appears to be restricted to the endodermis but the effect is not definite. It appears that pre-treatment with ribonuclease causes an increase in the diameter of the aggregates of grains above the cortex in the region of calcium accumulation. It is considered that this evidence is, in itself, insufficient grounds on which to base any conclusion as to the nature of the binding sites.

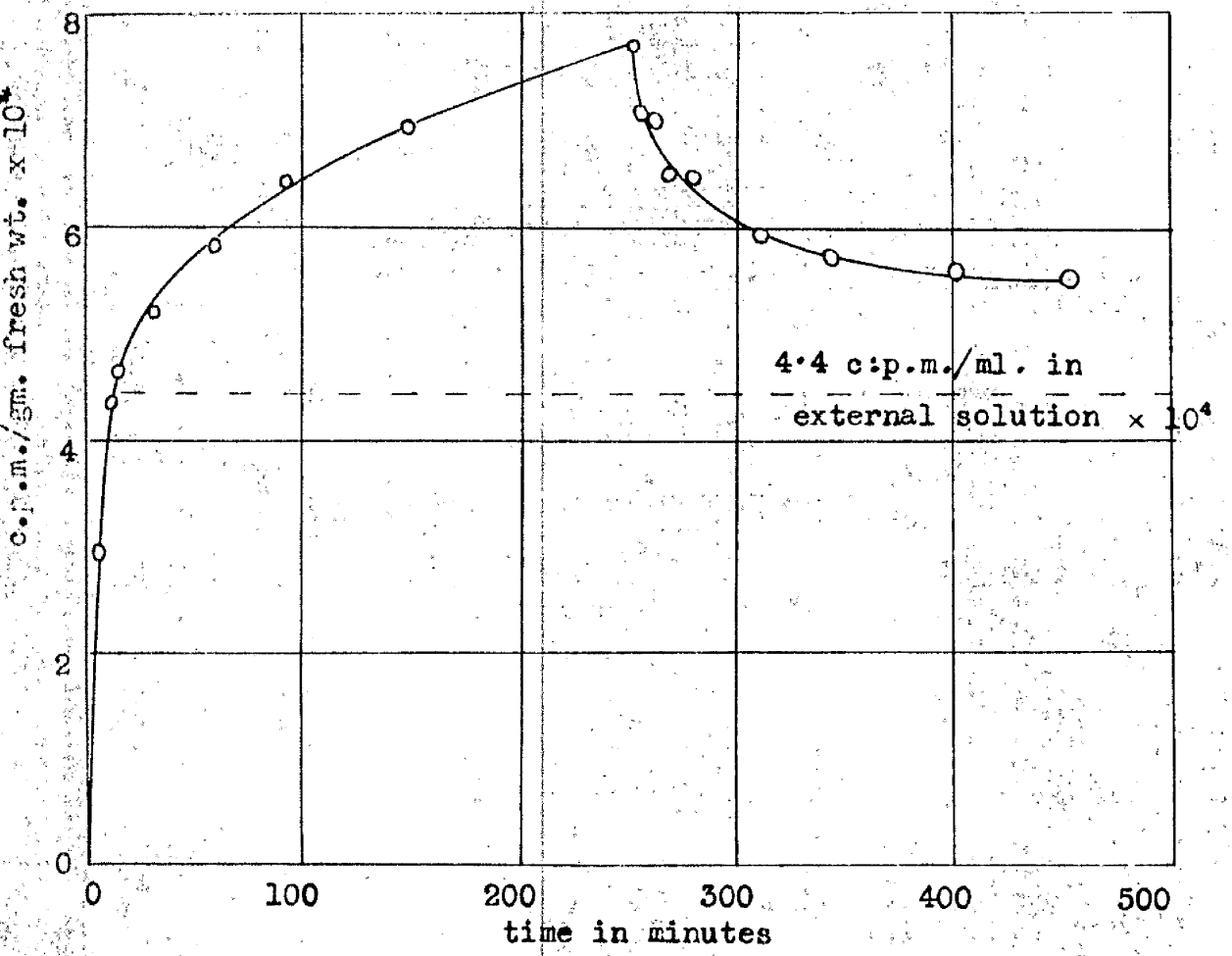


Figure 53. Uptake and exchange curve for rubidium ions with onion roots.

II. STUDIES WITH A MONOVALENT CATION.

It was felt that a comparison should be made between the uptake and retention of calcium and that of a monovalent cation. The available isotopes of monovalent cations are a mixture of β and γ radiations. Rb^{86} has a lower percentage of γ radiation than Na^{22} and K^{42} and has a half life of 18·6 days. It was found that a solution having an activity of 6 μc/ml. was required to give a satisfactory exposure time. The following experiments were carried out using the procedure adopted for calcium, treatment solutions containing 0·5 meq.Rb/l.

1. UPTAKE AND EXCHANGE OF RUBIDIUM IONS:-

The uptake and exchange curve for onion roots using a solution containing 0·5 meq. Rb / 1. is given in Figure 53. Although the concentration factor is not equivalent to that obtained with calcium after the same period of time, the shape of the curve is similar. A relatively small proportion of the total rubidium absorbed is exchangeable and the slope of the linear part of the curve is greater than the slope of the calcium uptake curve using the same material (Figures 7. and 53.).

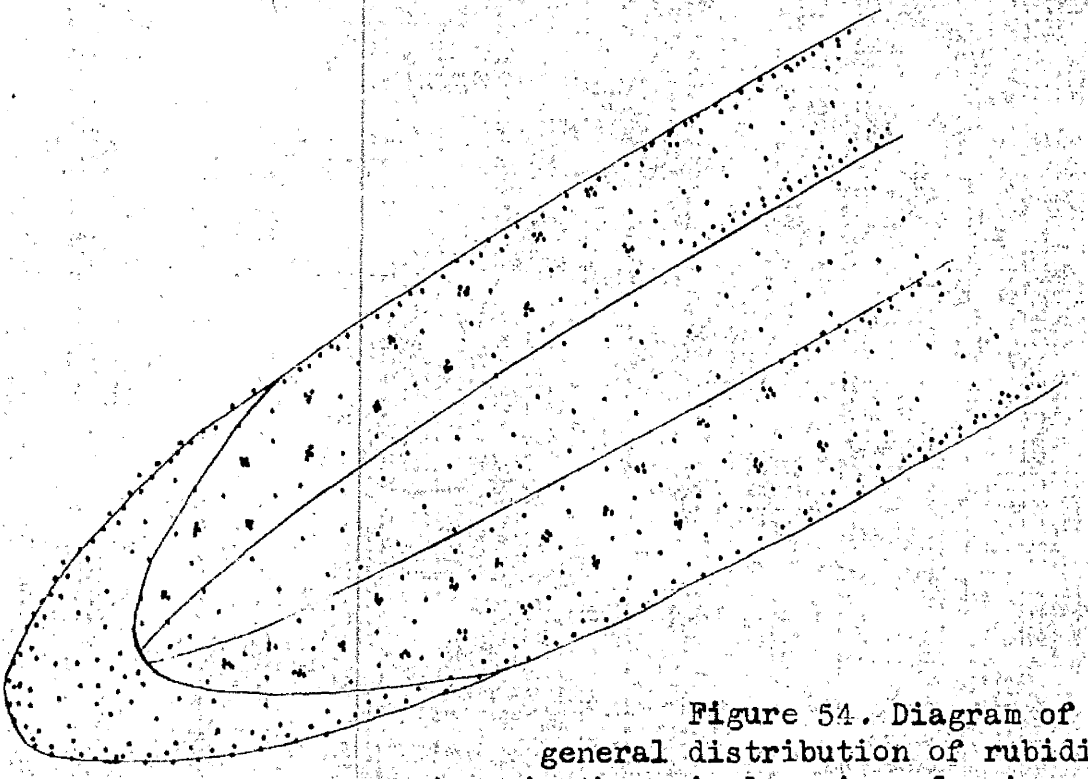


Figure 54. Diagram of the general distribution of rubidium ions in the apical region of onion root before exchange.

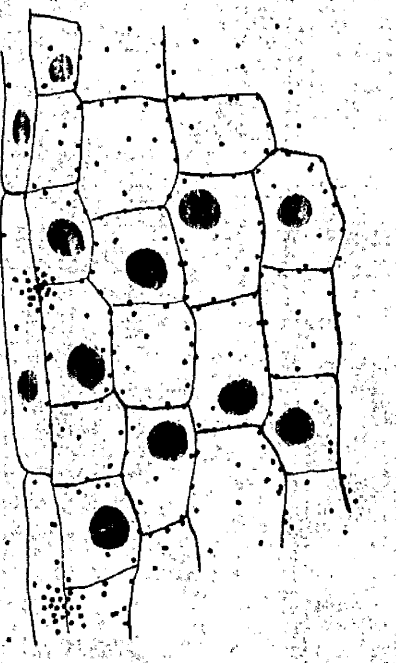


Figure 55. Longitudinal section of the apical region of onion root showing the localisation of rubidium in the epidermis before exchange.

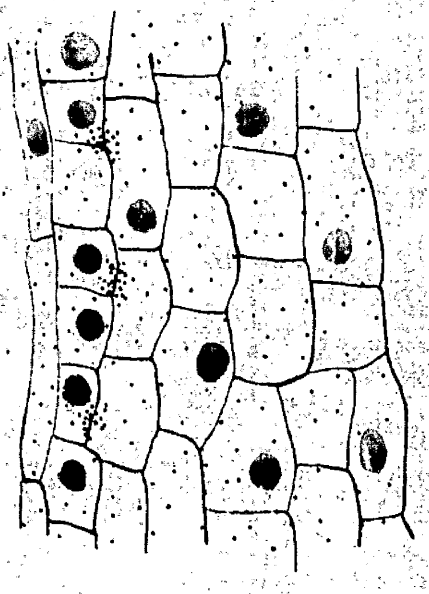


Figure 56. Longitudinal section of the apical region of onion root showing the localisation of rubidium in the hypodermis before exchange.

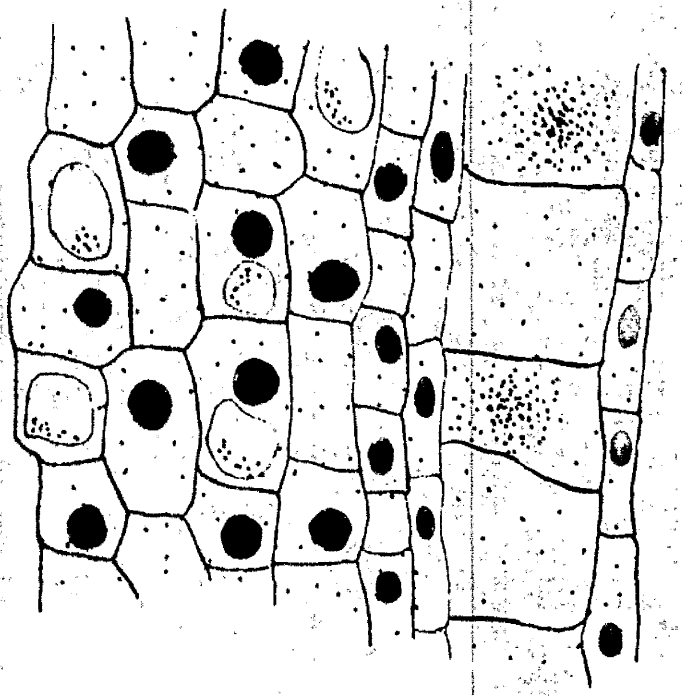


Figure 57. Longitudinal section of the apical region of onion root showing the distribution of rubidium in the inner cortex before exchange.

General Distribution of Rb^{86} in Non-exchanged Roots:-

The autoradiographs obtained with Rb^{86} were generally similar to those already described for calcium. (Figure 54.) There was a similar zone of uptake in the apical region of the root beyond which the hypodermis and the endodermis both demonstrated increased absorption of rubidium. The epidermis showed a lower absorption of rubidium and only a slight absorption was apparent in the central cells of the root cap.

Distribution at the Cellular Level:-

Root Cap: The uptake of rubidium appeared in the autoradiograph as a general darkening of the film above the central cells, no aggregates of grains being found.

Epidermis: As in calcium absorption, aggregates of grains were present above the cell walls. The aggregates were similar in shape and diameter to those observed with non-exchanged calcium treated roots but they were fewer in number and were less dense. The uptake was again restricted to the cells in the region of calcium accumulation.(Figure 55.)

Hypodermis: Similar aggregates of grains were found above the hypodermis beyond the region of calcium accumulation. They were positioned above the cell wall (Figure 56.).

Cortical Region: Distinct groupings of grains were evident above the parenchyma of the cortex. The reduced grains were not formed into circular aggregates but appeared as a line along the edge of the vacuole

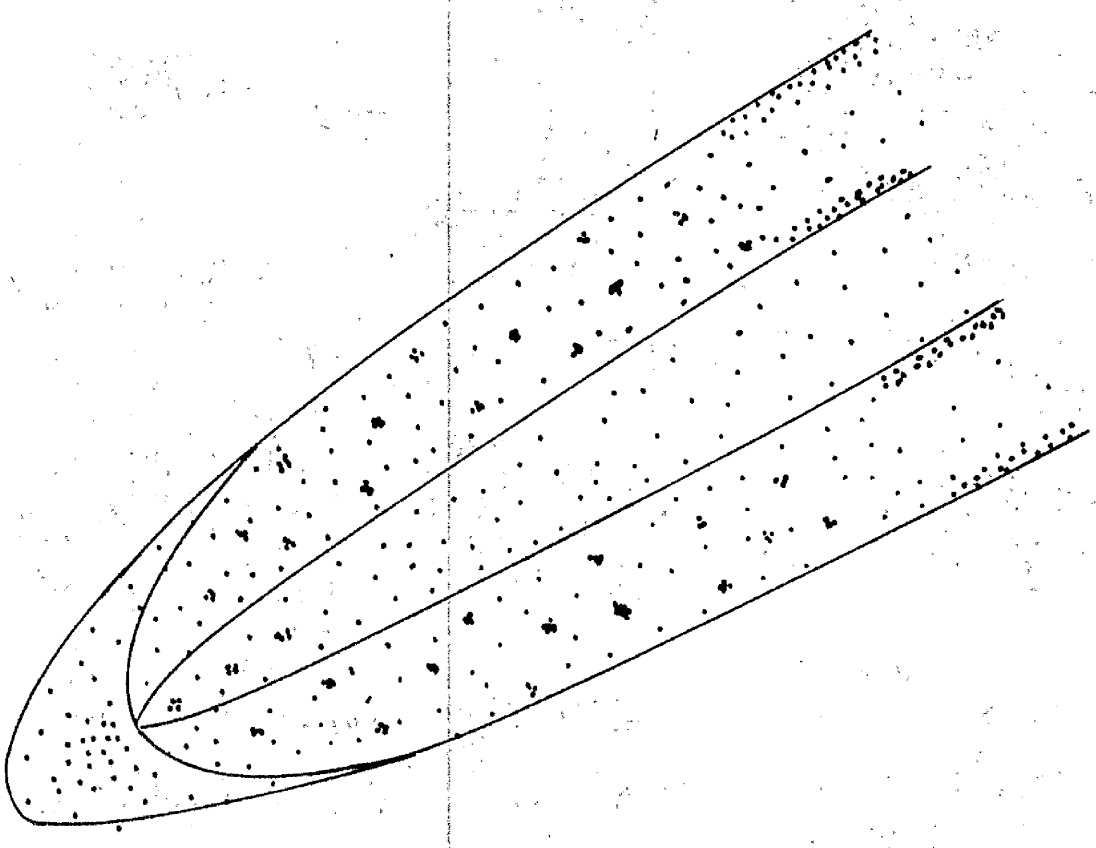


Figure 58. Diagram of the general distribution of rubidium ions in the apical region of onion root after exchange.

This localisation was restricted to the same area of the root as the region of calcium accumulation, again appearing above more cells in the inner cortex. The increased localisation in the meristematic cells which was found with calcium treated roots, was not repeated with rubidium. Some activity was present in the cell walls but considerably less than the calcium held in non-exchanged roots (Figure 57.).

Endodermis: Rubidium was found to be localised within the cell wall of those cells of the endodermis beyond the region of calcium accumulation.

Protoxylem Initials: A few aggregates of grains were found above these cells but this localisation was not always found to be present. Rb^{86} was localised within the cell wall (Figure 57.).

Distribution of Rb^{86} in Exchanged Roots:-

The autoradiographs of exchanged roots showed a slight decrease in the amount held in the cortex and a complete removal of epidermal rubidium. The material in the endodermis and hypodermis was retained (Figure 58.).

Distribution at the Cellular Level:-

Root Cap: A slight amount of rubidium was retained but no definite localisation was observed.

Epidermis: The rubidium was completely removed.

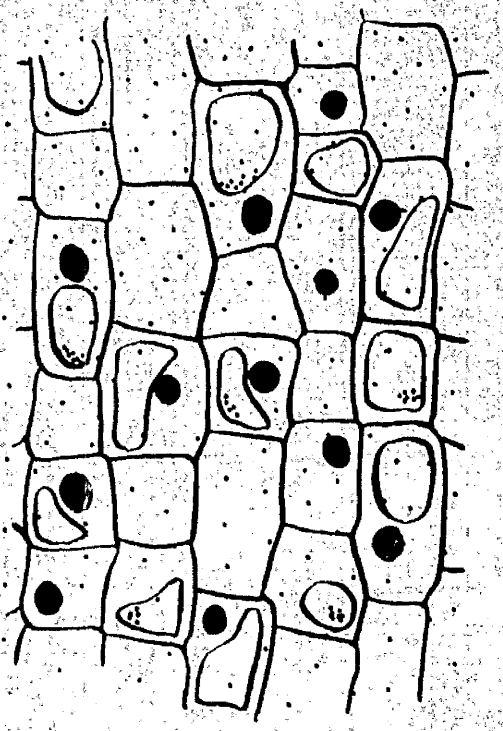


Figure 59. Longitudinal section of the apical region of onion root showing the rubidium localised in the cortical parenchyma after exchange.

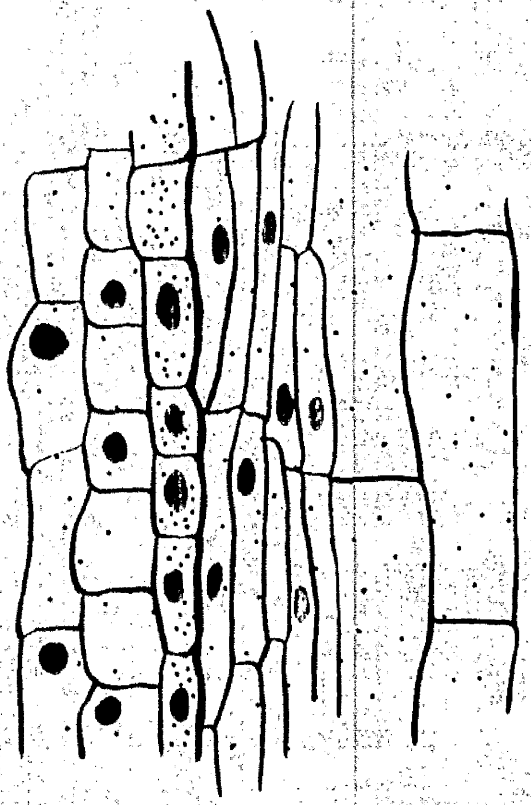


Figure 60. Longitudinal section of the apical region of onion root showing the rubidium retained in the endodermis after exchange

Hypodermis: The aggregates of grains were more distinct on exchange.

Cortical Region: The rubidium present in the cell walls was removed but the activity in the vacuoles was retained (Figure 59.).

Endodermis: The rubidium taken up by the endodermis was found to be non-exchangeable. The autoradiographs showed rubidium to be held generally in the cell cytoplasm, no aggregates of grains being present (Figure 60.).

2. DISCUSSION:-

Table XI summarises the distributions obtained with the divalent cation and the monovalent cation. It is apparent that most aspects of the absorption process are common to both ions. The region of calcium accumulation in the root, as well as the tissues in which localised concentrations of the cations occur, are identical indicating that a similar mechanism of uptake operates for both cations.

It is to be expected that the extent to which each component of the passive and active phases occurs, is dependent on the ion being absorbed. In this respect, slight differences are evident in the uptake of rubidium and calcium. A comparison of the uptake and exchange curves (Figures 7,16 cf. Figure 53.) shows that the proportion of passive uptake or exchangeable material, to total uptake at 240 minutes is

TABLE XI

Comparison of the Distribution of Rb^{86} and Ca^{45} in Non-exchanged and Exchanged Onion Roots.

TISSUE	NON-EXCHANGED		EXCHANGED	
	Rubidium	Calcium	Rubidium	Calcium
Root Cap	General uptake.	General and aggregates of grains in film.	Few aggregates of grains in film above central cells.	Aggregates above cells in centre of root cap.
Epidermis	Some small aggregates of grains in film.	Aggregates above cell walls in region of Ca accumulation.	Rubidium exchanged.	Calcium exchanged.
Hypodermis	General uptake.	Highest uptake in cells beyond 3·5 mm back from root tip.	Aggregates in film above cells beyond 3·5 mm back from root tip.	Aggregates more numerous than in equivalent rubidium treated roots.
Cortical Region	Line of grains in film above vacuoles in region of Ca accumulation	General uptake, most in cell walls Region of Ca accumulation	Distribution similar to non-exchanged roots. No Rb in cell walls.	Numerous aggregates. Localisation within cell walls.
Endodermis	General uptake, greatest in cells beyond region of Ca accumulation.	General uptake in cells beyond region of Ca accumulation	Rubidium retained.	Localisation of Ca within cell walls.
Protoxylem Initials	Aggregates of grains in film above some cells. Localisation not always present.	No localisation.	Localisation not positively identified.	Localisation within cell walls. Aggregates present in film.

1·0 : 1·1 in the case of calcium and 1·0 : 3·5 for rubidium. This relatively large amount of free calcium present in non-exchanged roots was apparent as a higher concentration in the epidermis and as freely diffusible calcium in the cell walls of all tissues of the root across to the endodermis. The free calcium tended to camouflage the calcium that was actively accumulated in the cells of the cortical parenchyma. In the case of rubidium ions, this free salt was much reduced and the localisation in the cortex was recorded in the autoradiographs prepared from non-exchanged as well as exchanged material.

The different grouping of the grains of the autoradiographic film above the cortical cells to those obtained with a calcium treatment, may signify a localisation of the cation attained by a different mechanism of uptake. However, it is possible by using an uptake solution of Rb^{86} having a considerably greater specific activity, that an almost circular aggregate of grains might be formed of an equal size and density to that obtained with calcium. It was considered that a longer exposure time than that already used (135 days), would not give a stronger autoradiographic image since several half lives had elapsed and the activity remaining in the sections was very low.

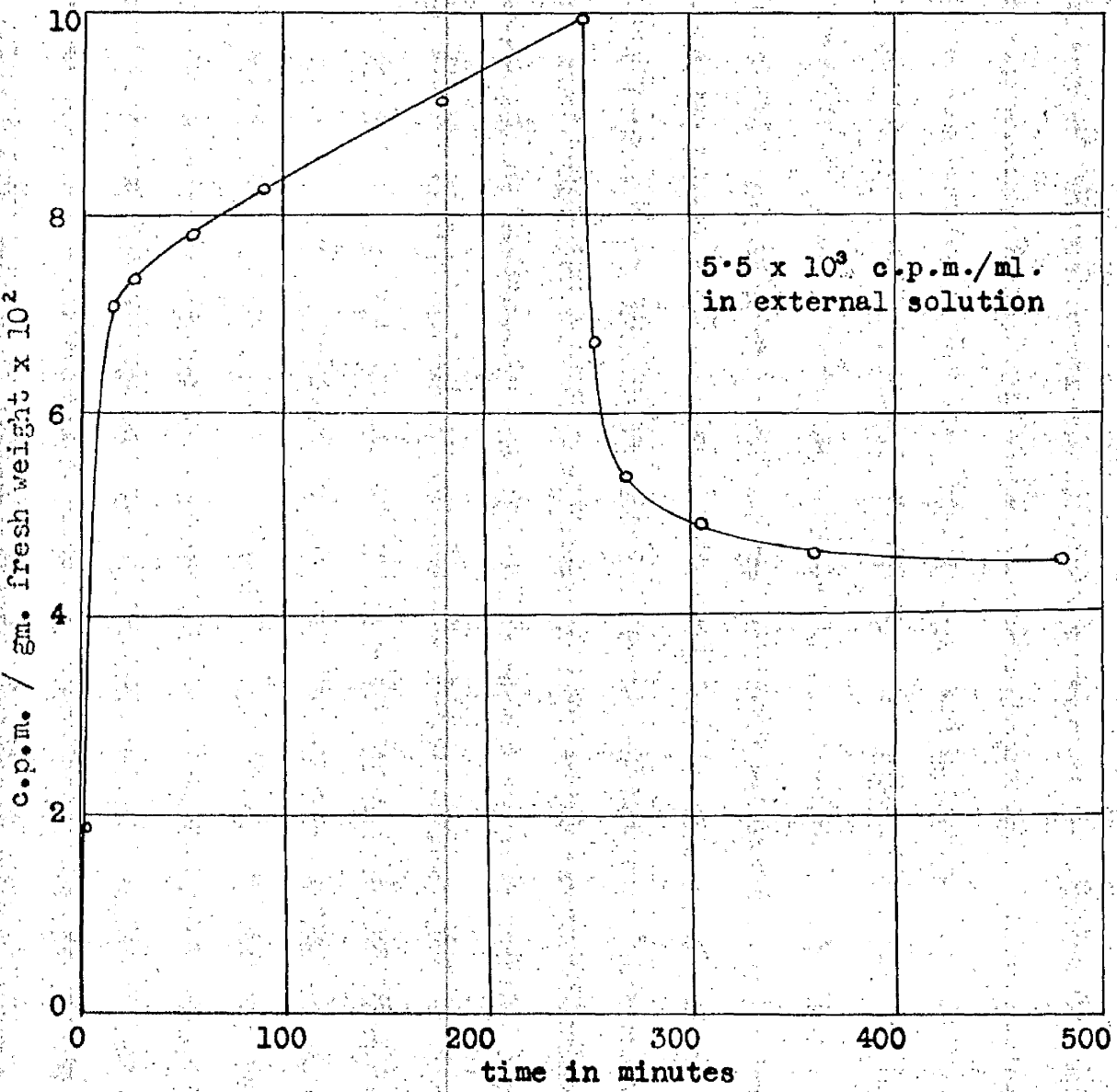


Figure 61. Uptake and exchange curve for sulphate ions with onion roots.

III STUDIES WITH ANIONS.

It is generally considered that the absorption of cations and of anions occur by two separate mechanisms but it is not known to what extent these two processes are interdependent. It was decided to apply the techniques already used with cations to a study of the behaviour of two selected anions. Sulphate was selected since S^{35} is a pure β emitter and is readily available with a high specific activity and has a half life of 87·1 days.

It was not known whether an anion which is not directly linked in a known way to a metabolic cycle, would follow the same pattern of uptake as sulphate ions. The halogens were considered in this respect and iodide finally selected since chloride could not be obtained with a sufficiently high specific activity and bromide has a very short half life. I^{131} has a half life of 8·04 days and the emission is a mixture of β and γ.

1.UPTAKE AND EXCHANGE OF SULPHATE IONS IN ONION ROOTS.

An uptake and exchange curve was prepared for onion roots by treating excised roots with a solution containing 0·5 meq. sulphate/litre. (Figure 61). A rapid initial uptake was observed over approximately

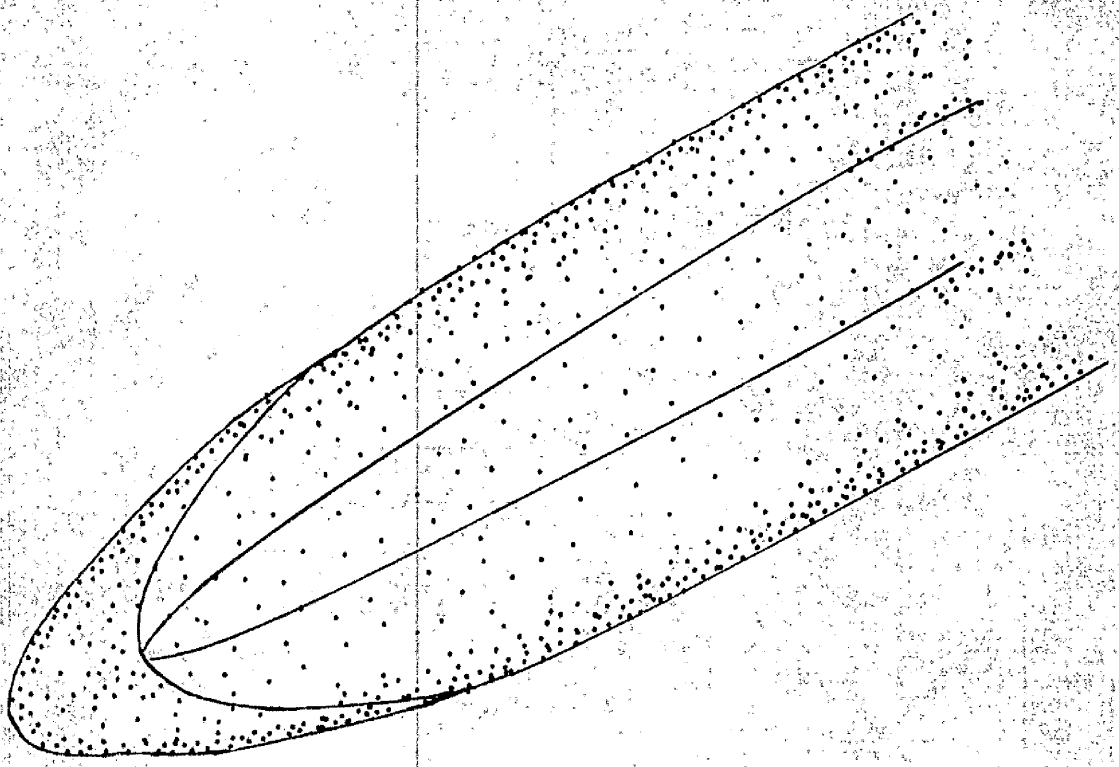


Figure 62. Diagram of the general distribution of sulphate ions in the apical region of onion root before exchange.

30 minutes, followed by a slower absorption which was linear with time. The general shape of the curve resembled that obtained with calcium and rubidium. Under the conditions of the experiment, the concentration factor at 400 minutes did not exceed one. Exchange did not appear to go to completion and the activity per gm. of root material (fresh wt.) remained higher than the c.p.m./ml. of the exchange solution.

The procedure laid down for calcium was followed, giving a 17 hour treatment period with the isotope (10 μc/ml.) for non-exchanged material and an uptake period of 7 hours followed by 17 hours exchange for exchanged material. Autoradiographs were prepared from freeze dried material in the standard way.

General Distribution of S^{35} in Non-exchanged Roots:-

The overall distribution of activity is illustrated in Figure 62. There was a general uptake in the outer cortex and around the tip of the root. An irregular distribution of sulphate was found in the epidermis and the endodermis showed an increased content of sulphate ions in those cells beyond the region of calcium accumulation (Figure 62.).

Distribution at the Cellular Level:-

Root Cap: A darkening of the film was observed above the outer layers of cells, the S^{35} being evenly distributed in the cell walls and cytoplasm.

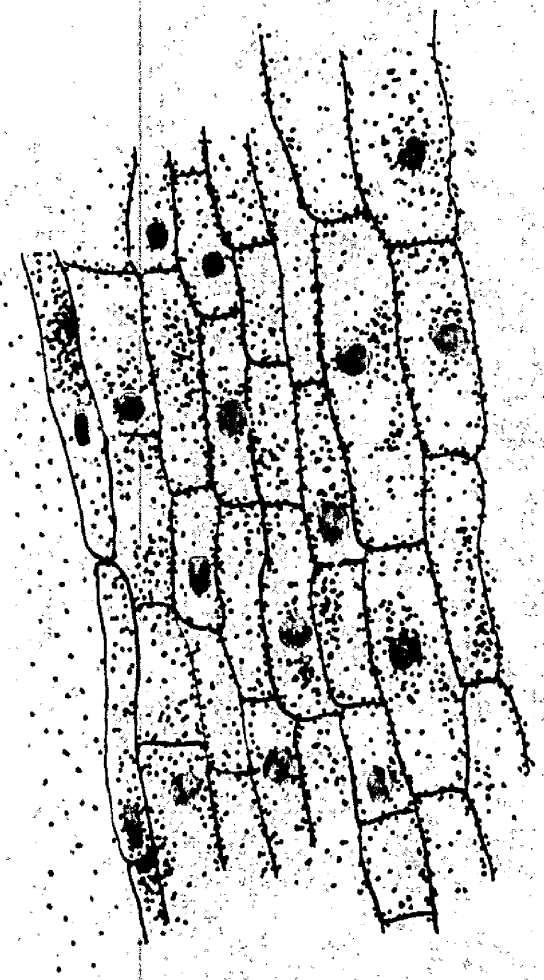


Figure 63. Longitudinal section of the apical region of onion root showing the sulphate held in the epidermis and parenchyma of the cortex before exchange.

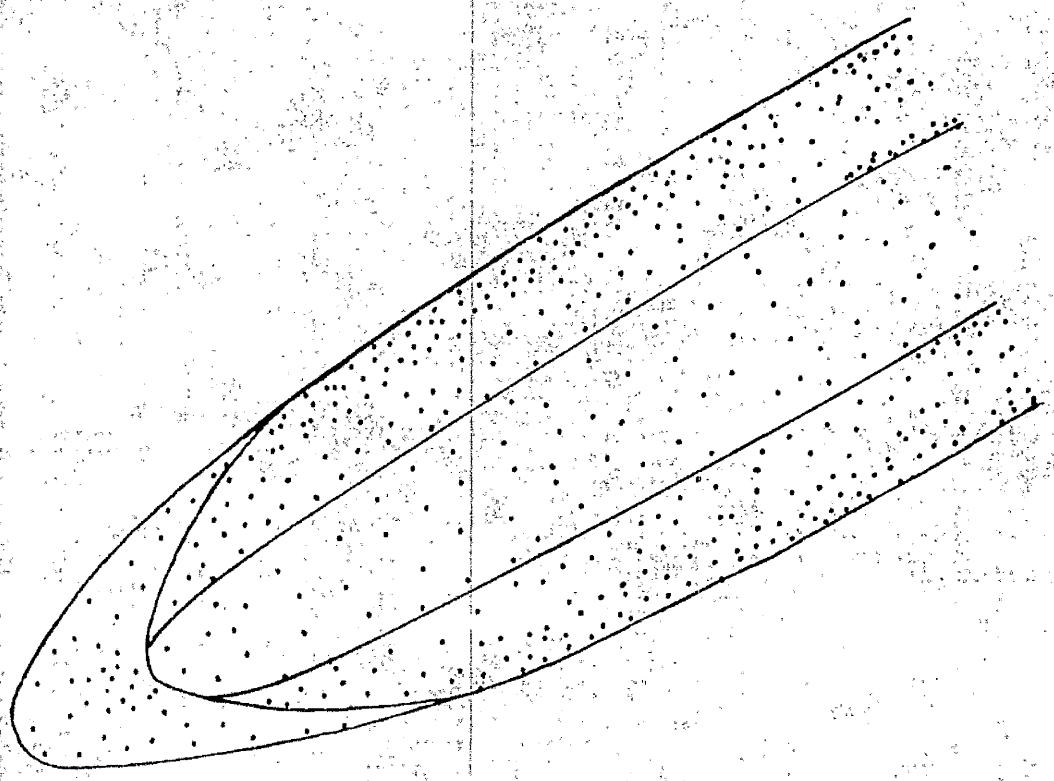


Figure 64. Diagram of the general distribution of sulphate ions in the apical region of onion root after exchange.

TABLE XII.

Distribution of S^{35} in Non-exchanged and Exchanged Onion Roots

TISSUE	NON-EXCHANGED	EXCHANGED
Root Cap	General uptake, greatest in outer layers.	No localisation.
Epidermis	Localisation within the cell walls.	No localisation.
Hypodermis	General uptake in cells beyond region of Ca accumulation.	S^{35} retained, no aggregates of grains were found.
Cortical Region	S^{35} was mainly in cell walls with a certain amount localised within the cell walls.	S^{35} was restricted to the region around the nucleus Some still present in cell walls.
Endodermis	General uptake in cells beyond region of Ca accumulation.	S^{35} retained, no aggregates of grains were found.
Protoxylem Initials	No localisation.	No localisation.

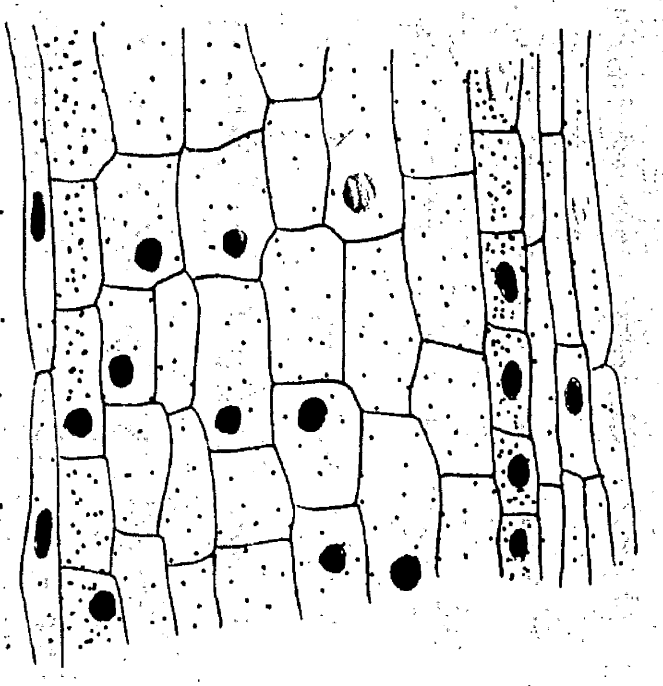


Figure 65. Longitudinal section of the apical region of onion root showing the sulphate retained in the hypodermis and endodermis after exchange.

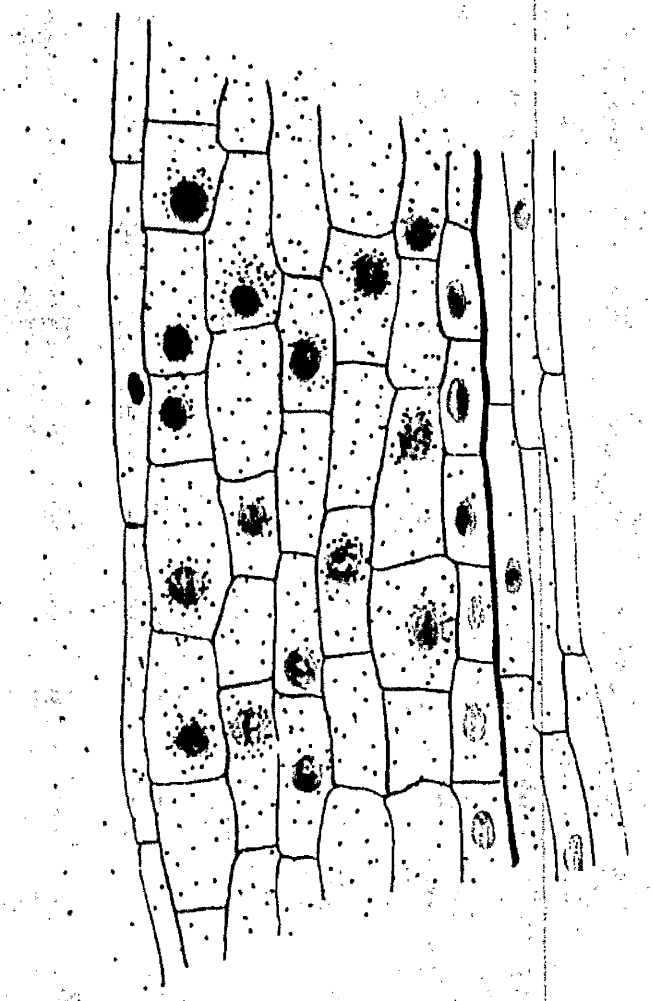


Figure 66. Longitudinal section of the apical region of onion root showing the sulphate retained in the cortical parenchyma after exchange.

Epidermis: Sulphate ions were localised within the cell walls and in a few instances; very irregular aggregates of grains were formed in the film above this tissue (Figure 63.).

Hypodermis: A small amount of sulphate was localised in those cells beyond the region of calcium accumulation.

Cortical Region: The grains of the image were found largely over the cell walls but a smaller number was present above the cytoplasm. No circular aggregates of grains were found. The cells showing the greatest concentration of S^{35} were those of the outer cortex (Figure 63.).

Endodermis: The uptake was greatest in those cells more than 3-4 mm. back from the root tip. The darkening of the film was more intense over the area within the cell wall.

General Distribution of S^{35} in Exchanged Roots:-

Figure 64. illustrates the distribution of sulphate ions after exchange. The sulphate held in the root cap and epidermis was found to be readily exchanged but the major portion of that in the cortical cells, endodermis and hypodermis was retained.(Table XII).

Distribution at the Cellular Level:-

Hypodermis: A general darkening of the film was found above this tissue (Figure 65.).

Cortical Region: The sulphate was clearly localised in the

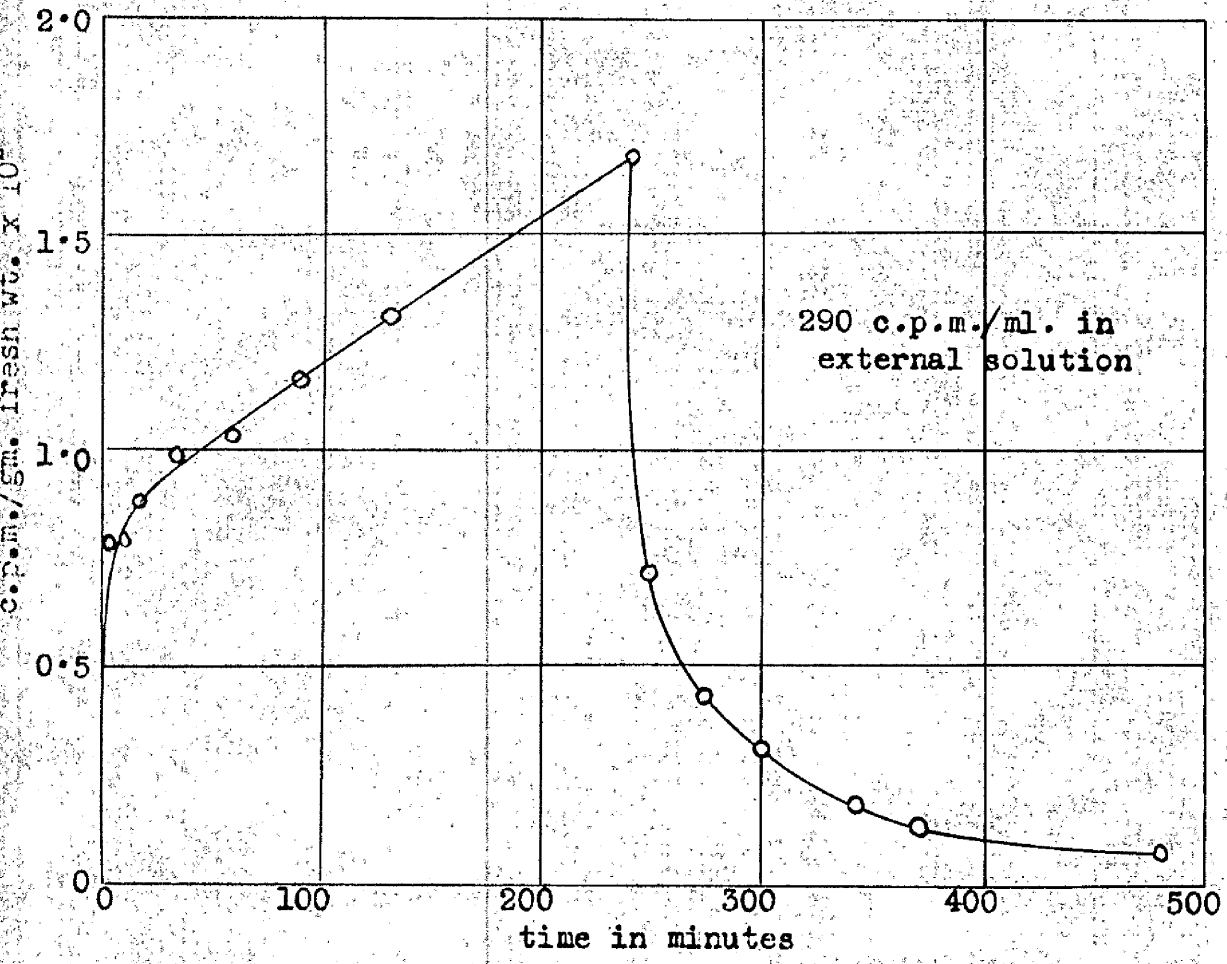


Figure 67. Uptake and exchange curve for iodide with onion roots.

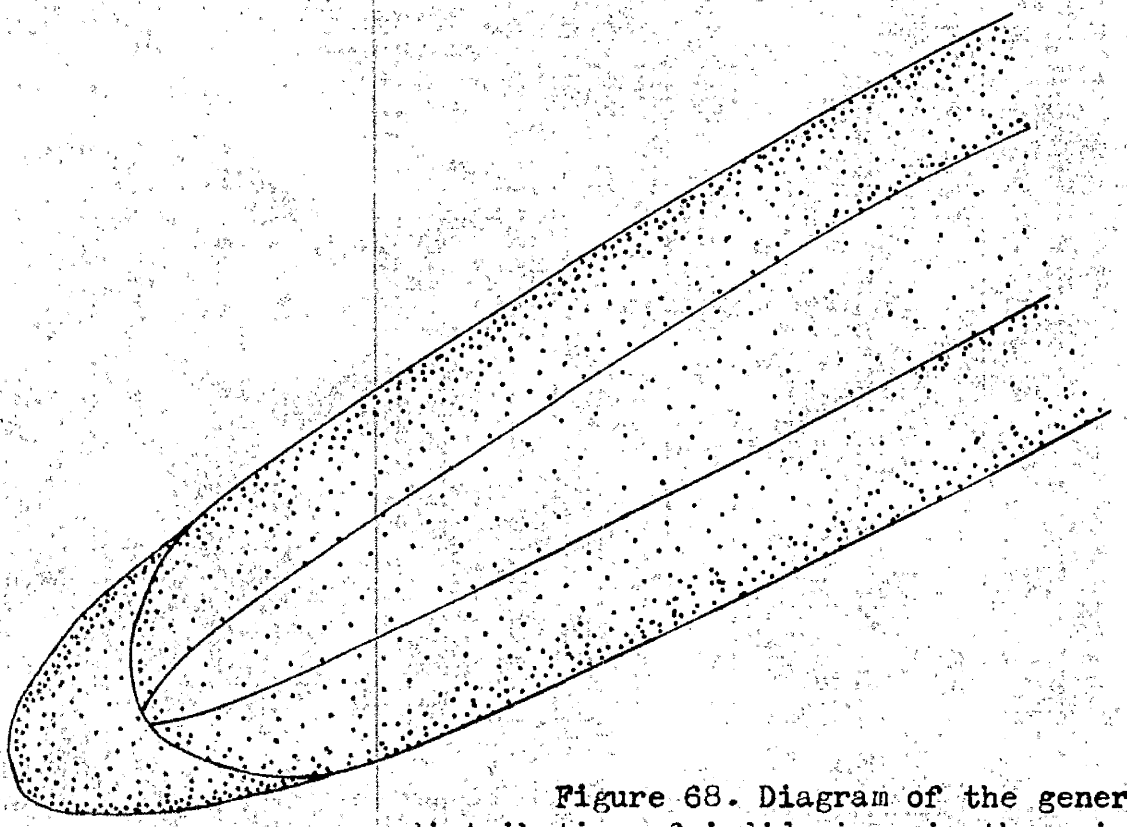


Figure 68. Diagram of the general distribution of iodide ions in the apical region of onion root before exchange.

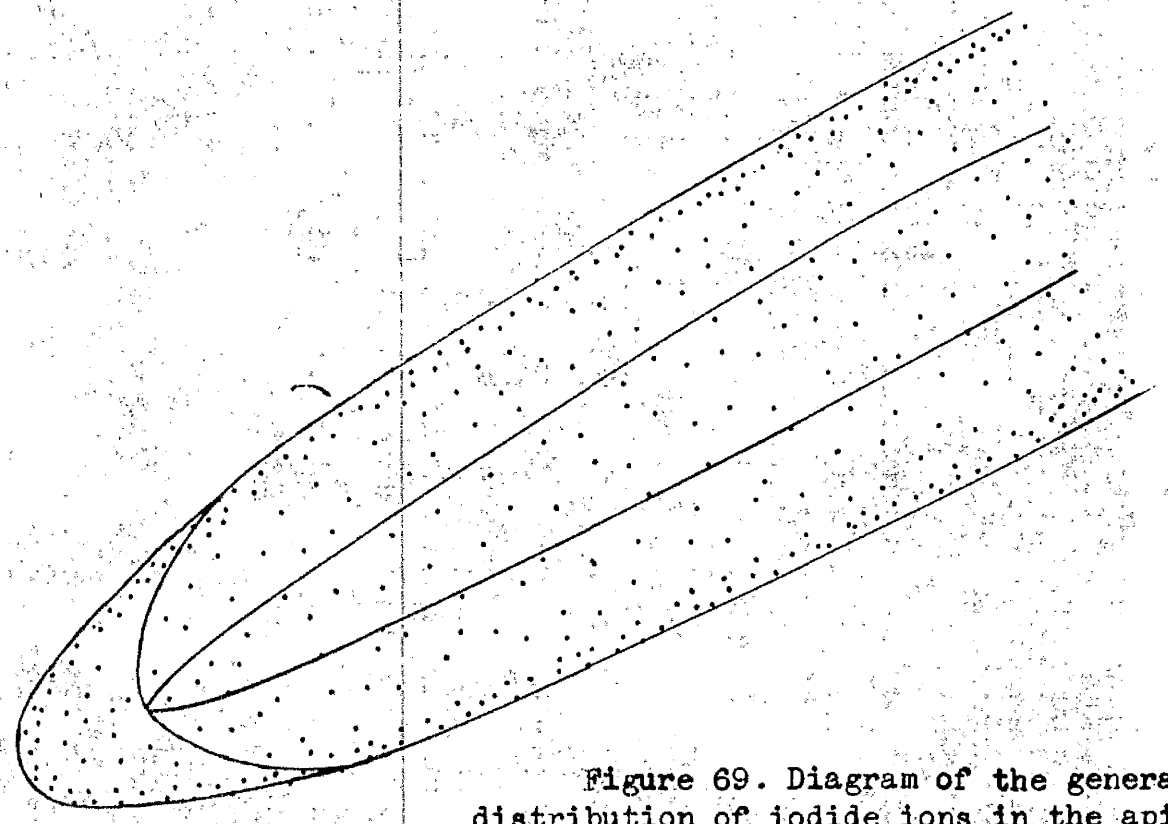


Figure 69. Diagram of the general distribution of iodide ions in the apical region of onion root after exchange.

region of the nucleus as shown in Figure 66. Most of the sulphate ions present in the cell walls were removed by exchange.

Endodermis: The isotope was retained as shown in Figure 65.

Protoxylem Initials: No localisation was found in either non-exchanged or exchanged onion roots.

2. UPTAKE AND EXCHANGE OF IODIDE IONS BY ONION ROOTS.

The uptake curve (Figure 67.) shows that a concentration factor of one was not obtained after 400 minutes in a solution containing 0·5 meq. iodide/litre. Exchange appeared to go to completion and the activity per gm. fresh weight of roots tended to equilibrium with the c.p.m./ml. of the exchange solution. Autoradiographs were prepared of non-exchanged and exchanged material.

General Distribution of I^{131} in Non-exchanged Roots:-

The autoradiographs showed that the uptake of I^{131} occurred mainly in the outer cortex (Figure 68.). The epidermis and hypodermis absorbed the ion equally and no regions of high uptake were observed. An even distribution of reduced grains was found in the film over the cell walls and the cytoplasm, no aggregates of grains being found above any tissue. A slightly increased absorption was found in those cells of the endodermis beyond the region of calcium accumulation.

TABLE XIII

Distribution of I^{131} in Non-exchanged and Exchanged Onion Roots.

TISSUE	NON-EXCHANGED	EXCHANGED
Root Cap	General uptake	No localisation
Epidermis	General uptake	No localisation
Hypodermis	General uptake	No localisation
Cortical Region	General distribution, mainly in the outer layers	Slight retention in the outer layers
Endodermis	Slight general uptake	No localisation
Protoxylem Initials	No localisation	No localisation

General Distribution of I^{131} in Exchanged Roots:-

The amount retained in the roots after 17 hours exchange was found to be negligible, the remaining iodide being restricted to the outer cortex. The isotope was evenly distributed over the cell wall and the cytoplasm (Figure 69.),(Table XIII).

3. DISCUSSION.

The uptake and exchange curves obtained with sulphate and iodide are similar to those obtained with calcium and rubidium. In the case of iodide,however, the curve levels off after the initial period of rapid absorption indicating that iodide ions are not actively absorbed. This fact is further supported by the continued exchange by inactive iodide after the normal exchange period. The results will be discussed in relation to a general hypothesis of salt absorption in the final section of this thesis. This discussion will be limited to a comparison of the absorption of cations and that of anions as shown by the autoradiographs.

In the case of sulphate ions, diffusion appeared to be limited to the wet cellulose walls and adsorption and ion exchange mechanisms were not found in the epidermis. In this tissue, the sulphate ions appeared to be held generally within the cell wall and after exchange the distribution of S^{35} in these cells was similar to that in the cells

of the cortex which suggests that no particular action is attributed to the epidermis as was the case with cation absorption.

The autoradiographs obtained with sulphate and iodide ions show that vacuolar accumulation of these ions does not occur. It is assumed that iodide is not actively accumulated in onion roots and that this may apply to all halogens since there is no evidence to relate the halogens to the metabolic processes known to occur in plant roots. Chloride ions have been reported to be required in photosynthesis (148) and it is possible that these ions will diffuse passively across the root for direct translocation to the leaves.

Sulphate accumulation is clearly associated with the nucleus of the cell and it is probable that this is also the site of protein metabolism.

The localisation in the endodermis is similar to that of absorbed cations and the region of sulphate accumulation in the cortex of the apical region of the root was similar, though less well defined, to the region of calcium accumulation.

DISCUSSION.

It is becoming increasingly clear that the absorption of salts is a complex process which does not depend on the operation of a single mechanism but on the co-operation of several. The existence of an 'active' phase of absorption has previously been based on the continued retention of ions after exchange and on the observed decrease in salt uptake under conditions prohibiting respiration. Further support is given to the operation of an active mechanism, in the case of cations at least, by the autoradiographs comparing living and dead onion roots. However, it is evident from these results that the material retained after exchange is not all absorbed by 'active' means since the accumulation into the endodermis and hypodermis was found in exchanged dead roots as well as living roots.

The question of a link with respiration has been approached from several viewpoints and in most cases, the relatively small amount of experimental evidence can be successfully applied to any hypothesis of salt absorption. It has been suggested that only anion absorption requires the expenditure of energy by the cell, in which case, cation absorption may occur by passive means such that the electrical neutrality of the cell is maintained. It is to be expected that the direct absorption of anions into metabolic cycles will depend on the maintenance of these cycles, on A.T.P. and hence directly on respiration. With regard to cations however, the link with respiration has not been elucidated. Since the dead onion roots did not demonstrate an accumulation of calcium in the

cortical parenchyma, it is assumed that an actively metabolising cell is required for cation accumulation. The separation in space of cation and anion accumulation sites in the cell, as shown in the case of calcium, rubidium and sulphate absorption by autoradiography, suggests that the absorption processes are not linked and possibly are not interdependent and it follows that separate links with respiration may exist.

In order that the electrical balance is maintained over the entire cell, it is possible that organic acid anions and hydrogen ions are absorbed into or released from the cell according to the mineral ions being accumulated.

Reports of an absorption of cations in apparent excess of the accompanying anions (52) have appeared in the literature but when the organic acid content of the cell (28) or carbon dioxide fixation (85) is also taken into account, the cation uptake is equivalent to the anions absorbed.

PASSIVE ABSORPTION.

It appears from the autoradiographic results reported in the previous sections and from published data, that several physical processes are involved in the passive phase of absorption. The evidence from both sources suggests that in most plant cells and tissues, ions diffuse readily in the wet cellulose walls and, in the case of cations, into the cytoplasm

as well. Passive movement of anions across the plasmalemma is not well established in the literature. However, the autoradiographs with iodide ions suggest that such a movement may occur since this ion was shown to be present in the cytoplasm as well as in the cell walls of non-exchanged roots. From experiments with sulphate ions, it was found that the site of active absorption was the nucleus of the cell and should the plasmalemma act as a barrier to diffusion of anions, a second site of active uptake would be formed at the cell wall.

It was not possible to distinguish between the sites of adsorption and ion exchange in the autoradiographs. Those obtained with dead non-exchanged and exchanged roots showed ~~that~~ the passive phase of absorption to be associated with the epidermis, hypodermis and endodermis. However, only that taken up by the epidermis is freely exchangeable. The aggregates of grains in the film were usually localised over the corners of the cells and the cell wall suggesting an exchange mechanism of calcium to pectate. The epidermal uptake was also found with rubidium but to a lesser extent and, in this case, the localisations were not associated particularly with the corners of the cells. Presumably adsorption and binding can occur to a variety of substances and the ions will be held with different degrees of exchangeability. Cations adsorbed by cellulose or held as pectates or proteinates are probably easily displaced by other cations from the medium or by hydrogen ions. Those ions forming stronger chemical combinations such as chelates with nucleic acids are probably only exchangeable with difficulty.

Helmy and Elgaby (48,153) have reported that the groups responsible for cation exchange are destroyed by heating as would occur in the experiments with dead roots. The distribution in the epidermis does not substantiate this statement.

Localisation of anions in the epidermis was slight and no aggregates of grains were found in the autoradiographs above this tissue which confirms the contention that ion exchange and adsorption mechanisms are predominantly cationic.

The uptake observed with all the ions studied into the endodermis, and, with the exception of iodide, also into the hypodermis, is not readily explained in terms of non-exchangeable binding. In the autoradiographs the localisation in the hypodermis appears as aggregates of grains in the case of the cations studied and as a general darkening of the film with sulphate and iodide. There is no apparent reason for the uptake into the hypodermis except that of a simple barrier which appears possible in the case of sulphate ions where a general uptake was observed. No particular importance has been attributed previously to the hypodermis (154).

The distribution in the endodermis is similar for both anions and cations and since the ions are held generally within the cell wall in each case, it seems probable that the mechanism is common to all ions. The general nature of the localisation does not suggest a binding mechanism and therefore it is proposed that the plasmalemma of the mature endodermal cells permits diffusion of ions into cells but prevents

exodiffusion. It is to be expected that the entire surface of the plasmalemma will act in this way, preventing diffusion of salts into the mature xylem elements and also rediffusion of salts into the cortex. Exchange will be slow even under conditions favouring exchange as in the experiments using strontium.

ACTIVE ACCUMULATION.

From the autoradiographs, it is not possible to say conclusively in the case of each cation investigated that vacuolar accumulation does occur but it seems most likely on the evidence available. On the other hand, it is very clear that a similar vacuolar accumulation of anions does not occur, at least in the case of sulphate and iodide.

The most likely mechanism of absorption of anions is their direct absorption into metabolic cycles rather than a vacuolar accumulation. Robertson (8) has suggested that anions may be accumulated by more than one mechanism since it is unlikely that all the anions taken in by the root could be absorbed immediately into the metabolic cycles. The rapid incorporation of supplied phosphate into adenosine triphosphate has been shown in a variety of plants (145,155). In 1956, Leggett and Epstein (26) showed that the sulphate absorption which they observed, was consistent with the view that the absorption involved specific sites in the cytoplasm. From the autoradiographs, it is possible to limit the site of sulphate absorption and hence of protein metabolism, to the

region around the nucleus of the cell.

It has been suggested by Lundegårdh, that ions such as chloride which are not incorporated, as far as is known, into the structural components of the cell, might be temporarily bound to respiratory enzymes such as cytochrome oxidase. The autoradiographs obtained with iodide do not show any accumulation of the iodide ions and the vacuolar accumulation visualised by Lundegårdh, Hoagland and Broyer (57,104) is not supported.

The absorption of anions into their respective metabolic cycles can be assumed to occur with a high degree of specificity. No direct competition between anions such as nitrate, sulphate and chloride would be expected since they are absorbed into different cycles and none has been reported. Competition may only exist between similar ions such as sulphate and selenate which can enter the same cycle (26).

Absorption of cations directly into metabolic cycles has not been supported except possibly in the case of potassium which might be bound to intermediates of glycolysis in the cytoplasm. Rubidium is assumed to follow the same path of absorption as potassium and it is to be expected that the autoradiographic responses will be similar. The autoradiographs obtained with rubidium do not show a localisation in the cytoplasm and therefore it is assumed that the absorption into glycolysis, if present, was insufficient to give an autoradiographic image. In comparison with the vacuolar accumulation observed, the localisation would be very slight.

The evidence for vacuolar accumulation of calcium has been discussed in the experimental sections. It is considered that the major part of the evidence is in support of this form of 'active' absorption and that it may apply generally to cations.

Active absorption has been shown to be highly specific and it is assumed that several different carrier molecules are required, each specific to a group of cations. In this way, the competitive inhibition of uptake can be attributed to ions within the same group competing for the same carrier molecule. No attempt has yet been made to identify these carrier molecules although several materials have been suggested such as ribonucleic acid (106), phosphatidic acid (107) and lecithins (108).

TRANSLOCATION.

The region of calcium accumulation is clearly defined in the autoradiographs obtained with calcium and rubidium and to a lesser extent with the anions studied. The importance of this region has been briefly discussed under the heading of Vacuolar Accumulation but not in its relation to the whole root.

The presence of an intense localisation of non-exchangeable material in the cortex, in a region of the roots in which the endodermis does not retain ions, suggests a link with translocation. It is most likely that an osmotic pump is formed which promotes the flow of ions

by simple diffusion from the external solution directly into the stele in this region of the root. It is conceivable that the function of the accumulated calcium in the root cortex is the formation of such an osmotic pump.

The extent of the region of the cortex in which calcium, rubidium and sulphate ions were accumulated, averaged 1000 - 4000 μ back from the root tip. This part of the root coincides with the region of water absorption and also it has been shown that the endodermis in this region does not accumulate salts. It is proposed, therefore, that the region of maximum salt absorption is also the region of maximum translocation.

The autoradiographs of exchanged calcium and non-exchanged rubidium material showed a localisation in the protoxylem initial cells which was virtually non-exchangeable under the conditions of the experiment. This result is in accordance with the 'bleeding phenomenon' described by Hylmo (105) in which he proposes that during the formation of the xylem elements, the 'test-tube' cells absorb ions as in active accumulation. The transverse end wall then ruptures and the vessels interconnect.

In discussing the evidence for salt absorption, a certain amount of confusion arises since it is general practice to relate the absorption characteristics of single celled plants to more complicated systems and vice versa. The experimental work on salt absorption can be

divided into three distinct groups ; on whole plants using techniques such as gross autoradiography and chemical anaysis of the plant parts, on excised roots by isotopic exchange and on large celled simple plants using microscopy as in the experiments of MacRobbie and Dainty. It is felt that the correlation of results from such varied experiments cannot be justified until the intermediate stage of absorption into each tissue structure of the root, has been fully elucidated.

CONCLUSIONS.

CONCLUSIONS.

1. A technique of autoradiography has been developed for use with water soluble isotopes, giving results at the cellular level with thin sections of plant material.
2. It has been shown by this technique that there are two phases in the process of salt absorption by excised plant roots. The existence of an 'active' phase has been established.
3. The results show that the material retained after exchange in an inactive solution of the same ion is not all actively absorbed.
4. The passive phase of absorption is associated with the epidermis, the hypodermis and the endodermis. The ions taken up by the epidermis were found to be readily exchangeable. The nature of the localisation in the hypodermis is assumed to be a binding of the ion which has a low degree of exchangeability. It is proposed that the endodermis acts as a barrier to diffusion in that part of the root beyond the region of calcium accumulation.
5. The active phase of absorption is primarily associated with the parenchyma of the cortex in the apical region of the root, this region being termed the region of calcium accumulation. Cations appeared to be localised in the protoxylem initial cells and in the central cells of the root cap.
6. Cation absorption occurs in the epidermis, hypodermis, endodermis,

cortical parenchyma of the apical region of the root and in the protoxylem initial cells. Anion absorption does not appear to occur in the epidermis, protoxylem initials or the central cells of the root cap. Two cations, calcium and rubidium, and two anions, sulphate and iodide, were used in these investigations.

7. On the autoradiographic evidence it is proposed that vacuolar accumulation of cations occurs in the cortical parenchyma in the region of calcium accumulation. Anion accumulation does not occur in the vacuoles but in the case of sulphate ions it is localised in the region of the nucleus of the cell. It is proposed that anions are absorbed into their respective metabolic cycles in the cytoplasm of the cell. The results obtained with sulphate ions suggest that the site of protein metabolism in the cell is restricted to the region of the nucleus.

8. The autoradiographs show that there is an intense localisation of non-exchangeable ions in the cortex in a region of the root in which the endodermis does not retain ions and it is therefore possible that these accumulated ions form an osmotic pump which promotes the flow of ions by simple diffusion from the external solution directly into the stele in this region of the root. The region of maximum salt absorption, therefore, is also the region of maximum translocation.

REFERENCES.

REFERENCES.

1. Robertson,R.N., Annu.Rev.Pl.Phys., 1951, 2, 1.

2. Lundegårdh,H., ibid, 1955, 6, 1.

3. Epstein,E., ibid, 1956, 7, 1.

4. Briggs,G.E. and Robertson,R.N., ibid, 1957, 8, 11.

5. Gauch,H.G., ibid, 1957, 8, 31.

6. Laties,G.G., ibid, 1959, 10, 87.

7. Sutcliffe,J.F., Biol. Rev., 1959, 34, 159.

8. Robertson,R.N., ibid, 1960, 35, 231.

9. Cowie,D.B., Roberts,R.B. and Roberts,I.Z., J. cell. comp. Physiol., 1949, 34,243.

10. Roberts,R.B., Abelson,P.H., Cowie,D.B., Bolton,E.T. and Britten,R.J., 'Studies in the Biosynthesis in E. coli', Carnegie Institution of Washington, Publication 607, 1955.

11. Whittam,R. and Davies,R.E., Biochem. J., 1954, 56, 445.

12. Sutcliffe,J.F., J. exp. Bot., 1954, 5, 313.

13. Hope,A.B. and Stevens,P.G., Aust. J. sci. Res., Ser. B., 1952, 5, 335.

14. Hope,A.B. and Robertson,R.N., Aust. J. Sci., 1953, 15, 197.

15. Höfler,K., Ber. deut. botan. Ges., 1952,65, 391.

16. Brooks,S.C., Protoplasma, 1953, 42, 63.

17. Burstrom,H., Physiol. Plant., 1957, 10, 741.

18. Briggs,C.E. and Robertson,R.N., New Phytol., 1948, 47, 265.

19. Briggs,C.E., J. exp. Bot., 1958, 9, 128.

20. Hope,A.B., Aust. J. Biol. Sci., 1953, 6, 396.

21. Krebs,H.A., Eggleston,L.V. and Terner,C., Biochem. J., 1951, 48,530.

22. Williams,D.E. and Coleman,N.T., Plant and Soil, 1950, 2, 243.

23. Hope,A.B. and Dainty,J., Aust. J. Biol. Sci., 1959, 12,395.

24. Levitt,J., Physiol. Plant., 1957, 10, 882.

25. Levitt,J., ibid, 1957, 10, 732.

26. Leggett,J.E. and Epstein,E., Plant Physiol., 1956, 31, 226.

27. Dainty,J., Personal communication, 1960.

28. Jacobson,L., Hannapel,R.J. and Moor,D.P., Plant Physiol., 1958, 33, 278.

29. Butler,G.W., Physiol. Plant., 1953, 6, 617.

30. Keller,P. and Deuel,H., Zeitschr. Pflanz. düng Bodenk., 1957, 79, 119.

31. Jansen,E.F., Jang,R., Albersheim,P. and Bonner,J., Plant Physiol., 1960, 35, 87.

32. Chasson,R.M. and Levitt,J., Physiol. Plant., 1957, 10, 889.

33. Diamond,J.M. and Solomon,A.K., J. Gen. Physiol. 1959, 42, 1105.

34. Walker,N.A., Nature, 1958, 181, 1288.

35. Etherton,B. and Higinbotham,N., Science, 1960, 131, 409.

36. MacRobbie,E.A.C. and Dainty,J., J. Gen. Physiol., 1958, 42, 335.

37. Haapala,E., Physiol. Plant., 1960, 13, 358.

38. Biddulph,O., Cory,R. and Biddulph,S., Plant Physiol., 1959, 34, 512.

39. Steward,F.C. and Harrison,J.A., Ann. Bot., Lond., 1939, 3, 427.

40. Higinbotham,N. and Hanson,J., Plant Physiol., 1955, 30, 105.

41. Sutcliffe,J.F., Symposia Soc. exp. Biol., 1954, 8, 325.

42. Epstein,E., Plant Physiol., 1955, 30, 529.

43. Kylin,A. and Hylmo,B., ibid, 1957, 10, 487.

44. Briggs,G.E., New Phytol., 1957, 56, 305.

45. Epstein,E. and Leggett,J.E., Amer. J. Bot., 1954, 41,785.

46. Viets,F.G., Plant Physiol., 1944, 19, 466.

47. Overstreet,R., Jacobson,L. and Handley,R., ibid, 1952, 27, 583.

48. Helmy,A.K. and Elgaby,M.M., Plant and Soil, 1958, 10, 78.

49. Rediske,J.H. and Selders,A.A., Plant Physiol., 1953, 28, 594.

50. Jacobson,L. and Overstreet,R., Amer. J. Bot., 1947, 34, 415.

51. Russell,R.S. and Ayland,M.J., Nature, 1955, 175, 204.

52. Hanson,J.B. and Bonnar,J., Amer. J. Bot., 1954, 41, 702.

53. Reichenberg,D. and Sutcliffe,J.F., Nature, 1954, 174, 1047.

54. Vervelde,G.J., Plant and Soil, 1955, 6, 226.

55. Sollner, K., Archiv. Biochem. Biophys., 1955, 54, 129.

56. Hoagland,D.R., Cold Spring Harbour Symp. Quant. Biol., 1940, 8, 181.

57. Hoagland,D.R. and Broyer,T.C., J. Gen. Physiol., 1942, 25, 865.

58. Robertson,R.N. and Wilkins,Marjorie J., Aust.J. sci. Res., Ser. B, 1948, 1, 17.

59. Lundegårdh,H.G. and Burstrom, H., Biochem. Z., 1933, 261, 235.

60. Epstein,E. and Hendricks,S.B., Peaceful Uses of Atomic Energy, 1955, 12, 98.

61. Hoagland,D.R. and Steward,F.C., Nature, 1939, 143, 1031.

62. Steward,F.C. and Preston,C., Plant Physiol., 1941, 16, 85.

63. Robertson,R.N., Aust. J. Biol. Med., 1941, 19, 265.

64. Ordin,L. and Jacobson,L., Plant Physiol., 1955, 30, 21.

65. Lundegårdh,H.G., Nature, 1946, 157, 575.

66. Lundegardh,H.G., ibid, 1939, 143, 203.

67. Lundegårdh,H.G., Nature, 1952, 169, 1088.

68. Robertson,R.N., Wilkins,Marjorie J., Hope,A.B. and Nestel,L., Aust. J. Biol. Sci., 1955, 8, 164.

69. Arisz,W.H., Koninkl. Ned. Akad. Wetenschap Proc., 1945, 48, 420.

70. Handley,R. and Overstreet,R., Plant Physiol., 1955, 30, 419.

71. Hill,R. and Hartree,E.F., Annu. Rev. Pl. Phys., 1953, 4, 115.

72. Honda,S.I., Plant Physiol., 1955, 30, 69.

73. Lundegårdh,H.G., Symp. Soc. exp. Biol., 1954, 8, 262.

74. Harman,J.W., Exp. Cell Res., 1950, 1, 382,394.

75. Miller,G.W. and Evans,H.J., Plant Physiol., 1956, 31, 557.

76. Shean,G.M., Plant Physiol. Suppl. 1956.

77. Middleton,L.J., New Phytol., 1956, 55, 117.

78. Robertson,R.N. et al, Aust. J. sci. Res., Ser. B, 1951, 4, 248.

79. Humphries,E.C., J. exp. Bot., 1951, 2, 544.

80. Sutcliffe,J.F., ibid, 1952, 3, 59.

81. Sutcliffe,J.F., ibid, 1957, 8, 36.

82, Sutcliffe,J.F., Potassium Symp. Int. Potash Institute Berne, 1956, 1.

83. Jacobson,L., Plant Physiol., 1955, 30, 264.

84. Jacobson,L. and Ordin,L., ibid, 1954, 29, 70

85. Hurd,R.G., J. exp. Bot., 1959, 10, 345.

86. Epstein,E. and Hagen,C.E., Plant Physiol., 1952, 27, 457.

87. Epstein,E., Nature, 1953, 171, 83.

88. Epstein,E., Science, 1954, 120, 987.

89. Jacobson,L., Overstreet,R., King,H.M. and Handley,R., Plant Physiol., 1950, 25, 639.

90. Osterhout,W.J.V., Botan. Rev., 1936, 2, 265.

91. Wohl,K. and James,W.O., New Phytol., 1942, 41, 250.

92. Epstein,E., Nature, 1960, 185, 705.

93. Overstreet,R. and Jacobson,L., Annu. Rev. Pl. Phys., 1952, 3, 189.

94. Hagen,C.E. and Hopkins,H.T., Plant Physiol., 1955, 30, 193.

95. Fawzy,H., Overstreet,R. and Jacobson,L., Plant Physiol., 1954, 29, 234.

96. Bange,G.J., Plant and Soil, 1959, 11, 17.

97. Hanson,J.B. and Kahn,J.S., Plant Physiol., 1957, 32, 497.

98. Fried,M. and Noggle,J.C., ibid, 1958, 33, 139.

99. Epstein,E., Amer. J. Bot., 1960, 47, 393.

100. Laties,G.G., Proc. nat. Acad. Sci., U.S., 1959, 45, 163.

101. Russell,R.S., Symp. Soc. exp. Biol., 1954, 8, 343.

102. Lundegårdh,H., Physiol. Plant., 1950, 3, 103.

103. Gonzalez,J.D.L. and Jenny,H., Science, 1958, 128, 90.

104. Lundegårdh,H., Physiol. Plant., 1958, 11, 342.

105. Hylmo,B., ibid, 1953, 6, 333.

106. Tanada,T., Plant Physiol., 1955, 30, 221.

107. Hokin,L.E. and Hokin,Mabel R., Nature, 1959, 184, 1068.

108. Bennet-Clark,T.A., 1955, 'The Chemistry and Mode of Action of Plant Growth Substances.', Ed. R.L.Wain and F.Wightman, London, Butterworth.

109. Steward,F.C. and Millar,F.K., Symp. Soc. exp. Biol., 1954, 8, 367.

110. Steward,F.C. and Street,H.E., Annu. Rev. Bioch., 1947, 16, 471.

111. Lundegårdh,H., Nature, 1960, 185, 70.

112. Kahn,J.C. and Hanson,J.B., Plant Physiol., 1960, 35, 621.

113. Shean,J. and Levitt,J., Physiol. Plant., 1959, 12, 288.

114. Conway,E.J., Intern. Rev. Cytol., 1953, 2, 419.

115. Jensen,W.A. and McLaren,A.D., Exp. Cell Res., 1960, 19, 414.

116. Bennet,H.S., J. Biophys. Biochem. Cytol., 1956, 2, 99.

117. Hutton,W.E., Nature, 1960, 185, 439.

118. Saltman,P. and Boroughs,H., Arch. Biochem. Biophys., 1960, 86, 169.

119. Saltman,P., Alex,T. and McCormack,B., Arch. Biochem. Biophys. 1959, 83, 558.

120. Bedford,D.S., Myers,A. and Preston,R.D., Nature, 1958, 181, 1251.

121. Preston,R.D. and Kuyper,B., J. exp. Bot., 1951, 2, 247.

122. Russell,R.S. and Shorrocks,V.M., United Nations Educational, Scientific and Cultural Organisation, International Conference on Radio-isotopes in Scientific Research,'The Effect of Transpiration on the Absorption of Inorganic Ions by Intact Plants'. NS/RIC/178.

123. Lundegårdh,H., Physiol. Plant., 1950, 3, 108.

124. Broyer,T.C., Botan. Rev., 1947, 13, 125.

125. Brouwer,R., Acta. Bot. Neerl., 1956, 5, 287.

126. Helder,R.J., ibid, 1958, 7, 235.

127. Stout,P.R. and Hoagland,D.R., Amer. J. Bot., 1939, 26, 144.

128. Gustafson,F.G. and Darken,M., ibid, 1957, 24, 615.

129. Biddulph,O.& Markle,J., ibid, 1944, 31, 65.

130. Fraser,D.F.,'The Translocation of Minerals in Trees' Technical Note No. 47, 1956, Dept. of Northern Affairs and National Resources,Canada.

131. Olsen,C., Compt. rend. Carlsberg, 1953, 28, 487.

132. Russell,R.S., Sanders,F.K. and Bishop,O.N., Nature, 1949, 163, 639.

133. Bartelmez,G.W., Anat. Rec., 1940, 77, 509.

134. Simpson, W.L., ibid, 1941, 80, 173.

135. Gersh,I. and Stephenson,J.F., Biological Applications of Freezing and Drying,Chapter 14, Edt. R.J.C. Harris, Academic Press,N.Y. 1954.

136. Glick,D. and Malmstrom,B.G., Exp. Cell Res., 1952, 3, 125.

137. Andrew,A.M. and Hale,A.J., Lab. Invest., 1954, 3, 56.

138. Brunauer,S., Emmett,P.H. and Teller,E., J. Amer. Chem. Soc., 1938, 60, 309.

139. Pelc,S.R., Proc. Phys. Soc., 1945, 57, 523.

140. Boyd,G.A., Autoradiography in Biology and Medicine, Academic Press Inc., N.Y., 1955.

141. Marinelli,L.P. and Hill, Ruth F., Amer. J. Roentgenol., Radium Therapy 1948, 59, 396.

142. Pelc,S.R., Intern. J. Appl. Radiation and Isotopes, 1956, 2, 172.

143. Florell,C., Physiol. Plant., 1956, 9, 236.

144. Pearse,A.G.E., Histochemistry, Churchill Ltd., London, 1953.

145. Celo,N., Marks,J. and Varner,J.E., Nature, 1957, 180, 1142.

146. Rodkiewicz,B., Exp. Cell Res., 1959, 18, 407.

147. Johansen,D.A., Plant Microtechnique, McGraw Hill Co., N.Y., 1940.

148. Van Lookeren,J., Campagne Proc. K. N. Akad. Wet. Amst., 1957, 60, 70.

149. Howard,A. and Pelc,S.R., Exp. Cell Res., 1951, 2, 178.

150. Taylor,J.H. and Taylor,S.H., ibid, 1955, 4, 164.

151. Thomas,M.D., Agr. Res. Proc., 1947, 105.

152. Ulrich,A., Amer. J. Bot., 1941, 28, 526.

153. Helmy,A.K. and Elgaby,M.M., Intern. Soc. Soil Sci., Trans. II and IV, Hamburg, 1958, 2, 174.

154. Heyward,H.E., The Structure of Economic Plants, Macmillan Co., N.Y. 1951.

155. Loughman,B.C., Plant Physiol. 1957, 32, Suppl., 37.

APPENDIX 1

STANDARD EXPERIMENTAL PROCEDURE.

The procedure has been detailed for calcium but it is applicable to any cation or anion. It has been used for studies on the absorption of calcium, rubidium, sulphate and iodide ions.

1. Treatment of the Root Material:-

Onions were grown for one week in constantly aerated tap water and half inch lengths of root were excised from one inch roots. The excised roots were equilibrated for 30 minutes in deionized water and then treated overnight at 30°C in a solution of 0·5meq. Ca/l of high specific activity (10μc-3μc/ml.). The material to be dried after treatment with Ca^{45} only (i.e. non-exchanged) was stained during treatment by adding neutral red to the uptake solution to give a concentration of 0·0001% neutral red.

The roots for exchange were rapidly rinsed three times in deionize water after treatment with Ca^{45} for 400 minutes and blotted before being placed in a solution of calcium chloride containing 0·5 meq.Ca/l at 30°C. The material was stained by adding crystal violet to the exchange solution giving a concentration of 0·0001% crystal violet. Exchange was continued overnight.

Both materials were rinsed rapidly in three changes of deionized water, blotted and immediately frozen in iso-pentane cooled with liquid nitrogen. The roots were frozen separately to prevent their adhering one

to another. Using a cooled scalpel and forceps, the roots were cut into three on a polythene stage cooled by acetone/drikold. The pieces were held in liquid nitrogen so that the segments were grouped according to their position from the tip of the root.

It was necessary that the time spent in washing, freezing and cutting was kept to a minimum to prevent displacement of calcium.

2. Freeze Drying:-

Paraffin wax (54°C) was first deaerated in the aluminium stage in the freeze drying apparatus and solidified under vacuum. The stage was cooled in liquid nitrogen and the roots transferred so that they were still surrounded by liquid nitrogen when placed in the apparatus. Liquid nitrogen was immediately added to the condenser tube and the vacuum applied so that the remaining liquid nitrogen around the specimens was slowly drawn off. A pressure of lower than 0·001 mm. mercury was attained in less than one minute from placing the material in the drying chamber. Liquid nitrogen was added every 60 minutes and drying continued for 30 hours, spread over three days, the apparatus being held in acetone/drikold overnight. Drying was continued for 100 hours with phosphorus pentoxide as the secondary condenser.

The material was embedded by heating the paraffin wax to 55-60°C without breaking the vacuum. After three hours the paraffin was solidified and the cylinders of wax were transferred to tubes which were then held at 55°C for 18 hours.

3. Autoradiography:-

Sections,10, 20 and 40μ thick were cut and pressed onto cleaned slides dipped in 0·5% wt./vol. solution of saran in methyl ethyl ketone. Slides supporting median sections were cleared of paraffin in xylol and coated with a 1% wt./vol. solution of saran in methyl ethyl ketone. By drying the slides in a vertical position an even film 1μ thick was obtained.

Kodak A.R.10 and A.R.50 autoradiographic films were floated out for three minutes on water in a darkroom fitted with a Wratten No.1 red filter. 30 seconds before applying the film the slides were dipped in a 1% wt./vol. solution of oelloidin in a 50/50 vol./vol. solution of ether and absolute alcohol. The films were dried by an air fan and exposed at 4°C.

The exposure times were determined by developing the 20μ and 40μ sections at different intervals of time. Development in Kodak D.19b developer was followed by staining for 24 hours in a 30/10 vol./vol. mixture 2% neutral red and Ziehl Nielsens carbol fuchsin. The gel of the autoradiographic film was destained in water and alcohol and the preparation was mounted in D.P.X. mountant.

APPENDIX 11

ISOTOPES USED IN AUTORADIOGRAPHIC EXPERIMENTS.

ISOTOPE	EMISSION	E_{max}	HALF LIFE in days
Ca^{45}	β	0·25	164
Rb^{86}	β	1·77	18·6
	γ	1·08	
S^{35}	β	0·167	87·1
I^{131}	β	0·61	8·04
	γ	0·36	

EXPOSURE TIMES REQUIRED FOR SECTIONED ROOT MATERIAL.

ISOTOPE	TREATMENT	CONCN.OF ION meq./l.	ACTIVITY OF SOLUTION	SECTION THICKNESS in μ	AVERAGE EXPOSURE TIME in days
Ca^{45} living onion	non-exchanged	0·5	5·5	10	50
	exchanged	0·5	5·5	10	75
Ca^{45} Dendrobium sp.	non-exchanged	0·5	5·5	10	35
	exchanged	0·5	5·5	10	55
Ca^{45} dead onion	non-exchanged	0·5	5·5	10	65
	exchanged	0·5	5·5	10	100
Ca^{45} living onion	Ca/Sr uptake	0·5	2·7	10	80
	Sr exchange	0·5	5·5	10	140
Rb^{86} living onion	non-exchanged	0·5	6·6	10	95
	exchanged	0·5	6·6	10	135
S^{35} living onion	non-exchanged	0·5	10·0	10	95
	exchanged	0·5	10·0	10	135
I^{131} living onion	non-exchanged	0·5	10·0	10	80
	exchanged	0·5	10·0	10	100