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SOME EFFECTS OF MINOR NUTRIENTS ON THE GROWTH AND METABOLISM OF PLANTS.

by

John V. Possingham

St. John's College

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ABSTRACT

Investigations are described which were carried out to analyse the way in which certain mineral element deficiencies restrict the growth and development of plants. The plant system used in this work was excised pea roots grown in sterile culture media, and the deficiencies studied were those of iron, magnesium and molybdenum.

Growth was measured at the cell level and related to other characteristics of the system; two different experimental designs being employed to assess the effects of deficiencies. In the first, roots were grown in full nutrient and in deficient media and growth was measured on samples taken after growing periods of 0, 3, 5, 7, 9 and 11 days; while in the second, roots were grown for 7 days in full nutrient and in deficient media and serial one centimetre sections taken from these roots were compared. The first approach assessed the effects of the deficiency on overall growth, and the second gave an indication of the effects of the deficiency on the longitudinal differentiation of pea roots.

Both experimental approaches were employed when examining iron and magnesium deficient roots, but only the second when examining molybdenum deficient roots. Roots deficient in iron and magnesium were obtained by culturing tips cut from germinated seeds in deficient media, but two successive tip passages were necessary to obtain roots deficient in molybdenum.

Growth was assessed basically in terms of length, cell number, cell volume, protein nitrogen, and rate of oxygen uptake. However with iron deficient roots measurements of invertase activity, sensitivity of the oxygen uptake to cyanide, and the frequencies of cells in the different stages of division were also made. The techniques involved in the culture of deficient and full nutrient roots, and the analytical techniques are described.

It has been shown that iron deficiency markedly affects the growth and development of excised pea roots. Growth in terms of length and cell number per root is stopped after 7 days and no further increases occur between days 7 and 11. Although iron deficiency stops cell division, measurements made at day 7 indicate that this deficiency does not restrict the process of cell expansion. In fact 7 day old iron deficient roots carry larger cells in the terminal centimetre than full nutrient roots. By 11 days the iron deficient roots have a pronounced swelling at the terminal end, and it is suggested that this is brought about by an abnormal expansion of the cells in the lateral direction.

Some cells containing mitotic figures are present in the tips of 7 and 11 day old iron deficient roots. However there are fewer cells in the division stages of prophase and metaphase and practically no cells in the stages of telophase and anaphase in the deficient roots when comparisons were made with full nutrient roots.

The protein nitrogen content of iron deficient roots is lower than that of full nutrient roots at day 7, but there is a considerable increase in both deficient and full nutrient roots between days 7 and 11. The trend of the derived quantity, average protein nitrogen content per cell, is the same in both groups of roots up to day 7, but from day 7 to day 11 it increases sharply in the deficient roots but does not change in the full nutrient roots. This result indicates that cell division was not stopped in the deficient roots by a shortage of protein nitrogen as such.

At the day 7 stage the distribution of protein nitrogen along the length of deficient roots is different to that in full nutrient roots. The front sections of the deficient roots contain an increased content and the back sections a decreased content when compared with full nutrient roots. On a per cell basis the situation is the same, as the cells

in the front sections of deficient roots have a higher average protein content and those in the back sections a lower content when compared with the cells of full nutrient roots.

The accumulation of protein nitrogen in the front sections of iron deficient roots is most probably associated with the cessation of active cell division in the meristem. Evidence is available which suggests that under normal conditions the formation and development of cells in the apex of the root is dependent on substrates synthesised in the mature regions of the root and translocated forward. It is considered that in iron deficient roots precursors of protein are no longer removed by the demands of the meristem and they condense to form protein in the regions adjacent to the apex.

Invertase activity per unit protein nitrogen is the same in both full nutrient and iron deficient roots at all stages. Further, there is no difference in invertase activity when the corresponding sections of full nutrient and deficient roots are compared at day 7. It is clear that in this one respect the protein of iron deficient roots is similar to that of full nutrient roots.

The rate of oxygen uptake per root of iron deficient roots is lower than that of full nutrient roots at the early day 3 stage, but there are large increases in the rates in both deficient and full nutrient roots between days 3 and 11. It is of some significance that iron deficiency clearly reduces the rate of oxygen uptake at a stage before the process of cell division is stopped. On a per unit protein nitrogen basis the rate of oxygen uptake of deficient roots is lower than that of full nutrient roots after day 3. It is suggested that the effects at days 3 and 5 are a direct effect of iron deficiency but the effects at days 9 and 11 are influenced by the fact that cell division stopped at day 7. The results from 7 day roots show that the effect of iron deficiency in reducing the

rate of oxygen uptake per unit protein nitrogen is confined to the front three sections of the root as iron deficiency does not alter the rates in the back three sections.

Iron recovery experiments show that iron deficient roots 7, 9 and 11 days old can resume cell division and grow when they are transferred to a full nutrient medium. It is of interest that in these experiments the recovery in terms of an increased rate of oxygen uptake is greater than the recovery in terms of length and protein nitrogen.

Experiments in which the rate of oxygen uptake of deficient and full nutrient roots were measured in the presence and absence of cyanide show that in both groups of roots there is a large fraction of the respiration insensitive to cyanide. The activity of this cyanide insensitive system increases considerably from day 3 to day 11 in both the full nutrient and iron deficient roots. Increases, after day 3 in the activity of this cyanide insensitive system, which would not contain iron, account for the large increase in the total rate of oxygen uptake of iron deficient roots between days 3 and 11.

The activity of the cyanide sensitive system involved in respiration decreases in both groups of roots between days 0 and 5. It increases from day 5 to 11 in full nutrient roots, but does not increase in deficient roots over this period. That synthesis of a cyanide sensitive system involved in respiration stops at or about the same stage as cell division in iron deficient roots is considered to be highly important.

This cyanide sensitive system most probably corresponds to the iron containing cytochrome/cytochrome oxidase system, and there is other circumstantial evidence that this system is important in the process of cell division. It is important to note that the activity of the cyanide sensitive system was the same in the tips of deficient and full nutrient roots at the day 7 stage. It may be that a certain minimum level of activity per cell is necessary to maintain division; a slight

reduction stopping cell division completely, but not being capable of detection by the method of measurement. However, although there is a correlation between the activity of a cyanide sensitive system involved in respiration and the rate of cell division this correlation may not be causal, and indeed iron may be important in other reactions essential for cell division and growth.

The experiments involving magnesium deficiency have established that this deficiency restricts growth in terms of length after 7 days. There is no abrupt cessation; growth in length continues to occur in the deficient medium after 7 days although the difference between the control and the deficient series also continues to increase. This deficiency reduces the rate of cell division but it does not stop division over the experimental period of 11 days. Seven day magnesium deficient root tips contain approximately the same number of cells as full nutrient tips and even at 11 days the deficient roots have an apparently normal apical meristem. There were no differences between the average volumes of the cells in the corresponding sections of magnesium deficient and full nutrient roots at day 7 and it is clear that this deficiency does not affect the process of cell expansion.

The protein nitrogen content of deficient roots was lower than that of full nutrient roots at days 7, 9 and 11, but on each occasion the reduction in protein nitrogen was equal to the reduction in cell number, so that the average protein nitrogen content per cell was the same in the both groups of roots. The same situation was found at 7 days in successive sections taken along the length of deficient and full nutrient roots, and this deficiency clearly does not affect the average protein nitrogen content per cell. Magnesium deficiency produces an organised slowing down of growth and it is possible that here the rate of cell division may be controlled by the rate of protein synthesis.

The rate of oxygen uptake per root in deficient and full nutrient roots shows the same general trend as that for cell numbers and protein nitrogen, however on a per unit protein nitrogen basis the rate of oxygen uptake is significantly lower in the deficient roots from the early day 3 stage. There is only a slight increase in the rate of oxygen uptake per unit protein nitrogen from day 3 to day 11 in deficient roots, indicating that the protein of magnesium deficient roots less effectively catalyses the changes associated with oxygen uptake than that of full nutrient roots. The 7 day experiments show that this effect applies uniformly throughout the entire length of the roots as the rate of oxygen uptake per unit protein nitrogen is lower in all sections of the deficient roots when compared with the rates in the corresponding sections of full nutrient roots. The average rate of oxygen uptake per cell is reduced at practically all stages, and also along the entire length of the deficient roots.

It is known that magnesium is an activator of many enzyme systems involved in the metabolism of carbohydrates, and it is also an activator of enzymes concerned with the transfer of energy in the cell. It is probable that a deficiency of magnesium restricts one or a number of these reactions and this is the basis of the effect on rate of oxygen uptake and growth. The essential feature of magnesium deficiency is that it produces an organised slowing down of growth.

The experiments in which the effects of molybdenum deficiency were studied are only preliminary, but they do show that it is possible to obtain roots deficient in this element if at least two successive tip passages are made. Seven day molybdenum deficient roots are shorter, have a lower cell number, protein nitrogen content, and rate of oxygen uptake than full nutrient roots of the same age. The reduction in cell number is equal to the reduction in protein nitrogen and

the average protein nitrogen content per cell is not affected by a deficiency of molybdenum. In view of the known importance of molybdenum in the metabolism of nitrogen it is suggested that the rate of cell division in molybdenum deficient roots may be controlled by the rate of protein formation. The overall rate of oxygen uptake per unit protein nitrogen was reduced in 7 day molybdenum deficient roots and this preliminary data suggests that the rate is reduced in practically all sections of the deficient roots. This deficiency in contrast to those of iron and magnesium brings about a reduction in the average volume per cell. The volume of the cells in all sections of the deficient roots are lower than those of the cells in the corresponding section of full nutrient roots.

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I INTRODUCTION

The purpose of this investigation has been the analysis of some effects of mineral deficiency on the vegetative growth and development of plants. This work has been based on two separate experimental approaches. In the first, plants grown in deficient and full nutrient media were sampled at intervals and measurements of growth were made on each sample. In the second, the distribution of growth within samples of deficient and full nutrient plants was analysed at an early stage of the development of the deficiency.

Frequently the effects of mineral deficiencies are analysed with plants sampled at a single stage of development, and the observations are made on the whole plant. In this experimental design the plants are usually sampled when they are showing advanced symptoms of deficiency. It is considered that this general procedure is open to several objections.

The devlopment of a plant involves not only a changing morphological structure but also probably a changing metabolic pattern. At different stages in growth the metabolic requirements of the system are likely to be different, and the effect of a mineral deficiency is therefore likely to vary with the stage of development. Secondly at any one stage the metabolic pattern is certainly not uniform throughout the plant, as the metabolic requirements of different tissues most probably vary. Thus the effect of a deficiency may differ according to the tissue in which it is established. Serial observations avoid the first objection, and determinations on different parts of the system at an early stage of the development of overall deficiency avoid the second.

It may be emphasised that observations during the early stages of the development of deficiency effects are important. As the effects of deficiency develop, two general consequences tend to obscure the general situation. A disturbance in one

phase of a metabolic system is likely to have secondary effects on others, and a disturbance in one part of a differentiated structure is likely to influence the activity of other tissues which are not immediately affected.

In this investigation the experimental material has been excised pea roots cultured in sterile media. The use of excised roots in a deficiency study is open to the objection that they represent a considerably simpler system than the intact plant. Also since they do not sustain some of the activities characteristic of the shoot, they do not on that account have the same metabolic requirements. On the other hand excised roots may undoubtedly be taken in certain important respects as a model for the intact plant.

The excised root is certainly a fully differentiated system which develops in the same general way as the intact plant. It does not carry lateral members which are unlike itself, but like the shoot, it does develop from a terminal meristem and it does extend by branching. Thus like the shoot it represents a system in which the tissues of the central axis are in a series of increasing age with increasing distance from the apex, and in which branches with secondary growth centres are carried along the flanks of the central axis.

In the general procedure used in the present investigation, two groups of cultures are set up, one with a full nutrient medium and the other with a medium deficient in a particular element. Samples are taken at intervals, and serial observations made with these show the earliest stage at which deficiency effects may be detected in the overall growth of the system. Subsequently a stage is chosen which shows an early phase of deficiency, and samples are taken at this stage and observations are made in different morphological regions. Both the full nutrient and deficient roots taken at this stage are dissected into successive one centimetre sections commencing at the apex

and separate observations are made on each. The successive sections represent a series of increasing age and they show the interaction between stage of development and deficiency. This latter procedure also shows whether the effects of deficiency are generalized throughout the system or only localised in one particular region.

In both phases of the investigation the same set of determinations are made. The following measurements are made on the whole roots in the successive samples and on the sections from the single sample.

a) Total Number of Cells.
b) Protein Nitrogen Content.
c) Rate of Oxygen Uptake.

In addition with whole roots the lengths are measured, and with the successive sections the breadths are determined.

For the analysis of growth, length determination is of limited significance. It has certain implications with regard to the extension of cells and the determination is in any case readily made. The measurement of breadth in the successive sections is important since from it an estimate can be derived of the total cell volume, and in turn average volume per cell in the different sections. The average volume per cell provides a valuable measure of morphological differentiation.

The determination of number of cells is important in two connections. The number of cells is an indication of the number of interacting metabolising units within the system, and secondly it provides an indication of the extent of cell division which is an important activity in the determination of growth and development.

The determination of protein nitrogen content is again important in two connections. Protein nitrogen content is a measure of the total mass of the metabolising system, and changes in this quantity are therefore an indication of a highly significant aspect of growth. It may be emphasised that protein content is a more reliable index of the growth of the

protoplasmic system than either fresh or dry weight which have not been determined in the present series of experiments. Secondly, the protein nitrogen content is, in relation with measures of metabolic activity, an index of metabolic differentiation.

Rate of oxygen uptake has been measured in both experimental series since it probably provides an integrated expression of a group of cytoplasmic activities. It is not a measure of overall activity, but it is a measure of a series of reactions within a particularly important aspect of metabolism, and as such it may be used as an approximate index of cytoplasmic activity.

From the primary group of determinations the following derivative quantities are calculated;

a) Average Protein Nitrogen Content per Cell.
b) Average Rate of Oxygen Uptake per Cell.
c) Rate of Oxygen Uptake per Unit Protein Nitrogen.

The average protein nitrogen content per cell is of some significance since it shows one aspect of the growth of individual cells and is also an indication of the distribution of an important product of synthesis. The average rate of oxygen uptake per cell is another measure of the development of the cell. The rate of oxygen uptake per unit protein nitrogen has been determined since it is an important index of metabolic status and in certain connections is therefore an index of metabolic differentiation.

In this series of experiments the effects of two deficiencies have been studied in detail and some preliminary data have been obtained on a third. Detailed observations have been made with iron and magnesium deficiencies and preliminary observations have been made with molybdenum deficiency.

Iron and magnesium deficiencies were studied as these elements are probably involved in different metabolic systems

and they are likely to have different effects on growth. Iron and magnesium present the further advantage for this investigation that deficiencies of these elements are readily established during culture of roots from inocula of seed root tips.

The effects of molybdenum deficiency have been examined as this element, in contrast to iron and magnesium, is available in the plant as an anion, and is likely to have effects distinct from the two cations. The molybdenum requirement of plants is much lower than is that for iron and magnesium, and it was found that deficiency effects with this element could only be determined in subcultures. Preliminary data only have been obtained with this element and these refer only to the effects of molybdenum deficiency within roots at an early stage of the induction of the overall deficiency.

II METHODS

1. Culture of Experimental Material

1) General Technique for Culturing Detached Pea Roots under Sterile Conditions

The basic techniques involved in the production of the experimental material in this investigation were similar to those outlined by White (1943). Root tips obtained from pea seeds germinated under sterile conditions were cultured in sterile media. In the conditions of the investigation the roots grew actively at the rate of approximately one centimetre per day when a full nutrient medium was supplied.

Commercial pea seeds (Variety Onward) were used throughout, and these were surface sterilized prior to germination. The sterilizing medium was the clear yellow fluid obtained on filtration of an 8% solution of calcium hypochlorite in water. Pea seeds presoaked for 15 minutes in water were placed in the sterilizing solution for 30 minutes and occasionally shaken to ensure wetting and intimate contact between the seeds and the solution. The hypochlorite solution was poured off and the seeds washed with two changes of sterile distilled water before being transferred to sterile petri dishes each containing 20 ml. of sterile water. Four seeds were placed in each petri dish and the dishes were placed in an incubator maintained at 25 ± 0.1°C. After germinating for 72 hours, the seeds carried roots approximately 2 to 3 centimetres long and these provided the material from which the tip inocula were excised. In the later experiments involving magnesium deficiency a different seed sample, but of the same variety, was used and here a germination time of 48 hours gave roots of a similar length. Root tips approximately 1 centimetre in length were cut from the sterile roots and transferred aseptically to sterile petri dishes each of which contained

20 ml. of sterile culture medium. Two tips were placed in each dish. The dishes were incubated at 25°C ± 0.1°C in the dark.

The petri dishes were of pyrex glass 4 inches in diameter and ½ an inch deep. They were sterilized by heating in an oven at 180°C for 3 hours; the culture media by heating in an autoclave for 10 minutes at a steam pressure of 20 lbs per square inch. Fungal and bacterial contamination was reduced to a minimum by carrying out the aseptic transfers of the seeds and root tips in a microbiological inoculating cabinet.

2) Composition of the Culture Medium

The basic culture medium which is a solution of inorganic salts and organic growth factors in 4% sucrose was similar to that described by Bonner and Addicott (1937). The procedure used in this investigation however involved a preliminary purification of the major constituents, and therefore in addition to these, a mixture of the micronutrient elements known to be essential for the growth of higher plants was also provided in the medium. The composition of the complete nutrient medium was as follows:-

Major Constituents		Minor Constituents	
Salt	Concentration (mg. per litre)	Salt	Concentration (mg. per litre)
$Ca(NO_3)_2 4H_2O$	236	$FeCl_3\ 6H_2O$	2.00
KNO_3	81	H_3BO_3	0.06
KCl	65	$CuSO_4\ 5H_2O$	0.04
$MgSO_4\ 7H_2O$	36	$MnSO_4\ 4H_2O$	0.04
KH_2PO_4	12	$ZnSO_4\ 7H_2O$	0.04
Sucrose	40,000	$NaMoO_4\ 2H_2O$	0.02
		Thiamin	0.10
		Nicotimic Acid	0.50

At intervals throughout the investigation concentrated stock solutions of each constituent were prepared. From the appropriate solutions a medium containing all the inorganic salts and the organic growth factors was prepared at double the concentration at which the substances would be present in the culture vessel. A solution of sucrose was also prepared; the concentration of which was double that of the culture medium. The inorganic salt and growth factor solution and the sucrose solution were autoclaved separately in 30 ml. test tubes, each of which was charged with 10 ml. of the appropriate solution. The contents of one tube of each solution was poured into each petri dish, and as a result the concentration of each constituent was reduced to half that of the two individual solutions.

Prior to autoclaving, the pH of the inorganic salt and growth factor solutions was adjusted so that when equal quantities of this solution and of 8% sucrose were mixed the final solution had a pH of 5.0. The initial pH of the culture medium was in all the experiments adjusted to 5.0 as Torrey (1954) had shown this to be optimal for the growth of pea roots. The adjustments of pH were made with 0.1N HCl and with 0.1N NaOH, and as the quantities added were small they did not appreciably affect the final salt concentration. It was found that no significant change in pH occurred when the solutions were autoclaved.

3) Preparation of Mineral Deficient Culture Media

To prepare culture media deficient in molybdenum, iron and magnesium respectively additional procedures besides omitting the salts containing these elements were involved. It was necessary to remove from the sucrose, major inorganic constituents and water that were used in the preparation of the media, the traces of molybdenum, iron and magnesium which were present as impurities. Only the major constituents of the media were purified, as the minor constituents were added

in such small amounts that the quantity of impurity that could be introduced was negligible. It was also essential to remove adsorbed metal impurities from the glassware which came in contact with the purified media.

a) Purification of Water

Distilled water of high purity was obtained by re-distilling in a pyrex glass still laboratory distilled water. The still constructed for this purpose was similar to that described by Ballentine (1954). In this assembly steam which is generated in a glass boiling flask, passes through a 90cm. vigreaux column, before it reaches a condenser from which water flows into a suitable receiver. The vigreaux column acts as a spray trap. The condenser and the vigreaux column are separated by a glass tube which slants upwards from the column and which is maintained above 100°C by an electrothermal heating tape wound over its external surface. This tube ensures that only steam reaches the condenser, and that fluid which might carry salts is prevented from passing from the vigreaux column to the condenser. The apparatus is equipped with a constant level device attached to the boiling flask, which allows for continuous operation.

b) Cleaning of Glassware

All the glass vessels to which the medium was exposed were cleaned by washing with synthetic detergents in tap water, treating with 50% 'analar' nitric acid for 24 hours, rinsing with tap water, and finally rinsing with glass distilled water. The acid treatment was particularly important as it removed all traces of metal cations and molybdenum compounds.

c) Purification of Sucrose

The sucrose was purified by passing an 8% solution through a fully regenerated column of mixed bed resin, Amberlite MB 1. Two columns in series were used each being 60cm. long and 2 cm. in diameter. The sucrose solution was

passed through the column at the rate of approximately 100 ml. per hour. This purification effectively removed the traces of molybdenum, iron and magnesium present in 'analar' grade sucrose.

d) Purification of Inorganic Salt Solutions

All the major inorganic components used in the preparation of the molybdenum and iron free media were treated to remove traces of these elements. The purification procedure was that described by Gentry and Sherrington (1950). In this acidified single salt solutions are shaken with a solution of oxine in chloroform. The metal impurities chelate with the oxine which after vigorous shaking remains in the chloroform layer. After separation of the two immiscible liquids, some chloroform may remain in the aqueous phase, but this is expelled by bubbling a stream of air through the solution. This procedure removes all traces of molybdenum and most of the iron.

The macronutrient salts used in the preparation of the magnesium free media were not specially purified, but were 'analar' grade reagents. However, the calcium nitrate used in these experiments was prepared by treating 'analar' grade calcium carbonate with 'analar' grade nitric acid. This provision was essential as commercial calcium nitrate was found to be heavily contaminated with magnesium salts.

e) Composition of Mineral Deficient Media

Molybdenum and iron free media were prepared by omitting the salts containing these elements from mixtures composed of purified sucrose, purified macronutrient salts and unpurified micronutrient salts and vitamins. Omission of the molybdenum and iron salts did not significantly alter the total salt concentration of the media, and no other changes in the basic media were made.

Magnesium free media were prepared by replacing magnesium sulphate with potassium sulphate in a mixture composed of purified sucrose and unpurified inorganic salts and organic growth factors. It was necessary to add potassium sulphate to supply the sulphate radical and to maintain the total salt concentration equivalent to that of the full nutrient mixture. Potassium sulphate was added to the medium at a concentration of 26 mg. per litre.

In the experimental series involving molybdenum and iron deficiencies, growth in the deficient medium was compared with growth in the full medium, and this was prepared by adding the appropriate compounds to the mixtures of purified constituents. When the control medium is prepared from unpurified constituents the possibility cannot be disregarded that depressed growth in the deficient medium is not due to impurities introduced by the purification procedure. On the other hand when purified salts are used in both media then differences in growth can only be attributed to deficiency.

2. Analytical Methods

1) Measurement of Rate of Oxygen Uptake

This measurement was made with the Warburg technique. The root tissue was placed in the annular space of the flask with 2.0 ml. of fresh culture medium, and 0.2 ml. of 30% caustic soda was added to the centre well to absorb carbon dioxide. A shaking speed of 100 cycles per minute was used in a constant temperature bath maintained at 25± .01°C. After allowing the gaseous and liquid phases in the flasks to equilibrate by shaking for a half an hour, the manometer taps were closed and readings taken over 90 minutes at 10 minute intervals. The absolute rates of oxygen uptake were obtained by multiplying the observed rates by the manometer constants. The manometer constants were determined by the method described by Dixon (1950).

The amount of root tissue placed in each flask was approximately 30 centimetres of root, eg. 4 roots each approximately 8 centimetres long. When measurements were made on root tips much less tissue was used as these have a higher rate of oxygen uptake. In these investigations the caustic soda solution was placed directly in the centre well of the flask and not absorbed on filter paper. This procedure was quite reliable with the relatively low rates of oxygen uptake that were involved with the root material.

2) Estimation of Protein Nitrogen

This measurement was made on the root tissue which had been used for the determination of the rate of oxygen uptake. The root sections were removed from the Warburg flasks and homogenised with approximately 5.0 ml. of water in an all glass Potter and Elverjhem homogeniser. The homogenate was transferred to a small 10 x 1 cm. centrifuge tube, and an equal quantity of cold 20% trichloraacetic acid was added to precipitate the protein fraction. The tubes were allowed to

stand for an hour in a refrigerator before being centrifuged at approximately 2000 x g for 10 minutes. The clear supernatant fraction was discarded and the pad of trichloroacetic acid insoluble material was washed once with 5 - 10 ml. of petroleum ether to remove fatty lipid material.

The dry precipitate was digested directly in the pyrex centrifuge tubes, 0.2 to 0.3 ml. of digest fluid being added to each tube. This mixture consisted of selenium, mercuric sulphate and potassium sulphate dissolved in nitrogen free concentrated sulphuric acid (Milton and Waters 1949). After heating for at least an hour the tubes were cooled and after adding 2 to 3 drops of 100 volume H_2O_2 they were reheated for a few minutes. The colourless digest was then diluted to 3.0 ml. with distilled water. In some cases when a large quantity of root material was involved the digests were made up to 5.0 ml.

The nitrogen content of the digests was estimated using a Markham steam distillation unit. The ammonia was distilled over and collected in a boric acid indicator solution. The boric acid solution was finally brought back to neutrality with standardised sulphuric acid. An 'Agla' micrometer syringe was used as a microburette and an electrical stirrer was employed to ensure mixing during the titration.

3) Estimation of Cell Number per Root and per Root Section

The cell maceration technique of Brown and Rickless (1949) was used. 10 to 40 centimetres of root material were immersed in 5.0 ml. of 5% chromic acid for 24 hours at room temperature. Maceration was effected by shaking and then squeezing the material through a fine capillary pipette according to the technique described by Brown and Broadbent (1951). The original cell suspension was diluted with further 5% chromic acid prior to making the actual counts. A Fuchs-Rosenthal haemocytometer slide was used and the best results were

obtained when the final suspension had a cell density of approximately 200 cells in the squared section of the haemocytometer. In the work with whole roots at least four roots per sample were used to avoid excessive variability between samples and accordingly a large dilution of the extracts was often necessary.

4) Estimation of Average Cell Volume

Estimations of average cell volume were made only in those experiments in which the deficient and the full culture roots were compared at day 7 by taking serial 1.0 cm. sections commencing at the growing point. In these experiments the sections which were used for the estimation of total cell number were also used for the estimation of total cell volume. From these two measures the average cell volume in the different sections was estimated.

The diameter of each 1.0 centimetre section was measured using a graduated scale inserted in the eyepiece of a binocular microscope. The eyepiece scale was calibrated against a known length on the microscope stage. Knowing the length and diameter of each section the total cell volume per section may be calculated by assuming the section to be a perfect cylinder. This method is open to some error as the individual roots are slightly tapered, but this error was reduced by taking at least two measurements of diameter on each section and using the mean value for the calculation of total volume.

5) Estimation of Invertase Activity

The invertase activity of root tissue grown in the presence and absence of iron was estimated by determining the hydrolysis of sucrose in conditions similar to those described by Robinson and Brown (1952). The roots were cut into approximately one centimetre segments and frozen in a small

volume of water for one hour. They were then thawed and washed free of internal and external sucrose by repeated washings with distilled water. The root sections were incubated for 4 hours at 25°C with 2.0 ml. of 2% sucrose substrate. The reaction was stopped by filtering off the root sections on a sintered glass filter. The segments were washed on the filter and these washings were added to the filtrate and this was made up to standard volume.

The reducing sugar produced was estimated by the Hagedoorn - Jensen technique (Strepkov 1957). The results are expressed as micrograms of hexose sugar produced per microgram of protein nitrogen.

Separate root cultures were used for the estimation of enzyme activity. After the tissue had been used for this measurement, the protein in it was determined by the method outlined above. The protein values obtained with this material were consistently lower than those obtained with fresh tissue, and it was therefore probable that some protein was hydrolysed and dissipated during the period of exposure to the sucrose solution.

The activities which are presented in the text are those derived from the hexose values divided by the corresponding protein nitrogen values obtained using the same sample of roots. The invertase activities derived from the hexose values divided by the mean protein nitrogen content established in earlier experiments are given in the appendix. These values, in absolute terms, are slightly lower than the values presented in the text, but they give differences between treatments which are relatively the same.

6) Cyanide Innibition of Oxygen Uptake.

Potassium cyanide was used as the source of the cyanide ion in these experiments and the cyanide alkali mixtures

elaborated by Krebs (1935) were used to maintain the cyanide concentration constant in the Warburg flasks. Initially unsatisfactory results were obtained as some of the cyanide in the external medium was absorbed by the cyanide alkali mixtures in the centre well of the flasks. This led to a changing concentration of cyanide and a changing rate of oxygen uptake with time. However this difficulty was avoided by presoaking the roots in the cyanide culture media mixture for an hour prior to placing them in the flasks which contained cyanide both in the annular space and in the centre well. When a short equilibration time of 15 minutes was followed by readings at 5 minute intervals over the next 45 minutes, a straight line rate of oxygen uptake was obtained in the presence of cyanide.

Two concentrations of cyanide were tested, 10^{-4} and 10^{-3} molar and the effects were assessed with culture media at pH 5.0. In those experiments involving 10^{-4} Molar KCN separate samples of roots were taken to determine the control rates and the rates in the presence of cyanide. The mean values of four replicate determinations in each case were used to establish one value for percentage inhibition.

This procedure was unduly extravagant with cultured root material, and accordingly in the subsequent experiments when using 10^{-3} molar KCN the control rates and the rates in the presence of cyanide were established on the same sample of roots. The technique was to determine the control rate after 15 minutes equilibration by taking readings at 5 minute intervals over 45 minutes. The root sections were then placed in petri dishes containing the cyanide/culture medium mixture for one hour, before transferring them back into flasks containing cyanide both in the centre well and in the external medium. As before the rate of oxygen uptake was

established by taking readings at 5 minute intervals over 45 minutes after an initial equilibration time of 15 minutes. Excellent results were obtained by this latter method as the variability was reduced, both measurements having been made on the same sample of roots.

7) Examination of Root Tips for the Relative Frequency of Mitotic Figures

Root tips 0.5 cm. long were taken from roots grown in sterile culture. They were fixed for 10 minutes in acetic acid: alcohol solution (1 : 3, V/V), washed, and transferred to normal hydrochloric acid and heated at 60°C for 10 minutes. The hydrochloric acid was washed off and the root tips were stained in aceto-carmine reagent for at least 6 hours.

Each of the stained root tips was taken separately and placed in a small drop of 50% glycerol on a microscope slide. A thin plastic cover slip was carefully placed over the root tip which was then squashed flat by gently pressure. The cells of each tip were spread out into a single layer by gently stroking the plastic cover slip with a glass rod. The technique was similar to that employed by Sunderland and Brown (1956) in their examination of the lupin apex.

The slides were examined using an oil immersion lens (x 100) fitted to a binocular microscope. The numbers of cells in each of the different mitotic phases were counted by taking transects across the slides. At least 1500 non-vacuolated cells were examined on each slide and the numbers of cells in interphase, prophase, metaphase, anaphase and telophase were recorded.

3. Methods of Statistical Analysis

The results presented in the following section, where necessary, have been examined by statistical procedures to determine whether the treatment differences reach significance. In general, direct comparisons of the results obtained from deficient and full nutrient roots, each harvested on a particular occasion, have been made.

The experimental designs used in these investigations are such that it has been possible to present the results in the form of trends with time, and this circumstance has reduced the need for extensive statistical analysis of the data. In many cases it is clear that the trends with time differ in the deficient and full nutrient series, but the precise stage of divergence is not always well defined. The actual trends with time are not simple straight lines but they are in general complicated polynomial functions. Therefore, it was decided not to group the data and establish the trends mathematically, but to determine directly the first occasion at which the treatment differences became significant in each instance.

The data from the iron deficiency experiments have been analysed using conventional analysis of variance tests to compare the differences between deficient and full nutrient roots. In some cases it was necessary to apply a log. transformation to facilitate this analysis. Assistance with these analyses was obtained from Mr. G.A.McIntyre, Division of Mathematical Statistics, C.S.I.R.O., Canberra, A.C.T., and this assistance is gratefully acknowledged.

The data from the magnesium deficiency experiments have been examined in a similar way, but here the less precise "t" test was employed as the level of replication was higher. The results obtained from the experiments involving molybdenum deficiency were not subjected to analysis. The low level of replication precluded this, and these data must be regarded as preliminary observations.

III EXPERIMENTAL DESIGN and RESULTS

1. Iron Deficiency Experiments

(1) Effect of Iron Deficiency on the Overall Growth of Pea Roots

The first series of experiments was designed to assess the effect of iron deficiency on the overall growth of detached pea roots grown in sterile culture media. The growth of this organised and differentiated plant system was characterised by taking the following measurements -

a) Length of the roots
b) Total number of cells per root
c) Total content of protein nitrogen per root
d) Total rate of oxygen uptake per root
e) Relative invertase activity per root

From these primary measurements it was possible to further define the growth of the system by deriving the following quantities -

f) Average rate of oxygen uptake per unit protein nitrogen
g) Average protein nitrogen content per cell
h) Average rate of oxygen uptake per cell

In these experiments root tips cut from germinated pea seeds were cultured at 25°C in full nutrient media and in media deficient in iron. Samples of roots were taken for analysis after growing periods of 3,5,7,9 and 11 days and the initial root tips ready for culturing were also analysed to provide the day 0 values. In preliminary experiments it was established that the root cultures derived from root tips cut from germinated seeds of a pure line variety of seed were very uniform. It was also found that

separate experiments involving the same seed sample, sterilization procedure, culture medium and growing period gave clearly reproducible results. Thus it was possible to assemble data on the growth of this system in a series of experiments that were conducted at widely separated intervals.

Each experiment usually involved approximately 32 roots grown in full nutrient media and 32 roots grown in iron deficient media. The 32 roots from each of the two nutritional treatments were at harvest divided into two equal groups. One group provided 4 samples, each consisting of 4 roots, which were used to estimate total cell numbers, while the other group provided 4 similar samples which were used first to establish a rate of oxygen uptake per root and then to provide data on the protein nitrogen content per root. The lengths of all the roots were measured. In general there was an internal replication of 4 within each experiment, and usually 3 replicate experiments provided the basic data on each occasion.

The activity of the enzyme invertase was estimated using separate cultures of roots and in these experiments protein nitrogen was again estimated. In these experiments the internal replication was usually 5 and the experiments were not repeated.

The mean values obtained in this series of experiments are presented in Table 1. The complete data are given in Tables 8 to 14 in the appendix, and the trends with time of the different determinations are presented in figures 1 to 8.

The effect of iron deficiency on growth in length is shown in figure 1, where it is clear that after 7 days, the growth in terms of length of the roots cultured in the iron deficient media is severely restricted. There is

some slight effect after 5 days but after 7 days little or no further increase in length occurs. The effect in the iron deficient roots, in terms of numbers of cells, is shown in Figure 2 from which it is clear that after 7 days there is no increase in the total number of cells in the iron deficient roots, but a large increase does occur in the full nutrient roots. The trend with time of both these measures in the full nutrient roots involves an initial lag phase between day 0 and 5, which is followed by large increases between days 5 and 9 with some reduction in the rate of increase between days 9 and 11.

Protein nitrogen content per root is presented in Figure 3. There is little change in the content of the full nutrient roots between days 0 and 3, but thereafter there is a very steep rate of increase which continues to day 11. The protein nitrogen content of the iron deficient roots is lower than it is in the roots supplied with iron at the 7, 9 and 11 day harvests. However the protein nitrogen content of deficient roots increases steadily between 7 and 11 days. There is no sudden stopping of protein nitrogen formation at or about the 7 day stage and there is almost a linear increase from the third to the eleventh day.

The total rate of oxygen uptake of roots grown in the presence and absence of iron is given in Figure 4. In the plus iron roots there is initially a slight decrease in the rate between days 0 and 3, but this is followed by a large increase over the next eight days. The increase in the rate of oxygen uptake of these roots is linear between days 5 and 9. The roots grown in iron deficient media have a lower rate of oxygen uptake per root than those cultured in a full nutrient media at all stages. An evaluation of the significance of the observed differences has shown that the

effect of iron at days 5, 7, 8 and 9 is significant at $P < .05$ and at day 3 it is significant at $P < .01$. Although the rate of oxygen uptake of the iron deficient roots is lower than that of full nutrient roots at the day 3 stage there is a considerable rise in the total rate of both groups of roots between days 3 and 7. From day 7 to day 11 there is only a slight change in the rate of the deficient roots, but a large change in the rate of the full nutrient roots.

The rate of oxygen uptake per unit protein nitrogen is given in Figure 5 and the trend shown by the full culture roots is important. There is a very pronounced decrease in the rate between days 0 and 3, which is followed by a gradual increase to day 7 and thereafter the rate remains fairly constant. The roots cultured in iron deficient media have a significantly lower rate of oxygen uptake per unit protein nitrogen than roots cultured in full nutrient media after a growing period of only 3 days. The rate in the deficient roots rises from day 3 to day 7 but then declines sharply to day 11.

The results of the two effects of iron deficiency in arresting the normal increase in cell number at 7 days, but not stopping the increase in protein nitrogen at the same stage are clearly shown in Figure 6, which gives the trend of the average protein nitrogen content per cell. In the iron deficient roots there is a large increase in the average protein nitrogen content per cell at 9 and 11 days. In the control roots this increase does not occur. The normal trend in the full culture roots is first a steep decrease from day 0 to day 3 and thereafter there is probably little change.

In Figure 7 the trend of average rate of oxygen uptake per cell with time has been presented. Once again the

control trend involves a large decrease between days 0 and 3 but thereafter there is only a slight increase in the rate to day 11. Iron deficiency here exerts a consistent effect and there is a lowering of the rate in the iron deficient roots after day 3 which continues to day 9. The mean rate in the 11 day old minus iron roots is apparently higher than the rate in the full nutrient roots, but this effect may not be real and could result from the slight errors associated with the determinations of total cell number and total rate of oxygen uptake per root.

The relative invertase activities of the roots after the different periods of growth are shown in Figure 8. The measure of invertase activity was the amount of hexose sugar formed in micrograms from a given amount of sucrose substrate divided by the protein nitrogen content of the roots in micrograms. In these experiments separate cultures of roots were used, and both the protein nitrogen estimations and the estimate of amounts of hexose formed were carried out on the same sample of root tissue. Iron deficiency has no effect here as there is no detectable deviation from the trend established for the full nutrient roots. There is a very pronounced drop in the invertase activity from day 0 to day 3, but thereafter the changes are slight with only a very gradual increase to day 11.

TABLE 1.

± IRON ROOTS - SUMMARY OF WHOLE ROOT DATA

Mean values from an experimental series which involved a treatment of ± iron and harvests of whole roots after growing periods of 0,3,5,7,9 and 11 days.

Day		0	3	5	7	9	11
Mean Root Length (cm)	-Fe	1.0	2.26	3.34	6.05	6.50	6.70
	+Fe		2.28	3.59	6.64	8.85	10.30
Cell No. /Root	-Fe	247,458	295,777	318,074	496,704	479,408	472,970
	+Fe		293,046	338,488	522,036	663,720	762,308
Protein N. /Root (µg.)	-Fe	59.20	57.89	72.27	88.4	101.9	113.7
	+Fe		57.31	71.58	97.5	122.8	141.6
O_2 Uptake /Root (cu.mm/hr)	-Fe	9.71	7.18	9.37	13.47	13.73	14.77
	+Fe		7.96	10.07	15.32	20.05	22.71
O_2 Uptake /unit Prot.N. (cu.mm/hr /µg.Prot. N)	-Fe	.1674	.1240	.1313	.1529	.1347	.1304
	+Fe		.1388	.1438	.1577	.1633	.1628
Prot.N. /Cell (µg. x 10^{-6})	-Fe	239.2	195.9	203.9	183.8	212.4	242.4
	+Fe		194.2	194.6	189.2	186.0	185.4
O_2 Uptake /Cell (cu.mm x 10^{-6}/hr.)	-Fe	39.24	24.36	27.51	28.27	28.68	31.12
	+Fe		26.89	29.22	29.84	30.39	29.75
Invertase Activity (µg.Hexose /µg.Prot.N)	-Fe	45.73	8.375	8.723	8.743	13.33	14.80
	+Fe		7.920	9.327	9.244	13.00	15.25

Figure 1. Change in Length with Time in Roots grown in Full Nutrient and in Iron Deficient Media.

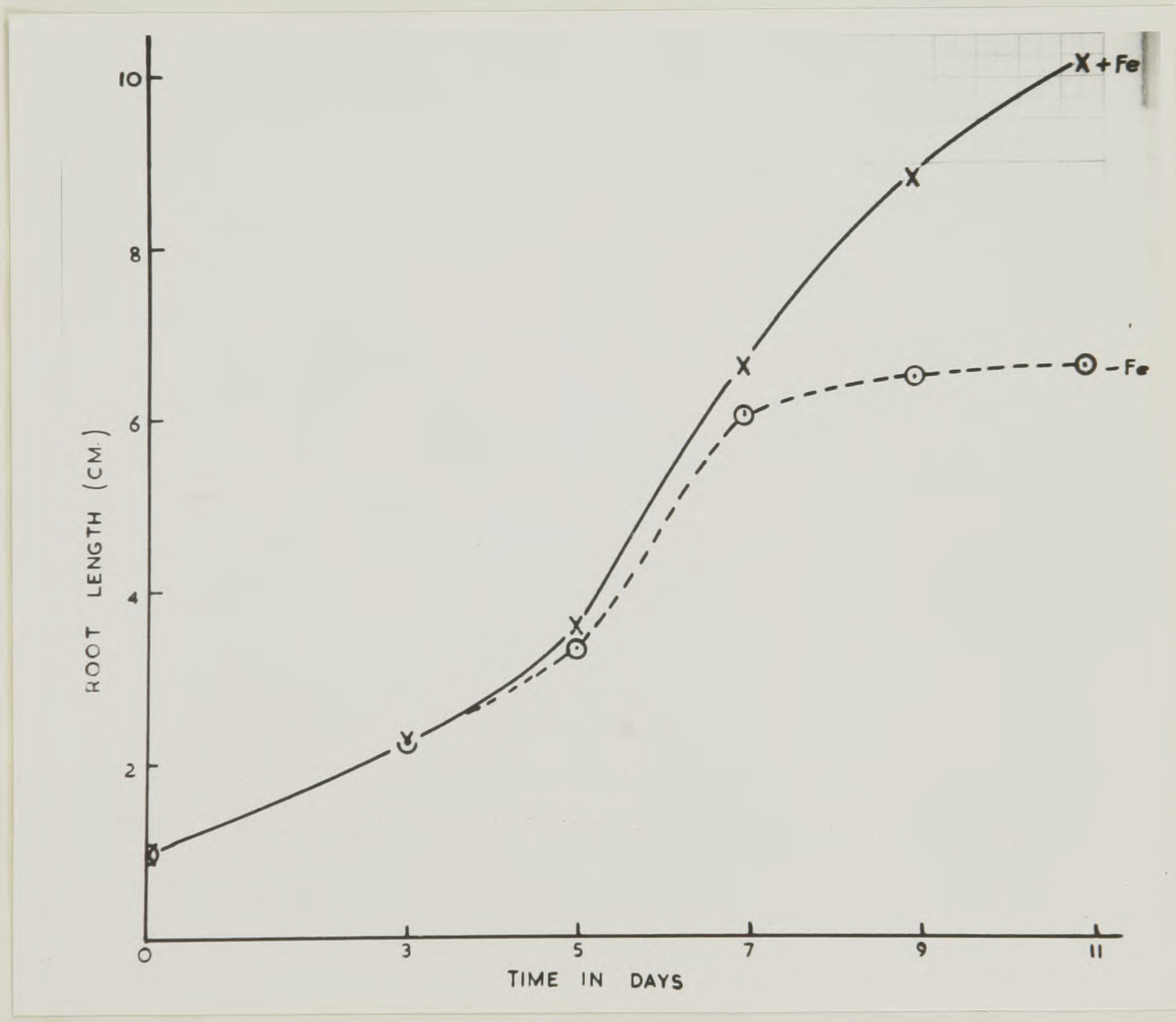
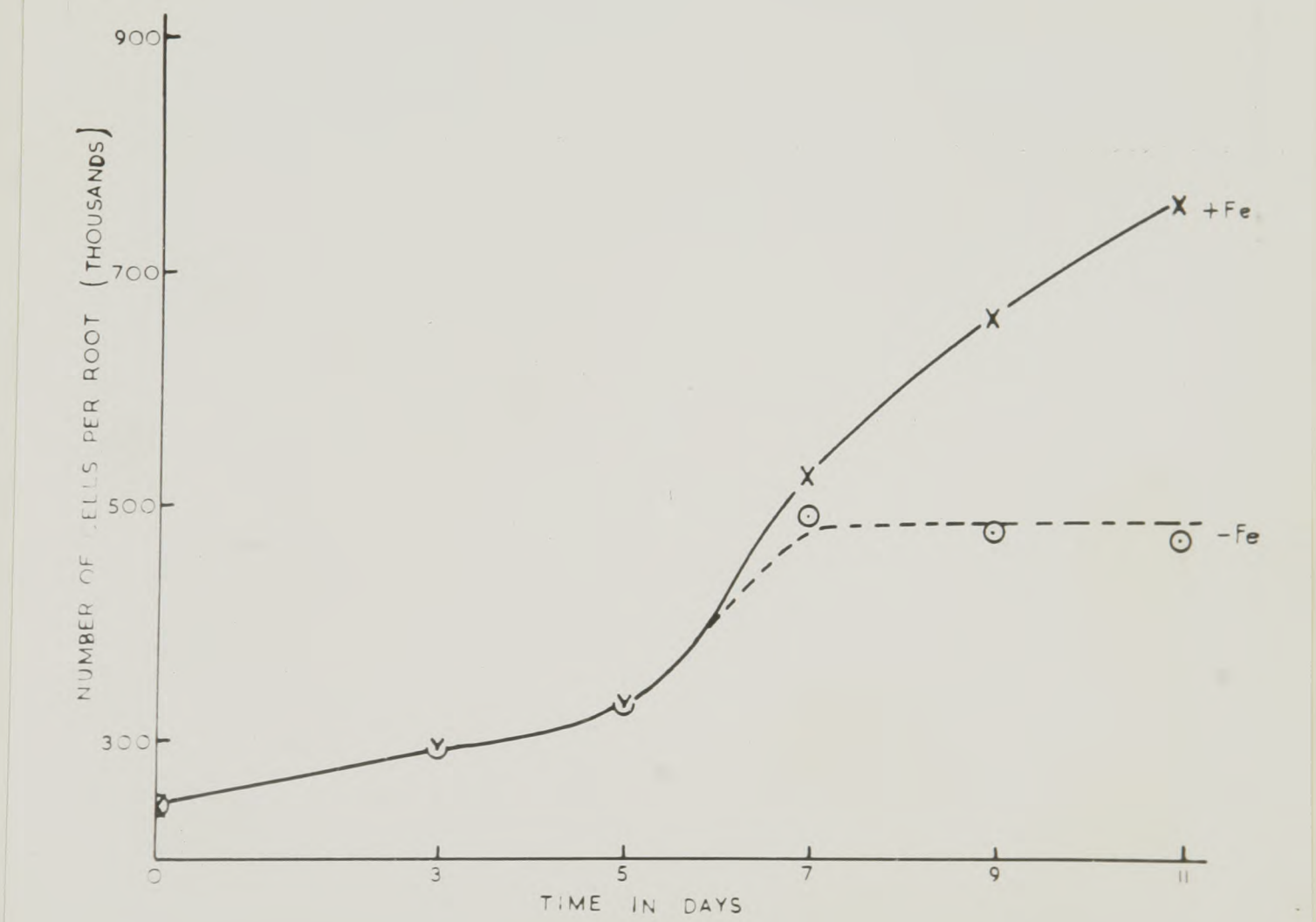


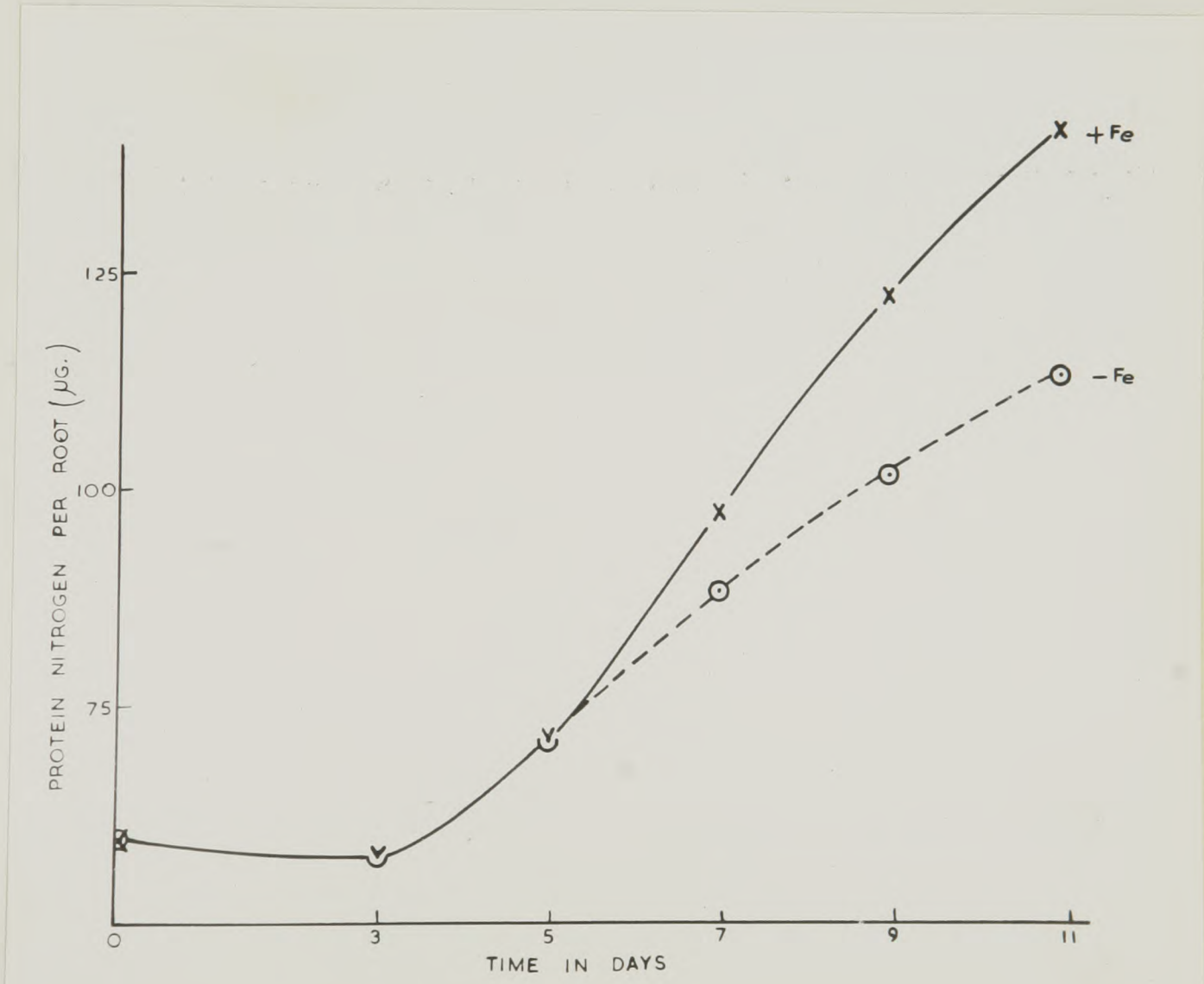
Figure 2. Change in Numbers of Cells with Time in Roots grown in Full Nutrient and in Iron Deficient Media.



Treatment Difference at Day 9 Significant at $P < .001$

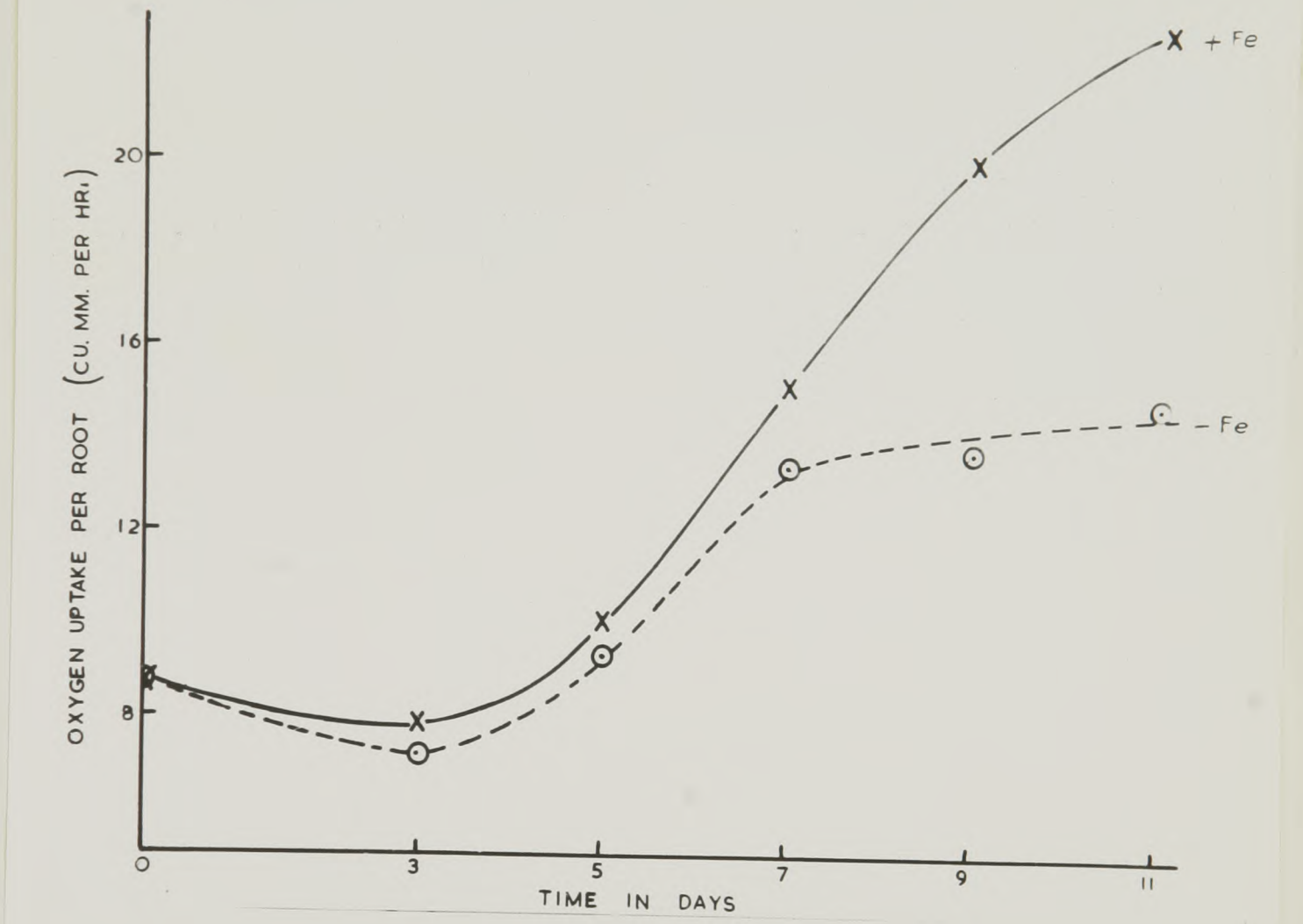
" " " Day 11 " " $P < .001$

Figure 3. Change in Protein Nitrogen Content with Time in Roots grown in Full Nutrient and in Iron Deficient Media.



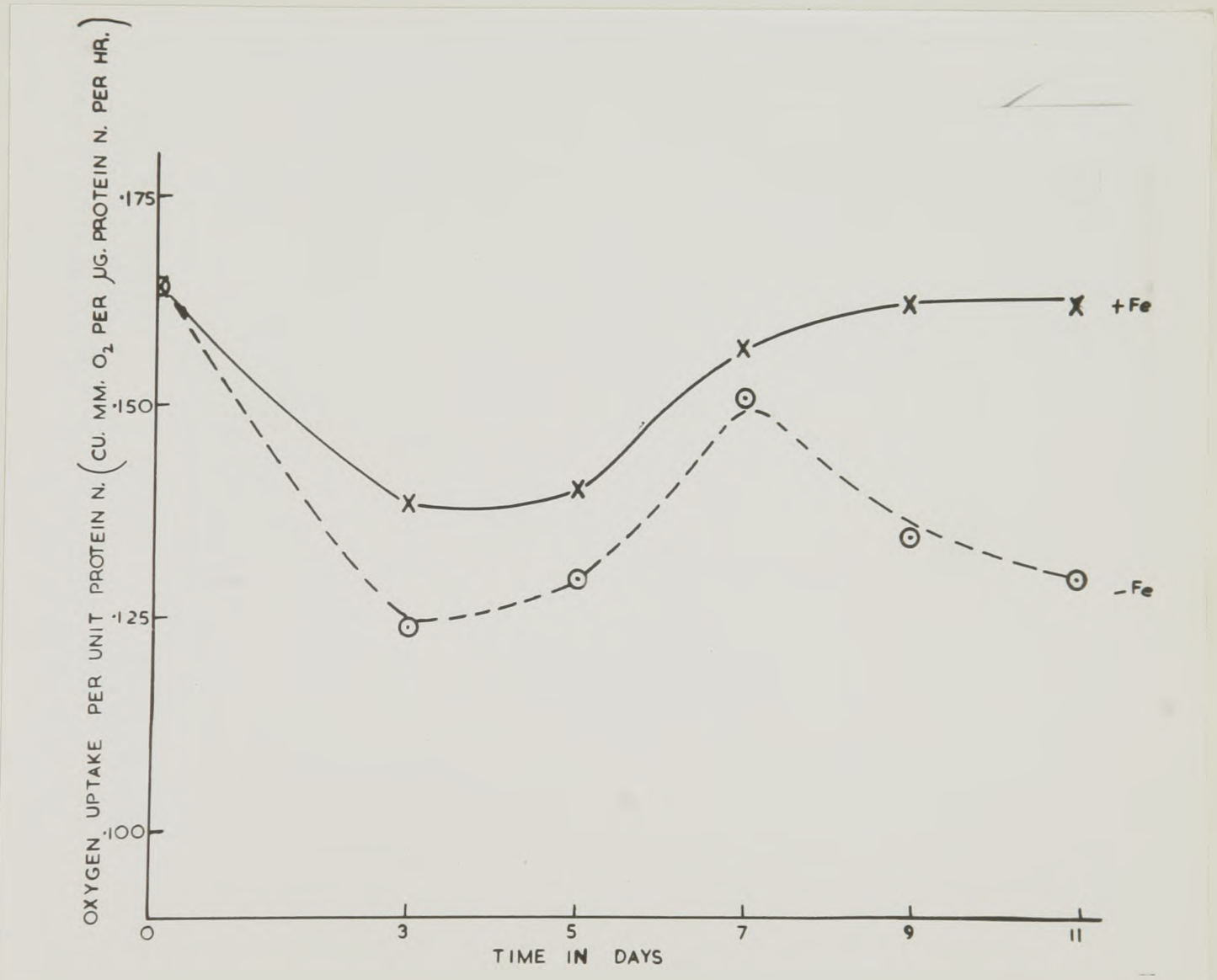
Treatment Difference at	Day 7	Significant at	$P < .05$
" " "	Day 9	" "	$P < .01$
" " "	Day 11	" "	$P < .001$

Figure 4. Change in Rates of Oxygen Uptake with Time in Roots grown in Full Nutrient and in Iron Deficient Media.



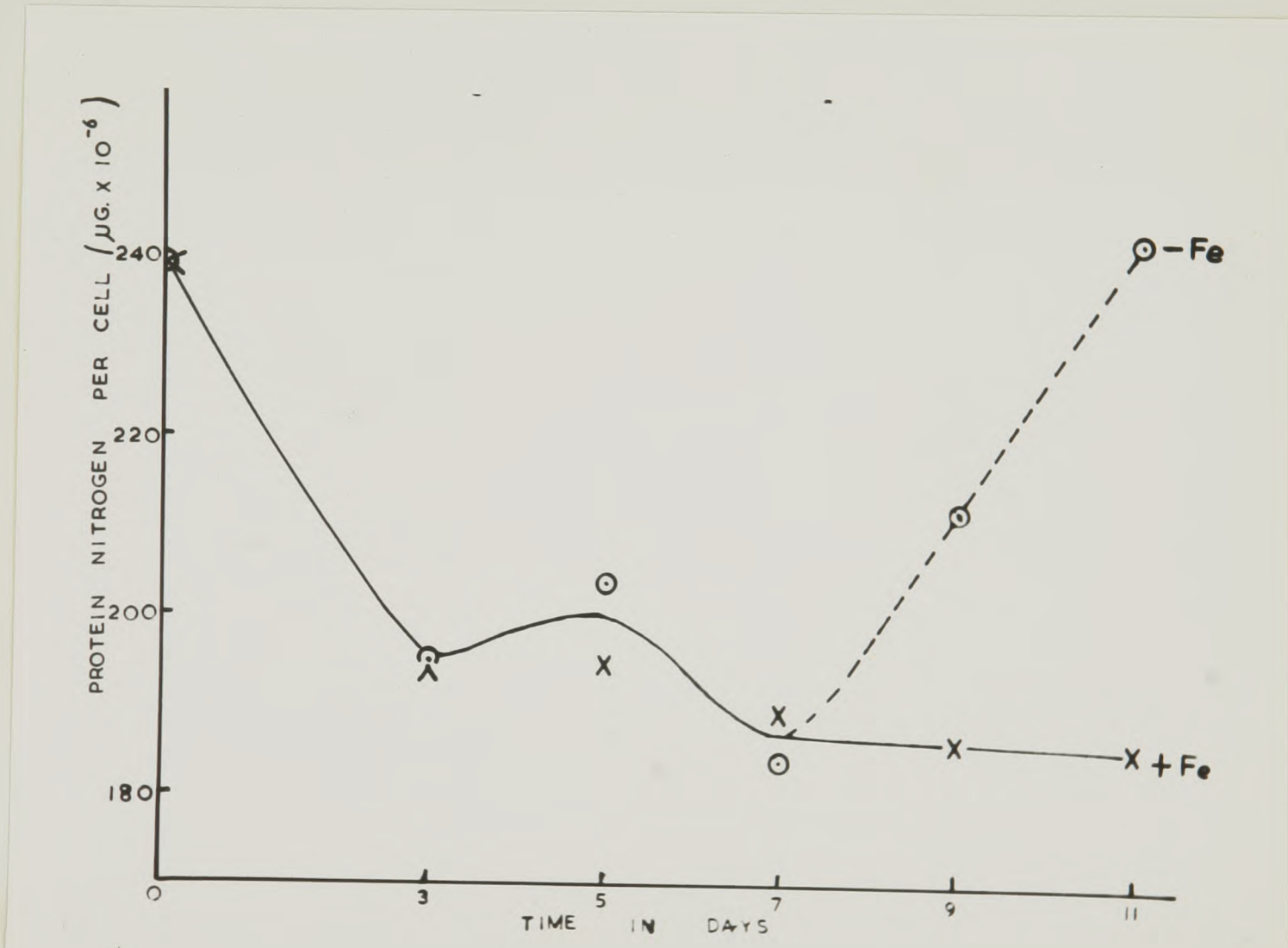
Treatment Difference at	Day 3	Significant at	$P < .01$
" " "	Day 5	" "	$P < .05$
" " "	Day 7	Not Significant	
" " "	Day 9	Significant at	$P < .05$
" " "	Day 11	" "	$P < .001$

Figure 5. Change in Rates of Oxygen Uptake per unit Protein Nitrogen with Time, in Roots grown in Full Nutrient and in Iron Deficient Media.



Treatment	Difference	at	Day 3	Significant at $P < .001$
"	"	"	Day 5	" " $P < .05$
"	"	"	Day 7	Not Significant
"	"	"	Day 9	Significant at $P < .001$
"	"	"	Day 11	" " $P < .001$

Figure 6. Change in the Average Protein Nitrogen Content per Cell with Time in Roots grown in Full Nutrient and in Iron Deficient Media.



Treatment Difference at Day 9 Significant at $P < .05$

" " " Day 11 " " $P < .05$

Figure 7. Change in the Average Rates of Oxygen Uptake per Cell with Time in Roots grown in Full Nutrient and in Iron Deficient Media.

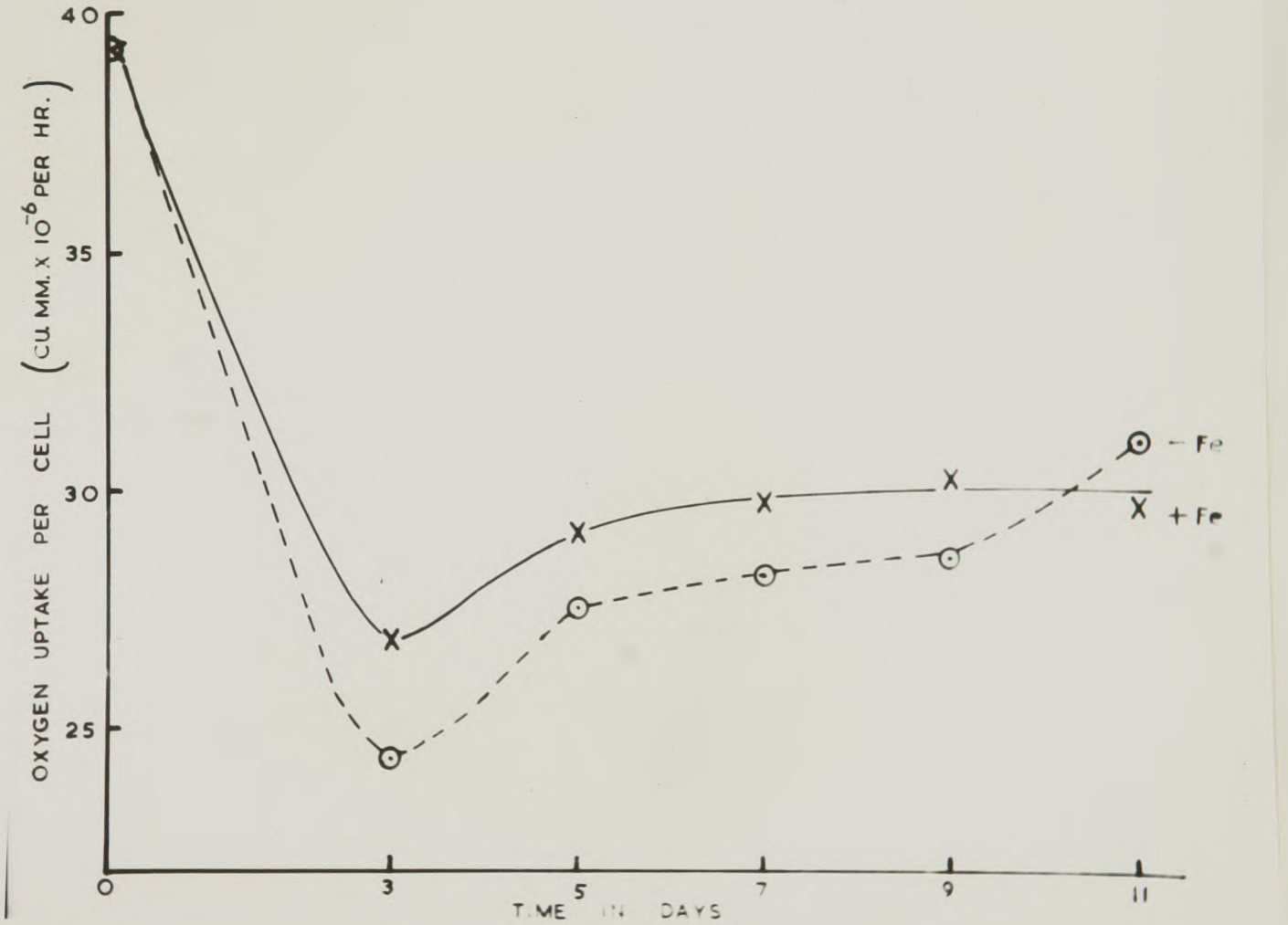
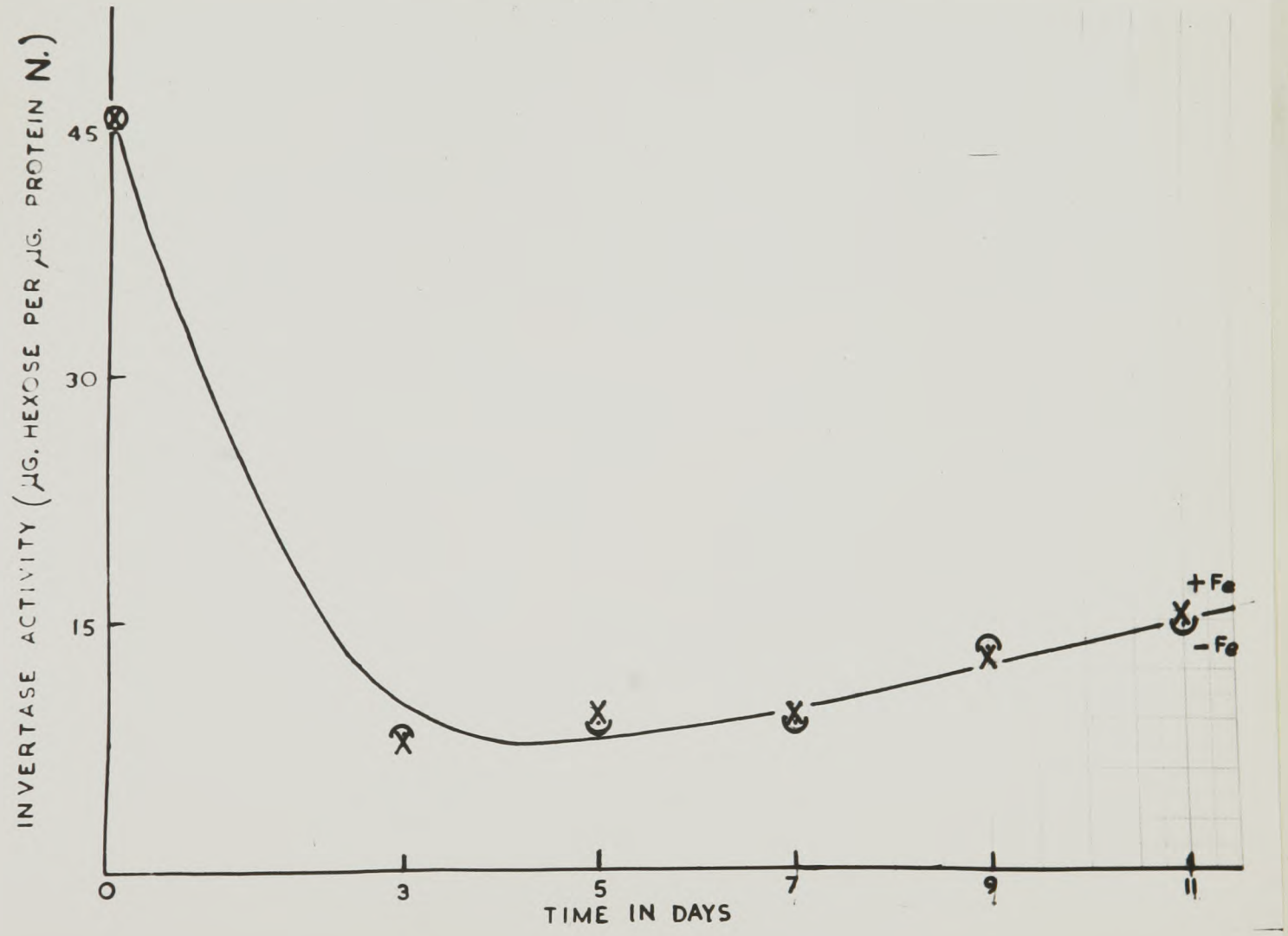


Figure 8. Change in Invertase Activity per Unit Protein Nitrogen with Time in Roots grown in Full Nutrient and in Iron Deficient Media.



(2) Effect of Iron Deficiency on the Longitudinal Differentiation of 7 Day Old Pea Roots

A second series of experiments was carried out using pea roots grown in full nutrient and iron deficient media. The purpose of these experiments was to establish whether the normal differentiation along the length of the roots was altered in the iron deficient group. In these experiments iron deficient and full culture roots were harvested and compared by taking serial one centimetre sections from each commencing at the apex. The corresponding one centimetre sections from the two groups of roots were compared. This method of comparison was a modification of the technique used in the growth studies of Brown and Broadbent (1950). In the present investigation, however, large one centimetre sections were used instead of the small one millimetre fragments of Brown and Broadbent.

The results of the previous series of experiments had shown that after 7 days, roots grown in media lacking iron are unequivocally iron deficient. There was some slight effect at day 5 but day 7 was the first stage when the roots were clearly iron deficient. Roots at this 7 day stage were harvested, they were cut into serial sections and the following measurements were made.-

a) Cell number per section
b) Total volume per section
c) Protein nitrogen content per section
d) Rate of oxygen uptake per section
e) Relative invertase activity per section

From these primary measurements the following quantities for each section were derived.-

f) Average volume per cell
g) Rate of oxygen uptake per unit protein nitrogen
h) Average protein nitrogen content per cell
i) Average rate of oxygen uptake per cell

After a 7 day growing period the length of the iron deficient roots was 6.0 centimetres and that of the full culture roots was 6.60 centimetres. At harvest, five accurately measured one centimetre sections were taken from each root and the remainder of the root was included in section six.

Each experiment harvested in this way usually involved approximately 24 iron deficient and 24 full culture roots. The sections from half the roots were used first to determine a rate of oxygen uptake per section, and then to establish the average protein nitrogen content per section. The diameters of the sections from the rest of the roots were measured using a microscope and this enabled total cell volume per section to be calculated. These root sections were finally placed in chromic acid and used for the estimation of cell number per section. Four replicate experiments with this design were carried out. A further four experiments were harvested by cutting the roots into sections and these sections were used to measure invertase activity. A summary of the results of these eight experiments is given in Table 2 and the complete data are given in Tables 15 to 27 in the appendix. In Figures 9 to 16 the trends along both the full culture and iron deficient roots are given.

Before considering the individual figures it is important to note that sections 1 to 5 are uniformly of one centimetre, but section 6 in the deficient roots is approximately one centimetre and in the full culture roots is approximately 1.6 centimetres. The graphs of the measures of absolute quantities show the differences per unit length in the first 5 sections but also show the total differences per root as section 6 includes all the root tissue beyond the 5.0 centimetre mark.

In roots grown for 7 days in iron deficient media there are fewer cells in sections 1 and 6 than in the corresponding sections of roots grown for a similar period in full nutrient media. The numbers of cells in the remaining sections is the same in the two series. This result is given in Figure 9. The distribution of cells in full nutrient roots is of some interest. The first section which includes the growing point contains approximately 100,000 cells and this is followed by section 2 which contains only about half this number or less than 50,000 cells. From section 2 there is a sharp increase in the cell number per section. Section 5 which is only a centimetre long contains almost 100,000 cells which is the same number as in section 1, and section 6 contains an even greater number of cells. In Figure 10 the average cell volume of the cells in the different sections is presented. The normal trend in cell size is from small cells in the first section to much larger cells in sections 2, 3, 4, 5 and 6. There is a gradual upward trend from section 2 to section 6. In the iron deficient roots the cells in section 1 have a significantly higher average volume than the cells of the corresponding section of full culture roots. Iron deficiency does not alter the average cell volume of the cells in sections 2,3,4,5 and 6.

The distribution of protein nitrogen along the length of the two groups of roots is shown in Figure 11 where the protein nitrogen content per section has been presented. It is known that at the 7 day stage iron deficient roots contain a reduced total amount of protein nitrogen when compared with full culture roots. Figure 11 shows that at this stage there is also a large alteration in the distribution of protein nitrogen along the length of iron deficient roots. The front 4 sections of the roots accumulate nigher than normal amounts while the overall decrease

results from the large reduction in section 6.

By contrast iron deficiency has little effect on the normal trend in the rate of oxygen uptake per section. This is shown in Figure 12 and the only effect of iron deficiency is a reduction in the rate in section 6. The full culture trend is first a decrease from the high rate in section 1 to the very low rate in section 2. Thereafter in sections 3, 4 and 5 there is an increase in the rate which is followed by a larger increase to section 6 which has a very high rate of oxygen uptake. However substantial differences are found between the full culture and iron deficient rates of oxygen uptake when these are expressed in terms of the protein nitrogen content per section. The trend for the rate of oxygen uptake per unit protein nitrogen is given in Figure 13. The rate of the full culture roots declines continuously and steeply from section 1 to section 6. There is a fall in the rate normally along the entire length of the roots. The iron deficient roots have a lowered rate of oxygen uptake per unit protein nitrogen especially in section 1. There are also decreases in the rate in sections 2,3 and 4, but the rates in sections 5 and 6 are the same in both groups of roots. The effect of iron deficiency is most marked in the front three sections and there is little effect in the last two.

The two derived quantities of protein nitrogen content per cell and rate of oxygen uptake per cell in the different sections are shown in Figures 14 and 15. The changed distribution of protein nitrogen in the iron deficient roots is clearly shown in Figure 14 where the average protein nitrogen content per cell is plotted. The average protein nitrogen content of the cells in the front 4 sections is increased, and that of the cells in the last 2 sections is decreased in the iron deficient roots, compared with the contents of

cells in the corresponding sections of control roots. In the control roots the protein nitrogen content per cell is low in section 1 but rises steeply to section 2. There is only a slight increase from section 2 to section 5, but there is a large increase from section 5 to section 6. Figure 15 shows the average rate of oxygen uptake per cell in the different sections of the two groups of roots. The trend of the full culture roots is complex but the first change is from a low rate in section 1 to a much higher rate in section 2. Thereafter there is a steady decline to section 5 and a final inflection in the curve results from the high rate of the cells in section 6. In the iron deficient roots cells in sections 1,2,3 and 4 have a higher rate of oxygen uptake but those in sections 5 and 6 have a lower rate than those in the corresponding sections of the full culture roots.

The characterization of the two groups of roots in terms of the relative invertase activity of the different sections is shown in Figure 16. It is clear that in the full culture roots there is a sharp downward trend in invertase activity from section 1 to section 2 but thereafter there is a more gradual decrease to section 5. In the iron deficient roots there is an indication of a slight overall reduction in invertase activity, and this reduction is approximately of the same order along the entire length of the roots. The reduction is not significant as the differences in total invertase activity per root are only very slight at the 7 day stage.

TABLE 2.

± IRON ROOTS - SUMMARY OF SECTIONED ROOT DATA

Mean values from an experimental series which involved treatments of ± iron, a 7 day growing period and harvests by sectioning.

Section		1.	2.	3.	4.	5.	6.
Cell No. /Section	-Fe	85,552	47,842	54,479	74.360	97,809	116,663
	+Fe	101,689	45,774	55.387	71,503	88.785	158,901
Average Cell Volume (cu.mm x 10^{-6})	-Fe	44.14	81.74	95.17	93.84	113.41	117.96
	+Fe	31.74	84.50	95.08	103.65	109.65	110.86
Protein N /Section (µg)	-Fe	13.77	7.89	10.15	13.62	16.89	26.09
	+Fe	11.29	7.02	8.70	12.12	16.44	41.68
O_2 Uptake /Section (cu.mm/hr)	-Fe	2.579	1.551	1.685	2.040	2.265	3.852
	+Fe	2.644	1.576	1.644	1.890	2.178	5.387
O_2 Uptake /unit Prot.N. (cu.mm/hr /µg. Prot.N)	-Fe	.1871	.1970	.1660	.1519	.1390	.1273
	+Fe	.2349	.2251	.1884	.1587	.1324	.1297
Prot.N. /Cell (µg. x 10^{-6})	-Fe	164.2	168.9	188.8	185.6	173.3	240.6
	+Fe	111.3	150.1	153.3	168.9	188.7	273.6
O_2 Uptake /Cell (cu.mm x 10^{-6}/hr)	-Fe	30.01	34.07	32.04	28.65	23.56	31.85
	+Fe	26.28	33.63	27.97	25.69	24.52	34.65
Invertase Activity (µg. hexose /µg. Prot. N)	-Fe	15.966	9.766	9.378	7.451	6.817	6.375
	+Fe	18.293	10.757	8.963	9.945	8.282	7.111

Figure 9. Numbers of Cells in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and in Iron Deficient Media.

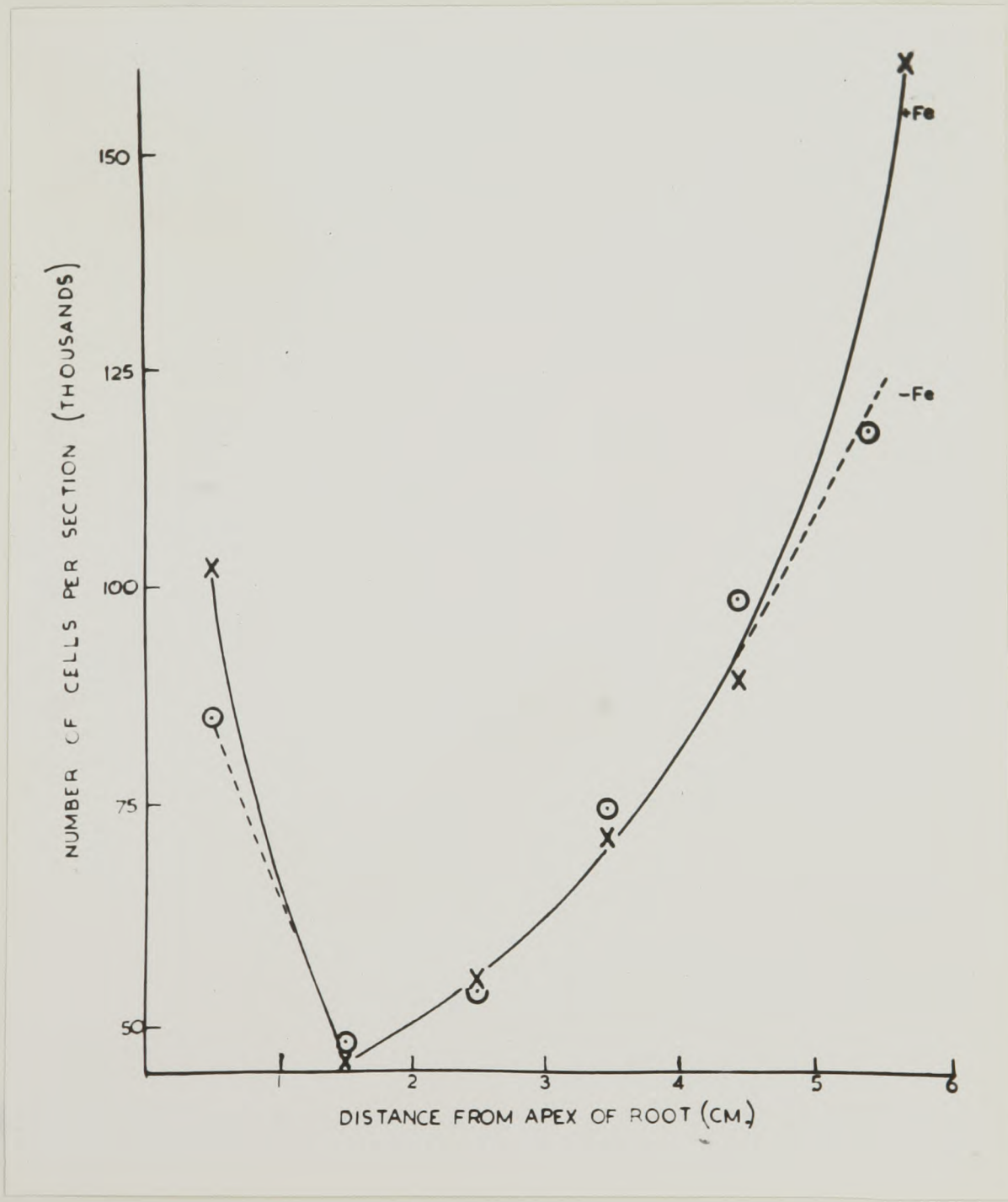
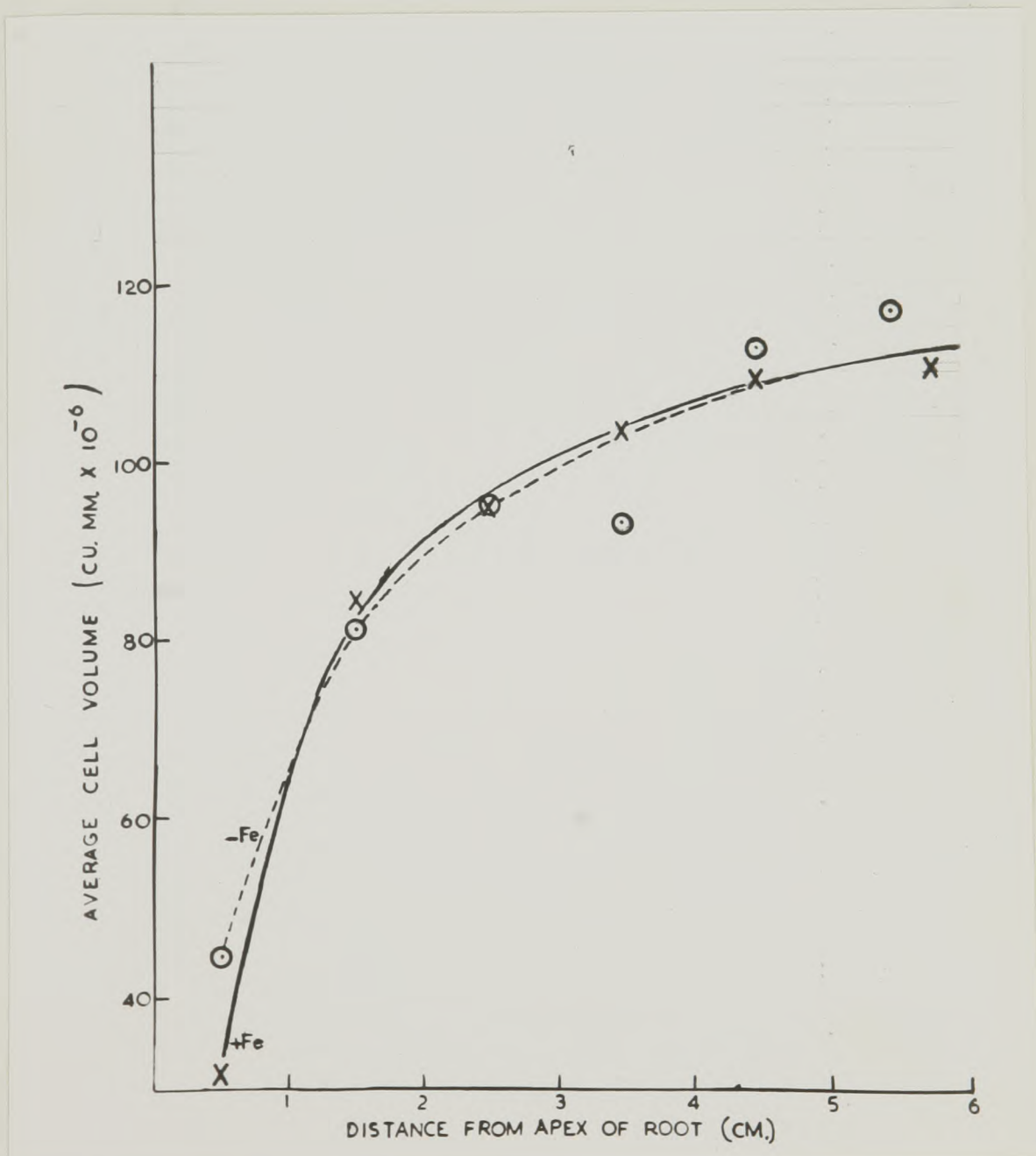
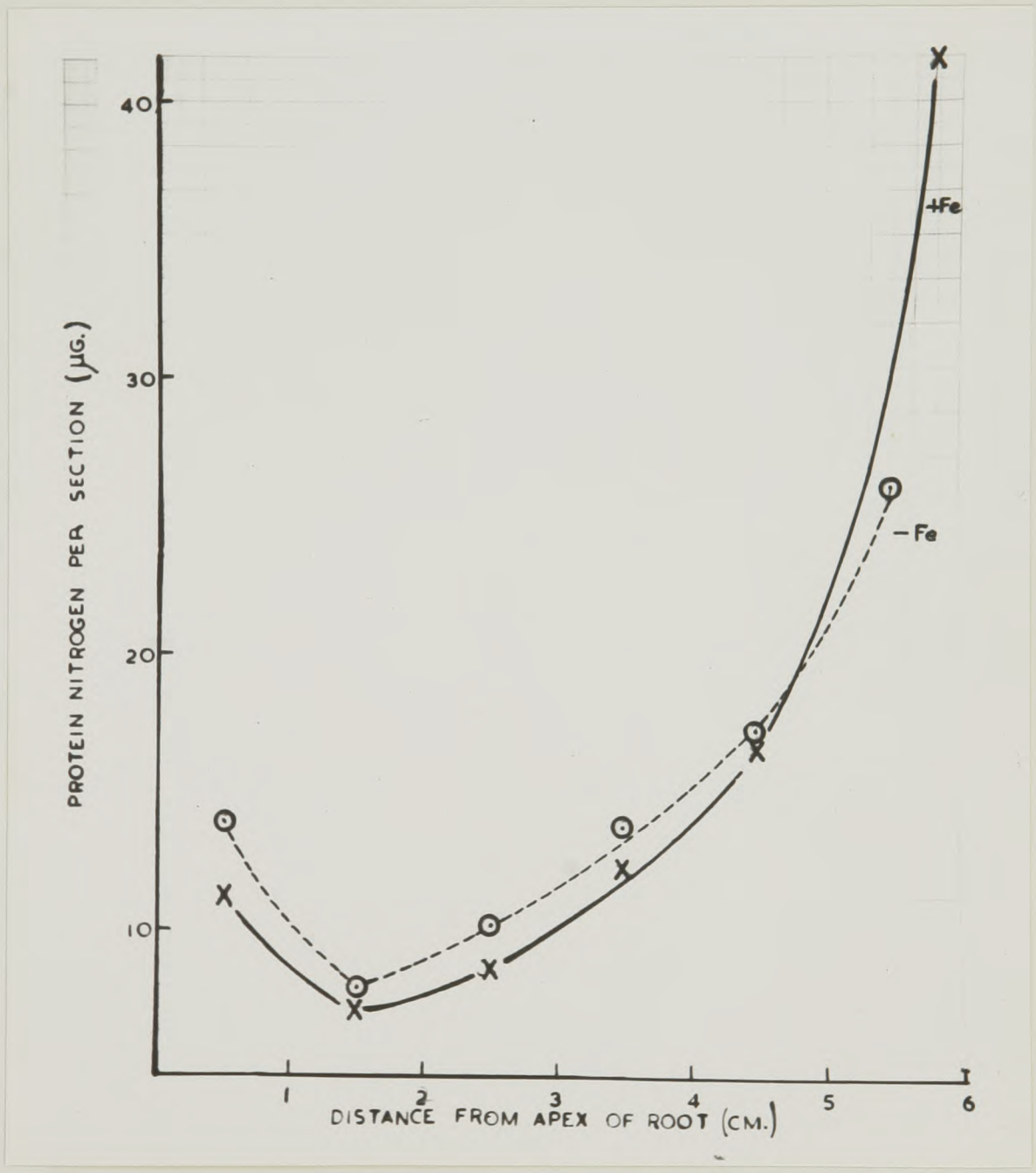


Figure 10. Average Volumes per Cell in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and in Iron Deficient Media.



Treatment Difference in Section 1. Significant at $P < .05$

Figure 11. Protein Nitrogen Contents in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and in Iron Deficient Media.



Treatment Difference in Section 1. Not Significant
" " " Section 2 Significant at $P < .05$
" " " Section 3 " " $P < .05$
" " " Section 4 " " $P < .05$

Figure 12. Rates of Oxygen Uptake in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and in Iron Deficient Media.

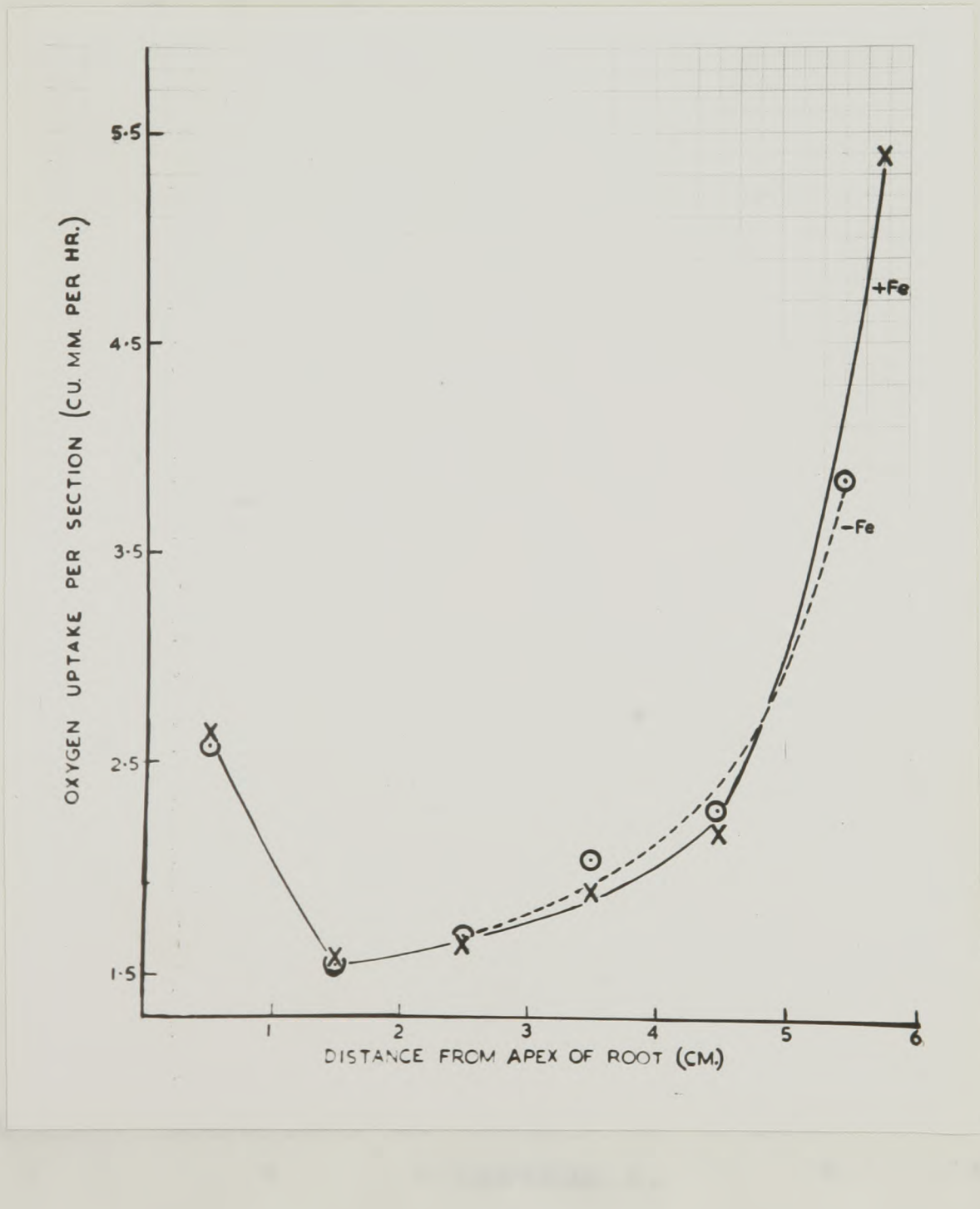
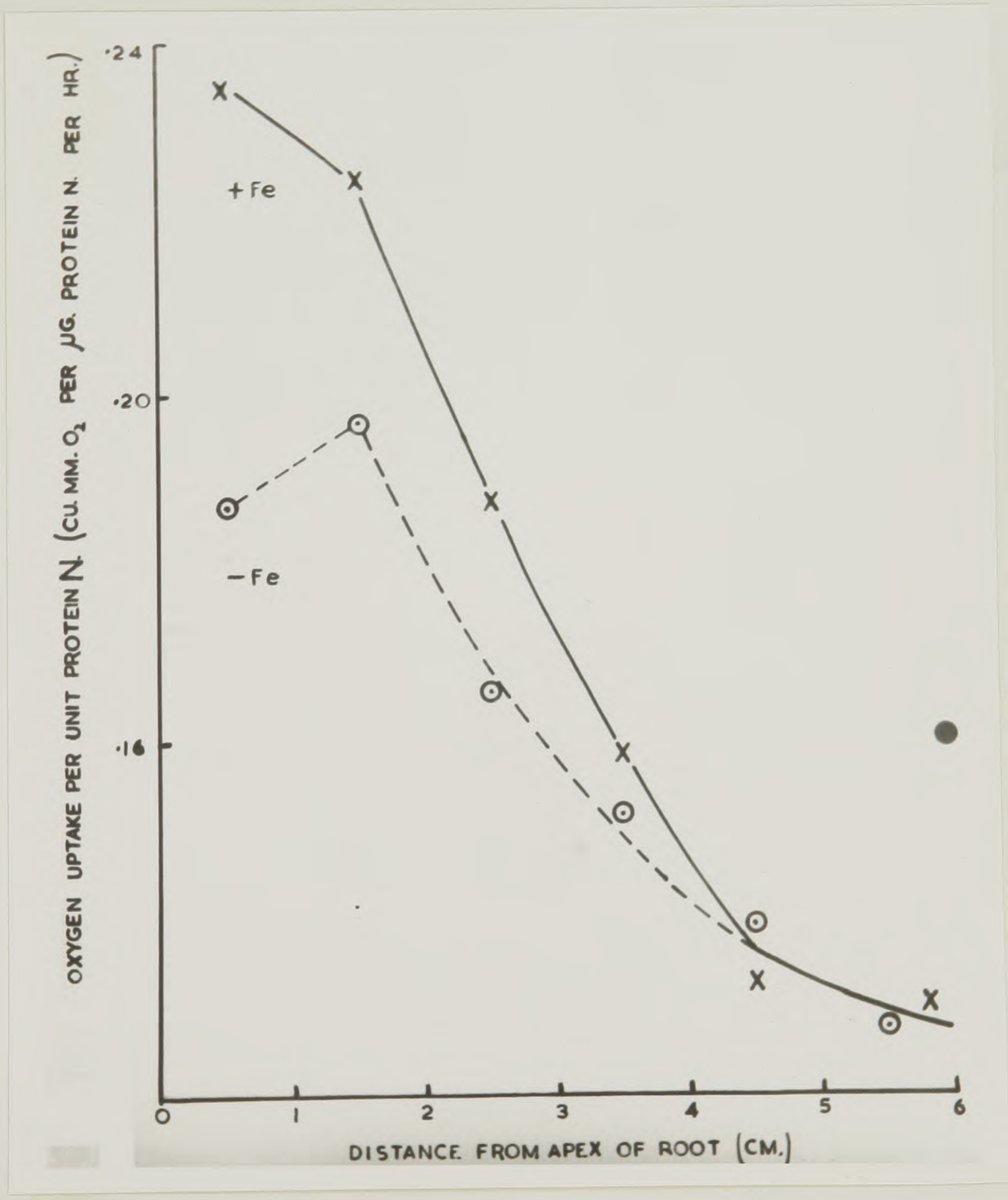


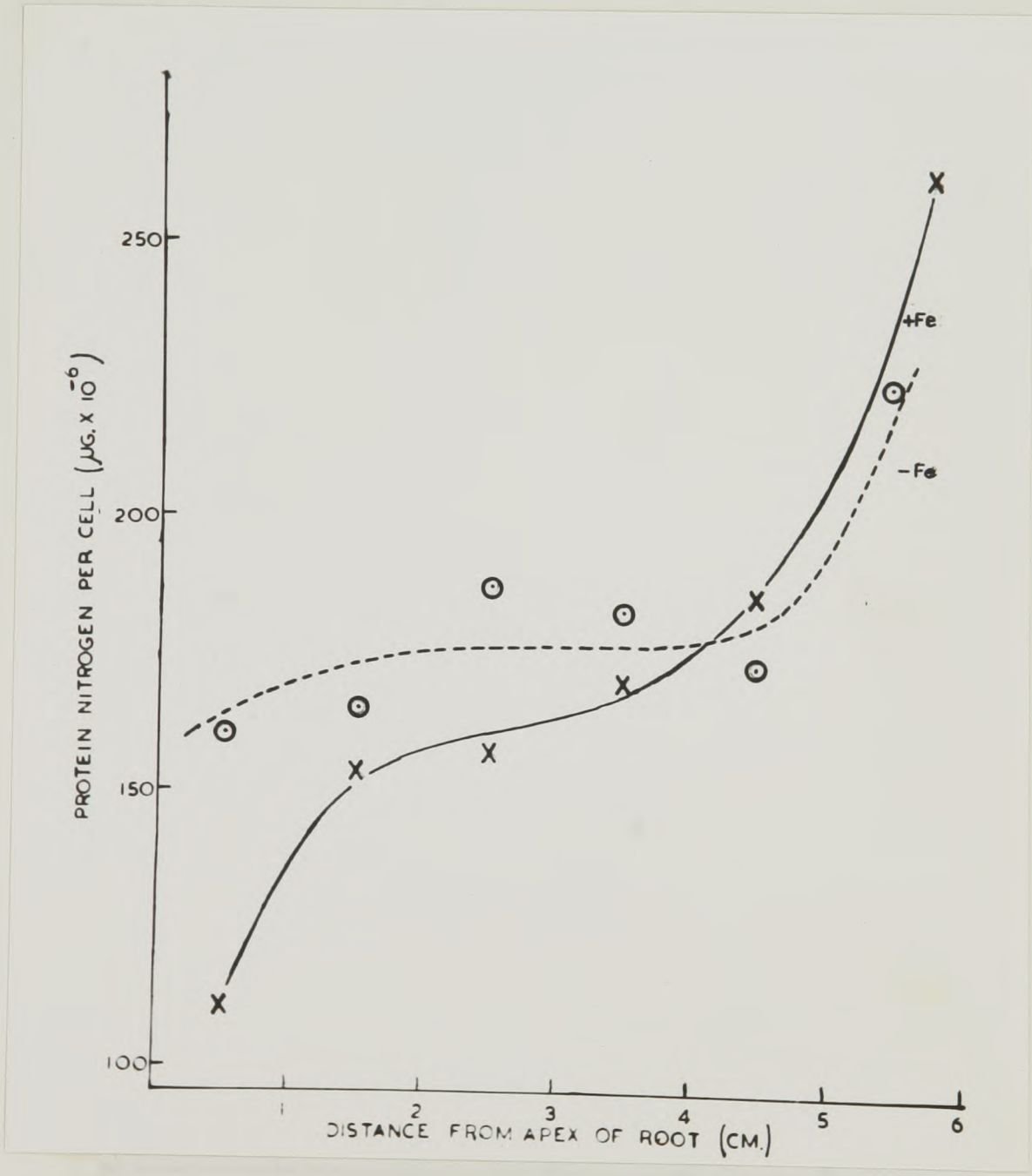
Figure 13. Rates of Oxygen Uptake per Unit Protein Nitrogen in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and in Iron Deficient Media.



Treatment Difference in Section 1. Significant at $P < .05$

" " " Section 2. " " $P < .05$

Figure 14. Average Protein Nitrogen Contents per Cell in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and in Iron Deficient Media.



Treatment Difference in Section 1. Significant at $P < .001$
" " " Section 2. Not Significant
" " " Section 3. Significant at $P < .01$

Figure 15. Average Rates of Oxygen Uptake per Cell in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Iron Deficient Media.

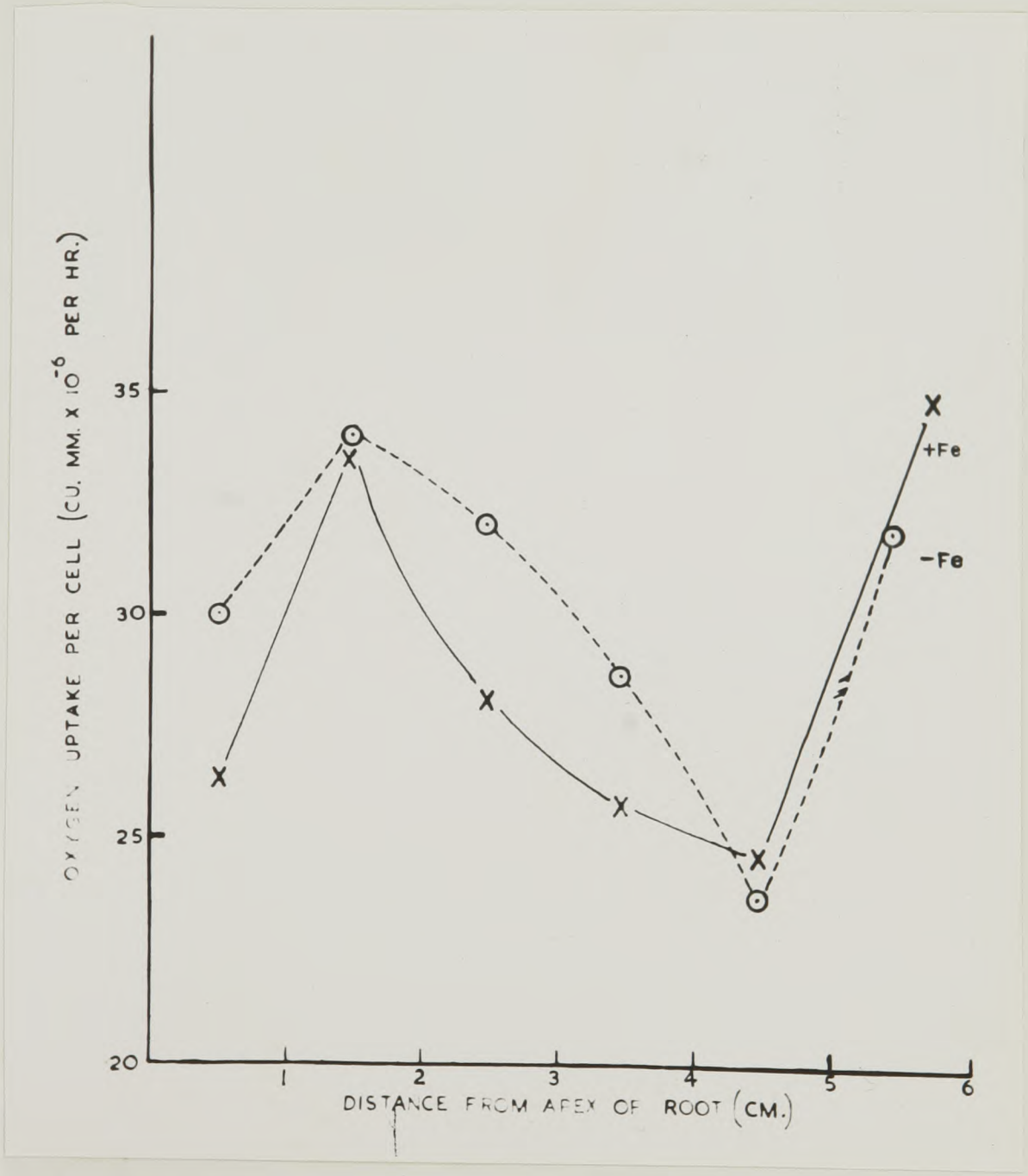
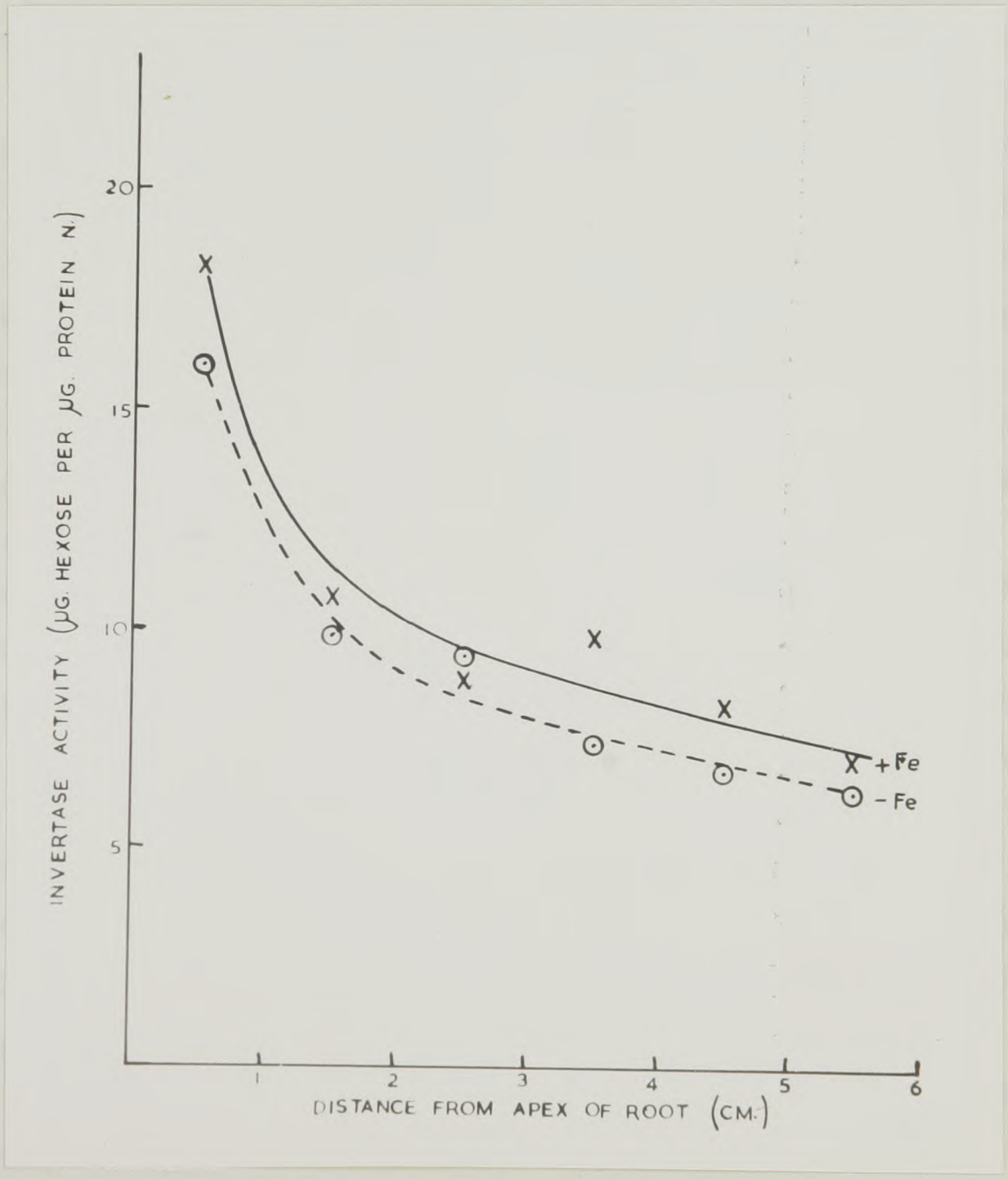


Figure 16. Invertase Activity per Unit Protein Nitrogen in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Iron Deficient Media.



(3) Sensitivity to Cyanide of the Oxygen Uptake of Pea Roots Grown in Full Nutrient and Iron Deficient Cultures

It is well known that iron is a component of the cytochrome system and this system has been shown to be quantitatively an important constituent of the terminal enzyme complement of pea roots (Fritz and Beevers 1955). This third series of experiments was carried out with a view to assessing the relative importance of the cytochrome system in roots grown with and without added iron. It was considered that the sensitivity of the oxygen uptake to inhibition by cyanide would give an indication of the proportion of the oxygen uptake catalysed by heavy metal systems. The most notably cyanide sensitive system is the cytochrome / cytochrome oxidase system and if this system was either reduced or replaced by another less sensitive to cyanide system in the iron deficient roots, then there might be a different sensitivity to cyanide with respect to oxygen uptake, in the two nutritional groups.

Preliminary experiments were conducted to ensure that a reproducible concentration of cyanide could be maintained in the Warburg flasks, and it was found that the best results were obtained if the roots were pretreated with cyanide for an hour prior to placing them in flasks which contained cyanide both in the external solution and in the centre well. Experiments were conducted using cyanide concentrations of 10^{-4} and 10^{-3} molar and the sensitivity of the oxygen uptake of roots cultured in the presence and absence of iron for periods of 0,3,5,7,9 and 11 days was established. In addition iron deficient and full culture roots were examined after seven days and the sensitivity of the oxygen uptake to 10^{-3} molar cyanide in the different sections was measured.

The results of inhibiting the oxygen uptake of whole roots are given in Figure 17 where the effects of 10^{-4} M. KCN are shown and in Figure 18 where the effects of 10^{-3} M. KCN are presented, while the actual values are presented in Tables 28 and 29 in the appendix. The rates of oxygen uptake of roots grown in full nutrient and in iron deficient media have been measured in the presence and absence of cyanide. In general terms the rates in the absence of cyanide were similar to those previously established, with the exception that the rate of iron deficient roots at day 3 was not here significantly lower than the rate of three-day full nutrient roots. It is clear that both concentrations of cyanide reduce the rate of oxygen uptake. The effect was greater when 10^{-3} M. KCN was used and the magnitude of the effect varied with the age of the roots.

In Figure 19 the relative effect of the two concentrations of cyanide on the rates of oxygen uptake are shown in terms of percentage inhibition. Considering first the effect of 10^{-3} M. KCN on the full nutrient roots there is clearly a pronounced trend with time. The initial one centimetre tips as they are first cut from the germinated seeds are very sensitive to cyanide and their rate of oxygen uptake is inhibited about 60%. However, there is a steep fall in the percent inhibition between days 0 and 5 after which it remains relatively constant with approximately 20% of the oxygen uptake being inhibited by this concentration of cyanide. The iron deficient roots show the same trend as the full nutrient roots when the results are expressed as relative inhibitions. In the experiments where 10^{-4} M. KCN was used the percent inhibitions of the oxygen uptake are lower, and it is clear that this concentration of inhibitor does not fully saturate the cyanide sensitive system. There is, however, a similar downward

trend with time, and once again the percent inhibitions of the oxygen uptake of iron deficient and full nutrient roots are the same.

In Figures 20 and 21 the results of these experiments have been presented in another form. Here the proportion of the oxygen uptake mediated by the cyanide insensitive and by the cyanide sensitive systems respectively are shown for each occasion. The rate of oxygen uptake in the presence of cyanide provides a measure of the activity of the cyanide insensitive system, and the difference between this and the uninhibited rate a measure of the cyanide sensitive system.

The results shown in Figure 21 are considered first as 10^{-3} M. KCN more nearly saturates the cyanide sensitive systems present in this root material, and so gives a more reliable estimate of their importance. The activity of the cyanide sensitive system declines from day 0 to day 5 in both groups of roots. In the full nutrient roots this system then increases steadily from day 5 to day 11 but in the iron deficient roots there is only a very slight increase in the activity of this system over the same period. The cyanide insensitive system, on the other hand, shows no change in activity between day 0 and day 3 in both groups of roots but thereafter increases considerably in both. There is almost a linear increase in the activity of this system in the full nutrient roots, but the increase is very much less in the iron deficient roots.

In Figure 20 the results of the experiments involving 10^{-4} M. KCN are presented in the same way. Here the activity of the cyanide sensitive system in both groups of roots shows little change between days 0 and 5 and thereafter in both groups declines to zero at days 9 and 11. The

activity of the cyanide insensitive system declines slightly between days 0 and 5 in both but rises steeply between days 5 and 11. The increase after day 5 is much greater in the full nutrient roots.

Figures 22, 23 and 24 present the mean results from two experiments in which the oxygen uptake per section of seven day old roots grown in full nutrient and in iron deficient media was measured in air and in the presence of 10^{-3} M. cyanide. The actual values are presented in the appendix in Table 30. The rates of oxygen uptake per section of both groups of roots in air are reasonably similar to the previously established rates for similar seven day old sectioned material. The roots used in these particular experiments were slightly shorter than before, especially the iron deficient sample. This is the reason for the low rate of oxygen uptake in section 6 of the deficient series.

The percent inhibitions presented in Figure 23 show that there is a far higher sensitivity of the oxygen uptake to cyanide in sections 1 and 2 than in the rest of the root. There is also a slightly increased sensitivity in section 6 when compared with section 3,4 and 5. There is no difference between the percent inhibitions obtained with full nutrient and with iron deficient roots.

In Figure 24 the relative proportion of the oxygen uptake catalysed by cyanide sensitive and cyanide insensitive systems in the different sections is shown. In both groups of roots the activity of the cyanide sensitive system is high in section 1 and declines to a lower value in sections 2,3,4 and 5. In the plus iron roots there is a pronounced rise in section 6. The amount of the oxygen uptake due to the cyanide insensitive system rises from section 2 to section 5

in both groups of roots. The rise continues to section 6 in the full nutrient roots but declines in the iron deficient roots. The low values in section 6 of the iron deficient roots for both the cyanide sensitive and insensitive systems reflects mainly the small amount of tissue in this section.

Figure 17. Changes in Rates of Oxygen Uptake, measured in the Presence and Absence of 10^{-4} Molar KCN, with Time in Roots grown in Full Nutrient and Iron Deficient Media.

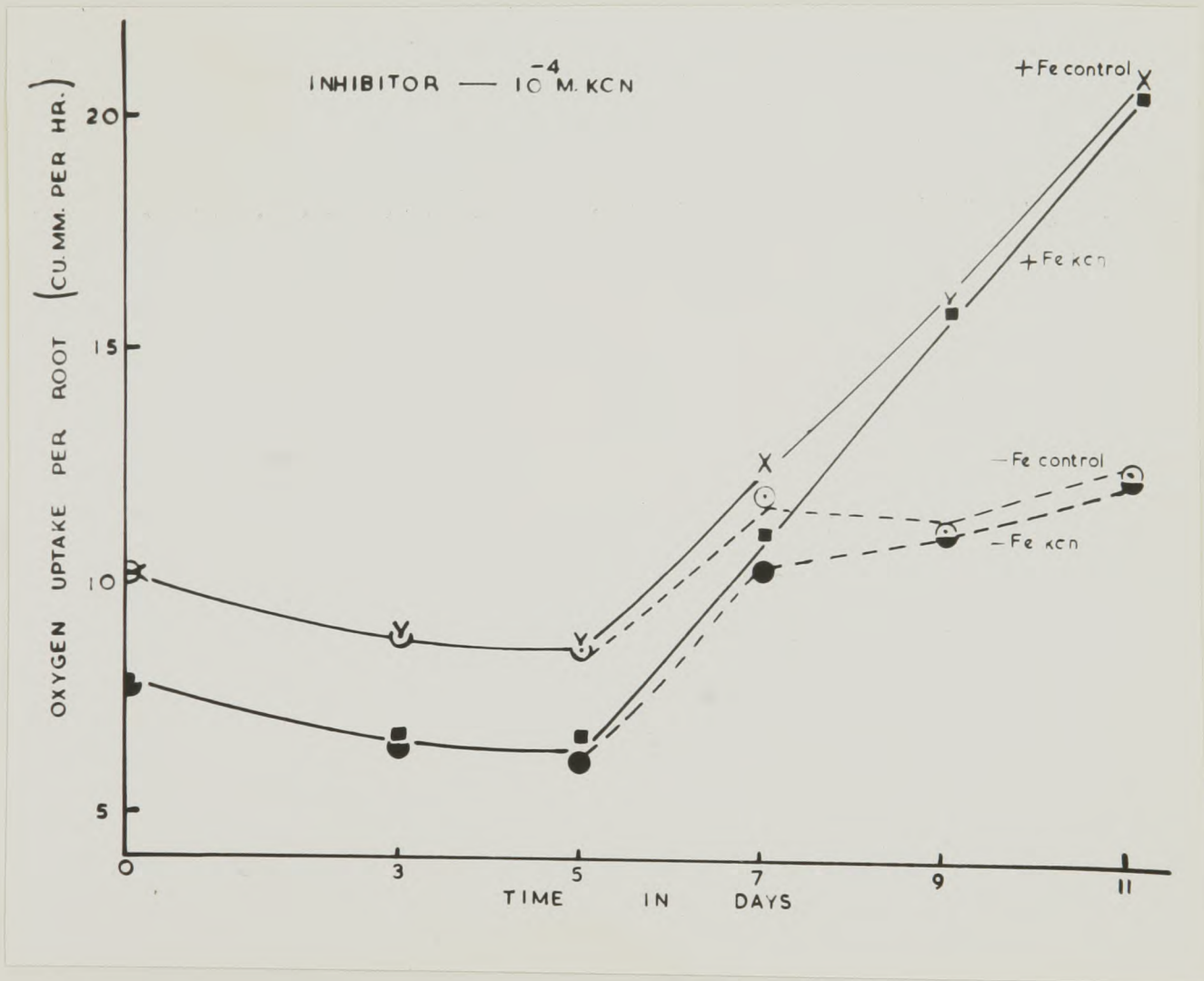


Figure 18. Changes in Rates of Oxygen Uptake, measured in the Presence and Absence of 10^{-3} Molar KCN, with Time in Roots grown in Full Nutrient and Iron Deficient Media.

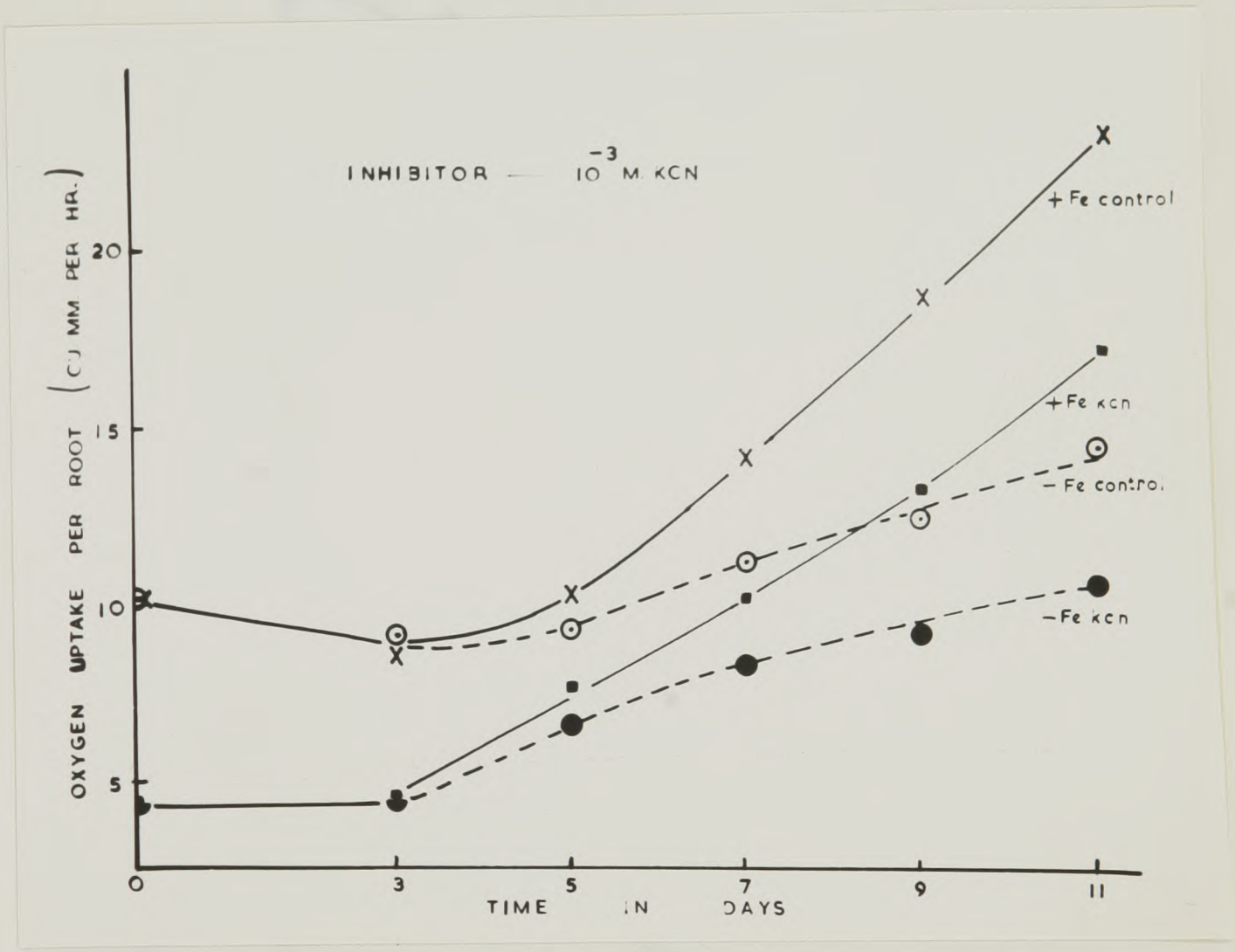


Figure 19. Changes in Percent Inhibitions of the Rate of Oxygen Uptake, by 10^{-4} and 10^{-3} Molar KCN, with Time in Roots grown in Full Nutrient and Iron Deficient Media.

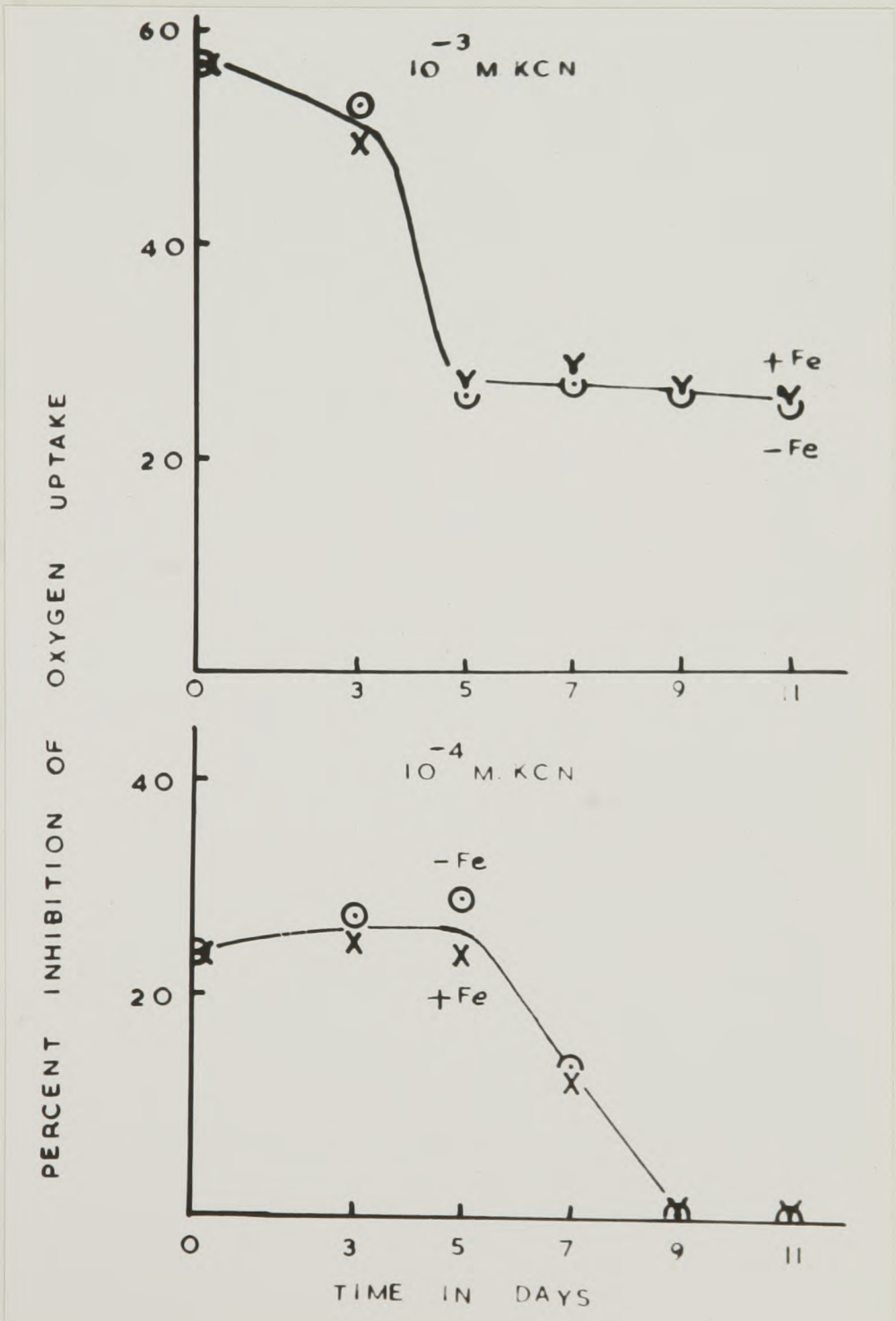


Figure 20. Changes in the Rates of Oxygen Uptake Mediated by the Systems Sensitive and Insensitive to 10^{-4} Molar KCN with Time in Roots grown in Full Nutrient and Iron Deficient Media.

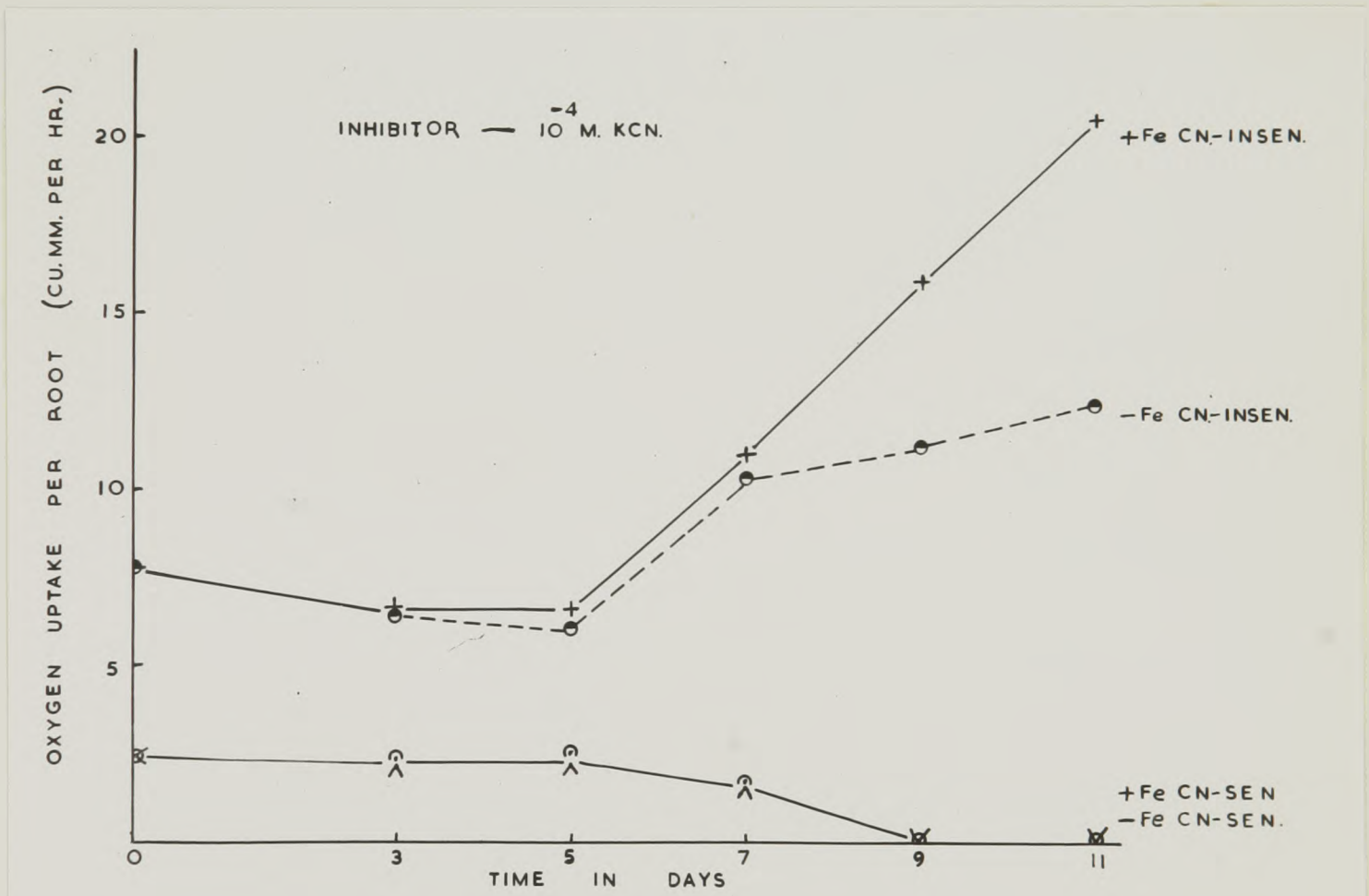


Figure 21. Changes in the Rates of Oxygen Uptake Mediated by the Systems Sensitive and Insensitive to 10^{-3} Molar KCN with Time in Roots grown in Full Nutrient and Iron Deficient Media.

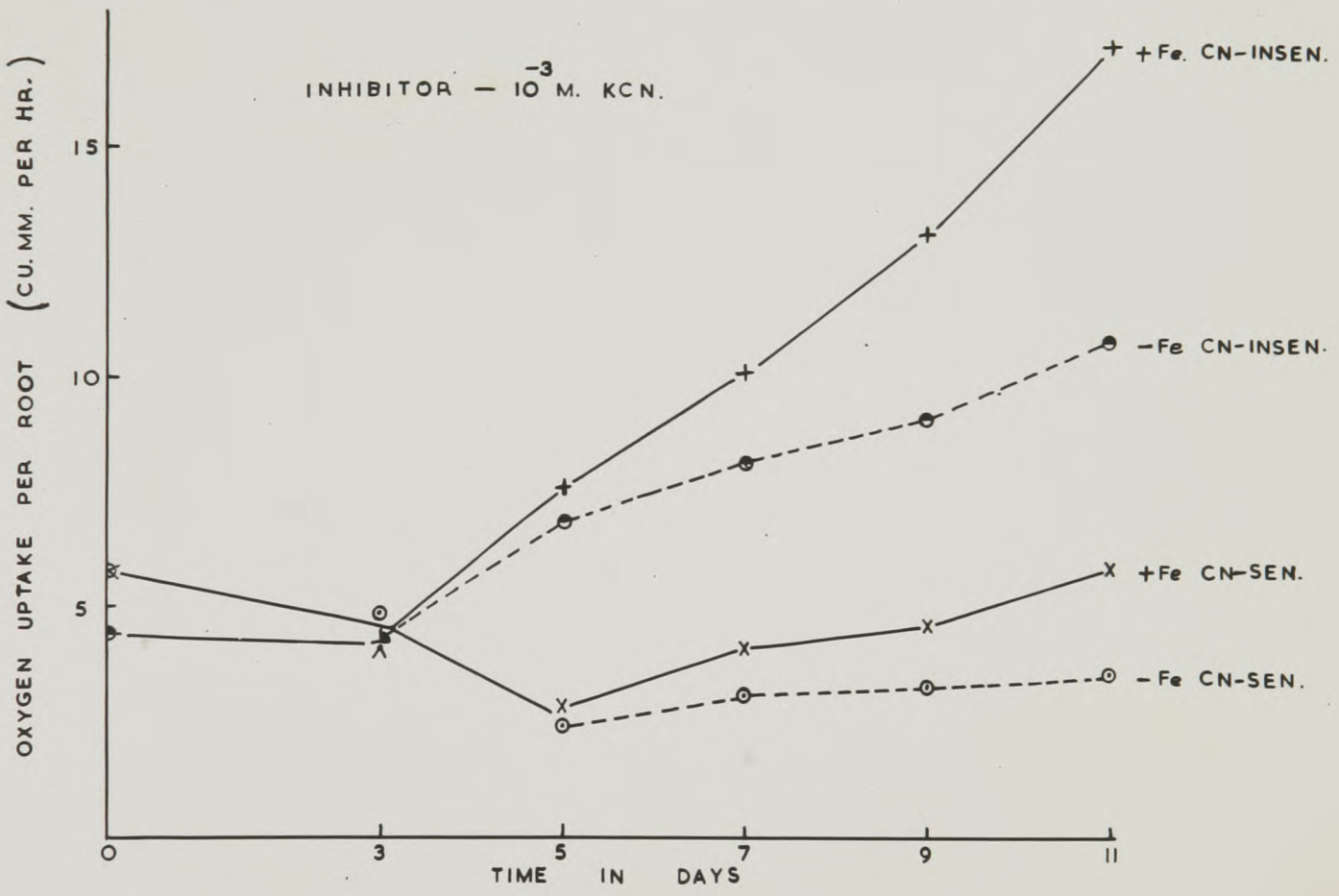


Figure 22. Rates of Oxygen Uptake, measured in the Presence and Absence of 10^{-3} Molar KCN, in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Iron Deficient Media.

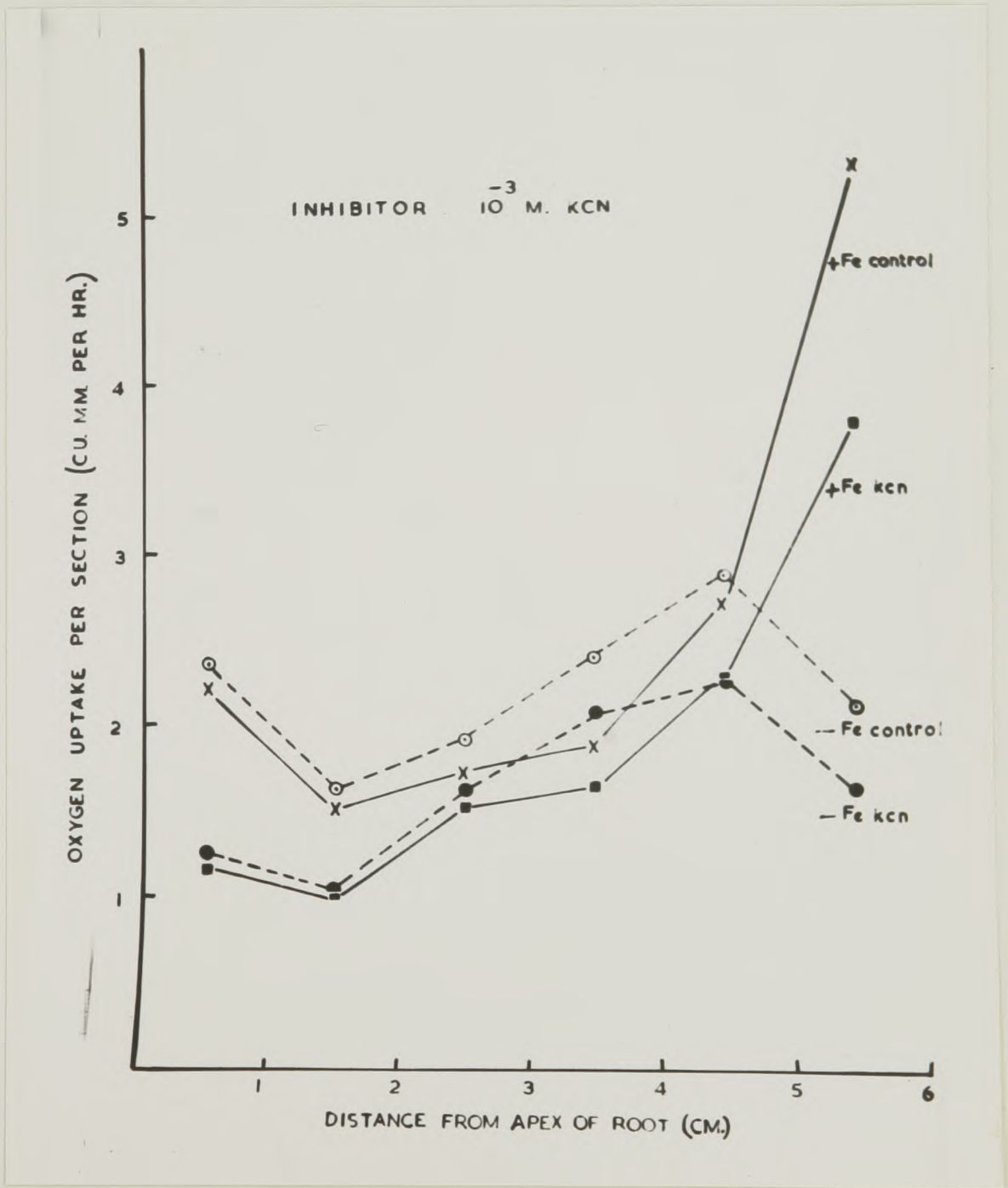


Figure 23. Percent Inhibitions by 10^{-3} Molar KCN of the Rates of Oxygen Uptake in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Iron Deficient Media.

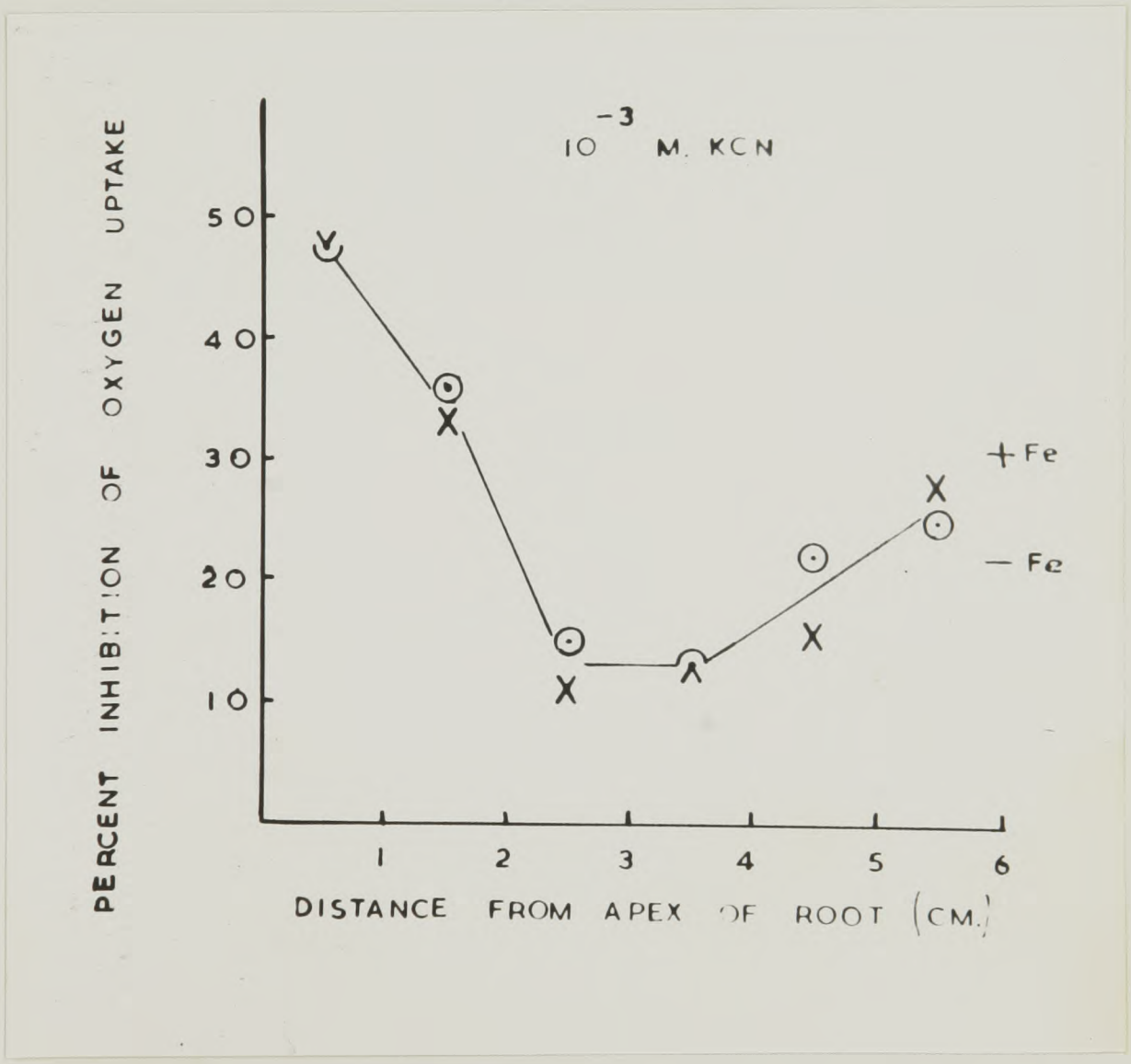
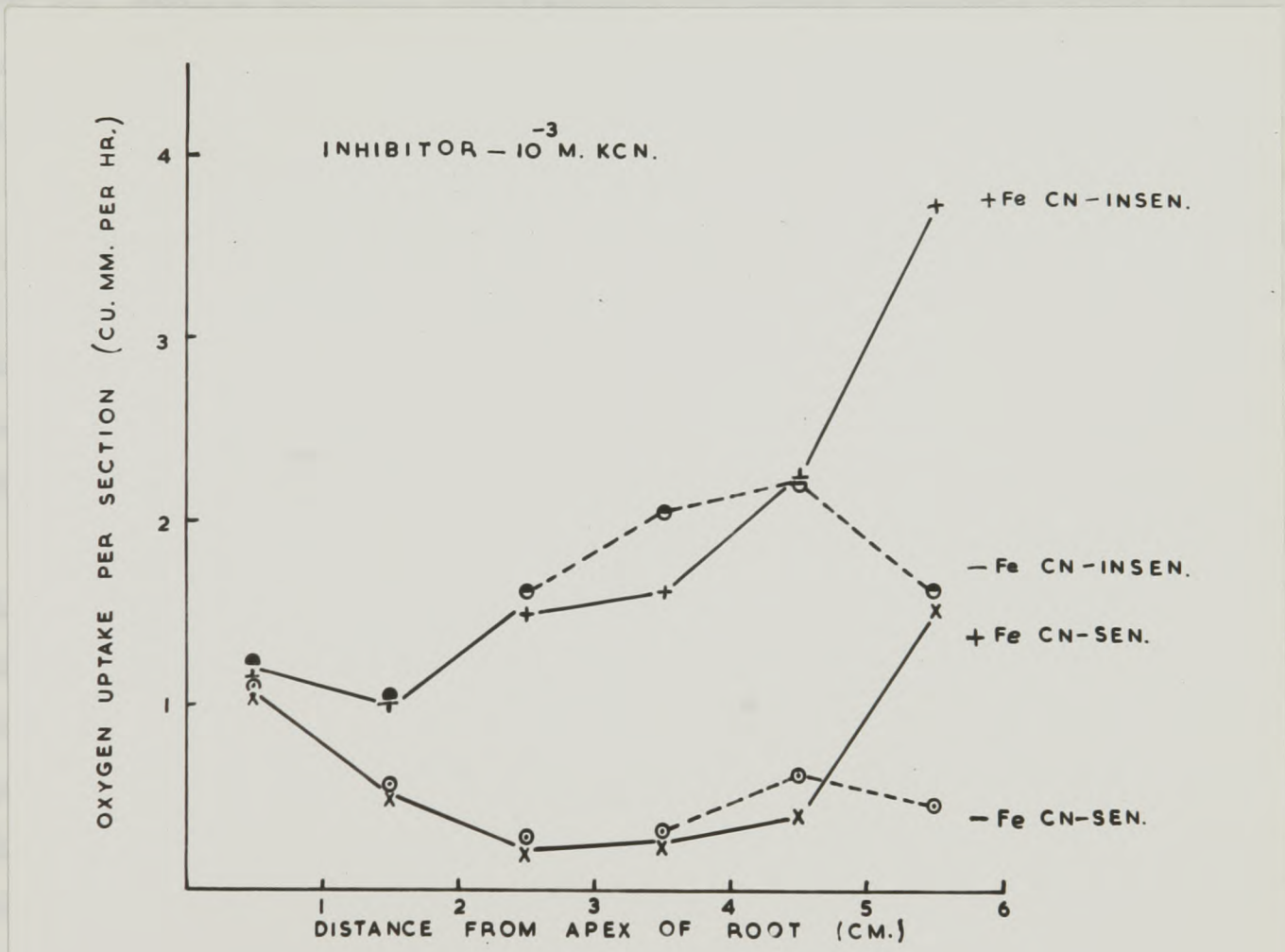


Figure 24. Rates of Oxygen Uptake mediated by the Systems Sensitive and Insensitive to 10^{-3} Molar KCN in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Iron Deficient Media.



(4) Relative Frequency of Mitotic Figures in the Tips of Full Nutrient and Iron Deficient Pea Roots.

The earlier experiments have quite clearly established that in the absence of iron, cell division virtually stops at or about the seven day stage. The following observations were made to determine whether cell division is interrupted at any particular stage of the mitotic cycle. Root tips from iron deficient and full culture roots were examined after seven and eleven days' growth, and the relative numbers of cells in the different mitotic phases were counted. The results of this survey are presented in Table 3 where the number of cells of each mitotic phase has been presented as a percentage of the total count of nonvacuolated cells.

A t day 7 in the iron deficient root tips the numbers of cells in prophase and metaphase are reduced to a half, and the numbers of cells in telophase and anaphase are reduced to a quarter of those present in the corresponding mitotic stages in the full nutrient root tips.

In iron deficient root tips after 11 days the number of cells containing mitotic figures is relatively less than the number in iron deficient tips from seven day old roots and also is less than the number in eleven day full nutrient tips. The eleven day iron deficient tips contain practically no cells in telophase and anaphase but some prophase and metaphase cells are present. The percentage of cells in the different mitotic stages is very similar in both seven and eleven day full nutrient tips. However, although no actual counts were made, it was observed that the total number of nonvacuolated cells was much reduced in iron deficient tips when compared with the numbers present in full nutrient tips.

TABLE 3. Frequency of Mitotic Figures in the Non-vacuolated Cells of Root Tips of ± Iron Roots

7 Day Old Root Tips

	Interphase		Prophase		Metophase		Anaphase		Telophase	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Numbers of Each Division Stage										
Sample (1)	1610	1570	20	27	9	19	2	6	2	5
(2)	1482	1750	14	39	6	22	2	15	2	10
(3)	1541	1620	17	34	10	20	3	13	2	12
Percentage of Each Division Stage										
Sample (1)	97.991	96.496	1.217	1.659	.548	1.168	.122	.369	.122	.307
(2)	98.406	95.315	.930	2.124	.398	1.198	.133	.817	.133	.545
(3)	97.965	95.350	1.081	2.001	.636	1.177	.191	.765	.127	.706
MEAN	98.131	95.720	1.076	1.928	.527	1.181	.149	.650	.127	.519

11 Day Old Root Tips

	Interphase		Prophase		Metophase		Anaphase		Telophase	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Numbers of Each Division Stage										
Sample (1)	1256	1571	6	24	4	17	0	7	1	8
(2)	1587	1594	4	31	1	22	0	11	0	9
(3)	1463	1633	5	33	2	26	0	13	0	7
Percentage of Each Division Stage										
Sample (1)	99.131	96.558	.473	1.475	.316	1.045	-	.430	.079	.492
(2)	99.685	95.620	.251	1.860	.063	1.320	-	.660	-	.540
(3)	99.523	95.385	.340	1.928	.136	1.519	-	.759	-	.409
MEAN	99.446	95.854	.355	1.754	.172	1.295	-	.616	.026	.480

(5) Iron Recovery Experiments

Three experiments were carried out to establish whether iron deficient roots were capable of responding to the addition of iron. In these experiments all the roots were cultured initially in iron deficient media, and then after a given time half of the roots were transferred to media containing iron while the other half were transferred to a fresh sample of iron deficient media. Roots were grown for 7, 9 and 11 days in the absence of iron and 4 days were allowed for recovery after they had been transferred to fresh culture solutions. The results of these experiments are presented in Table 4

In the first experiment, iron deficient roots seven days old were transferred to plus and minus iron media and after 4 days they were harvested. Measurements of length, total protein nitrogen and oxygen uptake per root were made. There was clearly a recovery of the iron deficient roots which had received iron in this experiment. These roots were longer, contained more protein nitrogen and had a higher rate of oxygen uptake per root and per unit protein nitrogen. The iron deficient roots which had been transferred to a fresh minus iron medium when analysed were very similar to the day 11 iron deficient roots which had been analysed in earlier experiments.

In the next two experiments which were of similar design iron deficient roots were supplied with iron at days 9 and 11. In each case there was a response when the iron deficient roots were placed in a plus iron medium when compared with the roots which had been placed in a minus iron medium. There was little increase in protein nitrogen in both these experiments and the dominant response was in terms of length and rate of oxygen uptake, both per root and per unit protein nitrogen. Although there was an unequivocal recovery of the eleven day roots when placed in the plus iron media the extent of recovery here was less than it was at 9 days which in turn was less than it was at 7 days.

TABLE 4. Iron Recovery Experiments

Expt. No.	No. of Roots		Mean Length per Root (cm)		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr)		O_2 Uptake per unit Prot.N. (cu.mm/hr. per µg.Prot.N)	
	=Fe	∓Fe	=Fe	∓Fe	=Fe	∓Fe	=Fe	∓Fe	=Fe	∓Fe
7 Days -Fe then 4 Days ± Fe										
C 18					126.6	167.8	17.28	20.71	.1333	.1233
					142.8	157.5	17.67	20.62	.1237	.1309
					136.9	163.4	17.08	20.24	.1247	.1238
					148.7	167.9	19.59	22.38	.1317	.1381
					139.9	182.6	15.78	24.96	.1128	.1367
					129.6	150.2	17.37	19.70	.1340	.1312
Mean	24	24	6.60	8.41	137.9	163.9	17.47	21.44	.1266	.1307
9 Days -Fe then 4 Days ± Fe										
D 18					174.2	169.3	16.68	22.68	.0958	.1340
					166.8	193.8	18.56	25.32	.1113	.1306
					166.8	171.7	16.90	20.40	.1013	.1188
					176.6	171.7	19.70	20.38	.1115	.1186
Mean	16	16	6.61	7.91	171.2	176.7	17.96	22.20	.1050	.1256
11 Days -Fe then 4 Days ± Fe										
D 18					203.6	191.4	22.34	23.22	.1097	.1213
					171.7	154.6	17.40	18.46	.1013	.1194
					171.7	171.7	17.68	19.89	.1029	.1158
					152.1	193.8	13.05	22.99	.0888	.1186
Mean	16	16	6.75	7.74	174.8	177.9	17.62	21.14	.1008	.1188

(6) Summary of Effects of Iron Deficiency

Effects of Iron Deficiency on the Overall Growth of Roots

a) After culture for 7 days, growth in length ceases and there is no further increase in the number of cells per root.

b) The protein nitrogen content of roots in deficient media is lower than it is in full media at 7 days, but it continues to increase from the seventh to the eleventh day. On the eleventh day the protein nitrogen content of deficient roots is lower than that of roots cultured with a complete medium.

c) At day 3 the rate of oxygen uptake of deficient roots is less than that of control roots, but between days 3 and 7 the rates of oxygen uptake of both groups of roots increase considerably. In the absence of iron there is little increase from day 7 to day 11, but over the same period there is a large increase in the presence of iron.

d) There is a significant reduction in the rate of oxygen uptake per unit protein nitrogen after 3 days' growth in an iron deficient medium. The rate increases to day 7 but declines steeply from day 7 to day 11. There is no fall in the full nutrient roots, a steady rate being maintained from day 7 to day 11.

e) The average protein nitrogen content per cell is the same in both groups of roots until day 7, but thereafter there is a large increase in the content per cell in the iron deficient roots. ~~to day 11.~~

f) The average rate of oxygen uptake per cell is lower in the iron deficient roots at all stages except possibly at day 11 where it is apparently higher.

g) There is no significant difference in the invertase activity of the two groups of roots at any stage of growth.

h) There is no difference in the two sets of roots in the relative sensitivity of oxygen uptake to 10^{-3} and 10^{-4} M. KCN. However the amount of oxygen uptake mediated by a cyanide sensitive system decreases from day 0 to day 5 in both groups of roots and thereafter remains almost constant in the absence of iron but increases considerably in the presence of iron.

i) The relative frequencies of mitotic figures are lower in deficient than in control roots at both the 7 and 11 day stages. In deficient roots when compared with control roots there are reductions in the frequencies of metaphase and prophase nuclei and large reductions in the frequencies of anaphase and telophase nuclei. The reductions were larger at day 11 than they were at day 7.

j) Roots grown in iron deficient media when transferred to a complete medium at days 7,9 and 11 recover and sustain renewed growth. The extent of the recovery is greatest at day 7, least at day 11. In a four day period of recovery the greatest relative change occurs in the rate of oxygen uptake while little change occurs in protein nitrogen.

Effect of Iron Deficiency on the Longitudinal Differentiation of Seven Day Old Pea Roots

a) Deficient roots have a smaller number of cells in sections 1 and 6 than do roots from a complete media. There is a large increase in cell number per section between sections 2 and 5 in both groups of roots.

b) The average volume of cells in the terminal centimetre of iron deficient roots is significantly higher than it is in the corresponding section of full culture roots.

c) The distribution of protein in the root is altered by deficiency at 7 days when compared with roots from a full medium. The deficient roots have a higher content in sections 1,2,3 and 4 and a lower content in section 6.

a) There is no effect of deficiency on the rate of oxygen uptake per section except in section 6 in which it is relatively low.

e) The rate of oxygen uptake per unit protein nitrogen shows a continuous downward trend from section 1 to section 6 in the full culture roots. In the iron deficient roots there is a large decrease in this rate especially in section 1 but also in sections 2 and 3. There is very little difference in the rate in both groups of roots in sections 4,5 and 6.

f) There are large increases in the average protein nitrogen content per cell in sections 1,2, 3 and 4 of the iron deficient roots and decreases in sections 5 and 6 when comparisons are made with full culture roots. In the full nutrient roots the trend is from a very low content in section 1, increasing steeply to section 2 more slowly to section 5 and again steeply to section 6.

g) In deficient roots the rate of oxygen uptake per cell is higher in sections 1,2,3 and 4 and lower in sections 5 and 6 than it is in full culture roots.

h) In the full culture roots invertase activity is highest in section 1, decreases to section 2 and thereafter remains more or less constant. Iron has little or no effect on this succession.

i) The sensitivity of the rate of oxygen uptake to cyanide innibition is greatest in section 1 and considerably lower in the rest of the root. There is little or no difference between the two series in this connection.

2. Magnesium Deficiency Experiments

As indicated in the introduction the effects of iron deficiency were compared with those of another cation, magnesium. The effects of magnesium deficiency were analysed in a second group of experiments, the results of which are presented here.

In the second group of experiments, pea roots were grown in full nutrient and magnesium deficient media. The techniques used in the purification and preparation of the media have been described elsewhere. In this connection, however, it may be noted that magnesium deficiency was induced by replacing magnesium sulphate in the complete medium with potassium sulphate at the rate of 26 mg. of this salt per litre of culture fluid. This substitution necessarily increased the total potassium content of the medium and the effect of this was examined by adding 26 mg. of potassium sulphate per litre to a complete medium which already contained the normal supply of magnesium sulphate. This treatment had no effect on growth. The additional provision of potassium sulphate did not depress growth, and the restricted growth when magnesium sulphate is omitted may therefore be attributed entirely to the absence of magnesium from the medium.

In this series of experiments a different sample of seed was used but of the same variety as that in the iron deficiency experiments. With these seeds germination was more rapid, and in the standard procedure the germination period could be restricted to 48 hours.

(1) Effects of Magnesium Deficiency on the Overall Growth of Pea Roots

Samples were taken at 0,3,5,7,9 and 11 days during the experimental period and similar analyses to those carried out on the iron deficient series were made. In these

experiments the estimation of invertase activity was omitted.

The complete series of results are given in the appendix in Tables 31 to 36, and a summary is presented in Table 5, the values of which are shown graphically in Figures 25 to 30.

Figure 25 shows the mean lengths in full nutrient and magnesium deficient media. The first difference occurs at 7 days when the magnesium deficient roots are 0.7 cm. shorter than the full culture roots. In contrast to the iron deficient roots the magnesium deficient roots continue to grow between days 7 and 11 and there is no sudden cessation of growth at or about the 7 day stage.

Figure 26 shows the total number of cells per root after different periods of growth. Here the first divergence occurs at 7 days when the magnesium deficient roots have a lower cell number per root than the full nutrient roots. Both the deficient and the control roots increase considerably in total cell number between days 7 and 11, but the difference between the two also increases. In this series of experiments both the deficient and control roots increase in cell number approximately linearly between days0 and 7. This immediate increase was not found in the previous experiments where there was a lag phase between days 0 and 5. With the exception of this early difference , which includes the obvious difference in the average cell number per tip in the two series of experiments, the general growth of the full nutrient roots was similar in both cases in terms of average cell number per root.

Again in Figure 27 showing the total protein nitrogen content per root the magnesium deficient roots are first significantly lower than the full culture roots at the day 7 stage. The protein nitrogen content of the magnesium deficient roots increases approximately linearly from day 0

while there is a steeper rate of increase in the full culture roots. In general the average protein nitrogen contents are higher in this series of experiments than in the earlier iron deficiency experiments, and, in addition, there is no lag phase between day 0 and day 3.

Figure 28 showing the total rate of oxygen uptake per root after different periods of growth is very similar to Figure 27. The magnesium deficient roots have a lower rate at the 7 day stage but there is a continued increase between days 7 and 11. The oxygen uptake per unit protein nitrogen is shown in Figure 29. Here the general trend for the full culture roots is very similar to that established in the earlier experiments. There is a steep decline in the rate between days 0 and 3 followed by a steady rise which in these experiments continues until day 11, but which in the earlier remained constant after day 9. Magnesium deficiency in the media brings about a difference in the roots even at the early day 3 stage as they have a significantly lower rate of oxygen uptake per unit protein nitrogen. From day 3 the rate of the magnesium deficient roots increases gradually to day 11, but at all stages the rate is very much lower than that found in the full culture roots.

Figure 30 shows the trend of the average protein nitrogen content per cell. The trend of the full culture roots in this experimental series differs from that found in the first series of experiments mostly with respect to the day 0 and day 3 values. In the present experiments the total fluctuation was low and all the values were between 190 and 210 micrograms x 10^{-6} per cell. There appears to be no effect which can be attributed to magnesium deficiency in this case and the slight increase of the mean values of the magnesium deficient roots at days 9 and 11 is certainly not significant.

Figure 31 shows the changes in the average rate of oxygen uptake per cell. Here again the fluctuations are slight but there is a consistently lower rate of oxygen uptake per cell in the magnesium deficient roots. The effect of magnesium is clearly defined at the 7,9, and 11 day harvests. On these occasions the values given with magnesium deficiency are clearly lower than those given with a full medium.

TABLE 5.

± MAGNESIUM ROOTS - SUMMARY OF WHOLE ROOT DATA

Mean values from an experimental series which involved a treatment of ± Magnesium and harvests of whole roots after growing periods of 0,3,5,7,9 and 11 days.

Day		0	3	5	7	9	11
Mean Root Length (cm)	-Mg	1.0	2.93	3.98	6.31	7.60	8.27
	+Mg		3.26	4.06	7.07	8.39	10.32
Cell No. /Root	-Mg	396,875	486,015	552,265	626,736	664,271	729,514
	+Mg		489,289	551,875	661,111	765,000	831,596
Protein N. /Root (µg.)	-Mg	80.79	95.3	112.4	121.2	133.7	145.0
	+Mg		94.1	110.2	127.4	151.5	162.1
O_2 Uptake /Root (cu.mm/hr)	-Mg	12.16	12.20	14.90	16.18	18.26	20.08
	+Mg		12.64	15.15	18.58	22.64	25.95
O_2 Uptake /unit Prot. N. (cu.mm/hr /µg. Prot.N)	-Mg	.1505	.1280	.1326	.1335	.1365	.1385
	+Mg		.1344	.1375	.1458	.1495	.1601
Prot.N. /Cell (µg. x 10^{-6})	-Mg	203.6	211.07	203.8	192.9	201.8	198.7
	+Mg		209.4	200.0	192.3	198.0	194.5
O_2 Uptake /Cell (cu.mm x 10^{-6}/hr.)	-Mg	30.64	27.34	26.98	25.86	27.40	27.48
	+Mg		28.72	27.41	27.86	29.57	31.12

Figure 25. Change in Length with Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.

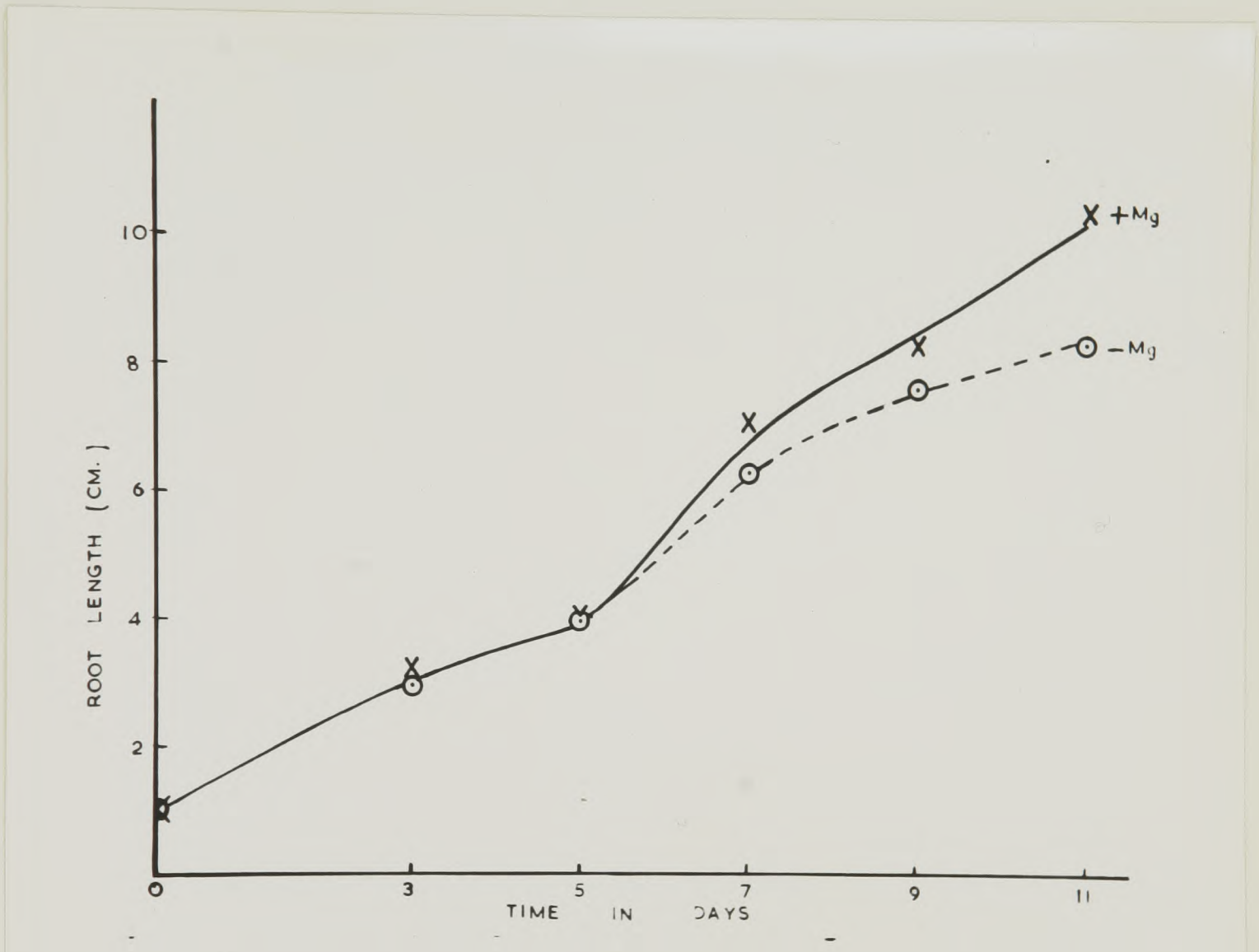
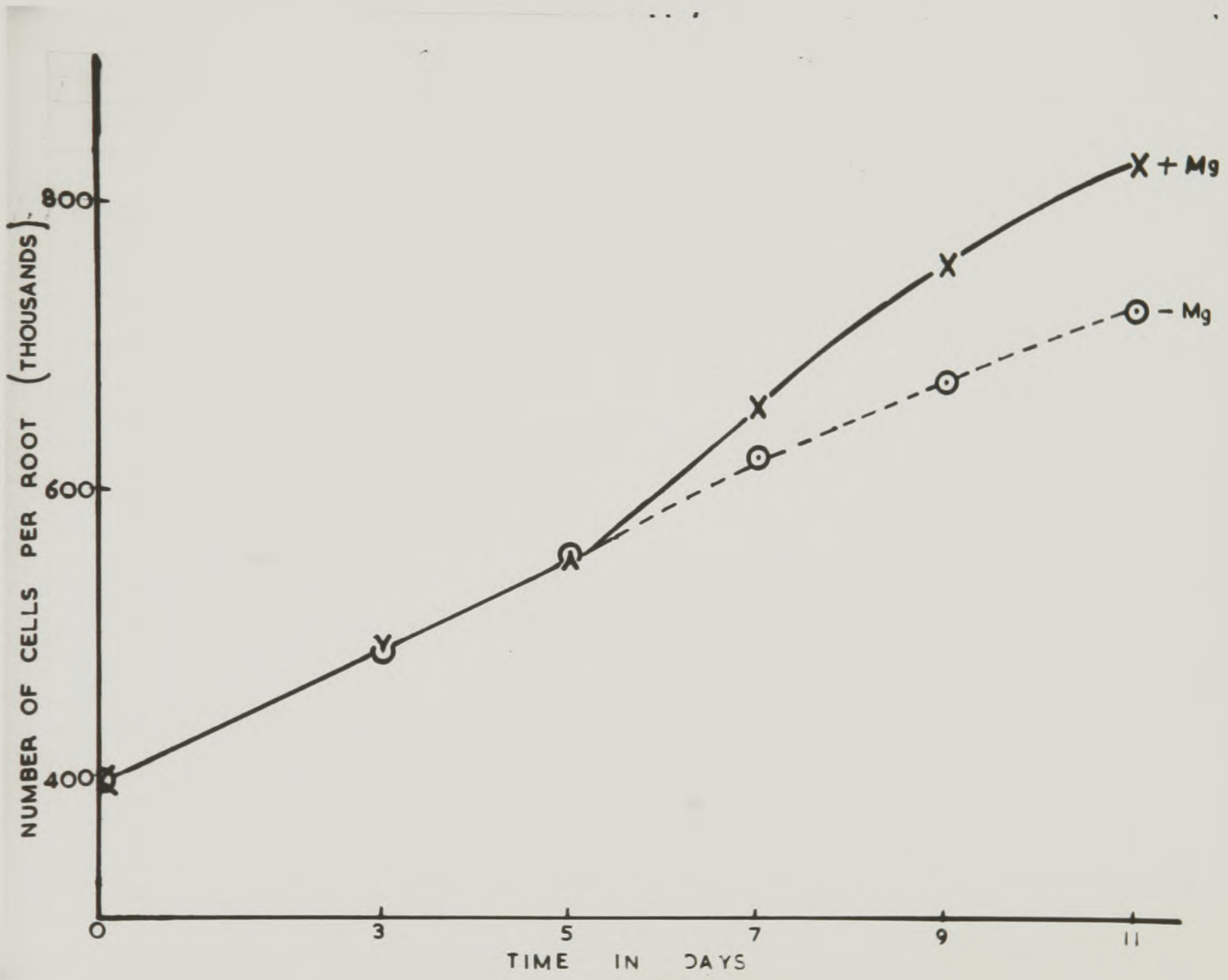


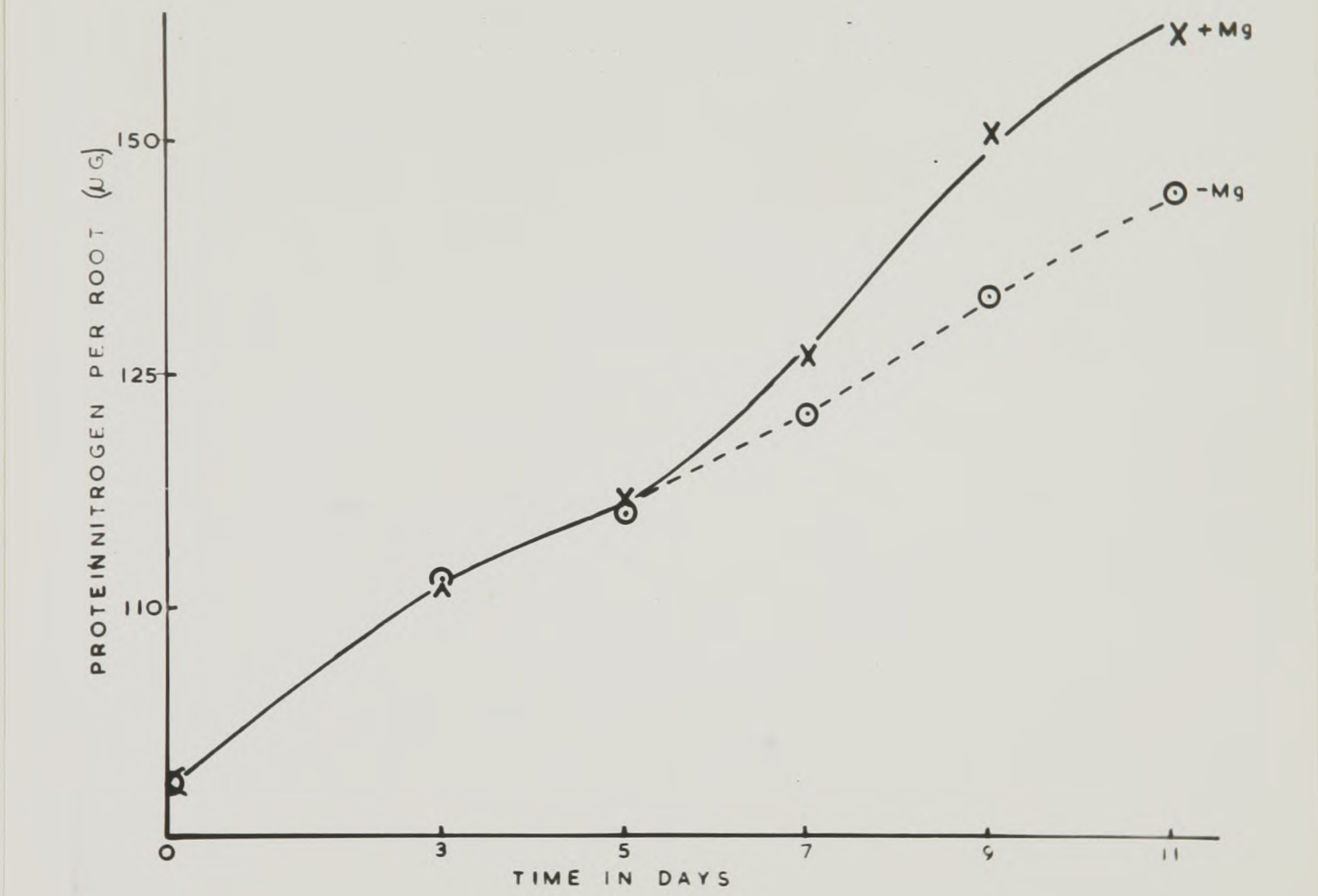
Figure 26. Change in Numbers of Cells with Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.



Treatment Difference at Day 9. Significant at $P < .01$

" " " Day 11 " " $P < .01$

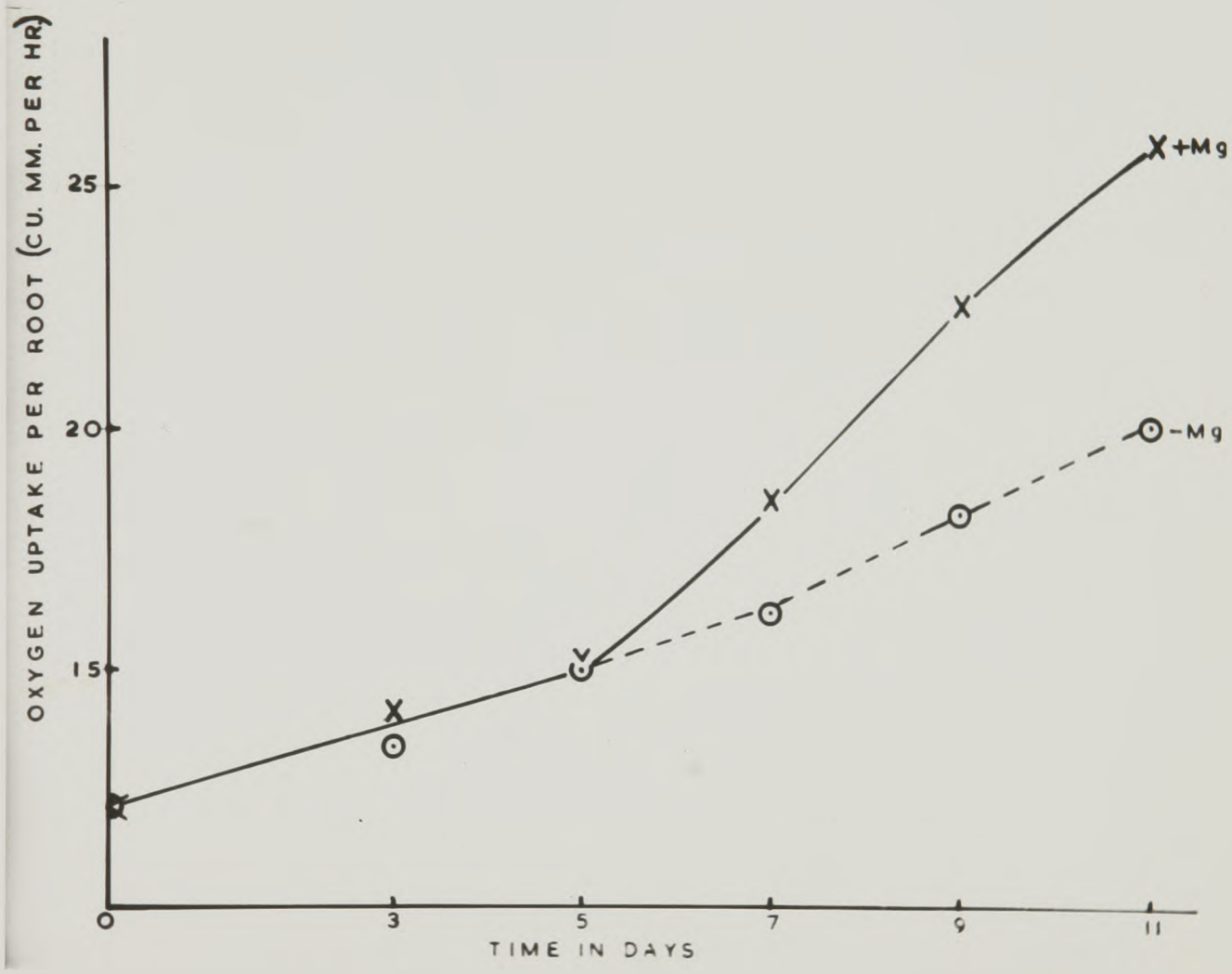
Figure 27. Change in Protein Nitrogen Content with Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.



Treatment Difference at Day 9. Significant at $P < .01$

" " " Day 11 " " $P < .01$

Figure 28. Change in Rates of Oxygen Uptake with Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.

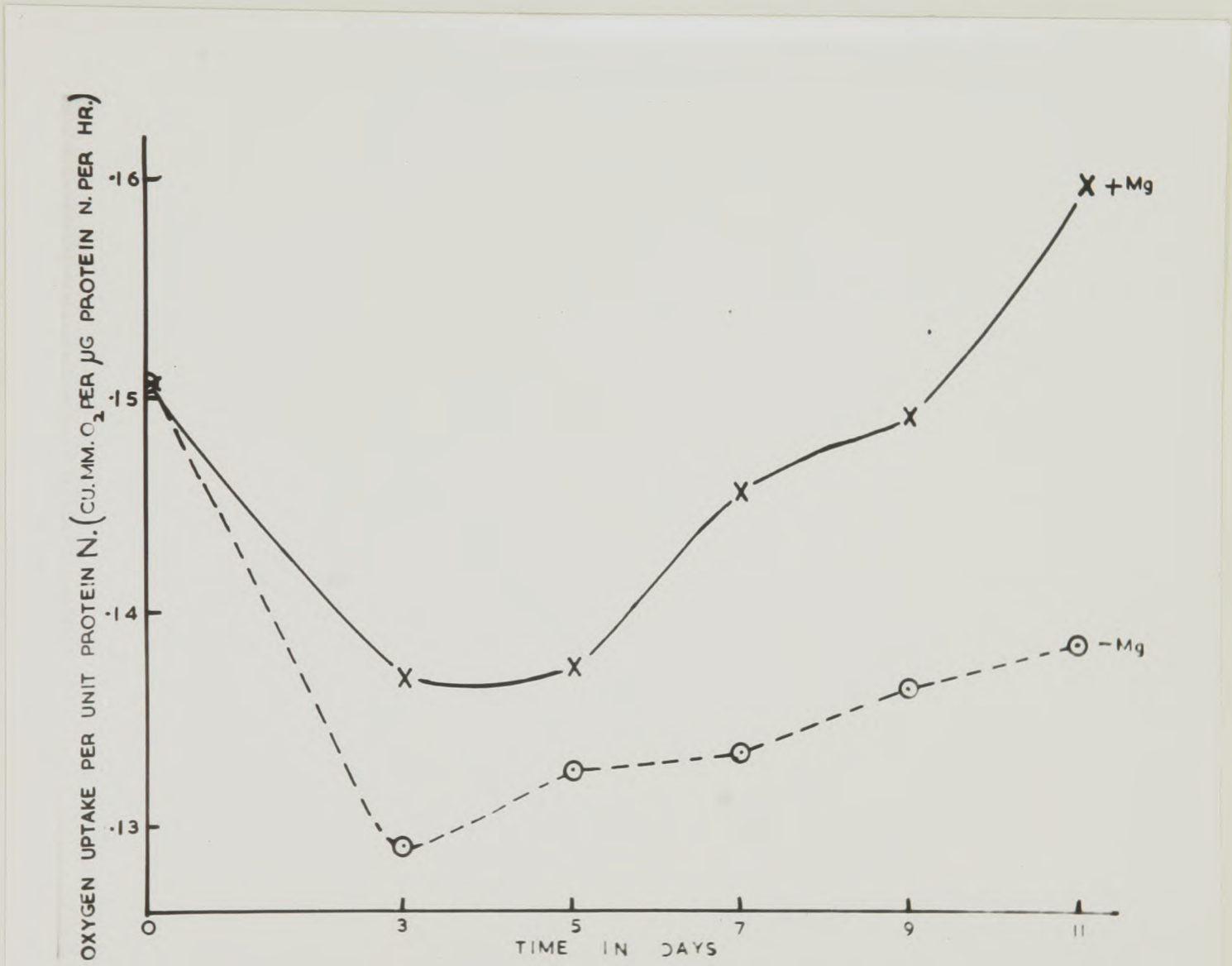


Treatment Difference at Day 7 Significant at $P < .01$

" " " Day 9 " " $P < .01$

" " " Day 11 " " $P < .01$

Figure 29. Change in Rates of Oxygen Uptake per Unit Protein Nitrogen With Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.



Treatment	Difference	at	Day 3	Significant at $P < .05$
"	"	"	Day 5	Not Significant
"	"	"	Day 7	Significant at $P < .01$
"	"	"	Day 9	" " $P < .02$
"	"	"	Day 11	" " $P < .01$

Figure 30. Change in the Average Protein Nitrogen Content per Cell with Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.

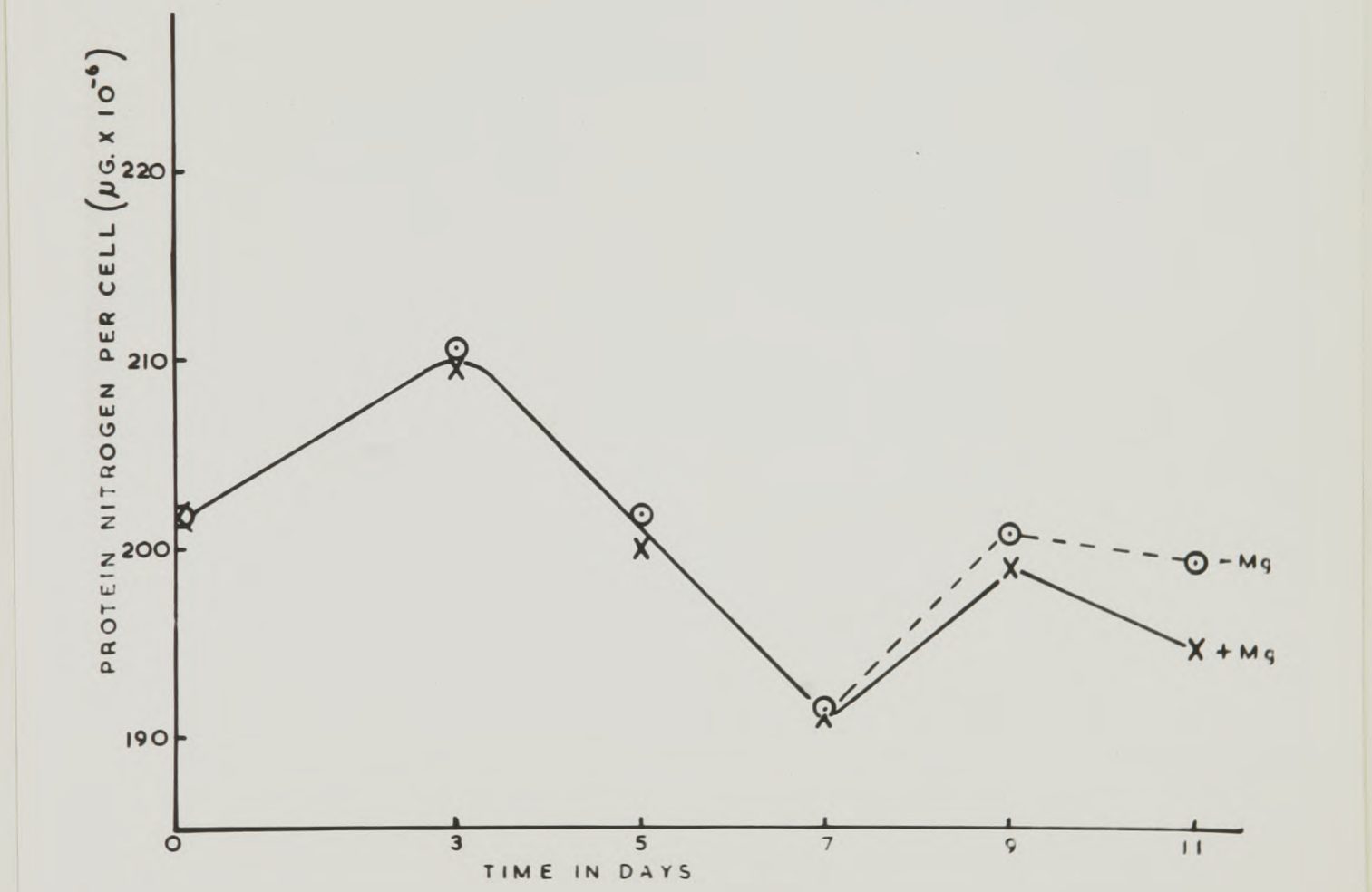
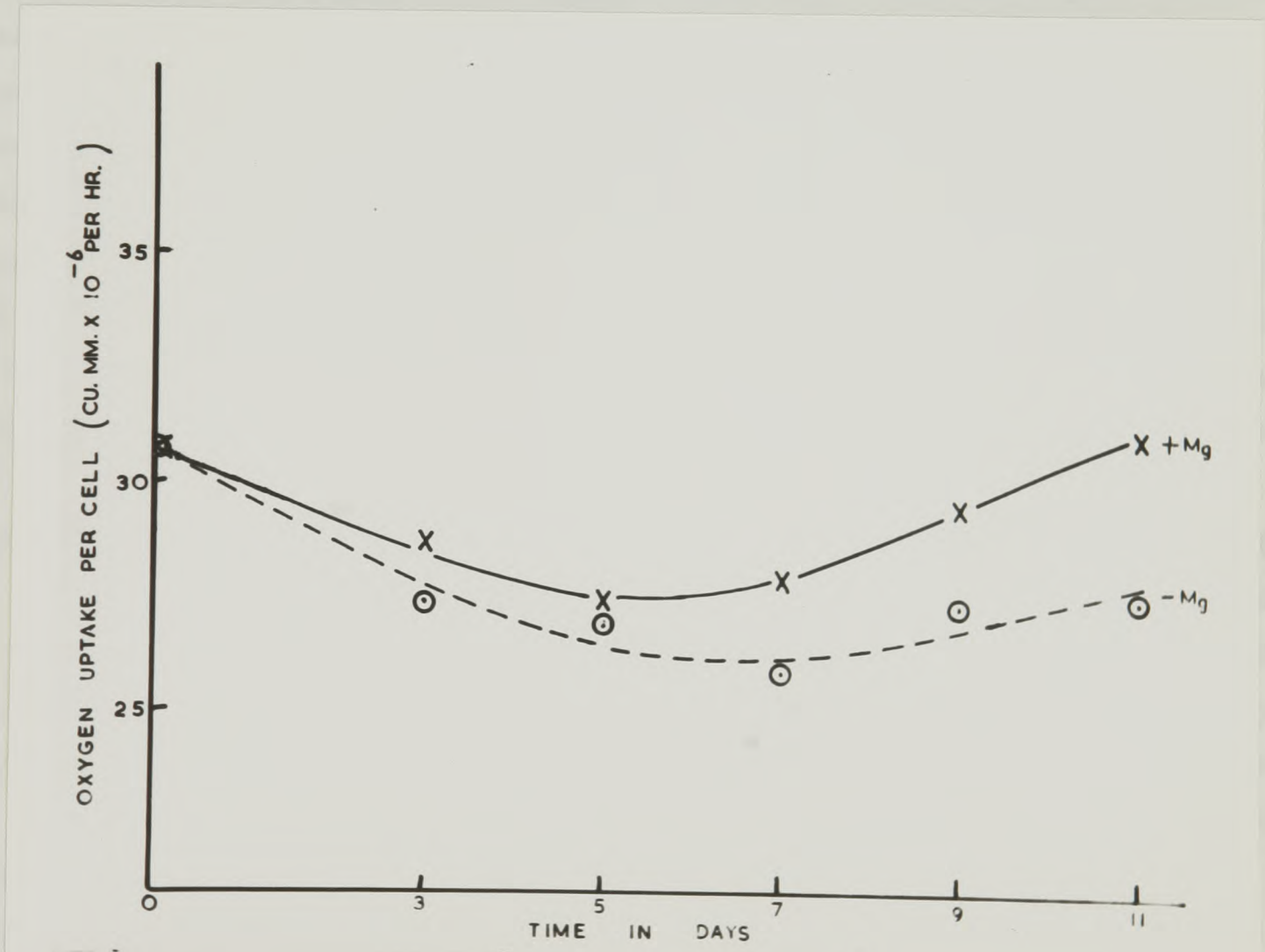


Figure 31. Change in the Average Rates of Oxygen Uptake per Cell with Time in Roots grown in Full Nutrient and in Magnesium Deficient Media.



Treatment Difference at Day 11 Significant at $P < .05$

(2) Effect of Magnesium Deficiency on the Longitudinal Differentiation of Seven Day Old Pea Roots

It is clear that magnesium deficiency restricts the overall growth of detached pea roots within 7 days, and the observed differences are of a similar order to those recorded with iron deficiency. In the case of iron deficiency although the overall changes were not large at the 7 day stage, many changes were found when the longitudinal differentiation within these roots was examined. Magnesium deficient and full culture roots were harvested at 7 days and examined by taking serial one-centimetre sections commencing at the apex. Four replicate experiments were conducted and in each measurements of cell number, cell volume, protein nitrogen content and rate of oxygen uptake per section were made. The mean values of these analyses, and of the derived quantities from them, are assembled in Table 6 and are also presented graphically in Figures 32 to 38. The complete data are given in the appendix in Tables 37 to 44.

The average cell number per section in roots grown for 7 days with and without added magnesium in the culture media is shown in Figure 32. In this case magnesium deficiency has no effect on the relative numbers of cells in sections 1 and 2, but there is an increased number in sections 3, 4 and 5. The number of cells in the final section is much lower in the magnesium deficient roots. The full culture roots again show the pattern of an apparent large increase in cell number between sections 2 and 6, while the number of cells in section 1 is considerably higher than the number in section 2.

In Figure 33 the average volume per cell in the different sections is presented and here the outstanding feature is the absence of any magnesium effect. The general trend of cell volume is similar to that established before

with the difference that the cells of sections 5 and 6 in both groups of roots are considerably larger than the cells of sections 1,2,3 and 4.

Figure 34 shows the mean protein nitrogen content per section in the two groups. There is no difference between the full culture and magnesium deficient roots in the first two sections but the deficient roots are higher in sections 3,4 and 5 and lower in section 6 giving a pattern of distribution very similar to that obtained in the case of mean cell number per section. The distribution of protein nitrogen in the full culture roots is identical with that obtained in the previous experimental series.

Figure 35 shows the total rate of oxygen uptake in the different sections. Magnesium clearly effects the normal trend by reducing the rate of oxygen uptake in sections 1 and 6 substantially. Once again the full culture trend is identical with that established in the previous experiments. On a per unit protein nitrogen basis however, the rate of oxygen uptake is much more uniformly disturbed by a shortage of magnesium. This is shown in Figure 36 from which it is clear that the rate of oxygen uptake per unit protein nitrogen is uniformly reduced in all sections of the magnesium deficient roots. The general position with the full medium is again similar to that established earlier but the consistent effect of magnesium in all sections is of considerable importance.

Figure 37 presents the data for the average protein nitrogen content per cell in the different sections. Here there is no consistent effect of magnesium deficiency and the trend is the same in both the full culture and the magnesium deficient roots. The general succession is similar to that established before as there is a low content of protein nitrogen in the cells of the first section, a high

but fairly uniform content in cells of sections 2,3 and 4, and finally a much higher content in the cells of sections 5 and 6.

Figure 38 shows the average rate of oxygen uptake per cell in the different sections. In the magnesium deficient roots there is a general lowering of the rate per cell in all sections except the last, but the general trend throughout the length of the root is similar in both the full culture and the magnesium deficient roots. This trend is complex in that there is an increase between section 1 and section 2, followed by a steep decline in the succeeding sections which does not continue as a pronounced rise is found in the rate per cell in the last two sections. With the exception that the final rise occurs in the last two sections instead of only the last section, the general trend along the full culture roots in these experiments is similar to that established in the first series.

TABLE 6.

± MAGNESIUM ROOTS - SUMMARY OF SECTIONED ROOT DATA

Mean values from an experimental series which involved treatments of ± magnesium, a 7 day growing period, and harvests by sectioning.

Section		1	2	3	4	5	6
Cell No. /Section	-Mg	76,901	57,382	86,302	134,453	139.739	111,354
	+Mg	80,781	51,130	72,560	108,541	124,197	186,089
Average Cell Volume (cu.mm x 10^{-6})	-Mg	38.20	75.97	77.47	90.89	129.79	147.48
	+Mg	42.10	77.17	75.98	85.04	111.49	158.42
Protein N /Section (µg)	-Mg	9.008	8.741	13.425	22.645	30.429	32.077
	+Mg	9.586	7.861	10.551	17.749	27.439	49.558
O_2 Uptake /Section (cu.mm/hr)	-Mg	2.047	1.617	1.893	2.678	3.171	3.328
	+Mg	2.500	1.718	1.812	2.361	3.309	5.467
O_2 Uptake /unit Prot.N. (cu.mm/hr µg.Prot.N)	-Mg	.2277	.1855	.1406	.1203	.1044	.1053
	+Mg	.2607	.2190	.1729	.1349	.1261	.1129
Prot.N. /Cell (µg.x 10^{-6})	-Mg	117.2	153.9	156.1	167.9	219.3	287.7
	+Mg	118.9	153.8	145.0	163.4	218.5	264.2
O_2 Uptake /Cell (cu.mm x 10^{-6}/hr.)	-Mg	26.60	28.56	21.96	20.08	22.83	30.23
	+Mg	31.02	33.63	24.95	21.74	26.53	29.60

Figure 32. Numbers of Cells in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.

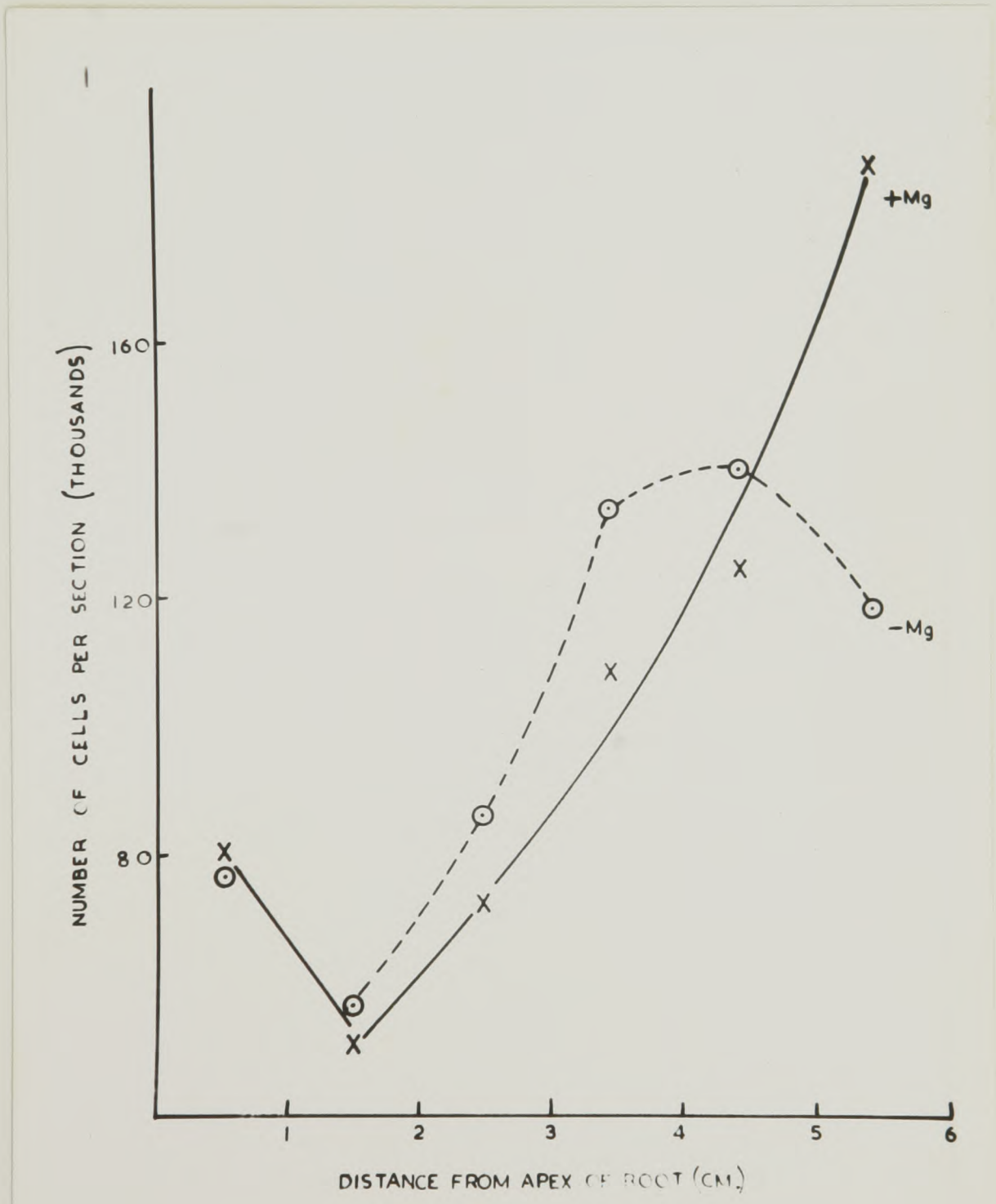


Figure 33. Average Volumes per Cell in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.

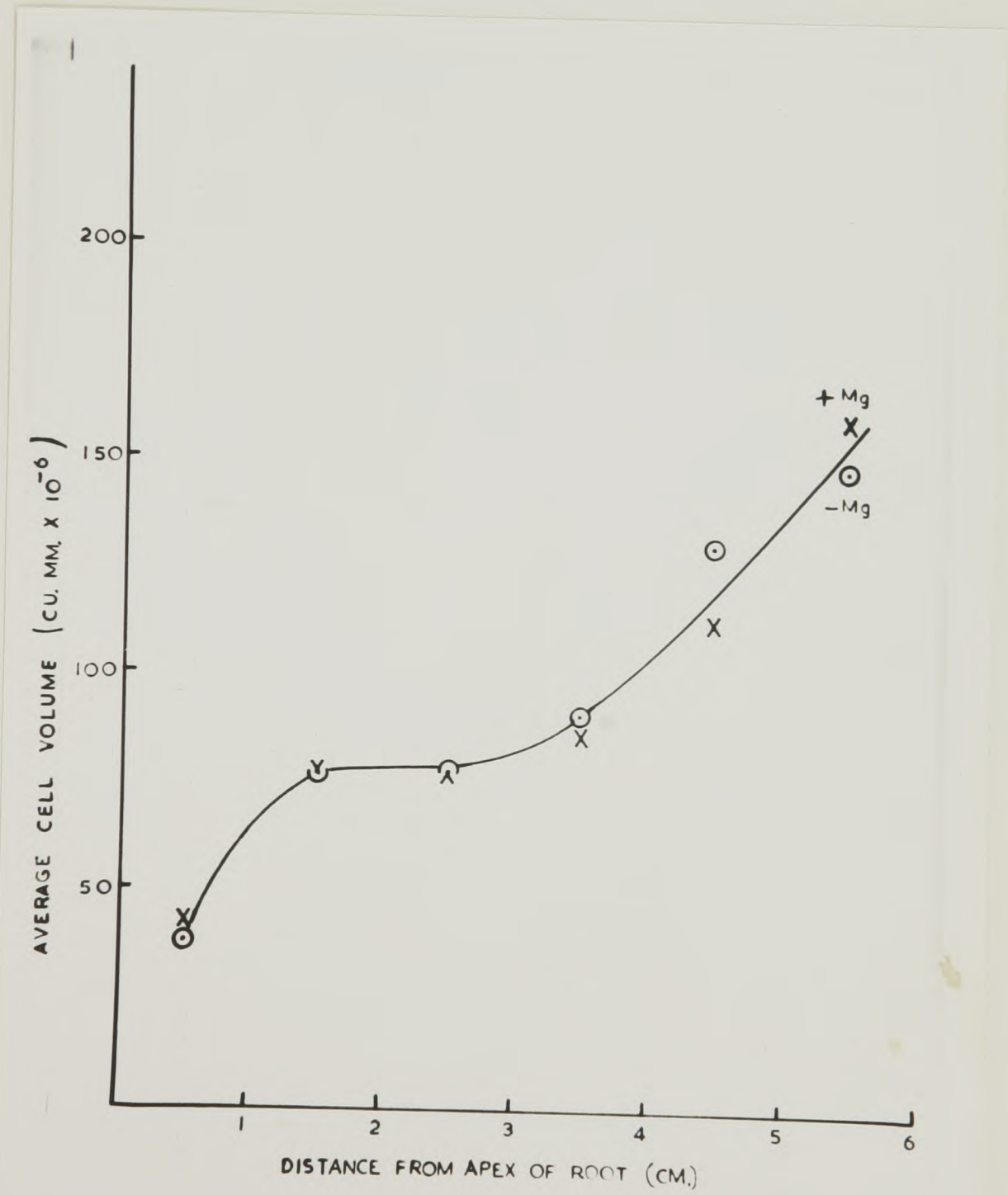


Figure 34. Protein Nitrogen Contents in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.

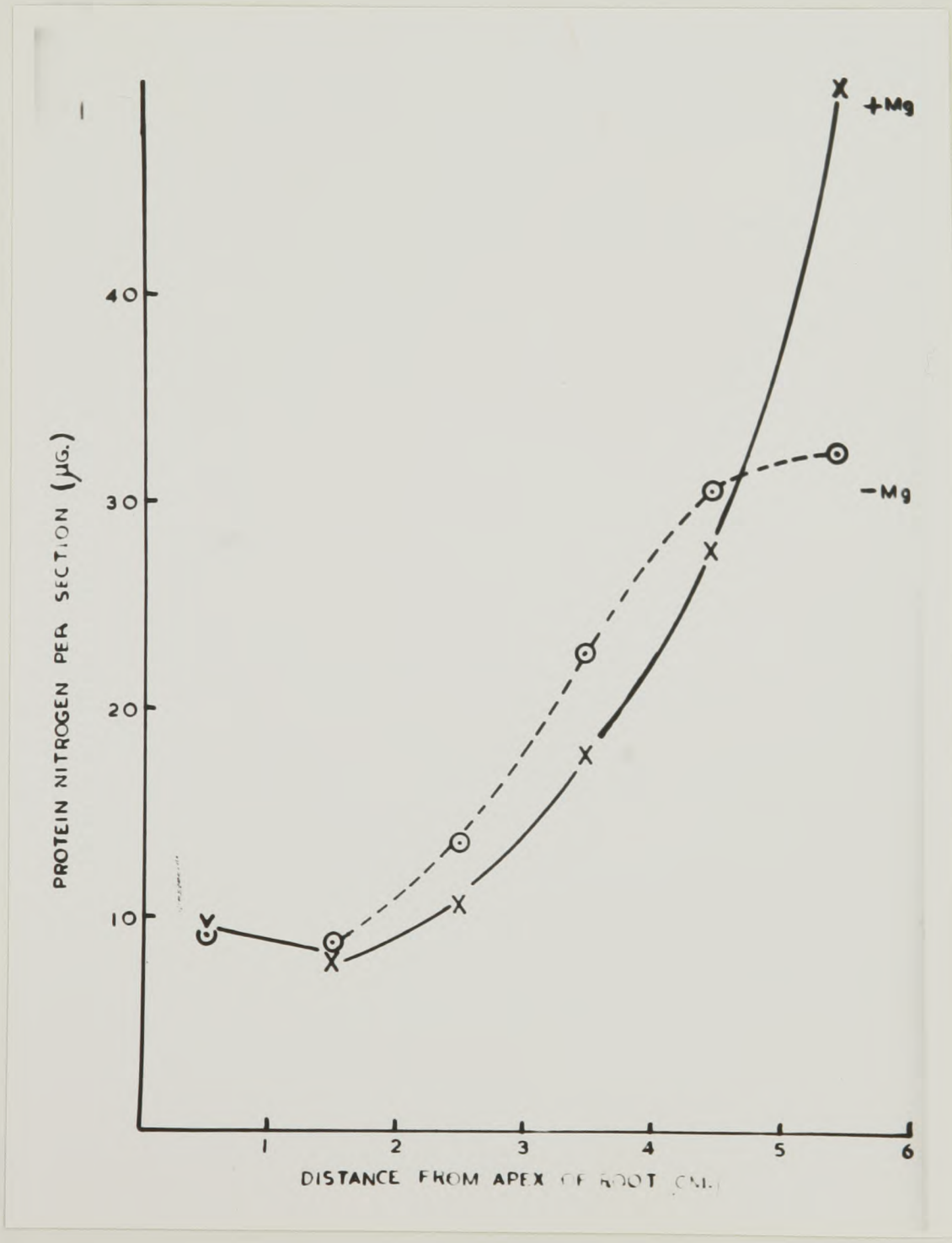
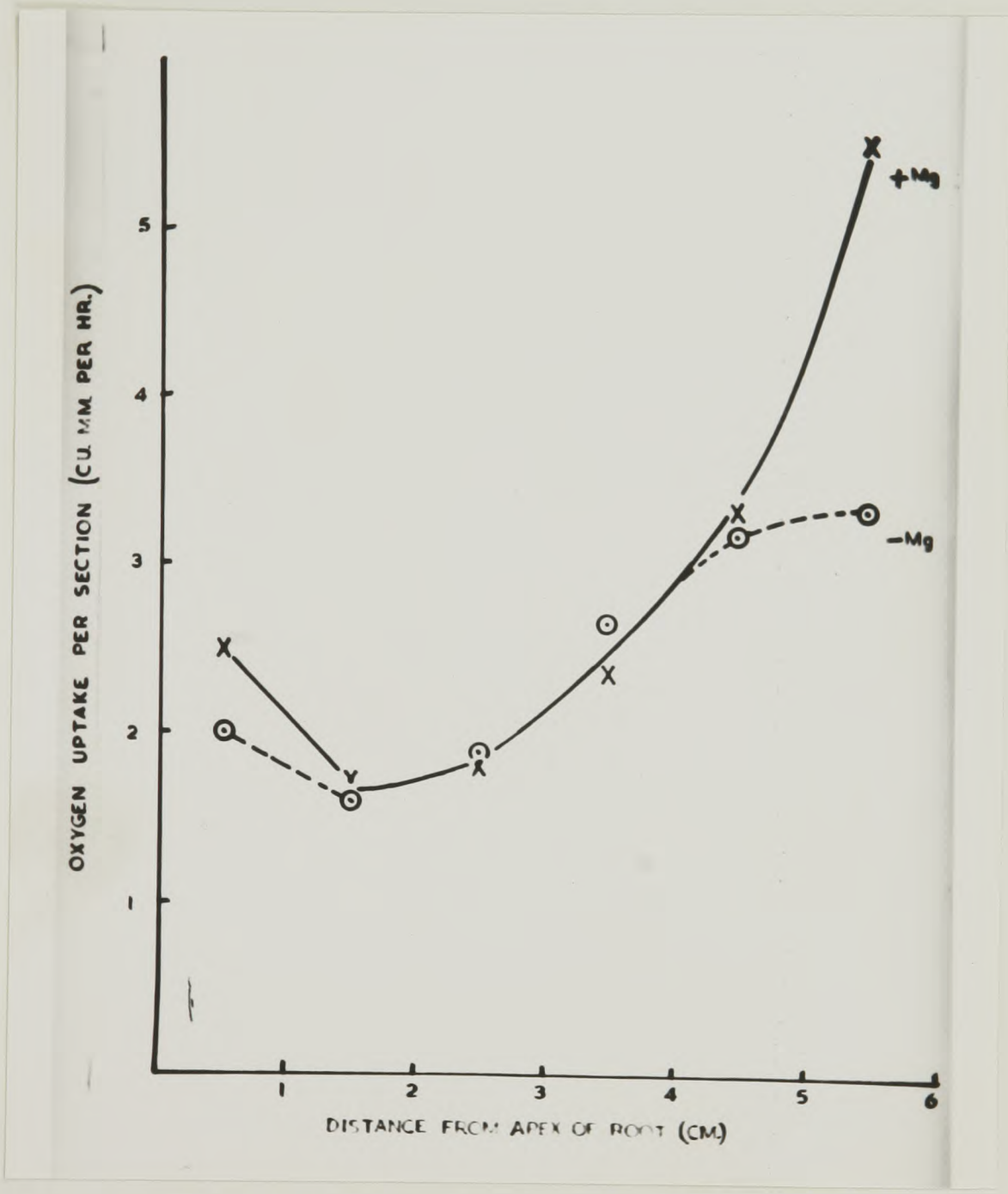
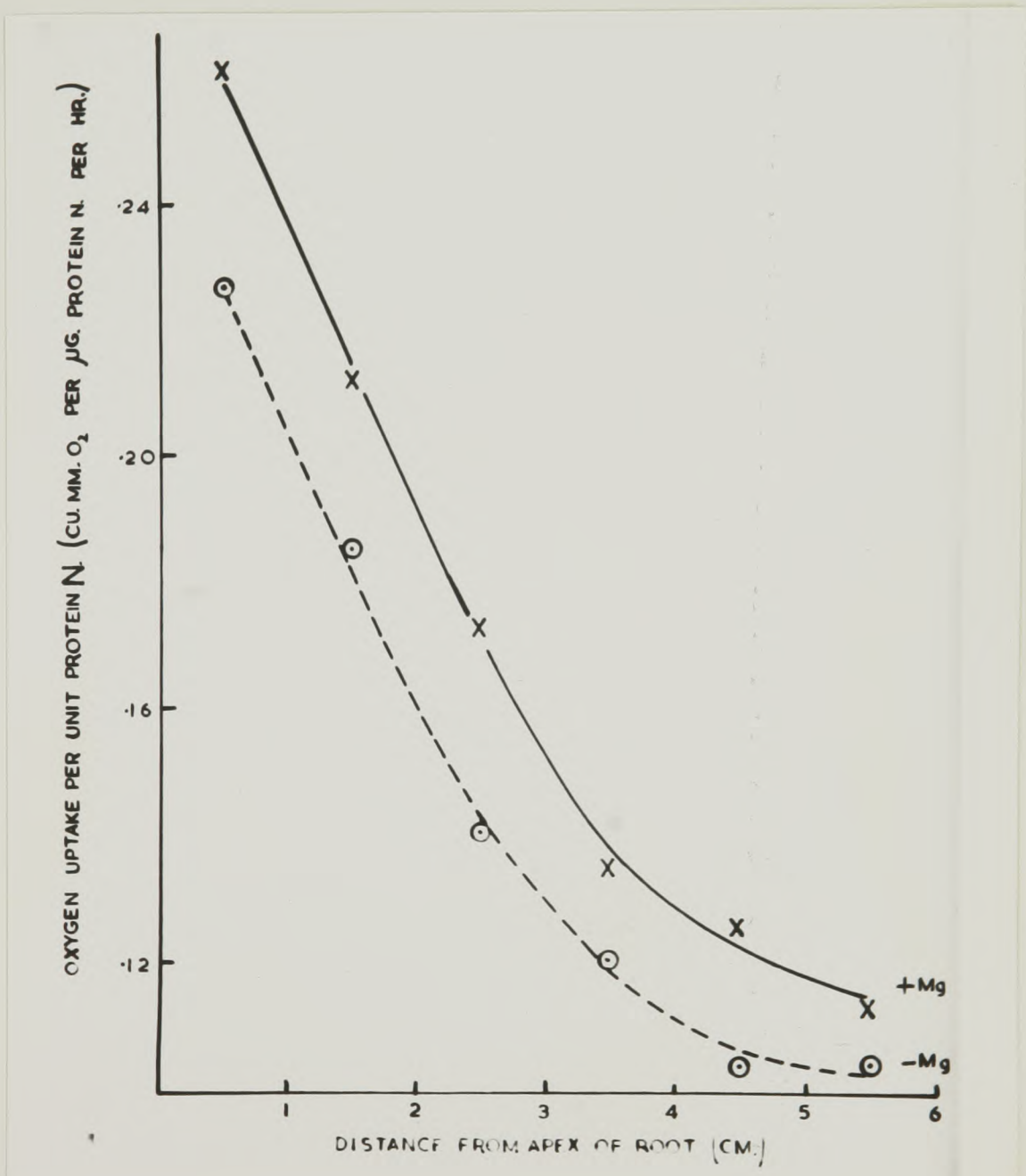


Figure 35. Rates of Oxygen Uptake in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.



Treatment Differences in Section 1. Significant at $P < .01$

Figure 36. Rates of Oxygen Uptake per Unit Protein Nitrogen in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.



Treatment	Difference in	Section 1	Significant at	$P < .01$
"	"	" Section 2	"	" $P < .01$
"	"	" Section 3	"	" $P < .01$

Figure 37. Average Protein Nitrogen Contents per Cell in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.

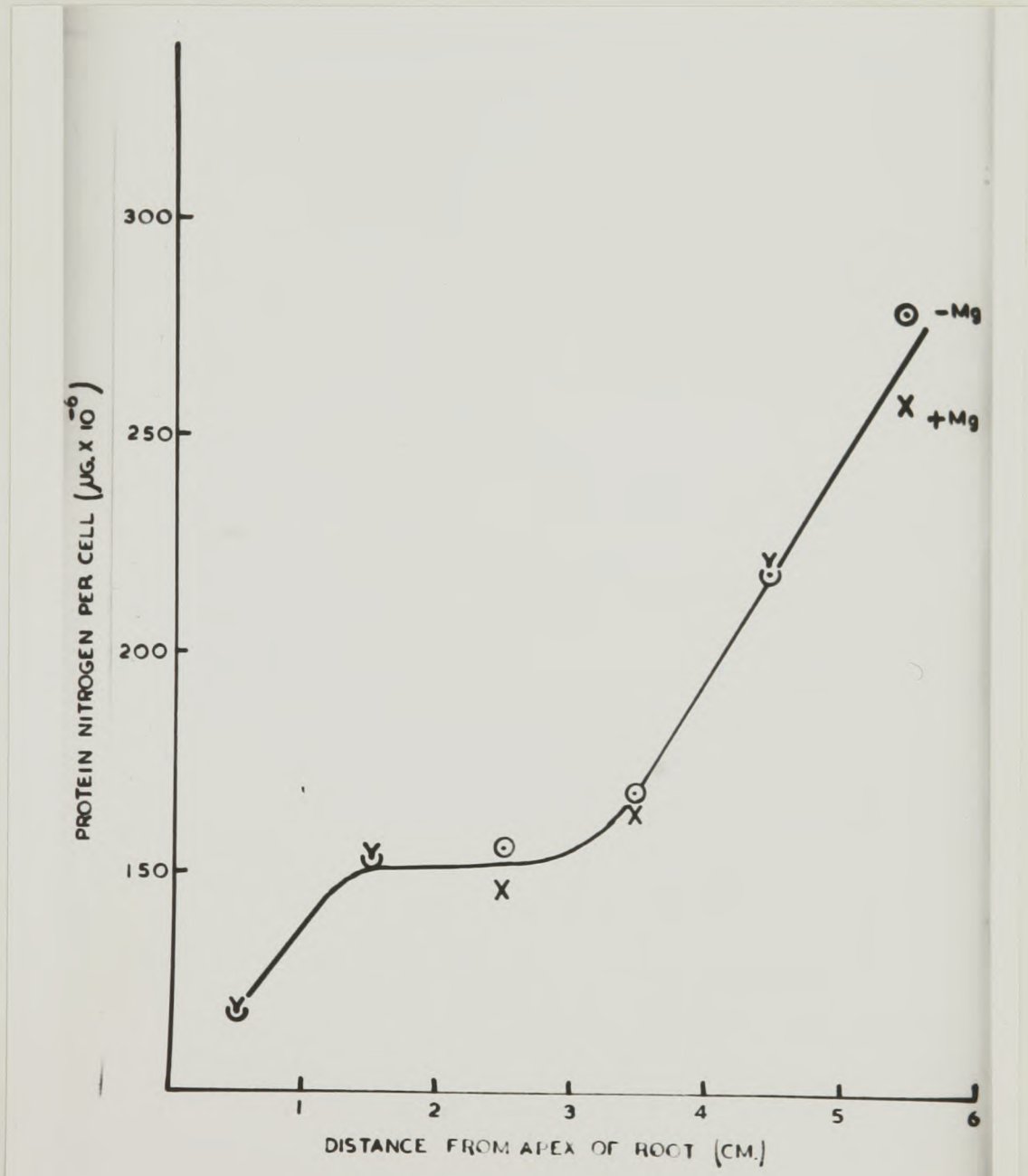
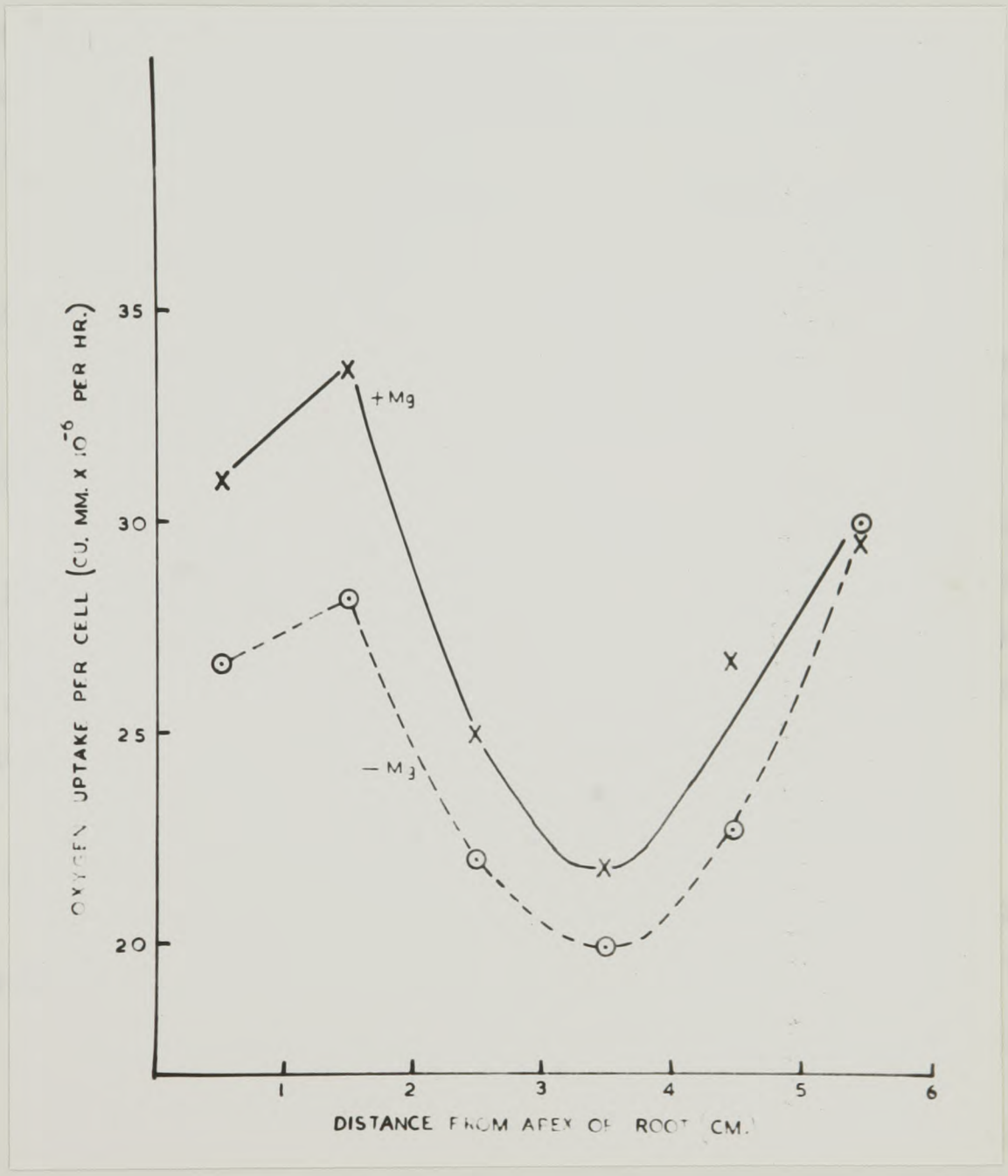


Figure 38. Average Rates of Oxygen Uptake per Cell in the Successive One Centimetre Sections from Roots grown for Seven Days in Full Nutrient and Magnesium Deficient Media.



Treatment Difference in Section 1 Significant at $P < .01$
" " " Section 2 Not Significant
" " " Section 3 Significant at $P < .05$

(3) Summary of Magnesium Deficiency Results

Effects of Magnesium Deficiency on the Overall Growth of Pea Roots

a) At 7 days length is less in the magnesium deficient than it is in the full culture medium. There is no abrupt cessation of growth, and length continues to increase in the deficient medium after 7 days although the difference between control and deficient experimental series also continues to increase.

b) A difference in cell numbers is first apparent at 7 days. Both series increase after this stage but the difference between the two also increases.

c) The same general situation is shown with protein nitrogen and with rate of oxygen uptake per root as with cell numbers.

d) The rate of oxygen uptake per unit protein nitrogen however is significantly lower in the magnesium deficient cultures from day 3. Thereafter there is only a very slight gradual increase in the rate in the absence of magnesium, but a considerable increase when it is supplied.

e) There is no significant effect of magnesium on the average protein nitrogen content per cell.

f) The average rate of oxygen uptake per cell is lower in magnesium deficient roots. The effect is largest at day 11.

Effects of Magnesium Deficiency on the Longitudinal Differentiation of Seven Day Old Pea Roots

g) There is no effect of magnesium deficiency on cell number in sections 1 and 2, but the values for sections 3,4 and 5 may be higher than those for control roots. The number of cells in the final section of the deficient roots is much lower than the number in the corresponding section of full nutrient roots.

h) There is no effect of magnesium on the average volume per cell in any of the sections.

i) The protein nitrogen distribution is very similar to that described for cell numbers per section. No effect in sections 1 and 2 but an increase in sections 3,4 and 5, and a decrease in section 6 of the deficient roots when compared with the normal roots.

j) The rate of oxygen uptake per section is reduced in sections 1 and 6 of the deficient roots but not altered elsewhere.

k) The rate of oxygen uptake per unit protein nitrogen is uniformly reduced in all sections of the magnesium deficient roots.

l) Protein nitrogen content per cell is identical in all sections of the two groups of roots.

m) The rate of oxygen uptake per cell is reduced in the magnesium deficient roots in all sections except in section 6.

3. Molybdenum Deficiency Experiments

1) The Effect of Molybdenum Deficiency on the growth of Seven Day Old Subcultured Pea Roots

The final series of experiments involved an analysis of the growth of pea roots cultured in full nutrient and in molybdenum deficient media. Although molybdenum is known to be essential for the growth of higher plants the amounts required are exceedingly low, and careful purification of the nutrient solutions was necessary to obtain roots deficient in this element. The procedure used for the purification of the inorganic salt constituents was known to reduce the level of molybdenum contamination to such an extent that tomato seedlings when grown in these solutions showed the symptoms of molybdenum deficiency within two weeks of transplanting (Possingham 1954).

Despite the use of such purified solutions only very slight differences in the length of pea roots grown ~~inocula~~ in full nutrient and molybdenum deficient culture media were recorded even after a ten day culture period. Further experiments were conducted in which instead of using root tips cut from the roots of germinated seeds, root tips were taken from roots which had been grown for 10 days in culture media. When such root tips were subcultured in full nutrient and molybdenum deficient media, a clear difference in length was apparent after a growing period of 7 days.

Therefore in this series of experiments root tips cut from germinated pea seeds were cultured for 10 days in full nutrient and in molybdenum deficient media and one centimetre tips cut from these roots were subcultured for a further 7 days in full nutrient and molybdenum deficient media respectively. These seven day subcultured roots provided the experimental material, and these were dissected into suc-

cessive one centimetre sections. The cell number, average cell volume, protein nitrogen content and the rate of oxygen uptake per section were measured in the corresponding sections of both groups of roots. The mean values from three replicate experiments are given in Table 7 and these are presented graphically in Figures 39 to 43. The complete results are given in the appendix in Tables 45 to 52.

Figure 39 shows the cell numbers per section in the corresponding sections of the full nutrient and molybdenum deficient roots. The distribution of cells in the full nutrient but subcultured roots is of importance as it differs from the distribution found in the full nutrient roots in the previous two series of experiments. Section 1 contains a relatively large number of cells compared to the numbers in sections 2,3,4 and 5. There is a large number of cells in section 6, but this section on an average measures 1.93 cm. in contrast to the one centimetre of every other section. The normal distribution of cells in these roots therefore involves a terminal centimetre of 80,000 cells but thereafter there is a relatively constant number of cells per centimetre of 35,000. The molybdenum deficient roots have the same number of cells per section in sections 1,2,3 and 4 when compared with the full nutrient roots, but there is a reduced number in sections 5 and 6.

The average volume of the cells in the different sections is shown in Figure 40. Molybdenum deficiency brings about a slight reduction in the average volume of the cells in all sections of the root. The trend in both groups of roots is similar with a low volume in section 1, followed by a more or less constant average volume per cell in sections 2,3,4 and 5, with finally a slight increase in section 6.

In Figure 41 the trend in protein nitrogen content per section is given. There is no effect of molybdenum in sections 1,2,3 and 4 but once again there is a reduction in

sections 5 and 6 when compared with the full nutrient roots. The normal distribution of protein nitrogen in these roots differs from that in the previous series of experiments as there is a nigh content in section 1 but thereafter a much lower but uniform content in sections 2,3,4 and 5.

The rates of oxygen uptake per section are shown in Figure 42. Here molybdenum deficiency exerts a considerable effect. The rate is reduced in all sections of the root and particularly in section 6. In Figure 43 the rate of oxygen uptake is presented on a per unit protein nitrogen basis. The previously established, continuous decrease in the rate of oxygen uptake per unit protein nitrogen from section 1 to section 6 in the full nutrient roots is also found in these subcultured roots. The rate is consistently lower in the molybdenum deficient roots, the effect being greatest in section 1. The differences in sections 5 and 6 of the two series are small.

The mean values for protein nitrogen content per cell and rate of oxygen uptake per cell have been presented in Table 8 but they are not shown graphically, since the results in both instances are variable. With protein nitrogen per cell there seems little doubt that molybdenum deficiency has no effect. The situation is not clear with respect to the average rate of oxygen uptake per cell as there is a slight reduction in the molybdenum deficient roots considered as a whole, and a consistent reduction in sections 1,2 and 3. These effects are probably valid although the large variability of the results with sections 4,5 and 6 obscures the situation.

TABLE 7.

± MOLYBDENUM ROOTS - SUMMARY OF SECTIONED ROOT DATA

Mean values from an experimental series which involved treatments of ± molybdenum, a 10 day period of culture, followed by a 7 day period of subculture and harvests of the subcultured roots by sectioning.

Section		1	2	3	4	5	6	Total
Cell No. /Section	-Mo	80.605	34.640	37.645	33.409	27.971	44.163	258.433
	+Mo	80.868	33.629	34.269	40.017	40.723	59.969	289.475
Average Cell Volume (cu.mm x 10^{-6})	-Mo	33.35	87.44	91.83	96.47	91.07	127.80	78.65
	+Mo	40.42	105.39	108.10	92.50	103.15	139.37	91.98
Protein N. /Section	-Mo	10.987	7.275	7.653	8.187	6.576	11.424	52.101
	+Mo	10.928	7.179	7.461	7.461	8.112	17.408	58.560
O_2 Uptake /Section (cu.mm/hr)	-Mo	2.101	1.304	1.240	1.250	0.944	1.694	8.533
	+Mo	2.431	1.402	1.329	1.247	1.149	2.603	10.161
O_2 Uptake /unit Prot. N. (cu.mm/hr µg.Prot.N)	-Mo	.1917	.1792	.1636	.1536	.1440	.1452	.1635
	+Mo	.2225	.1953	.1780	.1672	.1425	..1512	.1740
Prot. N. /Cell (µg. x 10^{-6})	-Mo	136.8	211.4	203.3	246.6	238.4	265.3	202.5
	+Mo	137.0	215.0	217.8	189.3	200.1	298.2	203.8
O_2 Uptake /Cell (cu.mm x 10^{-6}/hr)	-Mo	26.37	37.78	33.03	38.05	34.29	38.38	33.08
	+Mo	30.55	42.26	38.60	31.77	28.69	45.09	34.20

Mean Root Length (cm)

10 Days Culture Growth	-Mo	9.56
	+Mo	10.22
7 Days Subculture Growth	-Mo	5.92
	+Mo	6.93

Figure 39. Numbers of Cells in the Successive One Centimetre Sections from Second Phase Roots grown for Seven Days in Full Nutrient and Molybdenum Deficient Media.

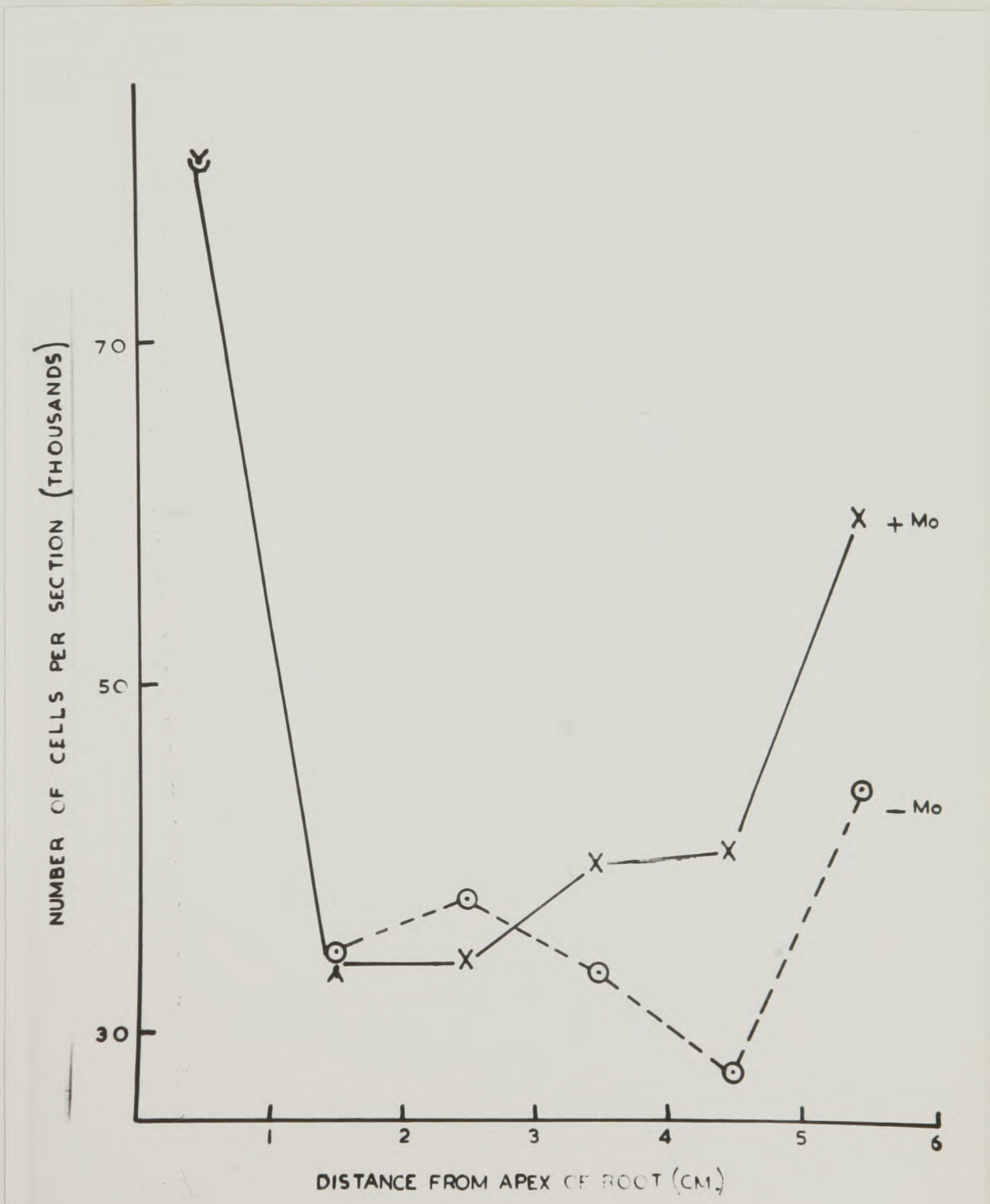


Figure 40. Average Volumes per Cell in the Successive One Centimetre Sections from Second Phase Roots grown for Seven Days in Full Nutrient and Molybdenum Deficient Media.

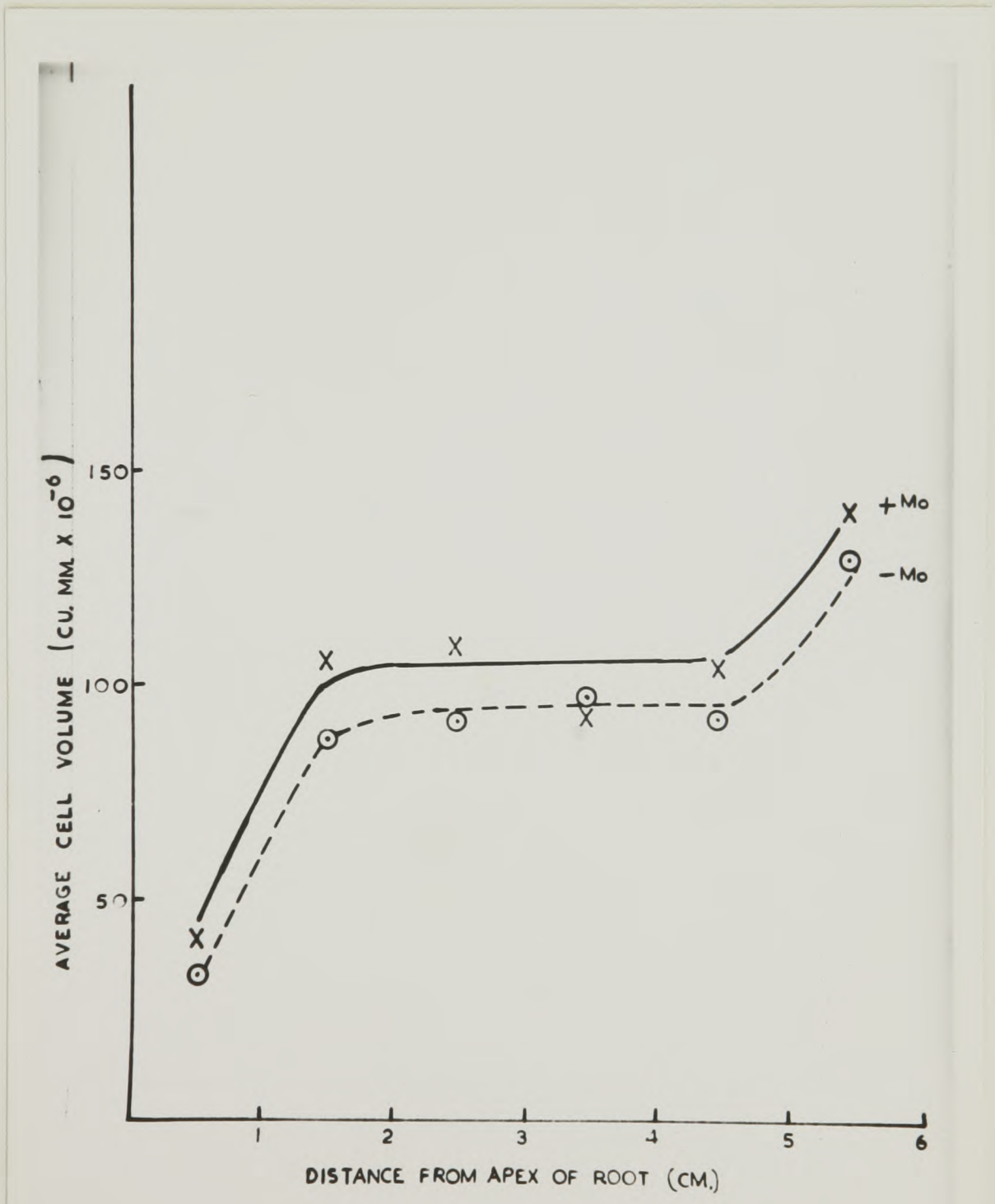


Figure 41. Protein Nitrogen Contents in the Successive One Centimetre Sections from Second Phase Roots grown for Seven Days in Full Nutrient and Molybdenum Deficient Media.

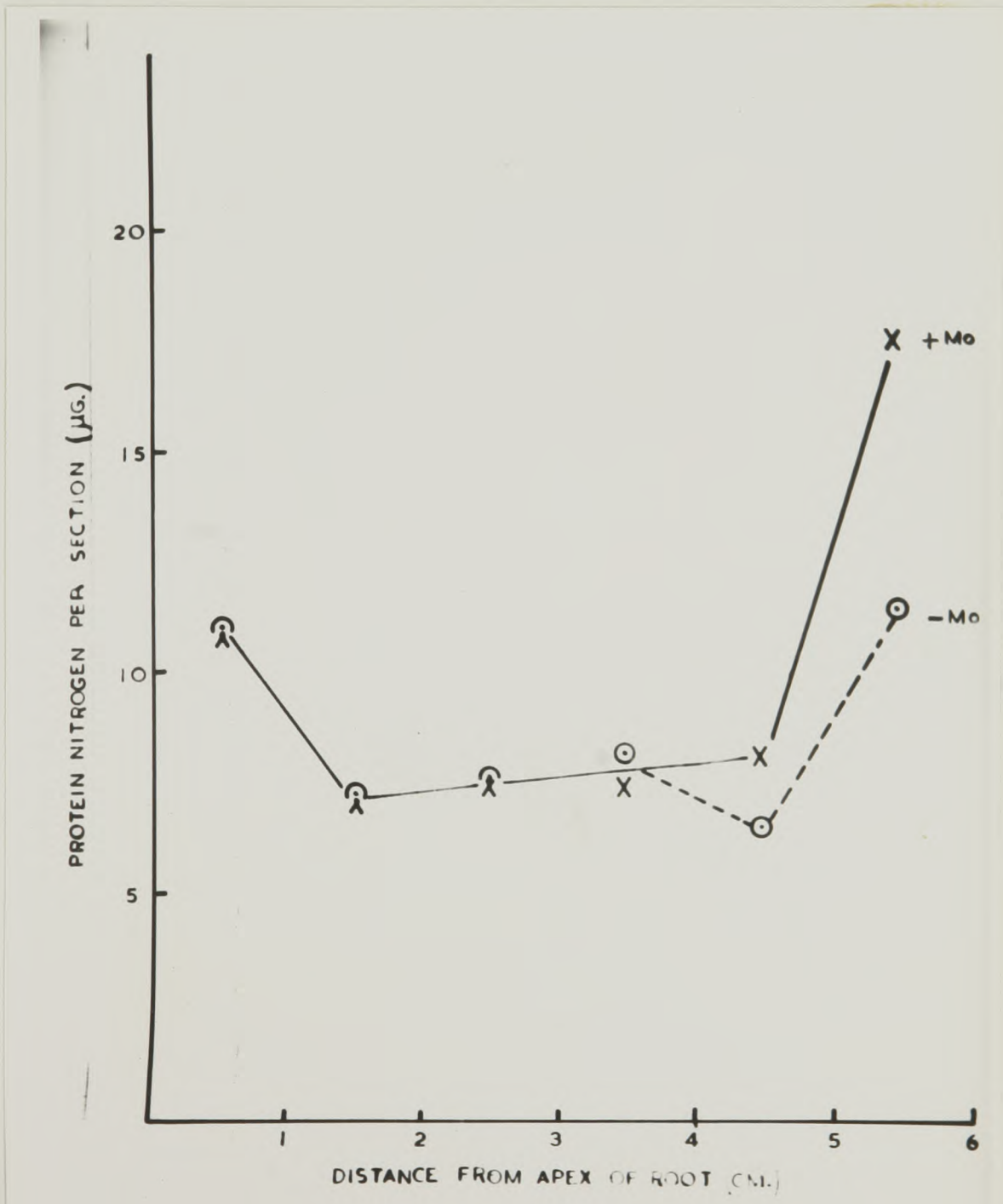


Figure 42. Rates of Oxygen Uptake in the Successive One Centimetre Sections from Second Phase Roots grown for Seven Days in Full Nutrient and Molybdenum Deficient Media.

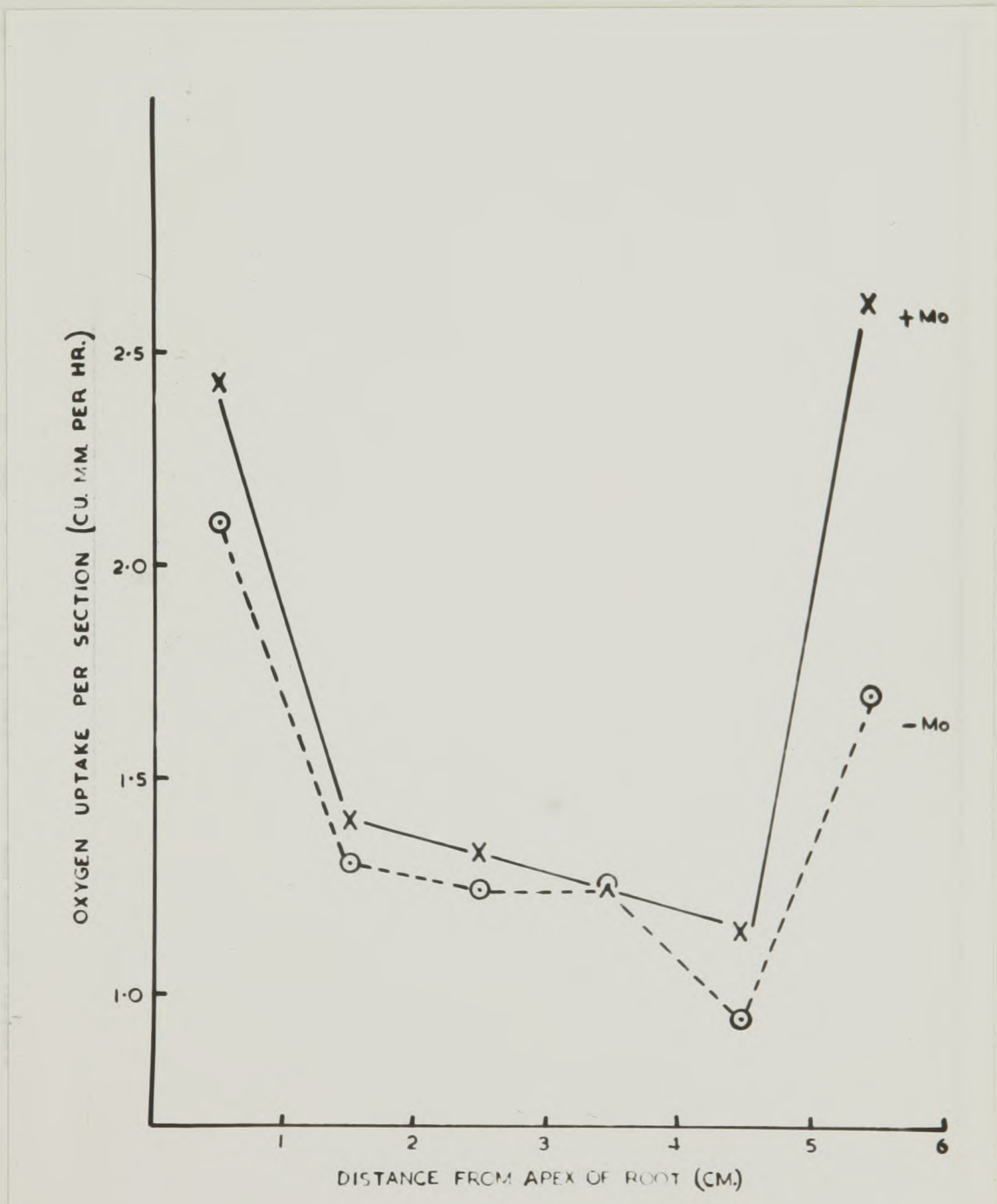
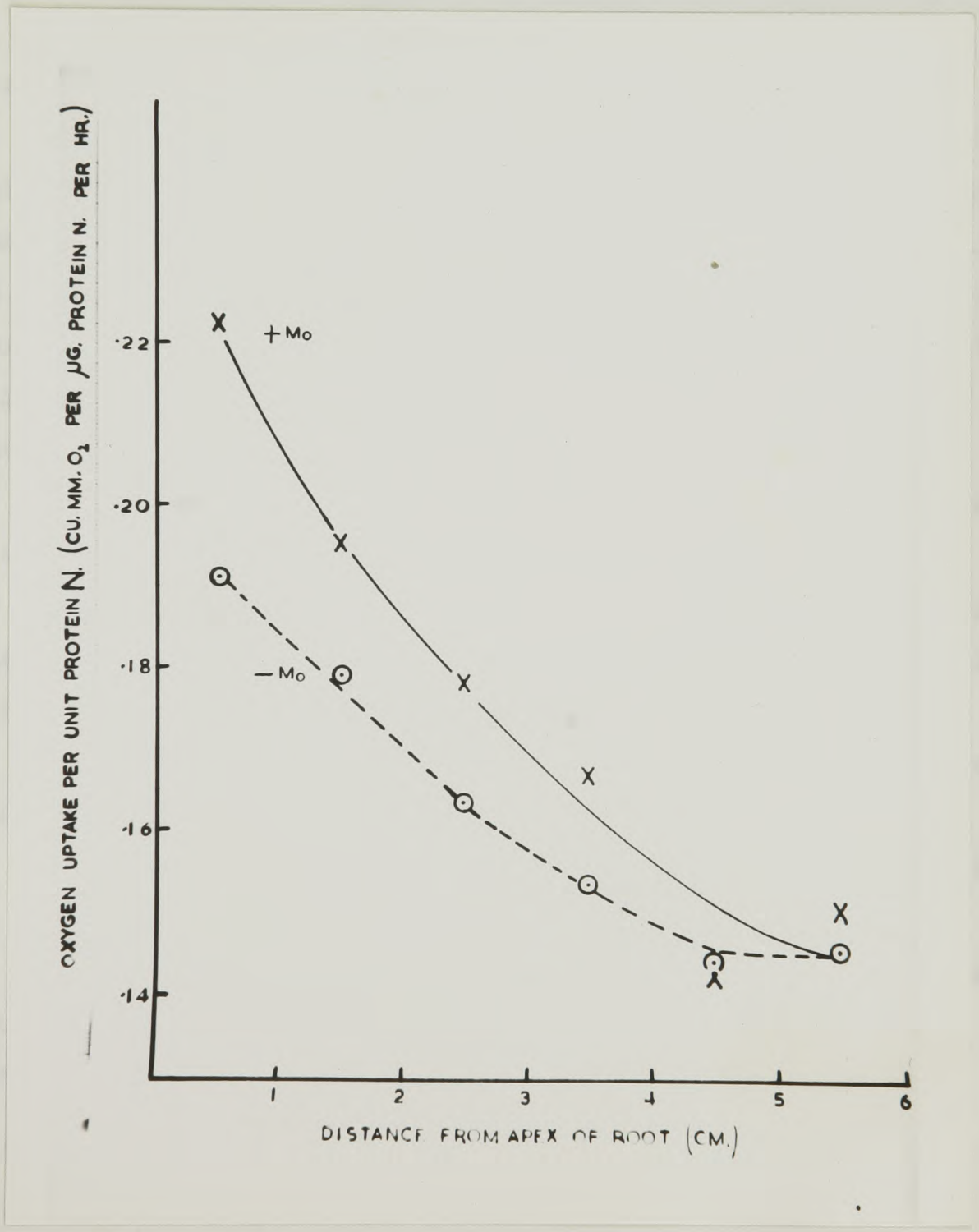


Figure 42. Rates of Oxygen Uptake per Unit Protein Nitrogen in the Successive One Centimetre Sections from Second Phase Roots grown for Seven Days in Full Nutrient and Molybdenum Deficient Media.



2) Summary of Molybdenum Deficiency Results

a) There is a slight reduction in the length of roots cultured for 10 days in a molybdenum deficient medium, but a considerable reduction in the length of roots subcultured for 7 days in the same medium.

b) On a whole root basis in the seven day subcultured minus molybdenum roots there is a reduction in total cell number, total protein nitrogen content, total rate of oxygen uptake and in average volume per cell.

c) Along the length of molybdenum deficient roots at this stage there is no change in the normal distribution of cells and protein nitrogen except a reduction in sections 5 and 6. However the distribution of cells and of protein nitrogen in the full nutrient roots was different to those found in the seven day full nutrient roots in the two earlier experimental series.

d) The rate of oxygen uptake per section is reduced in almost all sections of the minus molybdenum roots.

e) The rate of oxygen uptake per unit protein nitrogen is reduced in sections 1,2,3 and 4 of the roots subcultured in the molybdenum deficient media.

f) The average cell volume is reduced in all sections of the minus molybdenum roots.

g) There is no effect of molybdenum on the average protein nitrogen content per cell.

h) There is a slight reduction in the average rate of oxygen uptake per cell in the first three sections of deficient roots.

IV DISCUSSION

The results presented in the last section were assembled for the purpose of analysing the effects of iron, magnesium and molybdenum deficiency. In the course of the investigation, however, data were obtained with control roots supplied with a full nutrient which are of some significance in relation to the normal cycle of development in cultured roots. The data in this context are considered in the first section. In succeeding sections the effects of the different deficiencies are considered in detail.

1. Discussion of Results Obtained with Full Nutrient Roots

The design of the investigation provides two different sets of data. One set has been obtained from serial observations with cultures established from tips excised from germinated seeds. A second set was obtained from roots that had been cultured for 7 days and then dissected into successive one centimetre sections. This second set comprises three series of which two were based on cultures established from seed tip inocula, and one on subcultures set up from first phase cultured roots. The subcultured roots were used in the group of experiments involving molybdenum deficiency, and they show the longitudinal pattern of differentiation in conditions of subculture. First phase roots were those involved in the experiments with iron and magnesium deficiency.

In terms of overall growth the iron and magnesium controls were very similar quantitatively, but there were certain differences between the two experimental series. In the iron controls there was a lag phase between days 0 and 3 before substantial increases in length, cell number, protein

nitrogen, and rate of oxygen uptake occurred, whereas in the magnesium controls growth occurred immediately the tips were placed in the culture media.

The most likely basis for this difference was that the iron control roots were grown from root tips which had been cut from seeds that had been germinated for 3 days, while the magnesium controls were grown from thicker tips taken from seeds which had been germinated for only 2 days. The effect is probably similar to that described by Brown and Wightman (1952) who found a pronounced lag phase with short 0.3 cm. tips and no lag with long 1.0 cm. tips which grew directly they were transferred to culture media.

Although the different inocula gave different patterns of growth in the two series, the early differences were not maintained, and the overall growth of the two series was almost identical at the 7,9 and 11 day stages. The trends shown for the increases in length, cell number, protein nitrogen and rate of oxygen uptake per root were much as might be expected with a system that is increasing in size without restraint. These curves were similar to the well known growth curves obtained with bacterial cultures. The period of maximal growth was from day 3 to day 9 with slightly lower rates of increase between days 0 and 3 and between days 9 and 11.

The series of changes in the rate of oxygen uptake with time expressed on a per unit protein nitrogen basis were of considerable interest. The large initial decrease in the rate from day 0 to day 3 was undoubtedly a consequence of transferring the root tips from germinated seeds to culture media. Tips, when they are first cut from germinated seeds, have a relatively high rate of oxygen uptake per unit protein nitrogen, and in this phase they are metabolizing the reserve products contained in the seeds. Before growth can take place in the culture fluid the detached root tips must adjust their metabolism to the utilization of the relatively

simple primary substrates contained in the medium. It is during this period that the respiration rate falls considerably. From day 3 there was a steady increase with time in the effectiveness of root protein to catalyse the changes concerned with oxygen uptake. A similar series of changes with time in the respiration rate per unit protein nitrogen was observed by Petrie and Williams (1938) to take place in sudan grass grown with an adequate supply of nutrients..

The invertase activity, or amount of hexose formed from a standard amount of sucrose substrate per unit protein nitrogen, of cultured roots changed considerably with time. There was again a very large change between days 0 and 3 indicating that during this period the protein of full culture roots changes qualitatively. There was little further change in invertase activity between days 3 and 11.

With oxygen uptake per cell and average protein nitrogen content per cell the two series differed slightly. It is probable, however, that the trend in both these involved an initial fall between days 0 and 3, and thereafter very little change.

The results of the two experimental series in which successive one centimetre sections of seven day old roots, grown from tips cut from germinated seeds, were very similar. The outstanding feature was the unexpected distribution of cells along the length of the roots. There was a large number of cells in the first section, less than half this number in the second and from section 2 to section 6 there was a large increase in the cell number per section. Brown and Broadbent (1951) found that in pea roots growing attached to germinated seeds there was a large number of cells in the first four one millimetre sections, but thereafter there was a relatively constant number of cells per

unit length. Their roots can be considered as a simple system consisting of a terminal apex producing cells at a constant rate and so giving rise to roots which contain a constant number of cells per unit length except in the meristimatic region. This clearly is not the case in pea roots grown in culture media. The one centimetre tips cut from germinated seeds each includes between 300 and 400 thousand cells, but the one centimetre tips of seven day old roots each includes only approximately 100 thousand cells. It is clear that over the seven day experimental period there has been a large change in the size of the apical regenerating zone. Since this is the case the number of cells in any particular section must be regarded as reflecting the size and activity of the regenerating region at the time that particular section was formed.

In roots grown from root tips cut from germinated seeds, the changes in the apical meristem with time are so large that the numbers of cells in successive sections of the roots cannot be considered as representing a sequence in time. The progressive changes in cell number between sections 1 and 5 do not represent the changes with time that have occurred in section 6. This conclusion is in agreement with the observation by Richards (1934) that the changes between successive leaves of plants do not represent the changes that occur in one leaf with time.

The distribution of cells in the molybdenum control roots supports the conclusion that the distribution of cells along the length of cultured roots is influenced by the size and activity of the terminal meristem. The molybdenum control series, which were seven day old subcultured roots grown from root tips cut from ten day old cultured roots, had a terminal one centimetre section containing approximately 100 thousand cells. This was the same number of cells as in

the terminal one centimetre section of seven day old cultured roots grown from root tips cut from germinated seeds. The molybdenum control roots therefore had a fairly constant terminal section containing approximately 100 thousand from day 0 to day7, and these roots also had a relatively constant number of cells per unit length excepting in the meristematic region.

The changes in the protein nitrogen content per section and in the rate of oxygen uptake per section are also influenced in the iron controls and magnesium controls by the changes in the size of the meristem. They are in contrast to the changes found in the molybdenum controls where there was a high content of protein nitrogen and a high rate of oxygen uptake in the first section, but thereafter a relatively constant value per unit length of root.

Despite the effects of the meristem changing in size, the data from successive sections of seven day old roots nevertheless give some indication of the changes that occur with time during cell development. There is clearly a very large increase in the average volume per cell with time. This is shown by the differences between the average volumes of the cells in section 1 and section 2. A similar large increase in cell volume was demonstrated by Brown and Broadbent (1951) to occur between the apex and the five millimetre point in pea roots growing attached to germinated seeds. In the iron and magnesium controls there was an apparent increase in the average volume of the cells between sections 2 and 6, but in the molybdenum controls the average volume per cell was constant from section 2 to section 5. It is considered that in roots grown from tips taken directly from germinated seeds the first formed cells have a greater capacity for expansion than those formed subsequently. The situation is very similar with respect to the average protein

nitrogen content per cell. There is a large increase initially as shown by the changes between sections 1 and 2 in all three experimental series. The apparent increases in the average protein nitrogen content per cell between sections 2 and 6 in the iron and magnesium controls are probably due to differences in the size of the cells formed after different periods of growth. It is of interest that in the iron control series the average protein nitrogen content of the cells in section 6 of seven day old roots is very similar to the average content of the cells in the initial inoculum, indicating that only slight changes occur with time in the protein nitrogen content of fully expanded cells. Brown and Broadbent (1951) and Robinson and Brown (1952) have presented evidence to show that in roots of pea and broad bean growing attached to the seed the average protein nitrogen content of the cells in the first few millimetres is low, there is a sharp increase in the content as the cells expand and a slight decrease in the fully expanded cells. The results from the molybdenum controls, which more nearly approximate to a series in time, show that there is an initial large increase in the protein nitrogen content per cell, but thereafter this is relatively constant with time.

The changes in the average rate of oxygen uptake per cell in the different sections of roots cultured from germinated seeds are complex and only in part represent the changes with time in an average cell. The high rates of the cells in section 6 are probably associated with the fact that these cells were included with the initial inoculum. There is a close similarity between the rates of oxygen uptake per cell of the cells in section 6 of seven day old roots and of the cells in the initial day 0 tips. The cells included in the initial inoculum are very large cells, have a high protein nitrogen content and a high rate of oxygen uptake when compared with the cells actually produced in the culture medium.

The change in the average rate of oxygen uptake per cell between sections 1 and 2 of the iron and magnesium controls is clarified by reference to the data of Brown and Broadbent (1951). These workers showed that the rates for meristematic cells were low and that there were large increases during expansion. The first large one centimetre section of cultured roots contains all the meristematic cells, all the expanding cells and some fully expanded cells. The low average rate of the cells in the first centimetre is probably due to the very low rates of the meristematic cells. The high rate in section 2 indicates that fully expanded cells have a high rate of oxygen uptake per cell. The rate of oxygen uptake per cell of the mature cells falls considerably with time as shown by the change from section 2 to section 4. The results from the molybdenum control roots show that a similar downward trend in the rate of oxygen uptake per cell of the fully expanded cells occurs with time.

Estimations of the invertase activity in the different sections of the roots have shown that in this one characteristic root protein changes considerably between sections 1 and 2, but thereafter very little. Changes in invertase activity along the length of broad bean roots have been measured by Robinson and Brown (1952) and, using small one millimetre sections, they have found a very low activity in the region of cell division and a very high activity in the region of cell expansion. In broad beans there was initially a decrease in the activity of the fully expanded cells, but thereafter, even at increasing distances from the apex, the activity was relatively constant. The high activity in the first large one centimetre section of cultured roots is probably due to the high activity of the expanding cells. The slight changes in activity along the root from section 2 to section 6 indicate firstly, that changes in the size of

the meristem do not substantially alter in this respect the type of protein which is formed, and secondly, that changes with age in the activity of the protein are not large.

There were, however, large changes in the overall metabolic activity of the protein along the length of seven day old cultured roots. In both the iron and magnesium controls there was a progressive reduction in the rate of oxygen uptake per unit protein nitrogen from section 1 to section 6. A similar change occurred along the length of the molybdenum controls indicating that this change is not induced by the changing size of the meristem in the roots cultured from tips cut from germinated seeds.

It is considered that the changes in the effectiveness with which the root protein catalyses overall metabolic activity are associated with the age of the protein. Brown and Broadbent (1951), in their work with pea roots growing attached to germinated seeds, found little effect of stage of cell development on respiration. They did find some evidence that the rate of oxygen uptake per unit protein nitrogen was lower in meristematic cells than in mature cells. The present results suggest that one aspect of cell development includes a progressive reduction in the efficiency of the protein for overall metabolism after expansion has ceased.

The experiments in which the sensitivity of the respiration of pea roots to inhibition with cyanide was measured will be discussed in greater detail when considering the effects of iron deficiency. At this stage it is of interest that the percentage inhibition by 10^{-3} M. cyanide of the oxygen uptake of pea root tips, cut from germinated seeds, was of the same order as that found by Lundegardh (1949) for wheat root tips and by Henderson and Stauffer (1944) for tomato root tips.

The outstanding feature of the experiments was the decrease in the sensitivity to cyanide of the oxygen uptake of pea roots with increasing age. The sensitivity of eleven day old roots was much less than that of root tips cut from germinated seeds. This situation is similar to that described by James (1953) who investigated the sensitivity to cyanide of the oxygen uptake of barley root tips. He found that in soaked embryos of barley 80% of the respiration depends on the cytochrome system, but in root tips after ten days development cytochrome activity is not detectable. It appears that the overall metabolism of pea roots, like that of barley roots, becomes progressively less dependent on heavy metal systems with time.

The results of the experiments in which the sensitivity to cyanide of the oxygen uptake of successive sections of the roots was measured indicate that the terminal centimetre, which includes the meristem, is far more sensitive to cyanide than the remaining sections of the root. A similar result was found by Berry and Brock (1946) when they investigated the sensitivity to cyanide of the respiration of onion roots. If it can be assumed that the cyanide sensitive fraction of respiration is a measure of the activity of the cytochrome: cytochrome oxidase system, the present results may be taken as further evidence in favour of the conclusion of James (1953), 'that quantitatively cytochrome oxidase appears to be the most effective terminal oxidase in meristematic tissues and to be responsible for by far the largest part of the oxygen uptake. Satisfactory evidence of the activity of cytochrome oxidase in the respiration of adult plant tissues is much rarer and evidence of its absence or non-participation is more common.'

The last series of observations which provide information on the growth of full nutrient roots were those in which

the percentages of cells in the different stages of division were determined. The present data are of interest when compared with the results of Brown (1952) who measured the percentages of cells in the different stages of division in the root tips of germinated pea seeds. Although Brown used a slightly different technique it seems certain that in the apex of roots grown in culture media there are relatively fewer non-vacuolated cells containing mitotic figures than in the apex of roots attached to germinated seeds. This observation is compatible with a slower rate of division in the apex of cultured roots. The reductions in the numbers of mitotic figures in cultured roots do not apply to all division stages uniformly, and in particular there is a relatively large reduction in the number of cells in the stage of prophase. This effect may represent a real difference between cultured roots and roots growing attached to seeds, but on the other hand it might be a result of the different techniques used in each case.

2. Discussion of Iron Deficiency Results

The data assembled in the previous section show that iron deficiency profoundly affects the growth and development of excised pea roots. There was no further increase in cell numbers and only a slight increase in length in roots cultured in iron deficient media, after a growing period of 7 days. It is clear that in some way iron deficiency completely arrests cell division and growth. This result has not been described before. However, Burstrom (1954) has observed that wheat seedlings grown in flowing culture solutions require an adequate supply of iron before cell division and growth can proceed. This author has not presented any cell number data to support his claim that cell division ceases under conditions of iron deficiency.

The immediate reason for the suppression of cell division in iron deficient roots after a growing period of seven days is not clear. An examination of tips for the relative frequencies of cells in the different division stages showed that iron deficient roots at days 7 and 11 carried many cells in the stages of prophase and metaphase, but very few cells in the stages of anaphase and telophase. This result may be evidence that iron deficiency interrupts the process of cell division at some point between the stages of metaphase and anaphase. However, Brown, in unpublished work, found that when cell division was stopped by either anaerobic conditions or by the presence of cyanide, many cells in the stages of prophase and metaphase remained but no cells in the stages of anaphase and telophase. It may be that once mitosis progresses to the stage of early anaphase it automatically continues through to completion, and the present results may merely indicate that normal cell division has stopped.

The presence of some cells containing mitotic figures in the tips of eleven day old iron deficient roots, which had apparently stopped increasing in cell number, was surprising. It is possible that a slow rate of cell division, not large enough to be detected by the cell counting technique, was occurring even at this stage. Such a slow rate of division may have been the origin of the cells containing mitotic figures. It is considered that this possibility is unlikely and that it is more probable that when cell division is interrupted by iron deficiency, some cells remain in certain phases of mitosis for an extended period of time.

Although iron deficiency arrests cell division in some way, there is no evidence from the data that it restricts the process of cell expansion. In the successive sections of seven day old roots the volumes of the cells in corresponding sections of deficient and full nutrient roots are similar except in the terminal centimetre. In this section the average volume of the cells in the apex of deficient roots is enhanced by the circumstance that division is ceasing and the succession of small non-vacuolated cells is not being maintained. The evidence clearly indicates that iron deficiency does not restrict cell volume, and the limited increment in the length of deficient roots after 7 days can be attributed to the continuation of cell expansion in a system where cell division has ceased.

It was observed that after the 7 day stage the tips of iron deficient roots became swollen and obviously malformed. An extensive histological examination of these tips was not made, but 7 and 11 day old deficient tips were dissected under a binocular microscope. These root tips were swollen immediately behind the apex and this region contained greatly enlarged cells, while the meristem was greatly reduced.

The general effect was very similar to that described by Carlton (1954) for zinc deficiency in tomato roots. This worker emphasised the similarity of zinc deficient root tips and root tips from plants treated with naphthalene acetic acid. The further similarity of iron deficient root tips suggests that such a malformation may result from any treatment which stops cell division but permits the continuation of cell expansion. Carlton considered that the subterminal swollen zone resulted from an excessive expansion of the cells in the lateral disection. Thus in iron deficient roots although cell expansion is not restricted it is clearly an abnormal type of expansion.

The data for protein nitrogen show that there is a reduced content in the deficient roots compared with the full nutrient roots at day 7, but between day 7 and day 11 the content in both groups of roots increases considerably. There is almost a linear increase in protein nitrogen between day 3 and day 11 in the deficient roots and a higher rate of increase in the full nutrient roots. It is certainly possible that iron deficiency has no direct effect on the rate of protein synthesis and the linear increase in the deficient roots could result from a constant number of sites synthesizing protein at a constant rate. The full nutrient roots contain a greater number of cells and therefore a greater number of sites for protein synthesis after day 7.

The trend for the derived quantity, protein nitrogen per cell, shows that there is no shortage of protein nitrogen at the cell level in deficient roots. The large increase in average protein nitrogen content per cell in the deficient roots between days 7 and 11 indicates that it is not a shortage of protein nitrogen as such which limits the process of cell division in deficient roots. The experiments

in which seven day roots were examined by taking successive sections have shown that the distribution of protein nitrogen in deficient roots is different to the distribution in full nutrient roots. The front sections of deficient roots contain an increased content and the back sections a decreased content when comparisons are made with full nutrient roots. On a per cell basis, the situation is the same, and the cells in the front sections of deficient roots have a higher average protein nitrogen content and the cells in the back sections a lower content than the cells in the corresponding sections of full nutrient roots.

This accumulation of protein nitrogen in the front sections of iron deficient roots is most probably a consequence of the cessation of active division in the meristem. Brown and Wightman (1952) and Wightman and Brown (1953) have presented evidence which suggests that under normal conditions the formation and development of cells in the apex of the root is dependant on substrates synthesised in the mature regions of the root and translocated forward to the apex. It is indeed possible that normally protein precursors are moved forward and removed by the demands of the dividing apex. By the seventh day in iron deficient roots division has virtually stopped, but protein precursors undoubtedly continue to be formed. It is probable that these substances accumulate in the regions adjacent to the meristem and there condense to form proteins.

In this connection it is of interest that Possingham (1956) has shown that the precursors of protein, amino acids and amides, do accumulate in the tissues of iron deficient tomato plants. The important possibility emerges that mineral deficiencies which stop cell division may bring about the accumulation of intermediate compounds without specifically interrupting reactions in which those intermediate compounds are involved. Further, it is clear from

this result that a disturbance in one part of an organised growing system may bring about metabolic changes throughout that system.

The data obtained by Possingham (1956) have shown that iron deficiency alters the qualitative composition of the free amino acid pool of tomato plants as well as changing the total quantity of amino acids in the free pool. In addition Morgan and Keith (1954) have shown that the amino acid composition of the protein fractions isolated from different zones of bean roots vary considerably. Thus it is possible that in iron deficient roots the abnormal composition of the free amino acid pool influences the nature of the protein which is formed, and in particular, influences the metabolic characteristics of the protein. No direct evidence is available for this possibility.

The data for invertase activity per unit protein nitrogen show that in this one respect the protein of deficient roots is qualitatively similar to the protein of full nutrient roots at all stages. Furthermore the data from the seven day experiments indicate that iron deficiency has no effect on the invertase activity in any section of the root. A surprising feature is that the large cells with a high protein content in the tip of the deficient roots contain protein with a similar invertase activity to that in full nutrient tips.

It is an important feature of the deficient condition that when division has ceased this does not represent an irreversible change in the system. The cessation of division is in a sense only a suspension of activity and not a complete suppression of the mechanism on which the activity of cell division depends. This is shown by the results of the experiments in which deficient roots are transferred to a full nutrient medium after division has ceased. It is

evident that this treatment restores full activity and the growth of roots cultured in iron deficient media is limited solely by a shortage of iron. In view of this result it is not very likely that the growth of iron deficient roots is suppressed by an accumulation of toxic substances.

The oxygen uptake data, however, do suggest a reason for the failure of growth and cell division in roots cultured in an iron deficient medium. The rate of oxygen uptake of iron deficient roots was lower than that of full nutrient roots at days 3 and 5, and there is no doubt that iron deficiency reduces the rate of oxygen uptake at a stage before it stops cell division. The results of experiments in which the rate of oxygen uptake of deficient and full nutrient roots was measured in the presence and absence of cyanide show that at all stages in both groups of roots two terminal enzyme systems are operative. A system insensitive to cyanide, which would not contain heavy metals, and a system sensitive to cyanide which undoubtedly contains heavy metal components such as iron. The cyanide sensitive system most probably corresponds to the cytochrome/cytochrome oxidase system. In this connection Fritz and Beevers (1955) have recently shown that this system is quantitatively important in the respiration of pea seedlings.

The activity of the cyanide insensitive component of respiration is constant in both groups of roots from day 0 to day 3 and it increases steeply in both from day 3 to day 11. The increase in the iron deficient roots is less than the increase in the full nutrient roots. On the other hand the activity of the cyanide sensitive system decreases in both groups of roots from day 0 to day 5, increases in the full nutrient roots from day 5 to day 11, but remains almost constant in the deficient roots over the same period.

Although the total rate of oxygen uptake of deficient roots is less than that of full nutrient roots at days 3 and 5, there is a substantial increase in the rate of deficient roots from days 3 to 7 and a slight increase from day 7 to day 11. It is considered that iron deficiency restricts the total rate of oxygen uptake at day 3 and day 5 but the cyanide experiments show that the large subsequent increase in the total rate of deficient roots is due to increases in the cyanide insensitive system which is not dependent on iron.

However the most important feature of these cyanide results is that they show that there is no further increase in the activity of the cyanide sensitive system after day 5 in the deficient roots. It is of great significance that cell division stops at a stage between days 5 and 7. These results suggest the hypothesis that cell division may be influenced by the activity of a cyanide sensitive system involved in respiration.

Some circumstantial evidence is available for this hypothesis. James (1953) has concluded that in plants cytochrome oxidase appears to be quantitatively the most effective oxidase in meristematic tissues and to be responsible for by far the largest part of the oxygen consumption. He reports that satisfactory evidence of the activity of cytochrome oxidase in the respiration of adult tissues is much rarer and evidence of its absence or non participation is more common. Wigglesworth (1951) has summarized the work of many investigators who have shown that in insects the complete cytochrome system is essential for growth but not essential for maintenance. In the giant silkmoth Platysamia, it has been shown that cytochrome oxidase is very active in the growing larva and in the growing adult, but soon after pupation when diapause begins the cytochrome oxidase system is virtually out of action. A similar

result was found using Drosophila where during pupation the insects were not affected by the known inhibitors of cytochrome oxidase such as cyanide, but were very susceptible in the larval and adult stages.

It is possible that the sequence of events is that iron deficiency restricts the activity of the cytochrome oxidase system and this in turn arrests cell division and growth. However the results of the experiments in which the effects of cyanide on the respiration of the different sections of seven day old roots were assessed gave no indications that the activity of the cyanide sensitive system was lower in the tips of deficient roots than in the tips of full nutrient roots. It may be that a certain minimum cytochrome activity is required for cell division, a slight reduction stopping cell division completely, but not being capable of detection by the methods used.

The iron recovery experiments have shown that the recovery in terms of an increased rate of oxygen uptake was far greater than the recovery in terms of protein nitrogen and length. The magnitude of the increase in respiration suggests that significant changes in the existing protein of iron deficient roots occurred when iron was added to deficient roots, and it is possible that the recovery of respiration preceeded the new synthesis of cells and protein. In addition, it has been shown that when cell division is stopped by iron deficiency, anaerobic conditions or with cyanide, the array of cells containing mitotic figures is similar in each case.

The oxygen uptake data expressed both on a per unit protein nitrogen basis and on a per cell basis are also of some significance in relation to the present hypothesis. The results from the seven day old roots which were dissected

into successive sections show that iron deficiency primarily affects the metabolism of the regions containing newly formed cells. The rate of oxygen uptake per unit protein nitrogen was greatly reduced in the front three sections of deficient roots but practically unaffected in the last three sections.

The higher rate of oxygen uptake per cell of the cells in the front sections of deficient roots undoubtedly results from the large accumulation of protein nitrogen in these cells. The trends with time for whole roots show that iron deficiency reduces the average rate of oxygen uptake per cell at all stages except at the day 11 stage. Once again this effect results from the accumulation of relatively inactive protein nitrogen in the deficient roots.

The trend with time in rate of oxygen uptake per unit protein nitrogen has two inflections. It is considered that the depressions at day 3 and day 5 are a direct effect of iron deficiency, but the reductions at days 9 and 11 are in part a consequence of the fact that cell division has stopped at this stage but protein synthesis has continued.

As indicated earlier iron deficiency at day 7 reduces the rate of oxygen uptake per unit protein nitrogen of the newly formed tissues but does not affect that of the mature tissues in the back sections of the root. This effect is intimately connected with the fact that iron deficiency stops cell division, but it may also be related to the location of iron in the tissues of deficient roots. Biddulph (1953) has shown, using radio-active iron, that in plants this element is incorporated into most tissues but it is not subsequently translocated from mature to newly formed tissues to any great extent even under conditions of iron deficiency. It is thus possible that the tips of iron deficient roots have a much lower iron status than the mature sections of the roots. This possibility could contribute to failure of cell division in deficient roots, but if this were the

basis of the effect the reduction in division would probably be more gradual than was found. In addition, the cyanide data suggest that the iron containing cytochrome system is relatively abundant in deficient tips.

In conclusion it is emphasised that although the data obtained in this series of experiments fit the hypothesis that growth and cell division are arrested in iron deficient roots because of a restriction of the cytochrome oxidase system, there is no unequivocal proof of this hypothesis. It must be pointed out that the cyanide sensitive system involved in respiration has not been shown to be identical with cytochrome oxidase and the observed correlation may not be causal. The observed very abrupt cessation of cell division could also result if iron were a direct constituent of nuclei or if iron were involved in the synthesis of desoxyribose nucleic acid. Furthermore the relatively large quantities of iron required for normal growth suggest that this element has other important functions in plant cells besides acting enzymatically in the cytochrome oxidase system.

3. Discussion of Magnesium Deficiency Results

In the present experiments the rate of growth of pea roots cultured in a magnesium deficient medium was lower than that of roots cultured in a full nutrient medium. Although this deficiency reduced the rate of oxygen uptake at day 3, and reduced the protein nitrogen content and total cell number by day 7, it did not stop the growth of pea roots during the experimental period of eleven days.

As with iron deficiency, the primary effect of deficiency was a reduction in the rate of oxygen uptake. Magnesium has not been recognised as a component of any known terminal enzyme system operative in plants. However, McElroy and Mason (1954) have cited evidence which shows that this element may be an activator of at least twelve different enzyme systems involved in carbohydrate metabolism, and also that this element is concerned with the activation of a further eight enzyme systems involved in the citric acid cycle. It is clearly possible that the effect of this deficiency on oxygen uptake was established by restricting reactions concerned with carbohydrate metabolism.

In this connection it is of some interest that Petrie and Williams (1938) found that phosphorus deficiency in oat plants reduced the rate of carbon dioxide production per unit protein nitrogen. In their experiments they followed the drifts in respiration with time in oat plants grown under three different levels of phosphorus supply. The respiration rate per unit protein nitrogen in the low phosphorus plants was lower than that of the high phosphorus plants at the second harvest and remained almost constant with time over a further sixty days. The rate of the high phosphorus plants increased considerably over the same period. In the case of phosphorus it is known that this element is involved in carbohydrate metabolism as well as being con-

cerned in many energy transferring reactions. The effect of phosphorus deficiency on the respiration rate of oat plants was very similar to the effect of magnesium deficiency on the rate of oxygen uptake per unit protein of detached pea roots.

The most surprising feature of magnesium deficiency was that it permitted growth in terms of new cell production to continue for a long period after the rate of oxygen uptake was depressed. Further the reductions in length, cell number, and protein nitrogen in the magnesium deficient roots were of approximately the same order and the slowing down of growth took place in an essentially organised sequence. At all stages the average protein nitrogen content per cell was approximately the same in both groups of roots. It is probable therefore that in this case the rate of cell division was determined at least partially by the rate of supply of protein components.

The rate of cell division was reduced in the deficient roots but to a less extent than the rate of oxygen uptake, as there was a consistently lower average rate of oxygen uptake per cell in the deficient roots. This situation indicates that once again the primary effect of the deficiency is to reduce the overall rate of metabolic activity and this in turn restricts the rate of new cell formation and the rate of protein synthesis.

It is considered that the effect on cell division in these experiments was entirely indirect. It is of some interest that Steffenson (1953) has found that in magnesium deficient Tradescantia meiosis is abnormal, as he observed abnormalities between the bivalents in metaphase and also many chromosome aberrations and breakages. Fink (1950) has found mitotic anomalies in the root tips of Vicia faba, which were grown under conditions of a low magnesium supply,

while Finkle and Appleman (1953) directed attention to the fact that magnesium deficiency interrupts division in Chlorella cells without immediately bringing about a general inhibition of synthesis. Here may also be mentioned the findings of Webb (1953) on Clostridium welchii, which, like other gram positive rods, shows an inhibition of cell division and a resulting thread formation in cases of magnesium deficiency. All this evidence is indeed suggestive that magnesium is directly involved in cell division but the results of the present experiments show that this element becomes limiting for other metabolic reactions at a stage prior to reducing the rate of cell division.

The results of the experiments in which the longitudinal differentiation of seven day old roots was measured are consistent with the conclusion that magnesium deficiency brings about an organised slowing down of growth. The first three sections of the deficient roots contained approximately the same number of cells as the corresponding sections of full nutrient roots. The effect of magnesium deficiency in reducing the overall rate of division was shown by the large difference between the cell numbers in section 6 of the deficient and full nutrient roots.

This deficiency had no effect on the process of cell expansion as the average volume of the cells in the deficient and full culture roots was almost identical in the corresponding sections. The close similarity between the terminal one centimetre sections of deficient and full nutrient roots, both in respect of cell number and cell size, suggests that the meristem of both groups of roots was reasonably similar at this seven day stage. Even at the eleven day stage there was no visual evidence of abnormality in the tips of the deficient roots, and there was no swelling similar to that found in eleven day iron deficient tips.

Magnesium deficiency did not alter the normal distribution of protein along the length of the roots, and as a consequence the average protein nitrogen content per cell was the same in the corresponding sections of deficient and full nutrient roots. The measures of cell number, cell volume and protein nitrogen show that the deficient roots were similar to the full nutrient roots, at least over the first seven days.

However magnesium deficiency did considerably reduce the rate of oxygen uptake per unit protein nitrogen at the seven day stage. The rate was uniformly reduced in all sections of the deficient roots when comparisons were made with the rates in the corresponding sections of full nutrient roots. This result is compatible with the hypothesis that magnesium acts primarily as an activator of enzyme systems and as such is not bound to any particular section of the root. Under conditions of deficiency the magnesium of older tissues is free to move to newly formed tissues so that there is a uniform concentration throughout the length of the deficient roots. The general lowering of magnesium concentration as the system grows brings about the uniform reduction in the rate of oxygen uptake per unit protein nitrogen in all sections of the deficient roots.

There is some evidence in favour of the extreme mobility of this element in plant tissues as Phillis and Mason (1942) found a high diurnal fluctuation in the magnesium content of cotton leaves. Fudge (1939) has also shown that magnesium can be withdrawn from the near by leaves by developing citrus fruits to such an extent that deficiency symptoms can develop.

4. Discussion of Molybdenum Deficiency Results

The experiments which have been conducted to investigate the effects of a deficiency of molybdenum are only preliminary, but they do indicate that detached pea roots cultured in liquid media can be obtained deficient in this element. Of the elements known to be essential for the growth of higher plants, molybdenum is required in the smallest amounts, and in the present investigations deficient roots were only obtained after two successive tip passages. It appears that one centimetre root tips cut from germinated pea seeds contain sufficient molybdenum to sustain the growth of cultured roots for approximately ten days. This experience is similar to that of Hannay and Street (1954) who found that three successive tip passages were necessary to produce molybdenum deficient tomato roots.

In the present experiments, where a growing period of seven days was used, deficiency of molybdenum reduced the length per root, the cell number per root, the protein nitrogen content per root and the rate of oxygen uptake per root. Conclusions from such preliminary data are necessarily speculative, but it is probable that the observed changes were not initiated by a primary reduction in protein nitrogen. This was expected, as Mulder (1948) and Spencer and Wood (1954) have shown that a deficiency of molybdenum in higher plants leads to a reduction in the content of protein nitrogen. There was, however, no evidence of a consistent reduction in the average protein nitrogen content per cell in the deficient roots, a situation which would be expected if cell division and growth were restricted by a shortage of protein as such. It may be that such a change never occurs in this system and that no significant increase in the cell number is possible once the synthesis of protein is restricted.

The reduction in the rate of oxygen uptake was greater than the reduction in protein nitrogen, with the result that there was a large decrease in the rate of oxygen uptake per unit protein nitrogen in the deficient roots. Also the reduction in the rate of oxygen uptake was greater than the reduction in cell numbers as there was a slight decrease in the average rate of oxygen uptake per cell in the deficient roots. These results suggest that once again the primary effect of the deficiency was to affect the overall rate of oxygen uptake of the system.

It is not clear why molybdenum deficiency should restrict the rate of oxygen uptake, unless such an effect can result from any treatment which affects the rate of an essential metabolic reaction. Nicholas, Nason and McElroy (1954) have shown that molybdenum is essential for the activation of the nitrate reductase system of Neurospora, while Spencer (1954) has suggested that the activity of acid phosphatases isolated from tomato leaves may be controlled by molybdenum. It is possible that changes in the rates of many metabolic reactions may be associated with changes in the overall rate of oxygen uptake.

The present finding that molybdenum deficient roots have a lowered rate of oxygen uptake when compared with full nutrient roots is in agreement with the work of Nason, Oldewurtel and Propst (1952) who found that the endogenous oxygen uptake of homogenates of the apical leaves of molybdenum deficient tomato plants was lower than that of homogenates of the apical leaves of full nutrient plants. Their results were expressed per ml. of homogenate, and on a dry weight basis, but not on a per unit protein nitrogen basis. By contrast Loneragon and Arnon (1954) have reported that the endogenous oxygen uptake per gram dry weight was higher in molybdenum deficient cultures of Chlorella than the corres-

ponding full nutrient cultures. These workers also reported that Spencer, in unpublished experiments, found that leaf discs taken from the lower leaves of molybdenum deficient tomato plants had a higher rate of oxygen uptake per gram dry weight than those taken from the lower leaves of full nutrient plants. The reason for these contrasting results is not clear, but it is possible that an early onset of senescence in molybdenum deficient tissues could account for the effects observed by Spencer.

In pea roots the rate of oxygen uptake per section and per unit protein nitrogen was depressed in practically all sections of the deficient roots when comparisons were made with the rates in full nutrient roots. This situation suggests that molybdenum is uniformly deficient throughout the entire length of the roots, and that it is partially withdrawn from mature cells and translocated to the newly formed cells. Little information is available on the relative mobility of this element in plant tissues, but the author in unpublished experiments has observed that molybdenum deficient tomato plants continue to grow for an extended period in nutrient culture solutions by a process of the older leaves dying off before the next pair of upper leaves expand. The pattern of growth is consistent with the possibility that molybdenum from the lower leaves is utilized by the young expanding upper leaves.

One further effect of molybdenum deficiency in pea roots is that it reduced the average volume per cell. The deficiency reduced the average volume of the cells in all sections of the root, and per haps the most surprising feature was that it was not associated with a similar reduction in the average protein nitrogen content per cell. It is possible that the effect of molybdenum deficiency on cell expansion was an indirect one again brought about by the low rate of oxygen uptake of deficient roots. Nevertheless, it is surprising that the average volume of the cells in all sections of the root was reduced.

5. Comparison of the Effects of Deficiencies of Iron, Magnesium and Molybdenum

Comparisons between plants deficient in different essential nutrient elements are necessarily complicated and may be irrelevant. It is doubtful whether a plant deficient in one nutrient which catalyses certain reactions can ever be compared with another plant deficient in another nutrient which catalyses a different set of reactions. This is especially true where the levels of intermediate compounds are compared and also where the activity of particular enzyme systems are contrasted.

However it is possible to compare the way in which deficiencies of different elements restrict the overall growth of plant systems. It has been shown that iron deficiency brings about changes in pea roots which in a very short period completely stop the growth of the system in terms of new cell formation. Cell division in this case is abruptly stopped, but cell expansion continues. Active protein systhesis takes place in the deficient roots giving rise to large increases in the average protein nitrogen content per cell. It is probable that many intermediate compounds also accumulate in iron deficient plants once new cell formation has stopped.

On the other hand it has been shown that magnesium deficiency in pea roots reduces the rate of growth, but over an experimental period of eleven days this deficiency does not stop growth. In the deficient roots cell division continues at a reduced rate and once again cell expansion is unaffected. The rate of protein synthesis is reduced with this deficiency but to the same extent as cell division so that the average protein nitrogen content per cell is unaltered by the deficiency.

A third situation is found in molybdenum deficient roots. With this deficiency, as with magnesium deficiency, cell division and protein synthesis are reduced to the same extent. However molybdenum deficiency restricts the process of cell expansion as the average volume per cell is reduced in deficient roots.

Further analysis of the effects of these three deficiencies have shown other important differences. Iron deficiency does not affect the metabolism of the older mature cells, but it does specifically reduce the metabolism of the newly formed cells. It is possible that with this element once it is incorporated into cells it is not subsequently released and transported to meristematic regions even under conditions of extreme deficiency. However it is also possible that iron deficiency only affects the metabolism of dividing and newly formed cells. Magnesium deficiency, on the other hand, uniformly restricts the metabolism of all the cells of the system. In this case it is probable that this element is not bound to cellular constituents and is free to move from regions of high concentration to regions of low concentration. Although the experiments with molybdenum deficient roots were only preliminary, the indications are that a deficiency of this element affects all the cells of the system uniformly.

The three deficiencies of iron, magnesium and molybdenum all restricted growth by first reducing the rate of oxygen uptake. It might be expected that such a change would precede changes in the rate of cell division and in the rate of protein synthesis, but this situation has not been demonstrated before. It is suggested that the overall rate of oxygen uptake may be depressed in many cases of mineral deficiency at a stage before deficiency symptoms

develop. Steinberg (1951) and Steward and Thompson (1954) have suggested that protein metabolism and growth are so intimately connected that most mineral deficiencies must ultimately affect growth by their impact on protein metabolism. The results of the present experiments show that there was no shortage of protein nitrogen as such on a per cell basis in all three deficiencies. In the case of iron deficiency there seems no doubt that shortage of protein nitrogen did not restrict the overall growth of the system. This may not be true in the cases of magnesium and molybdenum deficiency where the rate of cell division was possibly adjusted to the reduced rate of protein synthesis. It is significant that none of the deficiencies brought about reductions in the average protein nitrogen content per cell, and it may be that for cell division to proceed, a certain minimum level of protein nitrogen must be available.

In conclusion, it has been shown that in the case of at least one deficiency, iron, overall growth was stopped by an arrest of cell division, while in the case of another deficiency, molybdenum, it has been shown that the restriction of growth was partially brought about by a restriction of the cell expansion process. It is clear therefore that investigations on the importance of the various mineral nutrients in the basic processes of cell division and cell expansion are essential for an understanding of the effects of mineral deficiencies on the growth and development of plants.

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VI APPENDIX

The primary data used in the preparation of Figures 1 to 45, which are presented in the text, are given in this section.

Roots ± Fe - WHOLE ROOT DATA

Tables 8 to 14 comparisons of Roots grown with and without added iron in the nutrient culture media. Root tips from germinated pea seeds cultured and harvests of whole roots made after growth periods of 0. 3. 5. 7. 9 and 11 days.

TABLE 8 Day 0 ± Iron Roots

Expt. No.	Cell No per Root	Protein N. per Root (µg)	O_2 Uptake per Root (cu.mm per hr.)	O_2 Uptake per unit Prot.N. (cu.mm/hr per µg. Prot. N.)	No. of Roots	Length per Root (cm)	Protein per Cell (µg x 10^{-6})	O_2 Uptake per Cell (cu.mm.x 10^{-6}/hr)
B 3.	273,958	58.88	9.98	.1692				
	222,917	60.64	8.79	.1451				
	245,833	56.80	10.73	.1890				
	239,583	56.00	9.81	.1752				
	255,000	56.00	9.13	.1631				
		63.52	10.42	.1641				
		62.40	10.23	.1639				
		51.20	8.82	.1722				
		58.24	9.50	.1629				
MEAN	247,458	59.20	9.71	.1674	100	1.0	239.2	39.24

TABLE 9 Day 3 ± Iron Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake per Unit Prot. N) (cu.mm/hr per µg. Prot. N)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 7.	223,804	257.785	47.84	48.64	7.02	6.73	.1467	.1384
	244,896	291,766	58.88	47.84	6.86	7.24	.1164	.1513
	263,644	277.705	47.84	58.88	6.12	7.76	.1279	.1318
	222,632	294,109						
Mean	238,744	280,341	51.52	51.84	6.66	7.26	.1304	.1467
B 9.	287,079	302,312	58.88	56.00	6.12	7.31	.1040	.1307
	278,876	278,876	56,64	56.00	6.84	7.24	.1207	.1294
	299,968	246,062	49.28	53.76	5.94	6.99	.1204	.1302
	288,250	203,885	50.88	44.16	5.80	6.21	.1143	.1406
Mean	288,543	257,784	53.92	52.48	6.17	6.94	.1150	.1343
B 27	346,875	331,250	66.26	61.84	8.17	9.05	.1234	.1464
	364,062	337.500	69.94	77.30	9.48	10.80	.1356	.1397
	378.125	315,625	65.52	64.05	8.56	8.96	.1307	.1400
	353,125	379,687	64.78	61.84	8.06	9.18	.1244	.1484
Mean	360,046	341,015	66.62	66.26	8.57	9.50	.1285	.1436
MEAN	295,777	293,046	57.89	57.31	7.18	7.96	.1240	.1388

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per cell ($cu.mm \times 10^{-6}/hr$)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 7.	28	28	2.06	2.07	215.8	184.9	27.89	25.90
B 9.	32	32	2.24	2.05	186.9	203.6	21.38	26.92
B 27	32	32	2.46	2.71	185.0	194.3	23.80	27.86
MEAN	-	-	2.26	2.28	195.9	194.2	24.36	26.89

TABLE 10 — Day 5 ± Iron Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake per Unit Prot. N) (cu.mm/hr per µg. Prot. N)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 6.	420,166	379,166	66.24	71.04	10.35	9.36	.1563	.1324
	339,583	339,583	69.92	62.56	10.31	10.38	.1475	.1659
	350,000	379,166	58.24	62.56	9.74	11.11	.1674	.1777
Mean	369.916	365.872	64.64	65.44	10.13	10.26	.1571	.1587
B 23.			80.98	67.73	8.45	10.02	.1043	.1480
			80.98	80.98	10.00	10.02	.1235	.1237
			83.92	80.98	9.74	11.76	.1160	.1452
			65.52	75.82	9.05	9.17	.1383	.1209
			79.50	91.30	10.26	11.38	.1291	.1247
Mean	-	-	78,18	79.36	9.50	10.22	.1223	.1325
B 26.	341,933	379,040	86.13	67.73	10.02	9.76	.1164	.1437
	320,312	281,250	76.56	64.05	9.18	9.95	.1199	.1552
	300,000	292,188	67.73	67.73	8.80	8.84	.1299	.1305
	353,125	365,625	68.46	70.67	8.20	10.12	.1198	.1431
	275,000	292,188	55.66	64.78	7.76	10.26	.1387	.1584
Mean	318,074	322,058	70.96	67.74	8.79	9.79	.1250	.1462
MEAN	337.514	338,488	72.27	71.58	9.37	10.07	.1313	.1438

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per cell ($cu.mm \times 10^{-6}$/hr)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 6.	21	21	3.60	3.90	174.7	178.9	27.38	28.04
B 23.	20	20	3.14	3.59	-	-	-	-
B 26.	40	40	3.30	3.44	233.0	210.3	27.64	30.40
MEAN	-	-	3.34	3.59	203.9	194.6	27.51	29.22

TABLE 11 Day 7 ± Iron Roots

Expt. No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr)		O_2 Uptake per Unit. Prot. N (cu.mm/hr. per µg. Prot. N)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
A 16.	524,219	558,214	95.7	107.7	12.52	16.39	.1308	.1522
B 1.	-	-	82.1	93.3	12.28	14.54	.1496	.1558
B 2.	528,416	506,208	83.9	88.9	14.23	15.37	.1695	.1728
B 4.	437,478	501,691	91.9	99.9	14.85	14.97	.1616	.1499
MEAN	496,704	522,036	88.4	97.5	13.47	15.32	.1529	.1577

Expt. No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell ($cu.mm \times 10^{-6}$/hr)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
A 16.	28	28	5.80	6.47	182.6	192.9	23.94	29.34
B 1.	16	16	5.70	6.60				
B 2.	20	20	6.57	6.90	158.8	175.6	26.92	30.35
B 4.	22	22	6.15	6.58	210.0	199.1	33.94	29.84
MEAN	-	-	6.05	6.64	183.8	189.2	28.27	29.84

TABLE 12 — Day 9 ± Iron Roots

Expt. No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake per Unit Prot. N) (cu.mm/hr. per µg. Prot. N)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 16.	471,491	719,409	115.1	146.0	17.11	24.90	.1486	.1714
B 23.			94.2	97.9	11.44	15.98	.1214	.1631
			80.2	136.9	11.27	22.14	.1405	.1617
			97.9	119.0	12.43	17.94	.1270	.1603
			84.7	103.1	11.70	16.32	.1382	.1583
Mean	471,491	719,409	94.4	119.2	12.79	19.45	.1352	.1630
B 28.	423,801	546,850	122.2	111.9	17.44	19.17	.1427	.1751
	453,096	555,053	110.4	136.9	15.89	21.95	.1439	.1603
	521,451	634,346	100.1	110.4	13.81	17.51	.1379	.1595
	472,626	628,878	78.0	110.4	11.70	19.41	.1499	.1758
Mean	467,743	591,282	102.7	117.4	14.71	19.51	.1436	.1676
B 29.	540,981	740,625	134.9	134.9	17.44	21.33	.1292	.1581
	517,545	646,875	103.0	122.7	13.84	20.64	.1343	.1682
	474,579	687,500	98.1	115.3	12.71	18.63	.1295	.1616
	462,861	646,875	98.1	154.6	10.82	24.12	.1102	.1561
Mean	498,991	680,469	108.5	131.9	13.70	21.18	.1258	.1610
MEAN	479,408	663,720	101.9	122.8	13.73	20.05	.1347	.1633

Expt. No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell ($cu.mm \times 10^{-6}$/hr)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 16.	26	26	6.58	8.97)				
B 23.	16	16	6.06	8.28)	200.2	165.7	27.13	27.04
B 28.	32	32	6.32	8.29	219.6	198.6	31.45	33.00
B 29.	32	32	6.82	9.61	217.4	193.8	27.46	31.13
MEAN	-	-	6.50	8.85	212.4	186.0	28.68	30.39

TABLE 13 Dey 11 ± Iron Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake per Unit Prot. N (cu.mm/hr per µg. Prot. N)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 18.	422,849	797,789	93.3	112.9	12.35	18.36	.1324	.1626
	416,600	735,299	90.3	110.9	11.67	17.42	.1292	.1570
	477,007	739,465	86.4	103.2	11.79	16.73	.1364	.1549
	431,181	729,050	91.3	110.9	12.78	17.97	.1400	.1614
Mean.	436,909	750,401	90.3	109.5	12.15	17.61	.1345	.1590
B 28.	433,566	775,000	122.2	159.0	16.08	26.09	.1316	.1641
	453,096	781,250	119.2	147.2	15.12	23.77	.1268	.1615
	468,720	781,250	108.9	135.5	14.64	21.73	.1344	.1605
	507,780	744,375	120.7	164.8	15.86	26.15	.1314	.1587
Mean.	465,791	767,969	117.7	151.6	15.42	24.43	.1310	.1612
B 29.	600,000	875,000	120.2	176.6	14.17	26.32	.1179	.1773
	499,219	839,844	127.6	147.2	15.63	25.08	.1225	.1704
	450,000	632,812	134.9	169.3	18.24	27.43	.1351	.1620
	421,875	726,562	149.7	157.0	18.98	25.57	.1268	.1629
Mean.	516,211	768,554	133.1	162.5	16.75	26.10	.1256	.1681
MEAN.	472,970	762,308	113.7	141.6	14.77	22.71	.1304	.1628

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell ($cu.mm \times 10^{-6}/hr$)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
B 18.	24	24	6.12	8.80	216.7	147.5	27.81	23.46
B 28.	32	32	6.58	10.10	252.7	197.4	33.10	31.81
B 29.	32	32	7.26	11.64	257.8	211.4	32.45	33.96
MEAN.	-	-	6.70	10.30	242.4	185.4	31.12	29.75

TABLE 14a Invertase Activity of ± Iron Roots

Whole Root Data

Expt. No.	No. of Roots		Mean Length per Root (cm)		Hexose per Root (µg)		Prot.N. per Root (µg)		Invertase Activity (µg hexose/ µg Prot. N)		Recalculated* Invertase Activity	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Day 0												
B 17	70				2233		47.12		47.40			
					2060		44.77		46.02			
					1790		44.18		40.53			
					1829		38.88		47.05			
					1579		35.34		44.68			
					1906		40.05		47.58			
					2098		44.77		46.88			
MEAN			1.0		1928		42.13		45.73		32.56	
Day 3												
B 12	35	35			385	440	42.88	41.92	8.974	9.961		
					394	339	50.56	42.08	7.809	8.064		
					412	431	43.68	58.08	9.428	7.423		
					394	339	46.72	42.08	8.442	8.064		
					389	348	46.72	62.24	8.345	6.089		
MEAN			2.78	2.65	395	379	46.08	48.32	8.375	7.920	6.823	6.613
Day 5												
B 14	25	25			475	488	54.77	73.62	8.671	7.737		
					577	488	62.43	63.02	7.843	8.448		
					513	593	57.71	63.02	8.222	9.491		
					699	513	57.71	73.02	11.098	9.578		
					449	603	53.60	53.01	7.782	11.381		
MEAN			3.57	3.83	543	537	57.25	65.14	8.723	9.327	7.513	7.502

* Invertase activity recalculated using the above hexose per root values but using the protein nitrogen values established in the previous experiments.

TABLE 14b Invertase Activity of ± Iron Roots

Whole Root Data

Expt. No.	No. of Roots		Mean Length per Root (cm)		Hexose per Root (µg)		Prot. N. per Root (µg)		Invertase Activity (µg hexose/ µg Prot. N)		Recalculated* Invertase Activity	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Day 7												
B 10	20	20	5.00	5.85	500	555	63.17	54.35	7.922	8.626		
B 11	20	20	5.78	6.76	635	821	73.79	77.01	8.824	9.165		
B 13	24	24	5.51	6.11	655	706	74.11	74.29	8.898	9.606		
B 15	14	14	4.95	6.07	600	733	64.37	76.43	9.328	9.578		
MEAN			5.31	6.07	598	704	68.85	73.02	8.743	9.244	6.764	7.220
Day 9												
B 21	24	24			1010	1107	72.14	88.30	14.00	12.53		
					1010	1131	73.62	86.14	13.87	13.13		
					989	1000	72.88	73.62	13.54	13.57		
					1071	1203	79.50	92.03	13.47	13.07		
					963	1071	72.88	88.30	13.21	12.12		
					794	1071	66.99	78.77	11.86	13.59		
MEAN			6.32	7.63	973	1097	73.01	84.53	13.33	13.00	9.549	8.933
Day 11												
B 22	20	20			1082	1227	72.14	83.18	15.00	14.75		
					1227	1227	88.34	72.14	13.89	17.00		
					1227	1467	80.98	91.28	15.15	16.08		
					1227	1371	83.18	83.92	14.75	16.34		
					962	1131	63.31	93.49	15.20	12.10		
MEAN			6.18	8.98	1145	1285	77.57	84.80	14.80	15.25	10.07	9.07

* Invertase activity recalculated using the above hexose per root values but using the protein nitrogen values established in the previous experiments.

7 DAY ROOTS ± Fe - SECTIONED DATA

TABLES 15 TO 27

Comparisons of roots grown with and without added iron in the nutrient culture media. Root tips from germinated pea seeds cultured for 7 days and the roots harvested by taking serial one centimetre sections commencing at the apex.

TABLE 15

Expt. No.	No. of Roots		Mean Root Length (cm)	
	-Fe	+Fe	-Fe	+Fe
A 16	28	28	5.80	6.47
B 1	16	16	5.70	6.60
B 2	20	20	6.57	6.90
B 4	22	22	6.15	6.58
Mean	-	-	6.05	6.62

TABLE 16

Number of Cells per Section

Expt. No.	-Fe				+Fe			
	A 16	B 2	B 4	Mean	A 16	B 2	B 4	Mean
Section 1.	76,674	92,803	87,178	85,552	99,777	110,144	95,146	101,689
2.	45,089	57,187	41,250	47,842	46,384	49,062	41,875	45,774
3.	60,938	50,312	52,187	54,479	58,036	57,562	51,562	55,387
4.	100,893	64,062	58,125	74,360	87,946	64,062	62,500	71,503
5.	112,500	94,677	86,250	97,809	97,768	70,774	97,812	88,785
6.	128,125	109,375	112,488	116,663	168,303	155,604	152,796	158,901
Total	524,219	528,416	437,478	496,704	558,214	506,208	501,691	522,036

TABLE 17 — Protein Nitrogen per Section (µg. Protein N.)

Expt. No.	-Fe A 16	-Fe B 1	-Fe B 2	-Fe B 4	-Fe Mean	+Fe A 16	+Fe B 1	+Fe B 2	+Fe B 4	+Fe Mean
Section 1.	12.31	12.88	15.31	14.48	13.77	11.36	11.41	10.61	11.78	11.29
2.	8.20	7.92	7.36	8.10	7.89	7.57	7.55	6.48	6.50	7.02
3.	11.36	9.76	9.42	10.06	10.15	8.83	9.39	8.24	8.35	8.70
4.	16.20	14.18	11.49	12.61	13.62	13.88	12.53	10.02	12.03	12.12
5.	21.67	16.56	12.37	16.96	16.89	18.51	16.21	15.31	15.71	16.44
6.	25.87	20.80	27.98	29.70	26.09	47.54	36.26	38.29	44.64	41.68
Total	95.71	82.10	83.94	91.90	88.41	107.70	93.34	88.94	99.87	97.26

TABLE 18 — Rate of Oxygen Uptake per Section (cu.mm per hr)

Expt. No.	-Fe A 16	-Fe B 1	-Fe B 2	-Fe B 4	-Fe Mean	+Fe A 16	+Fe B 1	+Fe B 2	+Fe B 4	+Fe Mean
Section 1.	2.083	2.589	2.725	2.920	2.579	2.681	2.565	2.808	2.520	2.644
2.	1.446	1.409	1.640	1.710	1.551	1.534	1.714	1.477	1.580	1.576
3.	1.835	1.519	1.547	1.840	1.685	1.767	1.849	1.428	1.530	1.644
4.	2.080	2.080	2.080	1.910	2.040	2.181	2.070	1.760	1.550	1.890
5.	2.187	2.263	2.130	2.480	2.265	2.464	2.292	2.027	1.930	2.178
6.	2.891	2.423	4.104	3.990	3.352	5.766	5.046	4.865	5.870	5.387
Total	12.522	12.283	14.226	14.850	13.472	16.393	14.536	15.365	14.970	15.319

TABLE 19

Average Cell Volume (cu.mm x 10^{-6})

	-Fe				+Fe			
Expt. No.	A 16	B 2	B 4	Mean	A 16	B 2	B 4	Mean
Section 1.	42.53	43.23	46.67	44.14	34.54	30.23	30.44	31.74
2.	76.91	79.06	89.26	81.74	90.35	79.86	83.30	84.50
3.	88.62	104.17	92.72	95.17	105.00	88.88	91.35	95.08
4.	97.83	93.19	90.49	93.84	93.23	111.72	106.01	103.65
5.	131.11	92.88	109.25	113.41	127.53	108.02	93.39	109.65
6.	122.17	121.55	110.16	117.96	98.72	119.73	114.04	110.86
Total	101.46	92.96	90.66	95.03	91.38	90.15	88.26	89.93

TABLE 20

Rate of Oxygen Uptake per Unit Protein Nitrogen

(cu. mm. per hr per µg. Prot. N.)

	-Fe					+Fe				
Expt. No.	A 16	B 1	B 2	B 4	Mean	A 16	B 1	B 2	B 4	Mean
Section 1.	.1678	.2010	.1780	.2017	.1871	.2360	.2248	.2647	.2139	.2349
2.	.1763	.1779	.2228	.2111	.1970	.2026	.2270	.2279	.2430	.2251
3.	.1615	.1556	.1642	.1829	.1660	.2001	.1969	.1733	.1832	.1884
4.	.1284	.1467	.1810	.1515	.1519	.1571	.1652	.1756	.1288	.1587
5.	.1009	.1367	.1722	.1462	.1390	.1331	.1414	.1324	.1228	.1324
6.	.1117	.1165	.1467	.1543	.1273	.1213	.1392	.1270	.1315	.1297
Total	.1308	.1496	.1694	.1615	.1528	.1522	.1557	.1727	.1498	.1576

TABLE 21 Rate of Oxygen Uptake per Cell (cu.mm. x 10^{-6} per hr)

Expt. No.	-Fe A 16	-Fe B 2	-Fe B 4	-Fe Mean	+Fe A 16	+Fe B 2	+Fe B 4	+Fe Mean
Section 1.	27.17	29.36	33.49	30.01	26.87	25.49	26.49	26.28
2.	32.07	28.68	41.45	34.07	33.07	30.10	37.73	33.63
3.	30.11	30.75	35.26	32.04	29.00	25.25	29.67	27.97
4.	20.62	32.47	32.86	28.65	24.80	27.47	24.80	25.69
5.	19.44	22.50	28.75	23.56	25.20	28.64	19.73	24.52
6.	22.56	37.52	35.47	31.85	34.26	31.27	38.42	34.65
Total	23.94	26.92	33.94	28.27	29.37	30.35	29.84	29.85

TABLE 22 Protein Nitrogen per Cell (µg x 10^{-6})

Expt. No.	-Fe A 16	-Fe B 2	-Fe B 4	-Fe Mean	+Fe A 16	+Fe B 2	+Fe B 4	+Fe Mean
Section 1.	161.8	164.9	166.0	164.2	113.8	96.3	123.8	111.3
2.	181.9	128.6	196.3	168.9	163.2	132.0	155.2	150.1
3.	186.4	187.4	192.8	188.8	152.2	145.8	161.9	153.3
4.	160.5	179.4	216.9	185.6	157.8	156.3	192.5	168.9
5.	192.6	130.6	196.6	173.3	189.3	216.3	160.6	188.7
6.	201.9	255.8	264.0	240.6	282.4	246.1	292.2	273.6
Total	182.6	158.9	210.1	183.9	1192.9	175.7	199.1	189.2

Invertase Activity of ± Iron Roots

7 Day Roots ± Fe - Sectioned Data

TABLE 23

Expt. No.	No. of Roots		Mean Length per Root (cm)	
	-Fe	+Fe	-Fe	+Fe
B 10	20	20	5.00	5.85
B 11	20	20	5.78	6.76
B 13	24	24	5.51	6.11
B 15	14	14	4.95	6.07
MEAN	-	-	5.31	6.07

TABLE 24

Hexose per Section (µg. hexose)

Expt. No.	-Fe					+Fe				
	B 10	B 11	B 13	B 15	Mean	B 10	B 11	B 13	B 15	Mean
Section 1.	123.5	131.5	173.7	160.4	147.3	141.2	157.2	195.4	132.9	156.7
2.	49.7	57.7	64.2	91.6	65.8	44.9	54.5	64.2	91.7	63.8
3.	78.6	70.6	85.5	123.7	89.6	49.7	70.6	78.7	82.5	70.4
4.	104.2	109.0	112.3	110.0	108.9	78.6	121.9	102.1	151.2	113.5
5.	85.0	115.5	125.7	68.7	98.7	117.1	131.5	116.6	155.8	130.2
6.	59.3	150.8	93.6	45.9	87.4	123.5	285.5	148.7	119.1	169.2
Total	500.3	635.1	655.0	600.3	597.7	555.0	821.2	705.7	733.2	703.8

Invertase Activity of ± Iron Roots

7 Day Roots Sectioned

TABLE 25

Protein Nitrogen per Section (µg. Protein N.)

	-Fe					+Fe				
Expt. No.	B 10	B 11	B 13	B 15	Mean	B 10	B 11	B 13	B 15	Mean
Section 1.	8.83	10.01	8.95	9.25	9.26	8.24	7.95	8.59	8.62	8.35
2.	6.63	6.18	7.12	6.94	6.72	5.00	5.44	5.64	6.73	5.70
3.	9.87	8.68	9.33	10.09	9.49	6.77	7.51	7.97	8.20	7.61
4.	14.72	12.36	14.35	18.09	14.88	9.72	11.04	10.79	12.62	11.04
5.	13.55	13.25	18.40	12.62	14.46	16.34	12.96	14.11	18.93	15.58
6.	9.57	21.50	15.46	7.36	13.47	18.25	30.33	20.24	21.45	22.57
Total	63.17	71.99	73.61	64.36	68.28	64.33	75.23	67.35	76.55	70.85

TABLE 26

Invertase Activity (µg. Hexose per µg. Protein N.)

	-Fe					+Fe				
Expt. No.	B 10	B 11	B 13	B 15	Mean	B 10	B 11	B 13	B 15	Mean
Section 1.	13.978	13.142	19.404	17.340	15.966	17.119	19.771	20.861	15.420	18.293
2.	7.502	9.345	9.016	13.200	9.766	8.971	10.009	10.428	13.630	10.757
3.	7.959	8.129	9.173	12.250	9.378	7.341	9.401	9.049	10.060	8.963
4.	7.082	8.819	7.823	6.080	7.451	8.091	11.042	8.668	11.980	9.945
5.	6.275	8.717	6.828	5.450	6.817	7.166	10.154	7.578	8.230	8.282
6.	6.202	7.014	6.054	6.230	6.230	6.765	9.413	6.735	5.550	7.111
Total	7.922	8.824	8.898	9.328	8.743	8.626	9.165	9.606	9.578	9.244

TABLE 27

Invertase Activity of ± Iron Roots

7 Day Roots Sectioned

Recalculated Invertase Activity

(µg. Hexose per µg. Protein N.)

	Mean Hexose per Root (µg)		Protein N. per * Root Section (µg)		Recalculated Invertase Activity (µg Hexose/µg. Prot. N)	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Section 1.	147.3	156.7	13.77	11.29	10.697	13.879
2.	65.8	63.8	7.89	7.02	8.340	9.088
3.	89.6	70.4	10.15	8.70	8.819	8.091
4.	108.9	113.5	13.62	12.12	7.996	9.372
5.	98.7	130.2	16.89	16.44	5.844	7.925
6.	87.4	169.2	26.09	41.68	3.350	4.059
Total	597.7	703.8	88.41	97.26	6.788	7.221

* Mean Protein Nitrogen contents of 1.0 cm root sections from ± iron roots 7 days old as established in experiments A16, B 1, B2 and B4.

TABLE 28

Cyanide Inhibition of Oxygen Uptake ± Iron Roots

Whole Root Results

10^{-4} molar KCN

Expt. No.	No. of Roots		Mean Length per Root (cm)		Control O_2 Uptake per Root (cu.mm/hr)		10^{-4} KCN O_2 Uptake per Root (cu.mm/hr)		Per Cent Inhibition of O_2 Uptake	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
					Day 0					
C 15	96				10.90		7.63			
					10.00		7.50			
					9.23		8.20			
					10.63		7.02			
Mean			1.0		10.19		7.77		23.75	
					Day 3					
C 5	42	42			9.03	9.65	6.48	6.99		
					8.57	8.34	6.33	6.58		
					8.81	8.45	6.42	6.27		
Mean			2.37	2.46	8.80	8.81	6.41	6.61	27.16	24.97
					Day 2					
C 4	36	36			8.36	7.86	6.48	6.61		
					8.54	9.58	6.05	6.39		
					8.75	8.70	5.63	6.83		
Mean			2.96	3.23	8.55	8.71	6.05	6.61	29.24	24.19
					Day 7					
C 2	16	16			11.17	13.07	10.20	12.42		
					12.81	12.15	10.45	9.65		
Mean			5.90	6.05	11.99	12.61	10.32	11.04	13.91	12.51
					Day 9					
C 7	24	24			10.90	16.20	12.15	15.74		
					12.50	14.77	10.92	16.92		
					10.25	17.80	10.65	15.39		
Mean			5.94	7.65	11.22	16.25	11.24	16.02	0	1.41
					Day 11					
C 6	30	30			12.21	22.46	12.06	21.74		
					14.00	21.10	15.17	20.70		
					11.48	19.25	10.21	19.30		
Mean					12.56	20.94	12.48	20.58	0	1.72

TABLE 29

Cyanide Inhibition of Oxygen Uptake ± Iron Roots

Whole Root Results

10^{-3} molar KCN

Expt. No.	No of Roots		Mean Length per root (cm)		Control O_2 Uptake per root (cu.mm/hr)		10^{-3} KCN O_2 Uptake per root (cu.mm/hr)		Per Cent Inhibition of O_2 Uptake	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
					Day 0					
C 15	48				10.50		4.68		55.33	
					10.40		4.33		58.37	
					9.58		4.26		55.53	
					10.14		4.27		57.89	
Mean			1.0		10.13		4.39		56.78	
					Day 3					
C 11	24	24			8.18	9.37	3.54	5.25	56.72	43.97
					10.00	7.80	5.00	3.40	50.00	50.00
					8.91	8.06	4.10	3.73	54.00	53.72
Mean			2.60	2.71	9.03	8.41	4.21	4.29	53.57	49.23
					Day 5					
C 9	18	18			8.73	10.68	6.18	8.38	29.21	21.54
					10.00	10.40	7.50	6.93	25.00	33.37
					8.88	10.29	6.83	7.58	23.09	26.34
Mean			3.07	4.10	9.20	10.49	6.84	7.63	25.77	27.08
					Day 7					
C 9	18	18			12.24	14.56	8.64	10.23	29.41	29.74
					10.20	12.97	7.74	9.83	24.12	24.21
					11.22	15.32	8.21	10.45	26.83	31.79
Mean			5.05	6.37	11.22	14.28	8.16	10.17	26.79	28.58
					Day 9					
C10	12	12			10.90	16.74	8.54	11.86	21.65	29.15
					12.50	17.76	9.14	12.66	26.88	28.72
					13.72	18.92	9.73	15.01	29.08	20.67
Mean			6.01	7.68	12.37	17.81	9.14	13.18	25.87	26.18
					Day 11					
C 11	12	12			15.26	23.21	12.38	16.77	18.87	27.75
					13.34	22.88	10.05	18.72	24.66	18.18
					14.86	23.43	10.12	16.36	31.80	30.18
Mean			6.99	10.69	14.49	23.17	10.85	17.28	25.11	25.37

TABLE 30

Cyanide Inhibition of Oxygen Uptake ± Iron Roots

7 Day Roots Sectioned

10^{-3} Molar KCN

Expt. No. C 14.

	Control O_2 Uptake /Section (cu.mm/hr)		10^{-3} KCN O_2 Uptake /Section (cu.mm/hr.)		Percent Inhibition of O_2 Uptake		No. of Roots.	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Sect.1.	2.452	2.295	1.250	1.215	49.02	47.06	16	16
2.	1.875	1.767	1.219	1.187	34.99	32.82		
3.	1.962	1.709	1.723	1.530	12.18	10.47		
4.	2.315	1.881	2.106	1.629	9.03	13.40	Mean Length	
5.	2.891	2.976	2.334	2.587	19.27	13.07	/Root (cm)	
6.	1.315	4.024	.902	3.132	31.41	22.17	-Fe	+Fe
Total	12.810	14.650	9.534	11.280	25.57	23.00	5.07	5.55

Expt. No. C 16.

							No. of Roots	
	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
Sect.1.	2.250	2.107	1.211	1.090	46.18	48.27	24	24
2.	1.342	1.250	.851	.833	36.59	33.36		
3.	1.870	1.708	1.537	1.496	17.81	12.41		
4.	2.457	1.842	2.033	1.611	17.26	12.54	Mean Length	
5.	2.842	2.427	2.126	1.993	25.20	17.88	/Root (cm)	
6.	2.905	6.479	2.375	4.312	18.25	33.45	-Fe	+Fe
Total	13.666	15.813	10.133	11.335	25.85	28.32	5.80	6.61

MEAN VALUES OF EXPTS. C 14 AND C 16

Sect.1.	2.351	2.201	1.230	1.152	47.60	47.61
2.	1.608	1.508	1.035	1.010	35.79	33.09
3.	1.916	1.708	1.630	1.513	14.99	11.44
4.	2.386	1.862	2.069	1.620	13.14	12.97
5.	2.866	2.701	2.230	2.290	22.23	15.47
6.	2.110	5.252	1.638	3.722	24.83	27.81
Total	13.238	15.232	9.833	11.302	25.71	25.61

Roots ± Mg - WHOLE ROOT DATA

Tables 31 to 36 comparisons of roots grown with and without added magnesium in the nutrient culture media. Root tips from germinated pea seeds cultured and harvests of whole roots made after growth periods of 0, 3, 5, 7, 9 and 11 days.

TABLE 31 Day 0 ± Magnesium Roots

Expt. No.	Cell No. per Root	Protein N. per Root (µg)	O_2 Uptake per Root (cu.mm per Hr)	O_2 Uptake per unit Prot. N. (cu.mm/hr per µg. Prot. N.)	No. of Roots	Length per Root (cm)	Protein per Cell (µg x 10^{-6})	O_2 Uptake per Cell (cu.mm x 10^{-6}/hr)
D 8.	423,437	75.38	11.46	.1521				
	392,187	81.27	12.01	.1478				
	368,750	76.56	12.04	.1572				
	390,625	92.25	13.48	.1460				
	414,062	78.51	11.82	.1505				
MEAN	396,875	80.79	12.16	.1505	100	1.0	203.6	30.64

TABLE 32 Day 3 ± Magnesium Roots

Expt. No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake per Unit Prot. N. (cu.mm/hr. per µg Prot. N)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 12	502,500	512,500	116.1	107.9	15.99	14.24	.1376	.1319
	517,500	515,000	121.0	109.6	15.10	14.96	.1247	.1365
	520,000	527,500	124.3	109.6	15.60	16.37	.1254	.1494
	472,500	480,000	81.8	134.4	11.00	16.56	.1346	.1406
	503,906	499,562						
Mean	503,281	506,912	110.8	111.2	14.42	15.53	.1301	.1396
D 13	404,166	495,833	99.8	86.7	11.81	11.60	.1183	.1338
	481,583	491,666	85.1	88.3	11.05	11.90	.1299	.1347
	479,166	431,250	101.4	98.1	13.87	13.24	.1367	.1348
	508,333	468,750	94.9	106.1	12.07	13.80	.1272	.1301
Mean	468,750	471,666	95.3	94.1	12.20	12.64	.1280	.1344
MEAN	486,015	489,289	103.1	102.6	13.31	14.08	.1290	.1370

Expt. No.	No. of Roots		Length per Root (cm)		Protein per cell ($\mu g \times 10^{-6}$)		O_2 Uptake per cell ($cu.mm \times 10^{-6}$/hr)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 12	40	40	3.03	3.48	220.2	219.4	28.65	30.64
D 13	40	40	2.83	3.05	203.3	199.4	26.03	26.80
MEAN	-	-	2.93	3.26	211.7	209.4	27.34	28.72

TABLE 33 Day 5 ± Magnesium Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake Per Unit Prot. N) (cu.mm/hr. per µg. Prot. N)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 10	530,469	596,094	149.5	143.9	18.09	17.93	.1396	.1450
	607,031	533,203	147.6	102.1	15.91	14.89	.1247	.1459
	634,375	585,156	100.1	145.6	14.35	18.18	.1433	.1447
	609,765	582,422	111.9	104.0	15.34	14.49	.1370	.1392
	566,015	634,375						
Mean	589,531	586,250	117.3	113.8	15.92	16.37	.1357	.1438
D 11	562,500	515,000	113.8	104.0	15.46	13.08	.1340	.1257
	467,500	465,000	111.9	100.1	14.14	12.65	.1264	.1263
	520,000	520,000	102.1	107.9	12.90	14.63	.1262	.1355
	507,500	570,000	102.1	113.8	13.20	15.55	.1293	.1363
Mean	515,000	517,500	107.5	106.5	13.88	13.97	.1291	.1312
MEAN	552,265	551,875	112.4	110.2	14.90	15.15	.1326	.1375

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell ($cu.mm \times 10^{-6}/hr$)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 10	40	40	4.28	4.28	198.9	194.2	27.00	27.92
D 11	40	40	3.67	3.84	208.7	205.8	26.95	26.90
MEAN	-	-	3.98	4.06	203.8	200.0	26.98	27.41

TABLE 34 Day 7 ± Magnesium Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake Per Unit Prot. N) (cu.mm/hr. per µg. Prot. N)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 1.			123.7	120.7	16.20	17.76	.1309	.1471
			124.4	127.4	15.82	19.63	.1272	.1541
			119.3	128.8	15.30	20.26	.1282	.1573
Mean			122.4	125.6	15.77	19.22	.1288	.1529
D 5.	537,500	568.750	129.6	108.9	16.89	16.74	.1303	.1536
	625.000	634.375	107.5	111.9	15.19	16.35	.1413	.1461
	637,500	653.125	126.6	138.4	15.34	19.48	.1211	.1407
	637.500	678.125	104.5	119.3	14.38	17.76	.1375	.1489
Mean	609,375	643,750	117.0	119.6	15.45	17.59	.1320	.1470
D 9.	575,000	675.000	122.7	147.2	16.35	18.99	.1332	.1289
	696,875	709,375	114.1	150.9	16.57	19.89	.1452	.1318
	643.750	612,500	126.4	125.1	16.12	20.12	.1275	.1607
	640,625	693,750	122.7	127.6	16.51	19.80	.1345	.1512
	650,000	687,500	134.9	122.7	18.90	17.59	.1400	.1434
Mean	640,625	675,000	124.1	134.2	16.89	19.18	.1360	.1423
MEAN	626,736	661,111	121.2	127.4	16.18	18.58	.1335	.1458

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell ($cu.mm \times 10^{-6}/hr$)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 1.	12	12	5.50	7.10				
D 5.	32	32	6.06	6.89	192.1	185.8	25.35	27.32
D 9.	40	40	6.76	7.20	193.8	198.8	26.36	28.41
MEAN	-	-	6.31	7.07	192.9	192.3	25.86	27.86

TABLE 35 Day 9 ± Magnesium Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr.)		O_2 Uptake per unit Prot. N) (cu.mm/hr. per µg. Prot. N)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 1.	558.594	787.500	121.2	164.9	14.72	25.29	.1189	.1533
	527.344	815.625	148.7	142.8	20.50	22.14	.1378	.1550
	683.594	660.937	117.8	153.1	14.64	21.63	.1242	.1412
	699.437	810.937	150.2	150.2	18.72	20.91	.1246	.1392
Mean	648.437	768.750	135.1	152.7	17.14	22.49	.1269	.1472
D 6.	640.625	718.750	117.8	152.1	19.44	21.80	.1650	.1433
	715.625	765.625	139.8	147.2	19.52	22.99	.1395	.1561
	625.000	796.875	139.8	147.2	19.17	22.88	.1371	.1554
	731.250	761.719	132.5	154.6	18.81	23.43	.1419	.1515
Mean	678.125	761.719	132.5	150.3	19.24	22.77	.1451	.1515
MEAN	664.271	765.000	133.7	151.5	18.26	22.64	.1365	.1495

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell (cu.mm $\times 10^{-6}$/hr)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 1.	28	28	7.26	8.15	208.3	198.7	26.43	29.26
D 6.	32	32	7.90	8.60	195.4	197.3	28.37	29.89
MEAN	-	-	7.60	8.39	201.8	198.0	27.40	29.57

TABLE 36 Day 11 ± Magnesium Roots

Expt.No.	Cell No. per Root		Protein N per Root (µg)		O_2 Uptake per Root (cu.mm/hr)		O_2 Uptake per Unit Prot. N) (cu.mm/hr. per µg. Prot. N)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 2.	740,625	833,333	151.6	150.2	22.68	23.44	.1495	.1560
	793,750	906,250	147.2	147.2	22.15	24.09	.1504	.1636
	765,625	921,875	144.3	166.4	19.38	25.97	.1343	.1560
	662,500	671,875	150.2	150.2	22.99	23.47	.1530	.1558
			144.3	170.2	21.48	29.29	.1488	.1714
Mean	740,625	833,333	147.5	156.9	21.74	25.24	.1473	.1607
D 4.	796,875	839,844	137.4	166.8	17.98	27.54	.1308	.1650
	678,125	847,656	159.5	152.1	23.57	23.74	.1478	.1560
	725,000	816,406	142.3	157.1	18.79	25.50	.1320	.1623
	665,625	839,844	157.0	184.0	21.84	29.78	.1390	.1618
	756,250	761,719	122.7	179.1	15.44	28.46	.1258	.1589
Mean	725,000	820,310	143.8	167.8	19.52	27.00	.1358	.1609
D 7.	781,250	886,719	149.7	166.8	19.62	25.33	.1310	.1518
	707,031	859,375	132.4	147.2	17.13	25.32	.1292	.1719
	738.281	832,031	159.4	164.4	19.76	24.48	.1239	.1505
	664,062	792,969	132.4	164.4	19.17	26.65	.1446	.1621
Mean	722,656	843,750	143.5	160.7	18.92	25.45	.1318	.1583
MEAN	729,514	831,596	145.0	162.1	20.08	25.95	.1385	.1601

Expt.No.	No. of Roots		Length per Root (cm)		Protein per Cell ($\mu g \times 10^{-6}$)		O_2 Uptake per Cell (cu.mm $\times 10^{-6}$/hr)	
	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg	-Mg	+Mg
D 2.	36	36	8.14	10.26	199.2	188.3	29.35	30.29
D 4.	40	40	8.53	10.45	198.3	204.6	26.92	32.91
D 7.	32	32	8.08	10.22	198.6	190.5	26.18	30.16
MEAN	-	-	8.27	10.32	198.7	194.5	27.48	31.12

7 DAY ROOTS ± Mg - SECTIONED DATA

TABLES 37 TO 44 Comparisons of roots grown with and without added magnesium in the nutrient culture media. Root tips from germinated pea seeds cultured for 7 days and the roots harvested by taking one centimetre sections commencing at the apex.

TABLE 37

Expt. No.	No. of Roots		Mean Root Length (cm)	
	-Mg	+Mg	-Mg	+Mg
D 14.	40	40	5.44	6.32
D 15.	31	36	5.98	7.11
D 16.	28	28	5.48	5.83
D 17.	28	28	5.50	6.32
MEAN	-	-	5.59	6.43

TABLE 38

Number of Cells per Section

Expt. No.	-Mg D14	D 15	D 16	D 17	Mean	+Mg D 14	D 15	D 16	D 17	Mean
Section 1.	74,687	70,416	81,250	81,250	76,901	76,250	78,125	82,812	85,939	80,781
2.	51,562	55,312	67,578	55,078	57,382	47,500	49,479	57,031	50,521	51,130
3.	79,062	89,583	80,469	96,094	86,302	67,500	71,180	81,250	70,312	72,560
4.	120,937	135,625	156,250	125,000	134,453	109,687	106,771	108,854	108,854	108,541
5.	141,250	145,833	127,344	144,531	139,739	121,250	97,917	161,735	115,885	124,197
6.	88,125	161,458	101,562	94,271	111,354	156,250	256,076	135,417	196,615	186,089
Total	555,623	658,227	614,453	596,224	606,131	578,437	659,548	627,099	628,126	623,298

TABLE 39

Protein Nitrogen per Section (µg. Protein N.).

Expt. No.	-Mg D 14	D 15	D 16	D 17	Mean	+Mg D 14	D 15	D 16	D 17	Mean
Section 1.	8.244	8.833	8.649	10.306	9.008	9.717	9.488	9.202	9.938	9.586
2.	8.097	9.570	8.466	8.833	8.741	7.950	7.852	9.018	6.625	7.861
3.	12.661	12.514	13.803	14.722	13.425	10.306	9.815	12.882	9.202	10.551
4.	16.783	21.716	27.421	24.660	22.645	17.667	15.050	23.188	15.081	17.749
5.	29.150	28.709	34.414	29.445	30.429	17.373	23.556	42.327	26.500	27.439
6.	24.145	46.744	28.709	28.709	32.077	37.395	73.605	37.543	49.688	49.558
Total	99.080	128.086	121.462	116.675	116.325	100.408	139.366	134.160	117.044	122.744

Table 40

Rate of Oxygen Uptake per Section. (cu.m.m. per hr.).

Expt. No.	-Mg D 14	D 15	D 16	D 17	Mean	+Mg D 14	D 15	D 16	D 17	Mean
Section 1.	1.872	1.908	2.160	2.248	2.047	2.538	2.580	2.316	2.565	2.500
2.	1.625	1.641	1.516	1.688	1.617	1.688	1.758	1.922	1.503	1.718
3.	1.763	1.658	1.913	2.238	1.893	1.698	1.757	2.072	1.721	1.812
4.	2.444	2.210	2.939	3.120	2.678	2.403	2.090	2.730	2.221	2.361
5.	3.514	2.729	3.378	3.062	3.171	2.895	2.788	4.659	2.895	3.309
6.	2.845	4.550	2.976	2.940	3.328	5.227	7.475	3.990	5.175	5.467
Total	14.063	14.696	14.882	15.296	14.734	16.449	18.448	17.689	16.080	17.167

TABLE 41

Average Volume per Cell (cu.m.m. x 10^{-6})

Expt. No.	-Mg D 14	-Mg D 15	-Mg D 16	-Mg D 17	-Mg Mean	+Mg D 14	+Mg D 15	+Mg D 16	+Mg D 17	+Mg Mean
Section 1.	38.77	40.21	38.66	35.17	38.20	44.68	44.38	35.29	44.07	42.10
2.	85.41	62.87	84.48	71.12	75.97	75.83	76.98	70.76	85.13	77.17
3.	85.88	65.96	93.66	64.39	77.47	75.77	63.34	81.21	83.61	75.98
4.	90.79	75.37	94.73	102.69	90.89	78.68	69.10	99.78	92.59	85.04
5.	117.90	114.67	142.46	144.15	129.79	121.12	82.91	118.28	123.66	111.49
6.	141.67	164.46	113.92	169.89	147.48	167.03	141.06	199.41	126.19	158.42
Total	97.56	99.80	99.11	105.07	100.38	106.39	96.13	112.51	100.60	103.91

TABLE 42

Rate of Oxygen Uptake per Unit Protein Nitrogen

(cu. m.m. per hr. per µg. Prot. N.)

Expt. No.	-Mg D 14	-Mg D 15	-Mg D 16	-Mg D 17	-Mg Mean	+Mg D 14	+Mg D 15	+Mg D 16	+Mg D 17	+Mg Mean
Section 1.	.2270	.2160	.2497	.2181	.2277	.2611	.2719	.2516	.2581	.2607
2.	.2006	.1714	.1790	.1911	.1855	.2123	.2238	.2131	.2268	.2190
3.	.1392	.1325	.1386	.1520	.1406	.1648	.1790	.1608	.1870	.1729
4.	.1456	.1018	.1072	.1265	.1203	.1360	.1389	.1177	.1472	.1349
5.	.1205	.0951	.0982	.1040	.1044	.1666	.1184	.1101	.1092	.1261
6.	.1178	.0973	.1037	.1024	.1053	.1398	.1016	.1063	.1041	.1129
Total	.1419	.1147	.1225	.1311	.1275	.1638	.1324	.1319	.1374	.1414

TABLE 43 Rate of Oxygen Uptake per Cell (cu.m.m. x 10^{-6} per hr.)

	-Mg					+Mg				
Expt. No.	D 14	D 15	D 16	D 17	Mean	D 14	D 15	D 16	D 17	Mean
Section 1.	25.06	27.09	26.58	27.66	26.60	33.28	33.02	27.96	29.84	31.02
2.	31.51	29.66	22.43	30.64	28.56	35.53	35.53	33.70	29.75	33.63
3.	22.29	18.50	23.77	23.28	21.96	25.15	24.68	25.50	24.47	24.95
4.	20.21	16.29	18.80	24.96	20.06	21.91	19.57	25.08	20.40	21.74
5.	24.88	18.71	26.53	21.19	22.83	23.88	28.47	28.81	24.98	26.53
6.	32.28	28.18	29.30	31.18	30.23	33.45	29.19	29.46	26.32	29.60
Total	25.31	22.32	24.21	25.65	24.37	28.43	27.97	28.20	25.59	27.55

TABLE 44 Protein Nitrogen per Cell (µg. x 10^{-6})

	-Mg					+Mg				
Expt. No.	D 14	D 15	D 16	D 17	Mean	D 14	D 15	D 16	D 17	Mean
Section 1.	110.4	125.4	106.4	126.8	117.2	127.4	121.4	111.1	115.6	118.9
2.	157.0	173.0	125.3	160.4	153.9	167.4	158.7	158.1	131.1	153.8
3.	160.1	139.6	171.5	153.2	156.1	152.6	137.9	158.5	130.9	145.0
4.	138.8	160.1	175.4	197.3	167.9	161.1	141.0	213.0	138.6	163.4
5.	206.4	196.9	270.2	203.7	219.3	143.3	240.5	261.7	228.7	218.5
6.	273.9	289.5	282.7	304.5	287.7	239.3	287.4	277.2	252.7	264.2
Total	178.3	194.5	197.6	195.6	191.5	173.5	211.3	213.9	186.3	196.2

7 DAY ROOTS ± Mo - SECTIONED DATA

TABLES 45 TO 52 Comparisons of roots grown with and without added molybdenum in the nutrient culture media. Root tips from germinated pea seeds cultured for 10 days, and then the terminal centimetre of these roots subcultured for a further 7 days. Subcultured roots harvested by taking serial one centimetre sections commencing at the apex.

TABLE 45

Expt. No.	No. of Roots		Mean Root Length after 10 days growth		Mean Root Length after 7 days subculture	
	-Mo	+Mo	-Mo	+Mo	-Mo	+Mo
A 4.	36	36	10.00	10.46	6.50	7.41
A 11.	30	30	8.82	9.70	5.91	6.61
A 12.	24	24	9.82	10.5	5.08	6.62
MEAN	-	-	9.56	10.22	5.92	6.93

TABLE 46

Number of Cells per Section

Expt. No.	-Mo				+Mo			
	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	88.229	72.857	80.729	80.605	83.766	68.214	90.625	80.868
2.	37.500	30.134	36.285	34 .640	35.723	28.929	36.236	33.629
3.	41.458	34.152	37.326	37.645	34.539	34.286	33.982	34.269
4.	34.271	27.589	38.368	33.409	47.566	36.027	36.458	40.017
5.	28.646	25.580	29.687	27.971	41.743	34.420	46.007	40.723
6.	67.604	43.705	21,181	44.163	91,546	47.388	40,972	59.969
Total	297.708	234.017	243.576	258.433	334.883	249.263	284.280	289.475

TABLE 47 Protein Nitrogen per Section (µg. Protein N.)

	-Mo				+Mo			
Expt. No.	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	11.408	10.672	10.880	10.987	11.040	10.864	10.880	10.928
2.	7.360	6.992	7.472	7.275	7.072	6.992	7.472	7.179
3.	8.176	6.624	8.160	7.653	7.360	7.552	7.472	7.461
4.	7.920	7.360	9.280	8.187	7.552	7.360	7.472	7.461
5.	5.520	8.096	6.112	6.576	7.920	7.360	9.056	8.112
6.	16.928	11.232	6.112	11.424	24.112	16.560	11.552	17.408
Total	57.312	50.976	48.016	52.101	65.056	56.720	53.904	58.560

TABLE 48 Rate of Oxygen Uptake per Section. (cu.mm. per hr.)

	-Mo				+Mo			
Expt. No.	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	1.976	2.316	2.012	2.101	2.295	2.506	2.492	2.431
2.	1.490	1.220	1.202	1.304	1.260	1.466	1.481	1.402
3.	1.377	1.239	1.105	1.240	1.340	1.371	1.275	1.329
4.	1.154	1.257	1.340	1.250	1.208	1.358	1.175	1.247
5.	0.899	1.176	0.758	0.944	1.036	1.206	1.206	1.149
6.	2.582	1.686	0.813	1.694	3.493	2.484	1.832	2.603
Total	9.478	8.894	7.230	8.533	10.632	10.391	9.461	10.161

TABLE 49 Average Volume per Cell (cu.mm. x 10^{-6})

	-Mo				+Mo			
Expt. No.	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	38.24	36.04	25.72	33.35	44.79	40.46	36.01	40.42
2.	100.18	88.27	73.86	87.44	110.65	112.47	93.04	105.39
3.	95.82	94.01	85.67	91.83	127.43	99.25	97.61	108.10
4.	101.58	99.08	88.76	96.47	88.02	92.05	97.43	92.50
5.	109.75	99.16	64.30	91.07	101.11	117.06	94.29	103.15
6.	125.33	125.76	132.30	127.80	148.53	117.28	152.30	139.37
Total	87.65	82.31	65.98	78.65	101.53	89.54	84.87	91.98

TABLE 50 Rate of Oxygen Uptake per Unit Protein Nitrogen

(cu. mm. per hr. per µg. Prot. N.)

	-Mo				+Mo			
Expt. No.	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	.1732	.2170	.1849	.1917	.2079	.2307	.2290	.2225
2.	.2024	.1744	.1608	.1792	.1781	.2096	.1982	.1953
3.	.1684	.1870	.1354	.1636	.1820	.1815	.1706	.1780
4.	.1457	.1707	.1443	.1536	.1599	.1845	.1572	.1672
5.	.1628	.1452	.1240	.1440	.1308	.1638	.1331	.1425
6.	.1525	.1501	.1330	.1452	.1449	.1500	.1586	.1512
Total	.1654	.1745	.1506	.1635	.1634	.1831	.1755	.1740

TABLE

TABLE 51 Rate of Oxygen Uptake per Cell (cu.mm. x 10^{-6} per hr.)

	-Mo				+Mo			
Expt. No.	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	22.40	31.79	24.92	26.37	27.40	36.74	27.50	30.55
2.	39.73	40.49	33.13	37.78	35.27	50.68	40.87	42.26
3.	33.21	36.28	29.60	33.03	38.30	39.99	37.52	38.60
4.	33.67	45.56	34.92	38.05	25.40	37.69	32.23	31.77
5.	31.38	45.97	25.53	34.29	24.82	35.04	26.21	28.69
6.	38.19	38.58	38.38	38.38	38.15	52.42	44.71	45.09
Total	31.55	38.01	29.68	33.08	31.64	37.68	33.28	34.20

TABLE 52 Protein Nitrogen per Cell (µg. x 10^{-6})

	-Mo				+Mo			
Expt. No.	A 4	A 11	A 12	Mean	A 4	A 11	A 12	Mean
Section 1.	129.2	146.4	134.7	136.8	131.7	159.2	120.0	137.0
2.	196.3	232.0	205.9	211.4	198.0	241.7	206.2	215.3
3.	197.2	194.0	218.6	203.3	213.1	220.3	219.9	217.8
4.	231.1	266.8	241.9	246.6	158.8	204.3	204.9	189.3
5.	192.7	316.5	205.9	238.4	189.7	213.8	196.8	200.1
6.	250.3	256.9	288.6	265.3	263.3	349.4	281.9	298.2
Total	192.5	217.8	197.1	202.5	194.3	227.6	189.6	203.8

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