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STUDIES on THE ROOT CAP AND ITS SIGNIFICANCE
IN GRAVIPERCEPTION

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A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science in the University of London.

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ACKNOWLEDGEMENTS

The role of the root cap in graviperception was studied in root tips of Haing seedlings.

My supervisor, Professor L.J. Audus, for his helpful advice and interest in this work.

It was shown that destarching and removal of the root cap causes the loss of geotropic reaction in the roots. The normal growth of the roots of Mr. S. White, Science Workshop, Bedford College, and Mr. J.C. Funnell, Chief Technician, Botany Department, Bedford College, for very helpful technical assistance. A study of recovery time was made. A relationship between the length of the roots at desecrating time and the length of recovery period was found. Mr. B. Chapman of the E.M. unit at the Botany School, Cambridge, for his help and advice.

Presentation time studies of normal roots and roots which were desecrated after stimulation indicate that the root cap can be removed after stimulating discussions. stimulation without influencing the subsequent response.

It was also shown that the stimulus must be transmitted within the presentation time and that the plasmatic connections between the cap and the root are necessary for this transmission.

The structural state of the root cap at recovery time was investigated with the Electron Microscope and the presence of starch grains shown. The results of this work are discussed in the light of the current theories on graviperception.

SUMMARY

The role of the root cap in graviperception was studied in root tips of Maize seedlings.

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It was shown that destarching and removal of the root cap causes the loss of geotropic reaction in the roots. The normal growth of the roots is not affected by this decapping and the roots regenerate their root caps during 36 hours. Geotropic reactivity reappears before the completion of regeneration and a study of recovery time was made. A relationship between the length of the roots at decapping time and the length of recovery period was found.

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Chapter I

Introduction

The problem of the perception of gravity by plants has occupied botanists and plant physiologists for more than a century. The subject has been reviewed extensively by Rawitscher (1932), Rufelt (1961), Larsen (1962), Audus (1962) and Wilkins (1966), and I shall not attempt to repeat these summaries unless necessary for the purpose of this work.

The first comprehensive hypothesis which was proposed to explain the perception of gravity by plants - "The Statoliths hypothesis" - was proposed by Nêmec (1900) and Haberlandt (1900) after a suggestion by Noll.

This hypothesis is still the main working hypothesis despite vigorous ups and downs. It has been succeeded by various other hypothetical mechanisms after the discovery of plant growth hormones, but it was reformulated by Audus (1962) in a modern context.

The geoelectric theory, that seemed to connect perception and response in a simple scheme (Brauner, 1924, 1928, 1965), and modifications of the statoliths theory by Cholodny (1922) and Larsen (1959), could not fulfil all the basic requirements posed by experimental evidence. The statoliths theory is still at the centre of controversy today.

"The receptor system for measuring linear accelerations by means of more dense particles inside a fluid system of lower density is almost universal among living organisms and is phylogenetically by far the longest established." (Trincker, 1962).

This statement is from a review in animal physiology where the statoliths theory of graviperception originated. In the animal kingdom, this perception mechanism is the basic one from primitive invertebrates to man, the principle of dense particles sedimenting in a fluid of lower density operates in many forms, the transmission of, and the response to the stimulus are mediated by separate and specialized systems. In order to

The elucidation of the perception mechanism in animals has been facilitated by the fact that they have specialized sensory organs, which plants do not seem to have, although sensitivity to various external stimuli might be partially localised.

This apparent "deficiency" complicated investigation in plant starching have varied. perception in general and in graviperception in particular.

Starvation of plants in order to make them utilize their starch was employed by Zollikofer (1918) and Protie (1928). The difficulty in separating the perceptive system in plants from the response mechanism may be the main obstacle to identifying it.

Treating the plants in the cold was tried by Haberlandt (1903), Némec and Haberlandt, after a wide survey of plant tissues, put forward the theory that starch grains, which are present in the sensitive regions (1936). Different chemical compounds were tried as well. Went and Farkel- and are redistributed under gravitational stimuli, are the means by which having (1908) treated roots in solutions of potassium alum. SO_2 was used the plant perceives gravity. Subsequent research was concerned with testing by Syre (1938). Gibberellin acid + kinetin solutions were used by Pickard- this theory in two ways: 1) Trying to destarch plants or plant organs and Gillespie and Thimann (1966). testing for sensitivity and response to gravitational stimuli.

2) Manoeuvring the starch grains into certain regions in the cell in a calculated sequence and observing their growth behaviour.

There are two recorded instances of plant organs which are geotropically reactive and have no starch (Tischler, 1905; Linsbauer, 1907). The reports are early ones and have not been re-investigated since. Recent investigations have shown that it is not always easy to detect starch by conventional

There are several cases though, in which the reported results are in staining techniques (Gillespie and Thimann, 1966) and see also Materials and disagreement with the starch statolith hypothesis.

Methods. It will be worthwhile to look again at these organs in order to

ascertain the absence of sedimenting particles (starch grains or other particles) by dipping them into potassium alum solution. They found that a

These workers found that staining with I-KI was unsatisfactory for certain percent of the roots showed geotropic curvature.

There have been numerous attempts to "destarch" plants or plant organs, since the first proposal of the starch grains as statoliths. Techniques of

achieving it and examining sensitivity and response to gravity after de-

starching have varied. Curvatures in starchless roots were genuine and not a

traumatic reaction. Attempts to repeat their experiments as shown later

Starvation of plants in order to make them utilize their starch was in the present work did not confirm their finding and the only explanation employed by Zollikofer (1918) and Protic (1928).

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Treating the plants in the cold was tried by Haberlandt (1903),

Hawker (1933) and von Bismarck (1959). A heat treatment was given by Syre

Syre (1938) used SO_2 (sulphurous acid) to destarch maize roots. He (1938). Different chemical compounds were tried as well. Went and Pikel-

reports that 53% of the roots that grew after the treatment and were starch-

haring (1908) treated roots in solutions of potassium alum. SO_2 was used less, showed a geotropic response. An attempt to repeat his work was un-

by Syre (1938). Gibberellic acid + kinetin solutions were used by Pickard-

successful (see Materials and Methods). SO_2 is a highly poisonous substance Gillespie and Thimann (1966).

and, as he offers no detailed description of the response, the curvatures observed

Decapitating roots, by slicing the root cap wholly or in part, was

tried by Younis (1954) and Syre (1938).

The majority of destarching experiments produced good correlation between the presence and quantity of sedimenting starch grains and geotropic response. found that this treatment caused the starch to disappear but geotropic response, though very slow as a result of retarded growth rate, was comparable to that in controls treated with water or sucrose. There are several cases though, in which the reported results are in disagreement with the starch statolith hypothesis.

The growth rate in Gibberellic acid and kinetin was 19% of the controls but Went and Pekarharing (1908) have destarched cress (*Lepidium sativum*) roots by dipping them into potassium alum solution. They found that a certain percent of the roots showed geotropic curvature.

These authors found that staining with J-KJ was unsatisfactory for this variety of wheat and they used the Per Iodic - Schiff reagent as the star. The authors say that most roots showed traumatic curvatures at concentrations which caused disappearance of starch but maintain that the observed geotropic curvatures in starchless roots were genuine and not a traumatic reaction. Attempts to repeat their experiments as shown later in the present work did not confirm their finding and the only explanation one can offer is that the curvatures that were observed by them were indeed of starch showed 50% elongation compared with controls but did not exhibit traumatic reactions. They also conducted an electron microscopical survey of one region in the coleoptile showing plastids without starch which did not show any redistribution under gravity. Those results were not obtained in all experiments. In a set of previous ones, the coleoptiles after depletion of starch showed 50% elongation compared with controls but did not exhibit any geotropic curvature.

Syre (1938) used SO_2 (sulphurous acid) to destarch maize roots. He reports that 53% of the roots that grew after the treatment and were starchless, showed a geotropic response. An attempt to repeat his work was unsuccessful (see Materials and Methods). SO_2 is a highly poisonous substance in maize roots (see Materials and Methods). and, as he offers no detailed description of the response, the curvatures observed might be due to injury effects as in the case of potassium alum.

Pickard and Thimann treated excised wheat (*Triticum vulgare*) coleoptiles with Gibberellic acid and kinetin solution at 30°C for 34 hours. They found that this treatment caused the starch to disappear but geotropic response, though very slow as a result of retarded growth rate, was comparable to that in controls treated with water or sucrose.

This is particularly important in this case because Gibberellic acid seems to promote the geotropic response of wheat coleoptiles at the concentrations used by these authors (see Norris et al., 1965) without affecting the growth rate. The growth rate in Gibberellic acid and kinetin was 15% of the controls but the ratio of curvature to growth was the same in both.

These authors found that staining with J-KJ was unsatisfactory for this variety of wheat and they used the Per Iodic - Schiff reagent as the starch indicator. It might be that some residual perception was still present in the coleoptiles and the presence of Gibberellic acid at stimulation and response.

They also conducted an electron microscopical survey of one region in the coleoptile showing plastids without starch which did not show any redistribution under gravity. Those results were not obtained in all trials and followed subsequent geotropic response. No reports that removal of starch showed 50% elongation compared with controls but did not exhibit any geotropic curvature. There is no microscopical evidence in his paper and there was most probably enough sedimenting starch left to enable the roots to respond.

The colour reactions of starch seem to be very complex and the exact chemistry of the staining reaction is somewhat uncertain. It was found that the PAS reagent does not always produce a distinct colour for instance usually resulted in an injury reaction and was not followed carefully by a microscopical study so that it is difficult to judge his results.

Byre (1938) tried removing various sections of the root cap but this usually resulted in an injury reaction and was not followed carefully by a microscopical study so that it is difficult to judge his results.

Von Dänemark (1959) working with Sphagnum species found that plants growing outside in winter or in the cold in the laboratory lost most or all

It is possible that residual starch grains do not always develop a distinct colour even in wheat and a rigorous investigation of the whole coleoptile under the electron microscope might be necessary in order to make sure that there are no sedimenting particles.

Von Bismarck quotes Benker (1914) as observing re-synthesis of starch. This is particularly important in this case because Gibberellic acid seems to promote the geotropic response of wheat coleoptiles at the concentrations used by these authors (see Norris et al., 1965) without affecting growth.

It might be that some residual perception was still present in the coleoptiles and the presence of Gibberellic acid at stimulation and response time promoted the response without affecting growth.

Younis (1954) sliced off various amounts of the root cap of *Vicia faba* and followed subsequent geotropic response. He reports that removal of 0.5 mm removes the root cap starch zone but does not affect the geotropic response. There is no microscopical evidence in his paper and there was most probably enough sedimenting starch left to enable the roots to respond.

Syre (1938) tried removing various sections of the root cap but this usually resulted in an injury reaction and was not followed carefully by a microscopical study so that it is difficult to judge his results.

Von Bismarck (1959) working with *Sphagnum* species found that plants growing outside in winter or in the cold in the Laboratory lost most or all

their starch but when stimulated in the Laboratory showed a geotropic response. This response was very slow even in comparison with the normal slow response of Sphagnum. The latter takes 1 - 1.5 hours and the former up to 14 days. The response stops completely below 2°C.

Zimmerman stimulated cross roots for a period exceeding the Von Bismarck quotes Ranker (1914) as observing resynthesis of starch presentation time and inverted them for a short time before allowing within a few hours of a more favourable temperature. the curvature to develop. This increased the response to a marked

extent Von Bismarck himself did not check starch resynthesis in his plants. He also shows one picture in which the starchless plastids seem to have sedimented in the sensitive zone but does not give further details about the plants that showed a geotropic response in the cold.

Von Ubisch repeated his experiments and got the same results, but then All the above reported destarching experiments have not succeeded in depleting the plants of starch totally or locally without impairing growth considerably. Under these circumstances it is very risky to give an unequivocal answer as to the nature of the perception mechanism, out on the klinostat after stimulation in the normal position i.e. they were put

Manoeuvring starch grains to selected positions in a predetermined with their tips downwards. sequence and observing geotropic response thereafter is a different approach which assumes that growth is not affected and the resultant response can be analysed. In this way it is possible to test various hypotheses as to the nature of graviperception. light to have reduced the

response because it removed the starch grains from the lateral walls. However,

The best known examples of this approach, to test the statoliths the mass bar interpretation of a shortened and reduced contact on micro-

theory, are the experiments by Zimmerman (1927) and von Ubisch (1928).

occluded preparations of roots that were fixed in boiling ethanol and stained with J in KJ. some of the statoliths were removed by the J in KJ (1927)

Zimmerman's results were interpreted by him as confirming the statoliths hypothesis whilst von Uebisch reported that her results were not explainable in terms of this theory. The accurate positioning of the starch grains. There are no numerical data of her experiments as well, Zimmerman stimulated cross roots for a period exceeding the presentation time and inverted them for a short time before allowing lateral wall membrane is the important factor in perception via statoliths the curvature to develop. This increased the response to a marked extent. The results were explained by him on the basis of the statoliths theory. He said that the inversion increased the extent of contact of the starch grains with the lateral wall, that it is the tangential shearing

force component of the statoliths contact which is the effective stimulus Von Uebisch repeated his experiments and got the same results, but (Brewer, 1891; Trincker, 1962), and not the pressure. then modified the experimental sequence. She inverted the roots for up to 20 minutes before stimulating in the horizontal position; then she re-inverted some of the roots before allowing the curvature to develop on a klinostat. The other part of the roots was not inverted again but put on the klinostat after stimulation in the normal position i.e. they were put with their tips downwards.

The reinversion after stimulation produced an increase in the response like in the original Zimmerman experiments. Von Uebisch said that if the

statoliths theory is correct then reinversion ought to have reduced the response because it removed the starch grains from the lateral walls. However, it is very important that these experiments are repeated with a careful check on the position of the starch grains at the end of each stage in the she bases her interpretation of a shortened and reduced contact on micro-manipulations. scopical preparations of roots that were fixed in boiling ethanol and stained with J in KJ. (John) (1922)

Her photomicrographs show a very marked shrinkage of cells and clumping of the starch grains. This is obviously an artefact due to the preparation method and cannot represent the accurate positioning of the starch grains. There are no numerical data of her experiments as well, and the assumption is made that the pressure of the statoliths on the lateral wall membrane is the important factor in perception via statolith sedimentation.

This is not necessarily a correct assumption and if we return to this effect consists of a Potential Difference of about 5-10 mV across a horizontal plant organ with the underside being positive. This potential force component of the statoliths contact which is the effective stimulus difference develops immediately and reaches a maximum in 10 minutes from the start of the horizontal stimulation. Brauner assumed that this p.d. (Breuer, 1891; Trincker, 1962), and not the pressure.

If the situation is similar in plants, re-inversion in von Uebisch experiments still provides an added shearing force despite the statoliths movement from the lateral wall.

As the inverted position itself can only be regarded as a metastable state and as the root tip shows random movements in their normal growth (Johnsson, 1966), a slight movement during the inversion period will tend to produce additional lateral stimulation.

It is very important that these experiments are repeated with a careful check on the position of the starch grains at the end of each stage in the manipulations.

Investigations into the time course of the G.E.R. in plants by Hertz (1960). Modifications of the statoliths theory were proposed by Cholodny (1922)

and Larsen (1959, 1961). They postulate that particles other than starch grains are the sedimenting particles in plant cells. time for this organ (4 minutes).

No experimental evidence is reported in support for either of those theories and they were criticised by Audus (1962) who showed that they are unacceptable on theoretical grounds. IAA to *E. coli* says coleoptiles produced

a P.D. of 10 mV in a similar way to the development of the G.E.E. in

The most comprehensive alternative to the statolith hypothesis was magnitude, direction and course of development. Grabar used the Hertz proposed by Brauner (1927, 1928) on the basis of the geoelectric effect. vibrating reed electrodes and Wilkins and Woodcock used a flowing solution. This effect consists of a Potential Difference of about 8-10 mV across a electrode but the results were the same.

horizontal plant organ with the underside being positive. This potential difference develops immediately and reaches a maximum in 10 minutes from the start of the horizontal stimulation. Brauner assumed that this p.d. was enough to cause redistribution of the IAA anion and trigger a differential growth response. These observations were followed by studies on cell models using KCl solutions separated by various membranes.

These cells gave rise to a p.d. across the membrane according to the particular experimental set-up in each investigation, but all in a pattern which enabled Brauner to claim that the results support his hypothesis concerning the mechanism of perception in plants. atial growth responses are no

better understood, so that there is no possibility of deducing the nature

The experimental set-up of these model cells was criticised severely of one from the others. For this reason it was thought advantageous to try on physical grounds (Wartenberg, 1957; Audus, 1962).

and find out if the root cap is a perception zone that can be "separated"

in Investigations into the time course of the G.E.E. in plants by Hertz (1960, 1961) show that this Geoelectric Effect indeed develops in Avena

coleoptiles but it has a lag period of up to 15 minutes - a fact which takes this effect outside the required presentation time for this organ (4 minutes).

Further research by Graham (1964) and Wilkins and Woodcock (1965), in which unilateral application of IAA to *Zea mays* coleoptiles produced a P.D. of 10 mV in a similar way to the development of the G.E.E. in magnitude, direction and course of development. Graham used the Hertz vibrating reed electrode and Wilkins and Woodcock used a flowing solution electrode but the results were the same.

A mica plate inserted into one side of the coleoptile, to prevent IAA transport on that side, caused a P.D. to develop beneath this barrier (Graham, 1964). This and similar evidence from phototropic responses (Johnsson, 1965) all point to the conclusion that the G.E.E. is a result of auxin redistribution and not the cause of it.

The above summary makes it evident that graviperception in plants is not yet well understood. This is due mainly to the difficulty in isolating perception from the response and studying it on its own. The transmission and translation of the stimulus into a differential growth response are no better understood, so that there is no possibility of deducing the nature of one from the others. For this reason it was thought advantageous to try and find out if the root cap is a perception zone that can be "separated" in some way from the rest of the root without affecting its growth. After

different methods of destarching the root cap "in situ" were found unsatisfactory as explained above, the decapping of primary roots of *Zea mays* was chosen as the best one for the following reasons:

1. Destarching

1. It is relatively simple and easy to remove the root cap without damage to the root tip owing to the special properties of the boundary wall between the two.

2. The removal of the root cap results in loss of the geotropic reactivity of the primary root.

3. The root cap is regenerated by the meristematic activity of the quiescent centre (Juniper et al, 1966). This regeneration takes place within 36 hours after decapping and during that period geotropic reactivity is recovered.

4. This method gives a uniform population of roots which contain no starch in the root cap without the need to examine each root individually.

A scale of starch content from 0 to 4 was constructed (Fig. 1) and it is clear that with such a method the plant is affected as little as possible, but the geotropic response is eliminated. Therefore the study of the significance of the root cap in the complex chain of perception and response is facilitated. The time course of recovery and its nature, and the state of the root cap at the time of recovery, can be studied in an attempt to ascertain what are the necessary elements for the regeneration of graviperception.

The root was traced on paper and two lines passed, one through the tip and one through the main axis of the root. The angle between these was measured with a protractor and taken as the curvature.

Chapter II

Materials and Methods

1. Destarching

Attempts were made to check the previous work on destarching of roots. *Lepidium sativum* (cress) roots were treated in various ways in order to destarch them. Syre (1938) used treatment with SO_2 and erythrosin and his experiments were repeated here together with those of Pekeharing and Went (1908) who used potassium alum solutions to destarch cress root tips. A low temperature treatment was used by Hawker (1933) and this was repeated on cress roots.

Alum and showing various amounts of starch
in the root esp.

The details of each treatment are described in subsequent paragraphs. The starch content and degree of curvature were determined in the same way for all the destarching experiments. Starch content was determined by staining in a solution of I in KI and then clearing with phenolchloralhydrate. Starch was seen clearly stained blue-black after clearing.

A scale of starch content from 0 to 4 was constructed (Fig. 1) and each root was compared and graded from the scale.

Root curvature was determined by two methods for this series of experiments.

- a. The root was traced on paper and two lines passed, one through the tip and one through the main axis of the root. The angle between these was measured with a protractor and taken as the curvature.

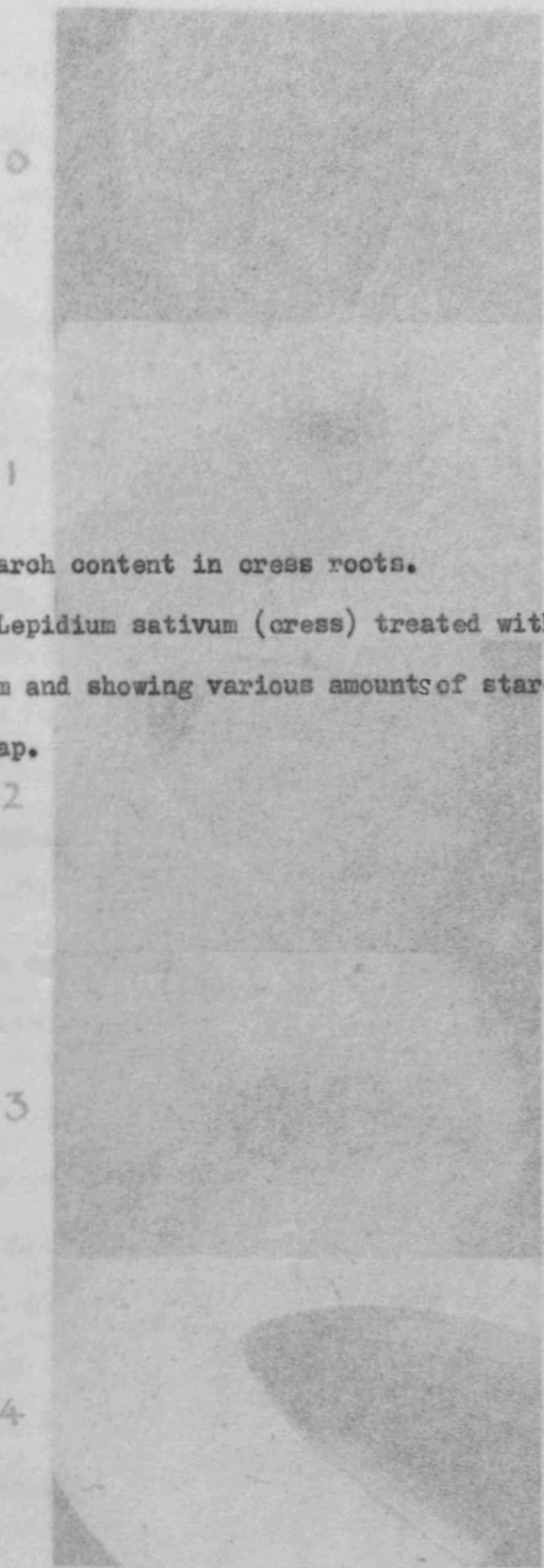
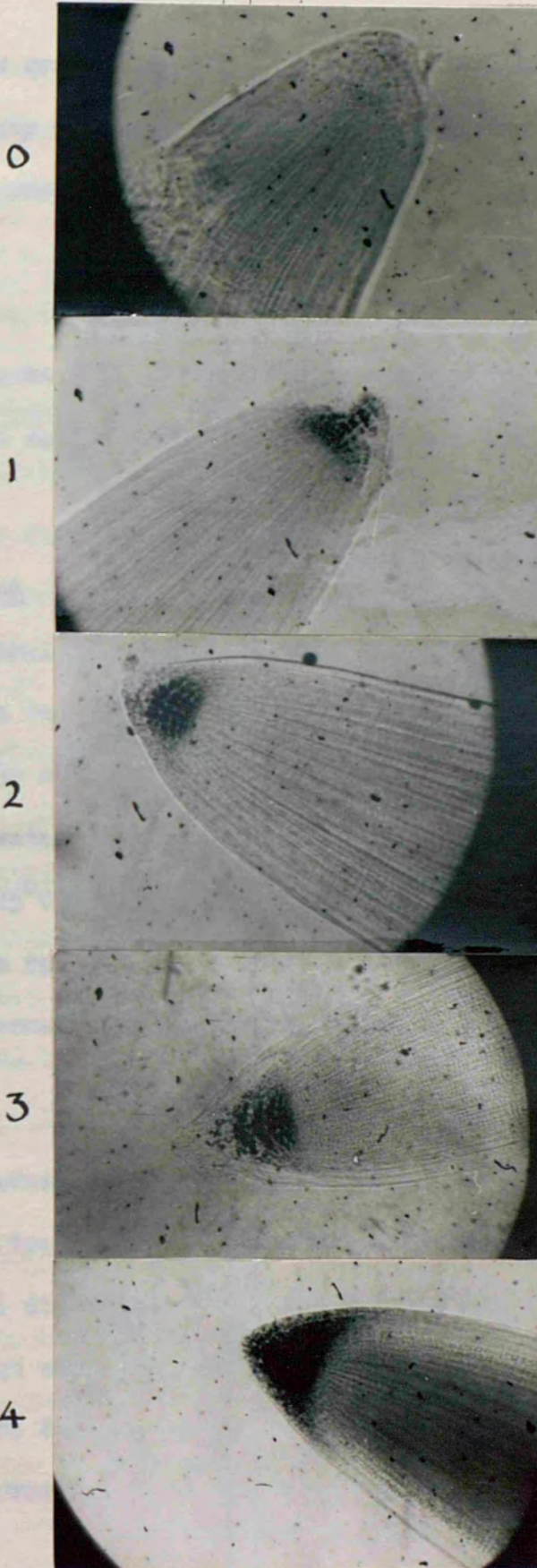


Fig. 1. A scale of starch content in cress roots.

Root tips of *Lepidium sativum* (cress) treated with Potassium Alum and showing various amounts of starch in the root cap.

Fig. 1.



b. The root was shadow graphed and the curvature measured on the shadow graph in the same way as in a. This latter method was employed in a few cases when the roots grew on agar. of curvature. This was done in order to see how a cold treatment after germination affects

i. SO₂ Treatment

The treatment of cress seeds and Zea mays seeds with SO₂ prevented germination even down to concentration of 0.1% SO₂ and was therefore abandoned.

ii. Erythrosin Treatment (0.1 - 0.6%) for different lengths of time.

Cress seeds were germinated on filter paper in petri dishes for 48 hours at 25 ± 1°C in the dark. The root tips were then dipped in a solution of 0.1% Erythrosin for 30 seconds (after Syre, 1938). They were then rinsed in distilled water and returned to the petri dishes for growth in the horizontal position and pretreatment time was chosen which gave reduction of starch without serious loss of growth. After treatment, the roots were re-orientated in a horizontal position and left for 24 hours to develop a curvature. In some cases the seedlings were put into petri dishes with 1%

iii. Cold Treatment

Cold is known to reduce starch in plants (Hawker, 1933; von Bismarck, 1959; etc.). Hawker's treatment was repeated on cress roots. Cress seeds were germinated in petri dishes at 5°C in the dark. When roots were about 10 mm long, the seedlings were re-orientated to a horizontal position. They were left for 24 hours to develop a curvature because the growth is slowed down in the cold and curvatures were not definite earlier. Curvature and

starch content were measured for each root as above. In one experiment the seeds were germinated at room temperature for 28 hours and then transferred to 5°C for growth and development of curvature. This was done in order to see how a cold treatment after germination affects starch content and geotropic response.

iv. Potassium Alum Treatment

Cress seeds were germinated in the dark at 25°C for 40-48 hours, in petri dishes on moist filter paper. They were then transferred to solutions of potassium alum (0.1 - 0.6%) for different lengths of time to the junction between the root cap and the root tip. The break was clean and sharp (see Fig. 2). Growth was determined by cutting the roots at the hypocotyl "node" and determining fresh weight of the sample. Roots grown on water were used as control. Starch content was assayed for each root. A combination of concentration and pretreatment time was chosen which gave reduction of starch without serious loss of growth. After treatment, the roots were re-orientated in a horizontal position and left for 24 hours to develop a curvature. In some cases the seedlings were put into petri dishes with 1% agar in water for the development of curvature, and were shadow graphed afterwards and not traced like the other ones. Starch content and curvature were determined for each root.

None of these treatments reduced starch content without impairing growth considerably (see results). Finally wheat and maize seeds were germinated in petri dishes in solutions of Gibberellic acid up to 1%

(after Thimann 1964). But this treatment had no visible effect on the starch content of the root caps.

v. De-capping

1. Growth Conditions and De-capping

After communication from Dr. B.E. Juniper, de-capping of maize roots was tried and found satisfactory as a way in which the whole root cap could be removed without any apparent injury to the plant (Juniper et al, 1966). The root cap was removed with a blunt scalpel under a low power binocular microscope by applying a pressure in a diagonal direction to the junction between the root cap and the root tip. The break was clean and sharp (see Fig. 2).

Fig. 2. Decapped root of Zea mays with the root cap near the tip showing clean break.

Zea mays var. White Horse Tooth (Carter's) was soaked in running aerated water at $25 \pm 1^{\circ}\text{C}$ for 48 hours. Seeds that had just begun to germinate were selected and put between two layers of moist filter paper in petri dishes. These were held upright in a wooden frame (Fig. 3) and put in the dark at $25 \pm 1^{\circ}\text{C}$ for approximately 24 hours, or until the required length of primary root was reached. The seedlings with straight roots were selected and de-capped.

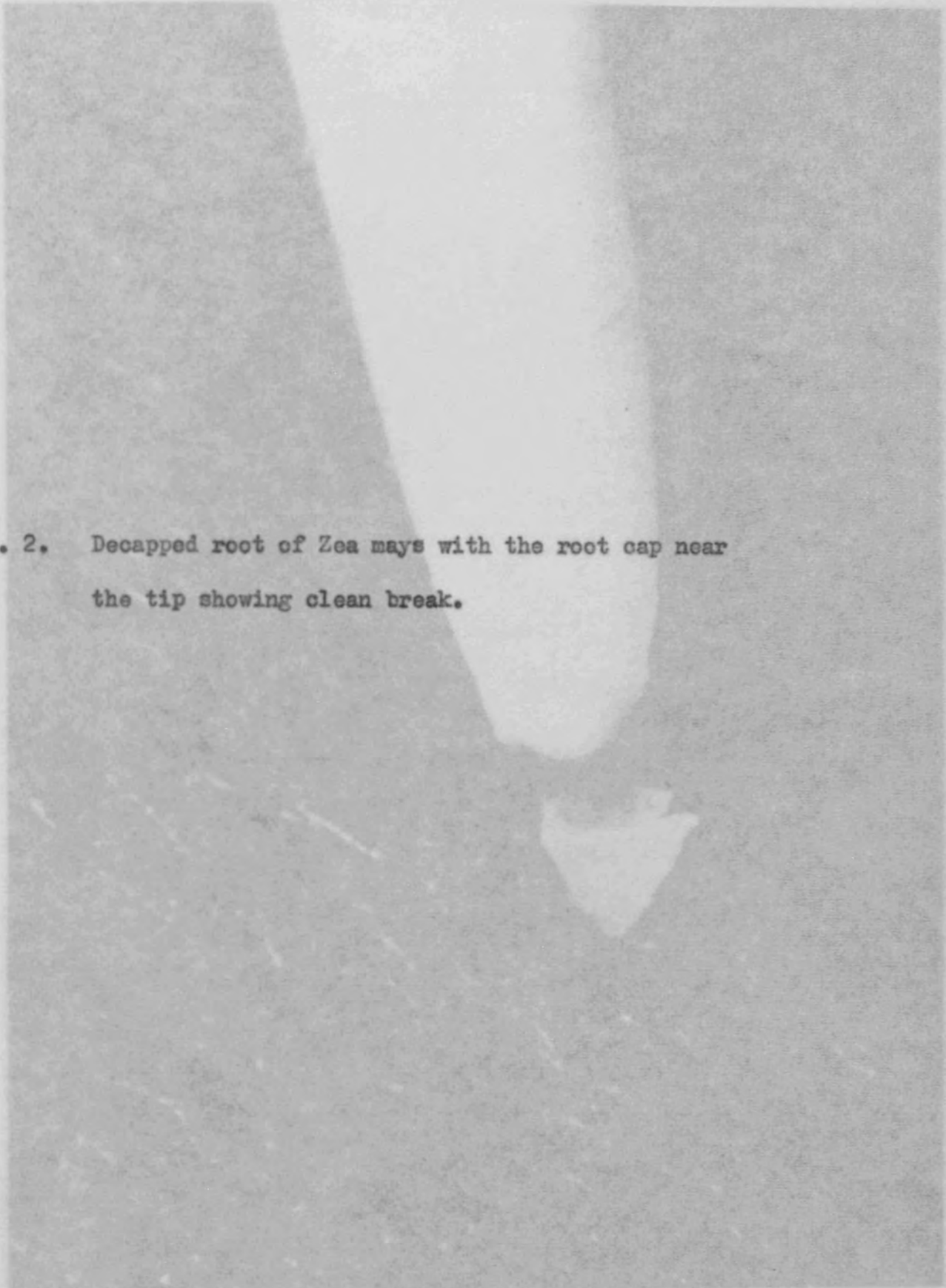


Fig. 2. Decapped root of Zea mays with the root cap near the tip showing clean break.

Fig. 2.



2. Growth Rate Measurements

To begin with growth rate measurements were made. The seedlings were put in a perspex chamber (Fig. 4a) and photographs were taken at 30 minute intervals. The film was developed and the length of the roots measured by putting the negative in a photographic enlarger and measuring the projected image. Growth rate of roots before and after de-capping was determined. In a different experiment the root cap was carefully replaced under the microscope immediately after detachment. Precaution was taken that contact was made through a moist junction and the fit was usually very good. Replacement was done also in some of the recovery time experiments. Student's *t* test was used to test the difference between de-capped and non-de-capped roots.

3. Recovery

As the roots showed recovery of the response to gravity, recovery time studies were undertaken.

Maize seedlings were grown as described above and roots falling within a certain length range were selected. This was done because preliminary experiments had shown that there might be some relationship between the root length at de-capping time, and the length at recovery time. A range of lengths was covered within the growth cycle of the primary root. The roots were de-capped, the seedlings numbered in black ink on the seed, and put to grow in the perspex chamber in a horizontal position. The chamber was in the dark at $25 \pm 1^{\circ}\text{C}$. Pictures were taken every hour for

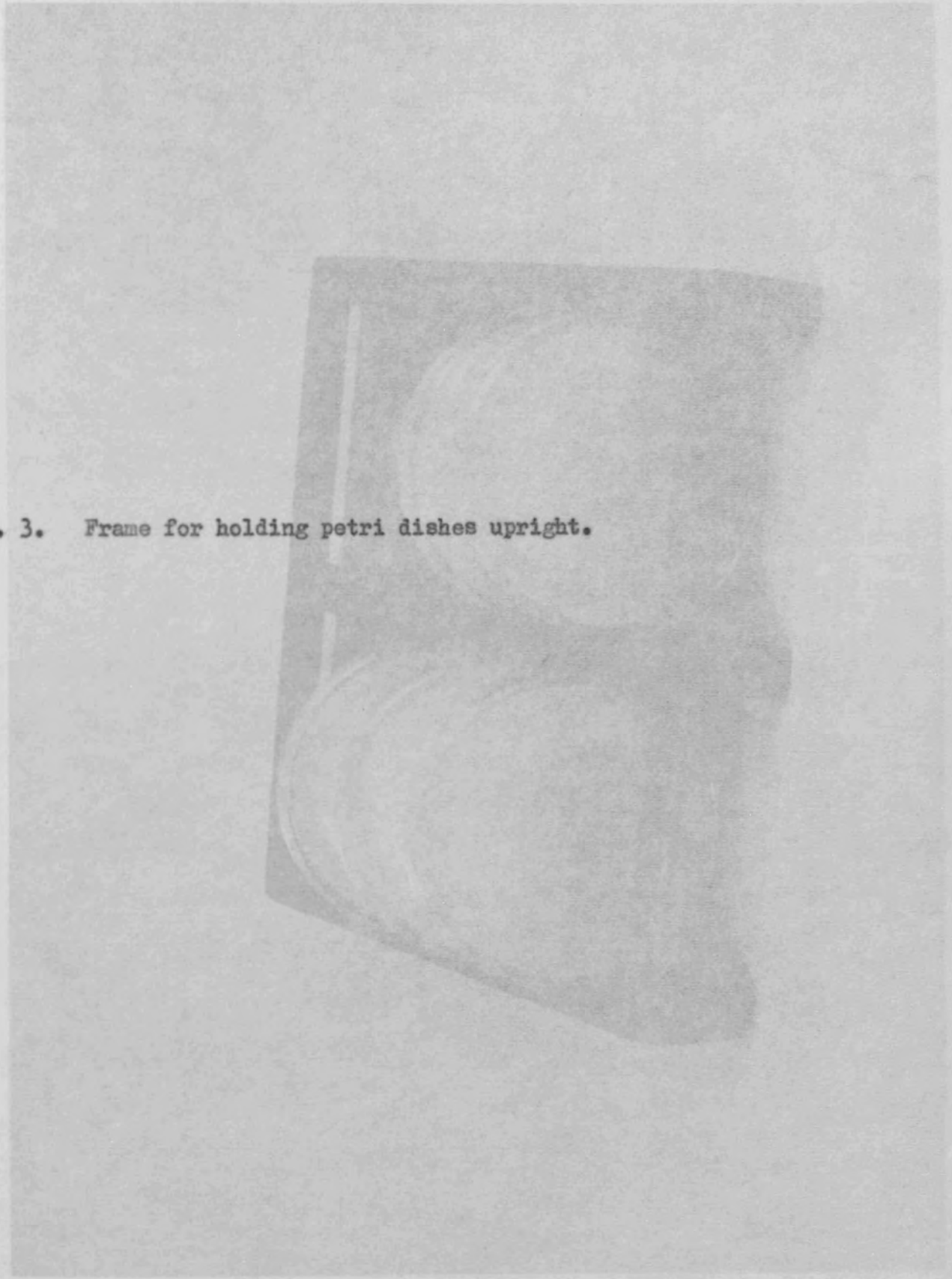
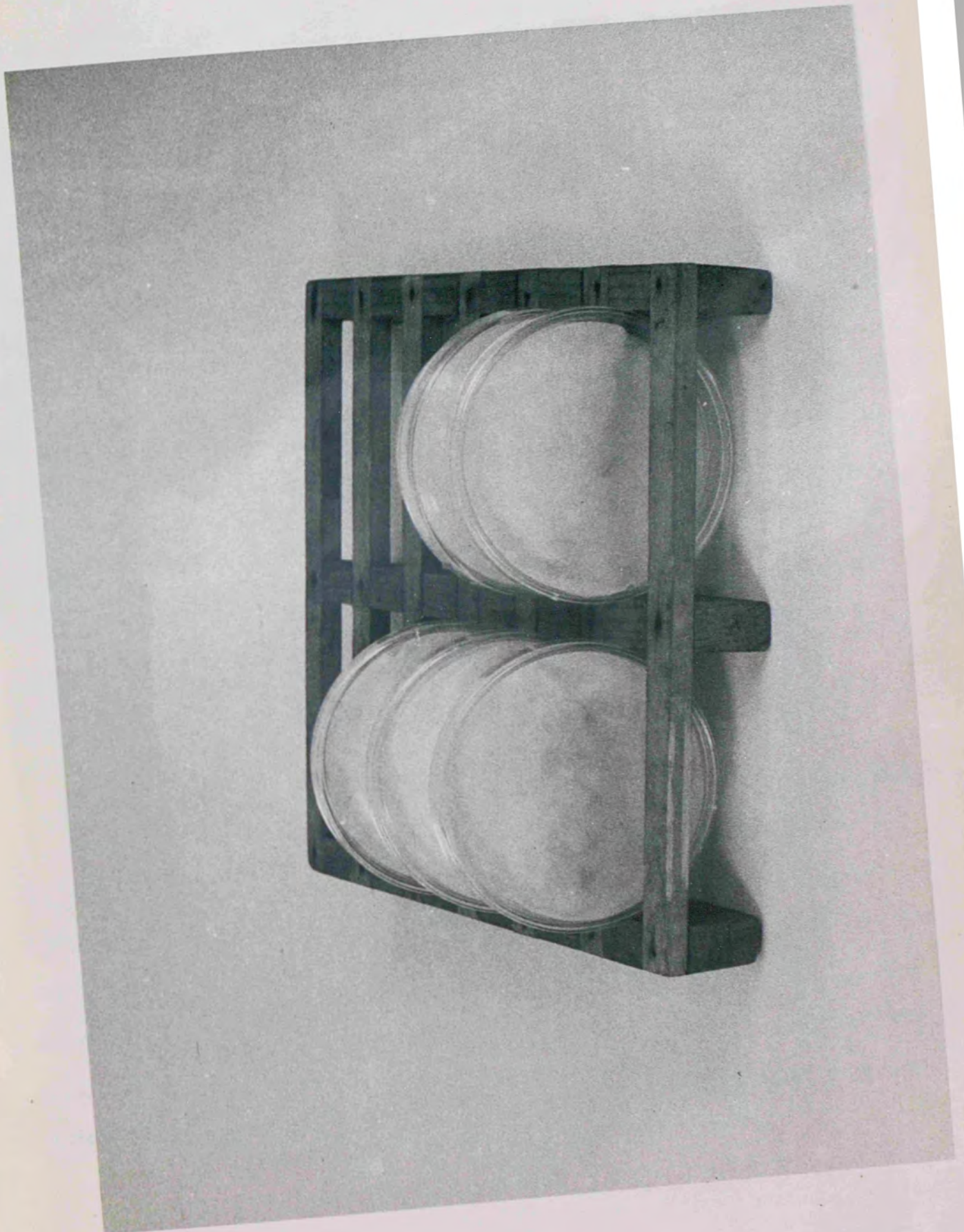


Fig. 3. Frame for holding petri dishes upright.

Fig. 3



36 hours or until positive curvature was established. The pictures were taken by means of a camera with a red flash attachment, which was fired automatically every hour.

After development the film negative was put in the photographic enlarger; the projected image of every root ($\times 15$) was then drawn. The angle between the root tip and the main axis of the root was obtained by passing two lines, one through the tip and one through the main axis. This angle was measured with a protractor and the average curvature of the sample against the time elapsed after de-capping. The recovery time was obtained as follows: regression lines were fitted to the two groups of Fig. 4. 1. Roots growing vertically. 2. Roots growing horizontally. points and the intercept between them was taken as the recovery time, i.e. the time when curvature started to develop (see Fig. 5). Each sample was averaged because the early experiments showed only a small variability within each sample (Juniper et al, 1966).

4. Presentation Time Studies

Further investigations into the role of the root cap in perception and transmission of the gravitational stimuli, and in control of the response, were necessary. It was thus decided to determine first the presentation time of the normal maize root, and when this was known, to see if the root cap could be removed after a stimulation for presentation time only, without affecting the response. This could be done by repeating the series of experiments for the determination of presentation time in normal

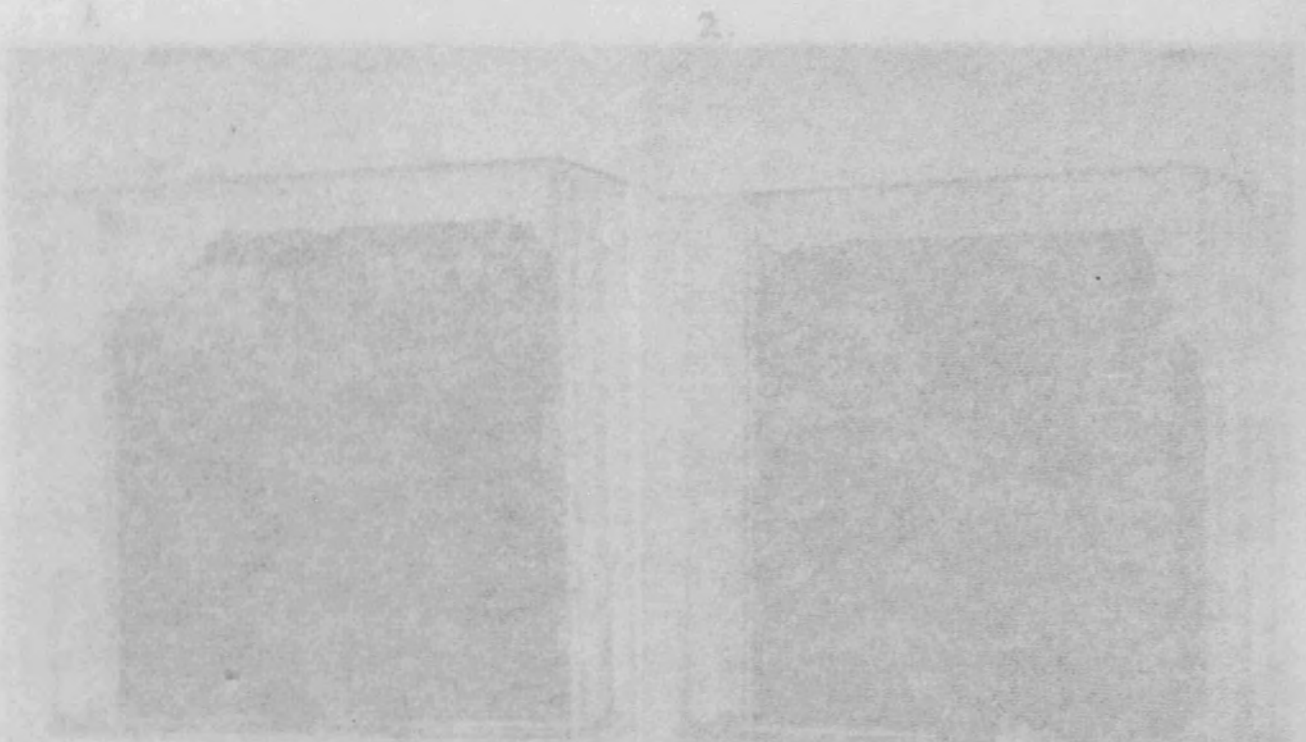


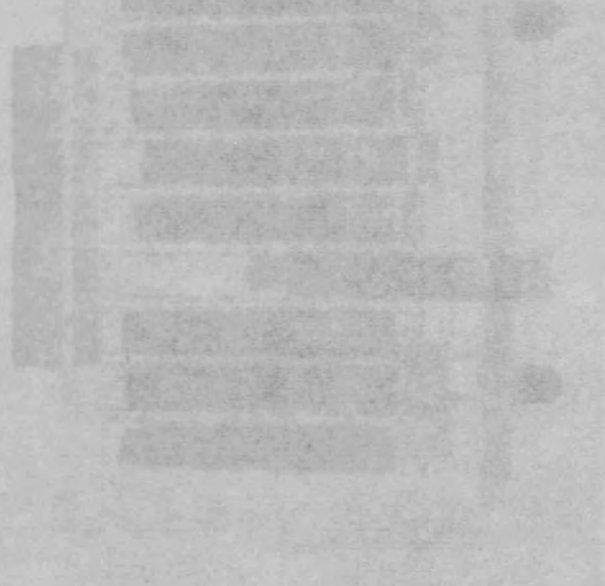
Fig. 4. a. Perspex chamber for growth rate and recovery time studies.

1. Roots growing vertically.

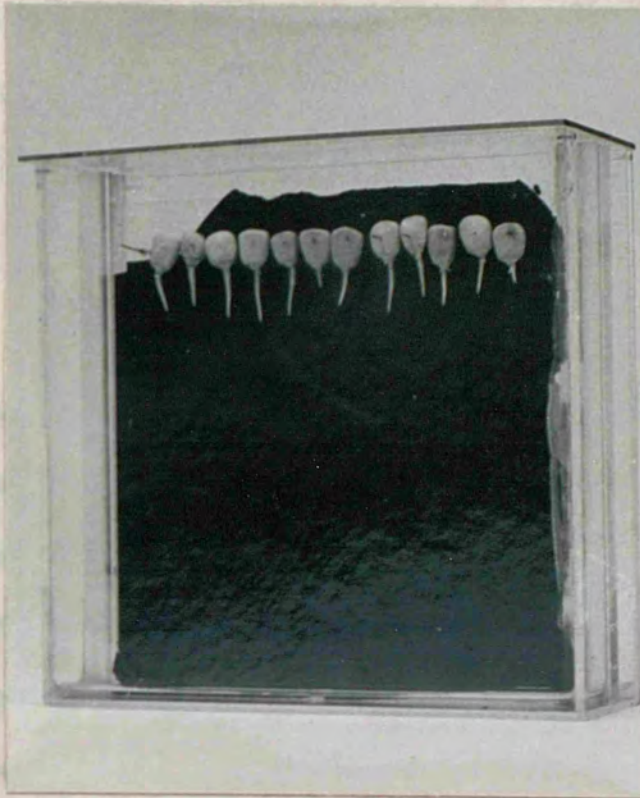
2. Roots growing horizontally.

Fig 4b

b. Perspex chamber with detachable holders used for presentation time studies.



1.



2.

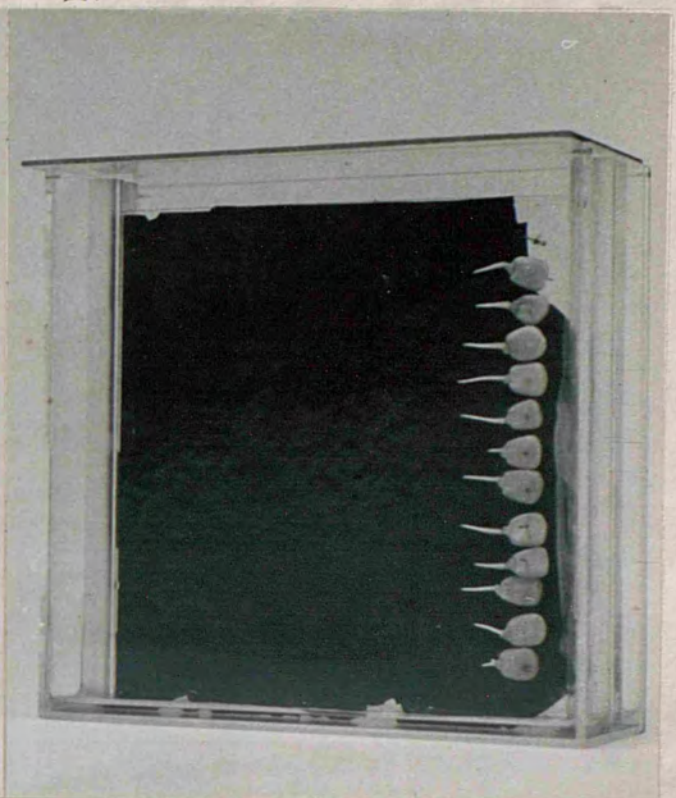
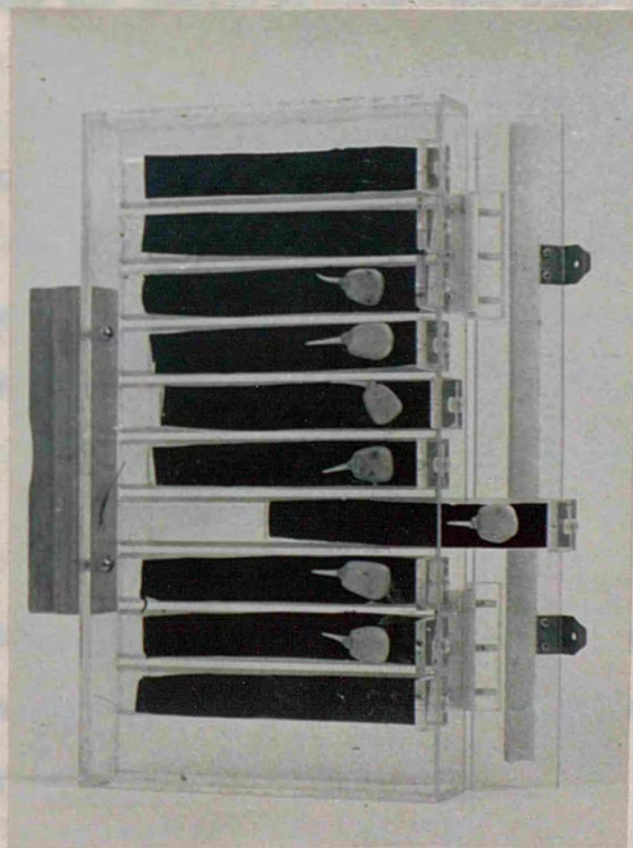


Fig 4b.



roots, but in this case removing the root cap straight after stimulation. If the presentation time calculated for this series was not significantly different from the normal one, then this indicates that the root cap does not control the response.

Presentation time is usually determined by exposing the plant or plant organ to increasing periods of stimulation and finding the stimulation time necessary to produce a visible curvature in 50% of the sample. Here a more sensitive method was used as well. Roots were grown as described above. Ten roots, of approximately even length, were selected and put on moist filter paper in a rectangular perspex chamber 4' x 6' x 0.5' with a glass plate for cover, which was secured by two rubber bands. This chamber was fitted to a klinostat and rotated at 1 rev. per minute along the long axis of the roots.

The klinostat triggered the camera and red flash attachment when the chamber was facing the camera. The whole apparatus was in the dark room at $25 \pm 1^\circ\text{C}$. The roots were first rotated for 30 minutes while pictures were taken every 5 or 10 minutes. After 30 minutes rotation was stopped so that the roots were in a horizontal position and subjected to a gravitational stimulus.

After the presentation time for normal roots was determined, a similar series of stimuli were in a series from 2 to 8 minutes (see results). After stimulation rotation was resumed and pictures taken as before for the subsequent $1\frac{1}{2}$ to 2 hours.

The root curvature was drawn and measured as described previously.

The average angle for the sample was plotted against time before and after stimulation. Regressions of angle on time were calculated for both phases (see Fig. 6).

The regression for the second phase was taken as the rate of curvature. The intercept between the two regression lines indicates the reaction time. After completion of the series of stimulation times, the rate of curvature for each was plotted against stimulation time. Again a regression was fitted and the intercept on the time axis was taken as the presentation time.

Fig. 5. The method used for obtaining recovery time.

From the same experiments, the percentage of roots that had shown more than 10° maximum curvature was found for each stimulation time and these percentages were plotted against stimulation time. Regression was fitted and the intercept at 50% was taken as the presentation time (see results).

Assuming that presentation time of a large population of roots follows a normal distribution curve, the first method used here to obtain presentation time gives a value at the short time tail of the curve, and the second, conventional, method gives a value at the peak, i.e. a mean value.

After the presentation time for normal roots was determined, a similar series of experiments was done, but the roots were de-capped after stimulation. A special chamber (Fig. 4b) was constructed from perspex. It was made so that each root was secured to a detachable holder that could be removed separately from the chamber.

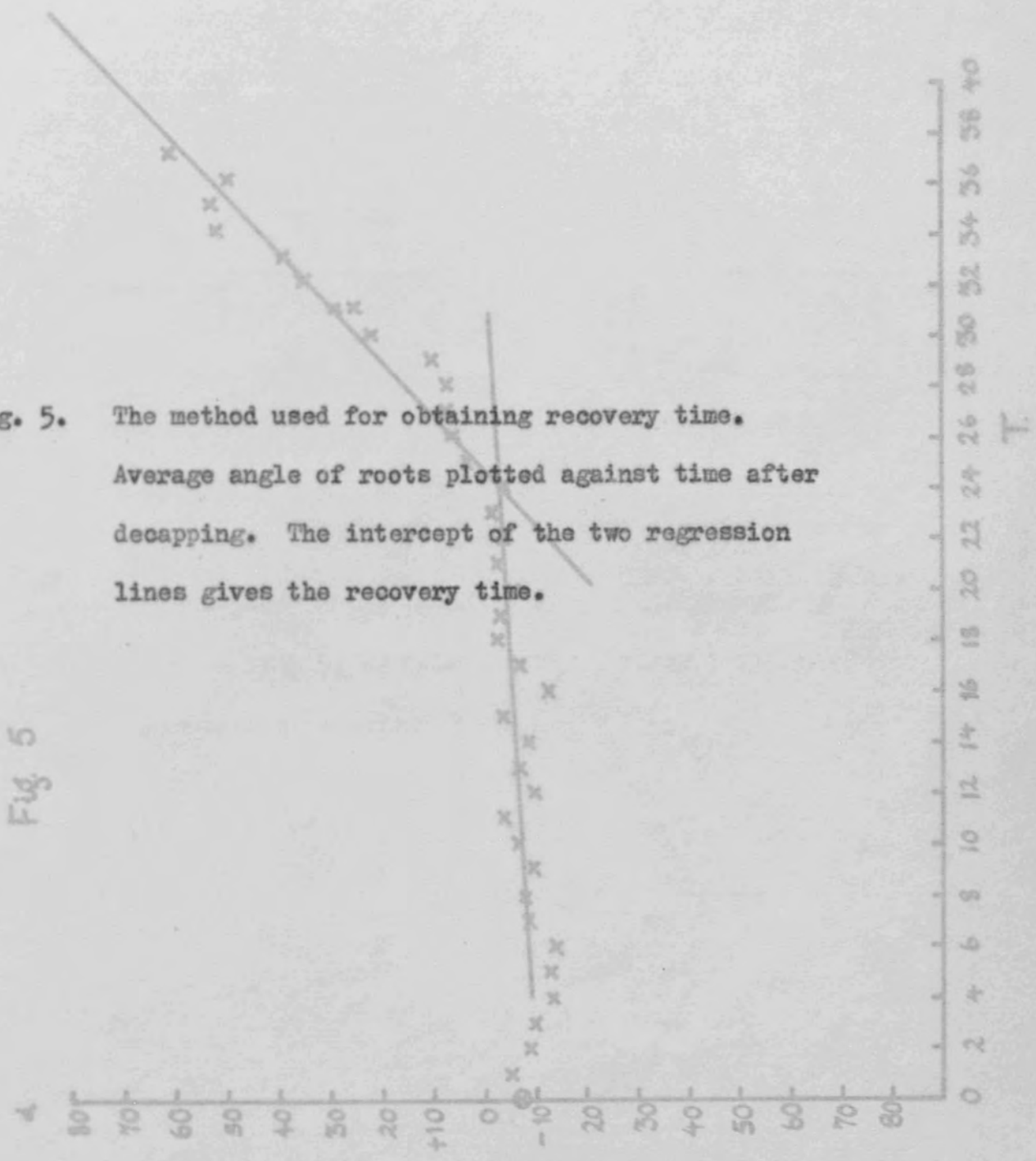


Fig. 5. The method used for obtaining recovery time. Average angle of roots plotted against time after decapping. The intercept of the two regression lines gives the recovery time.

Fig 5

Fig. 5

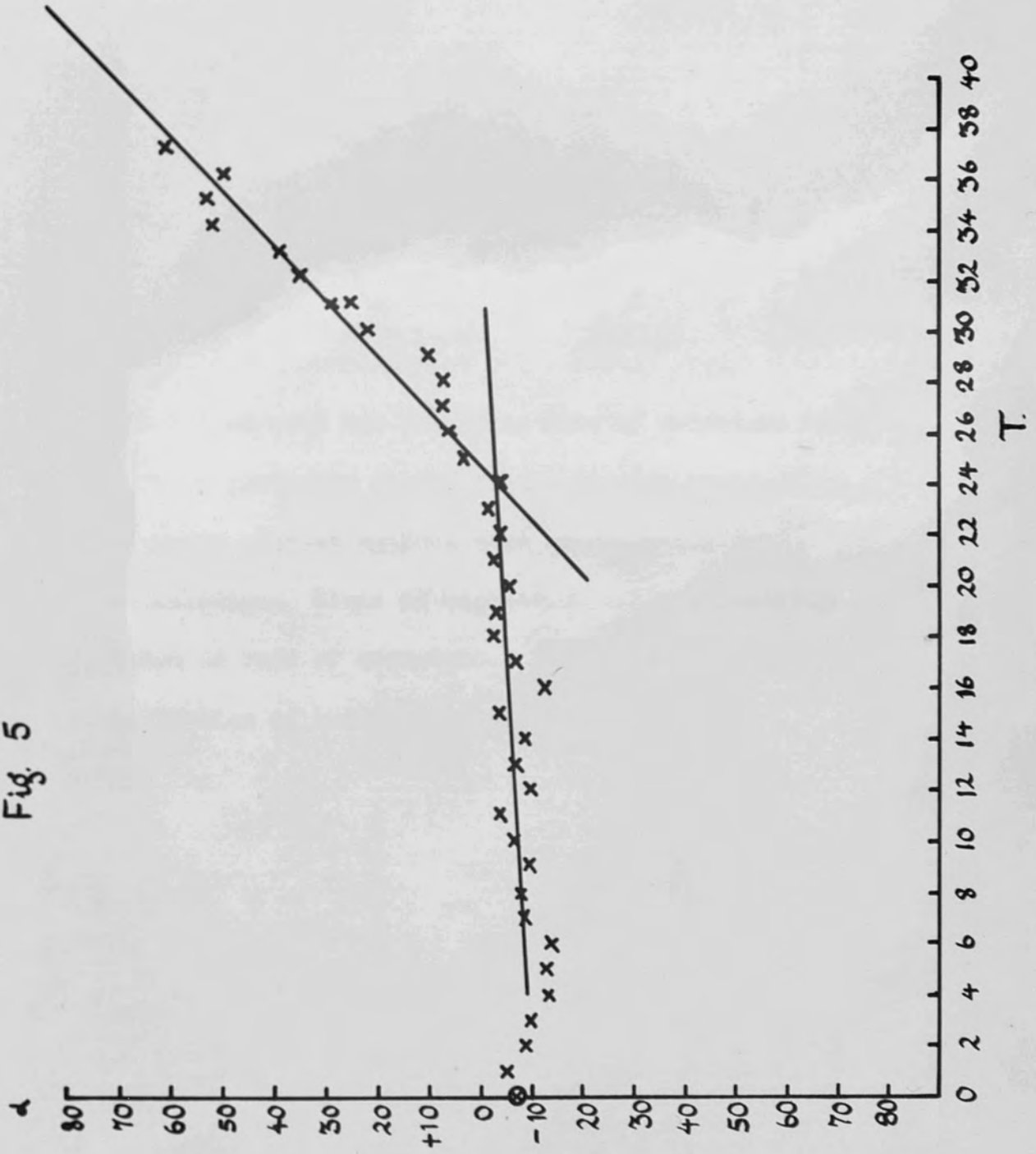


Fig. 6. The method used for obtaining rate of curvature after a short geotropic stimulation. Average angle α of roots plotted against time before and after stimulation. Slope of regression after stimulation taken as rate of curvature. The intercept gives an indication of reaction time.

Fig 6.

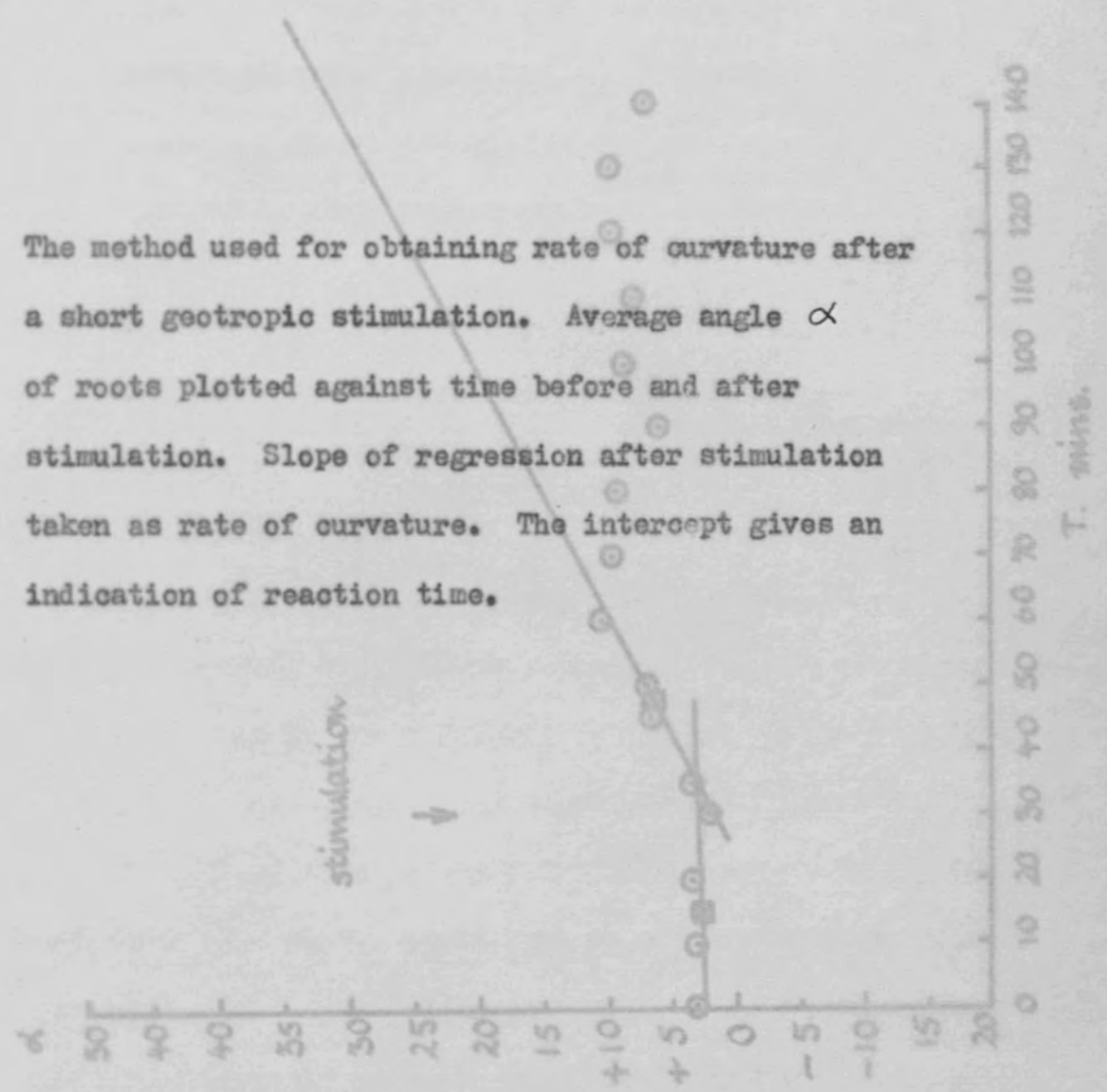
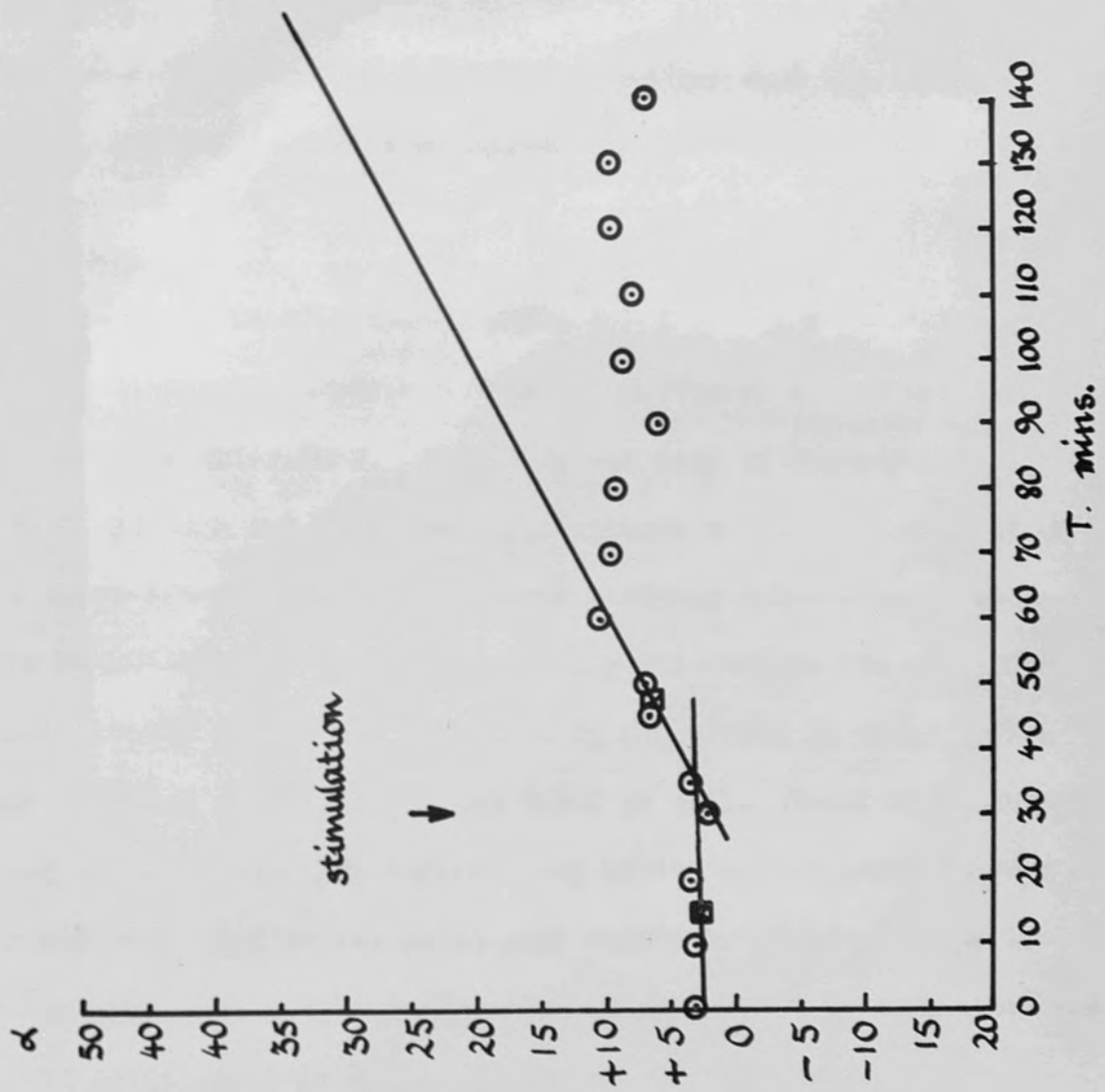


Fig 6.



After the first 30 minutes of rotation each root was removed, stimulated, then de-capped and returned to the chamber on the klinostat. The chamber was rotating during the whole experimental period and pictures were taken as before. Each root was numbered and the results were pooled at the end for calculating presentation time. These were done as for normal roots. - 7, 1 hour.

5. Microscopy in ethanol series followed by propylene oxide.

In order to find what was the state of the root cap cells at recovery time, various methods were tried.

Interference colours of pale gold to silver, 600-900 μ , were collected on copper grids, 75 or 100 mesh, with Light Microscopy. They were stained for 10-20 minutes in lead citrate

Roots were fixed in 4% Glutaraldehyde for 4-18 hours at different times after de-capping. Dehydration was in an ethanol series and then in an E.M. 100 electron microscope. Resin embedded material was also gradual transfer to chloroform. Embedding was done in wax-paraplast. Sections 6 μ thick were cut on a rocking microtome and the sections stuck to a glass slide after spreading. Several staining methods were tried.

I in KI was unsatisfactory for the recovering cap because the cytoplasm stained very densely and it was difficult to see starch in these cells. PAS technique (after Jensen, 1962) was tried as well. Wheat shoot apices sections and wheat coleoptiles sections and willow phloem sections were stained at the same time as the maize root sections. This was done to provide a control. The colour development of the starch in maize root was very weak and unsatisfactory compared with the controls.

It was then decided to use electron microscopical investigations in conjunction with phase contrast observations of resin embedded material.

ii. Electron Microscopy Chapter III

Fixation: Roots were fixed in 4% Glutaraldehyde buffered with 0.1 M phosphate buffer pH = 7. Fixation was for 4-18 hours. Then the roots

1. Destarching experiments
 were rinsed thoroughly in buffer and post fixed in either 2% OsO₄ buffered with 0.1 M phosphate buffer pH = 7, 1 hour, or 5% LiMnO₄ buffered with 0.1 M phosphate buffer pH = 7, 1 hour.

The various early methods used to destarch plant roots, as described in Materials and Methods, did not give very satisfactory results because of the considerable effect on growth. Fig. 7 and Fig. 8 show the effect of dehydration was in ethanol series followed by propylene oxide.

Embedding was in Epon. Longitudinal median sections were cut on an L.K.B.

No optimization of concentration and length of treatment was found, Ultratome with glass knives. Sections with interference colours of pale gold to silver, 600-900 Å, were collected on copper grids, 75 or 100 mesh, in these experiments there was never a case of a root without starch which with hexagonal holes. They were stained for 10-20 minutes in lead citrate showed a positive geotropic response (see Fig. 9).

(after Reynolds, 1963). Then they were coated with carbon and examined

in an A.E.I. E.M.6 electron microscope. Resin embedded material was also sectioned at 0.5 - 1μ on the L.K.B. Ultratome mounted in Canada balsam on glass slides and examined in a Zeiss phase contrast microscope.

reaction was due to in these experiments as it was not investigated further. Treatment with erythrocin gave essentially the same picture of no positive geotropic reaction in the absence of starch.

Results are given in Table I. In this table each root is numbered and its starch content, as graded from the scale, and its curvature in degrees after the time stated are given.

Chapter III

Results

1. Destarching experiments

The various early methods used to destarch plant roots, as described in Materials and Methods, did not give very satisfactory results because of the considerable effect on growth. Fig. 7 and Fig. 8 show the effect of treatment with potassium aluminium sulphate.

No combination of concentration and length of treatment was found, which reduced starch completely without severe injury to the plant. But Fig. 7. Graph showing the effect of treatment with Potassium Alum on growth of cross roots. in these experiments there was never a case of a root without starch which showed a positive geotropic response (see Fig. 9).

The same situation prevailed for treatment in the cold (see Fig. 10). There seems to be a reversal of the normal geotropic response of cross roots in the absence of starch in the cold. It is not known what this reaction was due to in these experiments as it was not investigated further. Treatment with erythrosin gave essentially the same picture of no positive geotropic reaction in the absence of starch.

Results are given in Table I. In this table each root is numbered and its starch content, as graded from the scale, and its curvature in degrees after the time stated are given.

X---X 7 hrs.
 O---O 5 hrs.
 Δ---Δ 3 hrs.

Fig. 7. Graph showing the effect of treatment with Potassium Alum on growth of cress roots.

Fig 7. Inhibition of Growth by Potassium Alum.

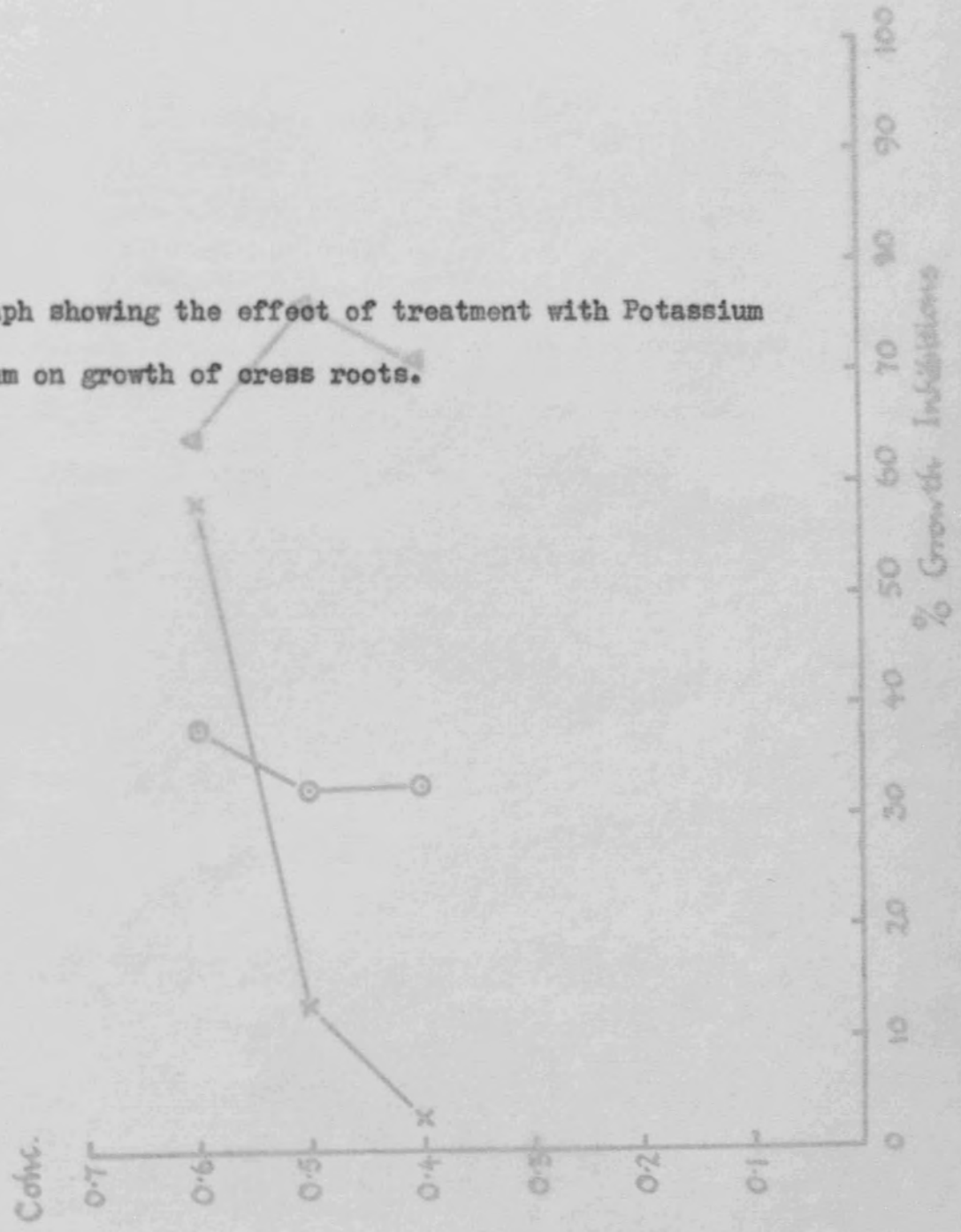
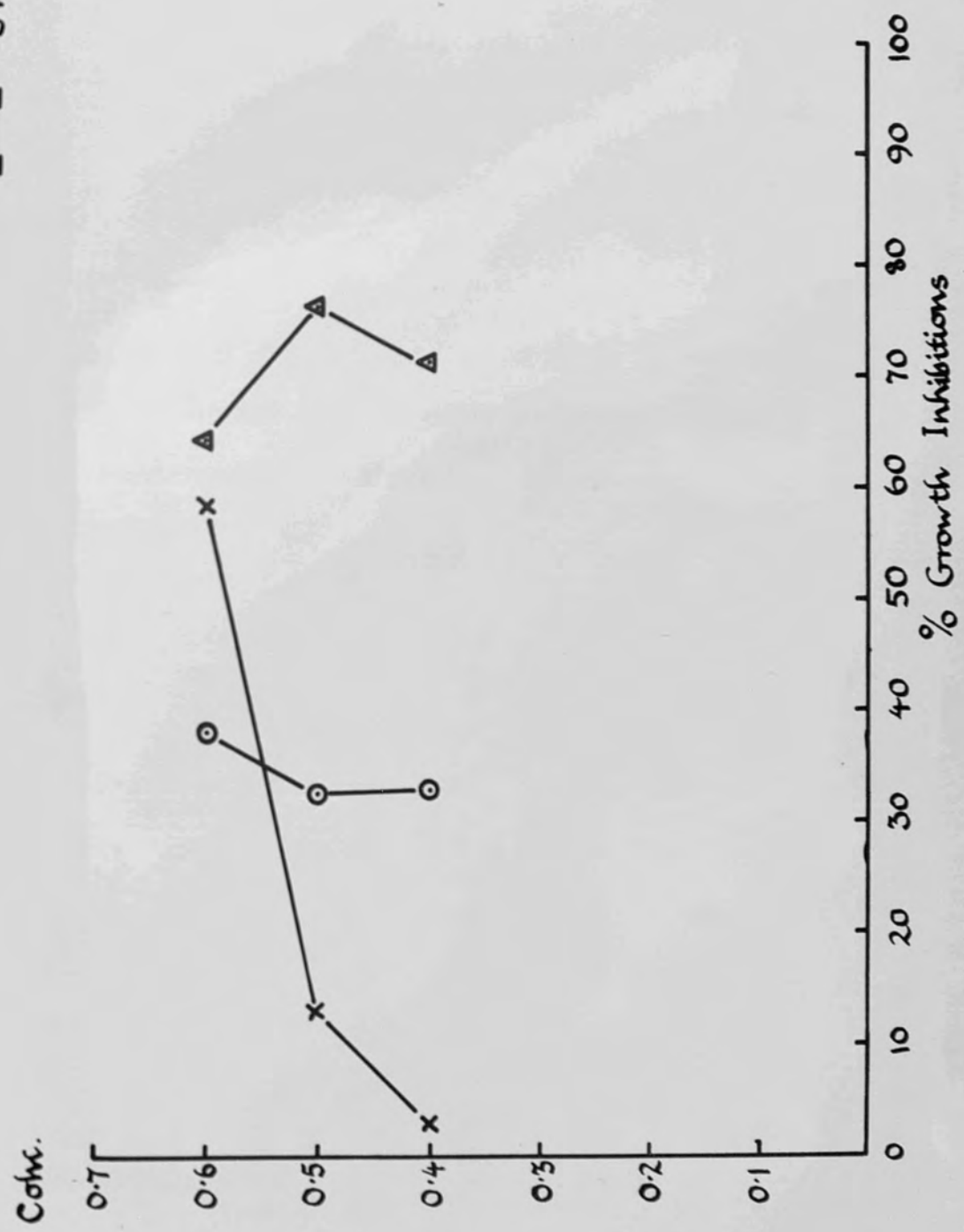


Fig 7. Inhibition of Growth by Potassium - Alum.

x—x 7 hrs.
o—o 5 hrs.
Δ—Δ 3 hrs



○—○ 0.4 %
x—x 0.5 %
△—△ 0.6 %

Fig 8. Inhibition of Growth in Potassium Alum.

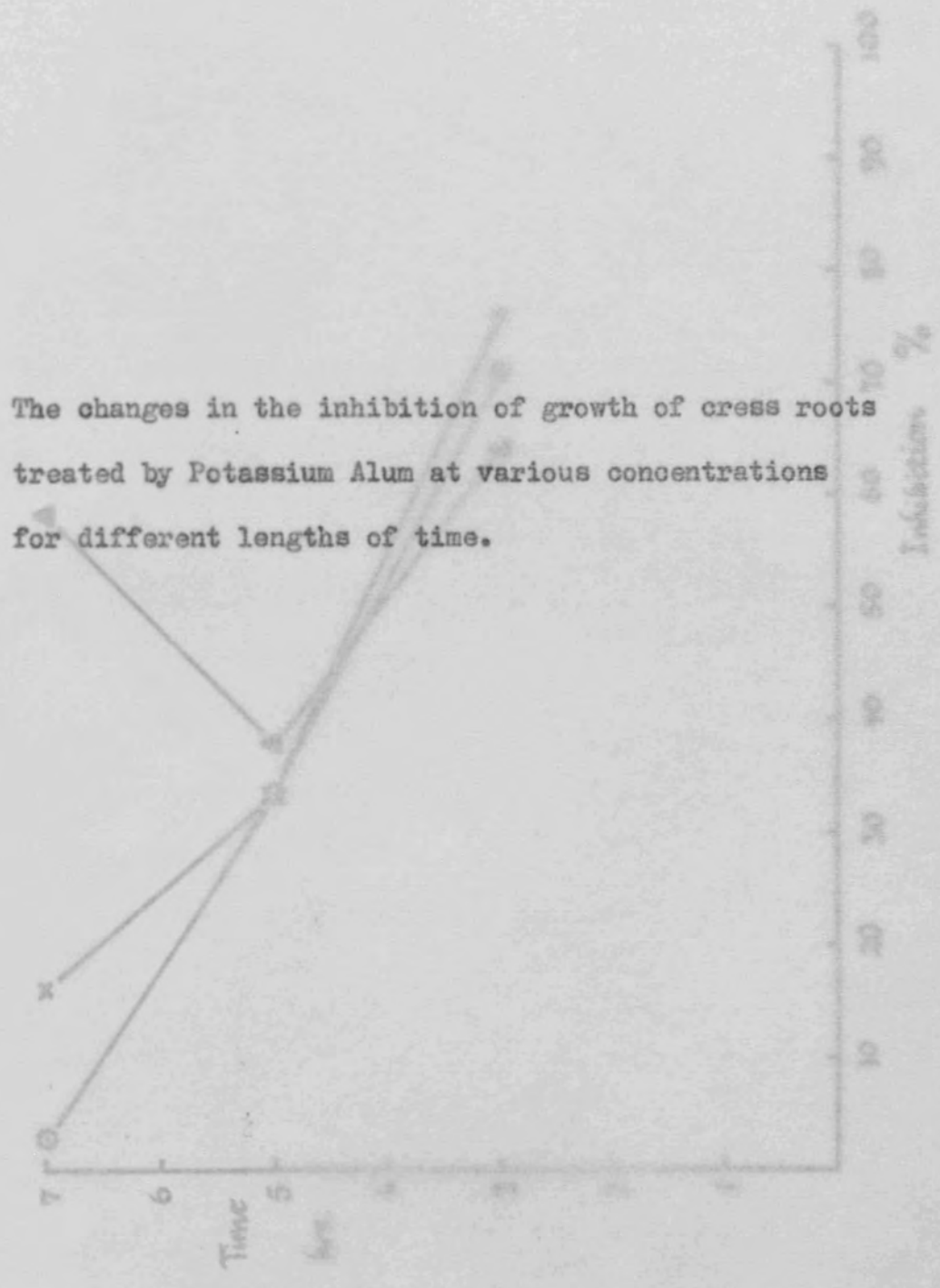


Fig. 8. The changes in the inhibition of growth of cress roots treated by Potassium Alum at various concentrations for different lengths of time.

Fig 8. Inhibition of Growth in Potassium-Alum.

○—○ 0.4%
 x—x 0.5%
 ▲—▲ 0.6%

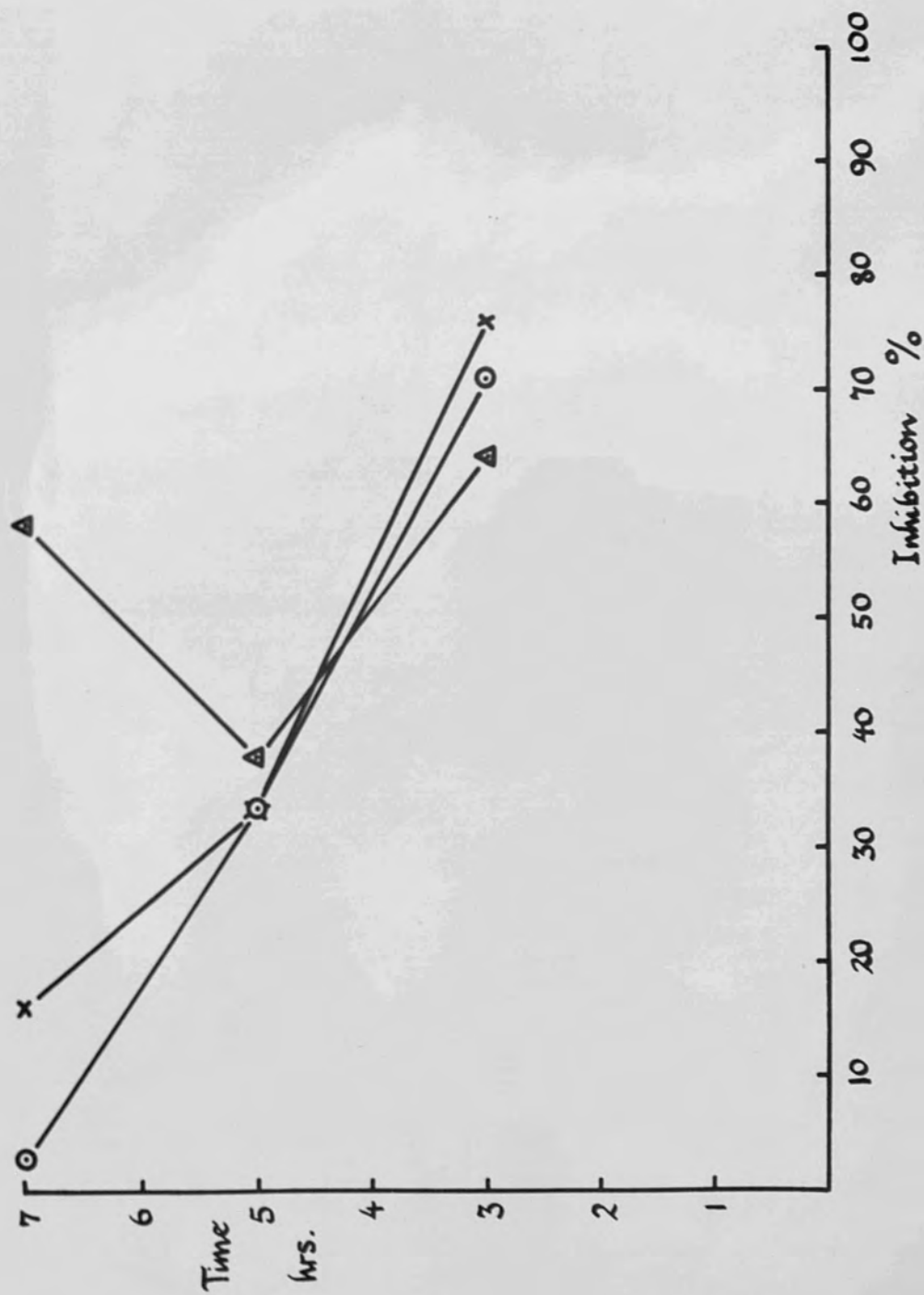
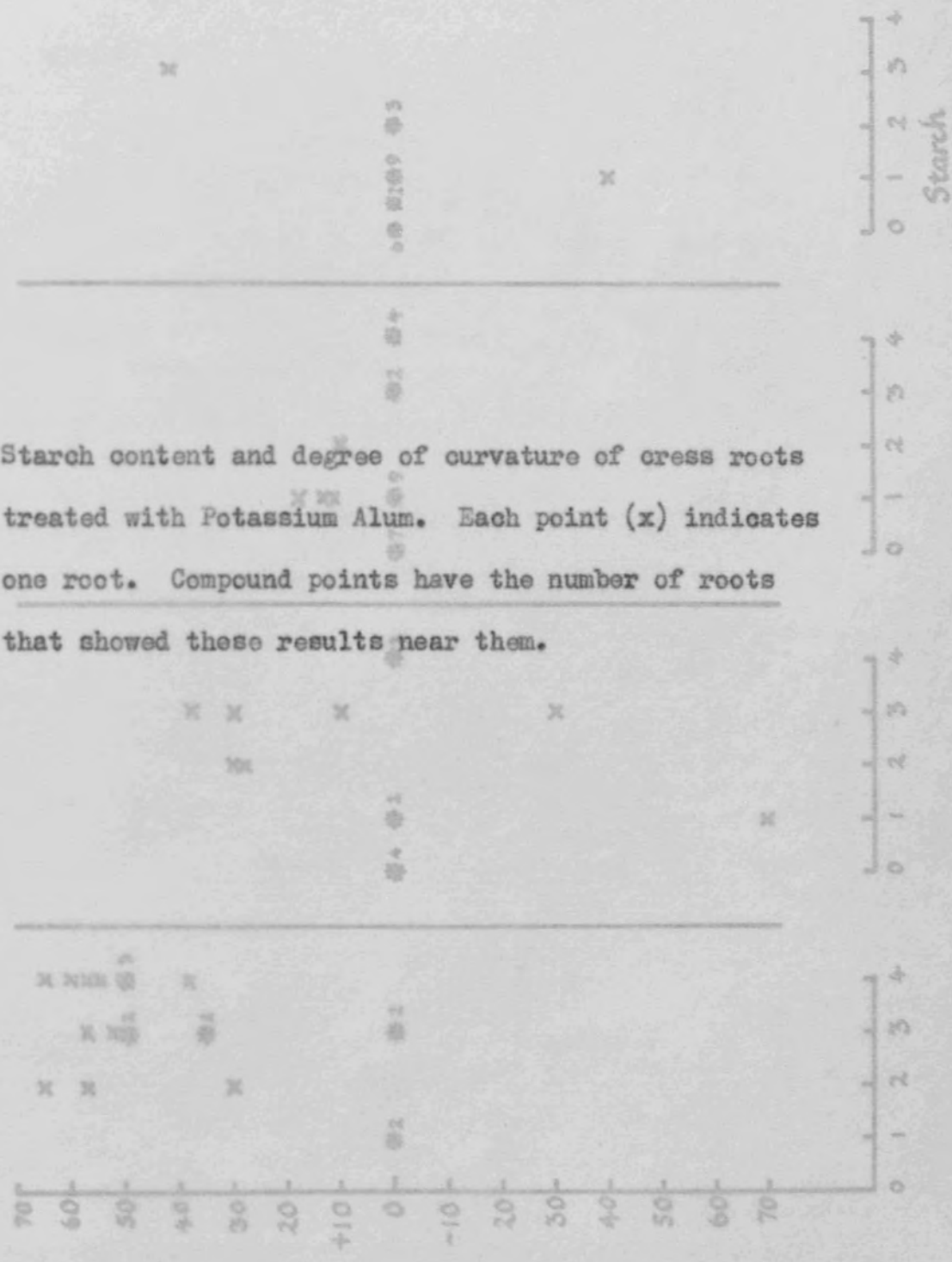


Fig 9. Curvature and Starch in Potassium Alum.
 H₂O 0.2% 0.3% 0.4%



Starch content and degree of curvature of cress roots treated with Potassium Alum. Each point (x) indicates one root. Compound points have the number of roots that showed these results near them.

Fig 9. Curvature and Starch in Potassium - Alum.

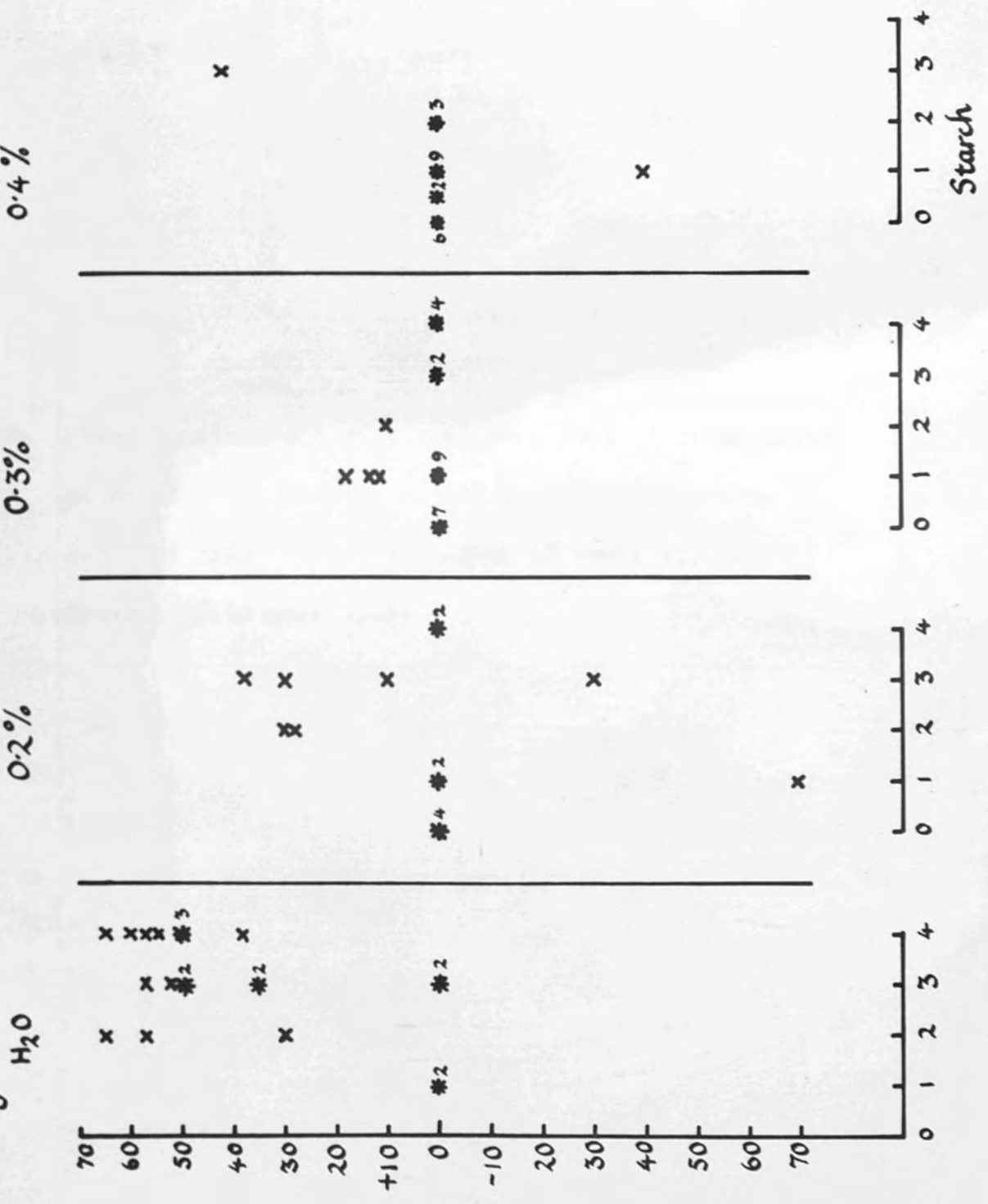


Fig. 10. Starch and Curvature at 4°C.

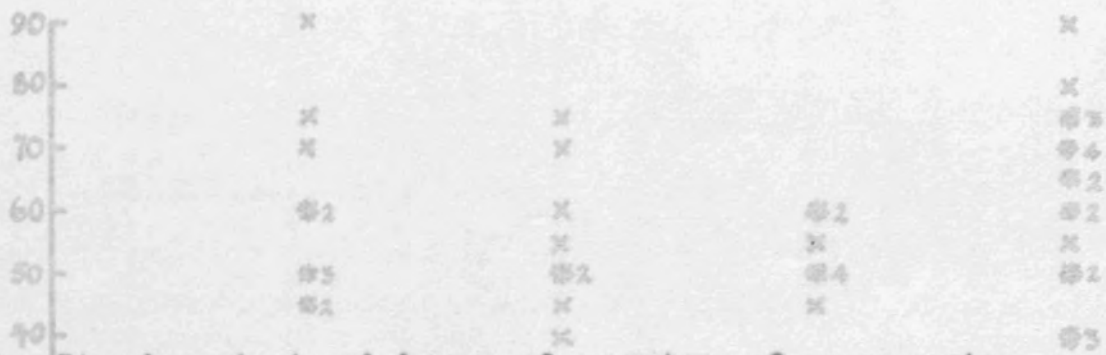


Fig. 10. Starch content and degree of curvature of cress roots grown at 5°C. Each point (x) indicates one root. Compound points have the number of roots that showed these results near them.

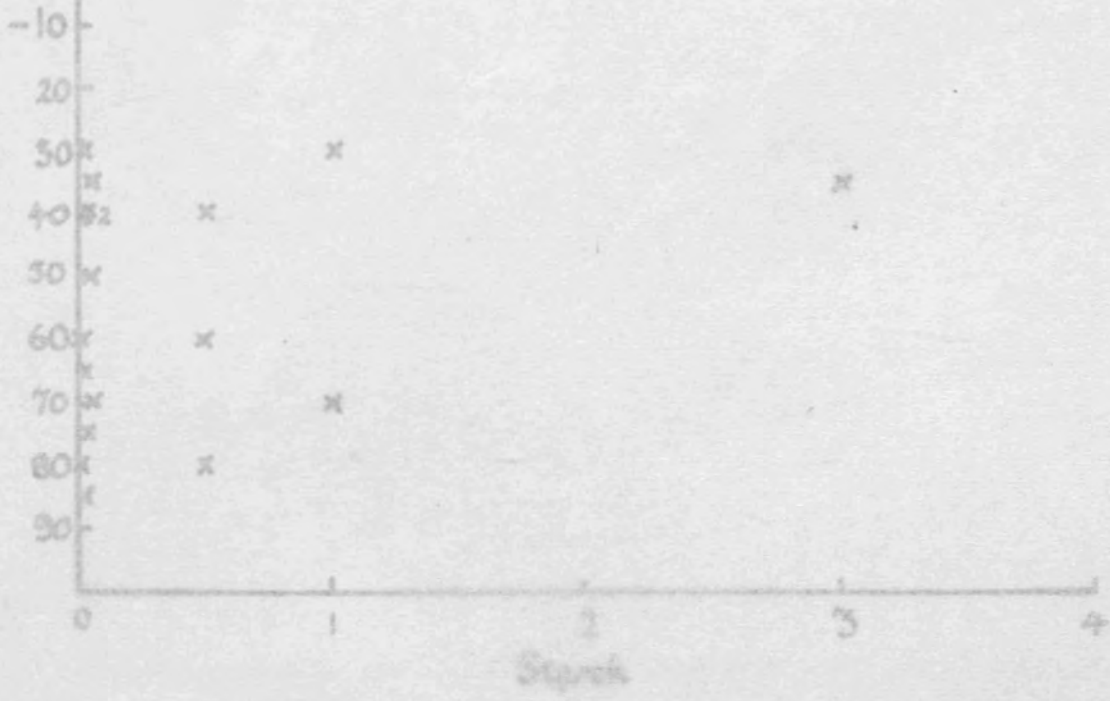


Fig. 10. Starch and Curvature at 4°C.

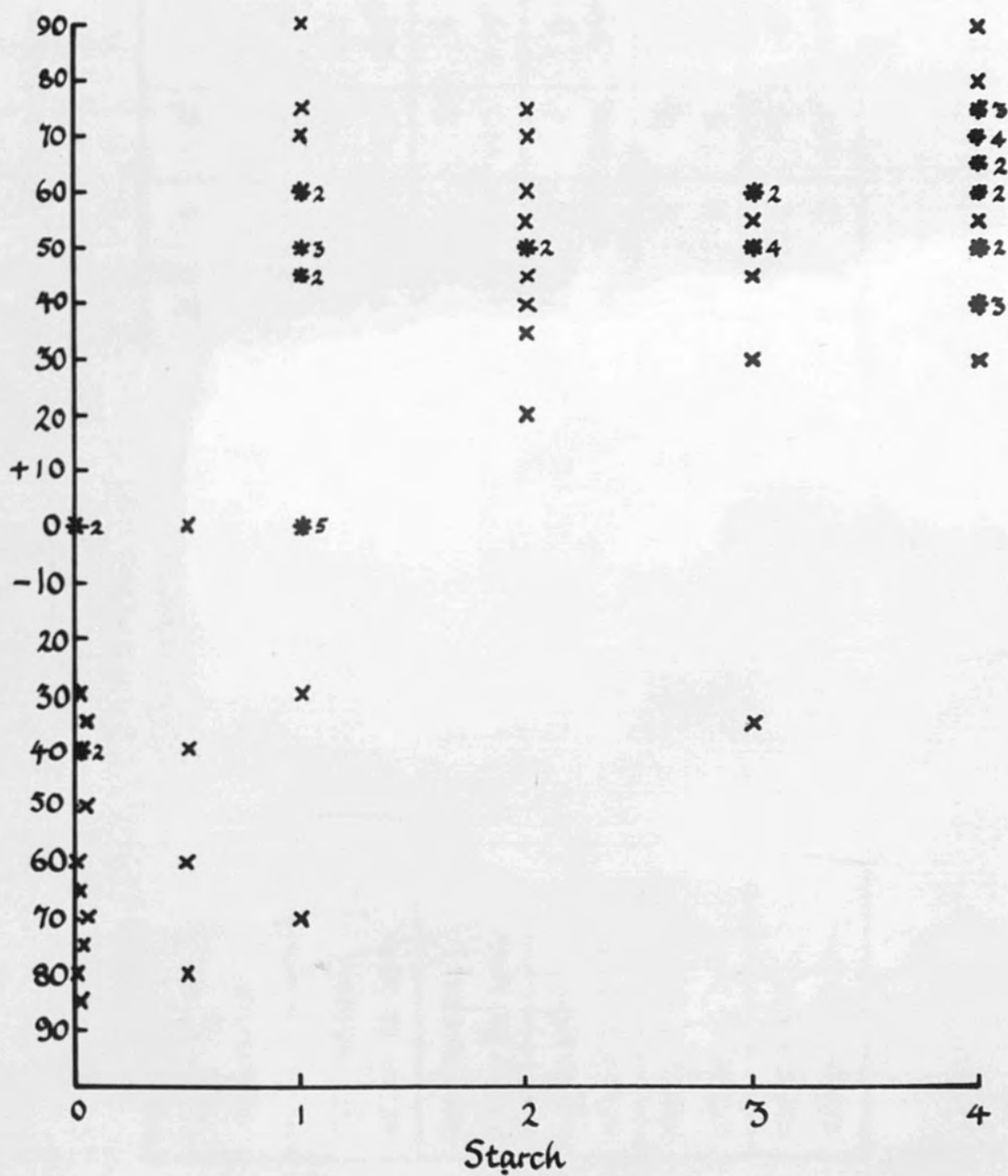


Table I
Starch content and curvature after treatment with erythrosin

Treatment	Root No.	1	2	3	4	5	6	7	8	9	10
H ₂ O control after 24 hrs.	starch	3	4	4	2	4	4	2	2	4	2
	curvature	+60°	+70°	+60°	+40°	+90°	+75°	+30°	+50°	+70°	+90°
Erythrosin after 24 hrs.	starch	4	4	3	1	4	3	2	3	1	4
	curvature	0	+10°	0	0	0	+10°	+15°	0	0	0
H ₂ O control after 24 hrs.	starch	4	2	2	4	4	4	4	4	4	4
	curvature	+80°	+65°	+80°	+90°	+85°	+75°	+65°	+80°	+90°	+80°
Erythrosin after 48 hrs.	starch	4	3	4	4	1	2	4	3	4	
	curvature	+40°	+45°	+40°	+75°	0	0	+50°	+45°	0	
Erythrosin after 24 hrs.	starch	4	4	4	3	3	1	4	4	4	4
	curvature	+50°	-40°	+60°	0	0	+45°	-70°	0	-70°	-85°

It is evident from the results that the erythrosin has no marked effect on starch content but affects geotropic response very strongly for 48 hours at least.

The results are given in Table III.

2. Decapping

Decapping Zea mays roots proved a very good way of removing the root cap completely to give primary roots which were still attached to the plants, but showed no geotropic response. It was decided first to investigate the effects of decapping on the growth rate of the roots.

1. Growth Rate Measurements

The growth rate of roots before and after decapping was compared.

First are given results from two experiments M_{52} and M_{53} in which the growth rate was followed for 3 hours before decapping, and 3 hours after decapping. Table II gives the t tests which show that there was no significant difference between the growth rate before and after decapping.

In order to confirm that the root cap had no effect on the root growth rate, a separate experiment was conducted in which the root cap

Table II

Students t test for significance of difference in growth rate before and after decapping (described in Materials and Methods) in half the number of the decapped roots. The others were

M_{52}	n = 10	t = 0.038	D.F. = 9	Not significant at P = 0.05
M_{53}	n = 11	t = 1.33	D.F. = 10	Not significant at P = 0.05

means for those saved at each time.

The results from several similar experiments were then pooled for the same test to be performed. *affected by decapping and replacing the root cap*

The results are given in Table III.

T in min.	Before Decapping		After Decapping	
	A Table III		A + cap	B - cap
		Non-decapped	Decapped	
Mean growth rate		0.78 mm/hr	0.75 mm/hr	
S.D.		± 0.256	± 0.282	
S.E.		0.041	0.045	
n		40	40	
t = 0.158 Not significant at P = 0.05				

t tests for significance of difference in growth rate

There is no significant difference in growth rate between non-decapped and decapped roots. *before and after decapping and replacing the cap t = 0.1099 D.F. = 10*
Not significant at P = 0.05.

In order to confirm that the root cap had no effect on the root growth rate, a separate experiment was conducted in which the root cap was put back onto the root tip after decapping (as described in Materials and Methods) in half the number of the decapped roots. The others were just decapped. Growth rate was measured every half hour for 3 hours before and after decapping. The results are presented in Table IV. In this table each group contains seven roots and the values for each time are the means for those seven at each time.

All the results on ~~comp~~ Table IV with rates point to the fact that the root cap Growth rate of roots as affected by decapping primary root. The and replacing the root cap loss of geotropic response is not therefore due to a reduction of its growth rate.

T in mins.	Before Decapping		After Decapping	
	A	B	A + cap	B - cap
0 - 30	1 mm/hr	1 mm/hr	0.8 mm/hr	0.8 mm/hr
30 - 60	1.2 "	1.2 "	0.6 "	0.4 "
60 - 90	0.6 "	0.4 "	0.4 "	1.0 "
90 - 120	0.6 "	0.8 "	0.8 "	0.6 "
120 - 150	1.2 "	0.4 "	1.2 "	0.4 "
150 - 180	0.4 "	0.6 "	0.4 "	0.6 "

recovery of the response. Therefore a range of root lengths was chosen, t tests for significance of difference in growth rate within the life span of the primary root, and recovery of each group followed.

A before and after decapping and replacing the cap $t = 0.1059$ D.F. = 10

The effect of replacing the root cap, straight after decapping, on Not significant at $P = 0.05$.

the recovery period was also followed. Results are presented in Fig. 11,

B before and after decapping and replacing the cap $t = 0.1769$ D.F. = 10

Not significant at $P = 0.05$.

is an indicator of its "developmental age" and that younger roots show a A after decapping and replacing B after decapping with no replacing higher rate of cell division and so they regenerate more quickly.

$t = 0.451$ D.F. = 10 Not significant at $P = 0.05$.

The replacing of the root cap did not have any marked effect on There is no significant effect of decapping, or decapping and replacing the recovery time (see Fig. 11). The three points indicated by solid circles cap, on the growth rate of the roots. in Fig. 11 possibly represent a slight shortening of the recovery period due

All the results on comparing growth rates point to the fact that the root cap does not affect or control the growth of the primary root. The loss of geotropic response is not therefore due to a reduction of its growth rate.

ii. Recovery Time Determinations

The next step was to follow the recovery of the geotropic reactivity of the decapped roots. It was known that they are capable of regenerating the root cap, and it was decided to try and determine, as accurately as possible, the time required for recovery of the geotropic response.

Fig. 11. Graph showing relationship between root length at

Preliminary experiments showed that there was some correlation between the length of the root at the time of decapping and the time needed for recovery of the response. Therefore a range of root lengths was chosen, within the life cycle of the primary root, and recovery of each group followed.

recover without the cap and the solid circles roots

The effect of replacing the root cap, straight after decapping, on which had their cap replaced straight after decapping, the recovery period was also followed. Results are presented in Fig. 11, and it can be seen that the relationship between root length, at decapping time, and recovery time is reaffirmed. It is probable that the root length is an indicator of its "developmental age" and that younger roots show a higher rate of cell division and so they regenerate more quickly.

The replacing of the root cap did not have any marked effect on recovery time (see Fig. 11). The three points indicated by solid circles in Fig. 11 possibly represent a slight shortening of the recovery period due

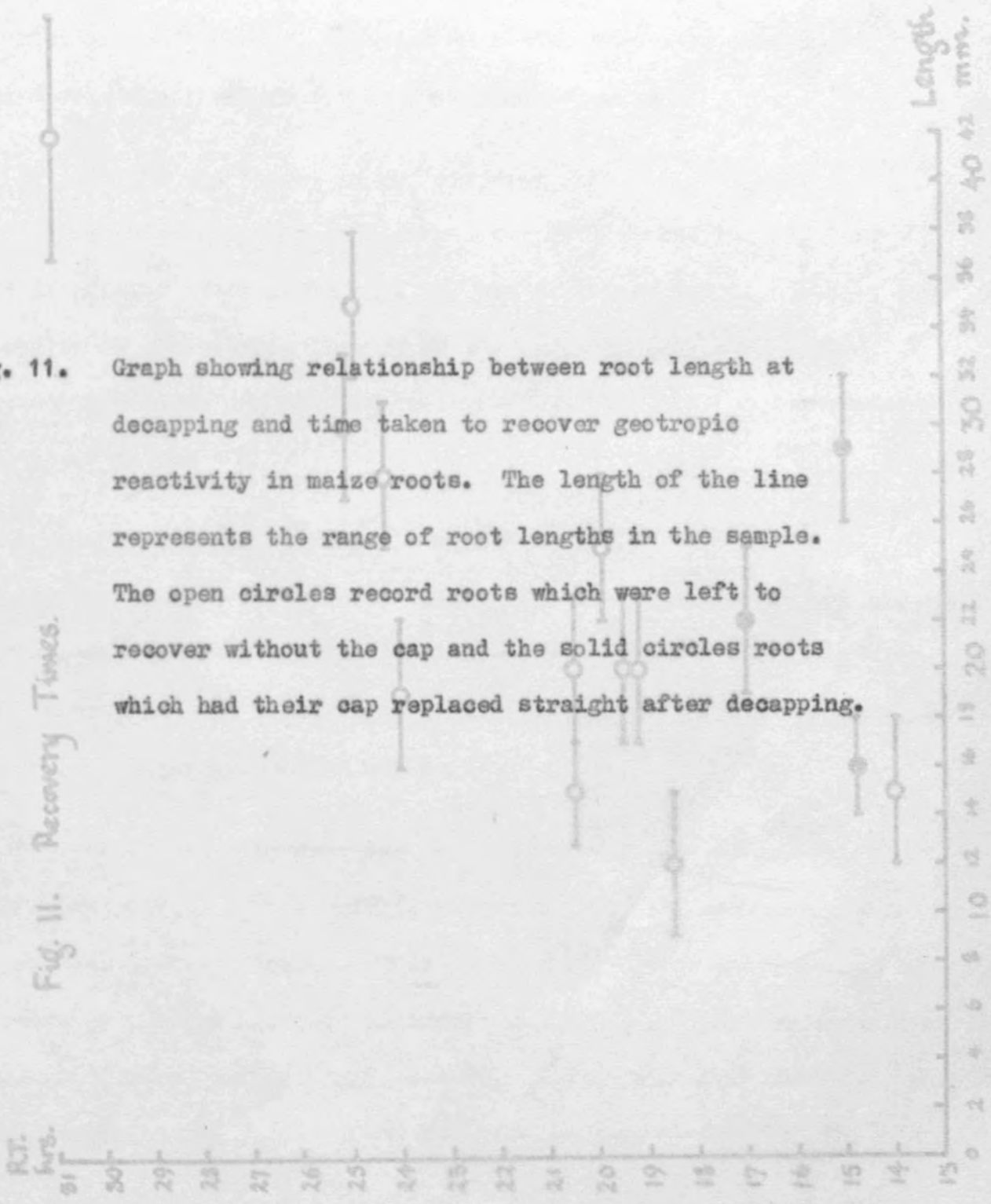
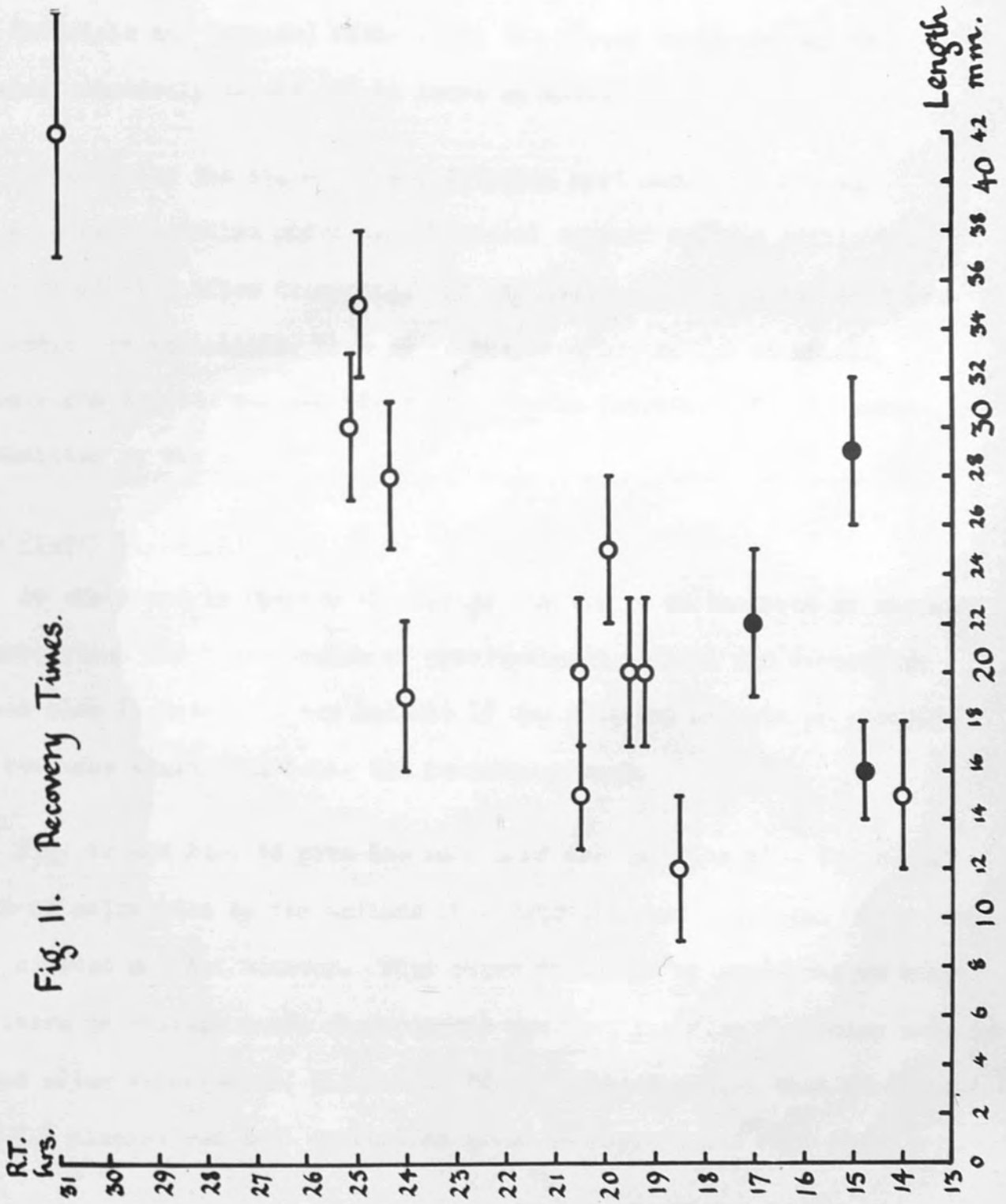


Fig. 11. Graph showing relationship between root length at decapping and time taken to recover geotropic reactivity in maize roots. The length of the line represents the range of root lengths in the sample. The open circles record roots which were left to recover without the cap and the solid circles roots which had their cap replaced straight after decapping.

Fig. 11. Recovery Times.



perhaps to a protection of the exposed root tip from exposure, afforded by replacement of the cap. There is certainly no transmission of a stimulus from the replaced cap (which was in good contact with the tip, see Materials and Methods) although in the closed humid chamber it remains relatively turgid for at least an hour.

By staining the starch in the detached root cap, in I KI, it did show sedimentation under gravitational stimuli applied within the first 30 minutes after decapping. If the starch grains in the root cap are acting as statoliths, then it is the severing of the plasmatic connections between cap and tip that prevents the stimulus from being transmitted to the tip.

iii. Presentation Time Studies

As explained in Chapter II, further knowledge on the role of the cap in perception and transmission of gravitational stimuli was desirable. It was also important to try and see if the root cap affects or controls the response apart from being the perception zone.

Fig. 12 and Fig. 14 give the values of presentation time for normal roots as calculated by two methods (see Materials and Methods). They are 1.75 minutes and 4.4 minutes. They cover the range of presentation time of sensitive to average roots as explained previously. When the roots were decapped after stimulation, the values for presentation time that were obtained were 2.8 minutes and 3.75 minutes as given in Fig. 13 and Fig. 15.

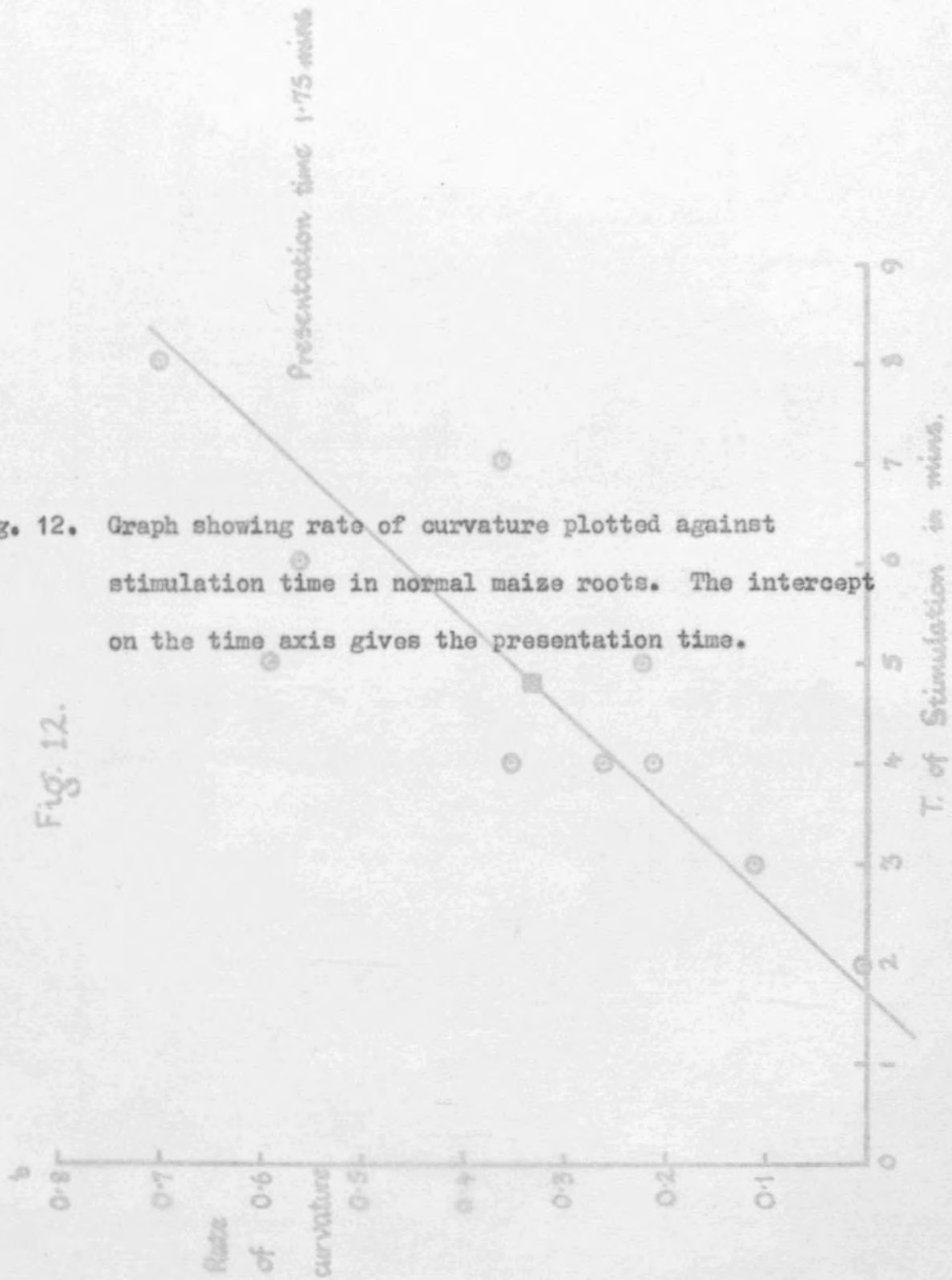
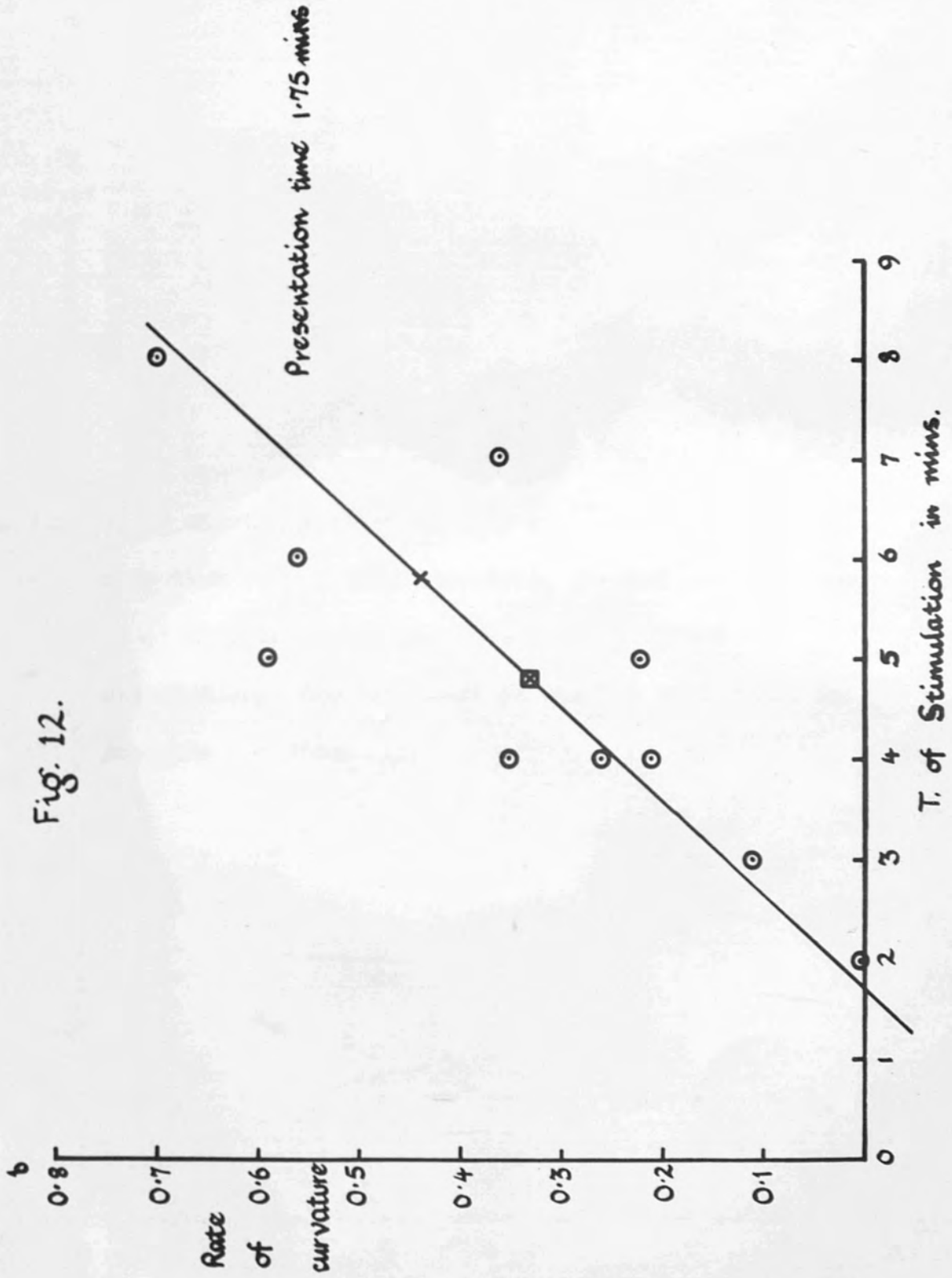
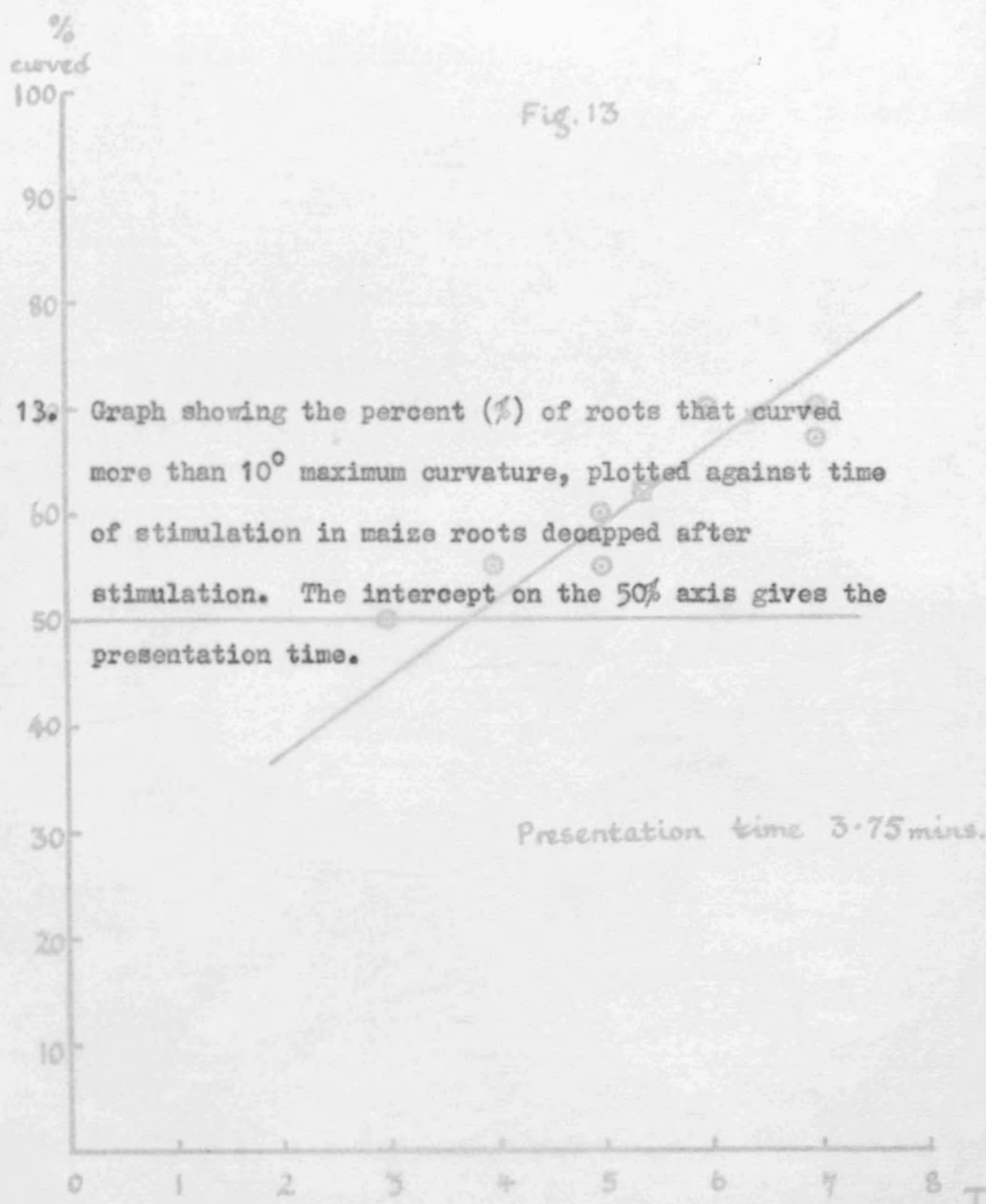


Fig. 12. Graph showing rate of curvature plotted against stimulation time in normal maize roots. The intercept on the time axis gives the presentation time.

Fig. 12.





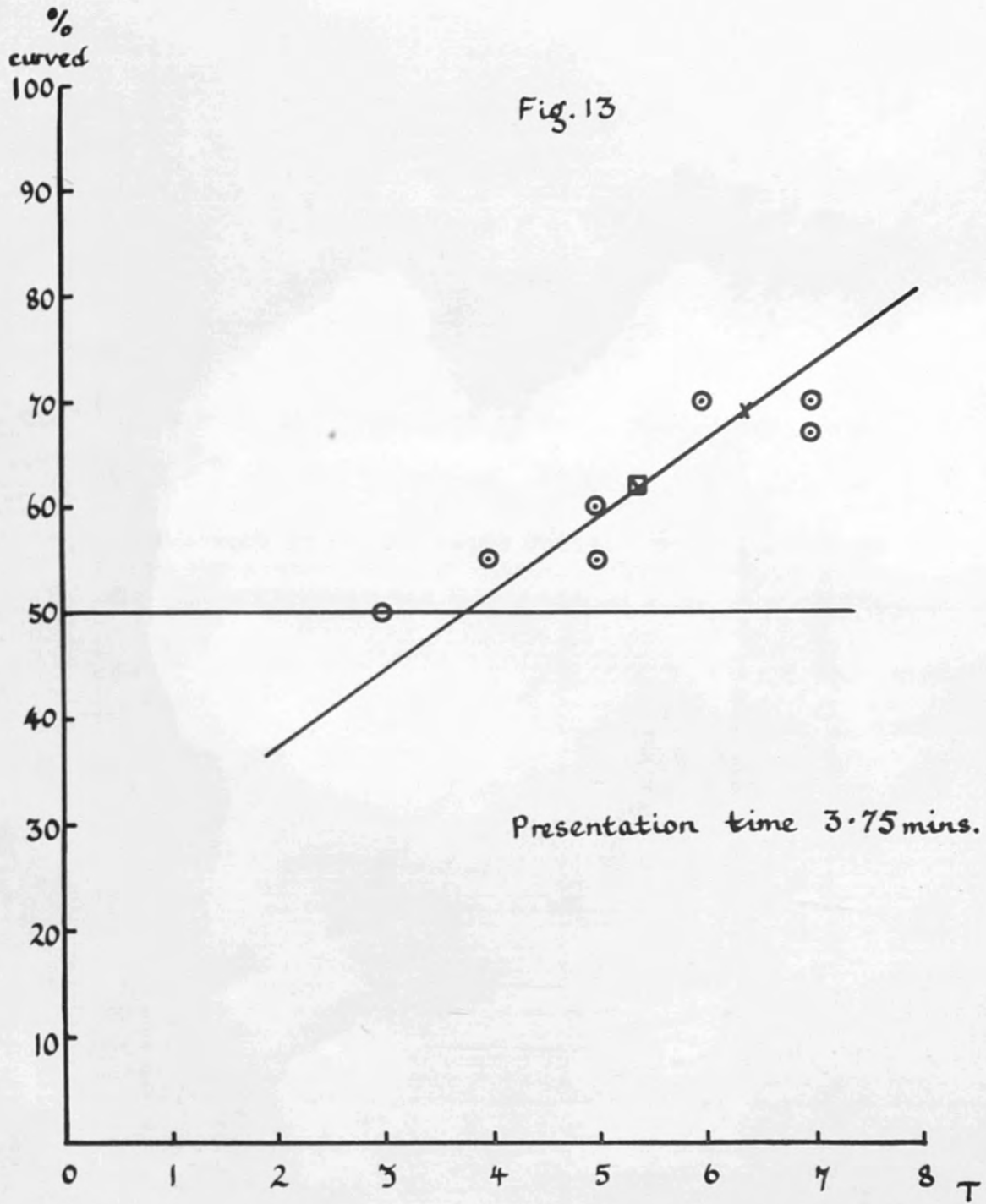


Fig. 14.

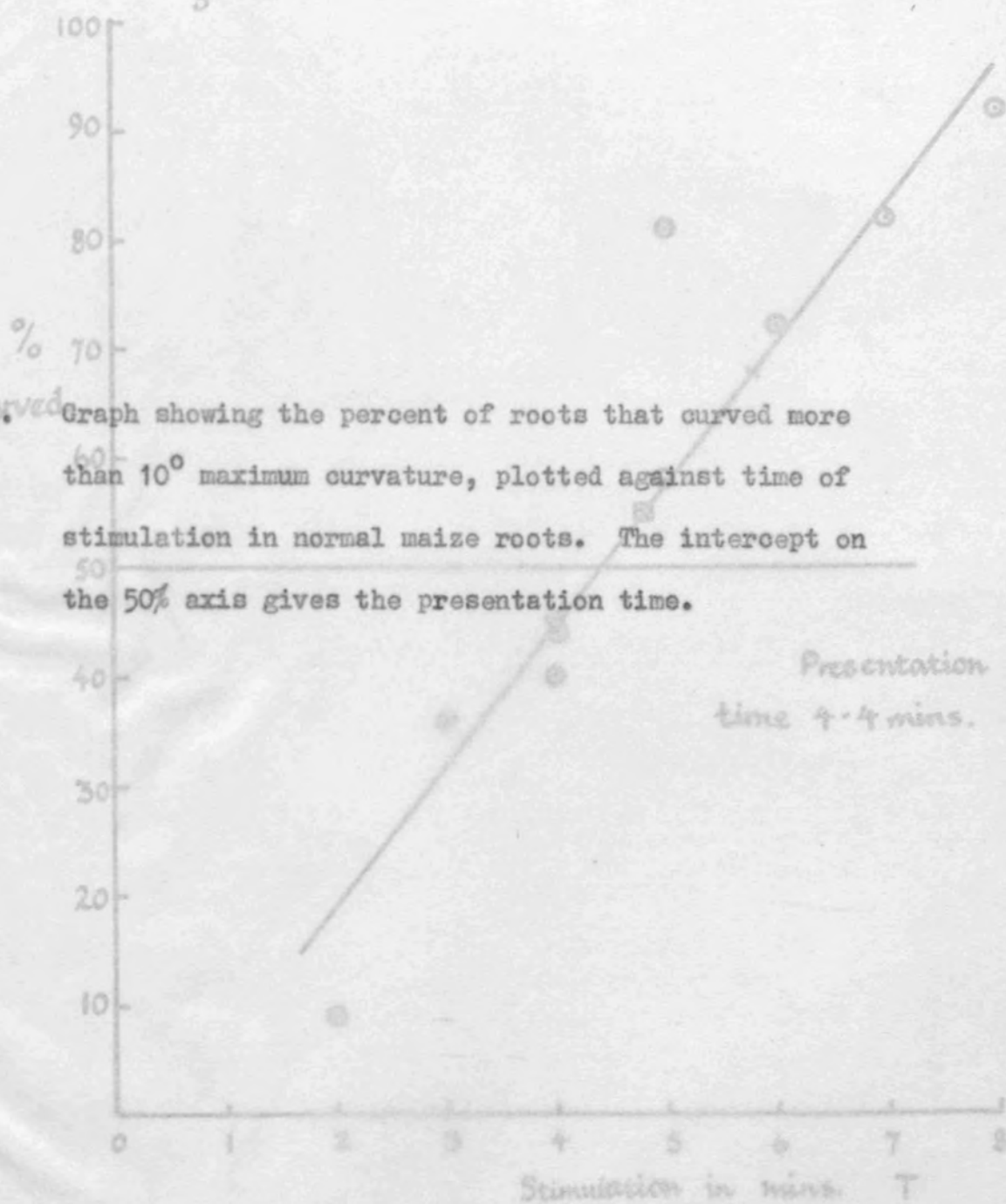


Fig. 14. ^{curved} Graph showing the percent of roots that curved more than 10° maximum curvature, plotted against time of stimulation in normal maize roots. The intercept on the 50% axis gives the presentation time.

Fig. 14.

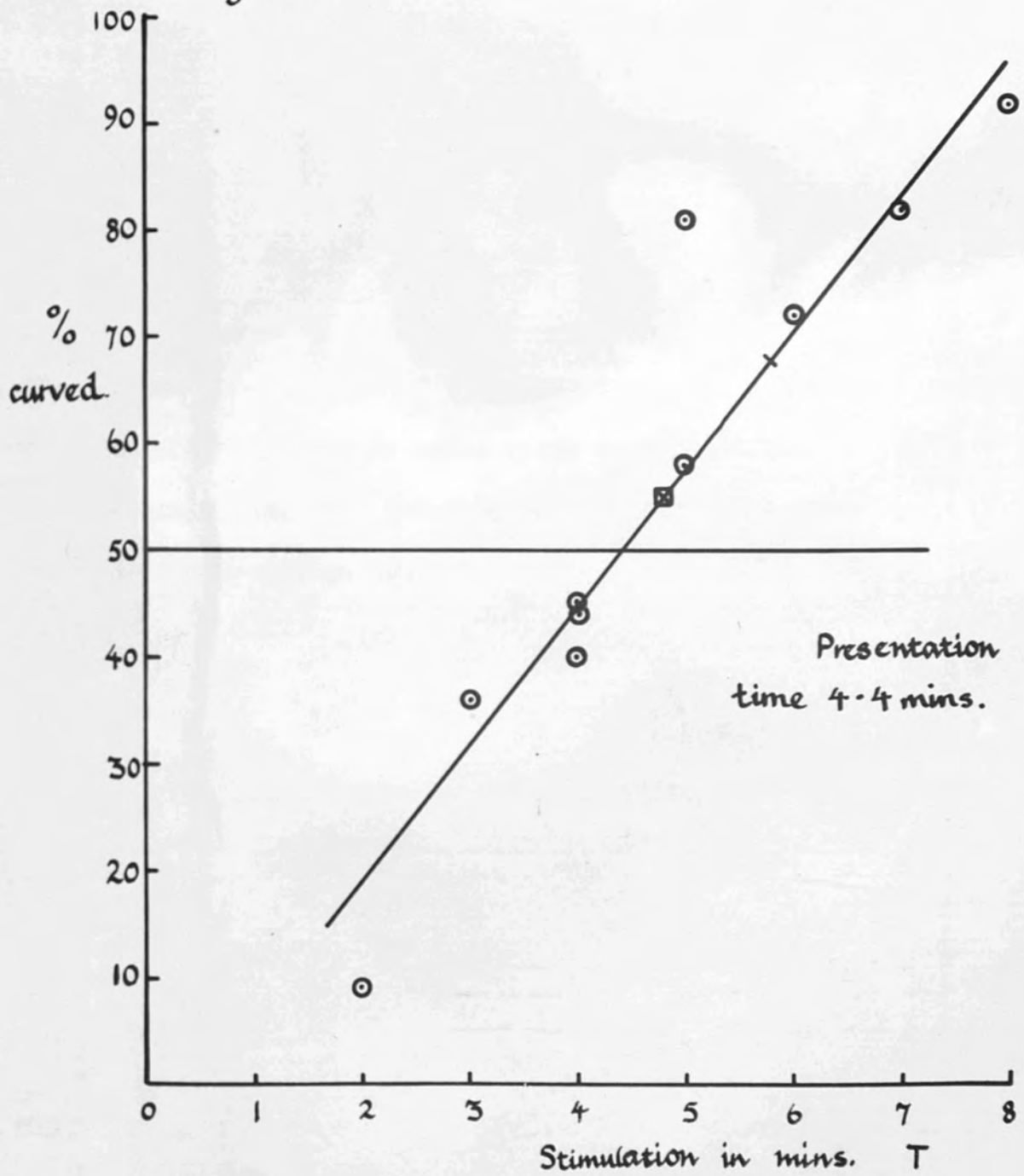


Fig. 15.
Rate of curvature

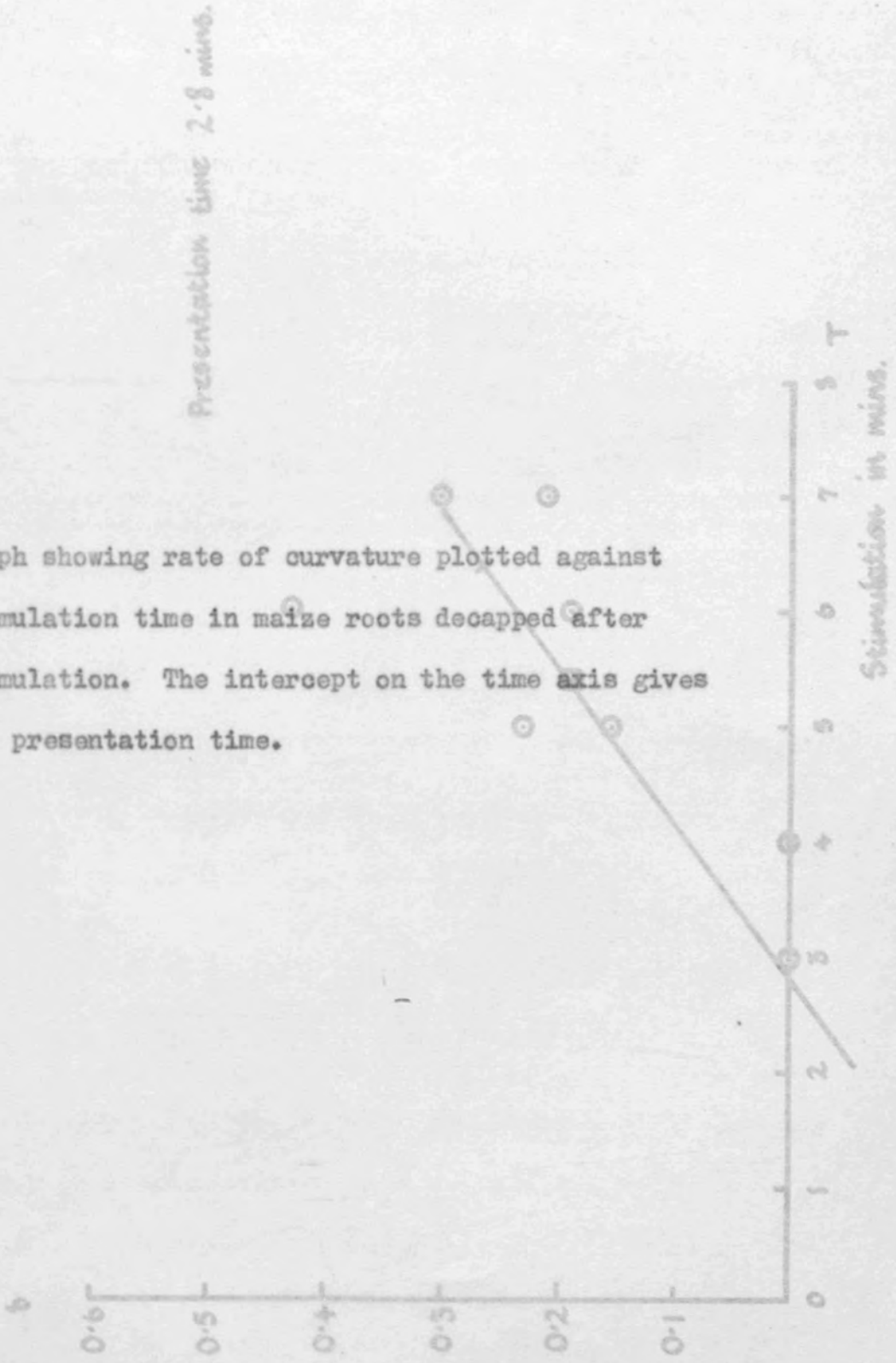
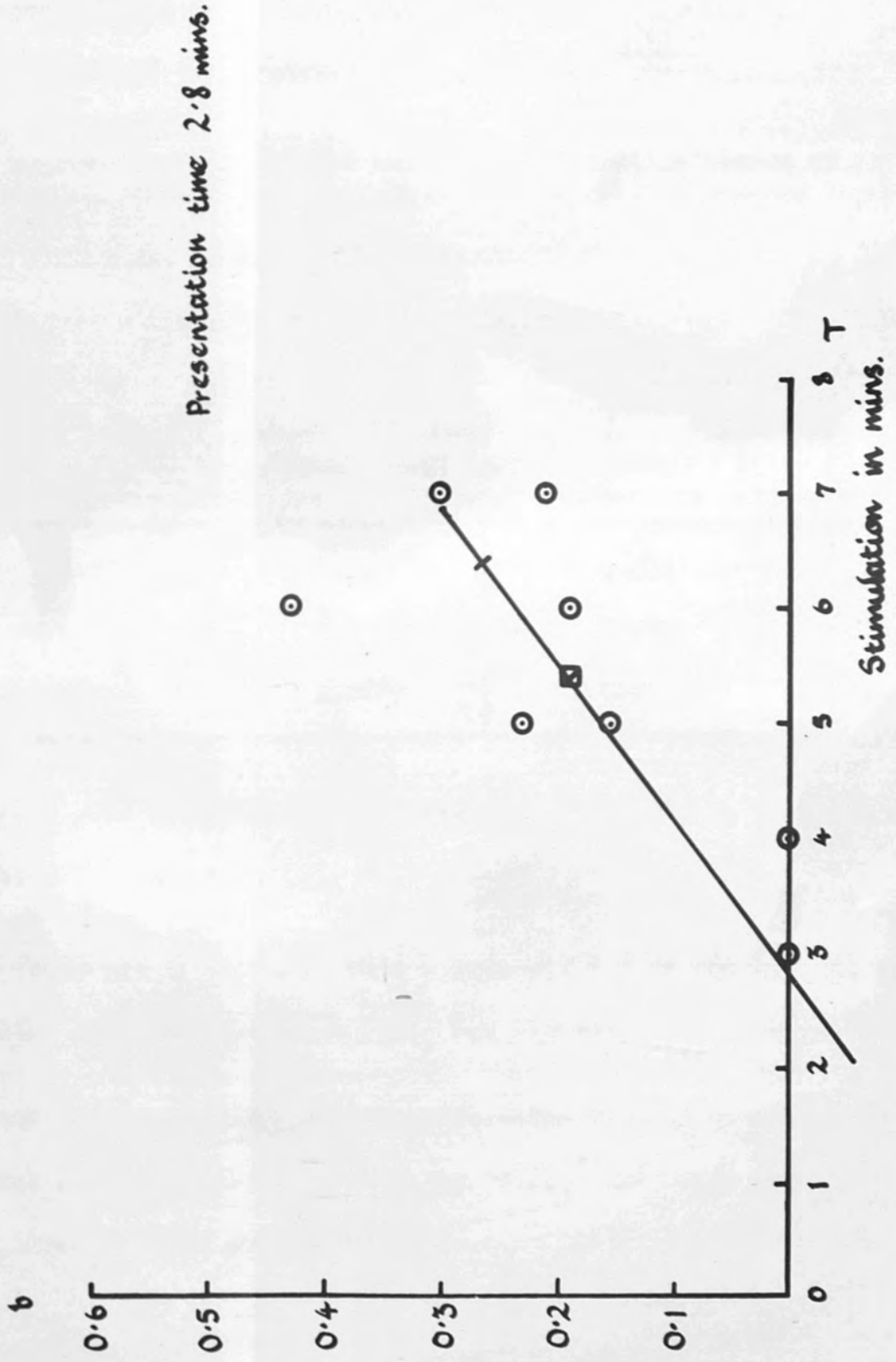


Fig. 15. Graph showing rate of curvature plotted against stimulation time in maize roots decapped after stimulation. The intercept on the time axis gives the presentation time.

Fig. 15.
Rate of curvature



The values are very close but nevertheless it was decided to test the two regressions, which were calculated on the basis of curvature rate, for significance of difference.

1.75 minutes and 4.4 minutes; the ones for roots decapped after stimulation are 2.8 minutes and 3.73 minutes. The latter values fall within the range of the former. From these results it can be reasonably concluded that the root cap acts only as a protective sheath. The stimulus must be transmitted from the cap to the tip within presentation time or at the end.

Table V

	Normal Roots	Roots decapped after stimulation
Regression coefficient b	0.11	0.077
Variance of b s^2	0.0092	0.0099
S.E. of b	0.0129	0.0133

The variances were examined for significance of difference by means of the variance ratio $F = s_1^2/s_2^2$.

They were found not to differ. Then a student's t test was applied to the two regression coefficients. $t = \frac{0.88}{\cancel{2.2}}$. Not significant at $P = 0.05$.

Then the 95% confidence limits of the intercepts of the regression lines on the time axis were calculated from the residual variances about the regression lines. These were as follows:

Normal roots 1.44 to 2.46 minutes.

Decapped roots 1.86 to 3.73 minutes.

These show considerable overlap.

From this it can be concluded that the presentation time values obtained for the two sets are not significantly different. This gets further support from the fact that the values for normal roots are 1.75 minutes and 4.4 minutes; the ones for roots decapped after stimulation are 2.8 minutes and 3.75 minutes. The latter values fall within the range of the former. From these results it can be reasonably concluded that the root cap acts only as a perception zone. The stimulus must be transmitted from the cap to the tip within presentation time or at the end.

It is not possible to judge from these experiments if the stimulus is transmitted from the cap in one parcel at the end of presentation time, or continuously throughout it.

We know, though, that sub-presentation time stimuli can be summed provided the lag between them does not exceed times ten of the stimulation time (see Fitting, 1905). This points to the fact that transmission of the stimulus might be continuous.

It is, of course, impossible to check this by the present experimental method because once the cap has been removed it cannot be replaced as a functional unit. Because of this it will be necessary to find a different way of measuring perception and/or transmission in order to follow it in greater detail. As explained in the next chapter, it might be possible to do this by means of electrophysiological measurements.

From the shape of the recovery curve (see Fig. 5), it looked as if the re-orientated before they recovered.

recovery was not a gradual, slow process but a somewhat abrupt one. This interpretation receives further support from the following data. In some experiments the roots were left to grow vertically after decapping and re-orientated to the horizontal position after various time intervals. Curvature development was followed and the recovery time calculated as before. In Table VII the results are presented in terms of the time that elapsed from re-orientation until curvature started to develop. This was obtained by subtraction of re-orientation time from the recovery time as given by the intercept of the regression lines as for the other recovery time experiments.

iv. Microscopy

Table VII

As explained before (see Materials and Methods), the light microscopy staining technique and the beginning of curvature investigation of the structural state of recovering root caps. Electron

Hours after decapping at re-orientation	Time from re-orientation to beginning of curvature	No. of roots
33	1.29 hours	5
34	1.29	5
32	1.29	5
30	1.29	5
30.66	1.33	5
30.16	1.33	5
29.91	1.30	5
29.33	1.89*	5

* The roots in this experiment might have been re-orientated before they recovered.

It seems from this table that the reaction time did not change, within the experimental period examined, after recovery has occurred. It might be that both presentation time and reaction time, or one of them only is longer than that in normal roots. To check this presentation time, studies at recovery time will have to be done. This is a very complicated task, and with the experimental techniques used here could be done only after recovery has been ascertained by means of curvature development. If it were possible to measure or correlate perception to electrical changes, it would offer a possibility of investigating the recovery process in greater detail.

iv. Microscopy

As explained before (see Materials and Methods), the light microscopy staining techniques that were tried were found unsatisfactory for the investigation of the structural state of recovering root caps. Electron microscopy in conjunction with phase-contrast studies of resin embedded material was chosen.

1. Normal and decapped roots

Fig. 16 shows a montage picture of a normal root cap of maize from a vertically growing root. The boundary wall between tip and root cap is very clearly recognizable, as is the distribution of starch grains in the cap cells. Fig. 17 shows the same root cap as seen under the phase contrast microscope.

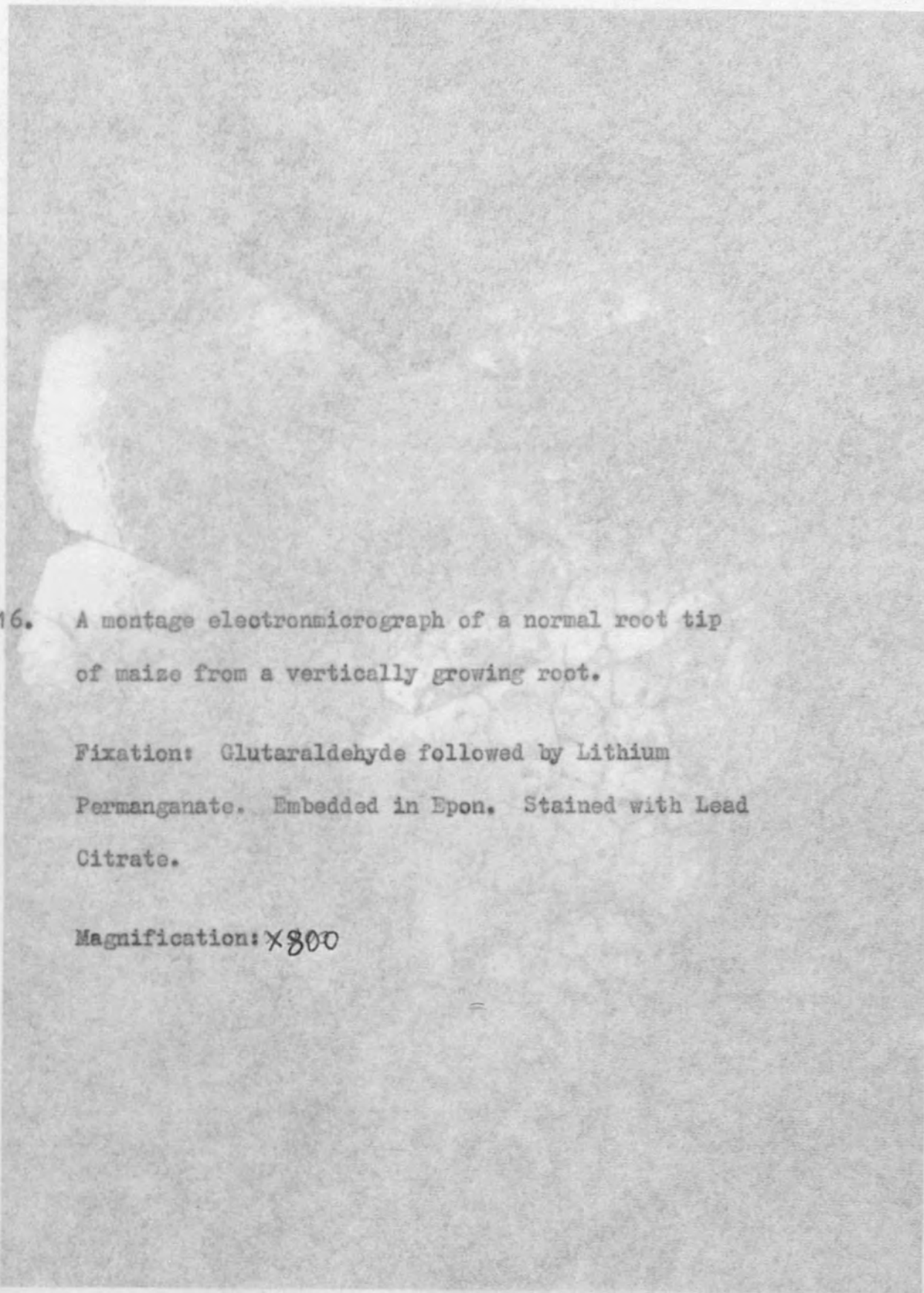
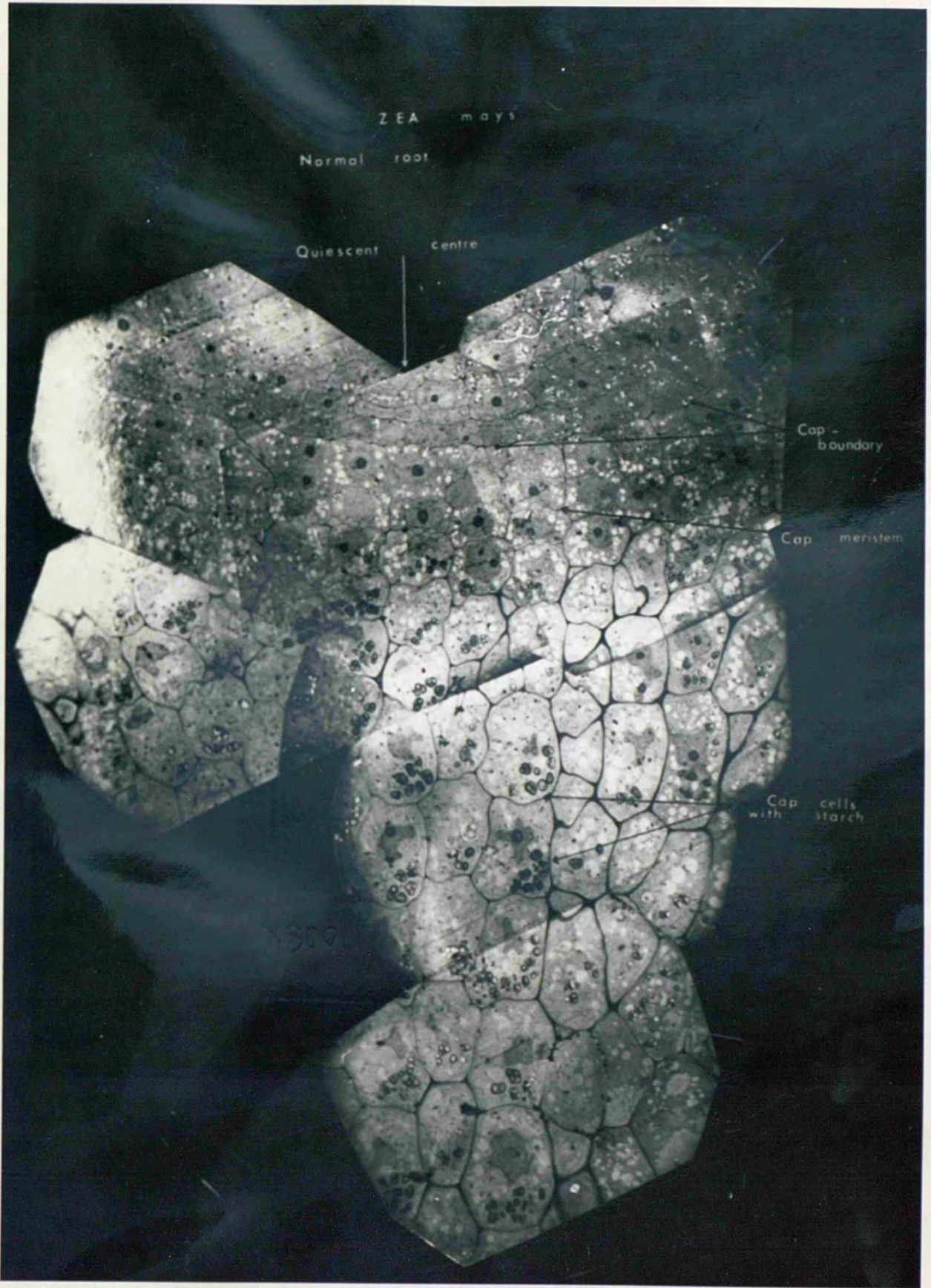


Fig. 16. A montage electronmicrograph of a normal root tip of maize from a vertically growing root.

Fixation: Glutaraldehyde followed by Lithium Permanganate. Embedded in Epon. Stained with Lead Citrate.

Magnification: X800

Fig. 16.



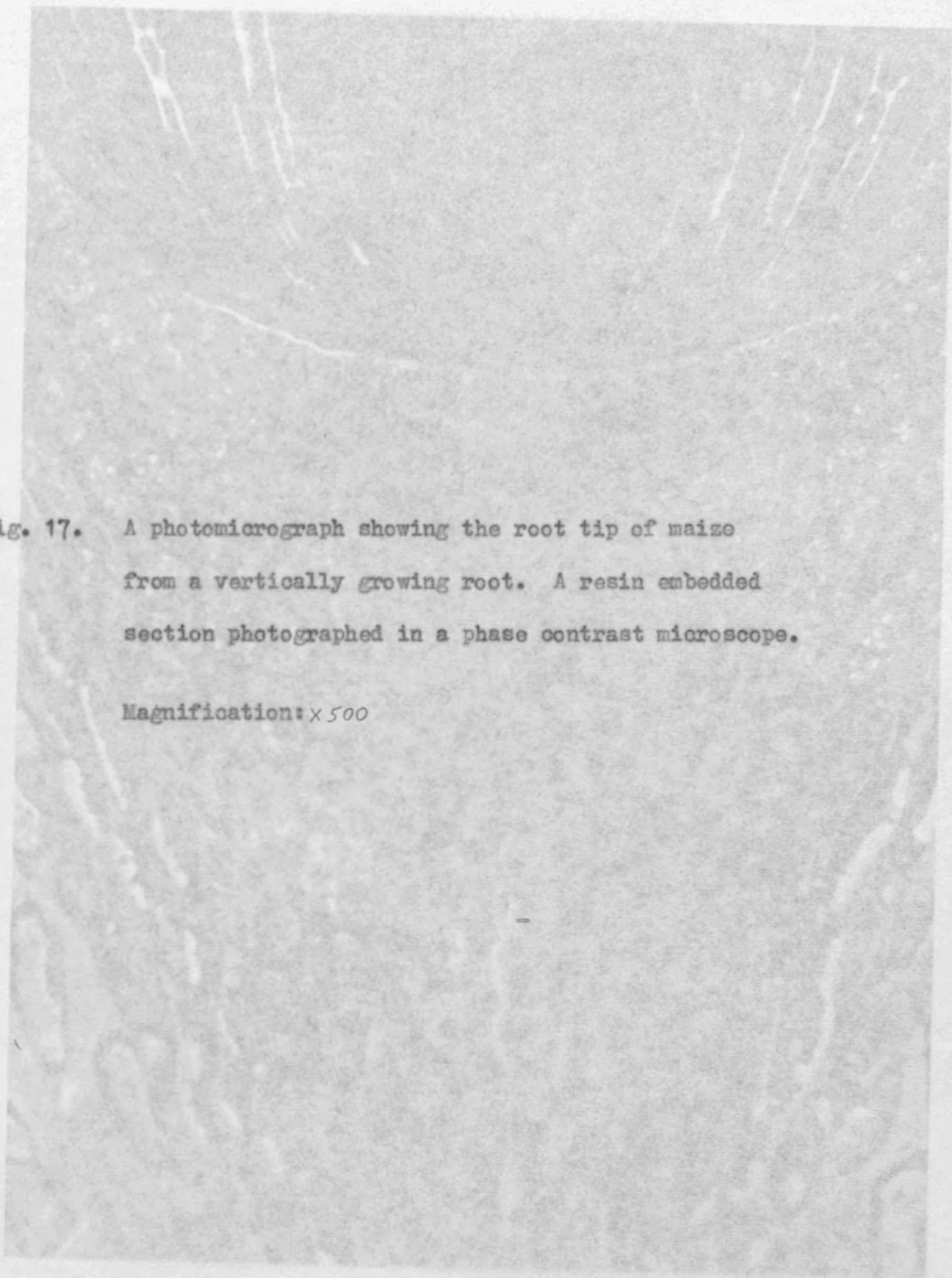


Fig. 17. A photomicrograph showing the root tip of maize from a vertically growing root. A resin embedded section photographed in a phase contrast microscope.

Magnification: $\times 500$

Fig. 17.

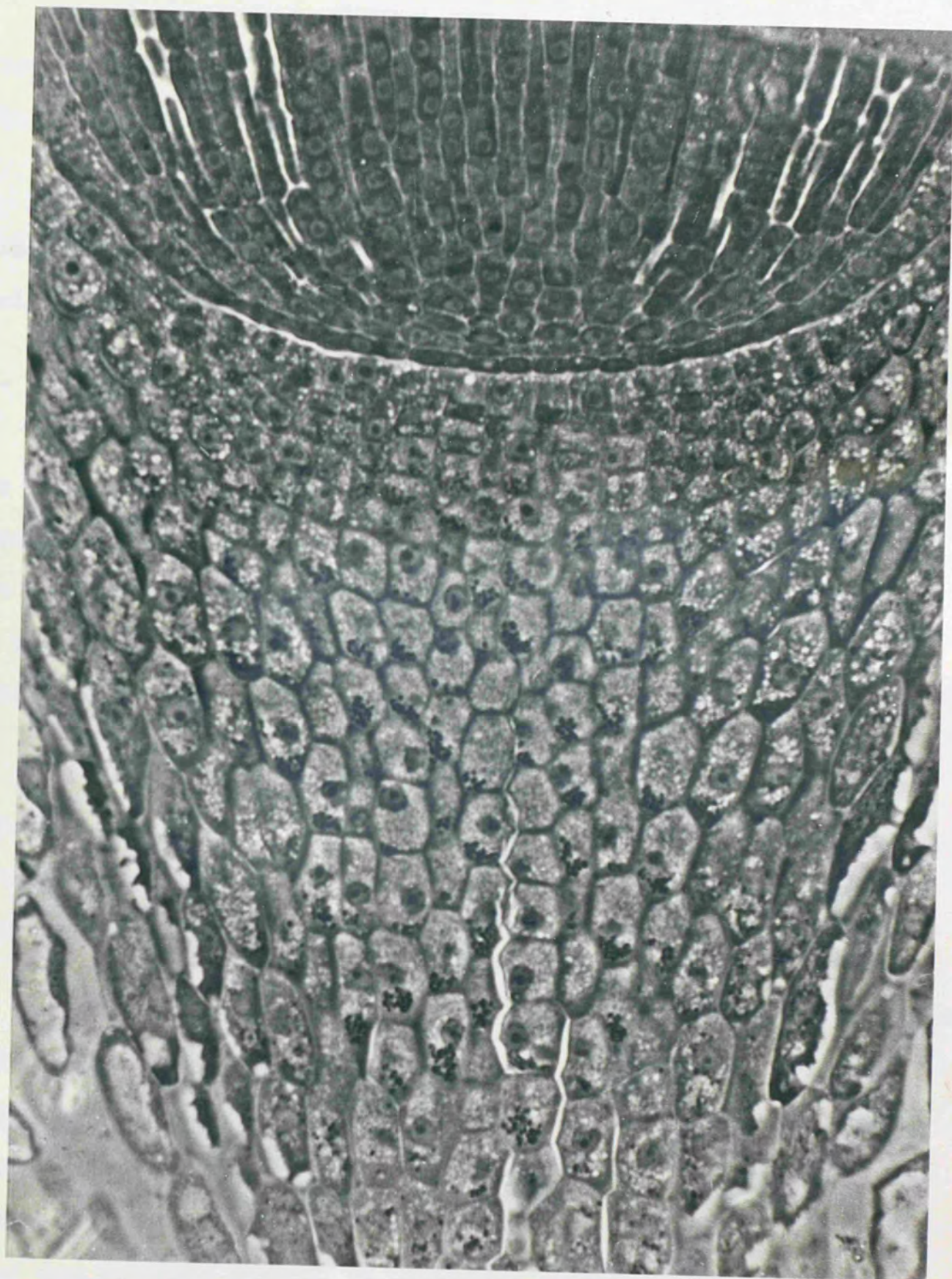


Fig. 18 is a montage electron micrograph of the root tip after decapping. There is no apparent damage to the tip and the break along the boundary wall is clean.

In higher magnification the plasmodesmata that were connecting the cap to the tip are shown protruding from the now exposed surface of the cell wall (see Fig. 19 and Fig. 20).

2. Recovering Roots

Roots after decapping were left to recover in a horizontal position so that it would be clear when they had recovered. When some of the roots had shown a positive curvature they were fixed for electron microscopy. At the same time other roots from the same group that had not shown yet a visible curvature at that time, There was always some variability within each sample. were fixed.

It was assumed that, if sedimenting starch was necessary for graviperception, a difference between these two groups might be detected on the sub-structural level. This difference, if observed, could indicate the requirements for perception.

It was further thought that it would be easier to detect sedimentation of starch at the bottom of cells than on lateral walls, because it is always easier to determine the longitudinal axis of the roots in a thin section. Therefore the recovered roots, and the ones from the same sample which had not shown curvature, were put in a vertical position for 10 minutes and then fixed and embedded as described.

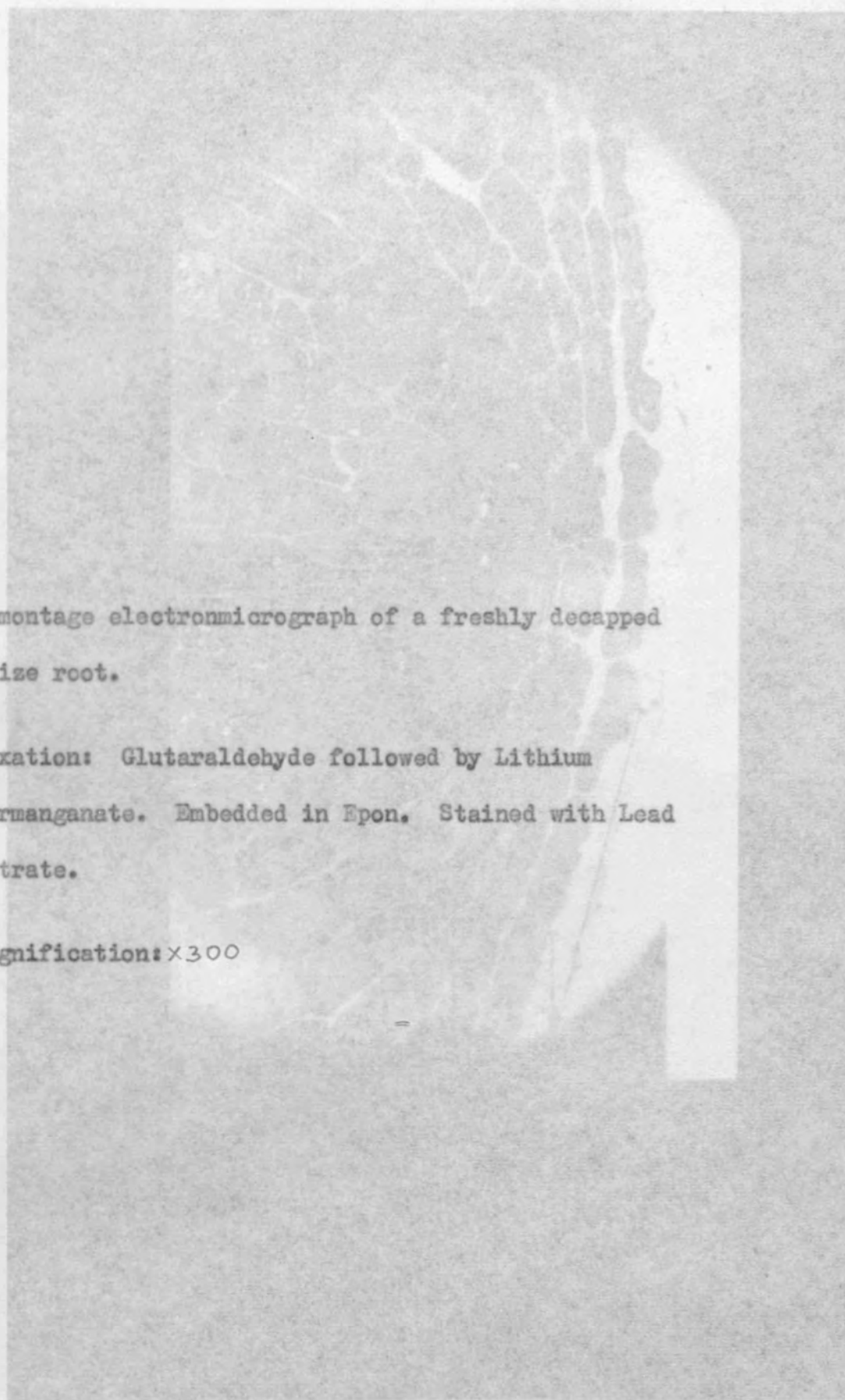
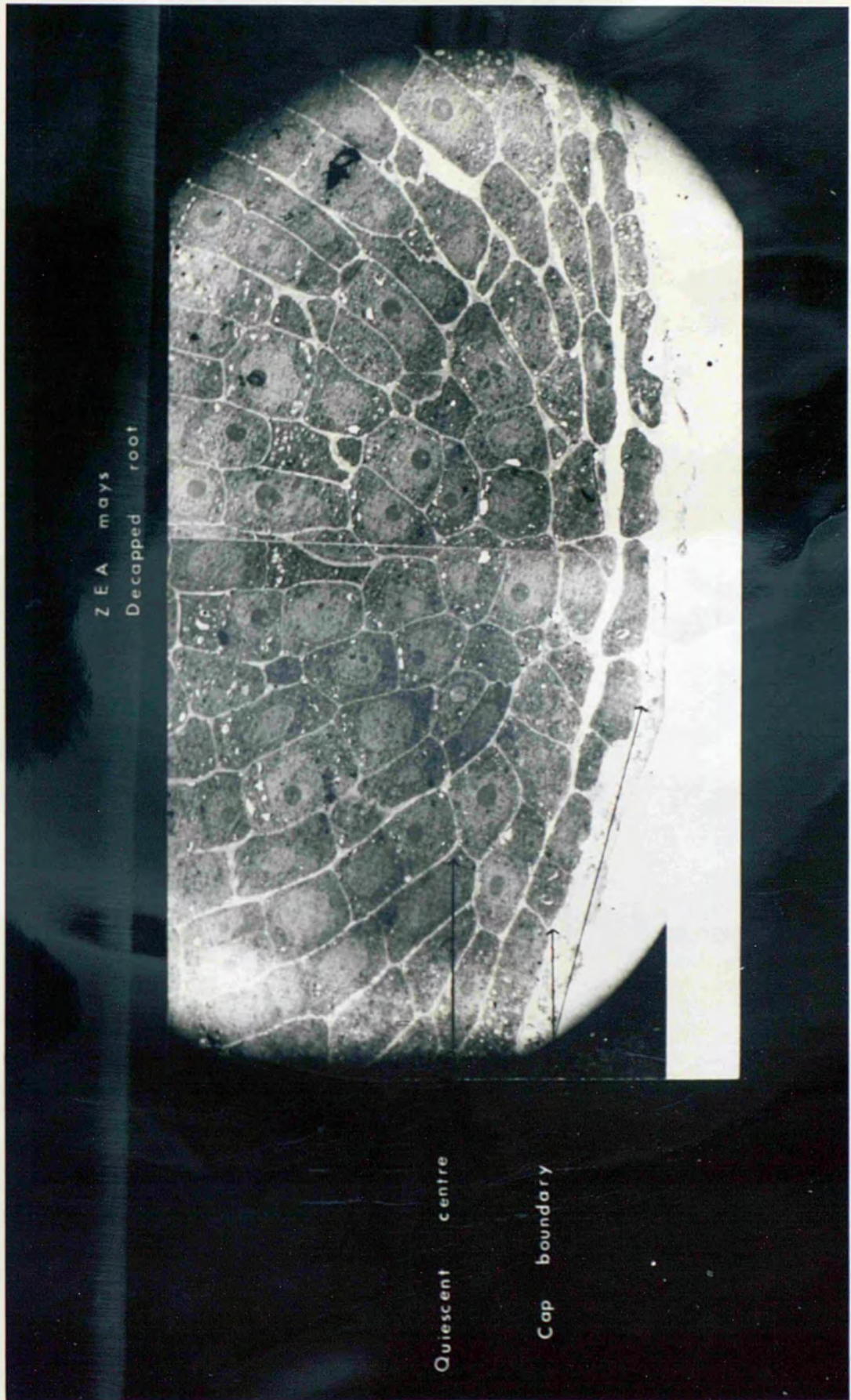


Fig. 18. A montage electronmicrograph of a freshly decapped maize root.

Fixation: Glutaraldehyde followed by Lithium Permanganate. Embedded in Epon. Stained with Lead Citrate.

Magnification: X300

Fig. 18



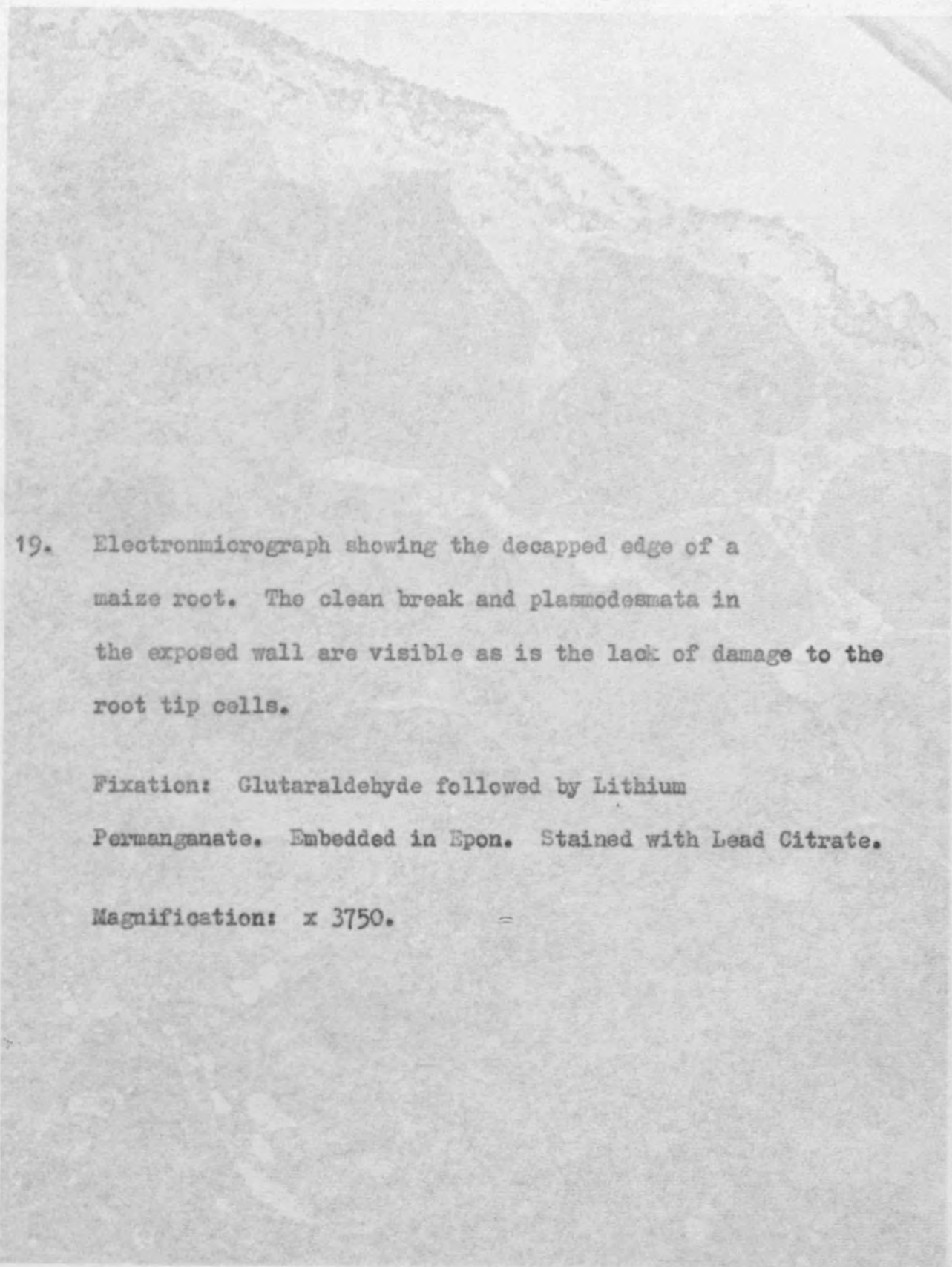
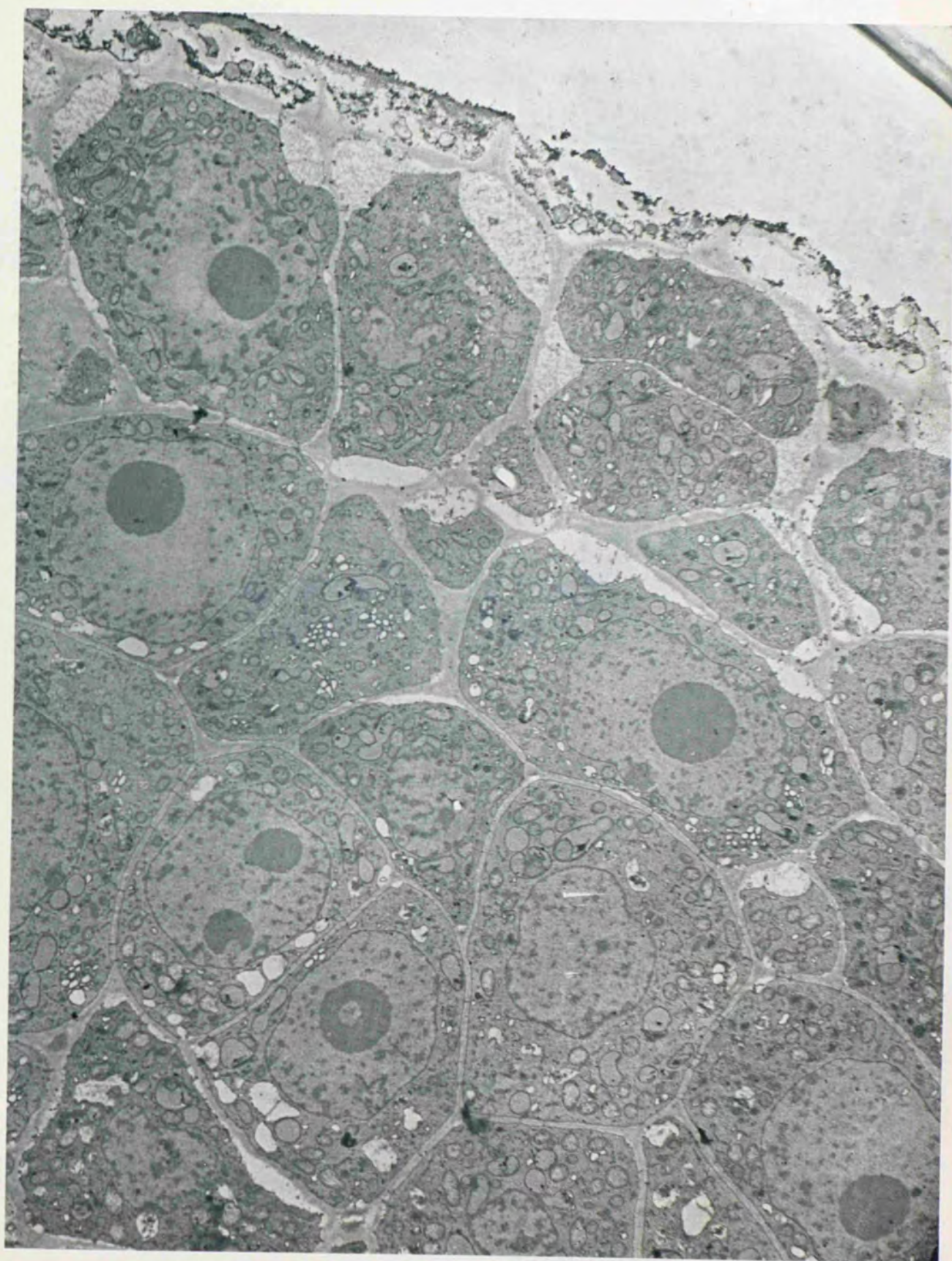


Fig. 19. Electronmicrograph showing the decapped edge of a maize root. The clean break and plasmodesmata in the exposed wall are visible as is the lack of damage to the root tip cells.

Fixation: Glutaraldehyde followed by Lithium Permanganate. Embedded in Epon. Stained with Lead Citrate.

Magnification: x 3750.

Fig. 19




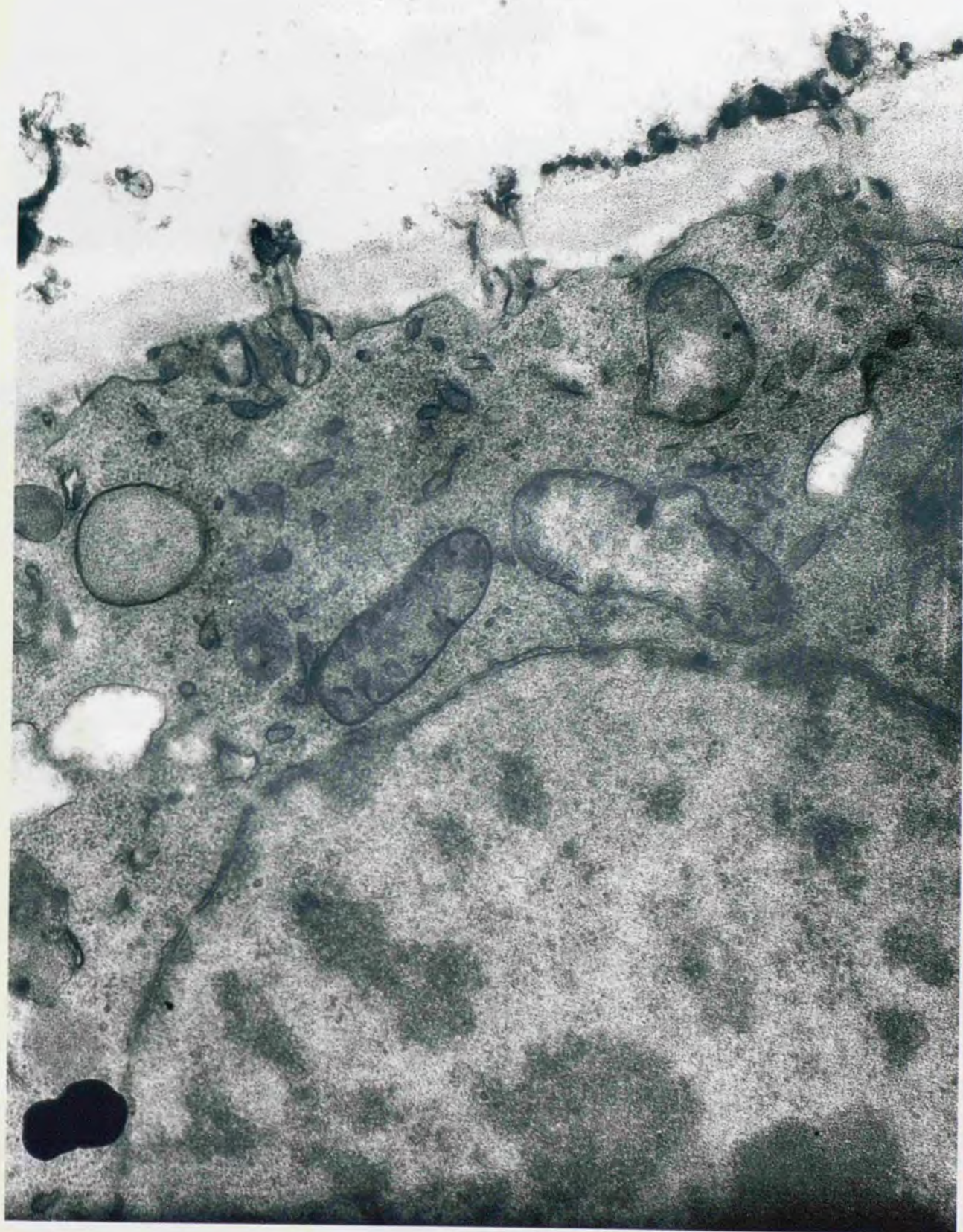


Fig. 20. Electronmicrograph showing edge of cell from the tip of a freshly decapped maize root. The severed plasmodesmata in the wall which is now exposed are still evident. There is no apparent damage to the cell otherwise.

Fixation: Glutaraldehyde followed by Lithium Permanganate. Embedded in Epon. Stained with Lead Citrate.

Magnification: x 16500.



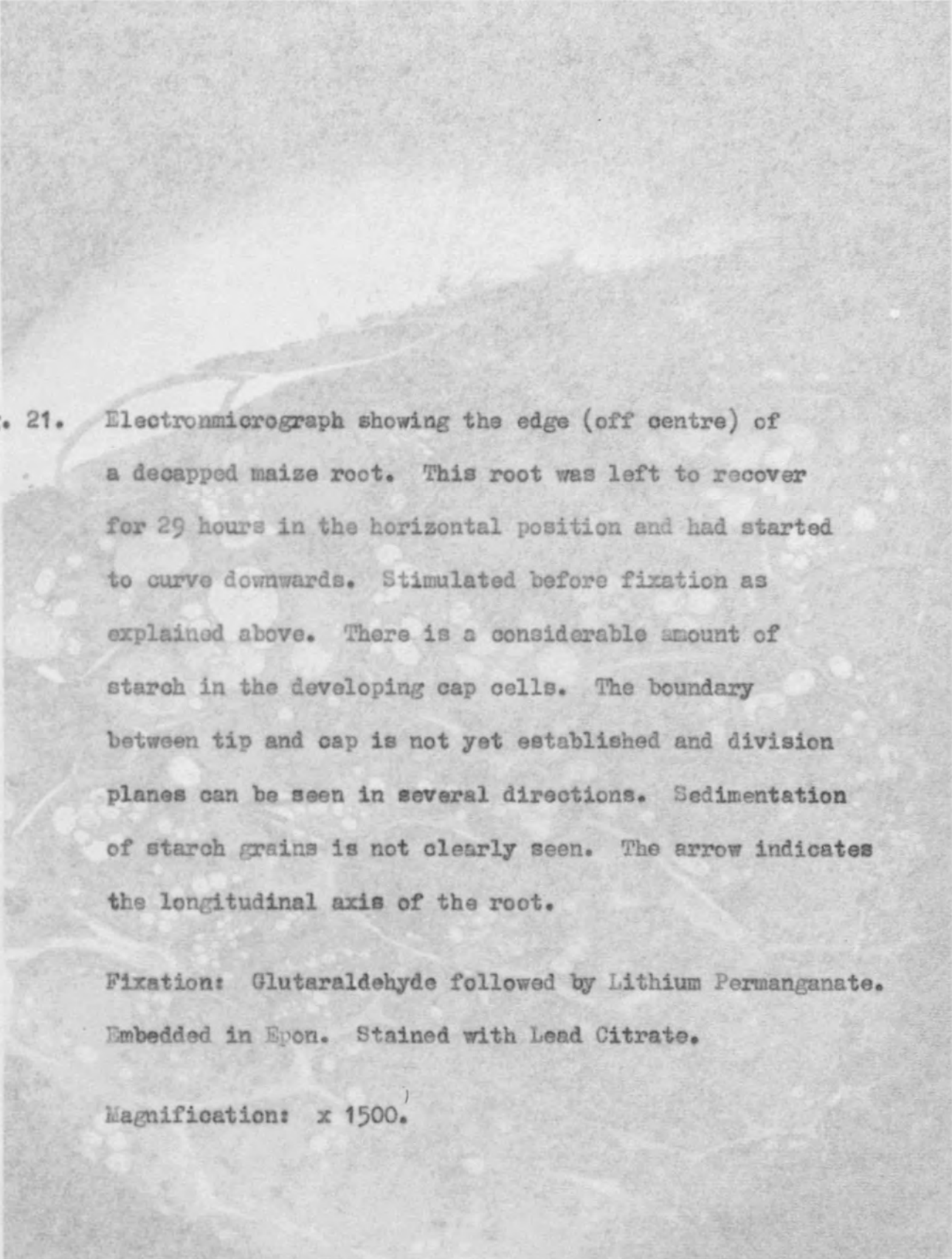
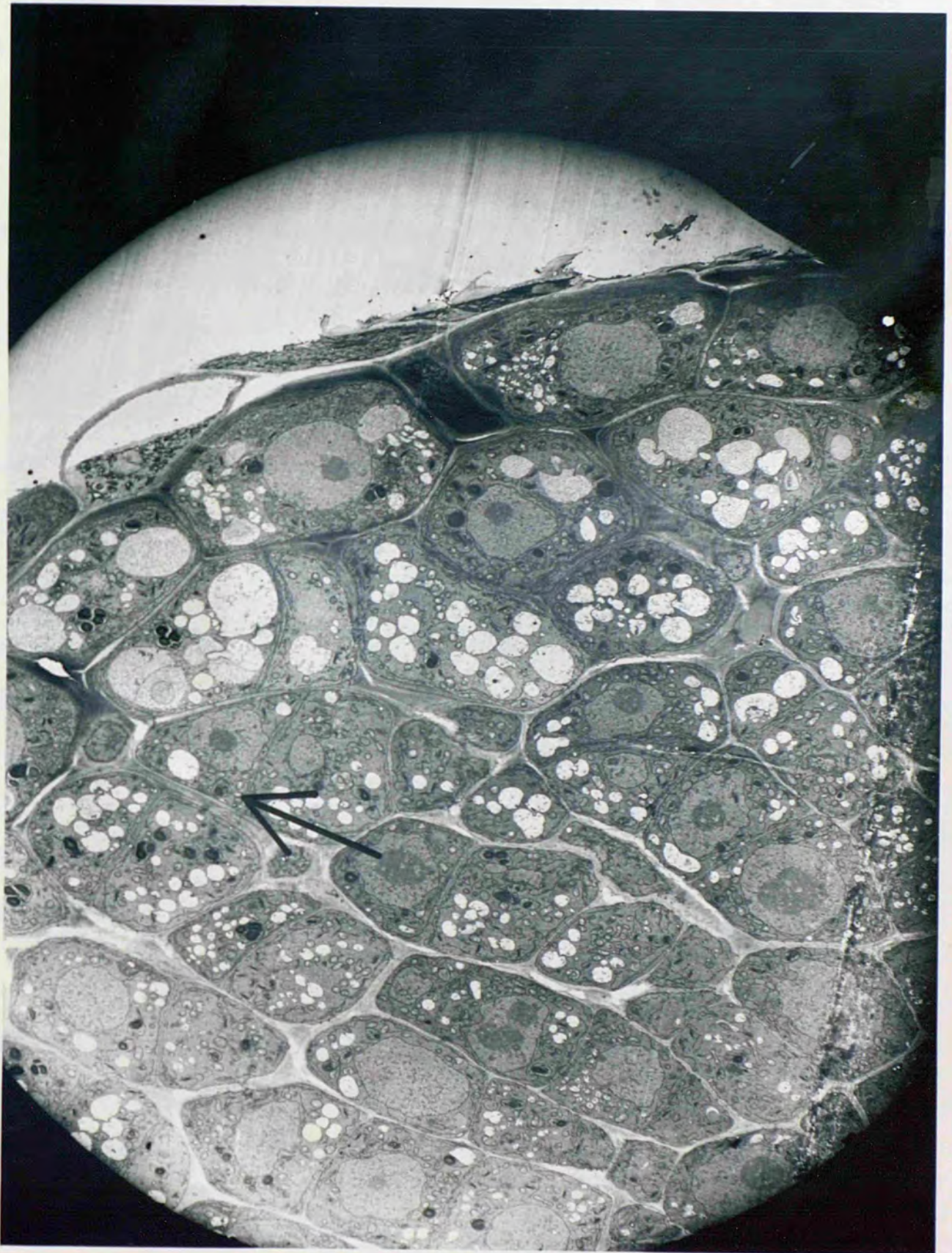


Fig. 21. Electronmicrograph showing the edge (off centre) of a decapped maize root. This root was left to recover for 29 hours in the horizontal position and had started to curve downwards. Stimulated before fixation as explained above. There is a considerable amount of starch in the developing cap cells. The boundary between tip and cap is not yet established and division planes can be seen in several directions. Sedimentation of starch grains is not clearly seen. The arrow indicates the longitudinal axis of the root.

Fixation: Gluteraldehyde followed by Lithium Permanganate.
Embedded in Epon. Stained with Lead Citrate.

Magnification: x 1500.



1 μ sections were cut and examined under a phase contrast microscope but it was very difficult to decide whether starch was present or not, and if it was redistributed.

When thin sections were examined in the electron microscope, the presence of starch in appreciable quantities was evident in both recovered roots and the ones that had not shown curvature when fixed (see Fig. 21 and Fig. 22).

Fig. The plastids containing starch seem, on the whole, to contain less starch than ones from normal roots (Fig. 23 and Fig. 24).

There is also a higher occurrence of vesicles and lamellae within the recovering amyloplasts, which might be interpreted as an indication of synthetic activity which is still continuing in these plastids. The starch does not show a clear sedimentation under the applied vertical stimulation as compared with normal roots grown vertically. A detailed statistical analysis was not done and therefore the accurate assessment of the distribution pattern is not yet possible. It is evident, though, that the situation in the recovered root is more complex than was at first estimated.

The fact that a clear sedimentation is not observed in the recovered roots might be due to a combination of any or all of the following factors.

- 1) A variability in size, and therefore in mass, among the amyloplasts is quite evident.
- 2) The arrangement, in three dimensional terms, of the cells in the recovering and rapidly dividing root, is much more complex than

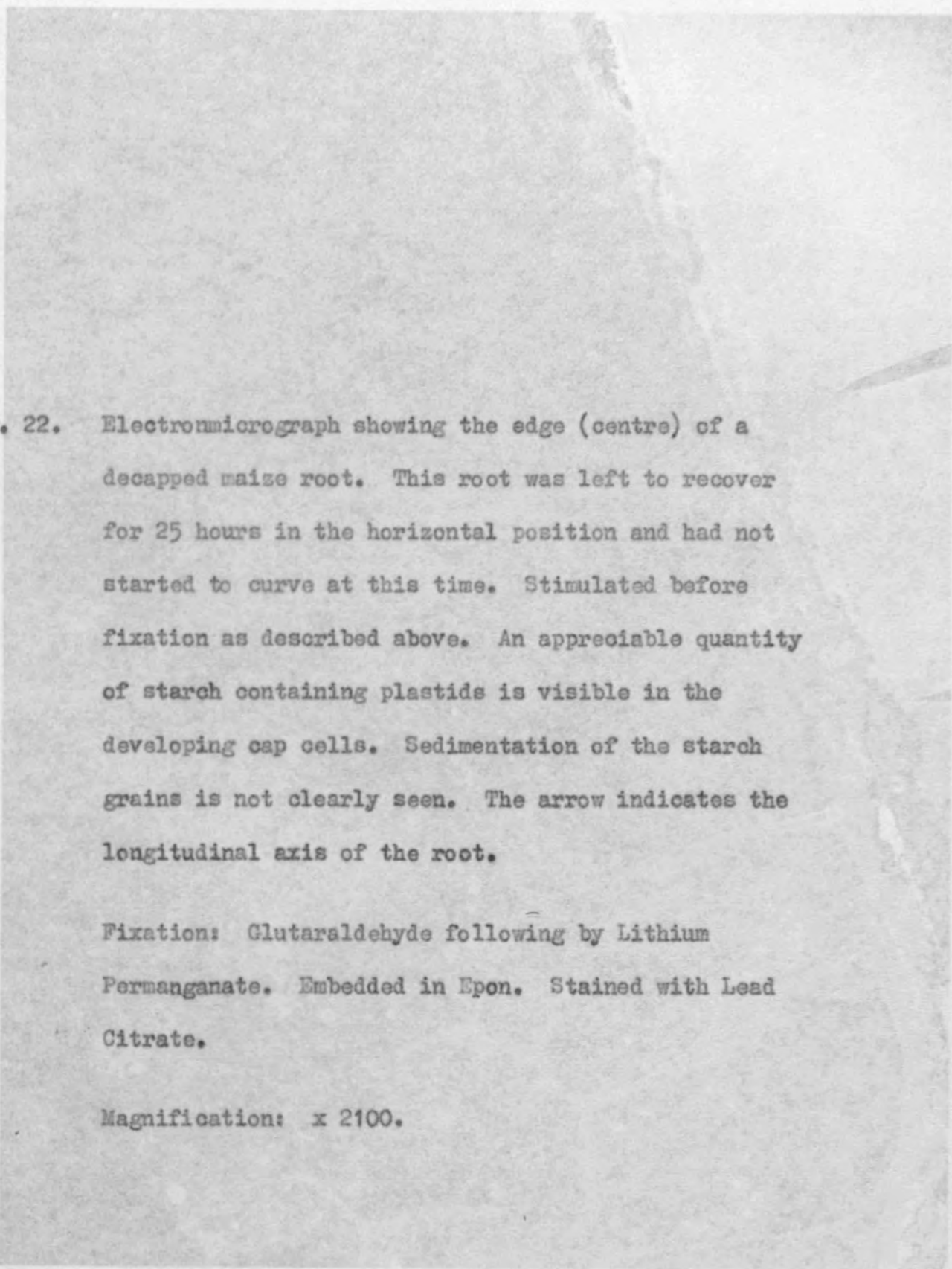


Fig. 22. Electronmicrograph showing the edge (centre) of a decapped maize root. This root was left to recover for 25 hours in the horizontal position and had not started to curve at this time. Stimulated before fixation as described above. An appreciable quantity of starch containing plastids is visible in the developing cap cells. Sedimentation of the starch grains is not clearly seen. The arrow indicates the longitudinal axis of the root.

Fixation: Glutaraldehyde following by Lithium Permanganate. Embedded in Epon. Stained with Lead Citrate.

Magnification: x 2100.

Fig. 22



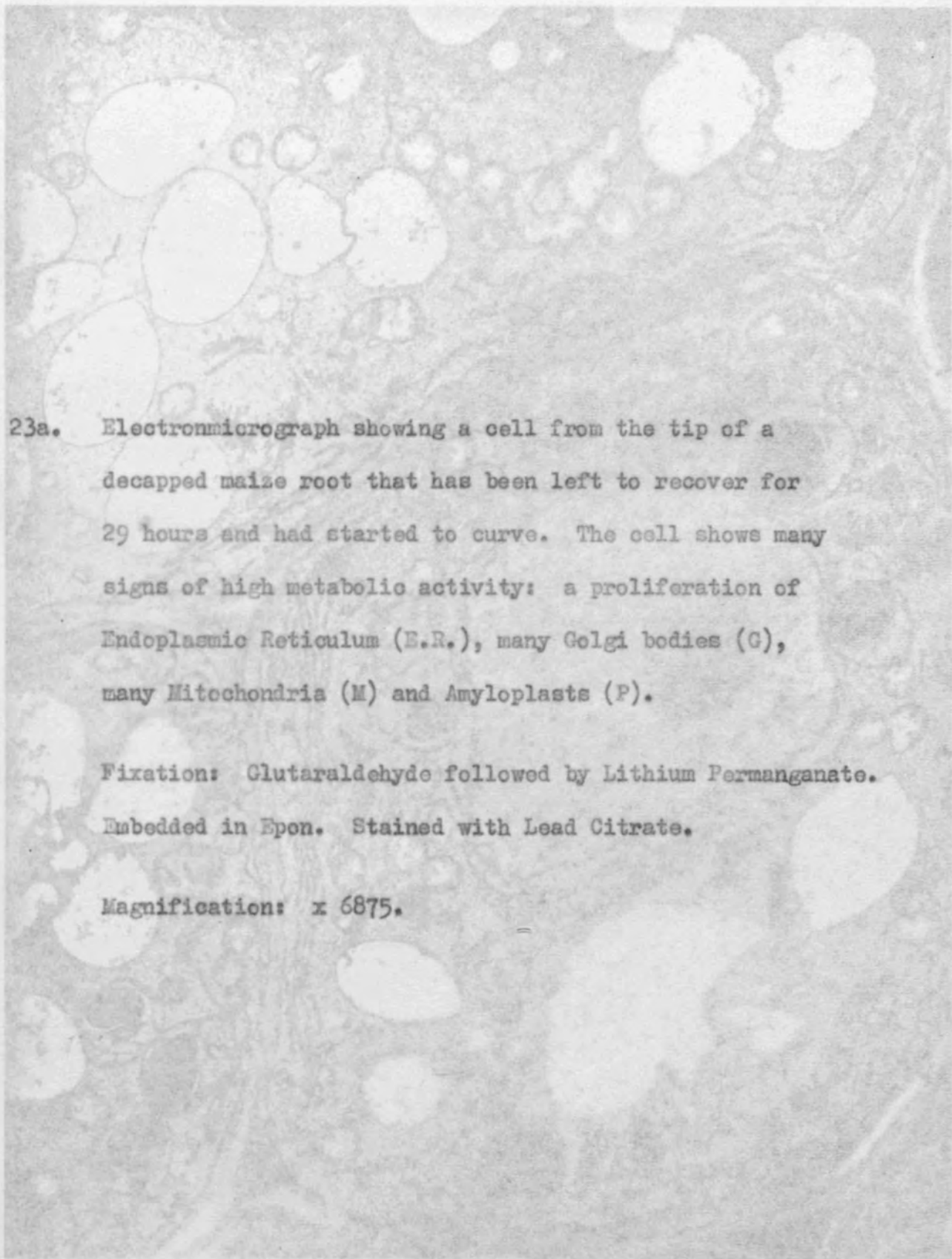


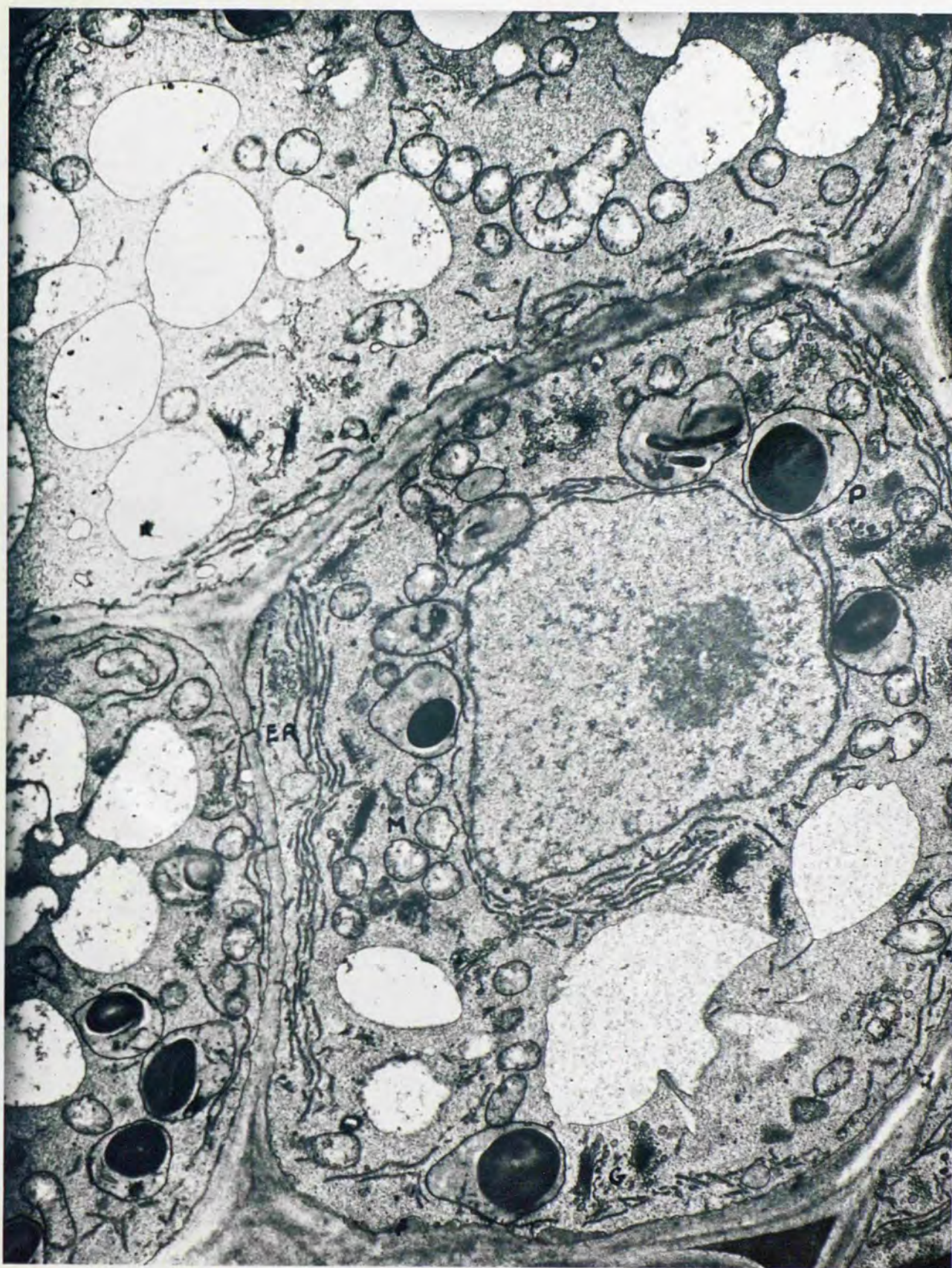
Fig. 23a. Electronmicrograph showing a cell from the tip of a decapped maize root that has been left to recover for 29 hours and had started to curve. The cell shows many signs of high metabolic activity: a proliferation of Endoplasmic Reticulum (E.R.), many Golgi bodies (G), many Mitochondria (M) and Amyloplasts (P).

Fixation: Glutaraldehyde followed by Lithium Permanganate.

Embedded in Epon. Stained with Lead Citrate.

Magnification: x 6875.

Fig. 23a.



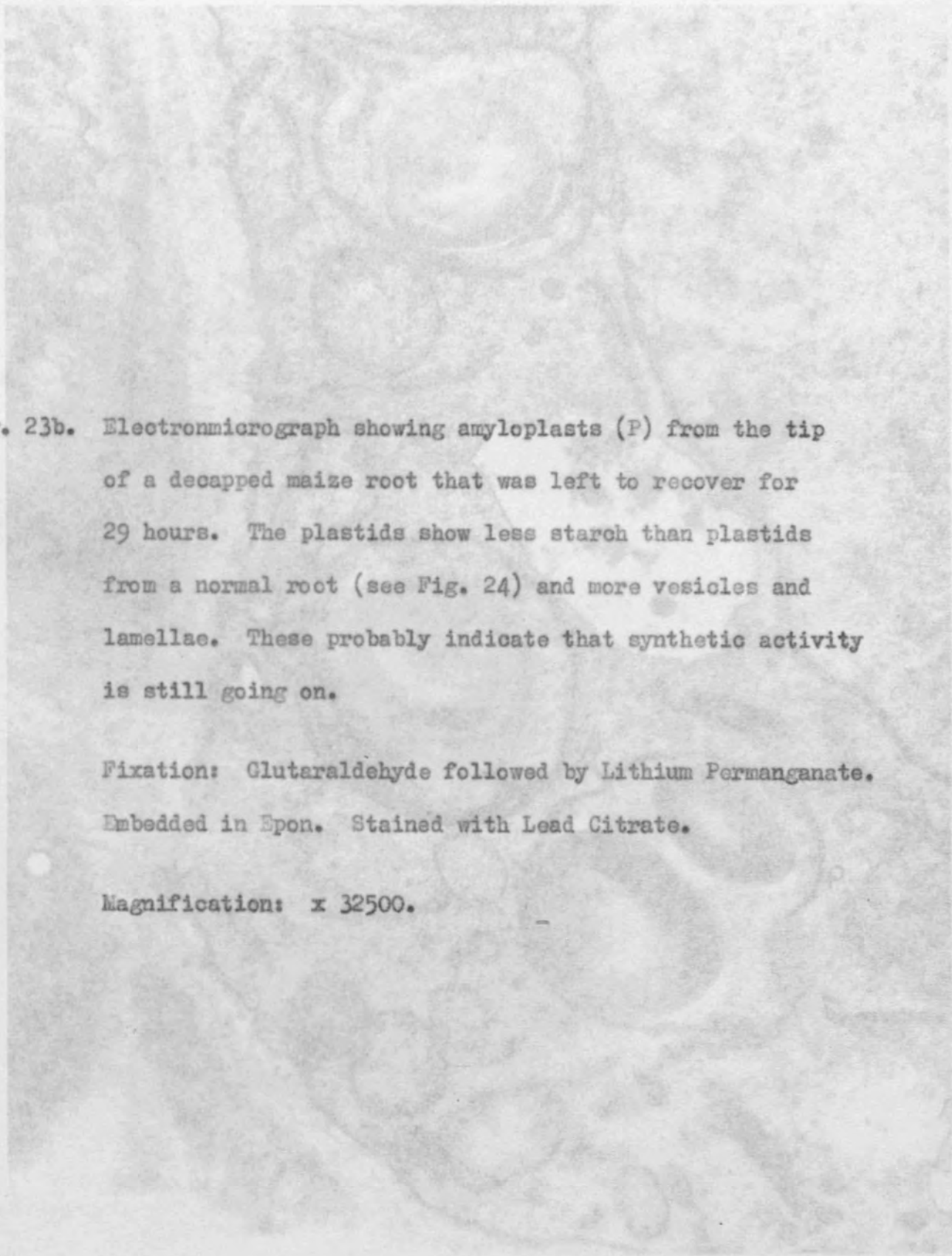
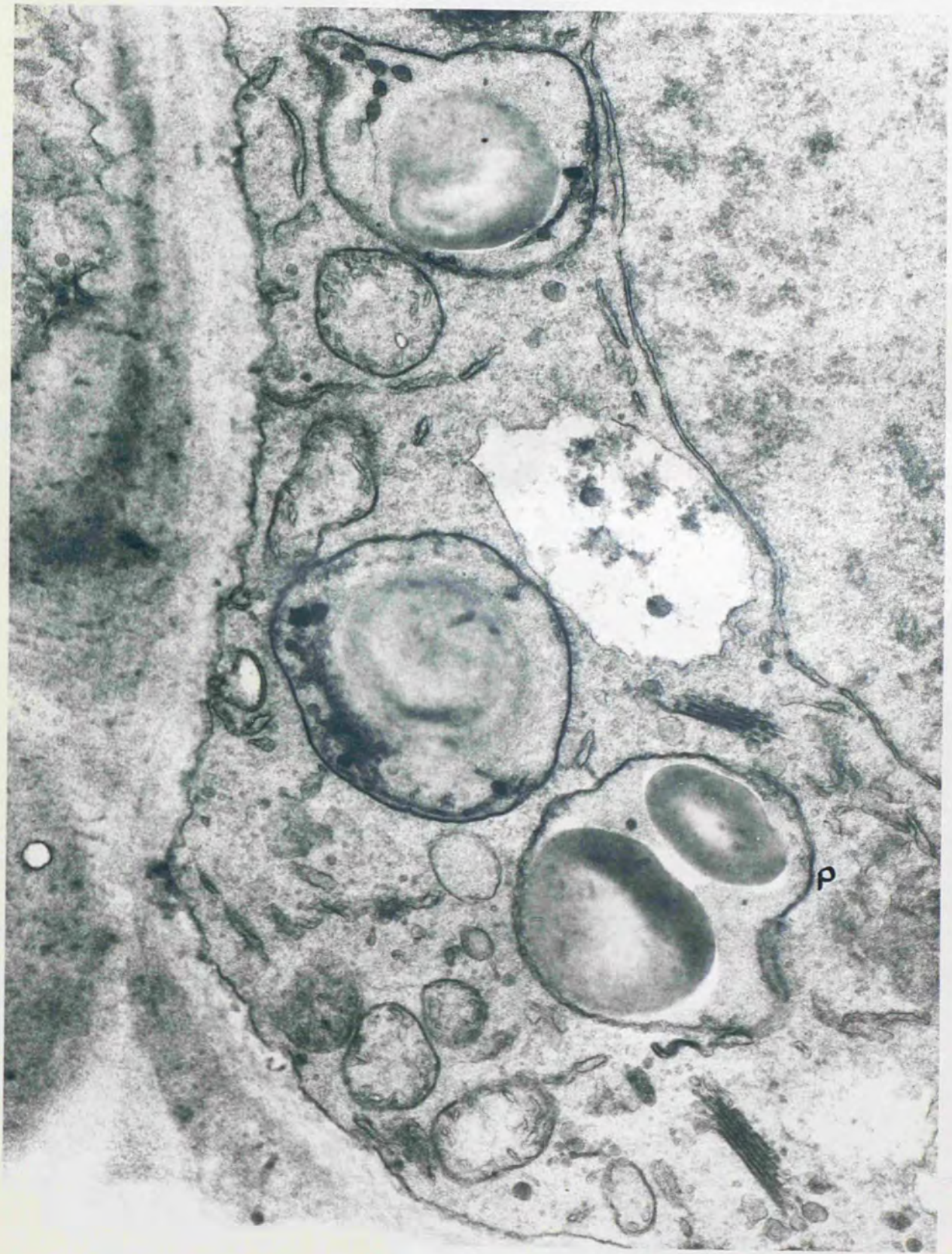
The image is a large, rectangular electron micrograph showing several amyloplasts, labeled with the letter 'P'. These organelles are roughly circular or oval in shape and contain internal structures such as vesicles and lamellae. The background is a light, grainy texture, typical of an electron micrograph. The amyloplasts are distributed across the field of view, with some appearing more prominent than others.

Fig. 23b. Electronmicrograph showing amyloplasts (P) from the tip of a decapped maize root that was left to recover for 29 hours. The plastids show less starch than plastids from a normal root (see Fig. 24) and more vesicles and lamellae. These probably indicate that synthetic activity is still going on.

Fixation: Glutaraldehyde followed by Lithium Permanganate.
Embedded in Epon. Stained with Lead Citrate.

Magnification: x 32500.

Fig. 236



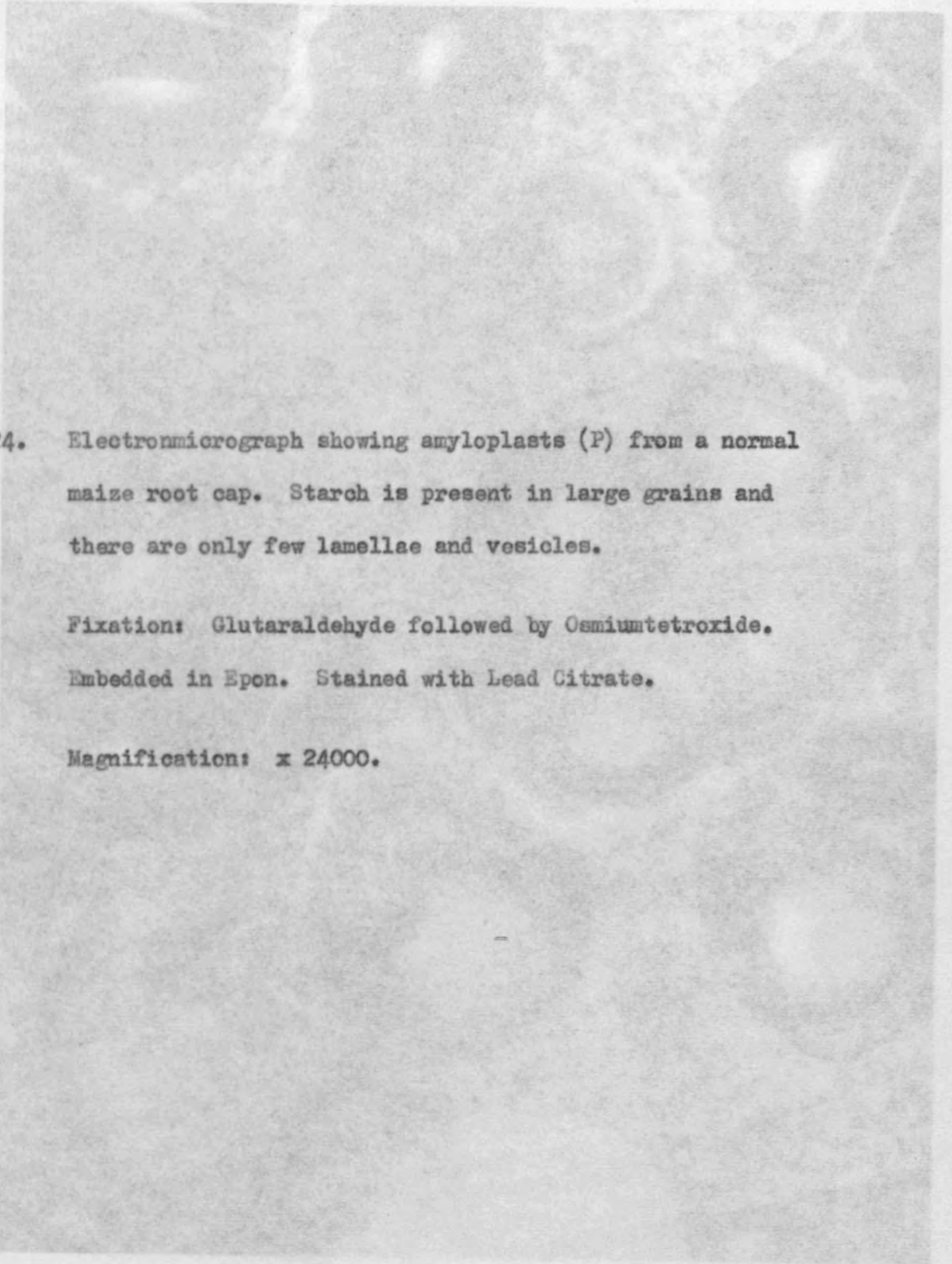
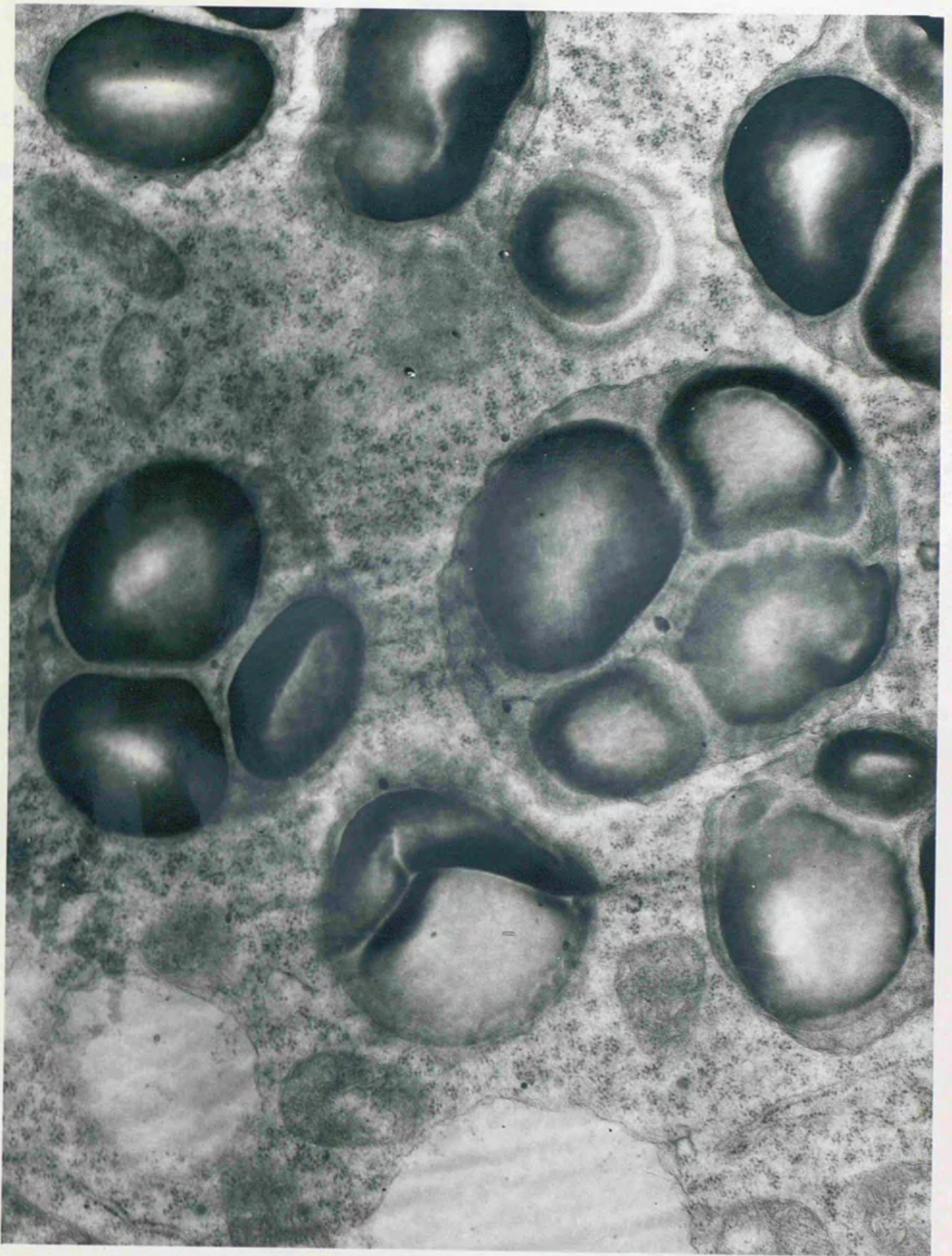


Fig. 24. Electronmicrograph showing amyloplasts (P) from a normal maize root cap. Starch is present in large grains and there are only few lamellae and vesicles.

Fixation: Glutaraldehyde followed by Osmiumtetroxide.

Embedded in Epon. Stained with Lead Citrate.

Magnification: x 24000.



the columns seen in the normal root. The planes of division seem to be less rigidly controlled in these circumstances. 3) Presentation time might be longer in these roots than the 10 minutes given as a vertical stimulation.

From all this it is clear that a careful and detailed investigation of the distribution of starch grains under gravity in recovering roots is needed to decide if they act as statoliths.

By removing the root cap growth is not affected and yet the root stops reacting to gravitational stimuli. There are small changes in the angle between the root tip and the main axis of the root but these changes fluctuate apparently in a random fashion (see Johanson, 1965).

The fact that growth is not affected seems to point immediately to the possibility of the root cap having no growth regulating role and therefore being mainly concerned with perception.

Further studies confirmed this as is seen from the presentation time studies. The root cap seems to be necessary only for the perception and can be removed after the presentation time without influencing the subsequent response.

Another important feature is that the stimulus is transmitted within the presentation time period. As the stimulus will not pass from the cap after it has been covered from the tip and replaced, it is probable that it is not a diffusible substance, for otherwise it would diffuse across the small water gap between cap and tip. Arisz (1958) reported the phenomenon

he called symplasmatic diffusion. Chapter IV This is not likely to be of a
strongly directional quality. Discussion

From the results presented above it is evident that the decapped
root offers a very convenient system for the investigation of perception
without complicating the issue with side effects.

the tip as a result of graviperception in the cap. On the other hand,

By removing the root cap growth is not affected and yet the root
transmission of some stimulus must occur, as the root cannot perceive
stops reacting to gravitational stimuli. There are small changes in the
without the cap, but the differential growth which constitutes the response
angle between the root tip and the main axis of the root but these changes
takes place in the elongation zone which is adjacent to the cap.
fluctuate apparently in a random fashion (see Johnsson, 1966).

There must be a living connection between the root cap and the root
The fact that growth is not affected seems to point immediately to
tip and not merely an aqueous diffusion zone. To put it more precisely,
the possibility of the root cap having no growth regulating role and
the plasmodesmata connecting the root cap to the tip would seem to be of
therefore being mainly concerned with perception.
primary significance in the transmission of the stimulus.

Further studies confirmed this as is seen from the presentation time
According to Dr. B.P. Juniver (private communication), statistical
studies. The root cap seems to be necessary only for the perception and
studies have established the fact that in the root cap there are more
can be removed after the presentation time without influencing the subsequent
plasmatic connections in the transverse walls than there are in the
response.

longitudinal ones, and the situation looks similar in the meristematic zone
of the root tip.
Another important feature is that the stimulus is transmitted within
the presentation time period. As the stimulus will not pass from the cap

after it has been severed from the tip and replaced, it is probable that it

It is tempting to postulate that the transmission of the stimulus
is not a diffusible substance, for otherwise it would diffuse across the
is dependent on membrane continuity and is some self propagating change in
small water gap between cap and tip. Arisz (1958) reported the phenomenon
the membrane itself. This would fit in with the fact that the stimulus

he called symplasmatic diffusion but this is not likely to be of a strongly directional quality. an asymmetrical or polarised state will

move longitudinally because of the small number of side connections as explained above.

It is possible to replace the tip of a decapitated coleoptile and obtain movement of growth hormones over the junction, and so it is unlikely that any substance of a growth regulating nature passes from the cap to the tip as a result of graviperception in the cap. On the other hand, transmission of some stimulus must occur, as the root cannot perceive without the cap, but the differential growth which constitutes the response

In the animal kingdom this is the normal process of transmission of perceived stimuli. The sensory cells of the labyrinthine apparatus show

There must be a living connection between the root cap and the root tip and not merely an aqueous diffusion zone. To put it more precisely, the plasmodesmata connecting the root cap to the tip would seem to be of

This excitability of membranes is not confined to animal cells; it has primary significance in the transmission of the stimulus.

has also been shown in giant algal cells by Wilkins (1948), Coffey and Mullins

(1952) According to Dr. B.E. Juniper (private communication), statistical studies have established the fact that in the root cap there are more

plasmatic connections in the transverse walls than there are in the

The electrostatic effect was investigated as a potential difference longitudinal ones, and the situation looks similar in the meristematic zone which develops across the whole organ and not as a moving charge which, of the tip. This might serve to promote transmission along the longitudinal axis, avoiding any attenuation by transverse movement.

possibly, develops at the end of the presentation period and moves along the organ presumably fairly rapidly.

It is tempting to postulate that the transmission of the stimulus

It has been proposed by Graham (1964) and by Wilkins and Woodcock (1964) is dependent on membrane continuity and is some self propagating change in that the G.E.R. in coleoptiles represents a later stage in the development of the membrane itself. This would fit in with the fact that the stimulus reaction and is possibly a result of auxin redistribution, as mentioned earlier.

moves from the cap to the tip possibly at the end of the presentation period. The transmission of an asymmetrical or polarized state will move longitudinally because of the small number of side connections as explained above.

There is also a paper by Small (1962) in which he describes a mechanism by which such a membrane change might be triggered, without the need to postulate any biochemical process in the root cap cells.

The sedimentation of statoliths provides a mechanism by which such a membrane change might be triggered, without the need to postulate any biochemical process in the root cap cells. The sedimentation of statoliths provides a mechanism by which such a membrane change might be triggered, without the need to postulate any biochemical process in the root cap cells.

In the animal kingdom this is the normal pattern of transmission of perceived stimuli. The sensory cells of the Labirinthian apparatus show a change in membrane potential which is measurable electrically and is transmitted to the nerve, and through it to the brain.

This excitability of membranes is not confined to animal cells; it has also been shown in giant algal cells by Blinks (1940), Gaffey and Mullins (1958), in higher plant cells by C.J. Bose (1927), in Mimosa by Sibacka (1962, 1966), in Dionea by Jackobson (1965).

It would be very interesting to follow this work with the more sophisticated techniques of modern electrophysiology.

The Geo-electric Effect was investigated as a potential difference which develops across the whole organ and not as a moving change which, after removing the root cap, the quiescent centre in the main root tip starts dividing, the cap is regenerated, and the boundary re-established the organ presumably fairly rapidly.

after approximately 36 hours (see Juniper et al, 1966).

It has been proposed by Graham (1964) and by Wilkins and Woodcock (1966) that the G.E.E. in coleoptiles represents a later stage in the geotropic reaction and is possibly a result of auxin redistribution, as mentioned earlier.

Newman (1963) used a measurement of an electrical change, the movement of a transverse p.d., as the indicator of auxin movement in coleoptiles.

There is also a paper by Small (1919) in which he describes a measured resistance change between two wire electrodes, which were inserted adjacently at 1 and 2 mm from the tip of a *Vicia faba* root, on geotropic stimulation. This change was very rapid and showed a sharp rise to a peak followed by an exponential decay.

Small's work was based on that of Fitting (1905); he followed the magnitude of the resistance change after geotropic stimulation from 0° to 90° , but unfortunately did not investigate it any further.

This reported resistance change might be an indication of a fast change that travels along the root from the cap. The shape of the curve in Small's paper somewhat resembles a typical slow action potential curve.

It would be very interesting to follow this work with the more sophisticated equipment available nowadays.

After removing the root cap, the quiescent centre in the maize root tip starts dividing, the cap is regenerated, and the boundary re-established after approximately 36 hours (see Juniper et al, 1966).

The recovery of graviperception is achieved before the whole cap is re-formed, and the exact time seems to depend on the length of the root at decapping time.

time. This is most probably a measure of the root's age in terms of the rate of growth.

Shorter roots are likely to be at a stage when cells have a shorter division period and therefore the regeneration is likely to proceed more quickly, at least in its first stages.

the presence of a considerable amount of starch-containing plastids.

The exact nature of the recovery process is difficult to determine. This fact lends support to the assumption that starch granules are necessary by the methods used in this work. From experiments in which roots were put horizontally after various time intervals from decapping, it looks as if it is somewhat abrupt.

There does not seem to be a large difference in the time that curvature takes to appear, after the recovery was established. Reaction time is long though (see results).

These experiments do not show, however, if the presentation times were identical in all the cases of recovery.

If perception could be closely correlated with a transmission of a change in an electrical state, then this would offer possibilities of following the changes during recovery in a more sensitive experimental set-up, without the necessity of measuring the curvature development.

As the cap, or at least part of it, seems to be necessary for the perception of gravitational stimuli by the maize root, it was thought interesting to try and find what structure the root cap cells had at recovery

time, and whether some indication as to the perception mechanism could be seen from the structural state. The distribution of starch grains in cells must be

many. The density and mass of the starch itself and the density and

As attempts to tackle the problem with light microscopy did not viscosity of the surrounding cytoplasm are no doubt important. The cyto- produce satisfactory results, an electron-microscopical investigation plastic properties i.e. viscosity, might change when the cell is not part was undertaken. The root at recovery time and closely before it, showed of the meristematic region. But what else determines the fact that in the presence of a considerable amount of starch-containing plastids. certain regions starch will sediment in some cells but not in others, is This fact lends support to the assumption that starch grains are necessary not clear. For instance, in *Vicia faba* only in the central zone of the for graviperception by the root cap, because this is the most striking root cap cells the starch sediments (see E. Griffiths, 1963). In the cytological feature of the root cap.

recovering root cap the asynchronisation of various processes might not be

complete. The amyloplasts have not shown a clear redistribution under gravity

in these recovered roots, as compared with a normal root growing vertically

vertically. This may be due to a variety of factors. Presentation time

might be prolonged in roots at recovery time. Presentation time seems to

be dependent on starch content as found by Hawker (1932, 1933), and the

amount of amyloplasts in the cap cells at recovery time is not yet as

In spite of the uncertainty about the sedimentation of starch, I think great as that in the normal roots. Also, the amyloplasts do not sediment that it is reasonable to say that the experimental results of this work can immediately after they are formed as can be seen in the meristematic be explained in terms of the statolith hypothesis which it would be region of the normal root cap.

Attempt to try and explain them in the terms of any other known theory

for the following reasons. The sedimentation hypothesis of the statoliths

is not supported by the following evidence.

The development of the C.M.E. in roots has not been investigated carefully, but even if it was shown that it develops in roots it might still be a result of auxin redistribution as in coleoptiles. It is also difficult to

The sedimentation is obviously a very complex mechanism, and the factors determining the sedimentation of starch grains in cells must be many. The density and mass of the starch itself and the density and viscosity of the surrounding cytoplasm are no doubt important. The cytoplasmic properties i.e. viscosity, might change when the cell is not part of the meristematic region. But what else determines the fact that in certain regions starch will sediment in some cells but not in others, is not clear. For instance, in *Vicia faba* only in the central zone of the root cap cells the starch sediments (see H. Griffiths, 1963). In the recovering root cap the synchronization of various processes might not be complete and some of the amyloplasts in a cell could sediment before others. All these considerations, and the fact that the geometry of the cells at this stage in the root cap is very complex in three dimensional terms, make a careful and detailed study of starch sedimentation in the root cap at recovery very necessary.

In spite of the uncertainty about the sedimentation of starch, I think that it is reasonable to say that the experimental results of this work can be explained in terms of the statoliths hypothesis whilst it would be awkward to try and explain them in the terms of any other known theory for the following reasons.

The development of the G.E.E. in roots has not been investigated carefully, but even if it was shown that it develops in roots it might still be a result of auxin redistribution as in coleoptiles. It is also difficult to

explain the special significance of the root cap in perception according to this theory.

The suggestion in Gillespie and Thimann's paper that the cells sense their own weight, by minute distortion of the membrane under pressure, is open to serious criticism. It requires a detection system of exquisite sensitivity, in fact 1 part in 10^6 (see Audus, 1962) and such a detection system would also have to possess a very low noise level.

If cells can sense their own weight, redistribution of the pressure is instantaneous upon geotropic stimulation. In this case it is difficult to explain a presentation time of 4 minutes.

A pressure change does not reach a maximum at $120-135^\circ$, and this is the angle at which geotropic stimulation seems to have the greatest effect.

The evidence from animal physiology suggests that it is the component of tangential shearing force on the membrane that is the effective stimulus and not the pressure.

If the changes in pressure and the changes in shearing force are plotted against the angle of tilt, it is the maximum change in shearing force that corresponds to the maximum in stimulation of the labyrinthian apparatus. (See Fig. 25a and Trincker, 1962).

An interesting plot is that of the function (S - P) against inclination (see Fig. 25b).

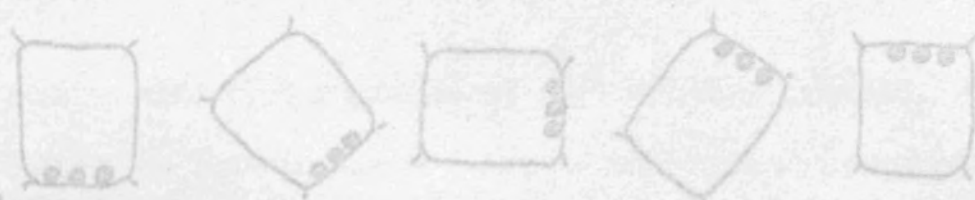


Fig. 25a. Initial changes in pressure (P) and shear (S) on the basal membrane of a statocyte during rotation.

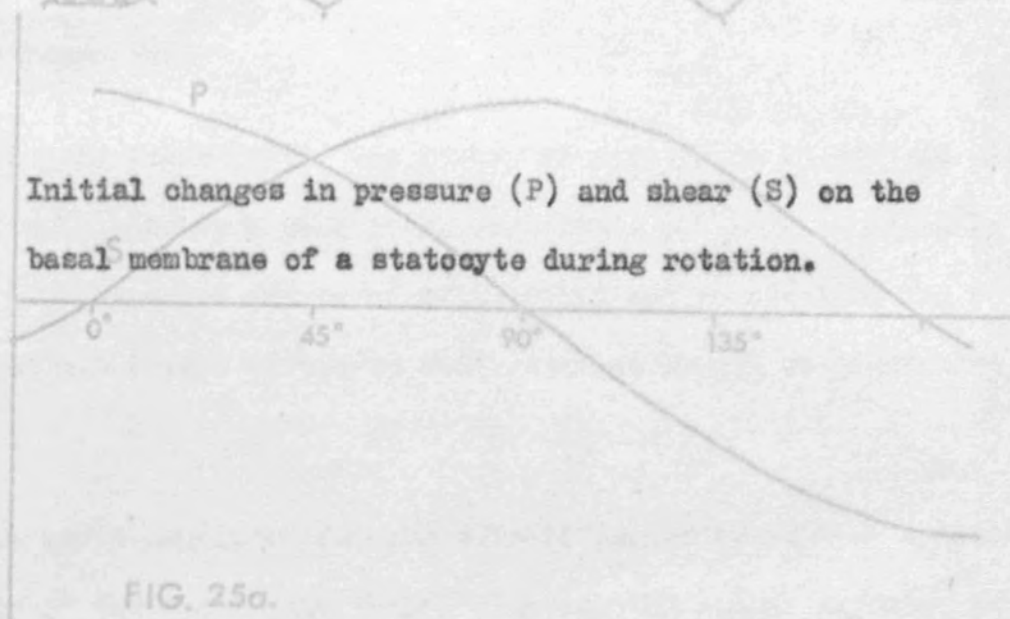
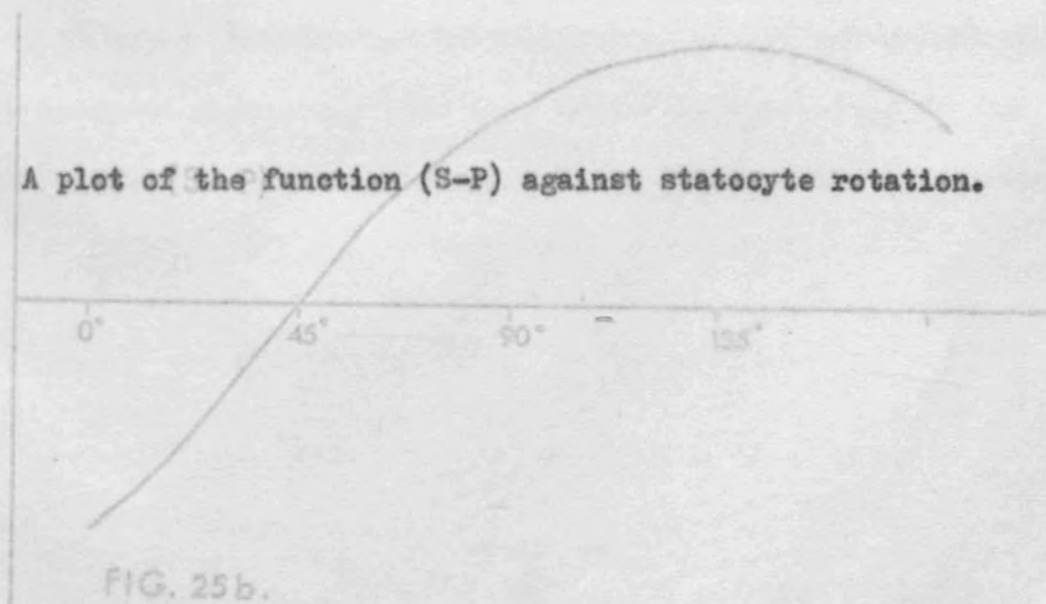


Fig. 25b. A plot of the function (S-P) against statocyte rotation.



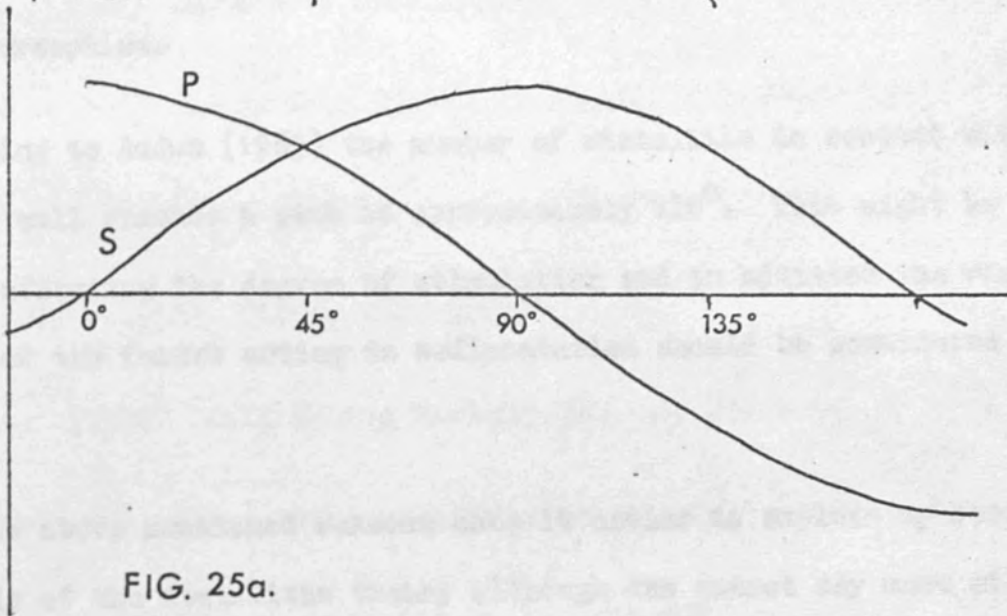
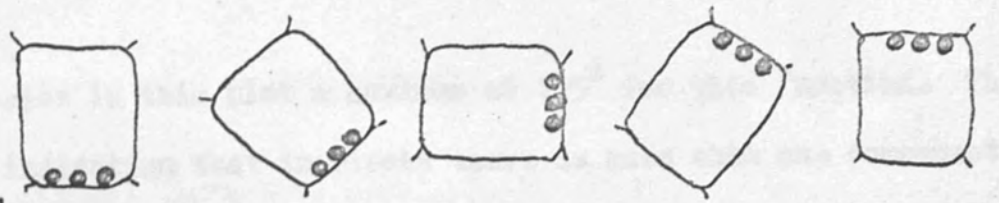


FIG. 25a.

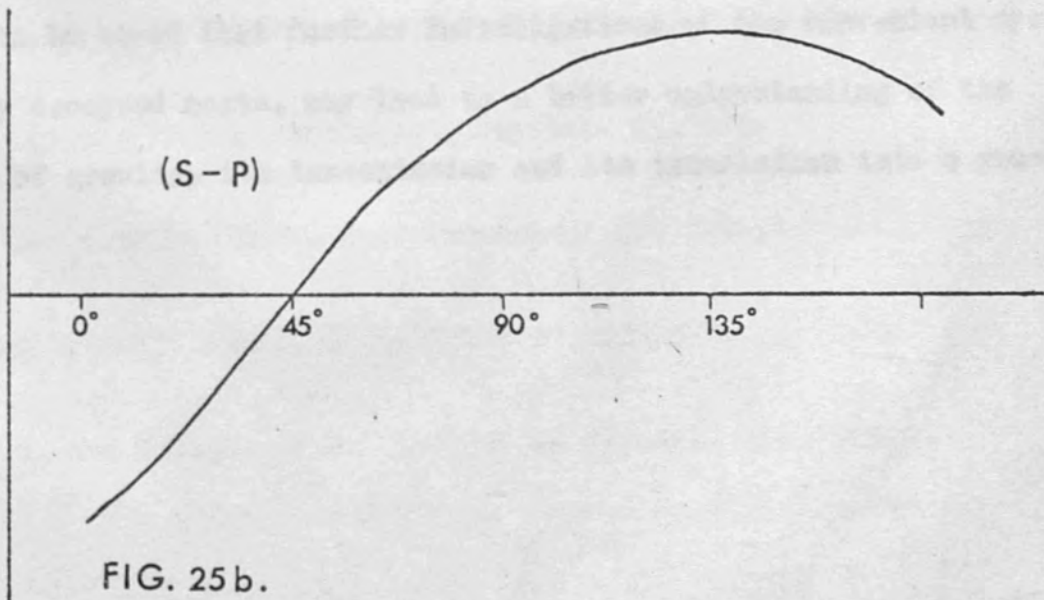


FIG. 25b.

One obtains in this plot a maximum at 135° for this function. This might be an indication that in plants there is more than one component affecting perception.

Audus, L.J. (1962) Symp. Soc. Exptl. Biol., 16, 197-226.

According to Audus (1965) the number of statoliths in contact with the lateral wall reaches a peak at approximately 120° . This might be one factor affecting the degree of stimulation and in addition the various components of the forces acting in sedimentation should be considered as well.

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All the above mentioned reasons make it easier to explain my results on the basis of the statoliths theory although one cannot say more at this stage.

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There are many questions unanswered as yet in this complex phenomenon, but it is to be hoped that further investigations of the convenient system provided by decapped roots, may lead to a better understanding of the perception of gravity, its transmission and its translation into a growth response.

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