

Vanadium uptake and transport in higher plants

by

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ABSTRACT

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The uptake and transport of vanadium in several higher plant species has been examined.

Studies utilizing excised *Hordeum* roots have revealed the uptake patterns of two different ionic species of vanadium (VO^{2+} and VO_3^-) to be similar. Both ionic forms exhibited a large extracellular component in their uptake. This extracellular component was shown to be exchangeable with a number of agents but was most effectively removed by a post uptake treatment in a solution of chromium(VI). This removal of extracellular vanadium by chromium was considered to be a genuine exchange process although the mechanism involved is unclear. The effect of temperature, pH, metabolic inhibitors and complexing agents were also examined and found to be similar for the two ionic forms considered. Concentration dependent uptake of the two ionic forms was also examined. Saturation kinetics were observed when a wide concentration range of vanadium (0.1-5.0 mM) was employed. These results were considered suggestive of conversion of vanadium to a common form within root tissues.

Whole plant studies utilizing *Zea* and *Vicia* also revealed highly similar uptake patterns for the two different ionic species of vanadium. Irrespective of the ionic form of vanadium supplied to the plant marked retention of vanadium within the root tissues was observed. Analysis of vanadium root-shoot transport patterns in plants supplied with different ionic species of vanadium suggested that a common form of vanadium was being transported in both cases. Possible interactions between vanadium and other ions within the plant are briefly considered.

ESR studies utilizing *Hordeum* clearly illustrated the ability of plant roots to reduce pentavalent vanadium to the tetravalent form.

Evidence from all three lines of study is discussed and considered to support the suggestion that vanadium within plant roots exists in the reduced tetravalent form. This is thought to be of prime importance in determining the mobility of vanadium within plant tissues.

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Contents	Page
Abstract	i
Acknowledgements	ii
Contents	iii
List of figures	v
List of tables	viii
List of abbreviations	ix
Note on graphs	x
 Chapter 1 Introduction	 1.
1.1 Geochemistry and presence in soils	2.
1.2 Vanadium in the biosphere	5.
1.2.1 Vanadium in lower plants	5.
1.2.2 Vanadium in higher plants	16.
1.2.3 Vanadium in aquatic organisms	23.
1.2.4 Vanadium in terrestrial animals	30.
1.2.5 Vanadium in selected enzyme systems	32.
1.3 Industrial uses of vanadium and its compounds	37.
1.4 Vanadium as a pollutant	38.
1.5 Aims of the study	40.
 Chapter 2 Vanadium chemistry	 42.
2.1 Vanadium chemistry - general	42.
2.2 Vanadium (+5)	43.
2.3 Vanadium (+4)	49.
 Chapter 3 Materials and methods	 54.
3.1 Introduction	54.
3.2 Plant materials	54.
3.3 Excised root studies	54.
3.4 Whole plant studies	57.
3.5 Analysis of plant material	57.
3.6 Data handling	62.
 Chapter 4 Excised root studies	 63.
4.1 Time course and desorption studies	63.
4.2 Temperature	68.
4.3 Hydrogen ion concentration	88.

	Page
4.4 Complexing agents	91.
4.5 Respiratory poisons	96.
4.6 Calcium and vanadium uptake	102.
4.7 Concentration dependent uptake of vanadium	107.
4.8 Discussion	111.
 Chapter 5 Whole plant studies	 115.
5.1 General	115.
5.2 Maize	116.
5.3 Broad beans	170.
5.4 Tomato	188.
5.5 Discussion	197.
 Chapter 6 ESR Investigations	 205.
6.1 Background	205.
6.2 Methods and materials	209.
6.3 Results and discussion	209.
 Chapter 7 Discussion	 215.
7.1 Discussion	215.
7.2 Future research requirements	230.
 References	 232.
 Appendix 1 Nutrient solution	 A.1
Appendix 2 Publications	A.2

List of figures	Page
1.1 Vanadium in the soil system	7.
1.2 Proposed scheme for ALA formation in higher plants	12.
1.3 Outline of tetrapyrrole biosynthetic pathway	15.
1.4 Postulated mechanism for accumulation of V by vanadocyte vacuoles	28.
2.1 Distribution of vanadate (+5) species as a function of pH and total V concentration	45.
2.3 Reduction potential versus pH for a solution of vanadium	45.
2.2 The equilibrium species present in a 1 μ M solution of V(+5) in water over the pH range 1 - 14	48.
2.4 Distribution of vanadyl (1V) species as a function of pH	52.
4.1a Root vanadium level v time (V+5)	65.
4.1b " " " " " (V+4)	67.
4.2a Desorption of root vanadium using various agents (10mM)	75.
4.2b " " " " " " " (50mM)	75.
4.3a " " " " " " " "	77.
4.3b Time course of vanadium desorption using the chromate anion	77.
4.4a Desorption of vanadium supplied as VOSO_4 by the uranyl cation	79.
4.4b " " " " " " " various cations	79.
4.5 " " " " " " " agents	82.
4.6a " " " " " " " "	84.
4.6b Time course for vanadium desorption of vanadium supplied as VOSO_4	84.
4.7a Time course for vanadium uptake, vanadium supplied as NH_4VO_3	87.
4.7b " " " " " " " VOSO_4	87.
4.8a Effect of temperature on vanadium (+5) uptake	90.
4.8b " " " " " (+4) "	90.
4.9a Effect of pH on uptake of vanadium	93.
4.9b Effect of pH on dialysability of vanadium in the presence of humified organic matter	93.
4.9c Effect of pH on uptake of vanadium by boiled roots	95.
4.10 " " " " " " (+4) by roots	95.
4.11a Effect of complexing agents on uptake of vanadium (+5)	98.
4.11b " " " " " " " (+4)	98.
4.12a The effect of metabolic inhibitors on vanadium (+5) uptake	101.
4.12b " " " " " " (+4) "	101.
4.13 Effect of altered Ca^{2+} levels on vanadium (+4) uptake	106.
4.14a Concentration dependent uptake of vanadium (+5)	110.
4.14b " " " " " (+4)	110.

	Page
5.1a Maize fresh weight production v applied vanadium (+5)	118.
5.1b " " " " v " " (+4)	118.
5.2a " dry " " v " " (+5)	120.
5.2b " " " " v " " (+4)	120.
5.3a Root vanadium levels in maize v applied level	124.
5.3b Shoot " " " " v " "	125.
5.4a Root/shoot vanadium levels for maize	130.
5.4b " " " " " "	131.
5.5a Tissue iron levels as affected by vanadium (+5)	136.
5.5b " " " " " " (+4)	136.
5.6a " manganese " " " " (+5)	138.
5.6b " " " " " " (+4)	138.
5.7a " calcium " " " " (+5)	140.
5.7b " " " " " " (+4)	140.
5.12a Dry weight production of maize v time	145.
5.12b " " " " vanadium (+5) treated maize v time	146.
5.12c " " " " " (+4) " " " "	146.
5.13a Shoot iron in maize v time	148.
5.13b " zinc " " " "	148.
5.13c Shoot manganese in maize v time	148.
5.13d " calcium " " " "	148.
5.13e " potassium " " " "	149.
5.14a Root iron " " " "	151.
5.14b " zinc " " " "	151.
5.14c " manganese " " " "	151.
5.14d " calcium " " " "	151.
5.14e " potassium " " " "	152.
5.15a Shoot iron levels in V (+5) treated maize v time	154.
5.15b " zinc " " " " " " "	154.
5.15c " manganese levels in V (+5) treated maize v time	154.
5.15d " calcium " " " " " " "	154.
5.15e " potassium " " " " " " "	155.
5.15f " vanadium " " " " " " "	155.
5.16a Root iron levels in V (+5) treated maize v time	158.
5.16b " zinc " " " " " " "	158.
5.16c " manganese levels in V (+5) treated maize v time	158.
5.16d " calcium " " " " " " "	158.
5.16e " potassium " " " " " " "	159.
5.16f " vanadium " " " " " " "	159.

	Page
5.17a Total vanadium accumulation in shoots of maize over 6 weeks	161.
5.17a " " " " roots " " " "	161.
5.18a Shoot iron levels in V (+4) treated maize v time	163.
5.18b " zinc " " " " " "	163.
5.18c " manganese levels in V (+4) treated maize v time	163.
5.18d " calcium " " " " " "	163.
5.18e " potassium " " " " " "	164.
5.18f " vanadium " " " " " "	164.
5.19a Root iron levels in V (+4) treated maize v time	166.
5.19b " zinc " " " " " "	166.
5.19c " manganese levels in V (+4) treated maize v time	166.
5.19d " calcium " " " " " "	167.
5.19e " potassium " " " " " "	167.
5.19f " vanadium " " " " " "	167.
5.20a Relationship between shoot vanadium, zinc and manganese (V+4)	169.
5.20b " " " " " " " (V+5)	169.
5.21a Bean dry matter production v applied vanadium (V+5)	175.
5.21b " " " " " " (V+4)	175.
5.22 Bean leaf and stem tissue vanadium levels v applied vanadium	177.
5.23 " root " " " " " " "	179.
5.24 Regressions to illustrate relationship between V tissue levels in different parts of bean plant	181.
X 5.25 Comparison of ^{present} data with that of other workers	190.
5.26 Tomato dry matter production v applied vanadium	192.
5.27 Tomato root vanadium levels v applied vanadium	199.
5.28 Tomato shoot vanadium levels v applied vanadium	201.
6.1a ESR spectra for roots exposed to V (+4) and CaCl_2	211.
6.1b " " " " " " V (+5) " "	211.
6.2a " " " " " " V (+4) after V free period	213.
6.2b " " " " " " V (+5) " " " "	213.
7.1 Vanadium in soils	226.
7.2 Possible uptake scheme for vanadium	229.

List of tables	Page
1.1 Unusually high concentrations of V in plants	19.
1.2 Vanadium in aquatic animals	25.
1.3 Vanadium in small terrestrial animals	25.
1.4 Enzymes affected by vanadium	35.
3.1 Analytical techniques available for vanadium determination	60.
4.1 Vanadium root tissue levels	69.
4.2 Effect of calcium on uptake of vanadium (+5)	104.
5.1 Anova of dry matter production in V treated maize	122.
5.2a Effect of ionic form and concentration on V uptake by maize roots	128.
5.2b Effect of ionic form and concentration on V uptake by maize shoots	128.
5.3 Comparison of regression coefficients (maize)	133.
5.4 Correlation matrices for various ions in maize tissue	142.
5.5 Ion levels in maize tissue as affected by calcium levels	172.
5.6 Comparison of regression coefficients (beans)	183.
5.7 Ion levels as affected by applied vanadium (+5)	185.
5.8 Ion " " " " " " (+4)	187

List of abbreviations

ALA	Aminolevulonic acid
ANOVA, AOV	Analysis of variance
CHM	Cycloheximide
DNP	Dinitrophenol
EDTA	Ethylenediaminetetra-acetic acid
d.w., d.wt.	dry weight
M	Molar
g	gram
UV	Ultraviolet
Q ₁₀	Temperature coefficient

Notes on graphs

The vertical bars on all figures illustrating original data represent \pm one standard deviation.

Data for excised root experiments were calculated on the basis of three independent samples. All experiments were performed a minimum of three times to verify their repeatability.

Whole plant data were calculated on the basis of three independent pots within a given experiment, the number of plants per pot varied depending upon the plant species involved and the duration of the experiment.

CHAPTER ONE

INTRODUCTION

1.0 Introduction

Over recent decades there has been a burgeoning of interest in the roles of heavy metals in the environment. One aspect of particular interest is the interaction of these metals with biological systems. Numerous metals have been shown to be essential for the growth and reproduction of living organisms, whereas others have been shown to exert a pernicious effect. Unfortunately these two groups are not mutually exclusive and a large number of metals have been shown to possess both characteristics, their effects depending upon the physical, chemical and biological environment. Vanadium is one such metal whose status as a nutrient is at present under review.

It has been inevitable that some metals would be more thoroughly investigated than others. Elements such as cadmium, lead, mercury and copper have, for decades, been considered of paramount importance in their interactions with living systems and have received close attention. Our understanding of the roles of the less "interesting" elements has as a result languished. Recently however, there has been a growing recognition of the need to extend our knowledge of these neglected elements.

Vanadium is one such element which has been largely neglected in the past. An incomplete understanding of its complex aqueous chemistry and a lack of suitable and precise analytical techniques has contributed to this neglect. Isolated reports exist relating to the effect of vanadium on biological systems but no coherent examination of vanadium's effects has been attempted. Data relating to plant systems are scarce and fragmented. The accumulation of vanadium by certain lower plant species is known and frequently cited, but similar data for higher plants are limited. Some algae are known to have a requirement for vanadium for healthy growth but no such requirement has been demonstrated for higher plants. Available data only highlights the huge gaps in our knowledge relating to the roles of this element in higher plants.

This study aims to obtain some basic information on the uptake and transport of vanadium in higher plants. Consideration will be given to the ionic species present and how uptake varies within chemical and biological systems. It is hoped that this information will prove useful in further studies on vanadium in plants.

A sine qua non for the understanding of vanadium in the environment is a knowledge of its chemistry which is of a particularly complex nature. At this juncture a brief introduction will be sufficient prefatory to a more rigorous discussion in the next chapter.

Vanadium, atomic number 23, is a group V transition metal. It has four common oxidation states, +2, +3, +4 and +5 of which only the latter three are thought to have any biological significance. It exhibits a tendency to form oxoanions, a property common to elements such as molybdenum, arsenic, tungsten and phosphorus. Biologically the most important species in the +5 oxidation state are the oxovanadium ions or vanadates. Vanadates exist as a number of ionic species, both cationic and anionic, depending upon concentration and solution pH. Vanadates are often considered to be the mobile forms in the environment although the +4 and +3 oxidation states are found. Cationic vanadium(+4) species are commonly found in the environment but the occurrence of vanadium(+3) is limited to one group of marine invertebrates. Interconversions between the different forms of vanadium readily occur.

1.1 Geochemistry and presence in soils.

Vanadium is an ubiquitous lithophilic element with a mean crustal abundance of 150 mg Kg^{-1} (Fleischer 1971). The mean abundance of vanadium in the earth's crust is of the same order as nickel, copper, zinc and lead (Bertrand 1950) although it is more dispersed than these metals. Therefore, in principle, this element is more accessible to plants than metals of the same abundance which are concentrated in discrete bodies. There are over 60 vanadium ores of which the most important is the polysulphide, patronite, found

in association with sulphur as well as with nickel and iron sulphides (Clarke 1968). Vanadium in igneous rocks is largely associated with basic magmas especially titaniferous magnetites (titanium bearing Fe_3O_4). The vanadium content of acidic, silicic, igneous rocks is much lower (van Zinderen Bakker and Jaworski 1980). Metamorphic and sedimentary rocks tend to have a vanadium concentration somewhere between the two igneous types. The ionic radius of trivalent vanadium is close to that of the ferric ion so it may replace iron in iron minerals. It is generally agreed that vanadium in rocks is present as an insoluble salt of the trivalent form and only in certain rare sulphide minerals does it occur in the bivalent form (Rose 1973).

The vanadium content of a soil is dependent on the rocks and minerals from which the soil parent material was derived and the pedogenic processes to which the parent material was subjected during formation and development; the more mature and developed a soil the less bearing the original parent material has on its vanadium content. Mitchell (1971) has reported a range of vanadium levels from 20 - 250 mg Kg^{-1} dry matter in Scottish soils and related these differences to the parent material from which the soil was derived. Swaine (1955) reports a normal range of soil vanadium concentrations as 20 - 500 mg Kg^{-1} dry matter. The mean soil level generally reported is 100 mg Kg^{-1} dry matter (Vinogradov 1959; Hopkins et al 1977). The proportion of extractable vanadium is almost invariably linked to the type of extractant used, soil type and drainage conditions. Berrow and Mitchell (1980) found extractable vanadium levels between 0.03 - 26 mg Kg^{-1} dry matter depending upon drainage and extractant used. The levels of vanadium extracted with EDTA were generally higher than those extracted with acetic acid, perhaps indicating an organically bound fraction of total soil vanadium.

The form of vanadium in soils is not known with any certainty at present. During soil formation the trivalent vanadium in the mineral lattice is oxidized to pentavalent vanadium (Yen 1972; Vinogradov 1959). The oxyanions of vanadium (+5) are soluble over a wide pH range and are generally considered

to be the mobile forms of vanadium in soils (Hopkins et al 1977). The degree of mobility is primarily determined by the prevailing physical and geochemical factors in the soil. Goldschmidt (1958) lists four groups of factors which may cause the precipitation of less soluble vanadium compounds;

- (a) Presence of reducing agents, such as organic matter, locally in the soils or sediments, for instance layers of carbonaceous plant remains in sandstones. In some cases the reducing agents act through the formation of hydrogen sulphide which may result in the immobilization of vanadous oxygen compounds by the precipitation of vanadium sulphide or sulphide salts.
 - (b) Local concentrations of heavy metals * which may form insoluble compounds with vanadate anions.
 - (c) A special case is the precipitation of vanadate ions in the presence of the divalent uranyl cation $(\text{UO}_2)^{2+}$ in such minerals as Carnotite $(\text{K}(\text{UO}_2)(\text{VO}_4)1.5\text{H}_2\text{O})$.
 - (d) Local precipitation of soluble vanadate ions as well as phosphate or arsenate ions is frequently caused by hydroxides of aluminium or of ferric ions.
- * in some cases calcium vanadates are precipitated.

The importance of reducing agents, such as organic matter, in the cycling of vanadium in soils has long been known. Szalay and Szilagy (1967) demonstrated the reduction of vanadate by humic acid preparations and subsequent geochemical enrichment. Goodman and Cheshire (1975) also demonstrated reduction of vanadium from oxidation state (+5) to (+4) when it was incubated with a peat humic acid preparation. Cheshire et al (1977) demonstrated the association of vanadium with the humic and fulvic acid fractions of a soil and made comparisons with other metals. The percentage of the total metals in the soil extracted by alkali (i.e. humic and fulvic acid fractions) decreased in the order:



This is in general agreement with a stability series for organic-metal complexes. Cheshire et al (1977) showed an

uneven distribution of metal between humic and fulvic acid fractions. Vanadium was largely found in the fulvic acid fraction. Suggestions have been made (Taylor and Giles 1970) that vanadium moves as a vanadyl complex in certain soils, especially when associated with iron oxides. Given the above evidence this might be particularly the case in less acid soils.

Several vanadium 'pools' can be envisaged to exist within any soil system (fig. 1.1). Exchange between the various pools depending upon physical, chemical and biological conditions.

1.2 Vanadium in the biosphere.

There have been few studies on the presence, form and function of vanadium in the biosphere. The paucity of the vanadium literature is apparent when compared with the body of data which has developed around such elements as copper, lead, cadmium and mercury. In consequence most studies relating to vanadium have been primarily concerned with levels of vanadium in the biosphere. Little or no attention has been given to the form, function and modes of transfer between various compartments within the biosphere. Here an effort is made to concisely report the available information on vanadium and where possible to relate the different aspects of vanadium in the biosphere.

1.2.1 Vanadium in lower plants.

Bertrand (1950) considered the first reliable report of vanadium in plants to be that of Ter Meulen (1931). In this work the presence of 3.3 mg Kg^{-1} vanadium in the tissues of the basidiomycete fungus *Amanita muscaria* was reported. No vanadium was detected in the six other species of toadstool examined. It is singularly appropriate that the first reliable report of vanadium in plants should concern *A. muscaria* since the fungus occupies a unique position in relation to vanadium in the biosphere. Byrne et al (1976) analysed 27 species of fungus and found an average vanadium content of 0.39 ppm (dw) (excluding *A. muscaria*). In

Fig.1.1 Vanadium in the soil system.

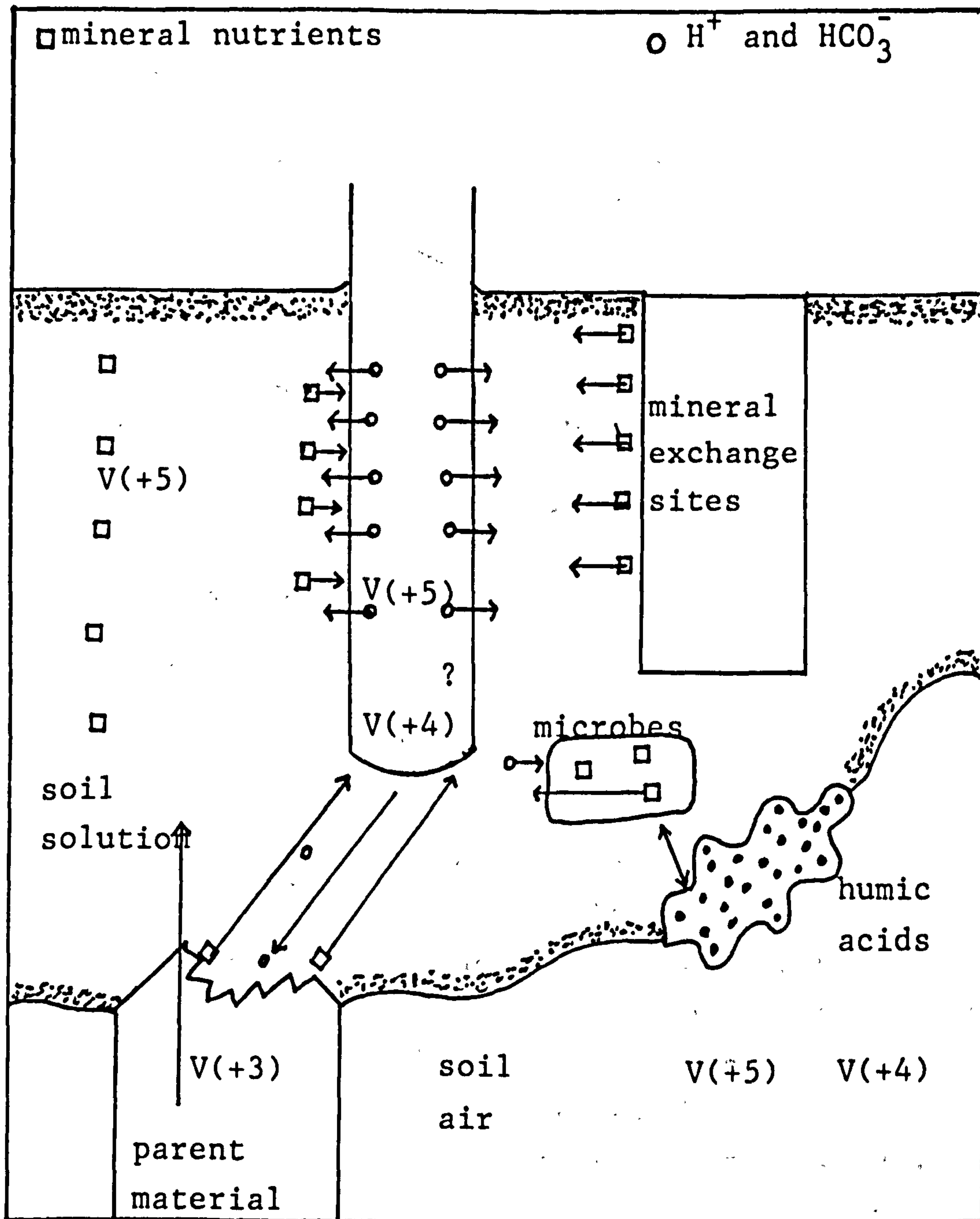
Several vanadium 'pools' can be envisaged;

(a) vanadium in the mineral lattice (+3,+4 and +5)

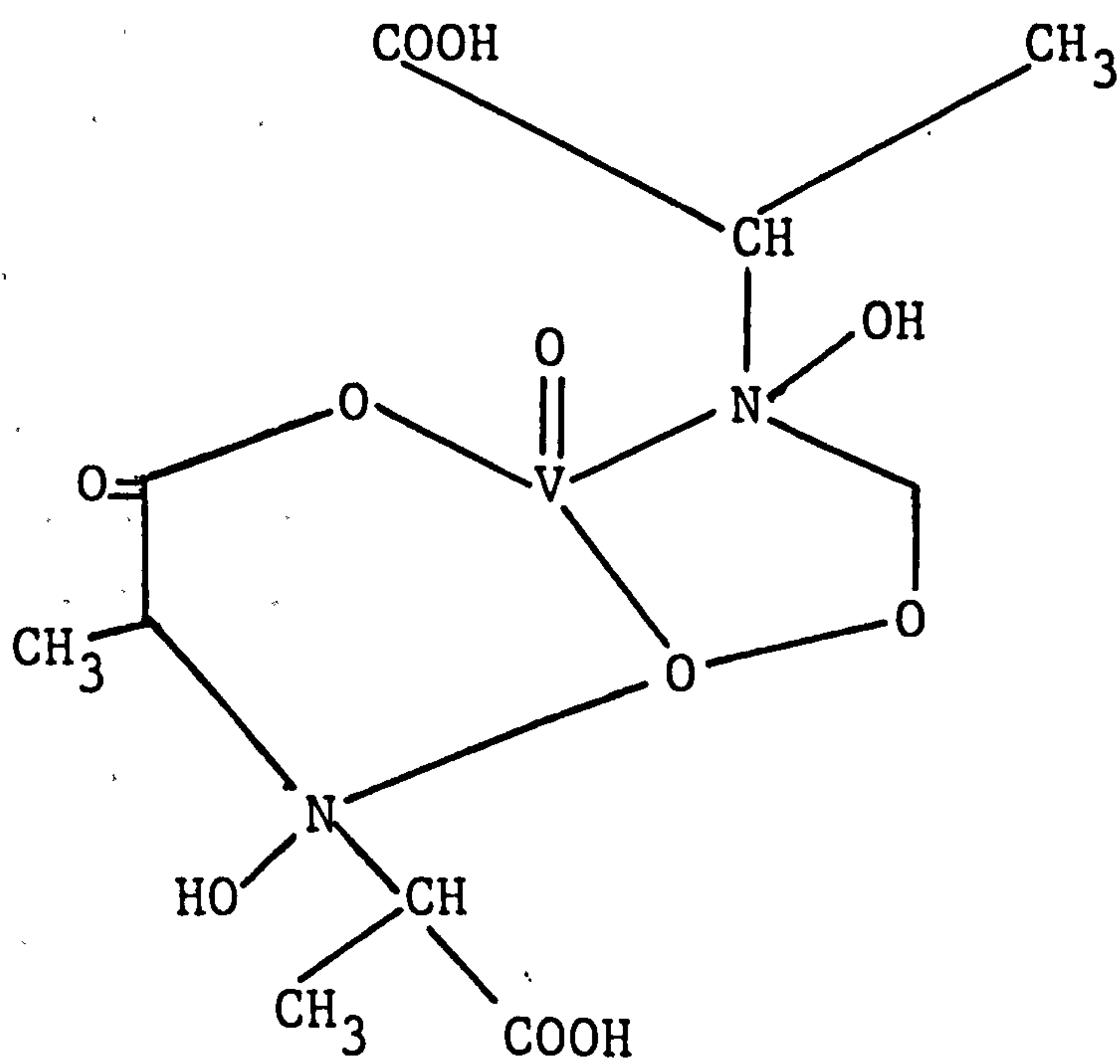
(b) free vanadates in solution (+5)

(c) mobile complexed vanadyl cation in solution (+4)

(d) immobile organically complexed vanadium (+4 and +5)



A. muscaria average values for cap and stalk were found to be 115 and 200 ppm (dw) respectively. Other reports confirm these high levels of vanadium in *A. muscaria* (Watkinson 1964; Meisch et al 1978). Bayer and Kneifel (1972) isolated a vanadium containing complex from *A. muscaria* by extraction with methanol. The structure of this complex, named Amavadin, is given below;



Later work (Lancashire 1980) suggests that the complex isolated and shown above is in fact the complex present in the mushroom and not an artifact of the extraction procedure. Studies using electron spin resonance (ESR alternatively known as electron paramagnetic resonance, EPR) on fresh tissue have shown that this complex is present in all parts of the mushroom i.e. cap, stalk and bulbous base although at differing concentrations. By comparing the spectra and ESR parameters obtained for segments of frozen mushroom with those of known alpha amino acid vanadyl complexes, Gillard and Lancashire (1983) were able to suggest that the chelating ligand was more likely a terdentate amino acid such as L-cystine or L-serine rather than a simpler bidentate amino acid such as glycine or L-alanine. Lancashire (1980) emphasizes the point that Amavadin is unusual in that it contains a N-hydroxy amino acid and not a hydroxamic acid or one of the many amino acids already known to occur in mushrooms. Whether or not vanadium plays a role in the metabolism of *A. muscaria* is unknown at present.

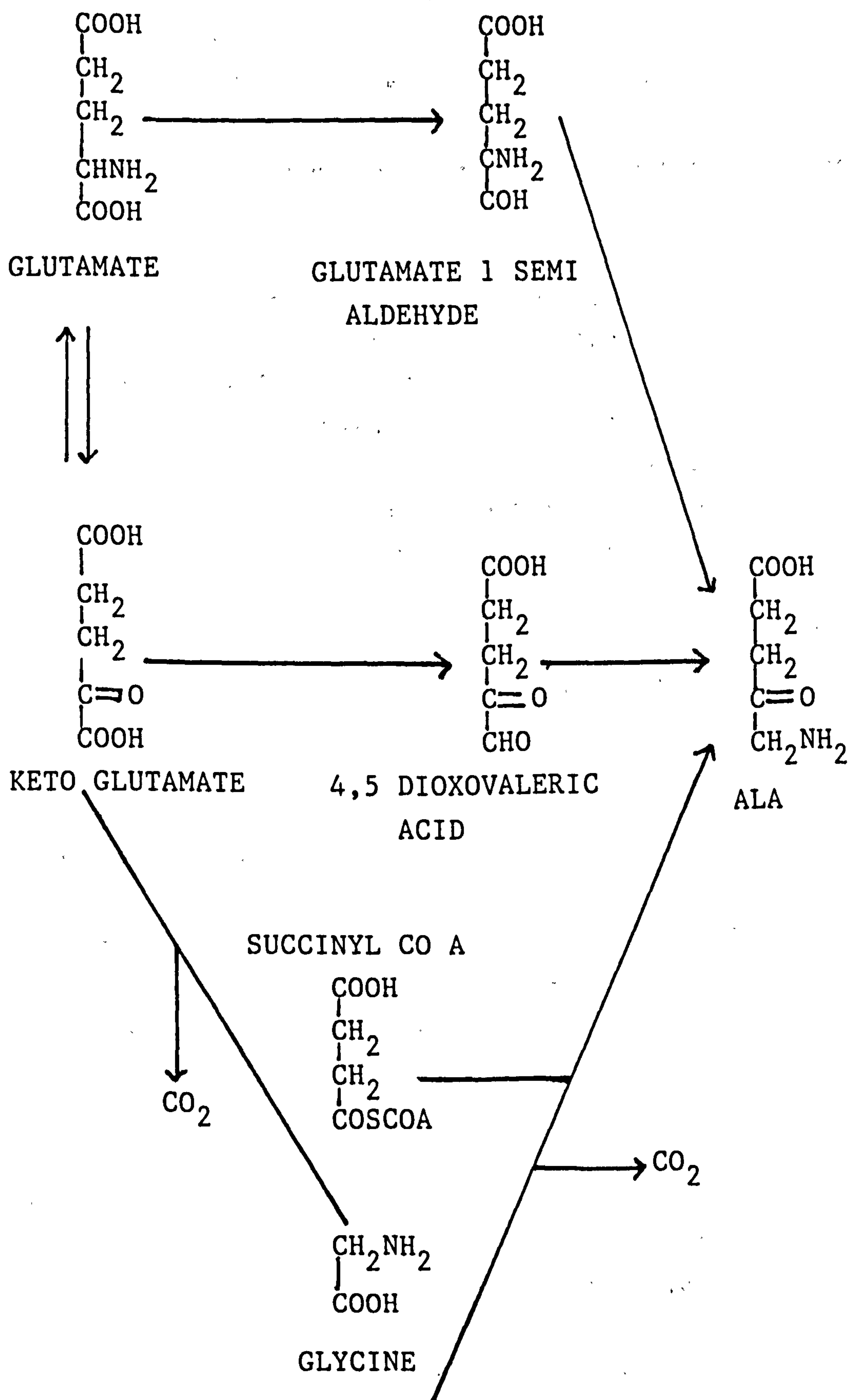
Many studies have shown that vanadium is able, to some degree, to replace molybdenum as the activator of nitrogenases in *Azotobacter* sp. and *Clostridium butyricum* (Bove et al 1957, Buczek 1973, Horner et al 1942, Jensen and Spencer 1946 and McKenna et al 1970). The exact nature of vanadium's influence is at present uncertain.

Data relating to vanadium in mosses, liverworts and lichens is limited although what there is has been reviewed by Puckett and Burton (1981). Both vanadate and vanadyl ions have been reported able to reduce phosphatase activity in whole thalli of *Cladina rangiferina* (Lane and Puckett 1979). Puckett and Burton (1981) attached great significance to this result because of the rising level of vanadium in atmospheric aerosols. Laboratory studies have shown that levels similar to those found in the environment can have serious deleterious effects on enzyme activity in some lichens (Leseur and Puckett 1980). The extent to which environmental factors may enhance or ameliorate these effects is uncertain.

Interactions between vanadium and algae have been studied extensively. The alga *Scenedesmus obliquus* requires a supplement of 100µgm. vanadium per litre (*sic*) to maintain maximal growth (Arnon and Wessel 1953) (not 100 ppm as reported by Bengtsson and Tyler 1976 and Peterson and Girling 1981). Of 16 other elements tested none was found capable of substituting for vanadium. This study led Arnon and Wessel (1953) to suggest that vanadium will be shown to be required by all green plants. However in the three decades since this paper first appeared vanadium's essentiality has been demonstrated for only a handful of algae and for no higher plants. The effects of vanadium on *Chlorella pyrenoidosa* and *Bumilariopsis filiformis* have been clearly detailed in numerous studies (Meisch and Bielig 1975, Meisch et al 1977, Meisch and Benzschawel 1978 and Meisch and Bauer 1978). Meisch and Bielig (1975) extended the observations of Arnon and Wessel (1953) on *S. obliquus* and also included *C. pyrenoidosa* in this study. Large increases in autotrophically produced dry weight occurred for both algae when vanadium was supplied. These

effects were however highly dependent upon culture conditions particularly light and iron levels. Increases in chlorophyll levels were also produced by vanadium but once again the degree of increase was dependent on culture conditions. This work led the authors to propose two sites of action for vanadium at different concentrations of vanadium and with different pH optima. The first site of action with low vanadium concentrations and a pH optima of 7 affected dry weight production. The second site of action required higher vanadium concentrations and had a pH optima of 7.5 - 8.0, this largely affected chlorophyll biosynthesis. Effects on chlorophyll biosynthesis and relationships with iron supply led the authors to investigate a possible role in porphyrin biosynthesis in general. To investigate this the yellow mutant 211 - 11h/20 of *C. pyrenoidosa* was employed. This mutant has lost the ability to produce chlorophyll but does produce a porphyrin precursor of chlorophyll (protoporphyrin). Mutants grown in a medium containing vanadium showed an increased production of protoporphyrin. To try and identify exactly where in the reaction sequence vanadium exerts its influence Meisch and Bauer (1978) investigated effects on delta - aminolevulonic acid (d - ALA) synthesis. For a long time ALA was thought to be the first intermediate that was unique to the tetrapyrrole pathway, although recent evidence suggests that in plants there may be other precursors which are specifically dedicated to this pathway (Castelfranco and Beale 1981). When vanadium was supplied in the medium a positive enhancement of the production of ALA was observed. In an attempt to elucidate more fully where vanadium has its point of impact Meisch and Bauer (1978) examined some of the pathways leading up to ALA formation. Fig. 1.2 summarizes the proposed pathways for ALA formation in plants. Previous work had shown vanadium to be capable of catalyzing the *in vitro* transamination of DOVA (Dioxovaleric acid) (Hoffman 1977). Meisch and Bauer (1978) thought it likely that it was through this step that vanadium exerted its influence. Unfortunately our knowledge of the pathways leading to ALA formation is poor and although the *in vitro* conversion of

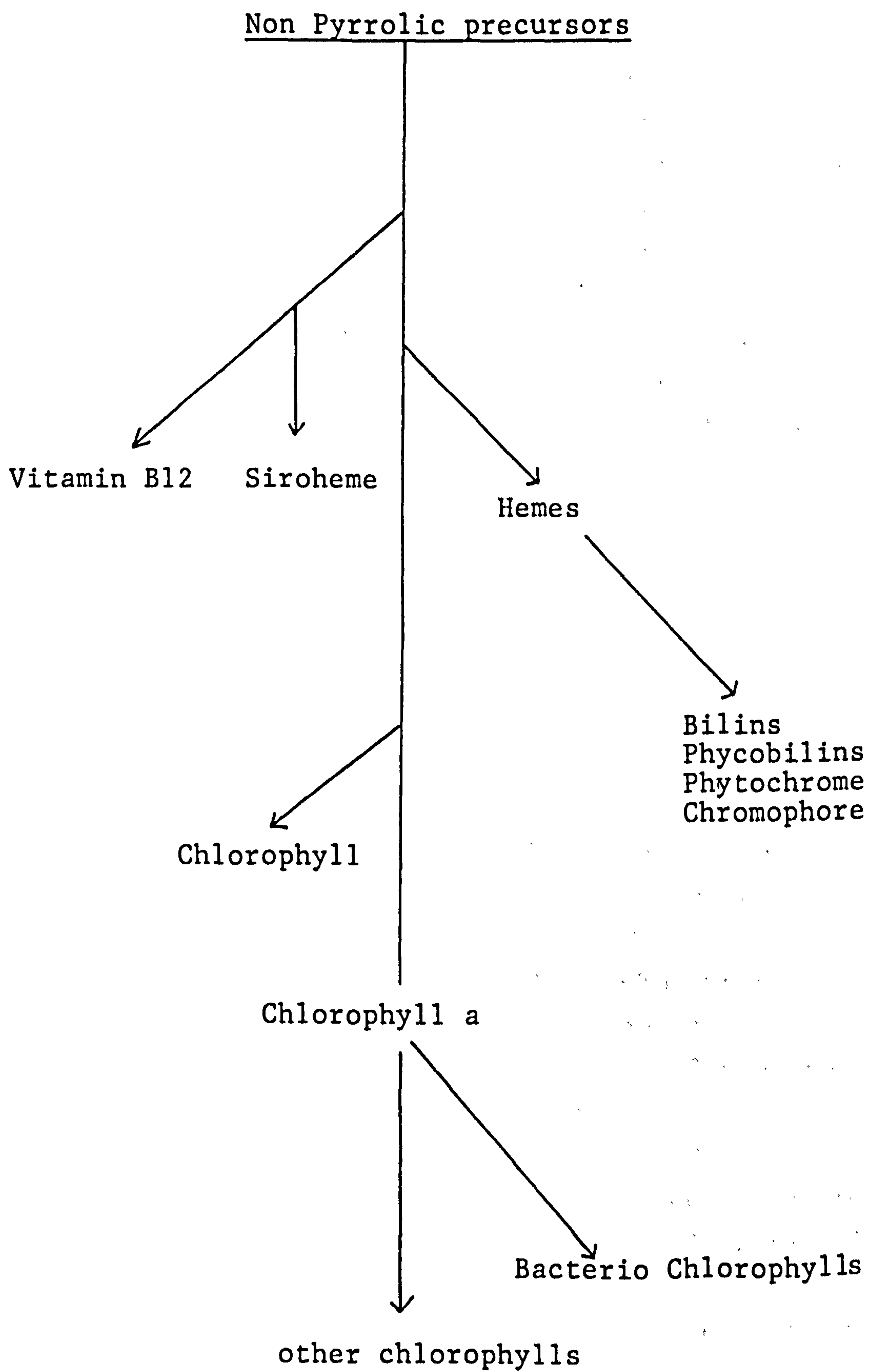
Fig.1.2 Proposed scheme for ALA formation in higher plants (from Castlefranco and Beale 1981)



5 carbon compounds to ALA has been demonstrated the operation of these steps for ALA formation *in vivo* has not yet been established for any plant tissue (Castelfranco and Beale 1981). Despite the fact that at present the exact nature of vanadium's influence cannot be determined the implications of work of this kind is clear. If vanadium does affect the synthesis of the primary intermediate in the pathway of tetrapyrrole biosynthesis then its potential to disrupt biological systems is immense (fig. 1.3).

Wilhelm and Wild (1980) reinforced much of the data already obtained with their work on *Chlorella fusca*. Vanadium had similar effects on this alga to those on *C. pyrenoidosa* and *S. obliquus*. Further to this a positive enhancement of Cyt - F and P - 700 production was demonstrated. Both of these molecules are important components of the electron transport chain and both are closely associated with iron. This led the authors to propose a link between vanadium and the metabolic processes involving the ferric ion. Simultaneously, Meisch et al (1980) were examining ultrastructural changes in *C. fusca* brought about through iron deficiency and vanadium treatment. Both iron and vanadium deficiencies caused a large increase in the amount of intracellular starch. Iron deficiency also brought about disruption in the chloroplast lamellar system and frequently total cell disruption. When vanadium was present iron deficiency symptoms disappeared. In vanadium grown cells with a sufficiency of iron little intracellular starch was accumulated, the main effect being an enlargement of the thylakoid system within the chloroplast. Later work (Becker and Meisch 1980) also showed an enhancement of carotenoid biosynthesis by vanadium and demonstrated that vanadium (vanadyl citrate (+4) and sodium metavanadate (+5)) was a powerful activator of algal photosynthesis. Work using isolated chloroplast systems revealed that increases due to vanadium occurred only in PS1, this enhancement being approximately equal to that of photosynthetic productivity in intact cells. This led to the suggestion that vanadium's point of impact is in

Fig.1.3 Outline of the tetrapyrrole biosynthetic pathway,
with its major products and their ontogenic
relationships.



PSI where it acts by donating electrons to the system thus undergoing valency change. This would seem to account for the dual effects proposed by Meisch and Bielig (1975) the other being on the synthesis of ALA as already discussed.

Lately there has been some work to suggest that vanadium may be beneficial to certain marine macro algae (Fries 1982). Both *Fucus spiralis* and *Enteromorpha compressa* produced large increases in fresh weight in response to vanadium treatment. These weight increases were accompanied by an increase in chlorophyll levels in the algae. No stimulatory effects were observed for red algae. Clearly vanadium is an important element for algae, especially unicellular freshwater species. However it seems likely that the effect of vanadium on growth and development and the activities of enzymes is different in higher plants and algae. Reasons for these differences will be discussed later.

1.2.2 Vanadium in higher plants.

The effect of vanadium on plant growth was investigated even before there was positive proof of the occurrence of vanadium in plants. Bechi (1879) was possibly the first to mention the presence of vanadium in plants although he produced no experimental details. The first work giving details is that of Demarcay (1900) who, using a spectrographic method, recognised the presence of vanadium in the ash of numerous plant species. Since that time interest has steadily developed usually accompanying developments in analytical technique. Many early workers, and indeed many subsequent ones, were concerned with the possible toxic effects of vanadium. This approach has meant that much attention has focused on dose-response relationships and little attention has been given to any qualitative changes which may occur in plants as a response to vanadium application. At present there are few detailed surveys of vanadium concentrations in plants from natural soils. Those that do exist suggest average values, for unpolluted sites, of between 0.5 - 2.0 ppm (dw) (Bertrand 1950, Schroeder et al 1963; Bengtsson and Tyler 1976).

Average values have very little meaning though for as Hopkins et al (1977) states,

"Vanadium in plants varies among the different parts of a plant, at different soil depths and with the seasons"

Vanadium levels in plants also vary considerably depending on edaphic factors such as pH, redox potential, presence of other ions, level of organic matter in soil and the parent material from which the soil was derived. Different plant species also vary considerably in the levels of vanadium they can tolerate within their tissues. Certain *Astragalus* species growing on the vanadium rich sandstone derived soils of the Colorado plateau contained an average of 144 ppm (dw) (Cannon 1963). Even some non-accumulator species contained remarkably high levels of vanadium when grown on this soil. Table 1.1 gives some indication of the concentrations which may be found in plants grown in soils with high vanadium levels. Some of the earliest experiments to demonstrate a potential phytotoxic role for vanadium were those of Brenchley (1932). In an attempt to increase the effectiveness of certain slag type fertilizers a finer grinding of the fertilizer was employed. It was considered likely that a finer grinding would release more phosphate and thus further stimulate growth. These finer ground slags however proved less effective and a diminution of the growth response was observed. This reduction in growth was attributed to 'impurities' in the slag, vanadium was singled out as a likely toxic agent and investigated. Tests revealed toxicity symptoms occurring at 40 ppm vanadium (VOCl_2). These symptoms were a general stunting of plant growth which was particularly severe in the root. Warington (1951) investigated possible interactions between vanadium, manganese and molybdenum in several plant species. This work demonstrated that at relatively high concentrations vanadium could counteract an excess of manganese in soya beans. Unfortunately vanadium concentrations of this level (10 ppm) produced toxicity symptoms in their own right. These symptoms took the

Table 1.1 Unusually high concentrations of vanadium
in plants grown on soils containing high concentrations
of vanadium (Hopkins et al 1977)

<u>SPECIES</u>	<u>V ppm (dw)</u>	<u>AREA</u>
<u>Unmineralized ground</u>		
<i>Carum</i>	4.5	Sweden
<i>Amanita muscaria</i>	181	France
<u>Near vanadium deposits</u>		
<i>Triticum aestivum</i>	5.0	USA
<i>Allium macropetallum</i>	133	Utah
<i>Oenothera caespitosa</i>	38	New Mexico
<i>Astragalus confertiflorus</i>	144	Utah
<i>Astragalus preussi</i>	67	"
<i>Aster venustus</i>	21	"
<i>Castilleja angustifolia</i>	37	"
<i>Chrysothamnus viscidiflorus</i>	37	"
<i>Eriogonum inflatum</i>	15	"
<i>Lepidium montanum</i>	11	"

Table 1.1

form of apical chlorosis and stunted root growth. It was concluded that vanadium could not act as a replacement for molybdenum in the nutrition of these plants. In an attempt to extend this work Warrington (1954) examined the effect of altering iron supply on vanadium uptake. It was concluded that the toxic effects of vanadium (and manganese) could be overcome by an increase in the plant's iron supply. Further work (Warrington 1956) seemed to point to an interaction of iron with vanadium outside the plant i.e. in the nutrient solution. The importance of the pH of the nutrient solution was discussed although no specific points were made. Hewitt (1953) also reported that vanadium could cause iron deficiency symptoms in sugar beet although no comment was made upon this fact. Singh and Wort (1970) examined the effect of foliar applications of vanadium as (VOSO_4) on sugar beet. Significant reductions in leaf growth occurred concomitant with an increase in sucrose content of the storage root. Accompanying these alterations were a higher rate of carbon dioxide fixation, a lower rate of respiration and a decreased rate of nitrate reductase, glutamic pyruvic transaminase, phosphatase and invertase activity. The enzymes of sucrose synthesis exhibited an increase in activity. No estimates of vanadium levels in plant tissue were provided. Levels of vanadium employed in this study were considerably higher than those used in any other study. The authors concluding remarks suggested that foliar applications of vanadium may have some part to play in future sugar beet production. This suggestion seems of little practical value as the levels required to bring about basic changes were so high. Singh (1971) examined the effects of vanadium on the growth, yield and chemical composition of maize. It was concluded that at applied levels above 0.25 ppm (nutrient solution) vanadium was injurious to maize but at lower levels vanadium was responsible for an increase in yield. In response to a growing suggestion that vanadium might be an essential element for higher plants researchers tried to produce evidence which would substantiate this hypothesis. Welch and Huffman (1973) demonstrated that both tomato and lettuce could be grown to full maturity on media

of various parameters on vanadium uptake by excised barley roots. The amount of uptake was shown to be dependent upon pH and presence of calcium ions. Over the concentration range employed (0.5 - 100 μ M) vanadium uptake appeared to be a linear function of concentration and did not follow saturation kinetics. Of eight anions tested none were found to interfere with vanadium uptake. Anaerobic treatment appeared to have little effect on vanadium uptake. Wallace et al (1977) examined the response of bush beans to applied vanadium. Once again plant tissue levels increased with an increased supply of vanadium, the major portion of the vanadium being retained within the roots. Despite no iron deficiency being present iron levels in roots and shoots were reduced - Mn, Cu and Ca levels were also reduced. The decrease in shoot Mn and Cu levels were compensated for by an increase in root levels. Some decreases in yield were reported but no mention of phytotoxicity was made. Davis et al (1978) in an investigation of the toxic properties of various elements proposed upper critical limits could be assigned for diagnostic purposes. The upper critical limit for vanadium was given as 2 ppm in the leaves and stems of actively growing plants. This level agrees well with one proposed by Hara et al (1976). Since the normally reported level in plant tissue is approximately 1 ppm (Bengtsson and Tyler 1976) it would suggest that vanadium might easily produce phytotoxicity problems in the future.

Vanadium has also been shown to effect rhythmic leaflet movement in *Albizia julibrissin* (Saxe and Satter 1979) bean leaf movement, barley leaf unrolling, respiration and phosphatase activity of bean (Saxe and Rajagopul 1981). Inhibition of stomatal opening in *Vicia* has also been demonstrated (Gepstein et al 1982). Vanadium has been detected in the phloem exudate of *Yucca flaccida* Haw. Levels of 0.71×10^{-3} μ moles g⁻¹ dw (0.36 ppm) were detected using the highly sensitive and reliable neutron activation technique (Wolterbeek and Van Die 1980).

It cannot be doubted that other effects of vanadium on higher plants will be discovered in the future. The debate as to whether or not vanadium is an essential element remains unresolved at present. The evidence suggests vanadium has some physiological role in plants but no unique and irreplaceable role has been described.

1.2.3 Vanadium in aquatic organisms.

In most aquatic organisms vanadium is present in only trace amounts (table 1.2). However, certain species of tunicate, notably those of the families *Ascidiidae* and *Cionidae*, can accumulate vanadium to extraordinary high levels (Swinehart et al 1974). Whole body concentrations in excess of 3000 mg Kg^{-1} (dry weight) are not uncommon (Van Zinderen Bakker and Jaworski 1980). Although this phenomenon has been recognized for decades few cogent attempts have been made to explain it. Bertrand (1950) dismissed the idea that Ascidians accumulated the vanadium from marine waters because of the extremely low concentrations found in unpolluted areas. Instead he suggested that Ascidians consumed a special vanadium rich food source although he gave no indication what this might be. More recent work has suggested direct accumulation from marine waters. Macara et al (1979) have proposed a mechanism of direct accumulation which explains 3 features of accumulation by ascidians;

- (1) The high specificity of the accumulation system
- (2) The large concentration gradients achieved
- (3) The large concentrations of acid found in the vacuoles of certain ascidian cells

In seawater vanadium is present as the V(+5) oxoanion. However, in Ascidians vanadium is known to exist in the reduced form as either V(+4) *Apousobranchia* or as a mixture of V(+4) and V(+3) *Phleobranchia* both of which are cationic (Macara et al 1979). Thus given a vacuolar membrane permeable to anions but not cations

Table 1.2 Vanadium in aquatic animals.

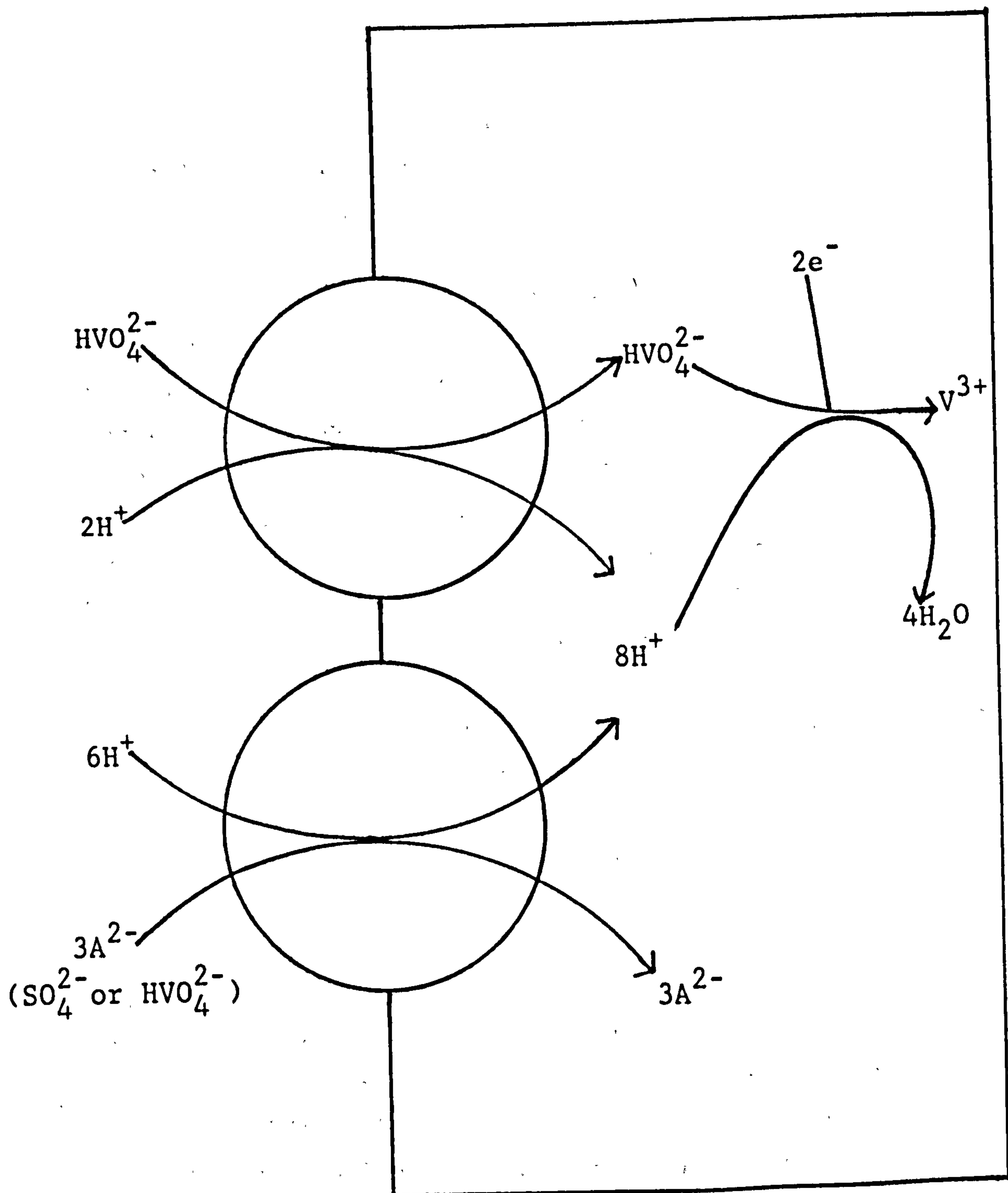
Table 1.3 Vanadium in small terrestrial animals.
(W.W. = Wet Weight; A.W. = Ash Weight; N.D. = Not Detectable;
D.W. = Dry Weight).

and a reducing agent within the vacuoles then vanadium will be accumulated. A diagrammatic representation of this mechanism is shown in figure 1.4. Since only those elements which are anionic in seawater and cationic upon reduction and stable in acid solution can be accumulated, the mechanism is highly specific. The large concentrations of acid within the vacuoles (0.1 M sulphuric acid; Carlson 1977) serve to stabilize the reduced vanadium and prevent reoxidation. The reduction appears to be mediated by an organic chromogen which has been given the trivial name tunichrome (Macara et al 1979). It is from this compound that the vanadocytes derive their green colour. Tunichrome is readily oxidized by V(+5) *in vitro* (Macara et al 1979) and is known to have an intra vacuolar concentration of around one Mol.dm⁻³ similar to that of the free vanadium. Further testing of this hypothesis, that tunichrome reduces V(+5) → V(+4)+V(+3) requires the isolation of transport competent vanadocyte vacuoles.

Despite these studies on the nature of the transport system no clear function has emerged for vanadium in Ascidians. Vanadium was once believed to function as an oxygen carrier in association with a protein. The vanadium - protein complex, haemovanadin being the functional form of vanadium *in vivo* (Goodbody 1974). It is now known however that haemovanadin does not exist and that the vanadium does not function as an oxygen carrier. More recently Macara (1980) has suggested that vanadium may have a role to play in sheath (test) formation although the exact role of vanadium in Ascidian metabolism is unclear.

Other studies have been addressed to the question of the potential toxicity of this element to marine species or the mechanisms by which vanadium is accumulated and cycled through the biosphere. Miramand et al (1980) examined vanadium uptake by Mediterranean mussels *Mytilus galloprovincialis* which showed slow uptake rates principally governed by passive absorption of the metal on the external surfaces in contact with seawater.

Fig 1.4 Postulated mechanism for accumulation of vanadium by vanadocyte vacuoles. The vacuolar membrane is assumed permeable to oxoanions but not to cations. Vanadate (or sulphate) moves into the vacuole diffusion. H^+ could be carried on the same porter, or separately. The reduction of the vanadate maybe by tunichrome or a precursor. The extent of accumulation is controlled by the concentration of the reductant.(Macara 1980)



(Stendhall and Sprague 1982). Surprisingly vanadium toxicity was relatively unaffected by either pH or water hardness, the authors ascribed this to vanadium's anionic state in natural waters.

1.2.4 Vanadium in terrestrial animals.

Vanadium is found at trace levels in most animals. Table 1.3 gives some indication of the range of levels encountered in animals under normal conditions.

For some years now there has been concern that vanadium may be an essential element for certain animal species and possibly man. Hopkins and Mohr (1971) noted that chicks fed on a purified diet containing low levels of vanadium had poor wing and tail feather development when compared with controls. In later studies utilizing rats, animals fed on low vanadium diets showed marginally reduced reproductive performance in the third generation and greatly reduced performance in the fourth generation (Hopkins and Mohr 1974). Accompanying this reduced reproductive performance was an increase in neo-natal mortality in the fourth generation. Other workers (Strasia 1971; Schwartz and Milne 1971) have obtained similar results. Villaume et al (1976) suggested that vanadium toxicity was dependent upon ionic form, toxicity decreasing along the series VO_3^- , VO_4^{3-} , $\text{V}_2\text{O}_7^{4-}$ and VO^{2+} . Other workers have however suggested that vanadium *in vivo* exists in only one form. Johnson et al (1974) found that rats injected with V(+5) later contained V(+4) in their tissues. Sabbioni et al (1978) described the similarity of metabolic pathways for different ionic species of vanadium. It was considered that the results supported the existence of a mechanism of conversion of the different cationic and anionic forms of vanadium to a common state. The nature of the ligand seems to be unimportant except in the case of thiovanadate (VS_4^{3-}). Another important factor in the metabolism of vanadium is the composition of the rest of the diet. Nielsen et al (1978) reported that altering the cystine - methionine balance in chick diets altered the appearance of vanadium

Interestingly vanadium differed from other metals in that higher levels were found in shell than soft parts. This difference came about not through high shell absorption but through poor uptake into the soft parts of the mussel. Certain environmental factors (temperature and salinity) were seen to influence vanadium uptake and loss in mussels and as a consequence it was considered unlikely that mussels would be useful as a bioindicator of ambient vanadium levels. Shrimp (*Lysmata seticaudata* (Risso)) and Crab (*Carcinus maenas* (L)) showed similar accumulation patterns to mussels, passive sorption on the external surfaces being of great importance. These organisms however showed significantly higher retention of ingested vanadium which led to the conclusion that the food pathway was also of major importance in the bioaccumulation of this metal in shrimps and crabs under natural conditions (Miramand et al 1981). The sabelliid Polychaete, *Eudistylia vancouveri* has been demonstrated to accumulate vanadium (and Titanium) to high levels when in close contact with a pollution source (Popham and D'Auria 1982). The possibility of using this organism as a bioindicator of vanadium pollution has been suggested but at present only scant information is available on its biology and as such its usefulness is limited. Unsal (1982) examined transfer of vanadium through a food chain to a terrestrial mammal (mouse). Mice fed contaminated mussels exhibited symptoms observed by other investigators in rats injected with vanadium. Few other studies have addressed themselves to the problem of vanadium accumulation through food chains. The importance of these pathways will increase as anthropogenic inputs are leading to an enrichment of this metal in marine waters (Duce and Hoffman 1976; Bertine and Goldberg 1971).

Data relating to freshwater species are at present scarce. Holdway et al (1983) found that bioaccumulation factors for vanadium were lower than for other metals and suggested that the danger to fish from vanadium was minimal. Studies on rainbow trout have revealed vanadium to be only moderately toxic when compared to other metals

toxicity symptoms. Other studies have also shown that diet composition significantly alters the response of chicks to elevated levels of dietary vanadium. Vanadium toxicity was mitigated by corn, dehydrated grass, cottonseed meal, ascorbic acid, EDTA and Chromate (Berg 1966; Berg and Lawrence 1971; Hathcock et al 1964; Hill 1976). Essentiality of vanadium for animals has not been conclusively established but it seems probable that vanadium plays some role in animal nutrition.

The role of vanadium in human nutrition is equally unclear. Compounds of vanadium have been administered therapeutically for a diverse variety of disorders; as antiseptic, spirochetocide, anti tuberculosis and anti anaemic agents; to boost resistance to infection and to improve appetite, nutrition and general health (Schroeder et al 1963). The doses employed were often very large and as such it seems unlikely that vanadium is particularly toxic to humans. In part this may be accounted for by poor absorption from the gastro intestinal tract as reported by some workers (Hopkins and Mohr 1971; Curran et al 1959). Most vanadium is unabsorbed and passes out of the body via the faeces. Residence time for the small quantity of vanadium absorbed is usually short e.g. 60% of absorbed vanadium lost within 24 hrs (Curran et al 1959) loss being via the urine. The most important pathway for vanadium toxicity in humans is the respiratory tract. Inhaled vanadium dust is known to cause pulmonary irritation, cough, sputum, wheezing, irritation of mucous membrane, injected pharynx and the green tongue which is one of the most visible symptoms of vanadium poisoning. Toxicity symptoms can be alleviated by removal from the source of contaminated dust. There are marked differences in the toxicity of different vanadium dusts depending upon particle size and chemical form of vanadium. In general terms toxicity increases with increasing solubility and decreasing particle size (Van Zinderen Bakker and Jaworski 1980). Waters (1977) noted that vanadium workers were more susceptible to colds and other respiratory illnesses than non-vanadium workers. This

problem could be related to the toxicity of vanadium to alveolar macrophages which had been demonstrated *in vitro*. It is believed that this might pre dispose individuals suffering vanadium exposure to respiratory infection.

Recently vanadium has been implicated in the etiology of manic depressive syndrome in humans (Naylor and Smith 1981). Unfortunately much of the evidence for vanadium's effect comes from clinical anecdotes and little hard data is available. Depression is linked to defects in water and electrolyte metabolism and is accompanied by a decrease in the red blood cell $\text{Na}^+ - \text{K}^+$ ATPase activity. Since vanadium (as vanadate) is a known inhibitor of certain $\text{Na}^+ - \text{K}^+$ ATPases (Macara 1980) it seemed worth investigating the effect of altering vanadium uptake through the diet. Reduced vanadium intake through the diet and increased vitamin C levels led to amelioration of symptoms. Vitamin C's efficacy stems from its ability to reduce vanadate (+5) into a form which does not affect $\text{Na}^+ - \text{K}^+$ ATPases. It appears manic depressives are particularly susceptible to vanadium effects since their diets did not contain high levels of vanadium (Anon 1982). The symptoms of melancholia and depression seen in victims of vanadium poisoning can now be understood more fully.

Other effects on human metabolism range from effects on the incidence of dental caries (Tank and Storvick 1960) to effects on cholesterol biosynthesis (Curran 1954). A full resume of effects on human metabolism is beyond the scope of this work but two useful reviews are 'Vanadium' (N.A.S. 1980) and 'Effects of vanadium in the Canadian environment' (Van Zinderen Bakker and Jaworski 1980).

1.2.5 Vanadium in selected enzyme systems.

Vanadium is known to affect a large number of enzyme systems and many of its physiological effects

(*vide supra*) can be traced directly to effects on these systems. Table 1.4 lists some of the enzyme systems which vanadium is known to affect but this is in no way comprehensive. Of particular importance to the plant ecologist/physiologist are the effects on nitrogenase, nitrate reductase and phosphatases.

The fixation of gaseous atmospheric nitrogen by free living and symbiotic microorganisms is paramount in the ecological cycling of nitrogen. Studies have shown that vanadium may have the potential to interfere in this fixation and as such it warrants close attention. It has been demonstrated that vanadium may replace molybdenum in the molybdoferredoxin component of nitrogenase with approximately 70% of normal activity (Hewitt and Smith 1975). This type of replacement has been observed in *Azotobacter* and *Clostridium butyricum* (Buck and Horner 1935; Horner et al 1942 and Jensen and Spencer 1946). There has also been a demonstration of increased N fixation in the blue green alga *Anabaena circinalis* when low concentrations of vanadium were supplied (Sahay and Sankaram 1968). Most of these studies have, however, demonstrated that molybdenum is a better activator of nitrogenase than vanadium. It has been suggested that increased environmental vanadium levels might lead to an overall decrease in N_2 fixation through competition with molybdenum (Bengtsson and Tyler 1976) but at present this seems unlikely.

Reports of vanadium interfering with nitrate reductase activity in several groups of organisms can be found (Notton and Hewitt 1972; Buczek 1973 (higher plants), Vaishampayan 1983 and Ramadoss 1979 (blue-green algae)). Buczek (1973) has reported inhibition of nitrate reductase from tomatoes both *in vivo* and *in vitro*. Ramadoss (1979) also demonstrated both *in vivo* and *in vitro* inhibition of nitrate reductase activity in *Chlorella vulgaris*. The author considered the mode of action of vanadium to be similar to that of HCN. Buczek et al (1980) suggested

Table 1.4 Enzymes known to be inhibited or activated by vanadium.

Table 1.4

<u>Enzyme</u>	<u>Valency (+4,+5)</u>	<u>Author</u>
Ribonuclease	+5	Ramasarma & Crane (1981)
Adenylate Kinase	+5	De Master & Mitchell (1973)
Adenyl Cyclase	+5	Schwabe et al (1979)
Squalene Synthetase	+5	Underwood (1977)
Nitrogenase	+5	Burns et al (1971) Sahay & Sankaram (1968)
Nitrate Reductase	+5	Ramadoss (1979) Buczck (1973,1980)
Acid Phosphatase	+5	Leseur & Puckett (1980) Tyler (1976) Van Etten et al (1974)
Alkaline Phosphatase	+4,+5	Lopez et al (1976)
(Na, K) ATPase	+5	Cantley et al (1977)
Ca ²⁺ ATPase	+5	O'Neal et al (1979)
(K ⁺ -Mg ²⁺) ATPase	+5	Cocucci et al (1980)
(Na ⁺ -K ⁺ -Mg ²⁺) ATPase	+5	Wingstrand & Lindberg (1980)

vanadium inhibits both biosynthesis and activity of nitrate reductase in cucumber and, to a lesser extent, nitrate uptake. This conclusion was supported by further work linking certain ATPases with nitrate assimilation. At present, the role of vanadium in nitrogen metabolism is unclear.

The influence of vanadium on soil phosphatase activity is one which gives cause for major concern. Tyler (1976) demonstrated a significant depression in phosphatase activity in soils incubated in vanadium concentrations found normally in urban/suburban areas. Since the mineralization of phosphorus, which is governed by phosphatases, is of major importance in maintaining the productivity of natural terrestrial ecosystems it can be seen how elevated soil vanadium levels might lead to decreased productivity.

Although early work of this type revealed the strong inhibitory effect of vanadium on phosphatases and suggested that vanadium may have several functions in biological systems, little attention was given to vanadium until 1977. At that time Cantley and co workers identified the impurity in commercially available ATP derived from equine muscle as orthovanadate, a potent inhibitor of $\text{Na}^+ - \text{K}^+$ ATPase. The inhibitory effect had been recognized for over a decade in equine preparations as opposed to laboratory synthesized ATP but the inhibitory agent had not been isolated. Since then it has been discovered that vanadium co purifies with sigma grade ATPase at a V:ATP molar ratio of about 1:3000. Vanadate acts by competing with the phosphate substrate for the binding site on the enzyme molecule. Effective competition is made possible by the ability of the vanadate to adopt a stable trigonal bipyramidal structure which resembles the state of phosphate during reaction (Macara 1980). Vanadate binds to the phosphorylation site of the enzyme from the cytoplasmic side of the plasma membrane. This binding is facilitated by Mg^{2+} which appears to lock

the enzyme in the E_2 state which binds potassium. ATP cannot bind to the vanadate trapped enzyme and will not phosphorylate it. The vanadate-enzyme complex has a high stability and therefore binding of Na^+ which requires the E_1 state cannot proceed.

Vanadate at concentrations of between 5 and 20 ppm has been shown to inhibit plasmalemma ATPases isolated from radish but not to affect mitochondrial ATPases also from radish (Cocucci et al 1980). Wingstrand and Lindberg (1980) have also shown vanadate inhibition of ATPases isolated from sugar beet roots.

Whilst vanadate appears to be the most effective species in relation to enzyme inhibition it must always be remembered that Vanadium (+4) can also inhibit certain enzymes. Vanadyl is known to inhibit ribonuclease (Lindquist et al 1973) and a vanadyl-thymidine complex has been demonstrated to inhibit staphylococcal ribonuclease (Tucker 1975).

It seems unlikely at present that any one specific role could be assigned to vanadium, indeed it is more likely that vanadium has several biochemical roles as indicated by its effect on numerous different enzymes.

1.3 Industrial uses of vanadium and its compounds.

Compounds of vanadium have many and varied industrial applications. Vanadium has been used as an additive for constructional steels in which it improves elastic properties, toughness and yield to tensile strength ratios. The metal has also been used in the production of tools, dies, springs and high strength tungsten alloys. Use as mordants in the dyeing and printing of cotton and in ceramics is in decline although use in the electronics industry is beginning to grow (N.A.S. 1973).

The catalytic properties which vanadium possesses have been employed in the synthesis of sulphuric and nitric acid and in the oxidation of numerous organic components

to commercial products. Vanadium has also been used as a catalyst in the synthesis of numerous polymeric plastics.

Use both as an ingredient and as a catalyst is small and seems unlikely to increase significantly although present information is incomplete.

1.4 Vanadium as a pollutant.

Concern that vanadium may act as a pollutant under certain conditions has prompted numerous investigations. Jastrow et al (1981) examined vanadium levels in plants grown on coal mine refuse which had been amended with various treatments. Levels were within the normal range for plants except when the ammendment was fly ash where elevated levels were observed. These elevated levels approached those which have been reported to be associated with toxic effects in some plants. Wallace et al (1977) examined the effect of fly ash on bush bean plants grown in solution culture. Despite slightly elevated levels of vanadium in the root no evidence of toxicity was found. Adriano et al (1980) suggested that vanadium may have potential as a hazardous agent because of concentration effects when plants are grown in fly ash amended substrate. All studies involving fly ash do however present serious difficulties in interpretation because of the complex nature of the substrate. Work to date does not suggest that major problems attributable to vanadium will arise with fly ash applications.

Bradford et al (1975) found that levels in sewage treatment plant effluents and sludges were highly variable depending upon the source of the effluent. No problems related to vanadium toxicity were found. Berrow and Webber (1972) reported similar findings and concluded that other metals in sewage sludge presented greater hazard than vanadium.

Goodroad and Caldwell (1979) examined the effect of

phosphorus fertilizer and lime on the levels of As, Cr, Pb and V in soils and plants. No indication of increased loading of the soils was found. Plant growth was unaffected and the study indicated that at present rates of application the use of phosphorus fertilizers would not significantly increase background levels of As, Cr, Pb and V in the soil.

The major anthropogenic source of vanadium is fossil fuel burning, most coals and oils containing at least trace amounts of vanadium. Vanadium is the major trace metal in petroleum products, especially in the heavier fractions. Crude oil has an average vanadium content of 50 mg Kg^{-1} with a range of $0.6 - 1400 \text{ mg Kg}^{-1}$ (Bertine and Goldberg 1971). Venezuelan oils are renowned for their high vanadium levels with an average of 112 mg Kg^{-1} and a maxima around 1400 mg Kg^{-1} (Bengtsson and Tyler 1976).. The vanadium is present as an organo metallic porphyrin complex which is concentrated during refining and distillation because of its low volatility. As a result residual oils tend to have a high vanadium concentration e.g. the average vanadium concentration in Venezuelan Bunker 'C' No. 5 and No. 6 residual oils is 870 mg Kg^{-1} compared with 112 mg Kg^{-1} for the crude (Zoller et al 1972). These residual oils are mainly used for domestic heating and electric power generation. During combustion most of the vanadium in the residual oils is released into the atmosphere in the form of V_2O_5 particles. An estimated one tonne of particles is produced for every 1000 tonnes of residual oil combusted (Zoller et al 1972). From this it has been estimated that emissions of vanadium to air by man through oil combustion are about equal on a global scale to the amount of vanadium in the atmosphere arising from natural sources. Vanadium may be transported in this form and high concentrations of metals such as vanadium can occur in so called "black episodes" (Brosset 1976).

Jacks (1976) has made attempts to trace the fate of deposited material in an area outside Stockholm. Approximately 5% of deposited vanadium was transported away from the area in run off water, but the bulk of the

deposited vanadium was retained in the soil, mainly in association with organic matter. Accumulation of vanadium in the soil at this slow rate was not considered to represent a problem.

In general, coal has a lower level of vanadium than oil. Coal in the United States has an average vanadium content of 25 mg Kg^{-1} (Bertine and Goldberg 1971; Zoller et al 1972). Unlike oil the trace elements in coal are largely transferred to the slag and fly ash during combustion and as such atmospheric emissions from coal burning is of minor importance. Subsequent disposal of these products may of course produce problems as has already been discussed. Small amounts of vanadium are also emitted during steel production although the contribution to atmospheric vanadium levels is minor.

Recently there have been reports of leaf and fruit necroses associated with vanadium rich ash emitted from a power plant burning fuel oil (Vaccarino et al 1983). Discrete lesions on both leaves and fruit of various plant species were observed. Similar lesions were reproduced on control material inoculated with vanadium. The results indicate some role for vanadium in the development of these necrotic lesions although it may be possible that other elements in the ash also have a role to play.

1.5 Aims of the study.

Preceding sections illustrate well the heterogeneous nature of the data relating to vanadium in the biosphere. The use of several different approaches has produced much data although information relating to higher plants is limited.

The project aims to produce useful information relating to the uptake and subsequent distribution of vanadium in a number of different higher plant species. Two distinct approaches will be employed; excised root studies and whole plant studies. It is envisaged that this dual approach will produce complementary information and allow

CHAPTER TWO

VANADIUM CHEMISTRY

2.1 Vanadium chemistry.

Vanadium was first discovered in 1801 by Andres Manuel del Rio who located it in a Mexican ore later known to be vanadinite. The element was originally named panchromium although this was soon changed to erythronium because of the red colour generated upon heating. Within two years however del Rio had retracted his claim considering after further examination that the new element was merely a chromate of lead (Clarke 1968). It was not until 1830 that the element was unequivocally identified when Sefstrom discovered it in an iron ore from Taberg, Sweden. The present name is derived from Vanadis the Norse goddess of beauty. Such beauty as the element holds for the chemist lies not only in the wide spectrum of colours its compound produce but also in the ease with which it catalyses chemical reactions.

Like other transition metals vanadium exhibits a wide range of oxidation states. In aqueous solution it can exist as V(+2), V(+3), V(+4) and V(+5) although compounds of lower oxidation states are readily oxidized by air to V(+5). Compounds of lower valence state (+1, 0 and -1) are known but are completely unstable in water. The outer electronic configuration in the ground state is $3d^3 4s^2$ thus the maximum oxidation state is +5 which corresponds to a d^0 configuration. Compounds in this oxidation state are therefore diamagnetic and colourless as compared to V(+2), V(+3) and V(+4) which produce coloured paramagnetic compounds (Nicholls 1974).

The vanadous ion, V^{2+} , slowly reduces water to hydrogen gas. It is of no known biological significance and will receive no further attention here. In the absence of complexing ligands the vanadic ion, V^{3+} , only exists in strongly reducing media being easily oxidized by air. The only living organisms known to contain V(+3) are the Tunicates (*vide supra*) where its function is unclear. Since V(+3) appears to be limited to this single group of marine invertebrates it shall not be discussed further here.

Undoubtedly the most important and common oxidation states of vanadium are V(+4) and V(+5). However within each oxidation state a number of ionic species are known to exist. The species present depends upon such factors as; ionic strength, pH, redox potentials, coordinating ligands and chelates, presence of other ions and the kinetics of formation of various vanadium species. Robinson (1981) details four types of chemical process which affect the chemical species present at equilibrium, these are;

- (a) Polymerization - depolymerization
- (b) Protonation - deprotonation
- (c) Complexation - decomplexation (inner sphere)
- (d) Oxidation - reduction

Since both V(+4) and V(+5) are affected by these processes it is worth considering each oxidation state separately.

2.2 Vanadium (+5).

In aqueous solution, in the absence of complexing ligands, V(+5) exists as a number of ionic species known as vanadates. The formation of the different ionic species is controlled to a large degree by the total vanadium concentration and the pH of the solution. Figure 2.1 summarizes which species will be present under a variety of conditions. At vanadium concentrations in excess of 10^{-4} m vanadium largely exists as polymeric species (except at the extremes of the pH range). Under conditions of extreme alkalinity orthovanadate (VO_4^{3-}) is the predominant species. With increasing acidity a series of protonations take place leading initially to pyrovanadate (HVO_4^{2-}) and subsequently to metavanadate (H_2VO_4^-). It is the metavanadate ion, usually written as VO_3^- , which extensively polymerizes at higher vanadium concentrations. Under extremely acid conditions the dioxo-vanadium cation, VO_2^+ , is the dominant species.

Figure 2.1 illustrates which ions will be present when equilibrium is attained. It in no way offers an explanation of the dynamics of change between the various species. Whilst the incipient species and concentrations

fig. 2.1 Distribution of vanadate(+5) species
as a function of pH and total vanadium concentration.
Taken from Pope and Dale (1968)

fig. 2.3 Reduction potential E(referenced to the
standard hydrogen electrode) versus pH for various
species of vanadium. Boundary lines correspond to
E.pH values where the species in adjacent regions
are present in equal concentrations. The short
dashed lines indicate uncertainty in the location
of the boundary. The upper and lower dashed lines
correspond to the upper and lower limits of stability
of water. Standard reduction potentials are given
by the intersections of "horizontal" lines with
the abscissa pH=0. Full details of the half reactions
involved are given in Robinson(1981). Not all known
species are represented on this diagram.

fig. 2.2 The equilibrium species present in a $1\mu\text{M}$ solution of $\text{V}(+5)$ in water over the pH range 1-14.

Vertical lines are points where equal quantities of the species on either side exist in the absence of other species.

(from Rubinson 1981)

may be known the attainment of equilibria may be slow. To enable a description of these dynamics to be made some knowledge of the exchange of ligands attached to the vanadium atom is required. A full description of the exchange of ligands is made impossible because of uncertainties over the structure of various aquated species. Figure 2.2 shows the hydrated monomeric species thought to exist over the pH range 1 - 14. V(+5) appears to change from four co-ordinate in base to six co-ordinate in acid although as already stated the exact nature of some of these species is uncertain. Robinson (1981) summarizes the available data which suggests that in general terms these exchanges are slow. As a result the equilibria shown in fig. 2.1 are only slowly attained. In practical terms this means that solutions should always be left for several days for equilibrium to be attained as has been suggested by numerous workers (Welch 1973, Robinson 1981, Chasteen 1983 and Macara 1980). When other ligands are involved complex formation will also be slow because the mechanism of complex formation is a dissociative one i.e. its rate depends upon the rate of cleavage of the bound ligand.

In biological systems where there are significant amounts of free ligands, it is highly unlikely that vanadium will exist as the free ion. Given normal conditions i.e. relatively neutral pH, it would be expected to be bound with common co-ordinating groups such as -SH, -SS-, -OH, -N-, -COO and PO_4^{3-} . Robinson (1981) has, however, demonstrated that when alkali cation metals such as calcium and magnesium are present, bonding between these groups is limited. In simple terms calcium and magnesium binds these groups more strongly than vanadium (it must also be remembered that in most environments the concentrations of these metals far exceed that of vanadium). This stronger bonding is particularly the case when higher number multidentate ligands are involved. The lower the dentation number the less the difference between the bonding of vanadium and these other metals. Robinson (1981) points to the various catechols as being of particular interest since they exhibit significant preferential binding of vanadium. When catechols bind with V(+5) they are oxidized and the vanadium is bound as V(+4), this is thought to be the mode

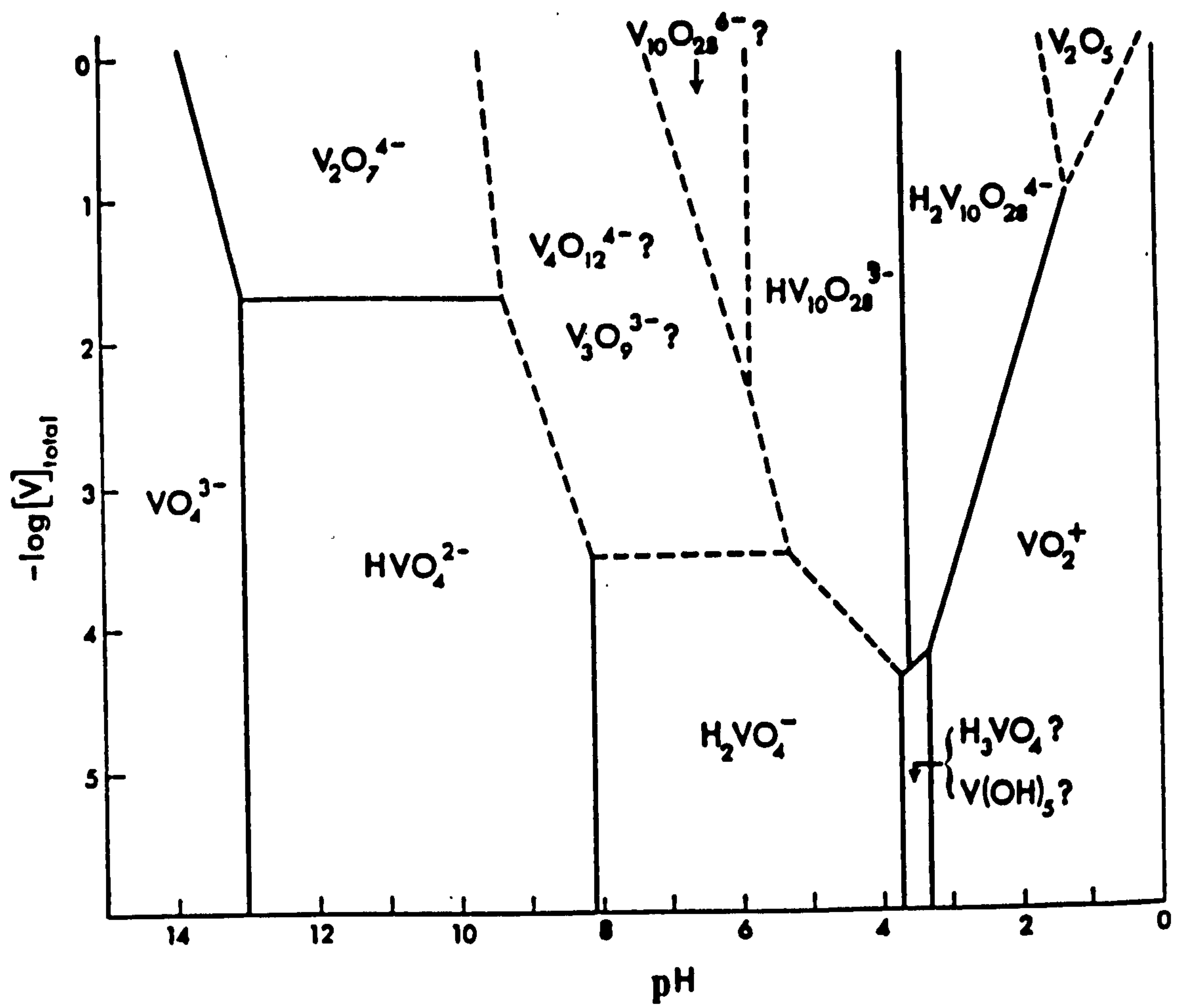


fig. 2.1

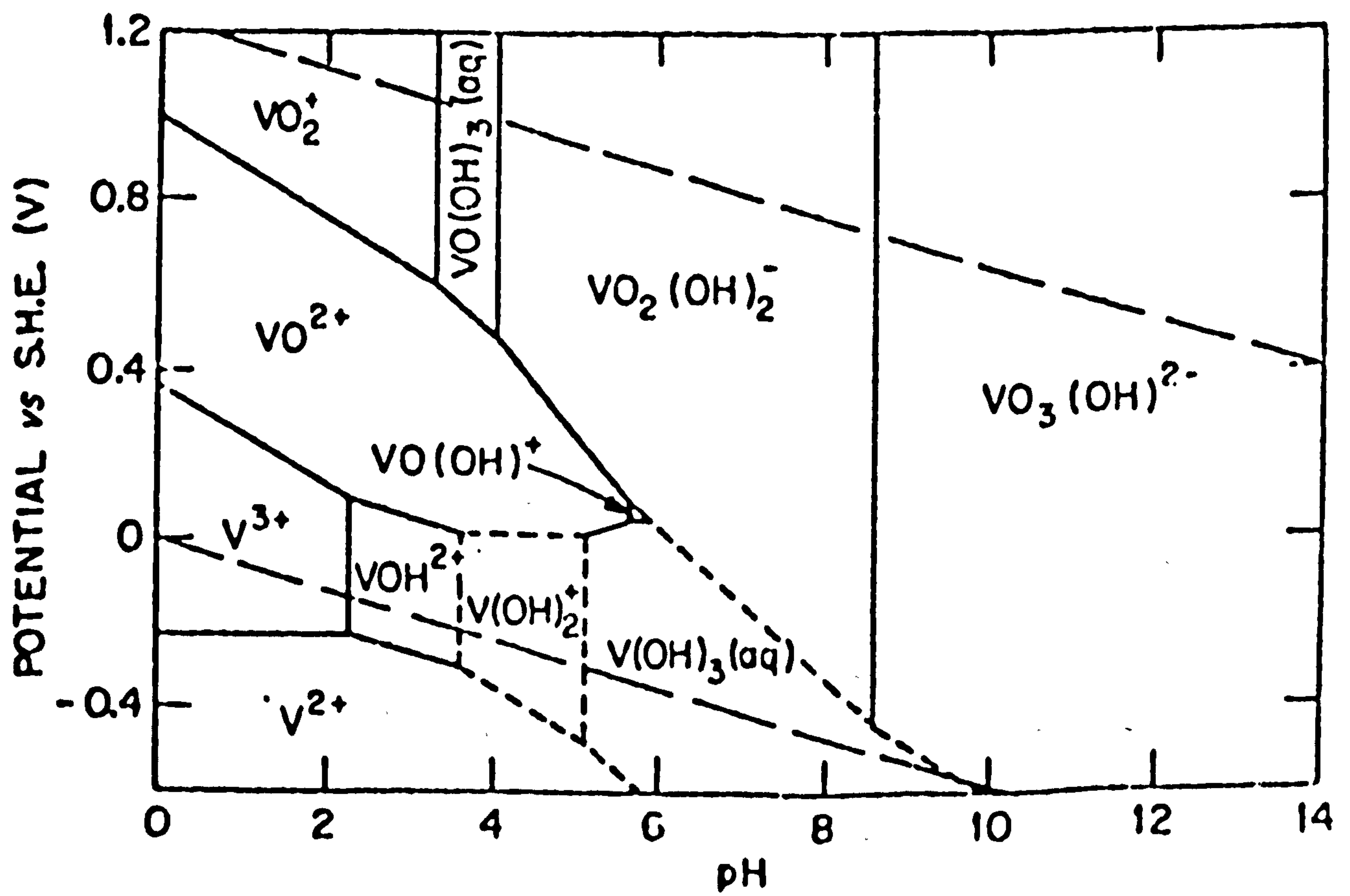
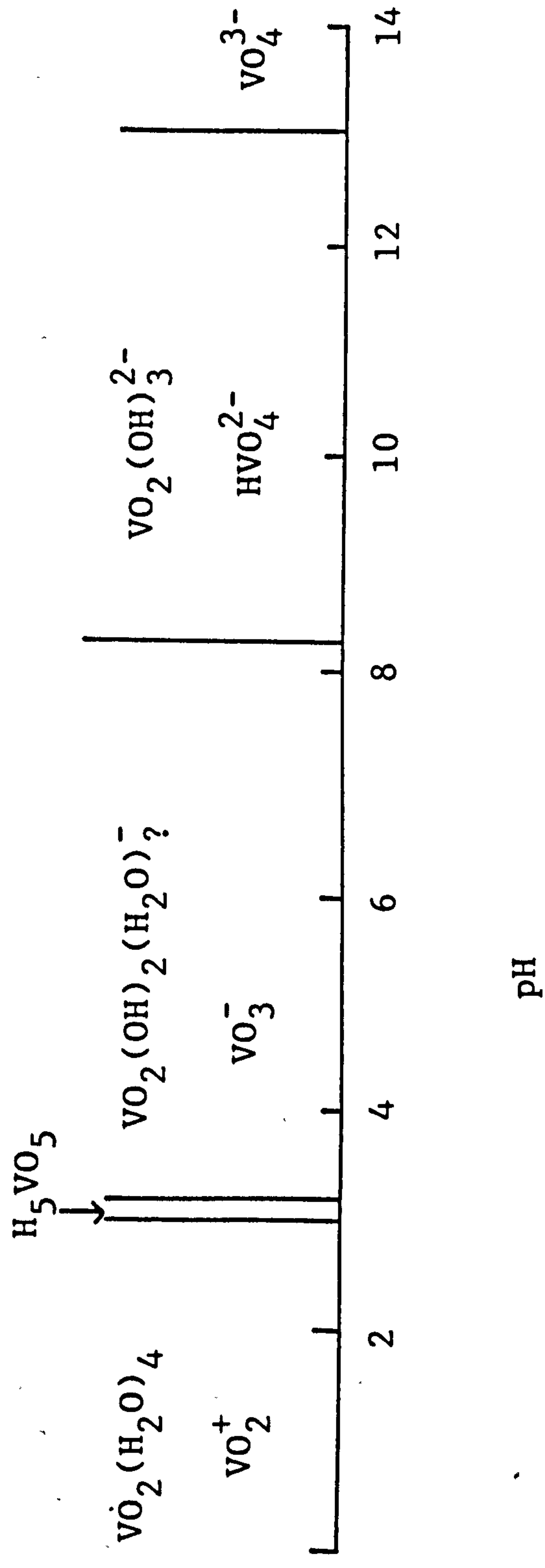


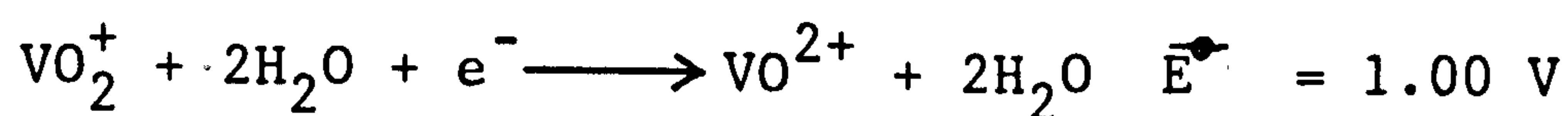
fig. 2.3

fig. 2.2



of action of catechols when employed in vanadium inhibition studies. Vanadate also preferentially forms complexes with compounds possessing cis glycol functions and EDTA, it can also form polynuclear species with phosphate (Macara 1980).

In alkaline, neutral or weakly acid solution vanadates are stable and dominate the solution chemistry of vanadium (see fig. 2.3). However, even at pH 7 vanadium can act as an oxidant. Reduction of V(+5) to V(+4) occurs in the presence of numerous compounds including L ascorbic acid, glutathione, norepinephrine, cysteine and possibly NADH (Chasteen 1983). At lower pHs the dominant species is the dioxovanadium ion. This ion is a powerful oxidant as the reduction potential indicates;



Clearly there is a potential for reduction of V(+5) to V(+4) although the extent of such reduction would be highly dependent upon the prevailing conditions.

2.3 Vanadium (+4).

V(+4) is the most important oxidation state for vanadium in aqueous solution; it is neither strongly oxidizing nor reducing, and acidified solutions are stable to air oxidation for long periods (Nicholls 1974). The hexa-aquo ion is far too acidic to exist in water; as a result the weakly acidic oxovanadium(+4) or vanadyl ion $(\text{VO}(\text{H}_2\text{O})_5)^{2+}$ is the dominant species. It is this ion (normally written as VO^{2+}) which gives solutions of V(+4) their blue colour.

The extent of polymerization in concentrated solutions of V(+4) is unclear but as with V(+5) it appears to exist as monomers at low concentrations i.e. 0.05M (Rossotti and Rossotti 1955; Robinson 1981). The effect of pH on V(+4) speciation has been studied by numerous workers (Francavilla and Chasteen 1975, Ianuzzi and Reiger 1975 and Selbin 1965). Francavilla and Chasteen (1975) explained their results in terms of the following equilibria;



This represents a pattern similar to that of V(+5) where a cationic monomer exists in strong acid and an anionic monomer ($\text{VO}(\text{OH})_3^-$ not shown in equilibria) exists in strong base. At intermediate pHs a dimer and precipitate are formed. Fig. 2.4 describes the distribution of these species in solution as affected by pH (this idealized distribution diagram is presumably under anaerobic conditions although the author does not state so).

Above pH 2-3 solutions of V(+4) undergo air oxidation (Fig. 2.2), hydrolysed species being particularly susceptible. When complexing ligands are present this oxidation is greatly retarded.

Unlike V(+5), V(+4) remains six coordinate throughout the entire pH range and this has allowed an easier examination of the kinetics of ligand exchange. In general terms exchange of ligated water and oxygen has been found to be extremely rapid and although exchange of other ligands has not been investigated thoroughly it is probably equally fast (Rubinson 1981).

V(+4) will readily combine with many different types of ligands to form complexes. In particular the ion has a high affinity for proteins, this combined with its EPR characters has made it an extremely useful tool for the investigation of the metal binding sites of various proteins. V(+4) will also form stable complexes with small ligands such as citrate, ATP, PPI, catecholamines and free amino acids. In view of this tendency to form complexes with common ligands and its air oxidation in non-acid conditions it is unlikely that the vanadyl ion will be found free in biological systems.

Complexing ligands can effectively retard the air oxidation of V(+4) although the extent of this depends upon the

fig. 2.4 Distribution of vanadyl(IV) species as a function of pH. Total vanadium concentration = 10 μ M. Dashed lines were not calculated but represent approximate behaviour in pH regions where reliable equilibrium data are not available. An unidentified species indicated by "?" is also present in the pH range 7-11. Taken from Robinson (1981).

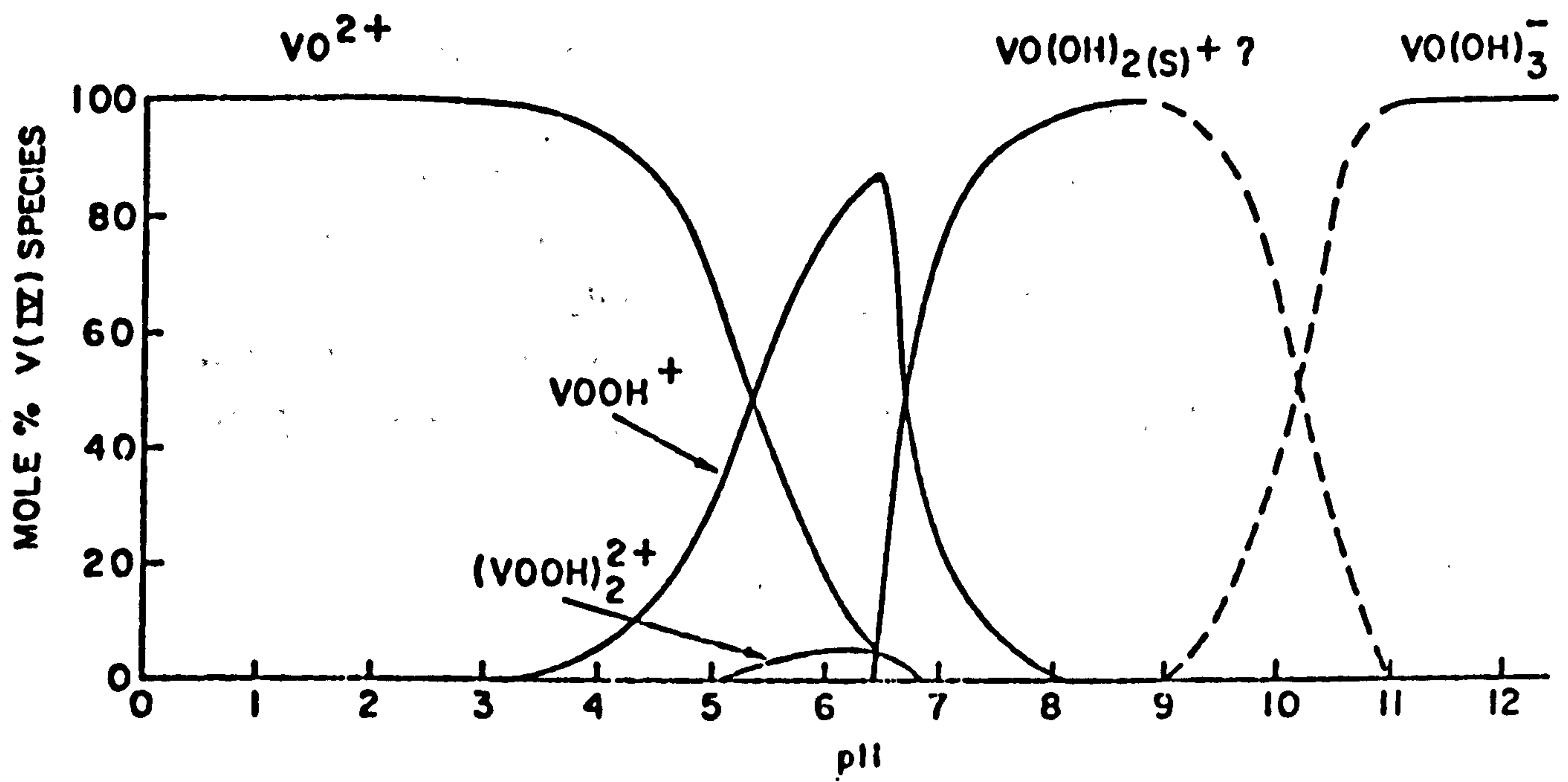


fig. 2.4

stabilities of the complexes of the oxidized and reduced species. For example if a V(+4) chelate is more stable than a V(+5) chelate and a free ligand is added to a buffered system, the system will attain greater stability if more V(+4) chelate forms. Thus a fraction of the V(+5) chelate and V(+5) free ions will tend to reduce to the V(+4) forms. Since in general V(+4) chelates do tend to be more stable than V(+5) chelates and there are often electron donors present in biological systems it is to be expected that vanadium will be present in biological systems as V(+4) chelates.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction.

The methods described below are common to many of the experiments reported in this thesis, and are largely based on the established and accepted techniques of other workers. Any modifications deemed necessary are detailed in the text, and the reasons for their introduction discussed.

All plants were grown, and experiments performed, in soil-less culture. This technique provided much greater control over the root environment compared with that available from soil based rooting media. The precedent for such an approach is well established and supported by the advances in our knowledge and understanding since the introduction of soil-less culture techniques.

3.2 Plant material.

Four species of plant were employed in this investigation; Barley (*Hordeum vulgare* c.v. Maris Mink), Maize (*Zea mays* c.v. Fronica), Tomato (*Lycopersicon esculentum* c.v. Moneymaker), and Bean (*Vicia faba* c.v. Aquadulce Claudia). These species were selected on the basis of several considerations;

1. All the species are known to be easy to grow and to produce an abundance of plant material in a relatively short period of time.
2. As common agricultural/horticultural crops their seed is both inexpensive and readily available; furthermore research findings may find some useful applications in these commercial industries.
3. Since all the species have been utilized by other workers there should be no difficulty in obtaining suitable comparable data.

3.3 Excised root studies.

The use of excised roots as an experimental tool in ion uptake studies dates back to the work of Hoagland and Broyer (1936). They offer numerous advantages over whole plants and have been adopted by many workers. Epstein and co-workers have used excised roots extensively in their experiments, and have

refined the original method proposed by Hoagland and Broyer into a technique requiring only short periods; minutes rather than hours, to demonstrate measurable uptake (Epstein et al 1963). The advantages offered by short periods of uptake can be described thus;

1. Uptake rates may vary over time for a number of reasons (Laties 1959); reducing uptake time minimizes the possible affects of this variation.
2. Short uptake periods offer less time for physiological changes to occur in the root.
3. The effect of proton extrusion and ion exchange liberation of H^+ on the pH of the uptake solution is reduced during short uptake periods.
4. Short uptake periods are less likely to lead to depletion of the ion in the uptake solution.
5. Loss of absorbed ion back to the bathing solution from the severed ends of the root is minimised by using short uptake periods.

The use of excised roots was adopted as a standard experimental technique. The following method was employed for the production of the root material; Barley seeds were soaked for two hours in aerated distilled water and then spread on a pre-soaked mesh of stockinette, stretched over a supporting frame of plastic coated wire and the mesh placed in contact with the surface of an aerated 0.5mM $CaCl_2$ solution in a 10 litre plastic bowl. Aeration was provided as a source of oxygen and stirring. The air supply was pumped through a KOH trap to remove CO_2 , then through a glass wool filter to remove particulate matter and finally bubbled into the culture solution through an aquarists air-stone.

The 0.5mM $CaCl_2$ culture solution used was found suitable for the production of healthy seedlings in a short period of time (6-10 days from sowing). Calcium was included in the media because of the evidence suggesting that calcium is required if selective cation transport is to continue unimpaired (Epstein 1961; Jacobson et al 1961). Seedlings were grown in the dark at a constant $23^{\circ}C$ by

placing them in an incubator. Evaporative losses of the media were minimised by covering the culture container with a second bowl. These growing conditions are similar to those used by many workers (Coombes 1977, Soldal and Nissen 1978, Harrison et al 1978, Vange et al 1974, Cutler and Rains 1974 and Logan 1985).

The normal procedure followed during an uptake experiment was as follows. Roots were excised from seven day old dark grown barley seedlings, then washed and thoroughly mixed in 0.5mM CaCl_2 . Sub samples of approximately 1.0g were removed and placed in uptake containers; these were then immersed in aerated 0.5mM CaCl_2 solution at ambient temperature until required. The time between excision and use was always as brief as possible, never exceeding 60 mins.

Roots were next placed in uptake solutions containing the appropriate concentration of the ion under study and 0.5mM CaCl_2 . This solution was always aerated, stirred and had a volume of 5 litres. Use of such a large volume ensured no concentration changes occurred during the period of uptake.

In experiments where time was the variable, 3 replicate samples were removed at each desired time. Control of pH was obtained by use of an Ammonium Acetate/Acetic acid buffering system. This allowed solutions being used for uptake experiments to be produced three days before required at pH's which remained constant. When temperature was a variable, this was controlled by placing the uptake container in a water bath at the required temperature. When metabolic inhibitors or complexing agents were being examined they were added to the uptake solution one hour prior to use.

All the uptake experiments were terminated in similar manner. Roots were washed in two changes of double distilled water and according to the particular experiment were placed in a desorption solution to remove exchangeable ion accumulations. This desorption solution was always cooled to less than 5°C to reduce uptake of ions liberated from the free space by exchange processes into the symplast.

3.4 Whole plant studies.

Whole plant studies were conducted on three species (Barley, Maize and Tomato). The procedures followed were similar for all three species. Seeds were soaked in distilled water for two hours and then spread onto moistened paper towel which was covered with a second sheet of moistened towel. Seeds were allowed to germinate and grow for two days, then uniform seedlings were selected for use in experiments. Plants were grown for varying periods in perlite. Perlite is a siliceous mineral of volcanic origin which, when crushed and heated, expands to form a light powdery substance of many times the original volume. Perlite is extensively used in horticulture as a plant growth medium. Lightness, uniformity, low background contamination and ease of root extraction contribute to its usefulness as an experimental growth medium.

A nutrient solution based on that of Hoagland and Arnon, as given in Epstein (1972 p.39) was used to meet the mineral requirements of the plants which were grown for longer periods than those used in excised root studies. Normally, 25ml aliquots of solution were supplied on a daily basis. Care was always taken to ensure that the supplied solution went directly on to the perlite and not on to the foliage. Vanadium was added to the nutrient solution on the day that the solution was supplied to the plant. The pH of the solution was generally unaltered by addition of these small amounts of ion. Plants were grown in randomly assigned blocks under green-house conditions providing a constant day length (16 hour light/day, 3000 lux).

After the required growth period had elapsed, plants were harvested by splitting into shoots and roots. Shoots were oven dried without further treatment. Roots had any adherent perlite washed off with tap water, then material was briefly washed in distilled water prior to being oven dried.

3.5 Analysis of plant material.

Numerous methods of analysing the elemental content of plant material exist; these include colorimetric, catalytic,

Neutron activation, polarographic and various spectrographic techniques, all of which can be usefully applied to the analysis of vanadium in plant materials. Several reviews have provided useful comprehensive lists of the techniques available for the determination of vanadium in biological samples (van Zinderen Bakker and Jaworski 1980; NAS 1974). Some of the techniques along with their reported detection limits are listed in table 3.1.

It was considered desirable that from the outset one technique should be selected and used throughout the study. In order to make this selection certain criteria were established, prior to any experimental testing of methods. Loosely, these criteria required that the technique should;

- (a) be sufficiently sensitive to detect vanadium in plant tissue at levels likely to be encountered in this work.
- (b) involve a minimum of sample pretreatment prior to analysis.
- (c) not suffer from interference problems.
- (d) allow analysis of large numbers of samples in short periods of time.
- (e) be relatively inexpensive.

Given these criteria, three methods appeared more attractive than others, these were atomic absorption spectrophotometry (AAS), neutron activation analysis (NAA) and the colorimetric technique of Budevsky and Johnova (1965). After a short series of tests involving these three methods, it was decided to use AAS, chiefly in the flame mode for all the subsequent determinations of vanadium. This was the method most suitable for the present work although the value of the other methods in different circumstances is recognised.

Solubilization of the plant material was achieved by a wet pressure digestion based on the method proposed by Williams (1978). The basic procedure was as follows; washed plant material which had been oven dried to a constant weight at 70°C was carefully weighed into disposable polystyrene digestion vessels. For excised

Table 3.1 Analytical techniques available for the determination of vanadium in biological samples.

<u>TECHNIQUE</u>	<u>REPORTED DETECTION LIMIT</u>	<u>REFERENCE</u>
<u>COLORIMETRIC</u>		
8- Quinolinol	1 µg *	Talvitie (1953)
4-(2-pyridylazo)-resorcinol	0.04 µg ml ⁻¹	Budevsky & Johnova (1965)
<u>CATALYTIC</u>		
Gallic acid & acid	5ng *	Welch & Allaway (1972)
Gallic acid & bromate	18 ng ml ⁻¹	Mottola (1975)
Gallic acid & bromate	100 ng ml ⁻¹	Fukusawa & Yamone (1979)
<u>PLASMA EMISSION SPECTROSCOPY</u>		
	0.015 µg ml ⁻¹	Greenfield et al (1975)
<u>NEUTRON ACTIVATION ANALYSIS</u>		
	0.01 µg ml ⁻¹	Salbu et al (1975)
<u>ATOMIC ABSORPTION SPECTROSCOPY</u>		
Flameless	0.0014 µg ml ⁻¹	van Zinderen Bakker & Jaworski (1980)
Flame	0.01 µg ml ⁻¹	NAS (1974)

* these figures represent absolute detection limits.

Table 3.1

root material, the weight was generally around 0.05-0.1g and to this one ml of concentrated (71%) nitric acid (Analar) was added. The caps on polystyrene vessels were then tightened and allowed to stand at room temperature for a minimum of four hours. After this, vessels were gently shaken then placed in a water bath at 80°C for four hours. Subsequent to this the pressure was released from the vessels and heating continued for two hours to allow expulsion of volatile acid fumes.

Root samples treated in this fashion required no filtration and were made up to a final measured volume with distilled water prior to analysis. For larger samples, or material not so readily digestible, proportionately larger volumes of acid were required. There was however an upper limit to the amount of material which could be digested in this manner. In this study there was never more than 1.0g of plant material with 5 mls of nitric acid. In some cases it was necessary to filter digested samples prior to analysis. Digests were diluted with a small quantity of distilled water and filtered through Whatman 541 filter papers. Filter papers were washed through with 5% nitric acid and the solutions made up to volume with distilled water.

All analysis for vanadium were performed using an Instrumentation Laboratory atomic absorption spectrophotometer Model 151. Instrumental parameters were those suggested by the manufacturers.

Analysis for other elements was performed using standard AAS conditions. Lanthanum was included in the determination of calcium to reduce chemical interferences. Standards for all analysis were prepared from commercially purchased stock solutions. All standards were matched with samples for acidity.

Errors in analysis have numerous possible sources. In all analyses attempts are made to reduce these errors to a minimum. One of the advantages of the analytical procedure employed was that it required minimal sample preparation.

However, as in all digestion procedures, losses may occur. In an attempt to estimate the extent of possible losses a recovery study was performed over a wide range of vanadium concentrations. Known volumes of vanadium standard solution were added to dry plant material prior to digestion. After digestion, the samples were made up to volume such that their concentration was in the range 0.5 - 5.0 $\mu\text{g ml}^{-1}$. The amount detected in these samples ranged from 92 - 100% of that added. Under the present experimental conditions this level of recovery was considered satisfactory.

With regard to the actual analysis, two potential problem areas were recognized, namely matrix effects and interference. It is thought that any problems with matrix effects would be overcome by the complete destruction of the matrix during the acid digestion. Interferences can prove problematical in some instances, as pointed out by Quickert et al (1974). Particularly problems may arise when large amounts of aluminium are present in the digest. However in all the present work concentrations of interfering metals such as aluminium were low and it seems unlikely that this element was interfering with vanadium analysis. Clearly however this possible interference must be acknowledged when determining vanadium in soils.

3.6 Data Handling.

All statistical analyses were performed on the DEC-20 mainframe computer at Liverpool Polytechnic. The three statistical packages used in this study were 'EASYSTATS', 'STATPACK' and 'GENSTAT' however the bulk of the data analysis was done using 'STATPACK'.

CHAPTER FOUR

EXCISED ROOT STUDIES

Excised root studies.

4.1 Time course and desorption studies.

To enable a characterization of the processes involved in the uptake of vanadium by barley roots to be achieved a series of experiments were performed to test for the classical features which indicate active absorption. The results of a series of time course experiments, which were conducted using varying concentrations of the vanadate anion, are given in fig. 4.1a. For each concentration used, a curvilinear response can be seen. Initially there was a period of rapid ion absorption, the magnitude of which was dependent upon the concentration of the ion supplied. This primary phase of uptake had a duration of 30-40 minutes after which a second slower phase of uptake occurred. This second phase of uptake continued for the duration of the experiment and exhibited a constant rate over the period examined.

The results of a similar series of experiments using the vanadyl cation are shown in fig. 4.1b. Once again a curvilinear response could be seen for each concentration used. The duration of the initial phase of rapid uptake was similar to that of the vanadate anion (30-40 minutes) and its magnitude was also dependent upon the concentration of the ion supplied. Similarly the second phase of uptake, which occurred at a reduced rate, remained constant for the duration of the experiment. Uptake of the vanadyl cation appeared to proceed at a greater rate than uptake of the vanadate anion, certainly in the initial phase; (Table 4.1).

Since the earliest extensive studies dealing with the course of salt accumulation in plant tissues, there has been a recognition of the fact that a relatively brief period of rapid uptake is normally followed by a slower but more prolonged period of uptake. Studies by many workers have produced results conforming to this model (Hylmo 1953, Epstein and Leggett 1954, Clarkson 1967, Colclasure and Schmid 1974, Veltrup 1978 and Holst et al 1980). Explanation of such results relies on an examination of the structure of the tissue in which they are produced.

fig. 4.1a Root vanadium level v time.

- (a) 590 μ M
- (b) 390 μ M
- (c) 196 μ M
- (d) 98 μ M

vanadium as NH_4VO_3

fig. 4.1a

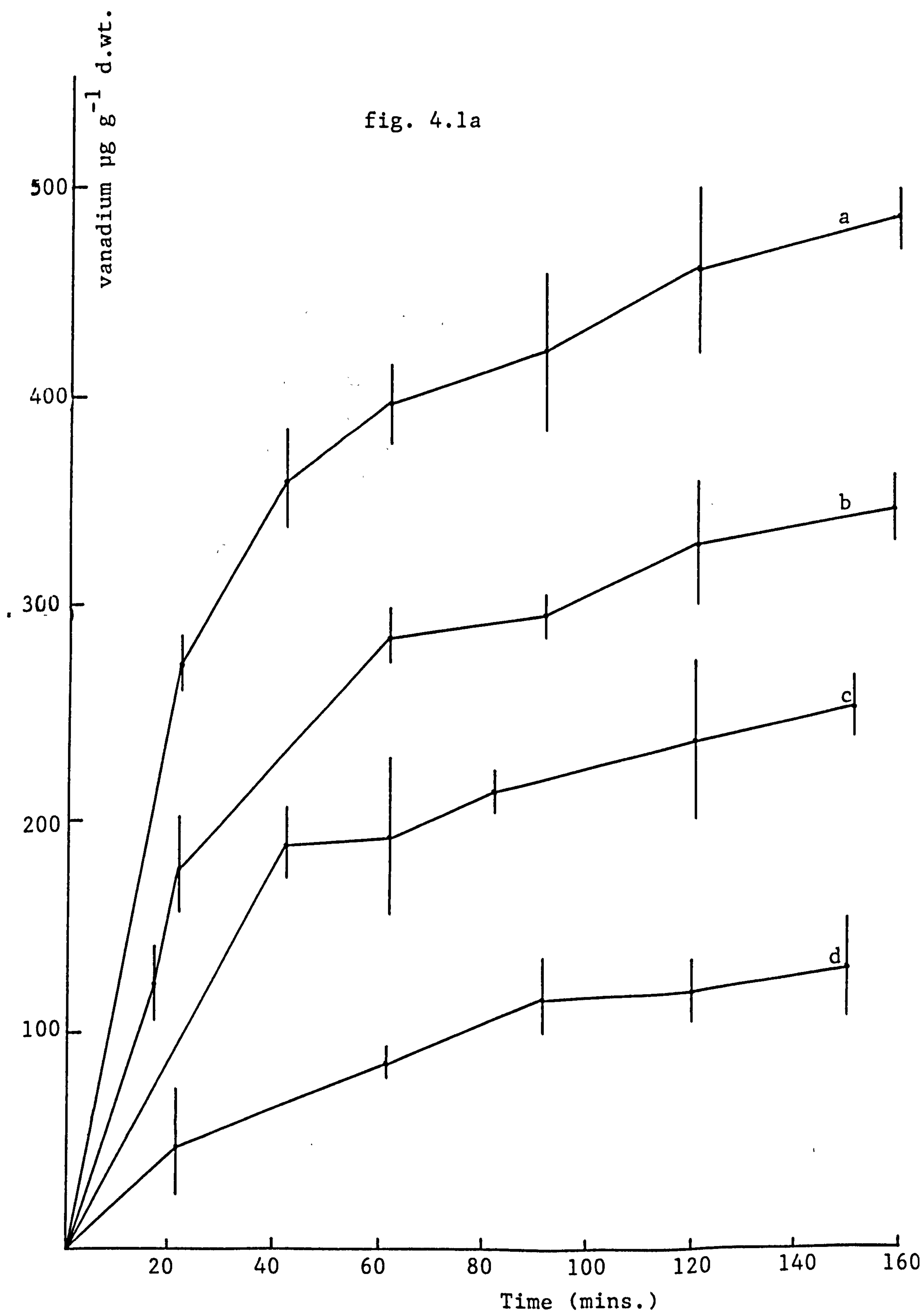


fig. 4.1b Root vanadium level v time.

- (a) 590 μ M
- (b) 390 μ M
- (c) 196 μ M
- (d) 98 μ M

vanadium as VOSO_4

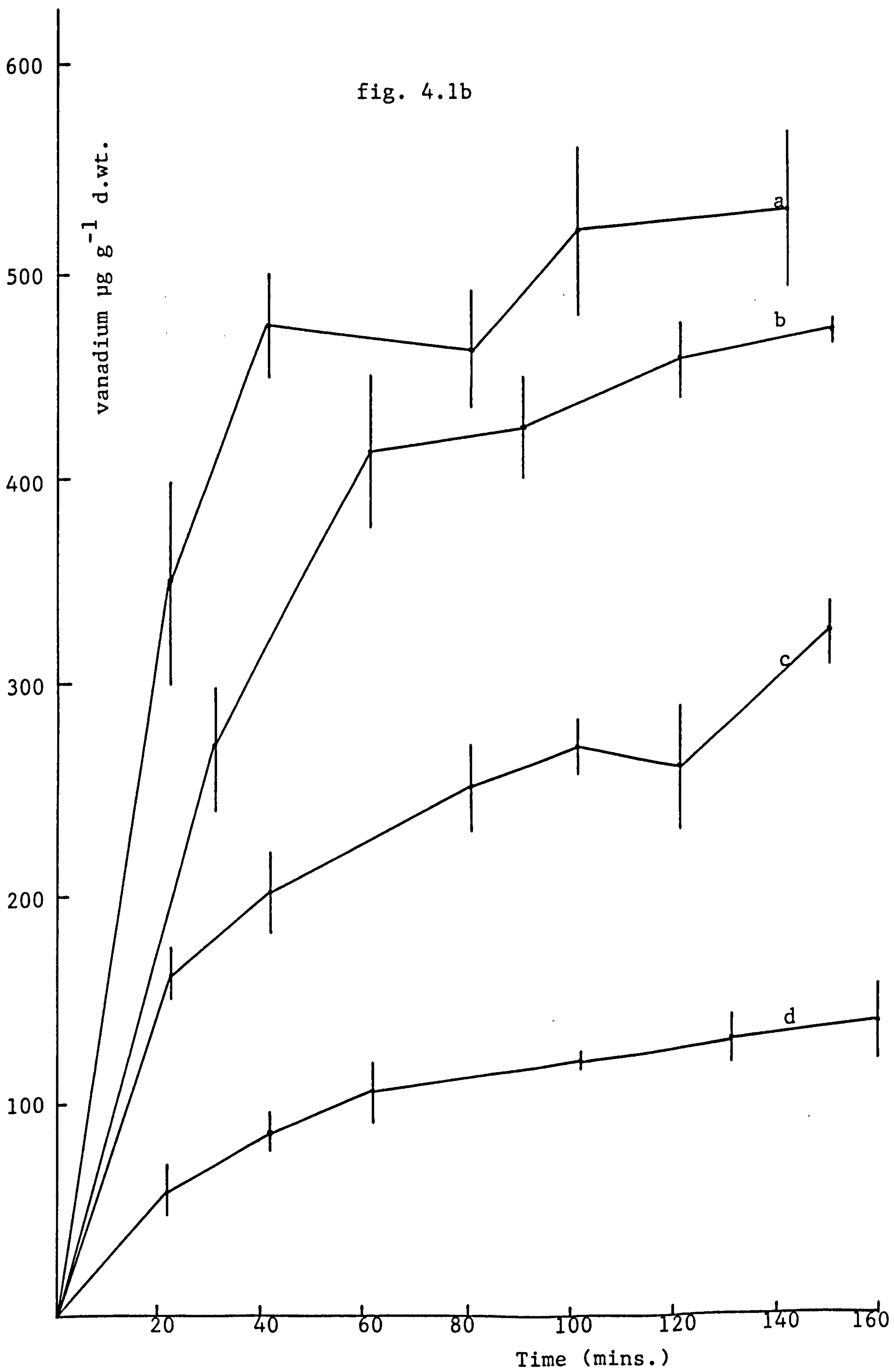


Table 4.1 Vanadium tissue levels after 20 mins.
uptake as affected by form and concentration.

Table 4.1

supplied ion	98μM		196μM		390μM		590μM	
vanadium form	VO ²⁺	VO ₃ ⁻	VO ²⁺	VO ₃ ⁻	VO ²⁺	VO ₃ ⁻	VO ²⁺	VO ₃ ⁻
tissue level μg/g d.wt.	60.3	55.1	161	121	205	176	349	276

Multicellular systems, such as excised roots, where a heterogeneous cell population is to be found invariably produce problems with interpretation of results. Even when a relatively uniform cell population is present, the extra cellular space can give rise to a variety of uptake phenomena. Therefore explanations of uptake patterns must consider not only the variety of cells present, but also the nature and size of the spaces between these cells. In plant tissues, such as excised roots, this space is largely composed of intercellular spaces and the plant cell wall which is readily accessible to solutes. Briggs (1957) brought into general usage the terms free space, apparent free space (AFS), water free space (WFS) and Donnan free space (DFS) in an attempt to characterize those distinct components of a tissue which are in free diffusive connection with the external solution. Any estimate of the free space of a tissue is termed the apparent free space. Values for the AFS of different tissues vary not only with the type of tissue but also with the nature of the solute, since different solutes will permeate tissues to different degrees. This is particularly the case when the solute under consideration is a charged ion. Monovalent cations, for example, would not only permeate the whole of the free space outside the membranes, they would also exchange with some of the cations, predominantly calcium, neutralizing the fixed negative charges of the uronic acids in the cell wall. Even within the divalent cations AFS can vary because of differing affinities for electrostatic binding sites. Thus divalent cations with a high affinity for binding sites will displace divalent cations with a lower affinity for the same binding sites. In general, the affinities of metal ions for binding sites have been shown to follow the same order as the stability series proposed for various metal ions by numerous workers notably Irving and Williams (1953).

The AFS can be considered as comprising of two parts; the WFS which is composed of the major part of the water outside the cell membranes and the DFS which is composed of that portion of the tissue which is under the influence of the fixed electrical charges in the cell wall. The DFS of all tissues tends to be dominated by cations and

Donnan equilibrium conditions are thought to apply in an approximate manner. It is worth recognizing however, that although the DFS is dominated by cations and considered largely in terms of cation exchange, other processes do occur within this root space. DFS has a small but measurable anion exchange component which is due to free amino groups of proteins which are important as cell wall matrix substances (Lauchli 1976).

Description of observed uptake patterns can now be attempted with regard to the known composition of the tissue under study. The primary phase of rapid uptake can be regarded as uptake into the free space of the root tissue, i.e. WFS and DFS is equally available to both cations and anions but DFS has only limited availability to anions, it is primarily a volume open to cations. This primary phase of uptake ceases when the WFS is composed of a solution identical in composition to the bathing solution and the DFS is in approximate equilibrium with this solution. The second phase of uptake can now be regarded as uptake across the plasmalemma and into the cytoplasm of the cell. The maintenance of a fixed rate of uptake over relatively long periods of time suggests that this is a process controlled to some degree by the cell, although the type or extent of control is unknown.

In order to resolve these two phases more lucidly, selective desorption has been employed. Desorption is a widely used technique which is particularly useful when studying uptake in systems with an extracellular component. When the ion under study is a radioisotope (or a radioisotope analogue has been used to substitute for the ion of interest), desorption can be simply achieved by the use of a 'cold' solution of the appropriate ion. However when radioisotopes are not available or not suitable for use, some other form of desorption has to be employed. The best alternative is to use a similarly charged ion which has a greater affinity (stability constant) for the root exchange sites. To facilitate this displacement it is usual to employ large excesses of the displacing ion which produces a more rapid

and complete exchange of ions. This type of technique was employed by several workers including Harrison et al (1979) who used lead to displace copper in excised barley roots and Brown and Beckett (1984) who used nickel to displace cadmium in lichen thalli.

Often when anions are being studied a simple water desorption is considered sufficient to remove all the extracellular ion (Epstein 1955 and Nissen 1974). Epstein (1955) when examining sulphate uptake found no difference between a water desorption and a desorption using a non radioactive solution of the sulphate ion. Presumably this is the result of a very small and labile exchange component. However it is not always the case that a simple water desorption is sufficient to remove all the extracellular anion present in a tissue. Bowen and Nissen (1976) demonstrated that extracellular borate could not be efficiently removed from a tissue by a simple water desorption procedure. To remove extracellularly bound borate it was necessary to employ a 30 minute post uptake desorption in 0.5mM calcium sulphate.

In the light of this knowledge a series of experiments were designed to examine how easily vanadium was removed from the free space of excised barley roots. The uptake period normally consisted of 150 minutes in a 0.4mM vanadium solution which also contained 0.5mM CaCl_2 . After uptake, the roots, in their containers, were removed from the uptake solution, spun dry and placed in a cold ($< 5^\circ\text{C}$) solution of the appropriate desorbing agent.

The most suitable desorbing agents are generally considered to be ions of similar size (ionic radius), geometry and charge to the ion under study. As a result when attempting to remove extracellularly bound vanadate anion, oxyanions such as chromate, phosphate and nitrate were tested. The results of a desorption test which employed these ions and water and acetate are given in fig. 4.2a. Nitrate and water were about equally effective in removing the vanadate anion from the tissue, the vanadium lost through these treatments is that which occupies the WFS. Chromate was the most successful desorbing agent, consistently removing

more vanadium than any other treatment. To test whether an increase in the strength of the desorbing agent would produce an increase in the amount of vanadium exchanged, a similar desorption experiment was performed using the desorbing agents at 50mM (fig. 4.2b). Once again the same pattern of effectiveness was observed with chromate being the most successful desorbing agent. The proportion of vanadium removed from the tissues was not altered substantially by the five fold increase in desorbing agent concentration (63% of the vanadium was removed with 10mM chromate; 55% removed with 50mM). This suggests 10mM is sufficient to produce complete exchange with all the exchange sites. Further studies tested the effectiveness of sulphate as a desorbing agent and also the effect of chromate concentrations less than 10mM. The results of these experiments (fig.4.3a) show sulphate to be of little value as a desorbing agent; this possibly relates to it having a small very labile exchange component as suggested above. Reduction of the chromate desorption concentration to 5mM leads to a less effective removal of extracellular vanadium. Finally, the progressive removal of vanadate anion from root tissue was examined with respect to time. All exchangeable vanadium was removed within 30 minutes (fig. 4.3b) and as a result a 30 minute post uptake desorption treatment was included in all subsequent experiments.

A similar series of desorption experiments were performed to determine a suitable desorbing agent for the vanadyl cation (VO^{2+}). The same logic that applied in the vanadate ion desorption experiments was used; initial trials concentrated on similarly charged oxyions. The uranyl cation (UO_2^{2+}) was tested first and fig. 4.4a presents the results of this experiment. Clearly this cation is unable to exchange with the vanadyl cation in the root. Subsequent to this it was decided to utilize other divalent cations, not necessarily oxyions, with known high affinities for root exchange sites i.e. high stability constants with organic ligands. Calcium and copper both proved of little value in removing vanadyl cation from the root (fig. 4.4b). It is doubtful that these ions were removing more than that vanadium occupying the WFS. Further experiments utilizing

fig. 4.2a Desorption of vanadium supplied as NH_4VO_3 by various agents at a concentration of 10mM.

- (a) no treatment
- (b) nitrate
- (c) water
- (d) acetate
- (e) phosphate
- (f) chromate

fig. 4.2b Desorption of vanadium supplied as NH_4VO_3 by various agents at a concentration of 50mM.

- (a) no treatment
- (b) nitrate
- (c) acetate
- (d) phosphate
- (e) chromate

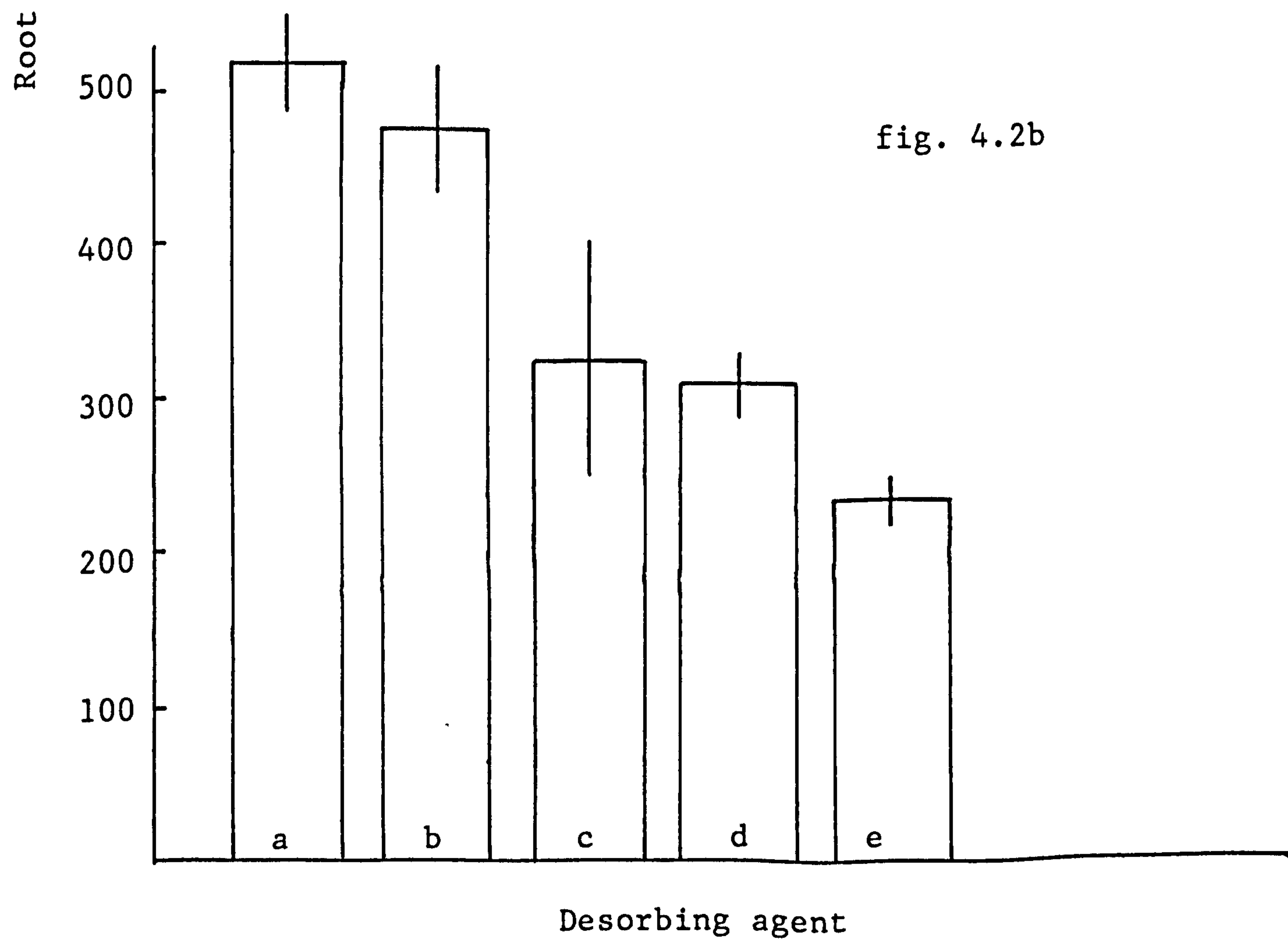
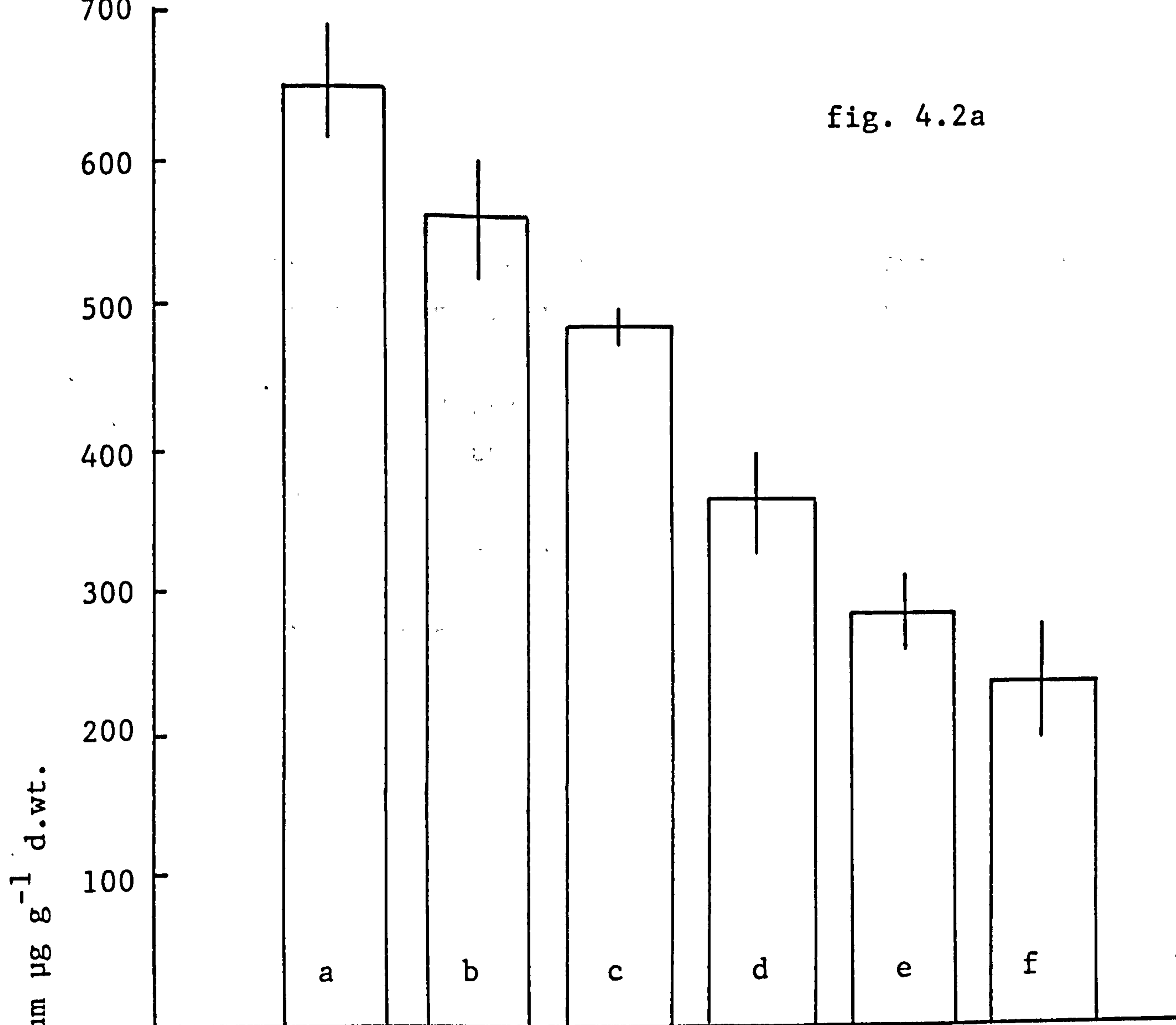


fig. 4.3a Desorption of vanadium supplied as NH_4VO_3 by various agents at various concentrations.

- (a) no treatment
- (b) chromate 10mM
- (c) chromate 5mM
- (d) sulphate 50mM
- (e) sulphate 10mM
- (f) sulphate 5mM

fig.4.3b Time course for vanadium desorption, supplied as NH_4VO_3 , by a 10mM chromate solution.

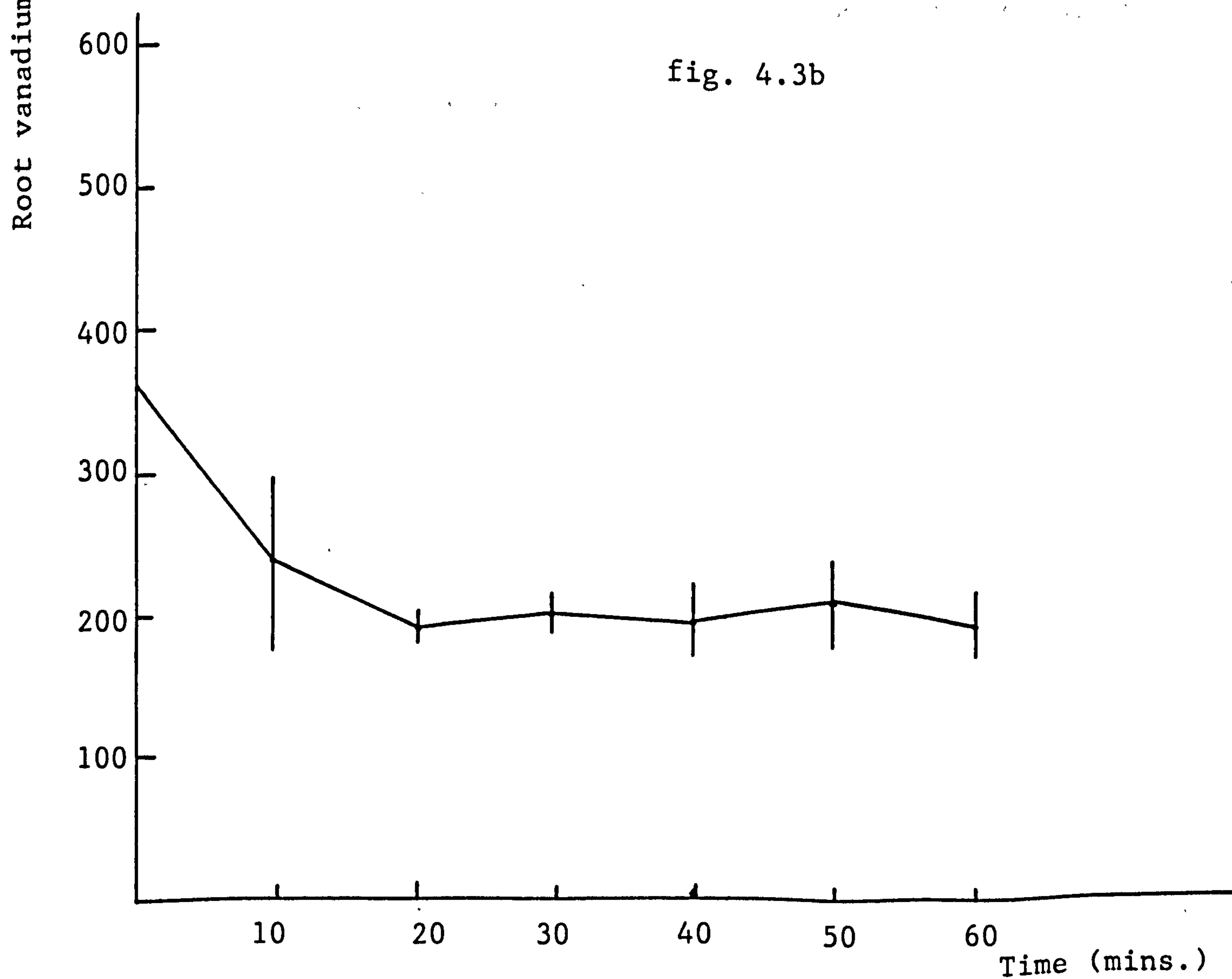
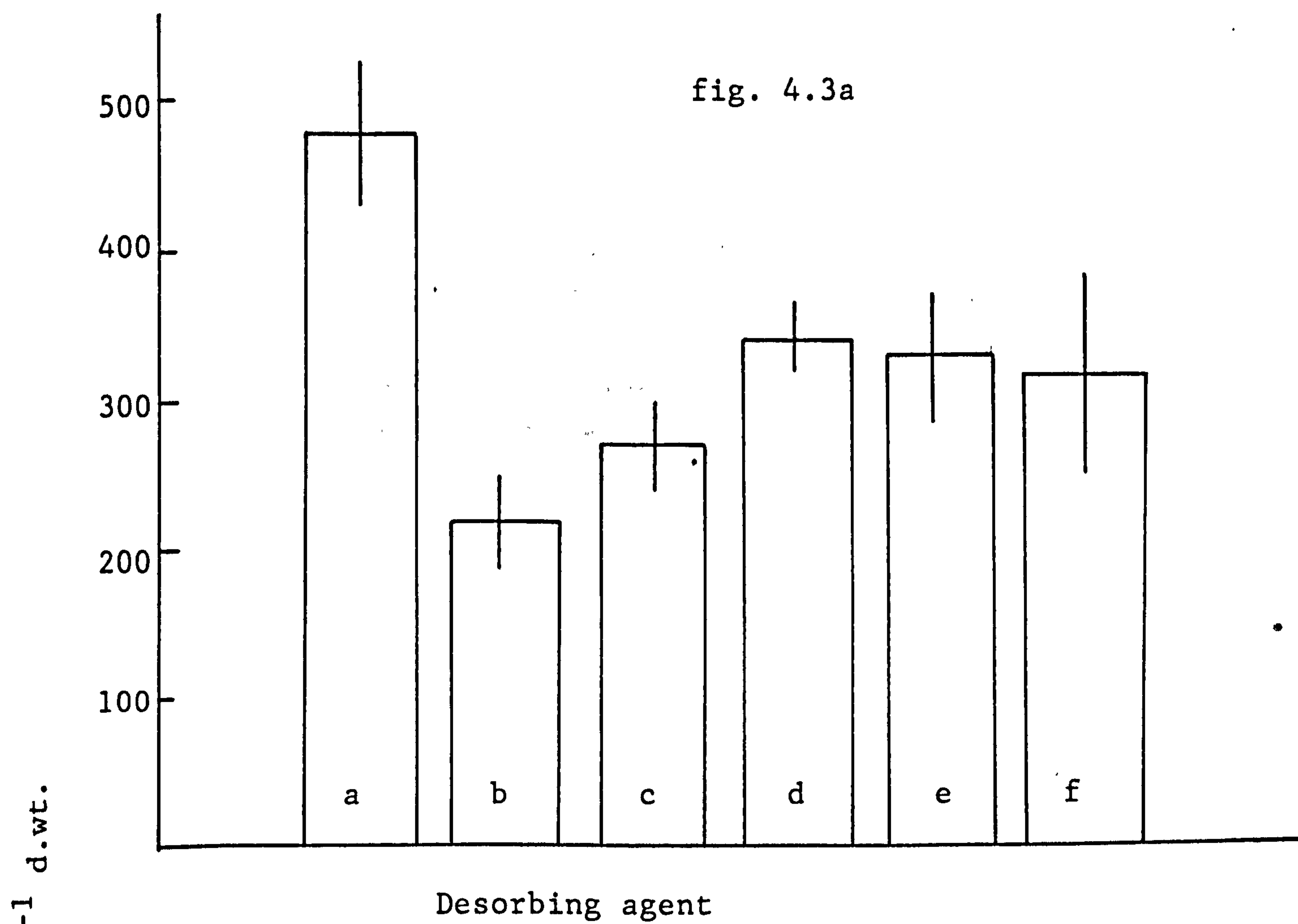
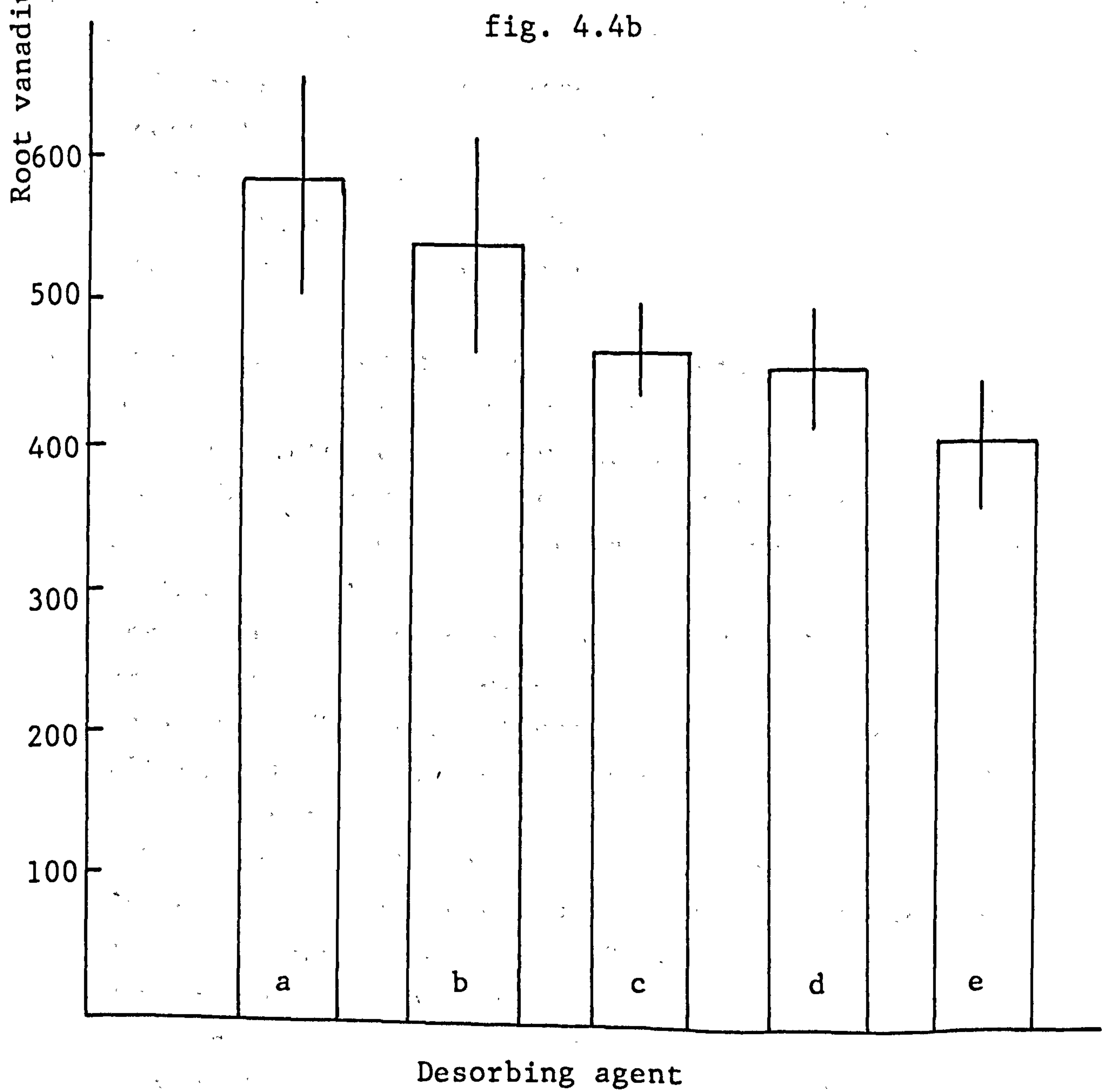
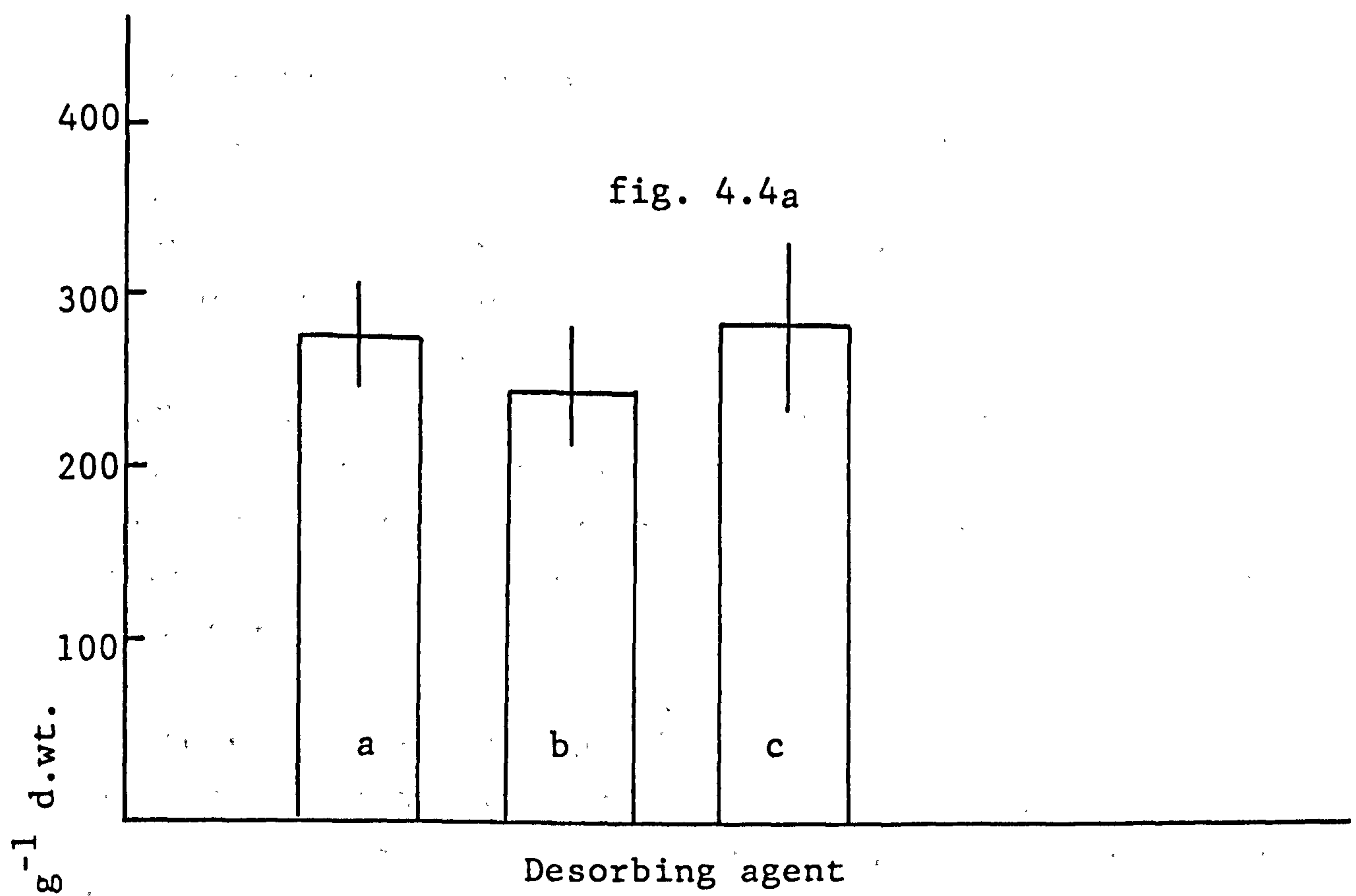


fig.4.4a Desorption of vanadium, supplied as VOSO_4 , using the uranyl cation at various concentrations.

- (a) no treatment
- (b) 0.5mM uranyl
- (c) 5.0mM uranyl

fig. 4.4b Desorption of vanadium, supplied as VOSO_4 , using various cations at a concentration of 5mM.

- (a) no treatment
- (b) uranyl
- (c) copper
- (d) calcium
- (e) water



a much expanded grouping of desorbing agents including anions and complexing agents gave rise to the results in fig. 4.5. Once again divalent cations seem unable to remove any more than the vanadyl cation in the WFS. Acetate, EDTA and citrate remove a larger proportion of the vanadium but surprisingly most vanadium is removed with the chromate anion (Potassium was the cation in the solution). This result was consistent throughout a number of desorption experiments even when other desorbing agents such as salicylate were employed (fig. 4.6a) and when the counter ion was altered.

The consistent success of the chromate ion in removing extra-cellular vanadyl cation is both surprising and difficult to explain in terms of recognized DFS phenomena. Conventionally, extra-cellularly bound ions are considered to be held purely by electrostatic forces thus the idea of a cation being displaced by an anion is difficult to accept. This difficulty in interpretation leads one to look for other possible causes of the exchange. One possibility is that the high concentration of the chromate ion used is causing disruption of the membrane and the exchange seen is nothing but leakage from the cell. Three lines of evidence can be used to refute this proposition;

- a) If membrane damage is occurring more exchange sites, both positive and negative, would become available, thus any inter-cellular vanadium released would be bound by these sites. Membrane disruption should not automatically be considered as leading to a decrease in total tissue content of an ion.
- b) Tests for membrane damage performed on this tissue proved negative. No evidence of potassium leakage or leakage of UV absorbing nucleotides could be found although microscopic examination of the tissue was not attempted.
- c) Other workers using high concentrations of metal ions for desorbing purposes found no evidence of membrane damage. Harrison et al (1978) used 5mM lead to displace copper and Brown and Buck (1979) used 20mM nickel to displace various extra-cellular ions; both found no evidence of membrane damage. Vange et al (1974)

fig. 4.5 Desorption of vanadium, supplied as VOSO_4 , by various agents at a concentration of 10mM.

- (a) no treatment
- (b) copper
- (c) magnesium
- (d) calcium
- (e) acetate
- (f) EDTA
- (g) citrate
- (h) chromate

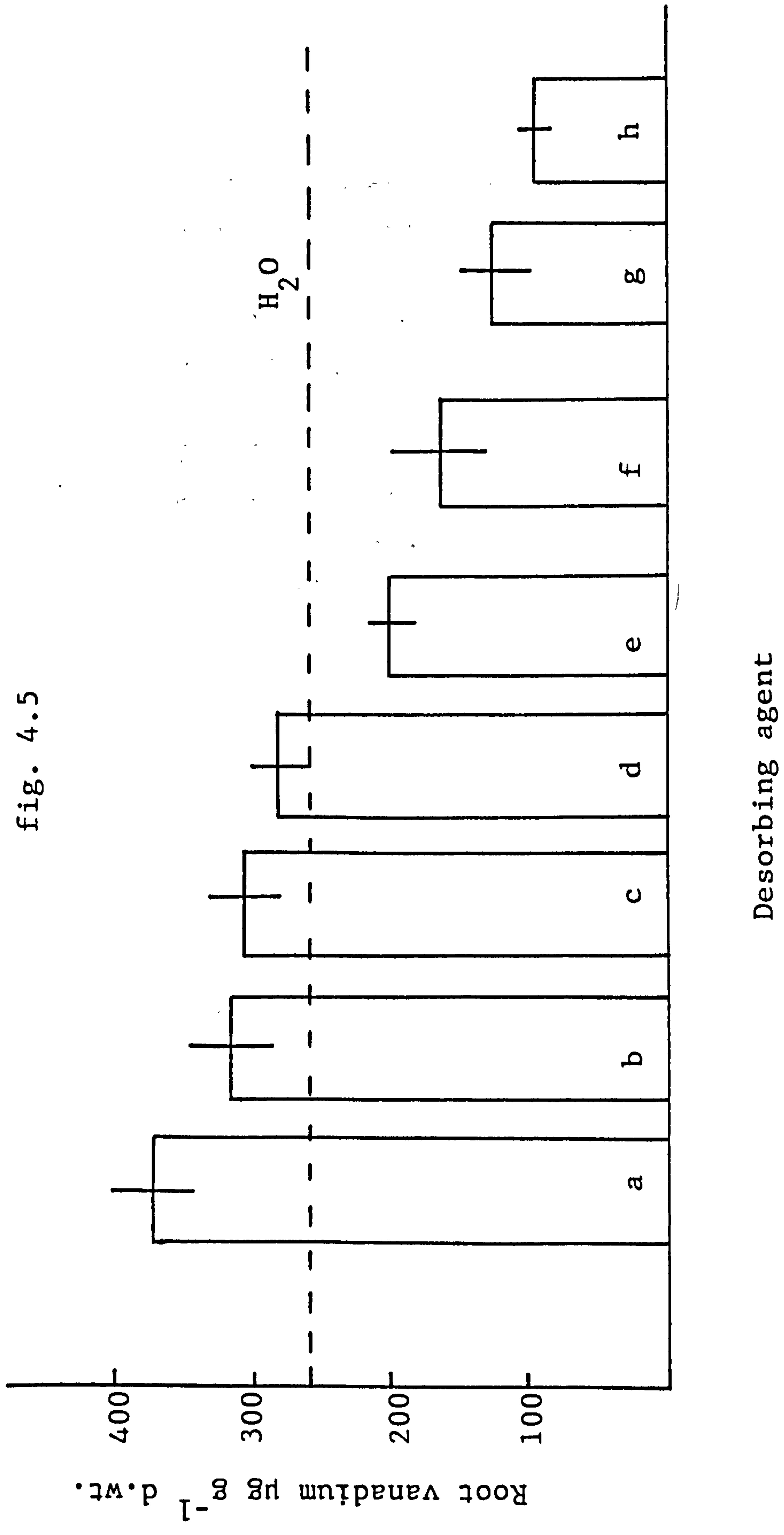
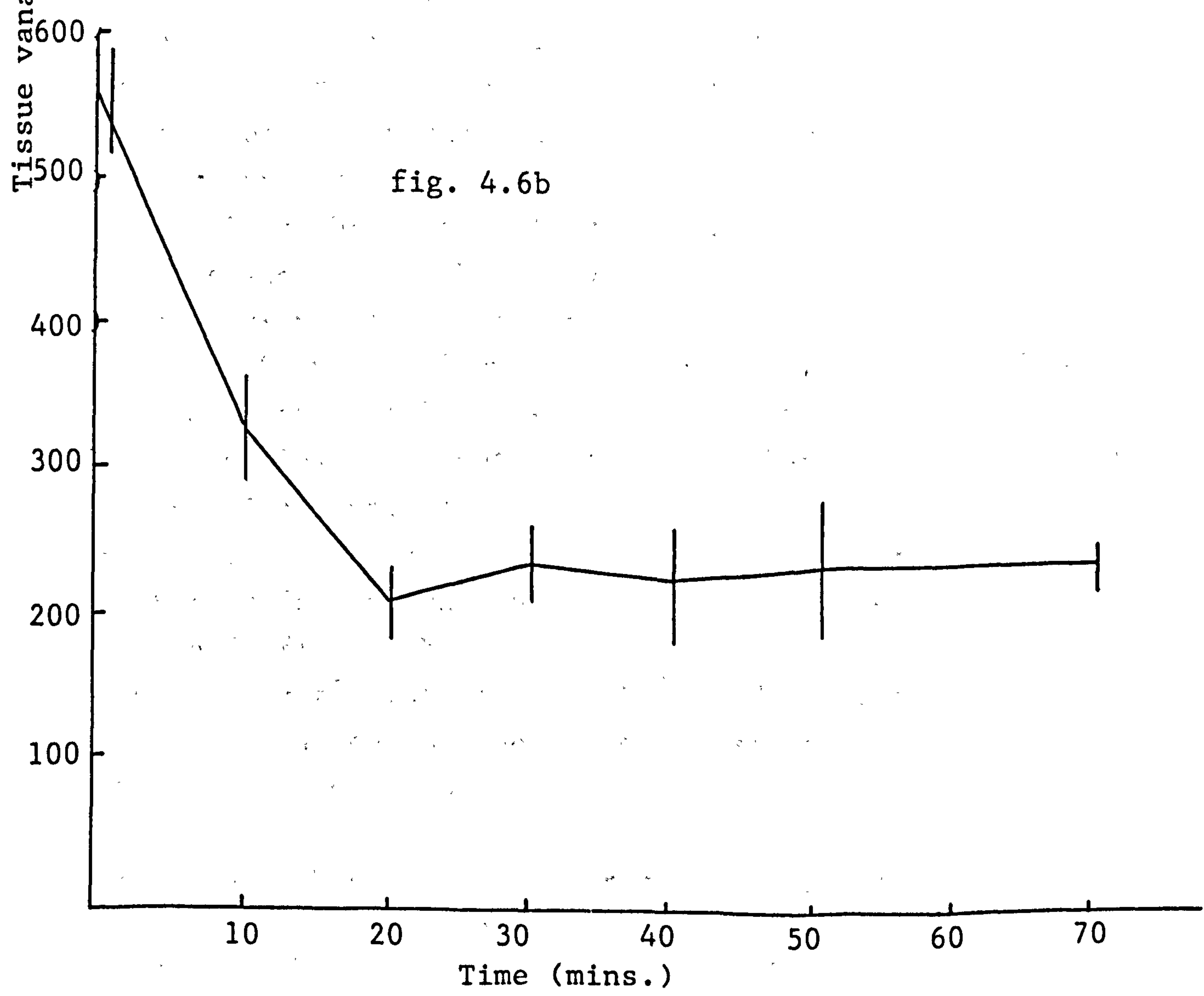
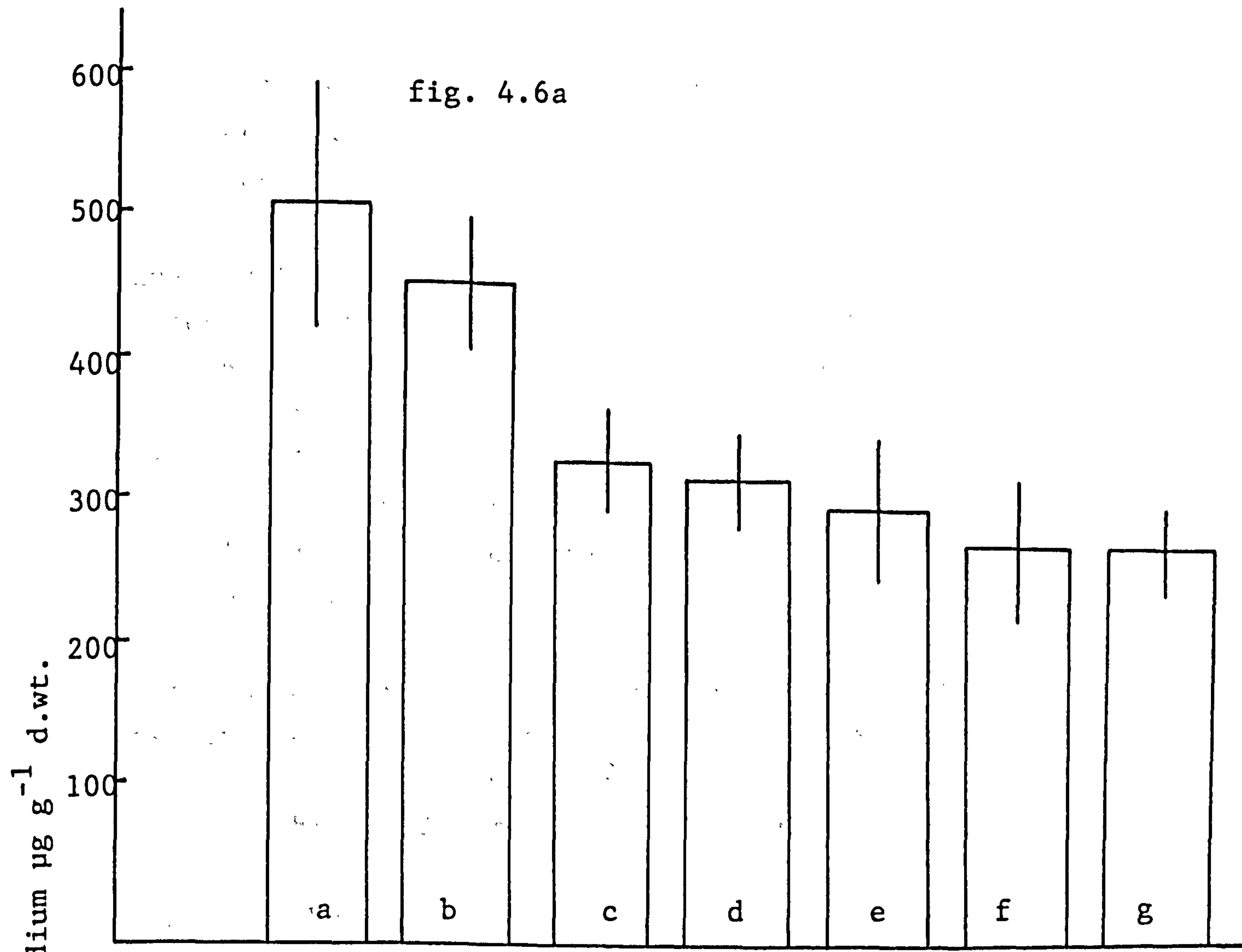


fig 4.6a Desorption of vanadium, supplied as VOSO_4 , by various agents at a concentration of 10mM.

- (a) no treatment
- (b) water
- (c) copper
- (d) EDTA
- (e) salicylate
- (f) chromate(Na_2CrO_4)
- (g) chromate(K_2CrO_4)

fig. 4.6b Time course for desorption of vanadium supplied as VOSO_4 .



used 10mM chromate as an inhibitor of sulphate uptake with no apparent harmful effects on the tissue.

Given that a 10mM desorption in chromate was the most successful way of removing vanadium from the tissue without apparently causing any damage to membranes it was decided to employ this as a standard post absorption treatment for the removal of vanadium as the vanadyl cation. A time-course of desorption showed that once again 30 minutes was sufficient time to remove most of the exchangeable vanadium (fig. 4.6b).

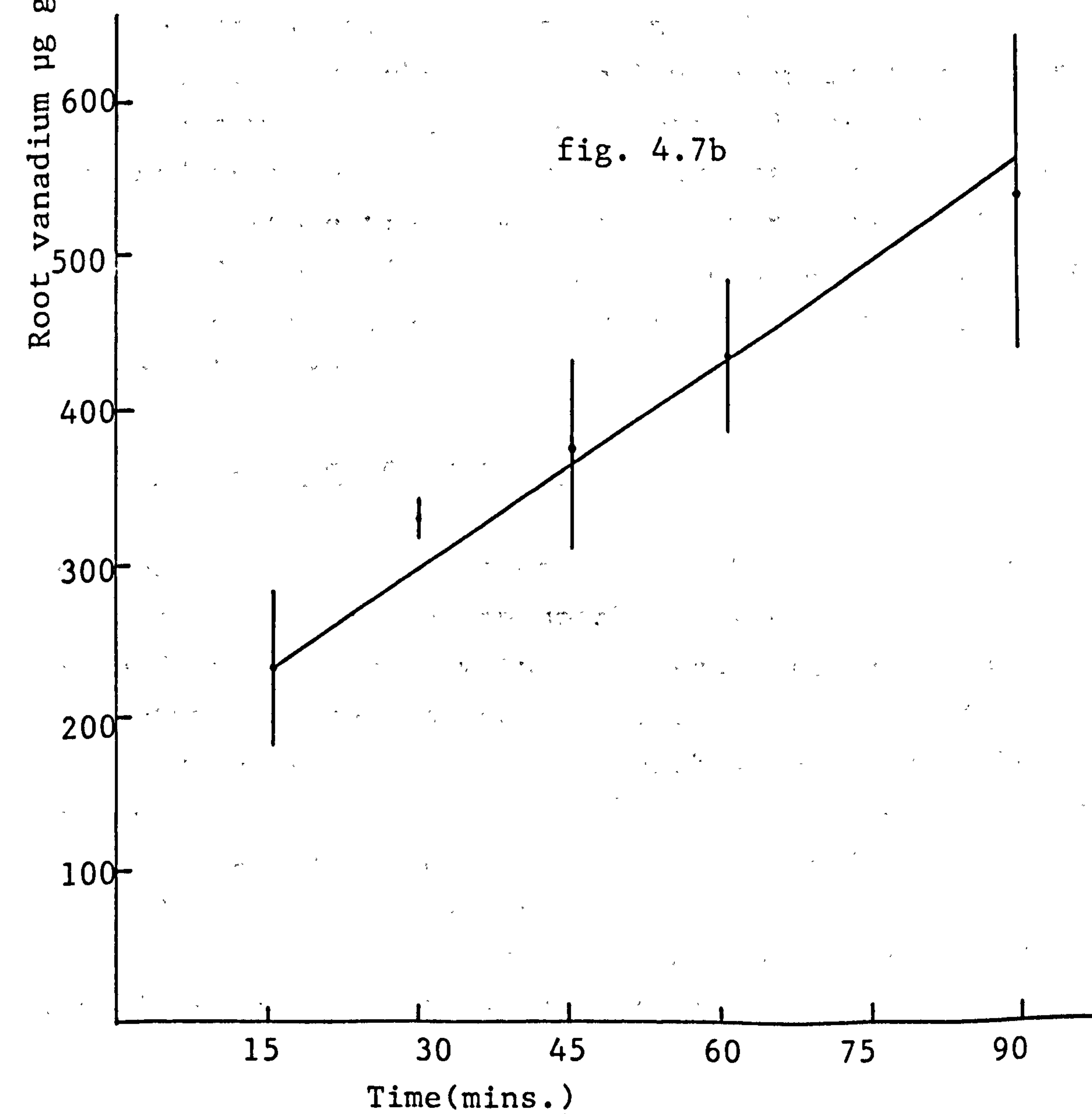
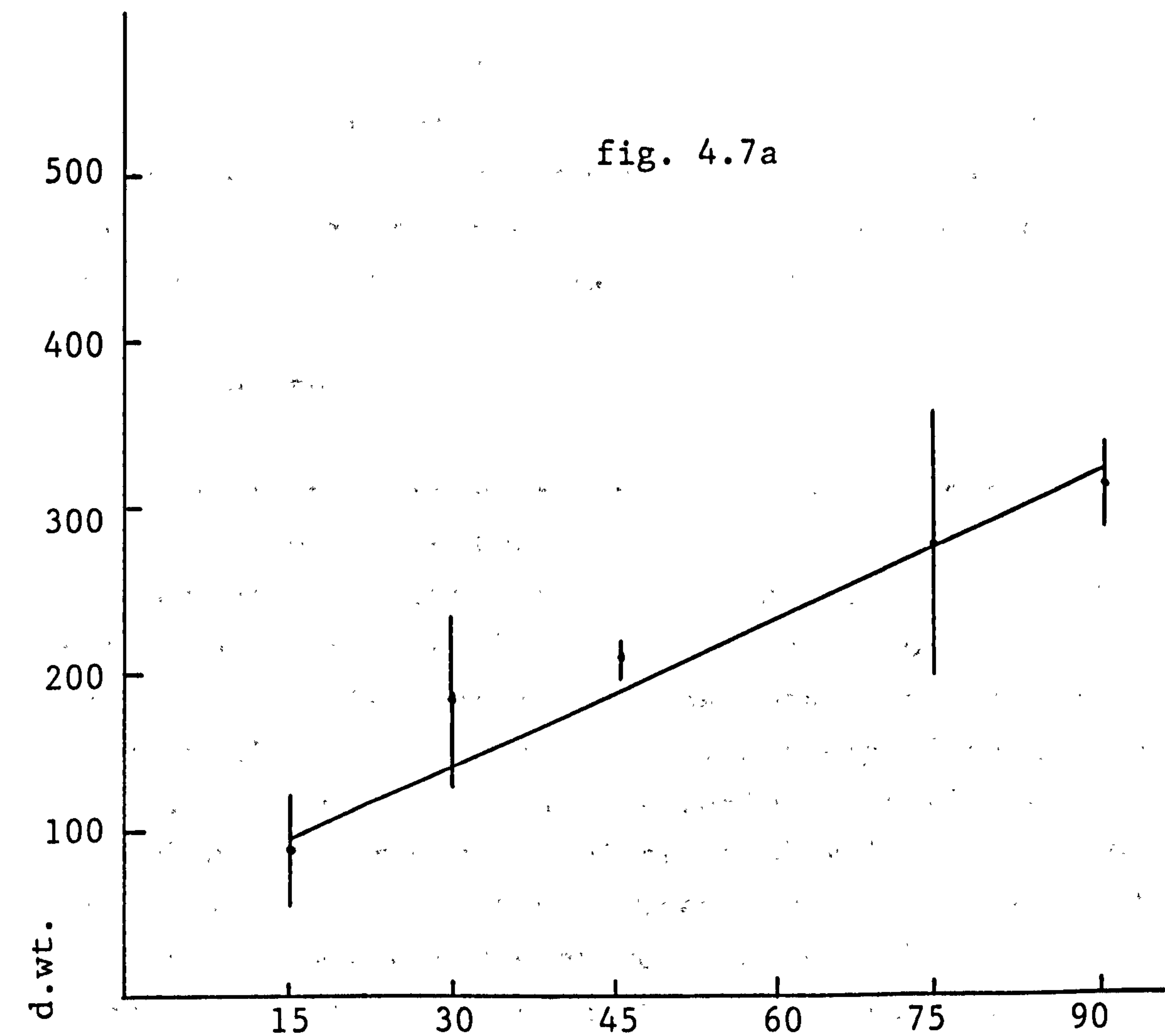
Time courses were once again measured however the 30 minute post uptake treatment in chromate solution was now employed to allow a distinction to be drawn between inter-cellular (exchangeable) and intracellular (non exchangeable) vanadium.

Uptake of the vanadate ion over a period of 75 minutes is shown in fig. 4.7a. Since the tissue was subjected to a post uptake treatment it must be assumed that this line represents uptake by processes other than simple exchange absorption. Although uptake appears constant over the period of the experiment it can be seen that if the line was extrapolated back it would not pass through the origin. Results similar to these have been obtained by numerous workers using different ions (Harrison et al 1978, Holst et al 1980 and Colclasure and Schmid 1974). Welch (1973) also obtained similar results for the vanadate anion but at a much lower concentration (5 μ M) than that used in this study. The significance of the linear phase of uptake will be discussed later. When a similar time course study was performed for the vanadyl cation, similar results were obtained (fig. 4.7b). Uptake rates are similar for both ions 4.3 μ g g⁻¹d.wt. min⁻¹ (VO²⁺) and 3.7 μ g g⁻¹d.wt. min⁻¹ (VO₃⁻) as can be seen from the slopes of the graphs (figs. 4.7a and 4.7b).

Once the exchangeable, "outer space" fraction has been removed from the tissue it can be seen that uptake of both ions shows a linear response. Although this suggests

fig. 4.7a Time course for vanadium uptake with desorption treatment employed. Vanadium supplied as NH_4VO_3 at a concentration of 0.2mM.

fig. 4.7b Time course for vanadium uptake with desorption treatment employed. Vanadium supplied as VOSO_4 at a concentration of 0.2mM.



a process under some form of metabolic control it does not automatically suggest active ion uptake. In an attempt to gain a clearer insight into these mechanisms a series of experiments were planned to examine the effects of various physical and chemical parameters on uptake.

4.2 Temperature.

If metabolic processes are involved in the uptake of vanadium it is to be expected that they will exhibit a high degree of dependence on temperature. The effects of temperature on the uptake of the vanadate anion are shown in fig. 4.8a. Regardless of the duration of the uptake period, temperature has no real effect on uptake (AOV not significant at 5% level). A similar series of experiments performed using the vanadyl cation produced similar results i.e. uptake of the ion was independent of temperature (fig. 4.8b). Results of this type where temperature appears to have little or no effect make the calculation of Q₁₀'s difficult. When a Q₁₀ has been calculated for other ions a variety of results has been observed. Colclasure and Schmid (1974) obtained a Q₁₀ of 2.2 for cobalt absorption by excised barley roots which is similar to the result obtained for zinc by Schmid, Haag and Epstein (1965). Other workers have obtained results similar to those presented here however. Holst et al (1980) determined a Q₁₀ of 1.2 for Beryllium uptake and considered this to be indicative of a physical rather than chemical process of entry.

4.3 Hydrogen ion concentration.

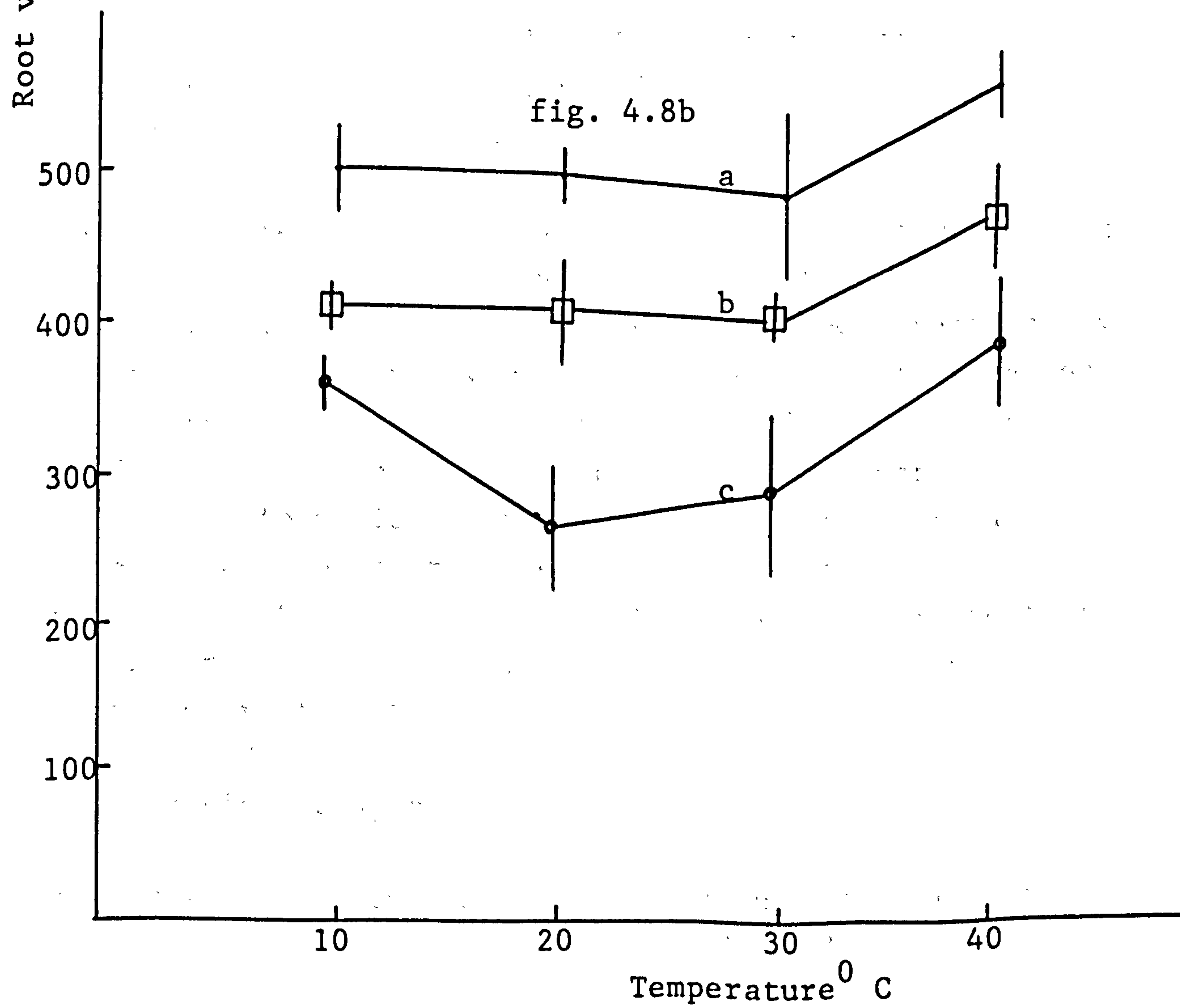
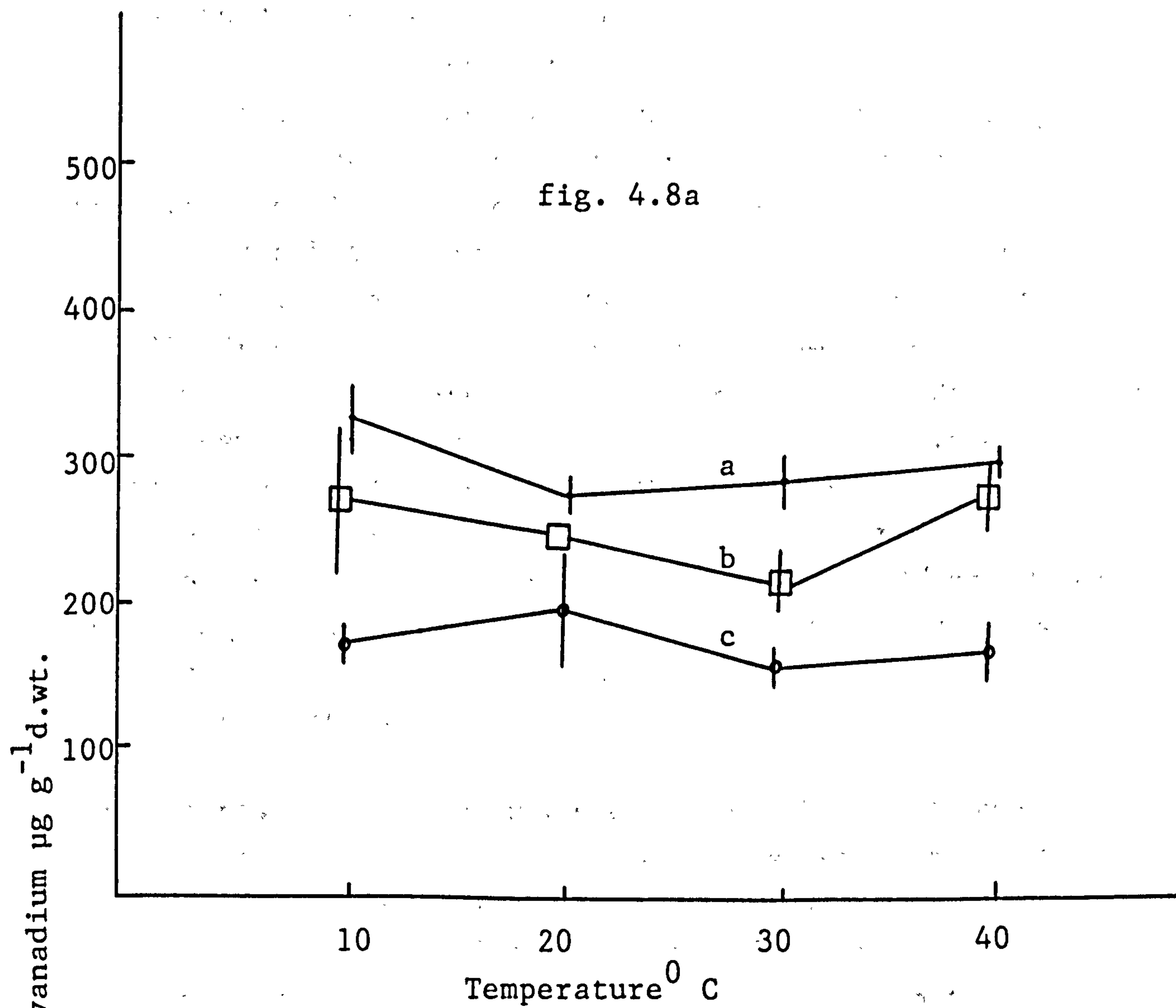
Numerous workers have investigated the effect of hydrogen ion concentration (pH) on uptake of ions. The results vary tremendously not only with the tissue used but also with the nature of the ion under study. Vange et al (1974) demonstrated the dependence of both sulphate and phosphate uptake on pH. Both ions (H_2PO_4^- and SO_4^{2-}) showed maxima of uptake at around pH 4, deviation from this pH produced sharp declines in uptake of both ions. In contrast Holst et al (1980) demonstrated increased uptake of beryllium with increased pH with a maximum at pH 8 and minimum at pH 4.

fig. 4.8a Effect of temperature on the uptake of vanadium supplied as NH_4VO_3 at 3 different time intervals.

- (a) 90 mins. uptake period.
- (b) 60 mins. uptake period.
- (c) 30 mins. uptake period.

fig. 4.8b Effect of temperature on the uptake of vanadium supplied as VOSO_4 at 3 different time intervals.

- (a) 90 mins. uptake period.
- (b) 60 mins. uptake period.
- (c) 30 mins. uptake period.



Welch (1973) examined the effect of pH on the uptake of low levels of vanadium as the vanadate anion. Uptake was maximal at pH 4, between pH 5 and 8 uptake showed only a slow decline but at pH's above this uptake dropped rapidly to a very low level. Results presented here show a similar relationship between uptake of the vanadate anion and pH (fig. 4.9a). A maxima of uptake was seen at pH 3 but this rapidly dropped as the pH increased. Some evidence of a plateau at pH 5 - 7 was seen which agrees with Welch (1973). These results were consistent even when other systems of pH adjustment were employed (NaOH/HCl). Acetate/acetic acid buffering was favoured because it required only small additions of buffer to produce a stable pH. Other systems examined required large additions of pH adjuster and were often very susceptible to change during the course of an uptake experiment.

Similar results have been obtained for the fixation of vanadium by humified plant organic matter (fig. 4.9b) (Bloomfield and Kelso 1973). In this instance a pH of 3 was optimal for retention of the vanadate anion. It is also worth noting that boiled roots show a similar pattern of ion absorption (fig. 4.9c). It is probable that pH may affect some chemical process prior to uptake which in turn affects the rate of uptake.

When the effect of pH on vanadyl cation uptake was examined a distinct peak of uptake was observed at pH 4.1 - 4.5 (fig. 4.10). Uptake of this ion was reduced dramatically if the pH varied in any way. Explanation of this pattern solely in terms of vanadyl cation hydrolysis products is difficult although it does seem that conditions which favour the formation of the VO^{2+} entity are those where greatest uptake occurs (i.e. more acid conditions). At maximal uptake (pH 4.1 - 4.5) other species will certainly be present (see chapter 2) and will no doubt alter the uptake characteristics for V(+4).

4.4 Complexing agents.

The presence of complexing agents in an uptake medium is likely to affect the absorption of any charged ion. The

fig. 4.9a Uptake of vanadium, supplied as NH_4VO_3 , as effected by pH. Buffering Acetate / Acetic acid uptake time 3hours.

fig. 4.9b Effect of pH on the dialysability of vanadium in the presence of humified organic matter. From Bloomfield and Kelso(1973).

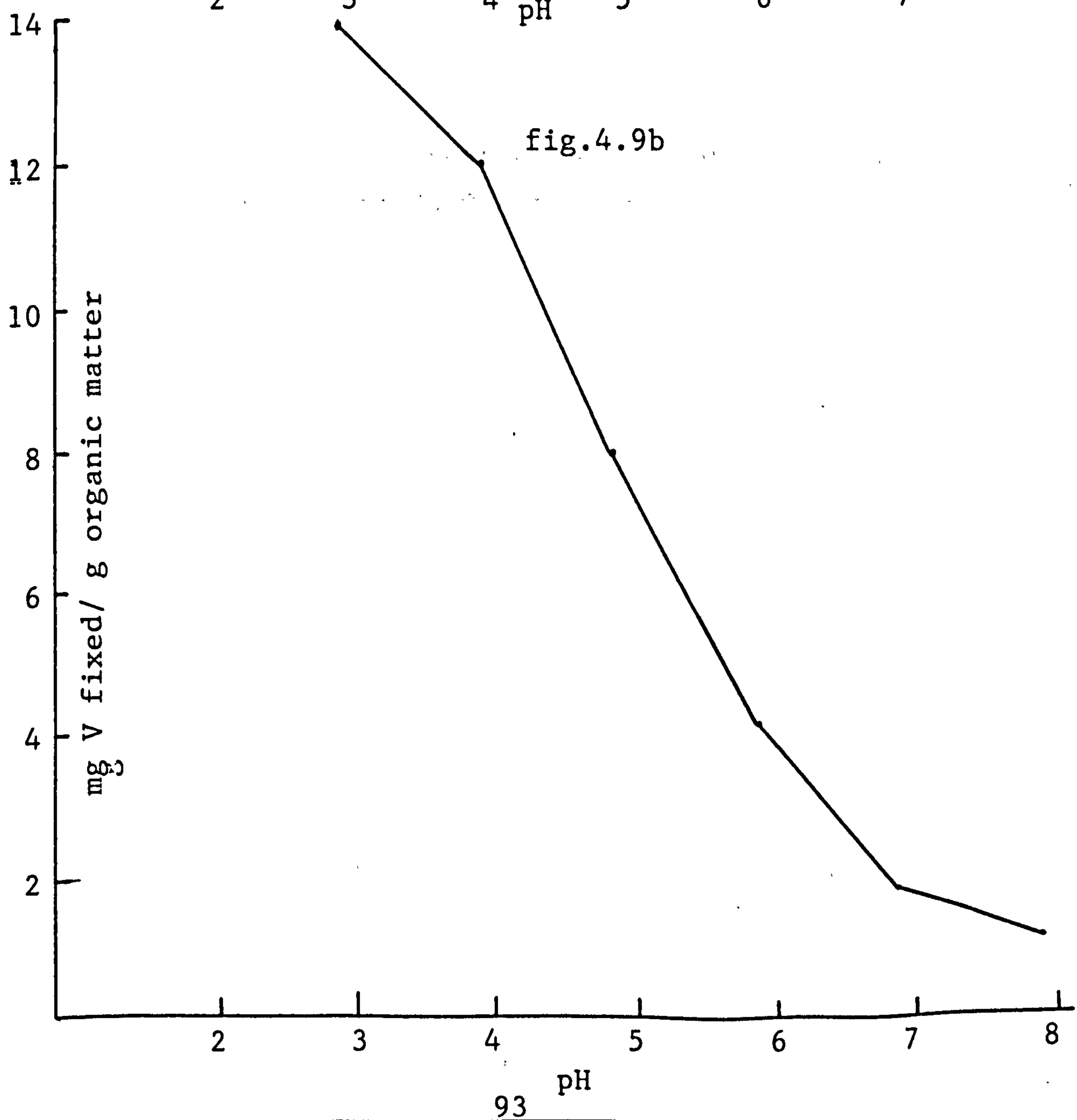
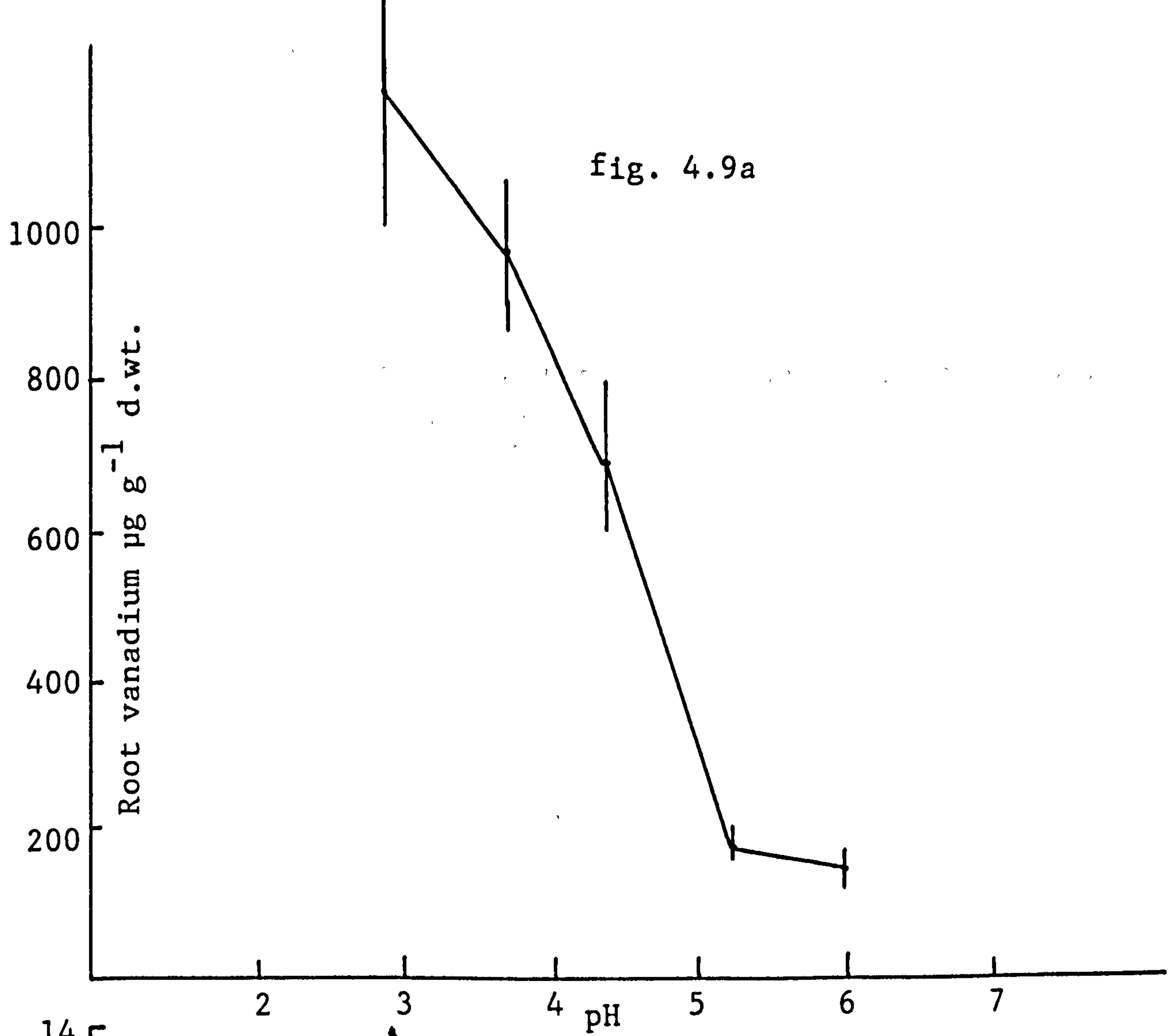
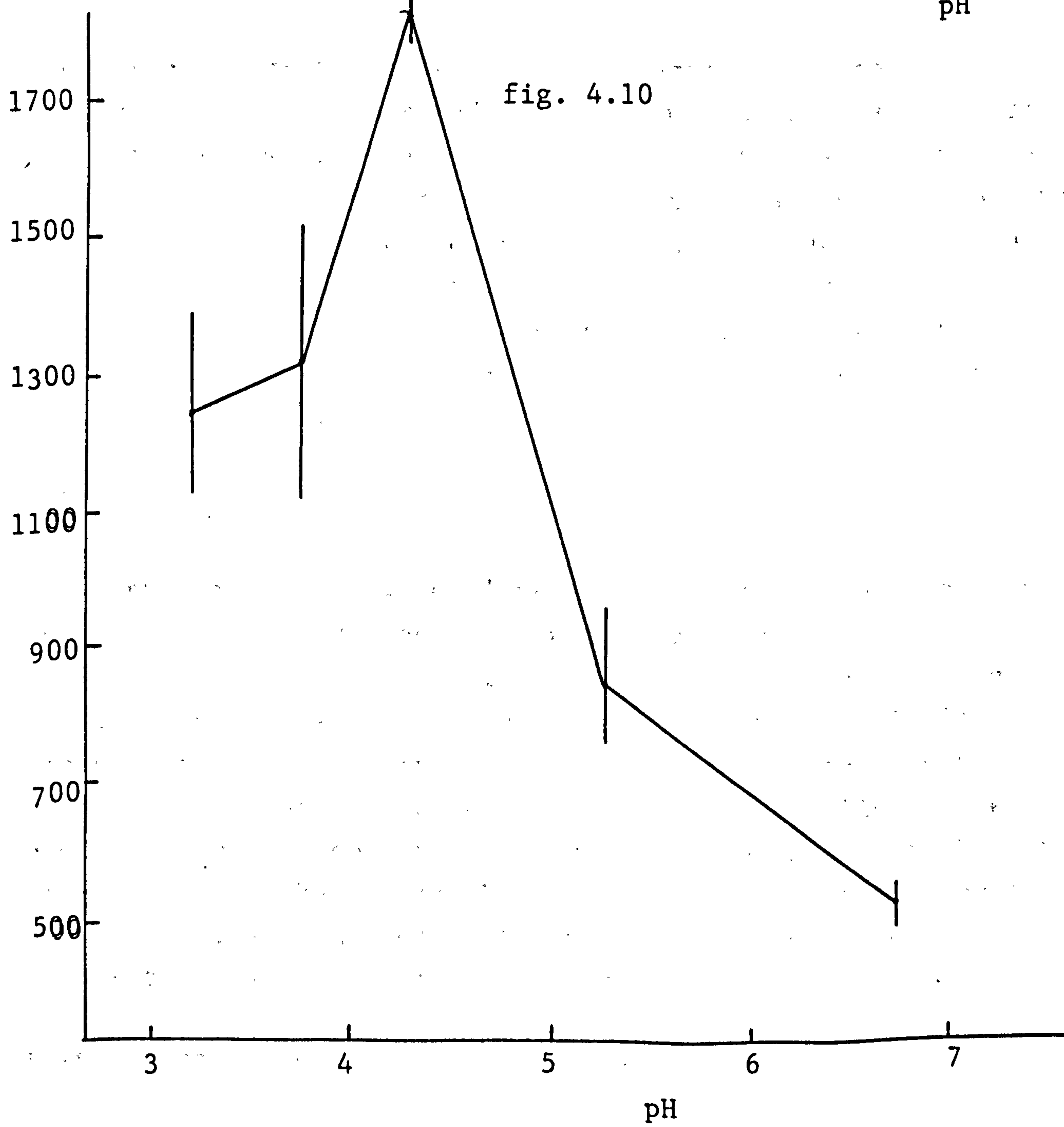
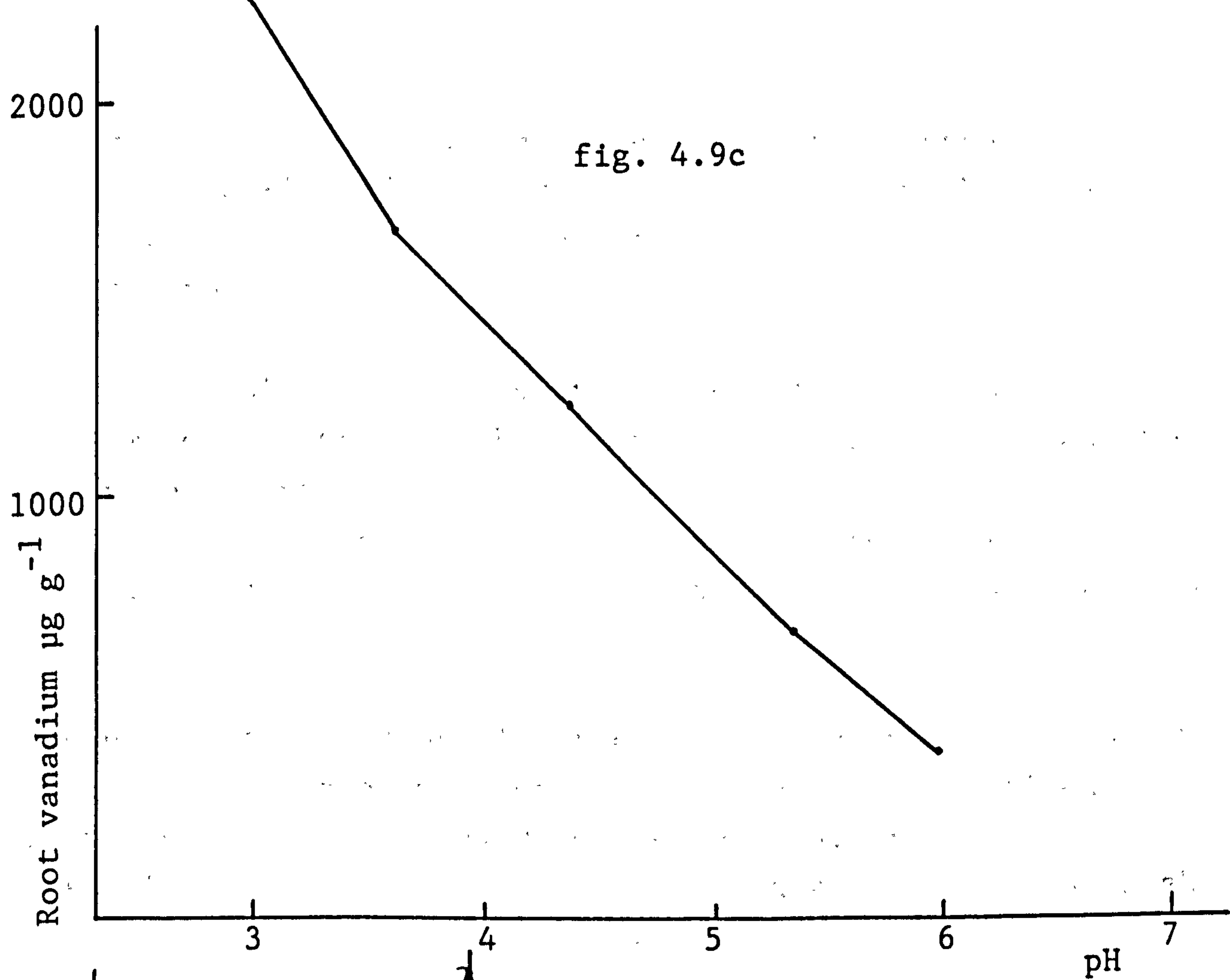


fig. 4.9c Effect of pH on uptake of vanadium,
supplied as NH_4VO_3 , by boiled roots.

fig. 4.10 Effect of pH on uptake of vanadium,
supplied as VOSO_4 , by barley roots.



nature of the effect being dependent upon the stability of the metal-ligand complex. Different ions might be expected to show different effects depending on their relationship to the complexing agent.

The uptake of the vanadate anion was reduced in the presence of complexing agents. The agents reduced uptake of vanadium anion in the order glycine - citrate - EDTA with EDTA causing the greatest reduction in uptake and glycine the least (fig.4.11a). This pattern was consistent even when the free space vanadium was not removed.

The uptake of the vanadyl cation is similarly affected by the presence of complexing agents. The order of effectiveness in reducing uptake is somewhat different however. EDTA seems less effective than citrate at reducing vanadyl cation uptake (fig. 4.11b) although the difference is only marginal.

Similar concentrations of complexing agents lead to proportionately greater reductions in uptake of the two forms. In roots from which the free space vanadium has been removed approximately 90% of vanadyl cation uptake is prevented by the presence of complexing agents. Reduction of vanadate anion uptake under similar conditions is only 80%. This difference may be accounted for by the ease with which the two ionic species combine with the complexing agent and the kinetics of complex formation.

4.5 Respiratory poisons.

Many investigators have employed respiratory poisons as a tool in eliciting information about uptake processes in plants (Colclasure and Schmid 1974, Van Steveneck and Van Steveneck 1972, Ordin and Jacobson 1955, Welch 1973 and Cram 1969). Inhibition of ion absorption by respiratory poisons such as cyanide, azide and carbon monoxide clearly established the need of aerobic respiration for nutrient absorption by higher plants. Up to the present however there has been no completely satisfactory explanation of the manner in which aerobic respiration is linked to ion transport.

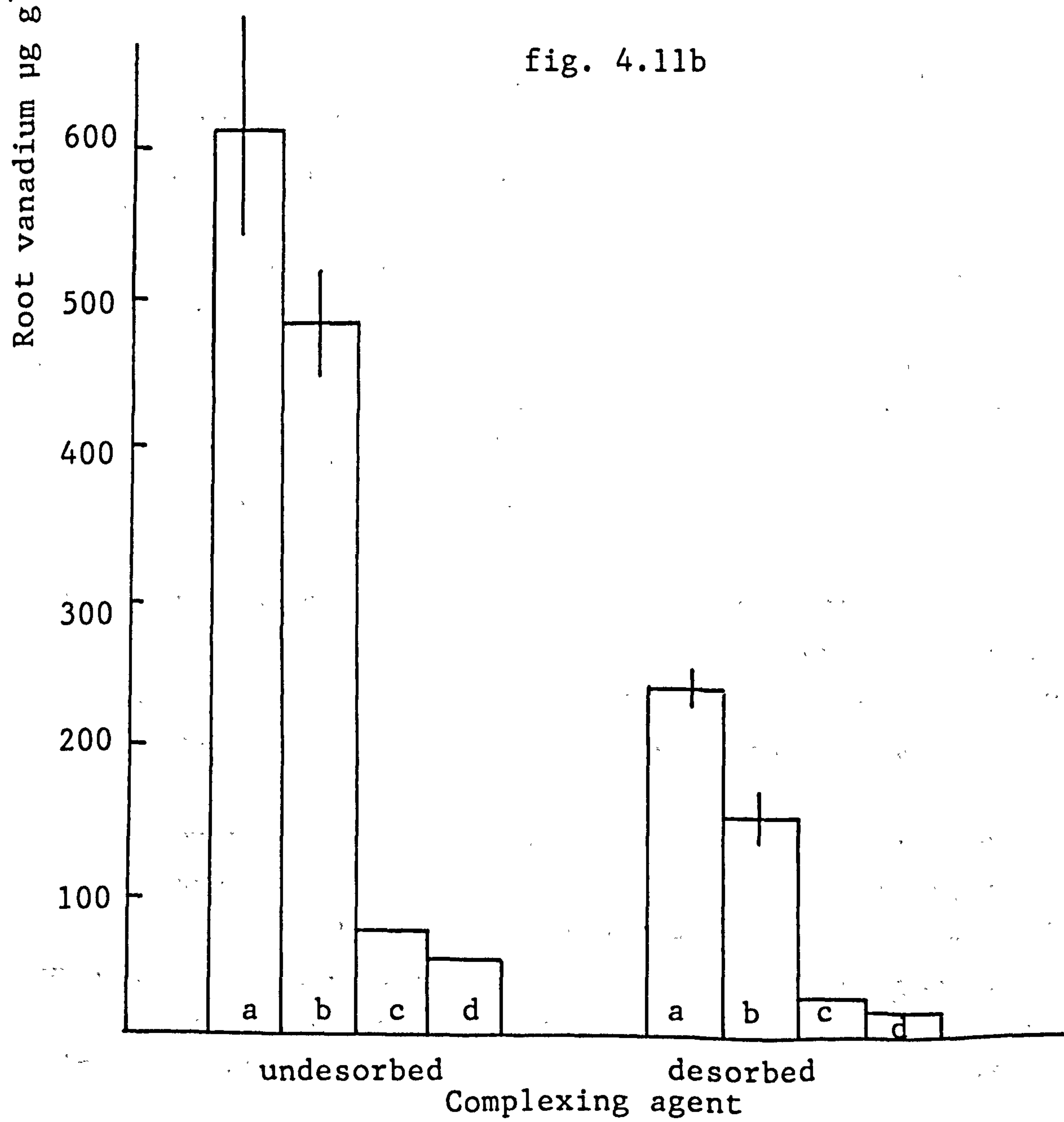
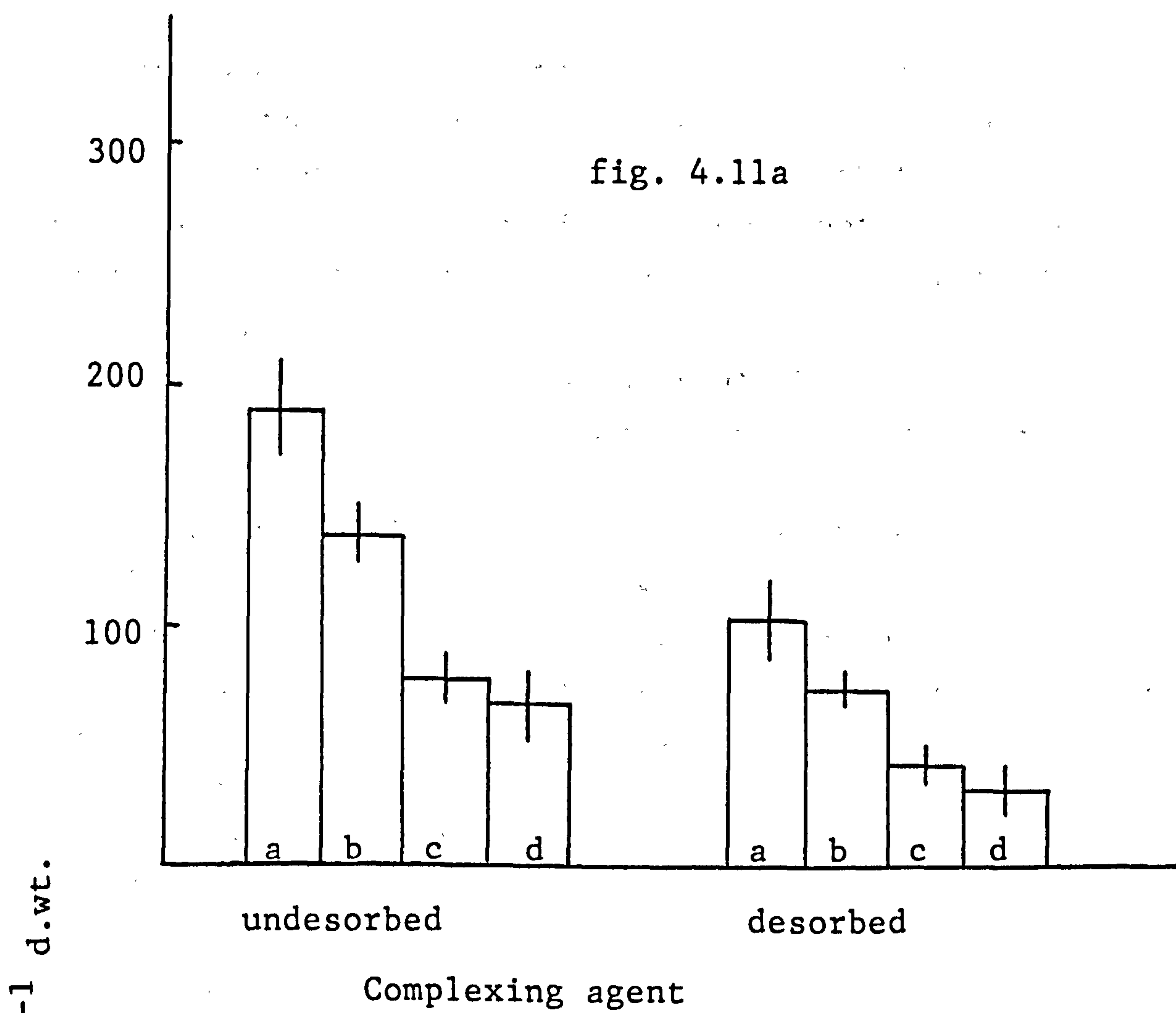
Initial suggestions were concerned with a counter flow of

fig. 4.11a The effect of complexing agents on the uptake of vandium supplied as NH_4VO_3 .

- (a) control
- (b) glycine
- (c) citrate
- (d) EDTA

fig. 4.11b The effect of complexing agents on the uptake of vanadium supplied as VOSO_4 .

- (a) control
- (b) glycine
- (c) EDTA
- (d) citrate



anions down the cytochrome chain during the oxidation of reduced carbon compounds (Lundegardh 1955). This view was however inconsistent with the evidence that DNP (dinitrophenol), which inhibits ATP formation but does not inhibit electron transfer down the cytochrome chain, is a potent inhibitor of ion absorption (Robertson et al 1951). This and more recent work has led to the conclusion that ATP is the intermediary link between aerobic respiration and ion transport.

Other poisons such as cycloheximide (CHM) which is an inhibitor of protein synthesis have been employed in studies on ion uptake (Wildes et al 1976). An ion which is transported via a protein carrier which is continually being synthesized and degraded should be inhibited by this poison and numerous workers have shown it to be effective in reducing uptake of ions (Polya 1968, Van Steveneck and Van Steveneck 1972 and Sutcliffe 1973).

In view of the difficulties known to be associated with obtaining consistent reliable results on vanadium uptake when metabolic inhibitors are employed (Welch 1973) it was decided to limit the study to two inhibitors; DNP and CHM. Even with this limited study difficulties in obtaining results with low variability were encountered. A series of experiments did however produce repeatable results although the high variability was a disturbing trend. Vanadate anion uptake appeared to be insensitive to CHM but reduced by DNP (fig.4.12a). The vanadyl cation also exhibited similar responses to both CHM and DNP (fig. 4.12b). These results were consistent regardless of the uptake period.

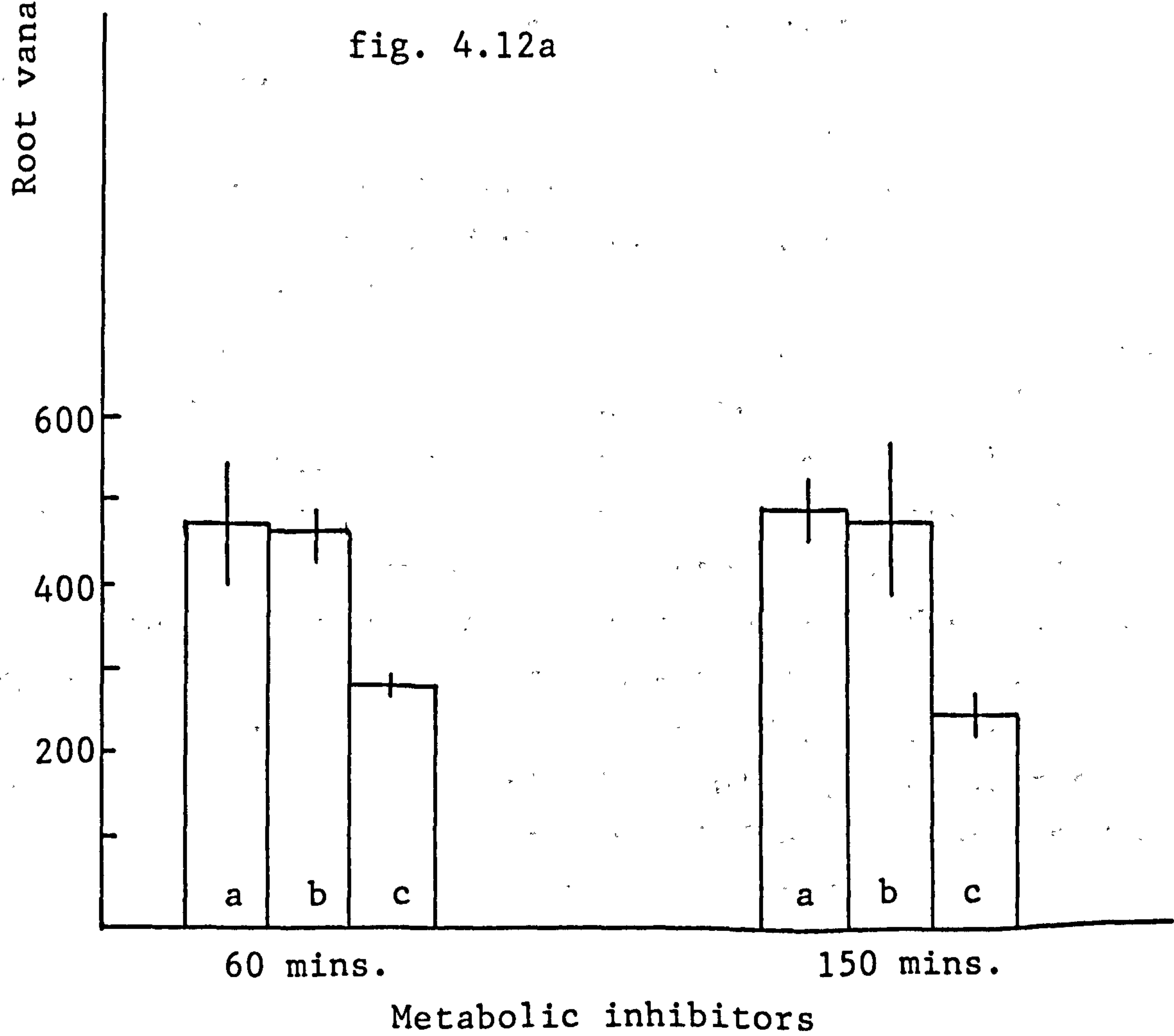
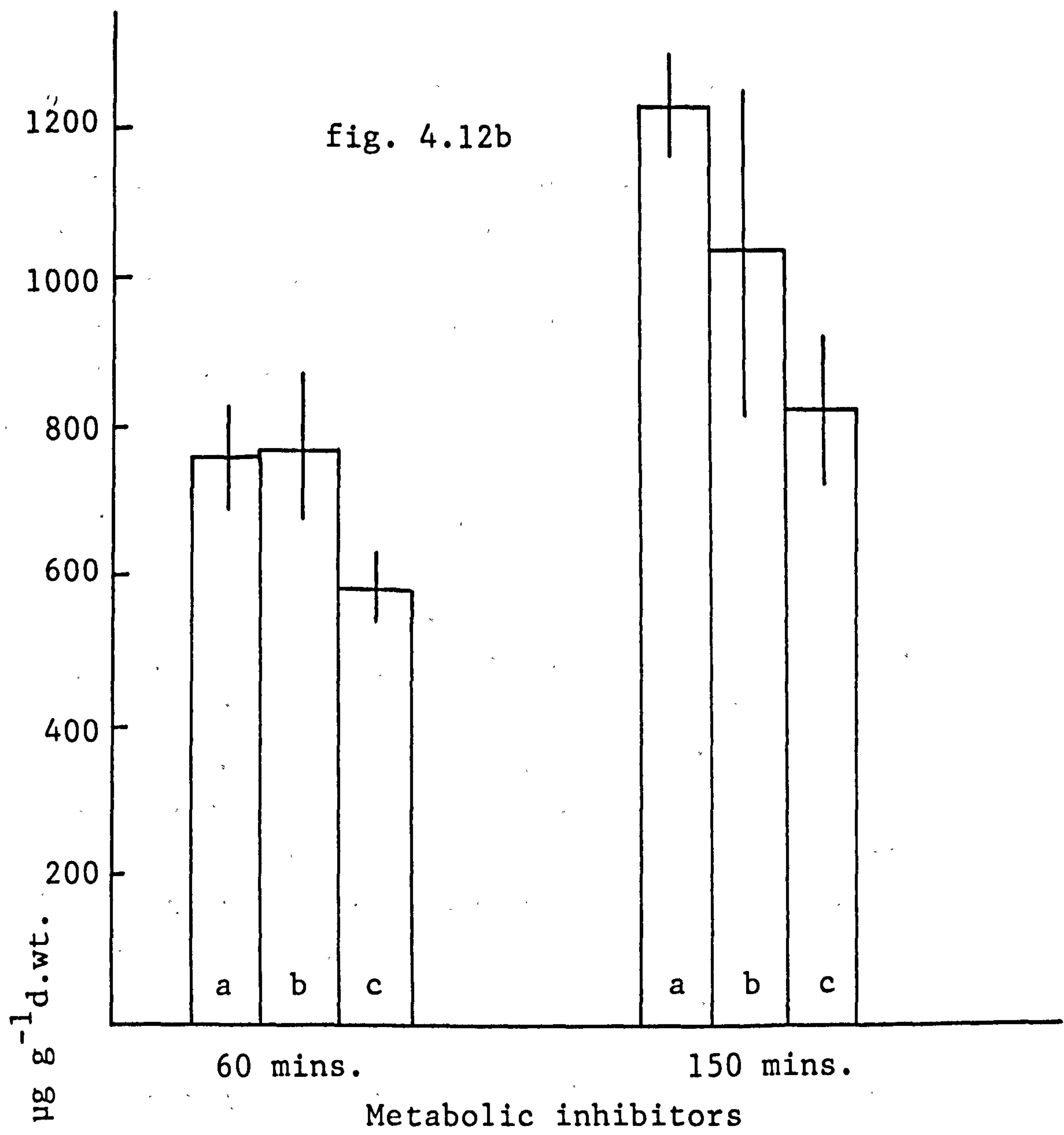
Interpretation of such results presents numerous difficulties. Welch (1973) felt inclined to attribute reductions in uptake by metabolic inhibitors to induced membrane deterioration rather than direct effects upon the uptake mechanisms. Indeed on the basis of these results it is difficult to point to a reason for the inhibition of uptake by DNP other than to say that it may be an energy dependent process deprived of its first source by the poison. The clear lack of effect of CHM might be seen as *prima facie* evidence that the transport of both ions does not involve a protein carrier. Other workers using CHM have shown however that inhibition of uptake into

fig. 4.12b The effect of metabolic inhibitors on the uptake of vanadium, supplied as VOSO_4 , during different uptake periods.

- (a) control
- (b) CHM
- (c) DNP

fig. 4.12a The effect of metabolic inhibitors on the uptake of vanadium, supplied as NH_4VO_3 , during different uptake periods.

- (a) control
- (b) CHM
- (c) DNP



the root develops only slowly with time (Wildes et al 1976). If the root possessed a large pool of the necessary protein then inhibition of synthesis of new protein might not manifest itself as a reduction in uptake unless long periods of uptake were investigated. The use of long periods of uptake would in itself produce problems and any effects observed might be considered secondary, as the product of the disruption of normal cell functioning.

4.6 Calcium and vanadium uptake.

Calcium is known to be essential for the maintenance of structural and functional integrity in plant cell membranes. Epstein (1961) showed it to be essential in maintaining selectivity of cation transport and also demonstrated its protective role in the presence of high concentrations of hydrogen ion (Epstein 1961). Welch (1973) demonstrated a 72% reduction in the uptake of vanadate anions by roots lacking calcium, but there is a paucity of data relating to its effect on the uptake of other ions.

Similar experiments to those of Welch (1973) employing higher concentrations of vanadate led to a similar reduction in vanadate anion uptake when calcium was absent from the uptake medium. The reduction in the uptake was much lower however, normally being in the range 16 - 25% (Table 4.2).

In contrast to this the uptake of vanadium supplied as the vanadyl cation increased when calcium was omitted from the uptake solution. This was the case both when roots were subjected to a post uptake treatment (desorption) and when they were not (fig. 4.13). Increase in total uptake of a cation is to be expected when calcium is omitted from the uptake solution since a greater number of root exchange sites will be available. Increased uptake into the cytoplasmic compartment could be the result of several effects. If the omission of Ca^{2+} was leading to a partial breakdown of the plasmalemma then the inner contents of the cell would no longer be under the control of cellular uptake mechanisms. It would be possible that a large influx of vanadium would occur to allow an equilibrium situation to develop. A second

Table 4.2 The effect of calcium level in the uptake solution on the uptake of vandium supplied as NH_4VO_3 .

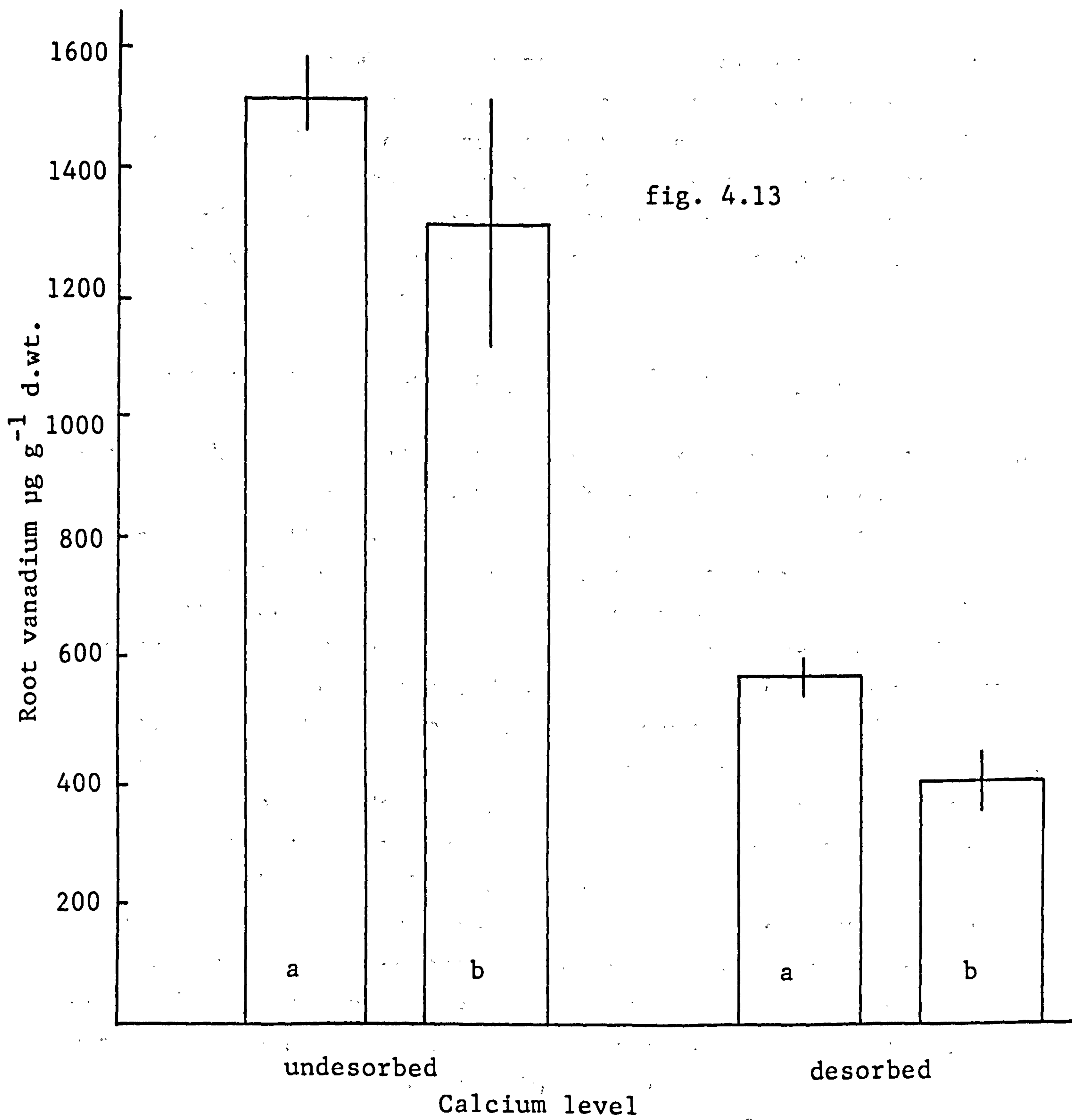
Table 4.2

Tissue vanadium level($\mu\text{g g}^{-1}$ d.wt.)		% Red ⁿ in uptake.
normal calcium	low calcium	
250	210	16%
190	140	26%
250	190	24%

Results are averages for three samples from replicate experiments.

fig. 4.13 The effect of altered calcium levels
on the uptake of vanadium supplied as VOSO_4 .

- (a) low calcium ($5.0\mu\text{M}$)
- (b) normal calcium (0.5mM)



mechanism through which increased uptake may occur when Ca^{2+} is omitted is through the removal of a competitive agent. Calcium ions might be competing with vanadyl ions for uptake sites and an omission of calcium from the uptake solution would lead to increased uptake. At present however no detailed evidence is available to provide a cogent explanation of this observed effect.

4.7 Concentration dependent uptake of vanadium.

Some of the most interesting recent work on uptake of inorganic ions has been concerned with the kinetics of the uptake mechanism. Absorption rates of inorganic ions by roots increase as the external ion concentration increases until an apparent saturation occurs. This saturation, along with the selective nature of ion absorption served as the basis of the ion carrier concept (Osterhout 1935 and Van den Honert 1937). In an attempt to produce an accurate quantitative description of this mechanism Epstein and Hagen (1952) employed Michaelis-Menten enzyme kinetic analysis. It was soon realized however that simple Michaelis-Menten analysis could not fully describe the uptake of ions over large concentrations since saturation did not always occur (Hodges 1973). The Michaelis-Menten model does however provide a useful basis for analysing uptake although recently more complex and potentially useful models have emerged (for a general review of this area see Epstein 1973 and Nissen 1974).

In this study no complex analyses of uptake have occurred. It was envisaged that this would be more of an exercise in gathering information on the pattern of uptake in relation to concentration of the supplied ion.

Welch (1973) working in the range 0 - 100 μM demonstrated that the vanadate ion does not exhibit saturation kinetics. This is in contrast to many other ions which do exhibit saturation kinetics in this range (e.g. K^+ , Rb^+ , Zn^{2+} , Cu^{2+} , Mn^{2+} and Cl^- see Welch 1973 and references therein). This kinetic evidence led Welch to conclude that vanadium is not accumulated in plant cells by normal active ion transport mechanisms as used by other ions.

In this work the concentration range examined was extended to 5mM and over this range it can be noted that the vanadate ion does exhibit saturation kinetics (fig. 4.14a). There also seems to be evidence of more than one mechanism is operating. This relatively high concentration of vanadate ion which is needed to produce saturation suggests that if a carrier is involved in vanadate ion uptake then the carrier has a low affinity for the ion.

A similar pattern of concentration dependent uptake can be observed for the vanadyl cation (fig. 4.14b) but saturation seems to be reached at a lower concentration than that required to produce saturation for the vanadate ion. Once again there is evidence for more than one mechanism operating in the uptake of vanadyl ion.

Whilst no analysis has been performed on this data it does provide useful information about the nature of the uptake processes for vanadium. A carrier of some sort does seem to be involved but the carrier ion affinity would seem to be low in relation to other ions.

fig. 4.14a Concentration dependent uptake of vanadium
supplied as NH_4VO_3 .

fig. 4.14b Concentration dependent uptake of vanadium
supplied as VOSO_4 .

fig. 4.14a

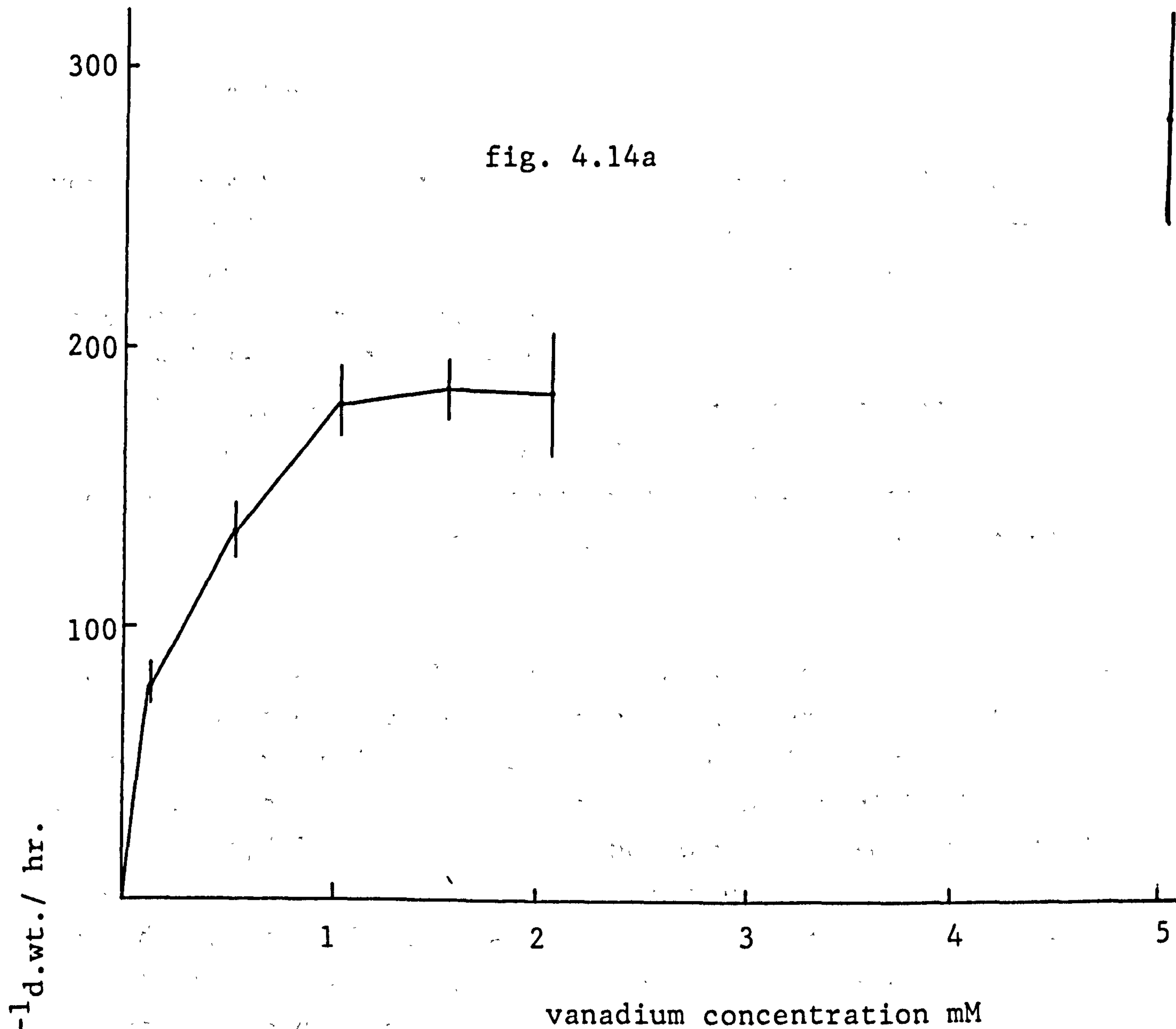
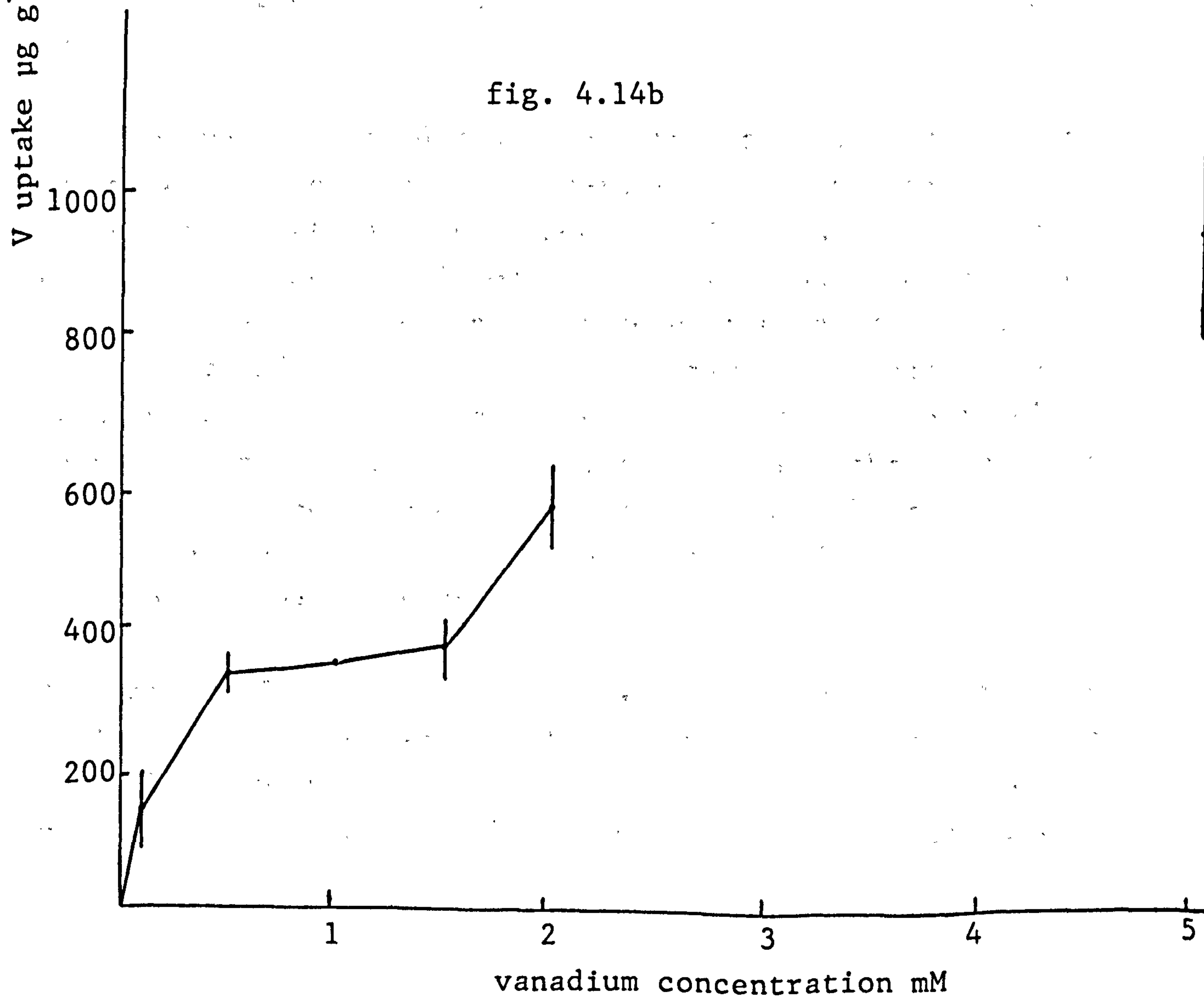


fig. 4.14b



4.8 Discussion.

One feature to have emerged from these uptake studies was the similarity of behaviour of the two different ionic species of vanadium. In terms of their response to altered ion concentrations, metabolic inhibitors and complexing agents they exhibited a close similarity. Most striking was the fact that the extra-cellular component of uptake for both ions could be most effectively removed by a post uptake treatment in the chromate anion. Such similarities have led to the suggestion that vanadium within root tissues adopts a common form.

The effectiveness of the chromate anion in removing the extra-cellular uptake component of both ionic species might be considered *prima facie* evidence for the oxidation of cationic V(+4) to anionic V(+5). Indeed air oxidation of V(+4) is known to readily occur in alkaline solutions (see chapter 2). However in the context of the current experiments it is considered more likely that reduction occurred. Certainly it is difficult to envisage why the extra-cellular component of uptake was so difficult to remove if it was present as an anion.

If V(+5) was reduced during the uptake process why was the chromate anion successful in removing the extra-cellular component of uptake? At present no clear answer to this question has emerged although it is felt that the chromate anion may be participating in some of the redox reactions in which vanadium is involved. Clearly the exchangeable vanadium was held in place by something other than simple physical forces. At present it is envisaged that some form of reduction and complexation occurs within the root tissues in a manner analagous to the complexation of borate with cell wall polysaccharides.

The nature of the ionic species which remain in solution and ultimately pass across the plasmalemma into the cytoplasm of the cell is by no means clear. It is however considered unlikely that free vanadyl ions will be involved in this

process given their tendency to form stable complexes with a variety of ligands. At present it is considered more likely that free or complexed vanadate ions will be more likely involved in the uptake of vanadium into the cell.

Prior to a discussion of the uptake processes involved in vanadium transport a brief note on the terminology employed is considered useful since workers tend to interpret terms in different ways.

Hodges (1973) makes the distinction between energy dependent and active transport. Energy dependent transport is considered to be any transport which depends, directly or indirectly, upon metabolism. Active transport is a special type of energy dependent transport where the ion is transported against an electrochemical gradient. The basis for the difference between energy dependent and active transport lies in the fact that an electrical potential difference exists across the membranes of actively metabolizing cells. Charged solutes such as inorganic ions move in response to this gradient as well as to the concentration gradient. Movement in response to these gradients is described as passive.

Epstein (1973) is reluctant to make such a distinction, suggesting ions may not move passively across membranes in response to an electrochemical potential because of the impermeability of the membrane to charged bodies. Epstein's view is that transmembrane ion movement in plant cells depends upon metabolically derived energy regardless of whether the movement is up or down an electrochemical gradient.

Hodges (1973) suggests that membrane impermeability is overcome by the combination of the ion with a carrier molecule in the membrane. The ion-carrier complex would then traverse the membrane although movement would be down the electrochemical gradient, and thus passive.

In the light of these comments certain points can be made about vanadium transport.

The linear pattern of vanadium uptake, exhibited when extra-cellular vanadium was removed, was constant for at least 90 minutes. Assuming that external factors, including solution concentration remain constant, and the driving force for transport remains constant, then this linear pattern can be considered to be commensurate with carrier mediated transport; that is an unchanging affinity of the carrier for the substrate maintains a constant rate of transport.

The relationship which exists between vanadium uptake and concentration (i.e. saturation kinetics) may also be used to indicate that a carrier is involved. One feature of these relationships is the high concentration of vanadium required in the solution before saturation occurs (0.5-1.0mM). One possible explanation of this feature is that at higher concentrations of vanadium polymeric species formation reduces the actual concentration of the metavanadate anion (VO_3^-) present such that at a total vanadium concentration of 1mM the actual concentration of metavanadate is much less.

Further evidence of the involvement of a carrier comes from Welch (1973) who demonstrated that no significant interference with vanadium uptake occurred in the presence of other anions.

Although it seems likely that a carrier is involved in the uptake of vanadium this in no way indicates whether the process is active or passive. In order to help establish this an examination of the effects of certain physical and chemical factors on uptake was attempted.

Temperature had little or no effect on uptake thus indicating a passive mode of entry. Similarly metabolic inhibitors had little effect, and although caution must be exercised when interpreting results from experiments with metabolic inhibitors these do seem to indicate a passive mode of entry for vanadium. The effect of calcium on vanadium uptake was one of the few effects which showed some degree of form dependence. However it offers little in relation to the resolution of the active/passive problem.

On balance the evidence seems to point to some form of carrier mediated passive movement of vanadium across the plasmalemma. This will be discussed later.

In all experiments pH was seen to have a pronounced effect upon the uptake of vanadium. In these experiments the two ionic forms differed slightly in their behaviour but it must be remembered that these uptake experiments were performed in buffered solutions.

The variation in the uptake of the vanadate anion with pH agrees well with the pattern found by Welch (1973). Welch suggested these changes could be attributed to either changing ionic forms of the ion or competition by OH^- for uptake sites but favoured the former for several reasons (see Welch 1973 p.831). At present there is no real evidence to suggest any other basis for this effect although the similarity between patterns of organic matter retention of vanadium, retention of vanadium by boiled roots and uptake of vanadium by live tissues suggests that the initial exchangeable phase of uptake may in some way contribute to the absorbed fraction.

Reduced uptake of vanadium from a solution of $\text{V}(+4)$ at pH's greater than 5.0 is probably best explained in terms of oxidation of $\text{V}(+4)$ at these higher pH's. The maximum uptake which occurs at around pH 4.5 may be explained as the optimum for carrier-ion complex formation. At more acid pH's hydrogen may become competitive and thus reduce uptake.

Whilst the incomplete nature of this work is recognized it is felt that it represents a more positive statement on vanadium uptake by root tissue than any previously available. Welch (1973) clearly rejected the idea that vanadium transport was an active process but refused to believe that any of the commonly recognized passive processes could account for the observed accumulation of vanadium within root tissues. The presently proposed mode of uptake (i.e. carrier-mediated passive absorption) does at least offer an explanation consistent with the available observations to date.

CHAPTER FIVE

WHOLE PLANT STUDIES

5.1 Whole plant studies.

The use of isolated tissue systems for the study of plant physiological problems has proved exceedingly useful. The advantages are manifold when compared with whole plant systems. Excised roots, leaves and storage organs have become favoured tools of investigators concerned with processes involved in mineral ion uptake. Much valuable information relating to the nature of uptake mechanisms has been gained and will continue to be gained from these types of experiments. There are however numerous problems associated with these techniques, the principal one being that isolated tissue systems in general only function 'normally' for a relatively short period of time. Even when short experimental periods are employed, the physical isolation of tissue systems can give rise to anomalous results. It is therefore of paramount importance that the functional unit with which experiments are concerned figures largely in any experimental series. Only in this way can the worth of results obtained for isolated tissue systems be assessed.

The objective of this section of the study was to produce data relating to the basic uptake processes in several different species of higher plant. Information on the relative toxicity of the different valence states and the amounts taken up by roots and subsequently transported to shoots was considered a prime objective. Secondly it was considered worthwhile to examine interactions between vanadium and other mineral nutrients especially calcium. In order to produce results representative of more than one species 3 plant types were employed; Maize, *Zea mays* L., cv. Fronica, Broad bean, *Vicia faba* L., cv. Aquadulce claudia and Tomato, *Lycopersicon esculentum* Mill., cv. Money maker.

Initially results will be presented for each of the three species and then drawn together in a more general discussion.

5.2 Maize.

Few reports exist relating vanadium to maize. Singh (1971) reports some effects of vanadium on growth, yield and chemical composition of maize. Low levels (less than one ppm) of vanadium appeared to stimulate growth whereas higher levels (1.25 and 6.25 ppm) caused growth reductions (based on plant height, leaf number and area). No vanadium tissue levels were reported. Initial experiments were designed in a similar fashion although it was felt important to obtain data on tissue vanadium levels.

Plants were pre-germinated on moist tissue paper, grown for one week in seed trays and then transferred to 4 inch pots when treatment began. Nutrient solutions were supplemented with vanadium at five levels (0, 2, 20, 100 and 200 μM), supplied as either the vanadyl cation or vanadate anion. After 6 weeks, plants were harvested. Fresh weight measurements were made for both roots and shoots and are illustrated in figs. 5.1a (VO_3^-) and 5.1b (VO^{2+}). Although these figures clearly show decreasing fresh weight production with increasing applied vanadium none of the differences were significant ($P < 0.05$). In repeat experiments dry matter production was measured, (figs. 5.2a (VO_3^-) and 5.2b (VO^{2+})). Root dry matter production appeared unaffected, although certain qualitative differences were apparent. Shoot dry matter production was significantly reduced by applied vanadium. Treatments which vary significantly from one another as determined by an SNK test are shown in table 5.1.

These results show similar trends to those of Brenchley (1932) who, using barley, demonstrated that shoots were much more susceptible to damage by vanadium than roots. In roots no sign of adverse effects was observed in concentrations below 40 ppm in the nutrient solution but depressions in shoot dry weight production were evident at much lower concentrations.

Tissue vanadium levels were also measured and are represented graphically in figs. 5.3a (root) and 5.3b (shoot). Root vanadium levels increase with an increased supply of vanadium to the tissue. No difference could be detected in the

fig. 5.1 Maize fresh weight production as affected by applied vanadium.

- (a) vanadium applied as NH_4VO_3
- (b) vanadium applied as VOSO_4

fig. 5.1a

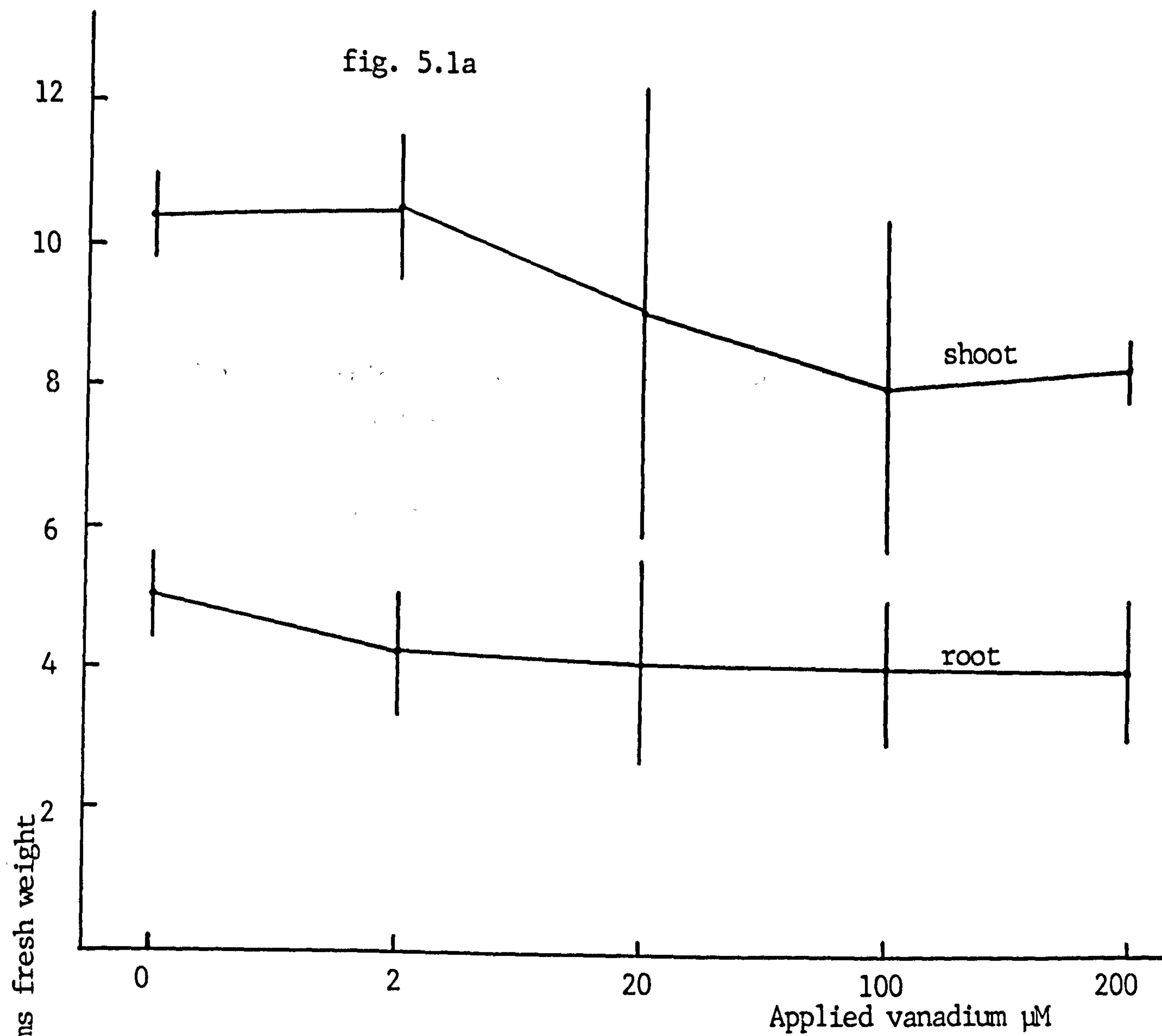


fig. 5.1b

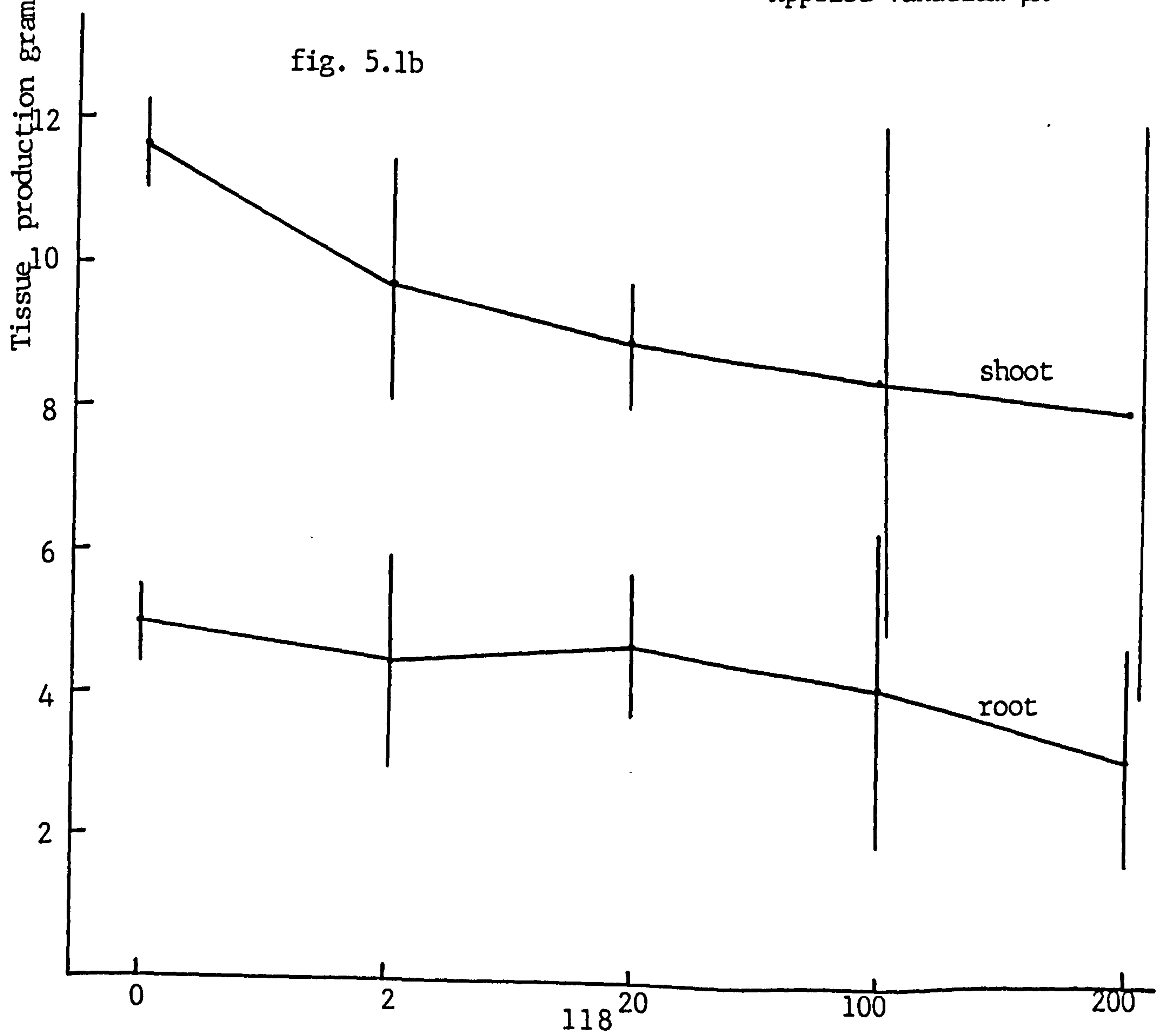


fig. 5.2 Maize dry weight production as affected by applied vanadium.

(a) vanadium applied as NH_4VO_3

(b) vanadium applied as VOSO_4

fig. 5.2a

Tissue production grams dry weight

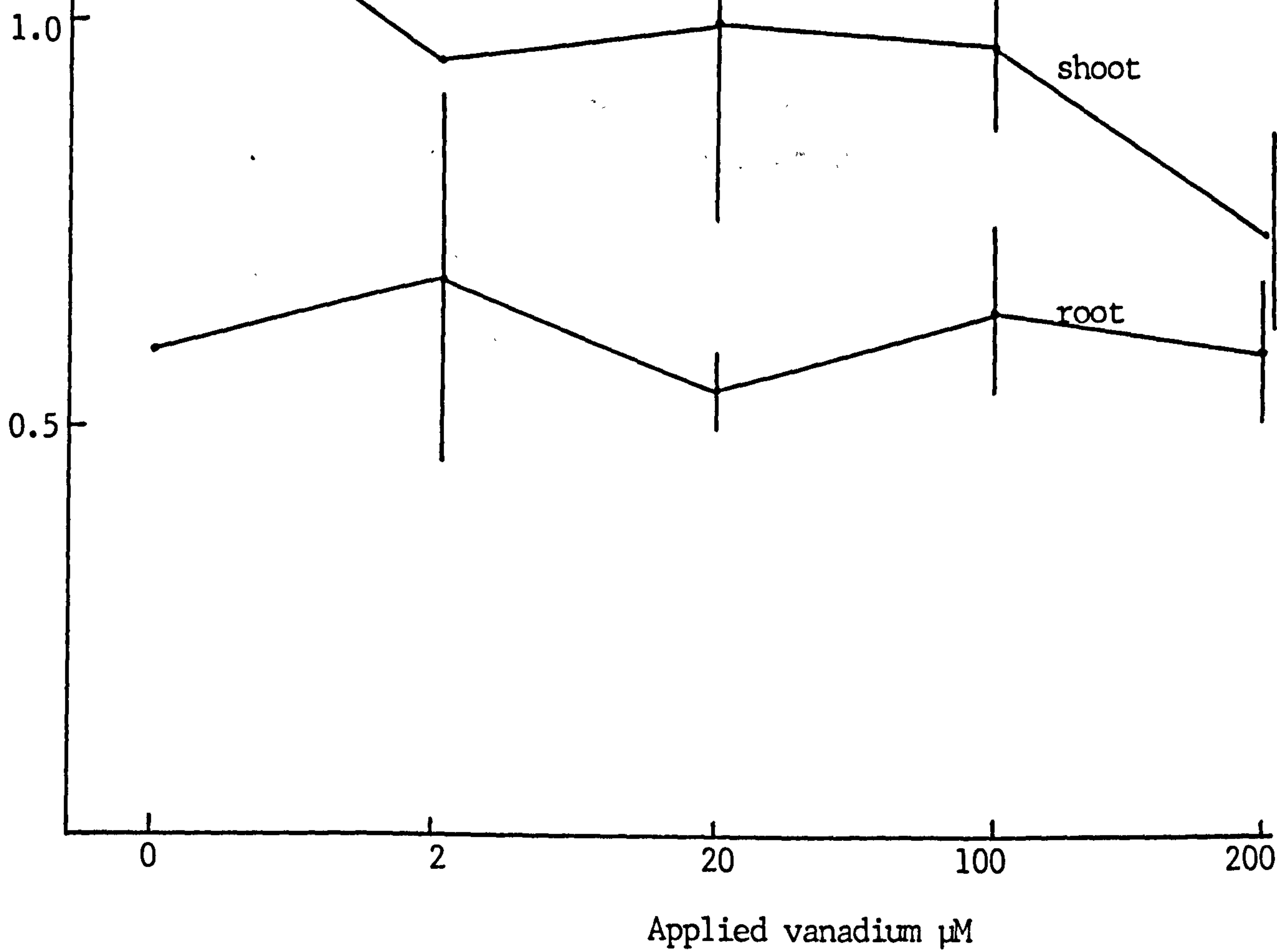


fig. 5.2b

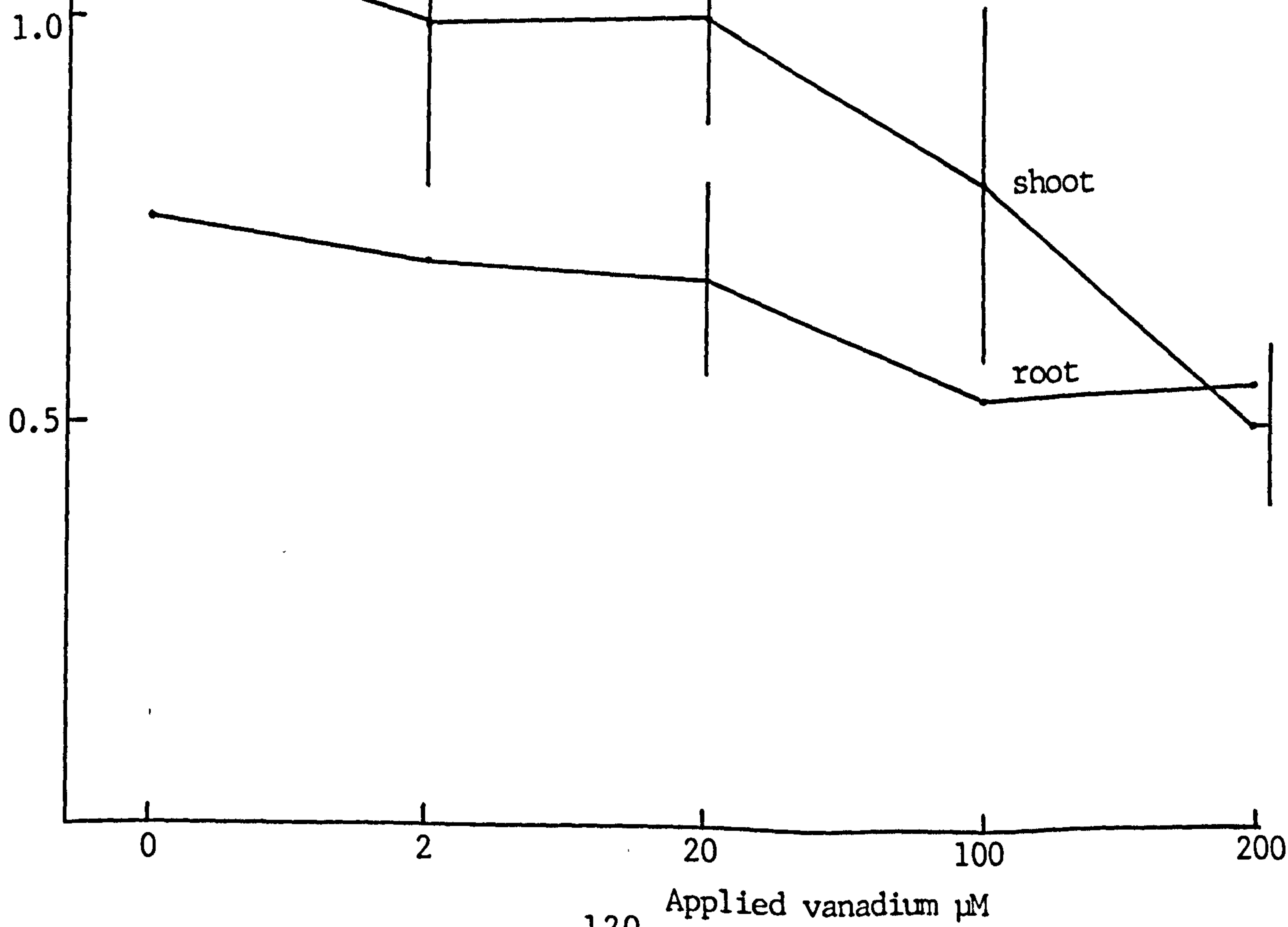


Table 5.1 Analysis of variance of dry matter production in shoots of maize plants treated with two different forms of vanadium at four different concentrations.

Table 5.1

Anova for shoots treated with different levels of the vanadate anion.

Source	SS	DF	MS	F
Between	0.38017	4	0.909504	4.169*
Within	0.22800	10	0.02280	
Total	0.60817	14		

A D C B E

Anova for shoots treated with different levels of the vanadyl cation.

Source	SS	DF	MS	F
Between	0.799	4	0.1997	6.306*
Within	0.317	10	0.0312	
Total	1.116	14		

A C B D E

Where A = control, B = 2 μ M, C = 20 μ M, D = 100 μ M and E = 200 μ M.

Any two treatments joined by a line are considered not to be significantly different from one another.

fig. 5.3 Vanadium levels in maize plants treated with vanadium at four different concentrations and in two ionic forms.

(a) roots

(b) shoots

x — x vanadium applied as VOSO_4
• — • vanadium applied as NH_4VO_3

Fig.5.3a

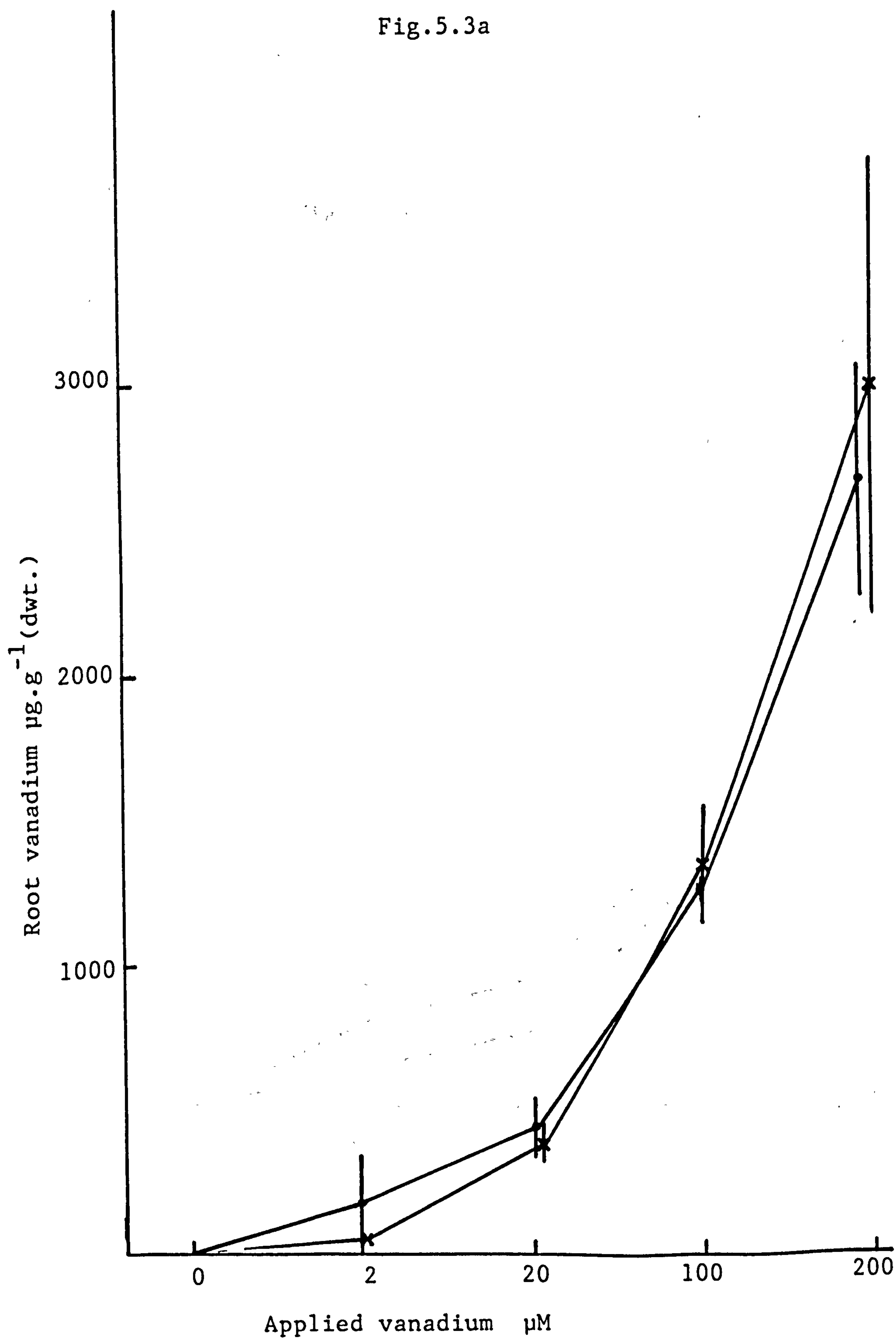
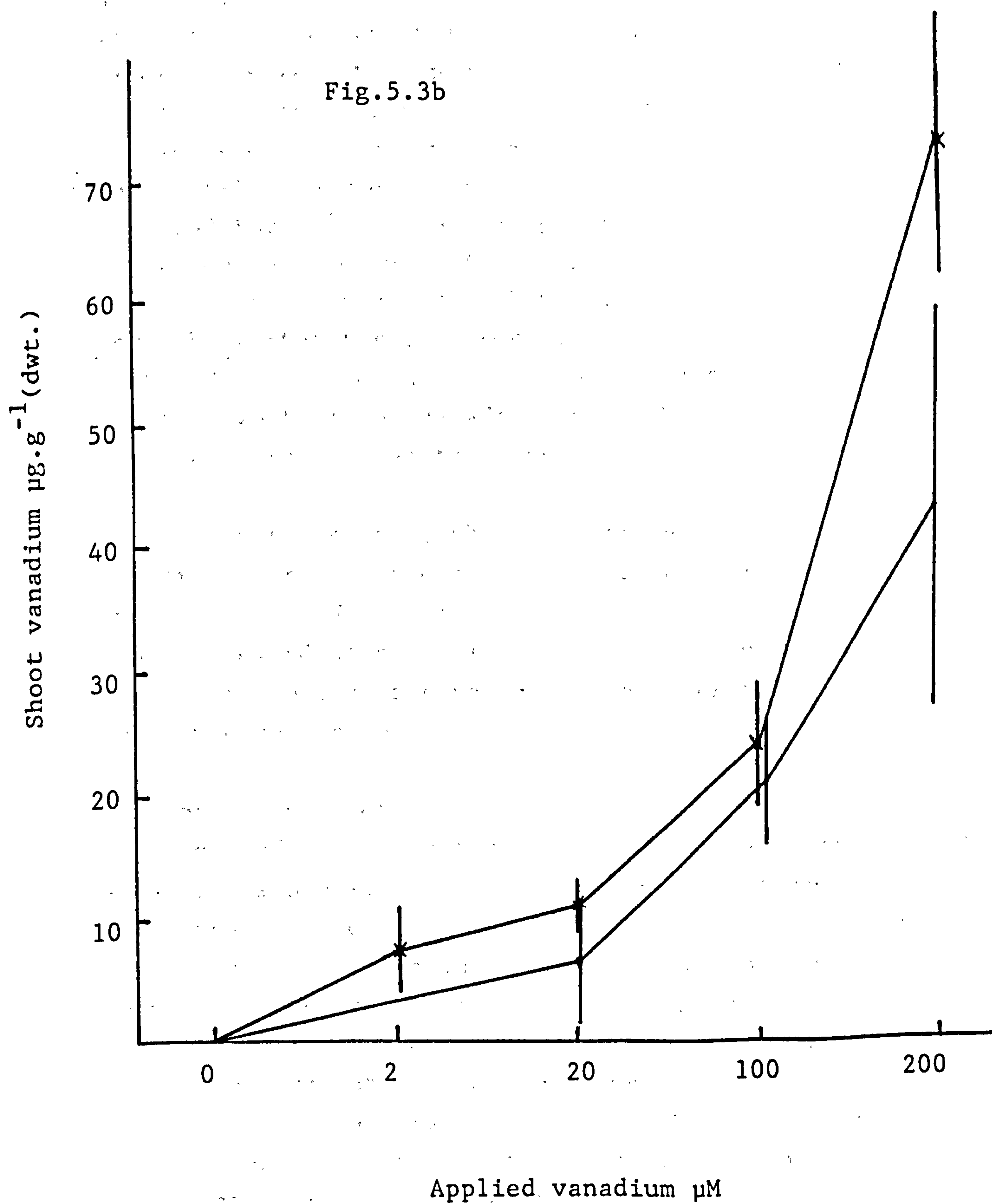


Fig.5.3b



root uptake of the two different forms in this experiment (table 5.2a) although this was not always the case. Shoot levels can also be seen to increase with an increased supply of vanadium to the plant. Statistical analysis reveals this to be a significant effect and also shows some degree of interaction (table 5.2b). This interaction is only marginally significant, the tabulated value being 2.24($P < 0.05$).

Because of the variable nature of these experiments each was repeated several times. Results from these repeated experiments were then pooled to try and reach some conclusions about root/shoot relationships for the two different valence states used. As was expected both forms exhibited high correlations between root and shoot vanadium levels ($r^2 = 0.74$ and 0.68 for VO_3^- and VO^{2+} respectively) levels. Most of the variation in shoot vanadium levels could be explained by variation in root vanadium levels. Significant regressions could be fitted for both forms; fig. 5.4a (VO_3^-) and fig. 5.4b (VO^{2+}). A comparison of the regression coefficients for the two forms of vanadium shows them not to be significantly different from one another (table 5.3).

These results indicate that similar levels of the two different forms in the root will produce similar levels in the shoot. No definite conclusions have as yet been reached regarding the initial acquisition of vanadium by root tissue which appears to be a complex process dependent upon numerous factors.

The antagonistic effect of one trace element upon another may profoundly influence plant mineral balance and growth. Vanadium is one such element which may exert an antagonistic influence on other trace elements. Some of the inter relationships between vanadium and elements such as manganese, molybdenum and iron were examined by Warington (1951, 1954 and 1956). The following conclusions were reached;

- (a) Vanadium can counter an excess of manganese in plants and reduce the toxicity of manganese.
- (b) Vanadium is unable to replace molybdenum in the nutrition of plants.
- (c) Vanadium toxicity can be reduced by alteration of iron supply.

Table 5.2a.

The effect of ionic form and concentration on vanadium uptake by Maize roots.

Table 5.2b.

The effect of ionic form and concentration on vanadium uptake by Maize shoots.

Table 5.2a.

Source of variation	df	SS	Ms	F	Sig.
Cells	7	27890816			
Ionic form	1	6646.8	6646.8	0.06	NS
Concentration	3	27777083.0	9259027.9	84.63	**
Ionic form X Concentration	3	107086.3	35695.4	0.33	NS
Within	16	1750588.0	109411.8		
Total	23				

Table 5.2b.

Source of variation	df	SS	Ms	F	Sig.
Cells	7	12470.8			
Ionic form	1	726.3	726.3	99	**
Concentration	3	11025.4	3675.1	49.9	**
Ionic form X Concentration	3	719.1	239.7	3.24	*
Within	16	1177.7	73.6		
Total	23	1348.5			

fig. 5.4 Regression lines illustrating the relationship between root vanadium levels and shoot vanadium levels.

(a) vanadium applied as NH_4VO_3

(b) vanadium applied as VOSO_4

Fig. 5.4a

$r^2 = 0.741$
 $b = 0.015$
 $P = 0.01$

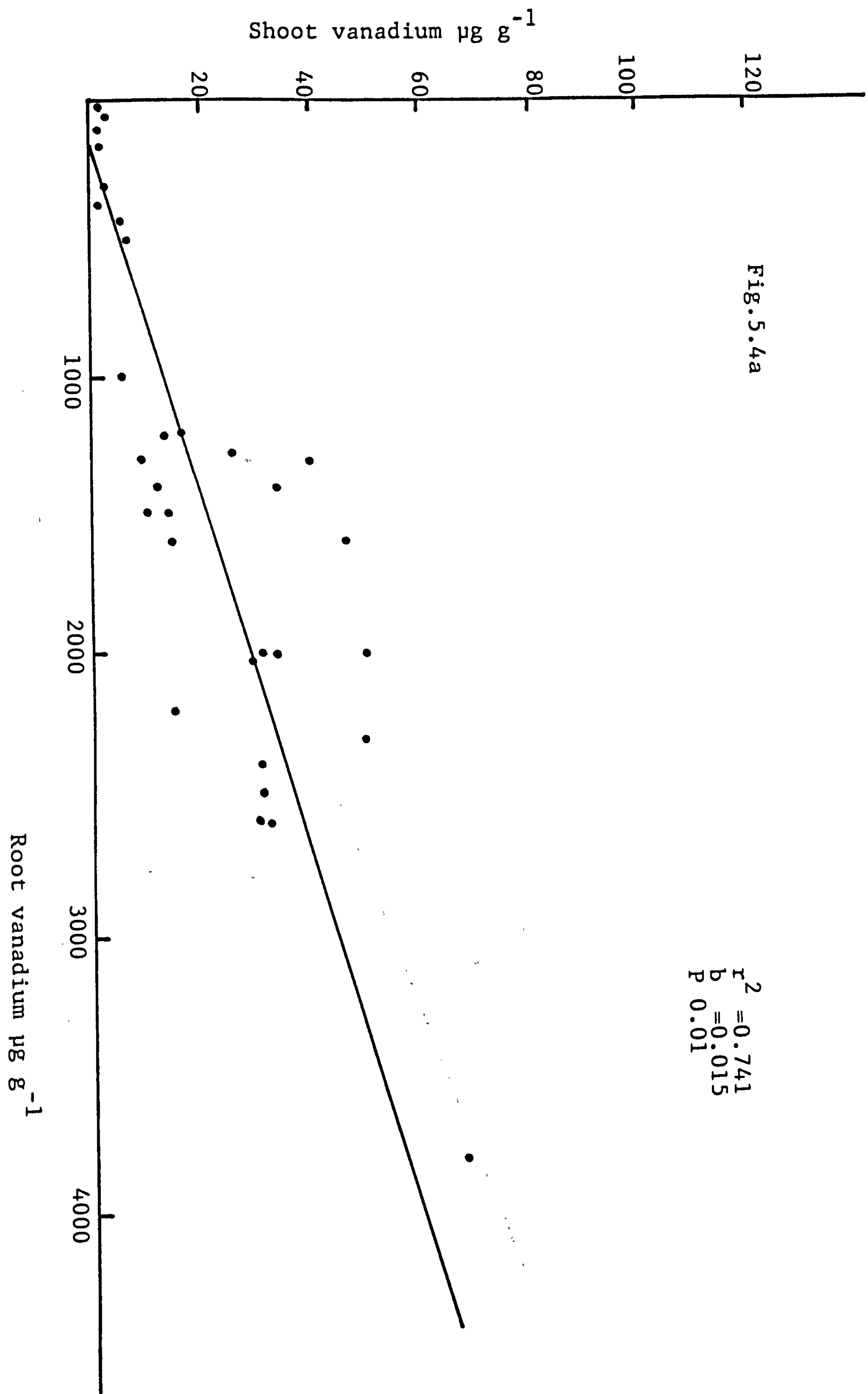


Fig. 5.4b

$r^2 = 0.682$
 $b = 0.019$
 $P 0.01$

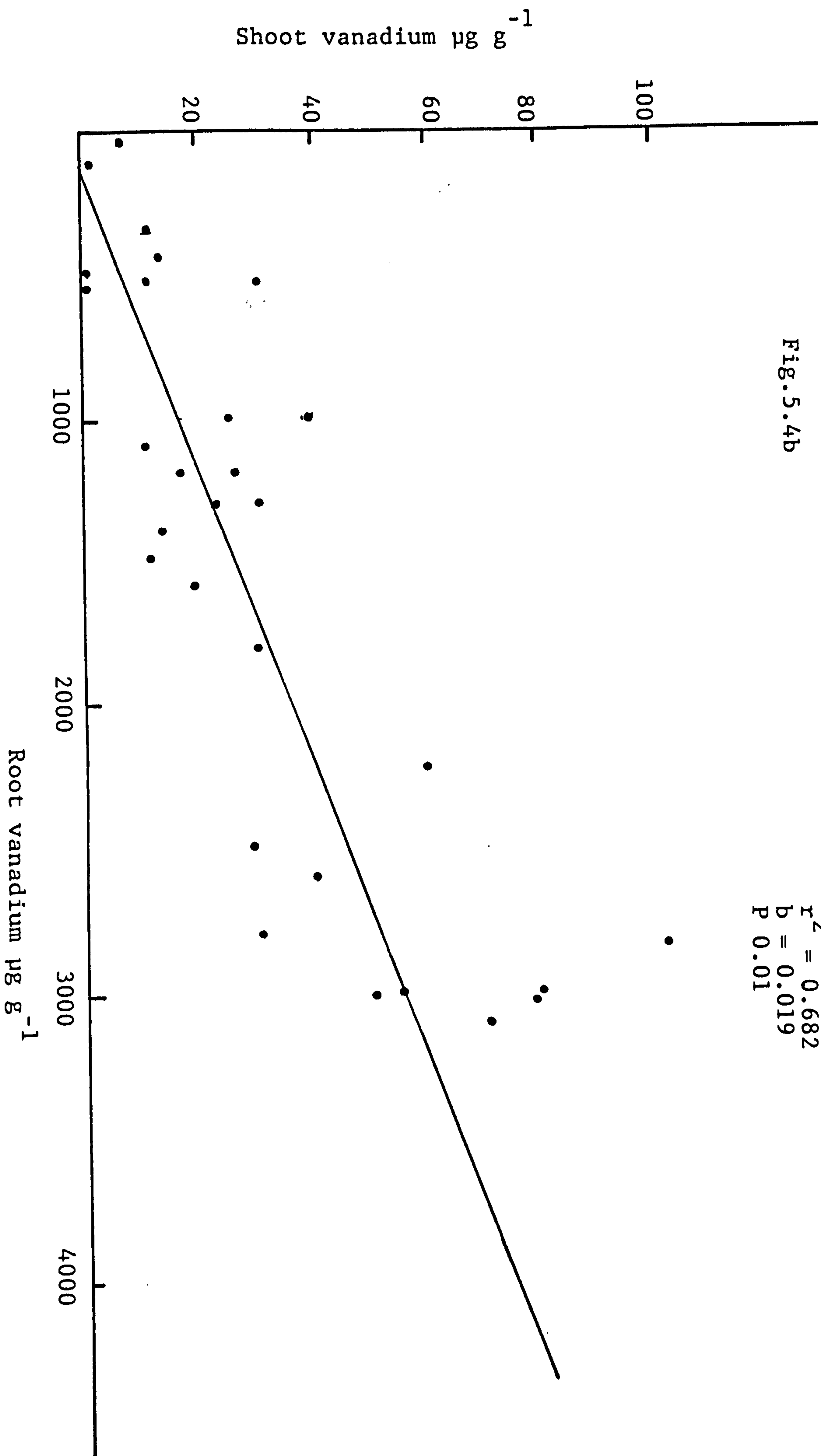
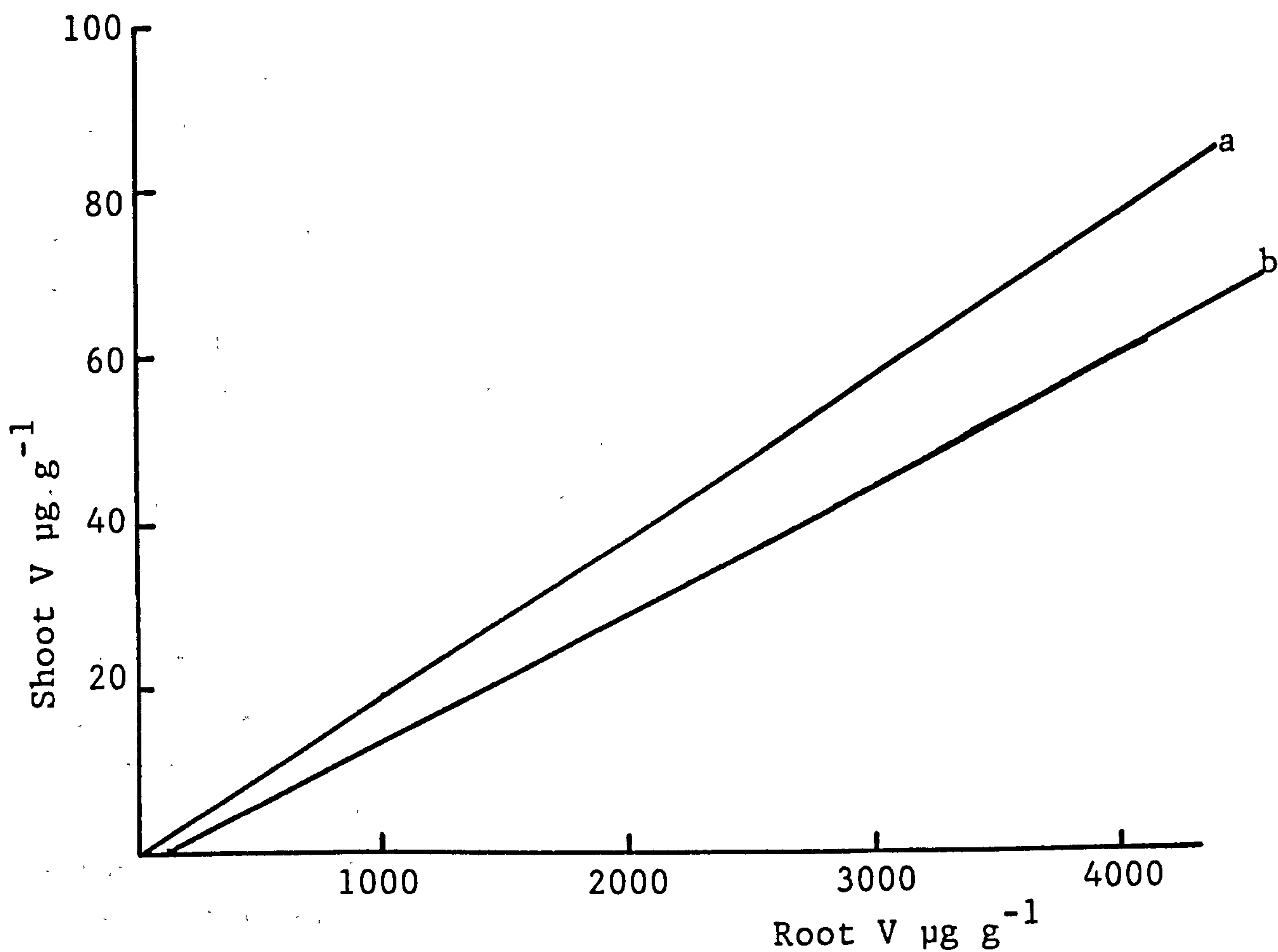


Table 5.3 A comparison of regression coefficients
which describe the relationship between root
and shoot vanadium levels for maize supplied
with vanadium in two different forms.



$\text{VO}^{2+}(\text{a})$

$$r^2 = 0.68$$

$$Y = 0.019X - 0.022$$

$\text{VO}_3^-(\text{b})$

$$r^2 = 0.74$$

$$Y = 0.015X - 1.58$$

Comparison of regression coefficients.

n	41	41
$\text{sum}(x - \bar{x})^2$	4.60×10^7	3.81×10^7
variance	202.23	79.86
b	0.019	0.015

$D = 1.154$ not significant $P = 0.05$

Since little or no further work has occurred on the nature of vanadium - nutrient interactions it was considered prudent to at least attempt a basic re-examination of the area. To this end plants grown earlier were analysed for iron, manganese and calcium content. Tissue levels for each of the elements (iron, manganese and calcium) have been plotted against the applied level of vanadium and where significant difference in ion tissue levels has been observed this is indicated on the graph.

Regardless of the applied form of vanadium, no significant differences were produced in iron levels of root or shoot tissue (fig. 5.5a and fig. 5.5b). In contrast both shoot and root manganese levels were significantly affected by vanadium applied as VOSO_4 (fig. 5.6b) and root levels significantly affected by vanadium applied as NH_4VO_3 (fig. 5.6a). Interpretation of these effects is difficult since no direct linear relationship appears to exist. In the case of calcium, vanadium applied as VOSO_4 significantly affects root levels (fig. 5.7b) but no other significant affects were apparent (fig. 5.7a).

The correlation matrices (tables 5.4a and 5.4b) suggest numerous possible relationships but as might be expected on the basis of results already presented few of these represent simple linear relationships. The highest r^2 value was 0.51 suggesting that more than one factor affects the level of the ion in a tissue. The only relationship which appeared significant irrespective of applied vanadium form was that between root vanadium and shoot calcium. The nature of this relationship will be discussed later.

Uptake of numerous metal ions has been shown to vary with time (Jones et al 1973, Jarvis et al 1976 and McGrath 1982). Examination of tissue levels on a concentration basis can sometimes produce misleading results especially when uptake rate and growth rate vary. At present little data relating to temporal variations in vanadium uptake are available. Singh (1971) gave some indications of mineral content changes to be expected over a period of time but made no reference to vanadium levels over this period.

fig. 5.5 Tissue iron levels as affected by applied vanadium.

(a) vanadium applied as NH_4VO_3

(b) vanadium applied as VOSO_4

Where a significant difference in tissue iron levels is detected by AOV it is marked on the graph (*).

fig. 5.6 Tissue manganese levels as affected by applied vanadium.

(a) vanadium supplied as NH_4VO_3

(b) vanadium supplied as VOSO_4

Where a significant difference in tissue manganese levels is detected by AOV it is marked on the graph (*).

fig. 5.6a

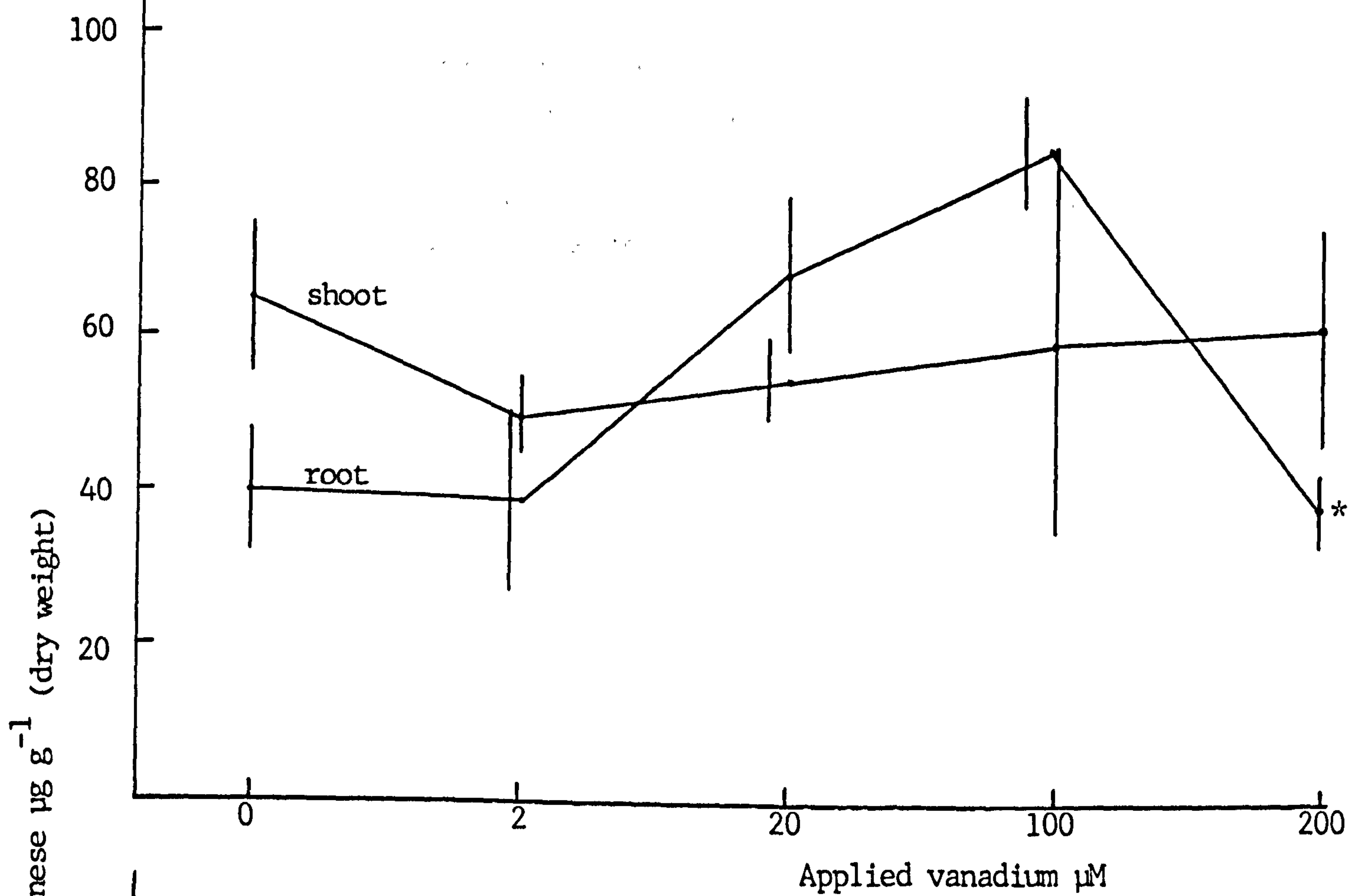


fig. 5.6b

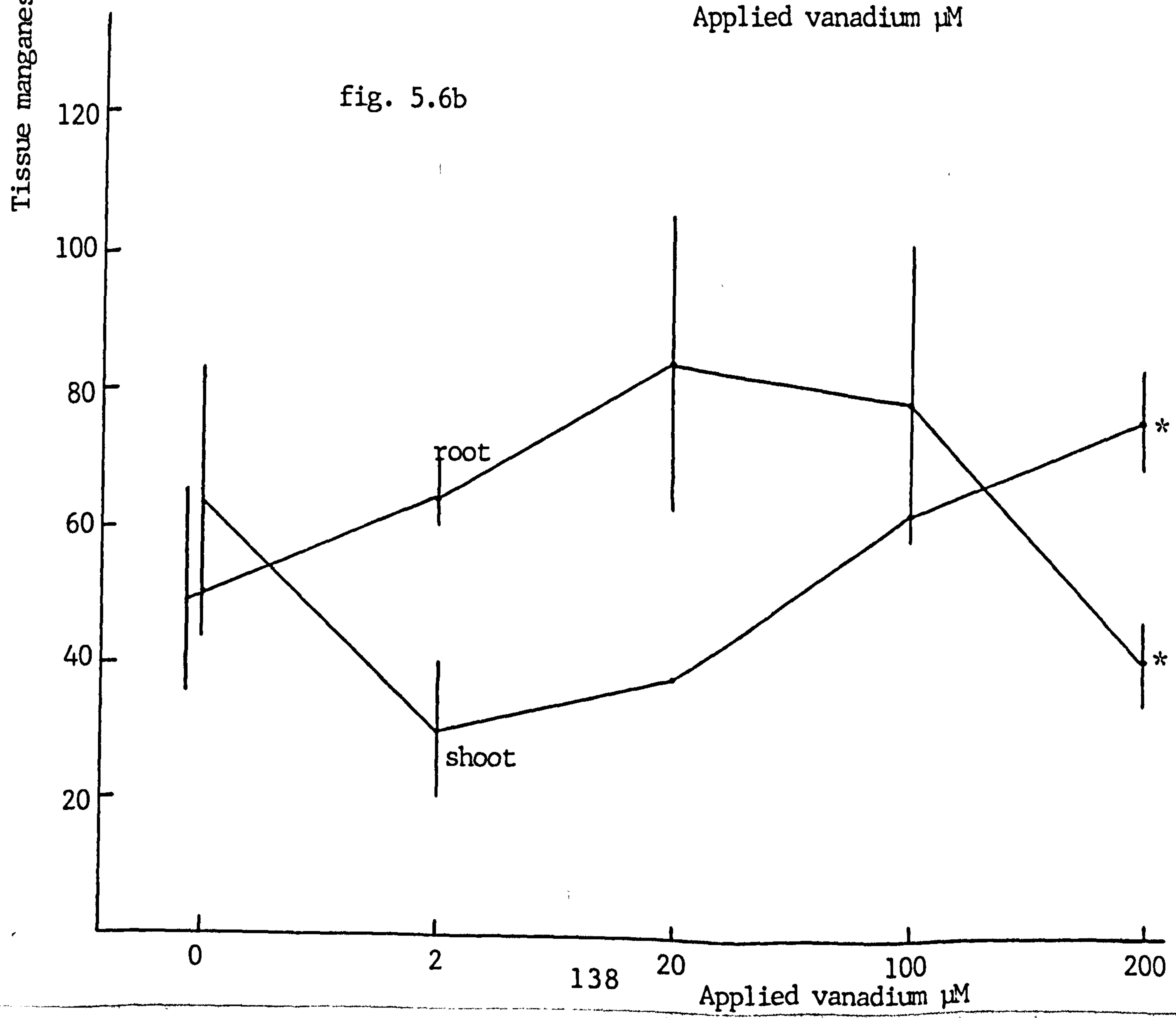


fig. 5.7 Tissue calcium levels as affected by applied vanadium.

(a) vanadium supplied as NH_4VO_3

(b) vanadium supplied as VOSO_4

Where a significant difference in tissue calcium levels is detected by AOV it is marked on the graph (*).

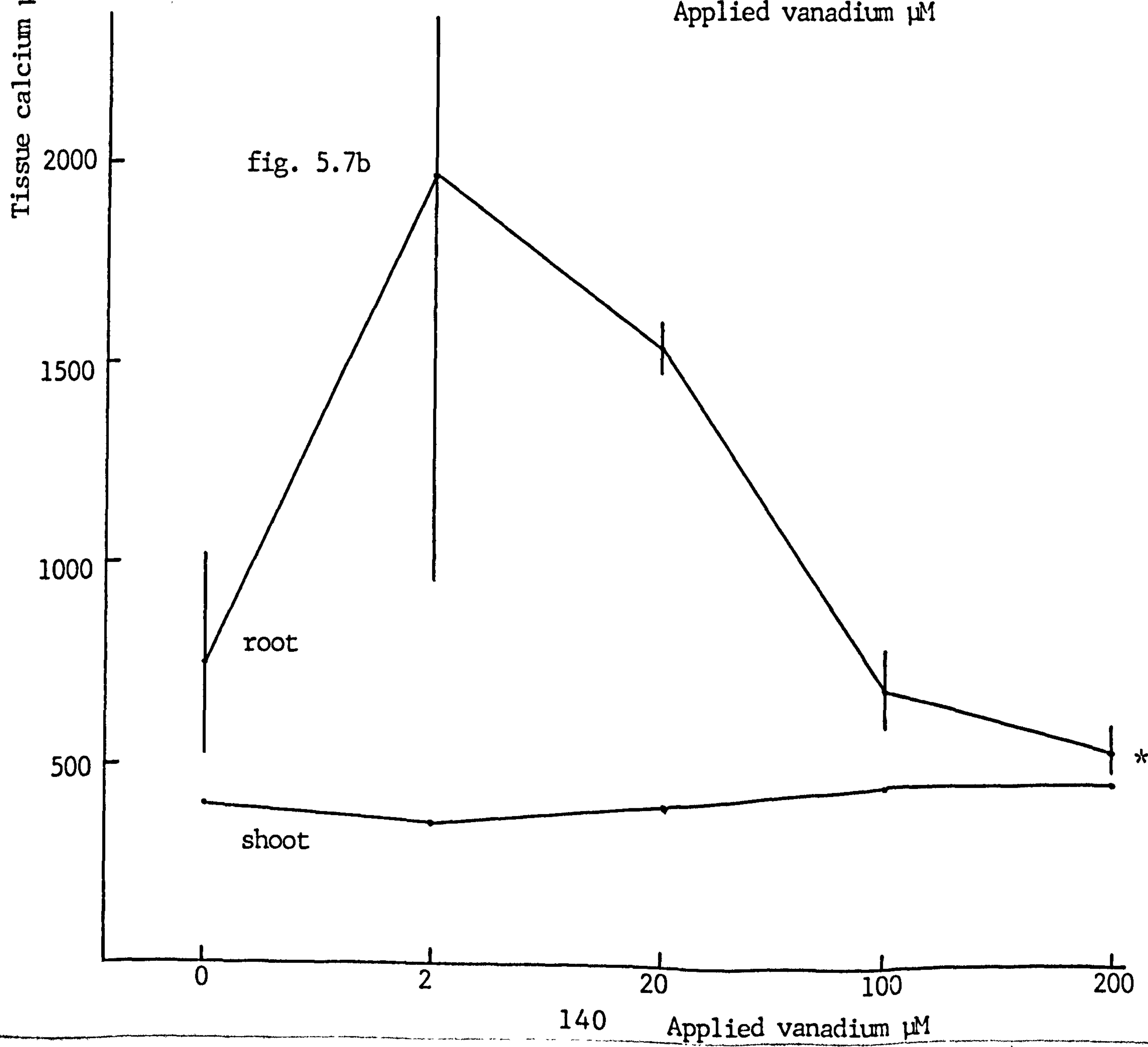
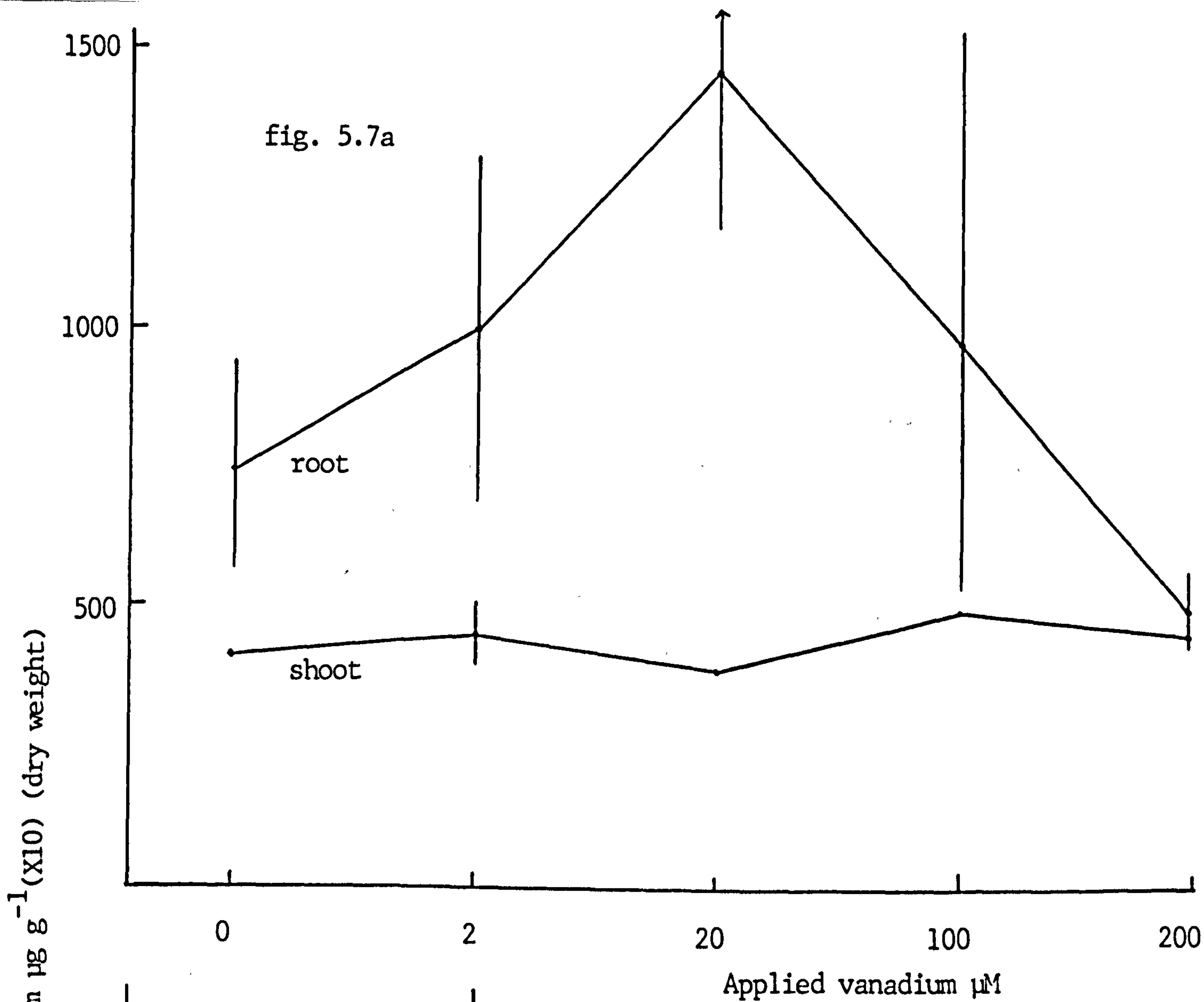


Table 5.4 Correlation matrices to illustrate relationship between various ions in root and shoot tissues of maize.

(a) vanadium applied as VOSO_4

(b) vanadium applied as NH_4VO_3

Table 5.4a

Tissue nutrient levels as affected by applied vanadyl cation.

CORRELATION MATRIX

Variable

Root V	1.0							
Shoot V	**	1.0						
Root Fe			1.0					
Shoot Fe				1.0				
Root Mn		*			1.0			
Shoot Mn	*	*		**		1.0		
Root Ca				*		**	1.0	
Shoot Ca	*			*		**	*	1.0
	RV	SV	RFe	SFe	RMn	SMn	RCa	SCa

Table 5.4b

Tissue level as affected by applied vanadate anion.

CORRELATION MATRIX

Variable

Root V	1.0							
Shoot V	**	1.0						
Root Fe	*	*	1.0					
Shoot Fe				1.0				
Root Mn				*	1.0			
Shoot Mn				**	1.0			
Root Ca						1.0		
Shoot Ca	**	**	*		*		1.0	
	RV	SV	RFe	SFe	RMn	SMn	RCa	SCa

* = significant (P. 0.05)

** = significant (P. 0.01)

In an attempt to produce information on the type of temporal effects an experiment was planned during which the uptake of vanadium and other ions was monitored over a six week period. Maize was germinated and grown for a three week period during which no treatment was applied. After this initial treatment-free stage, vanadium was added to the nutrient solution of treatment plants at a concentration of 100 μM , as either VOSO_4 or NH_4VO_3 . Two weeks after the treatments commenced, the first plants were harvested and in each following week a further three plants from each treatment were harvested, for a six week period. Dry weights were recorded and plants were analysed for vanadium, calcium, manganese, iron and zinc.

To facilitate description of the results they will be dealt with in three groups, controls, NH_4VO_3 treated plants and VOSO_4 treated plants.

Controls.

The dry weight production of these plants increased over the six week period (fig. 5.12a). Levels of ions within the different plant tissues are given in fig. 5.13a-e (shoots) and fig. 5.14a-e (roots). Shoot levels of iron, zinc, manganese, calcium and potassium do not significantly alter over the six week period ($P < 0.05$). Similarly root levels show no significant variation over the six week period. Comparison of these levels with others obtained for maize is difficult since experimental conditions vary widely; however zinc and manganese are within the ranges reported for maize by Nair and Prabhat (1977); calcium and potassium are higher (2-3 times) than those reported for maize by Singh (1971) and iron levels are lower than those reported by Clark (1977).

NH_4VO_3 (V+5) Treated plants.

Dry weight production increased over the six week period (fig. 5.12b). The levels of iron, zinc and manganese in the shoots all vary significantly with time (fig. 5.15a, b + c $P < 0.05$). Calcium and potassium levels do not

fig. 5.12 Dry weight production of maize as affected
by applied vanadium and time.

- (a) control
- (b) NH_4VO_3 treated plants
- (c) VOSO_4 treated plants

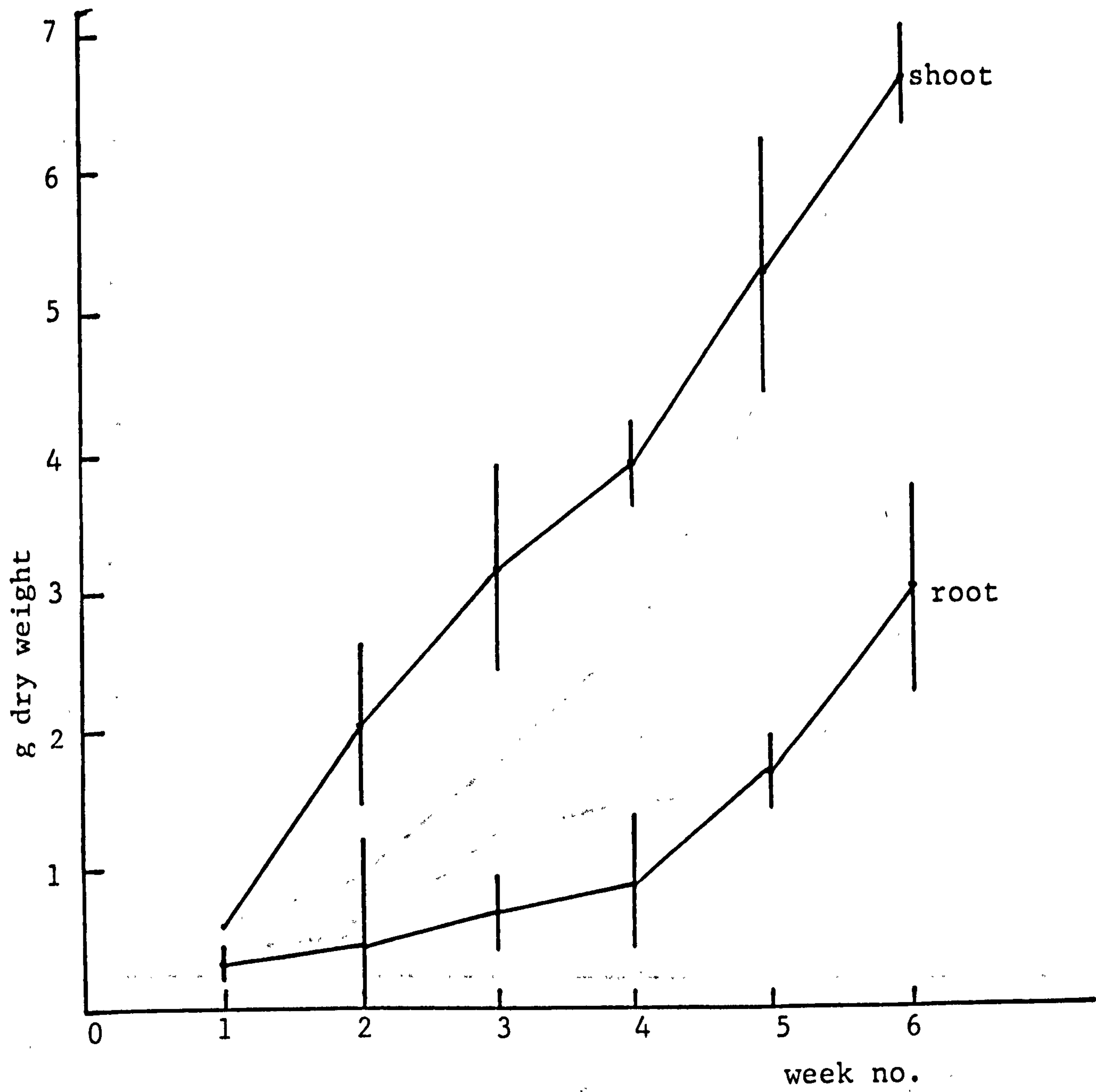


fig. 5.12a

fig. 5.12b

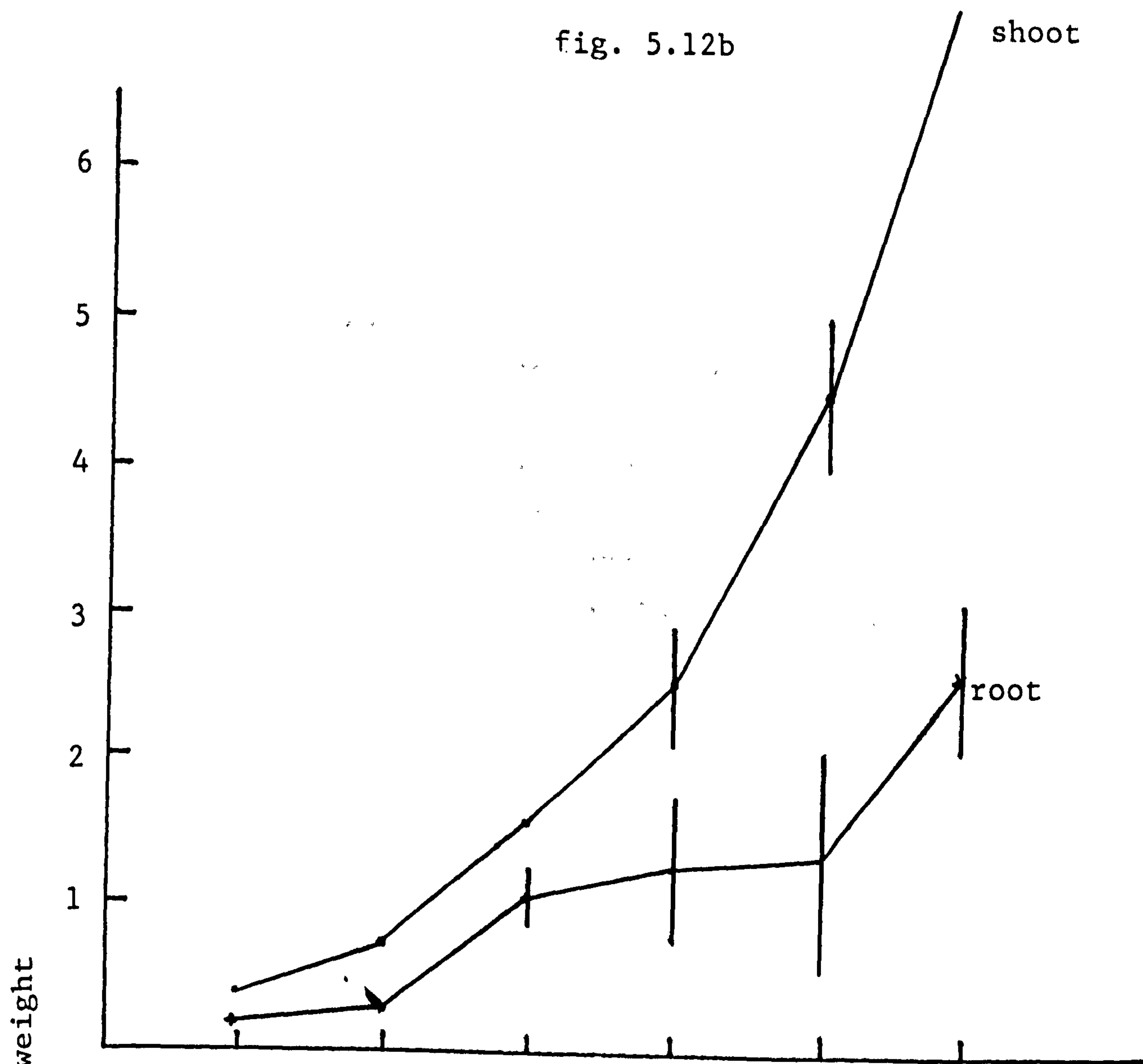


fig. 5.12c

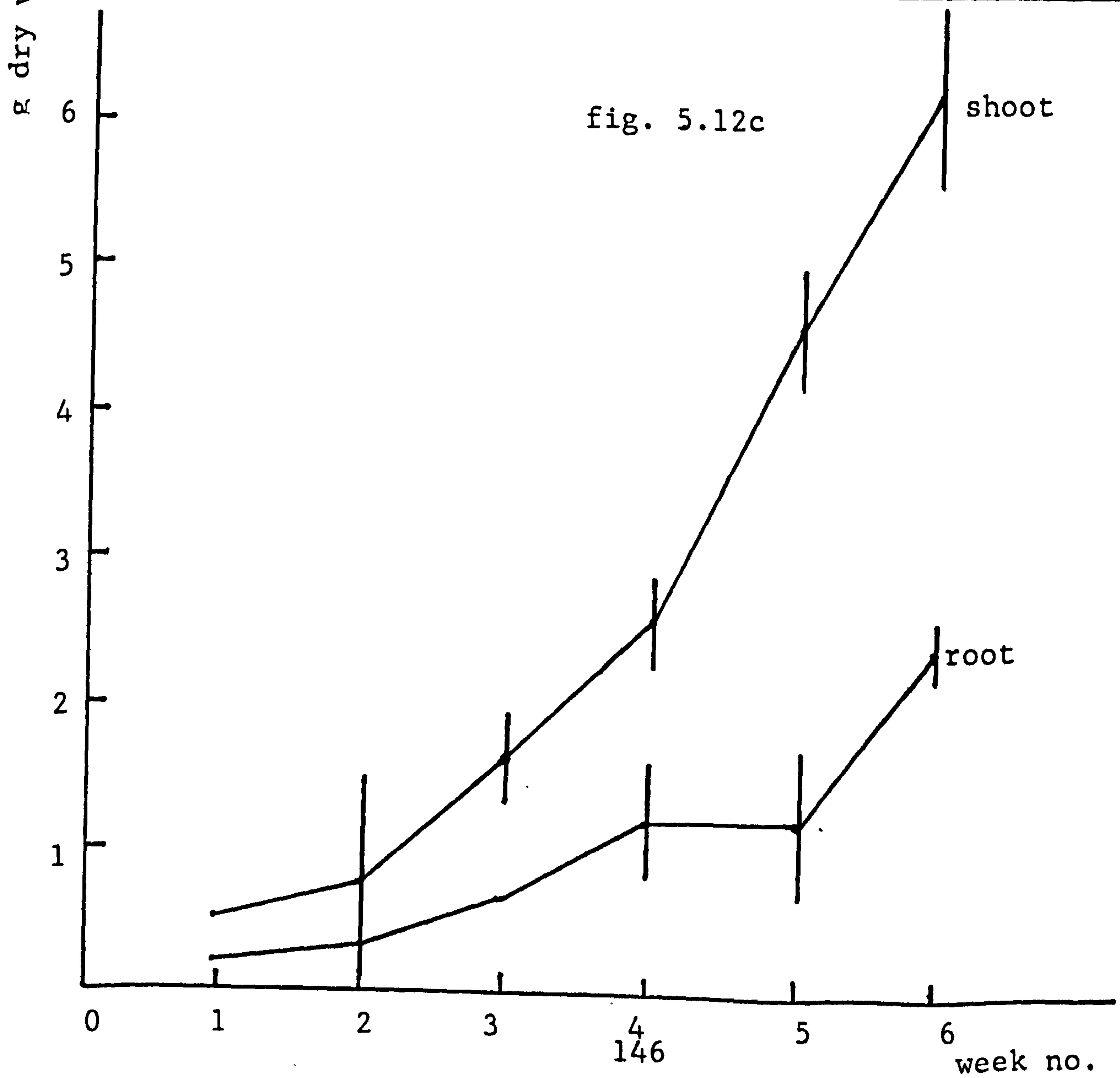
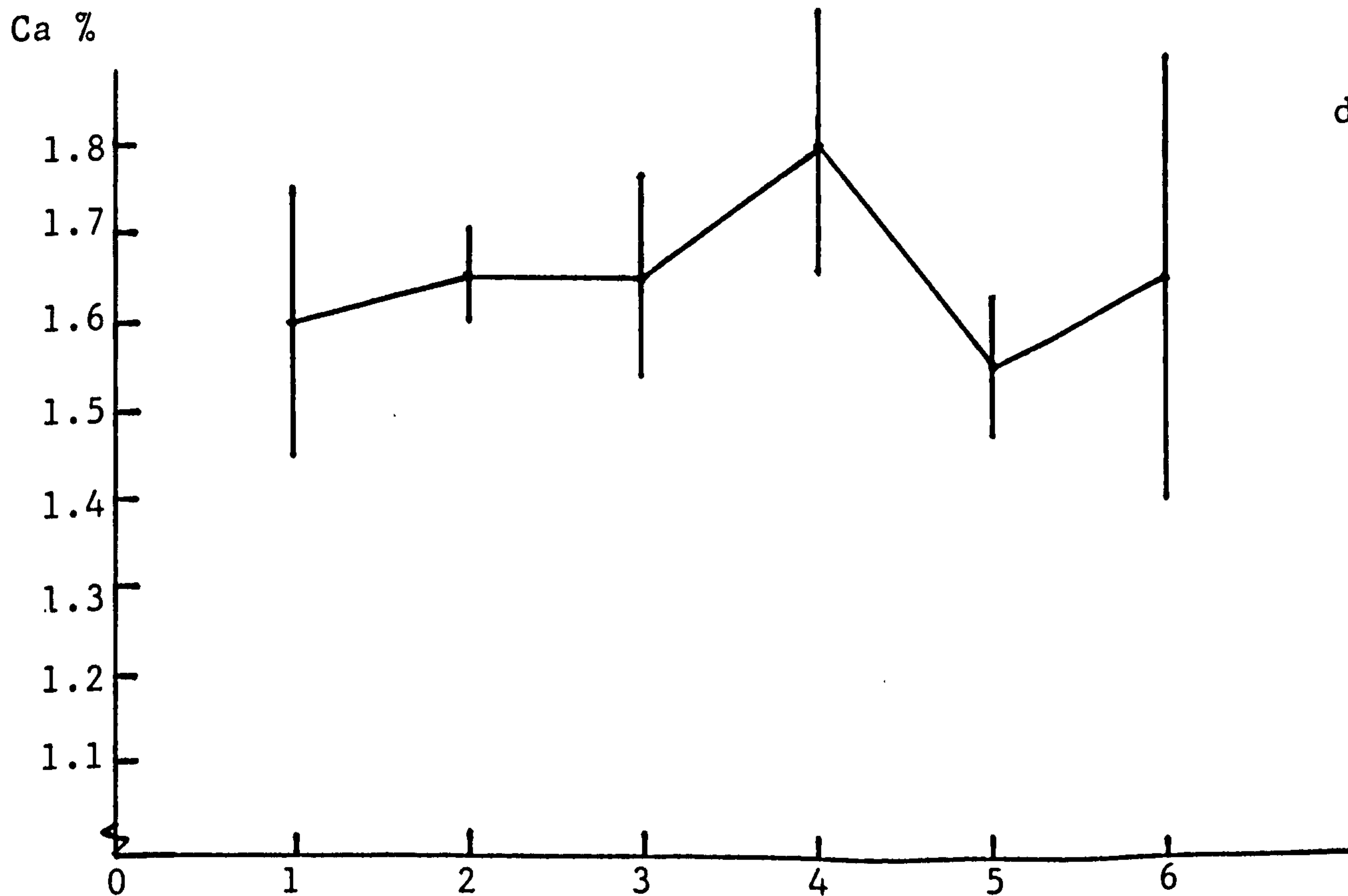
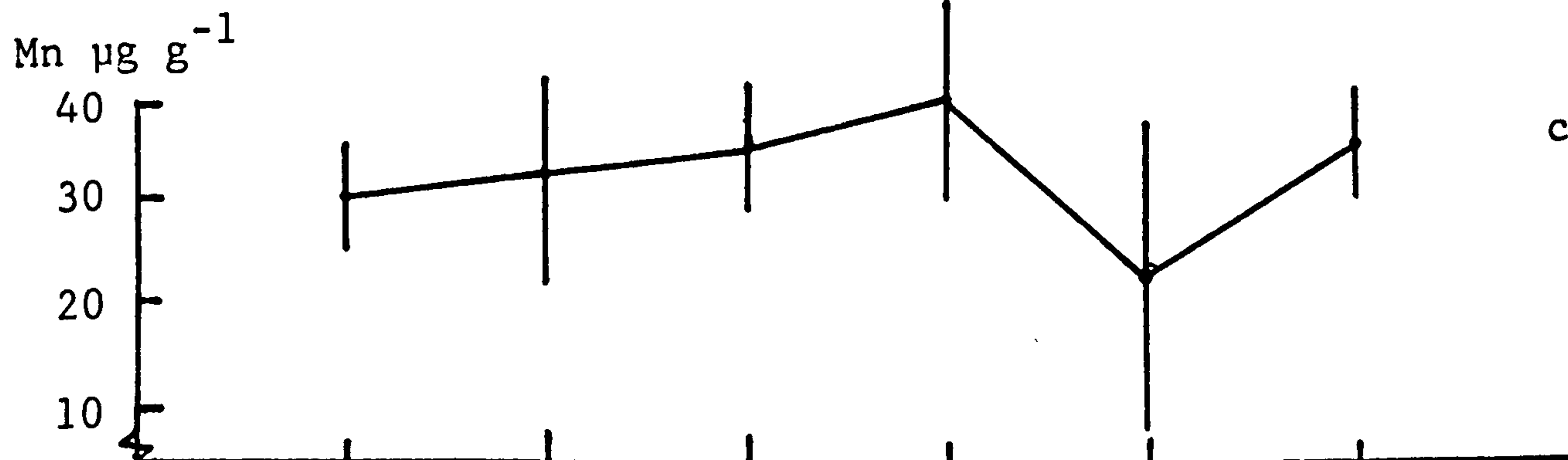
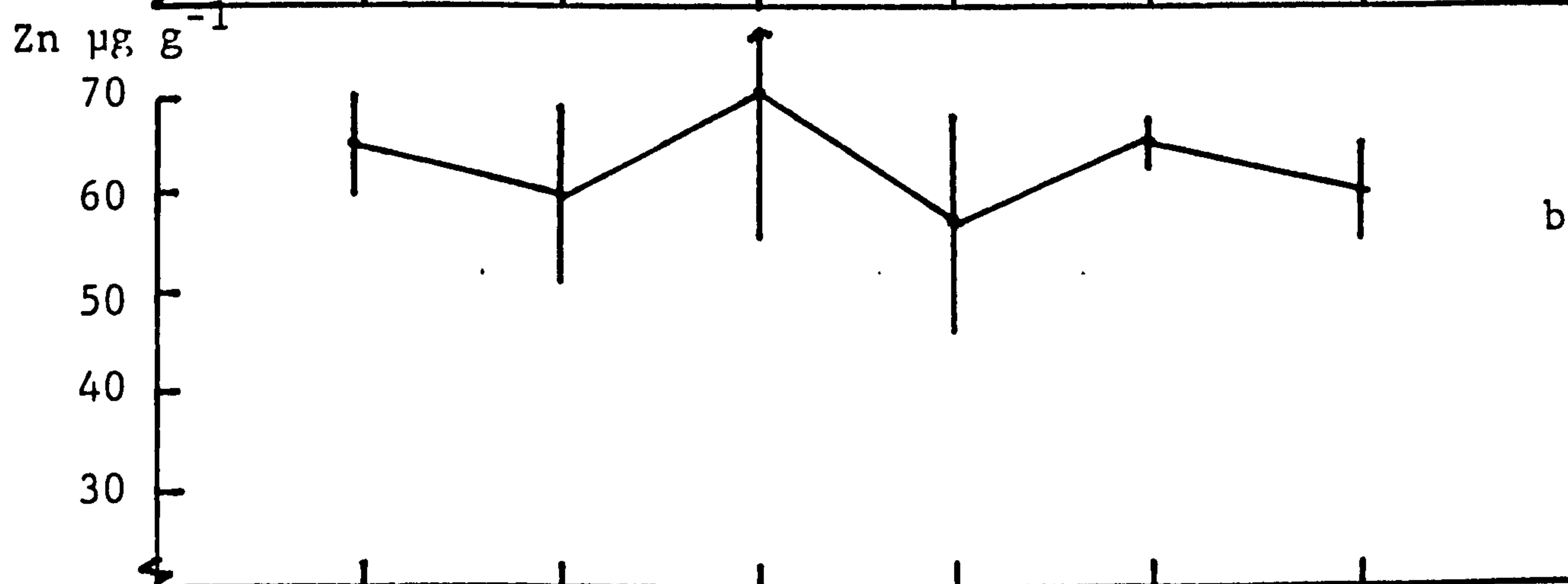
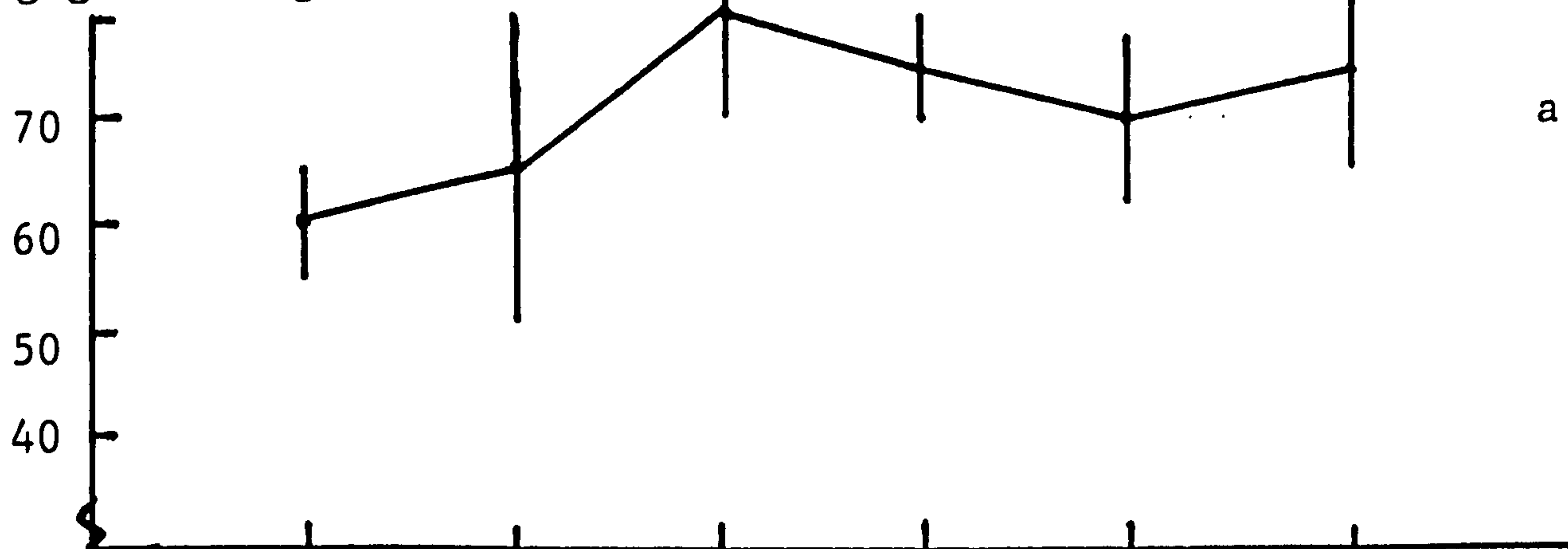


fig. 5.13 Ion levels in shoots of control plants
over a six week period.

- (a) iron
- (b) zinc
- (c) manganese
- (d) calcium
- (e) potassium

Fe $\mu\text{g g}^{-1}$ fig 5.13



week no.

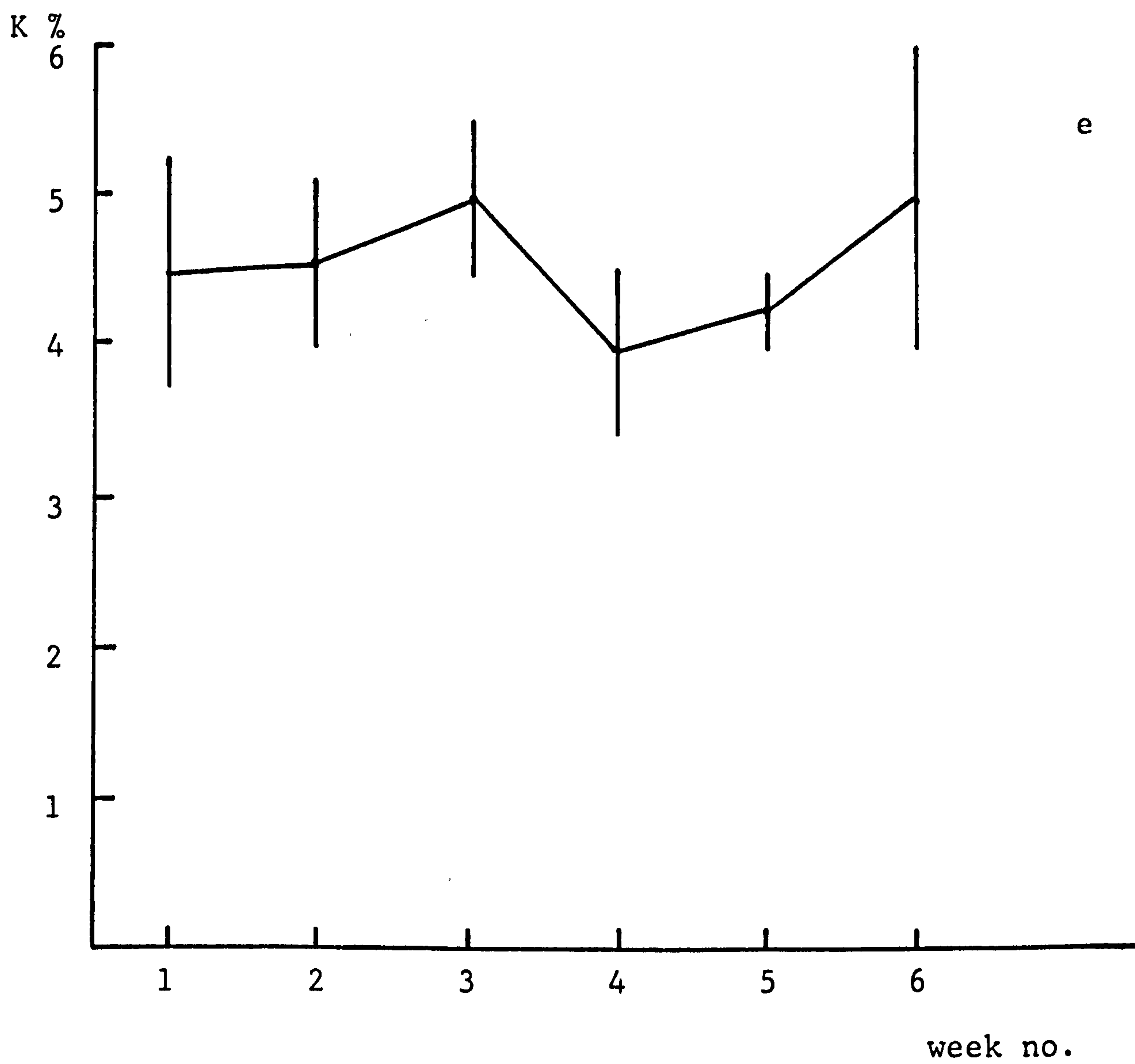
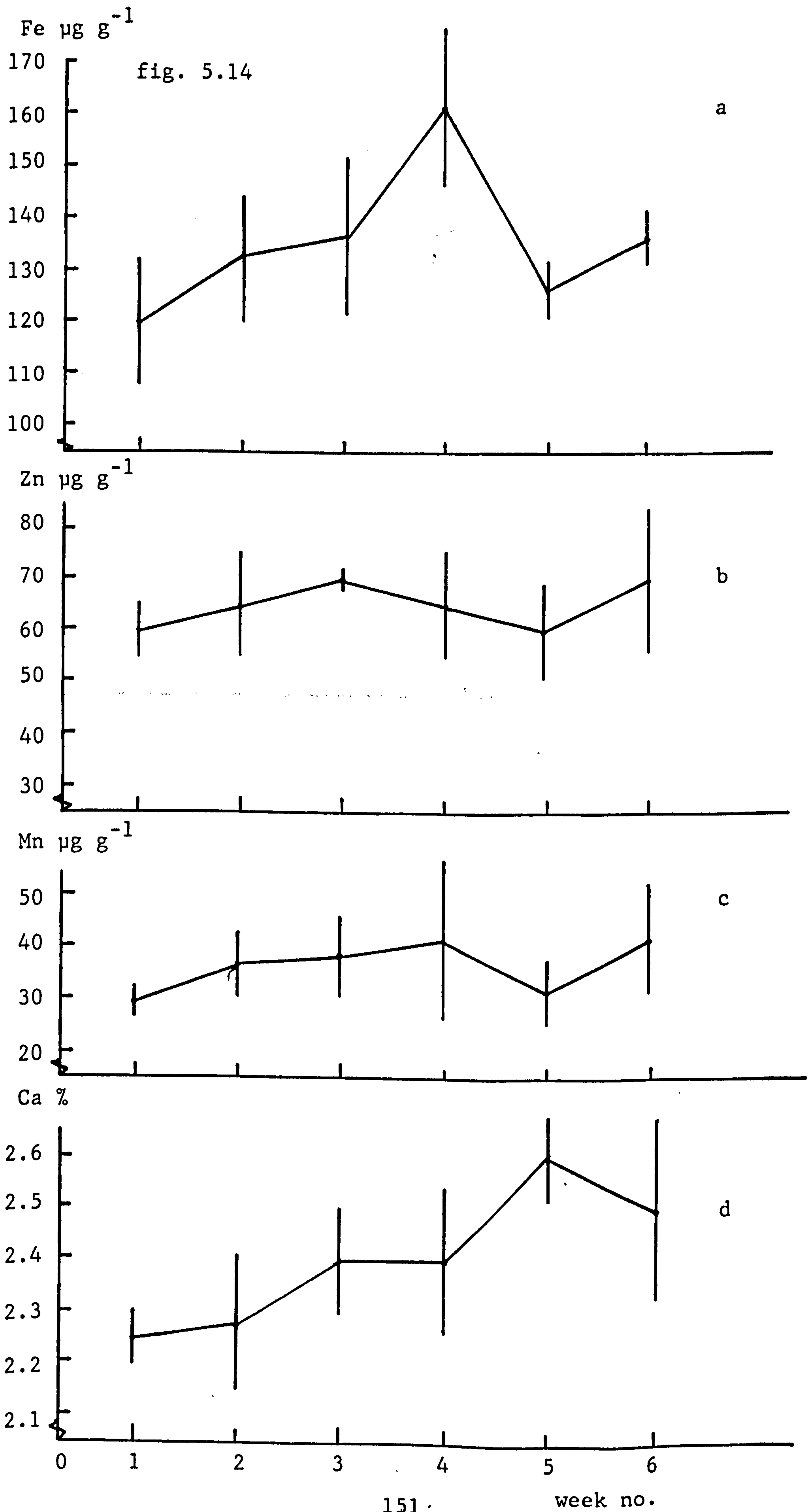


fig. 5.14 Ion levels in roots of control plants
over a six week period.

- (a) iron
- (b) zinc
- (c) manganese
- (d) calcium
- (e) potassium



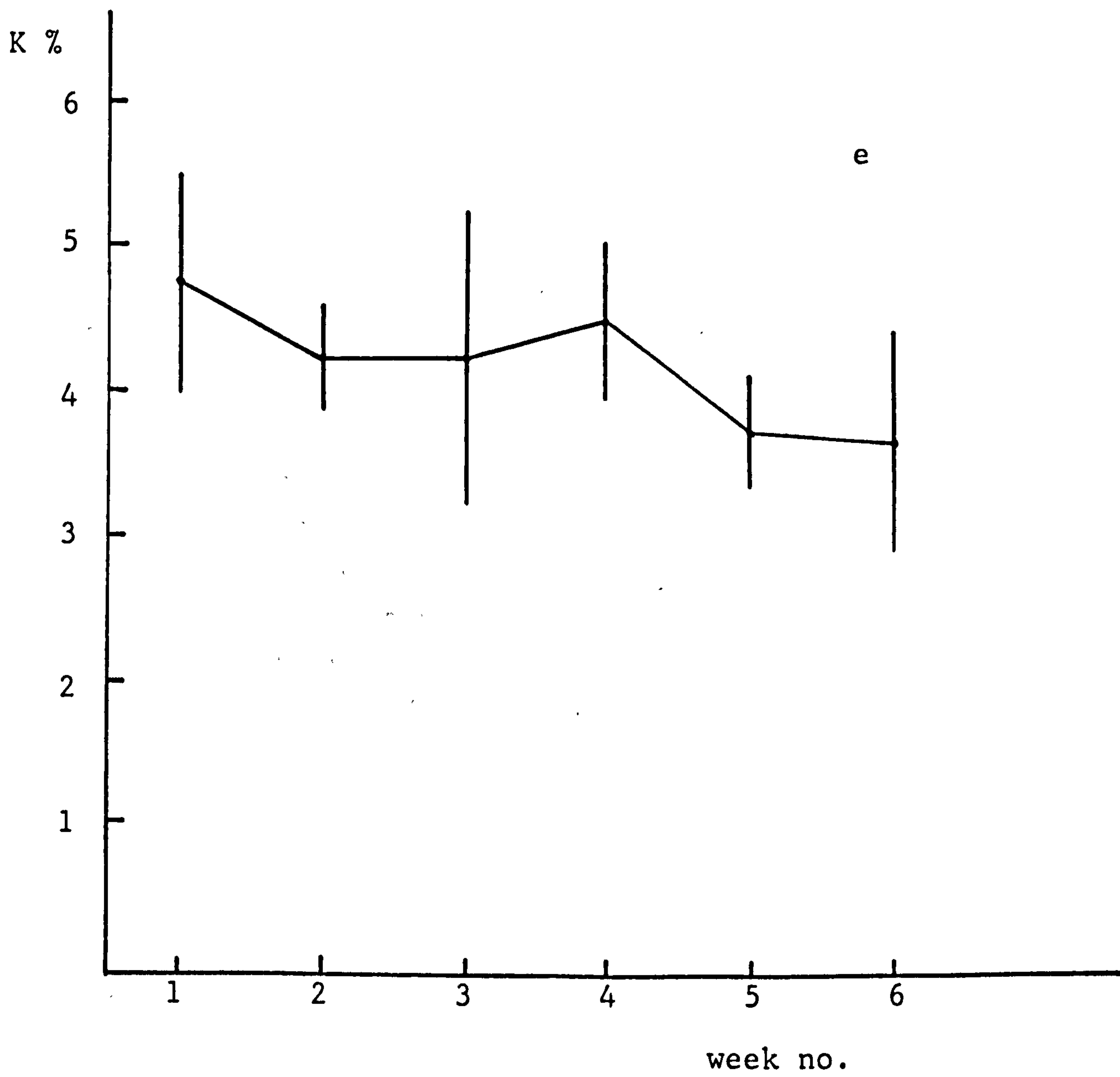
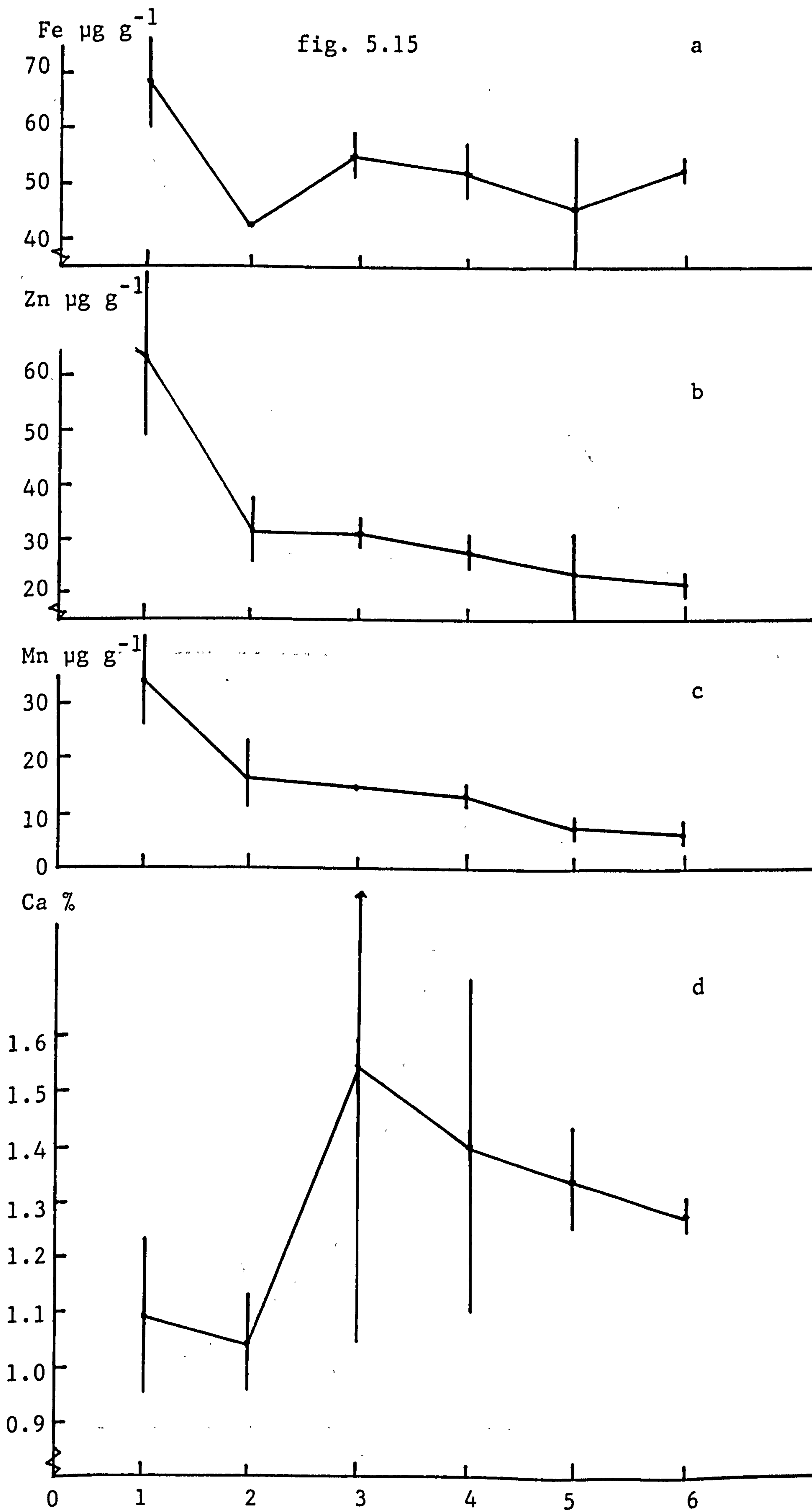


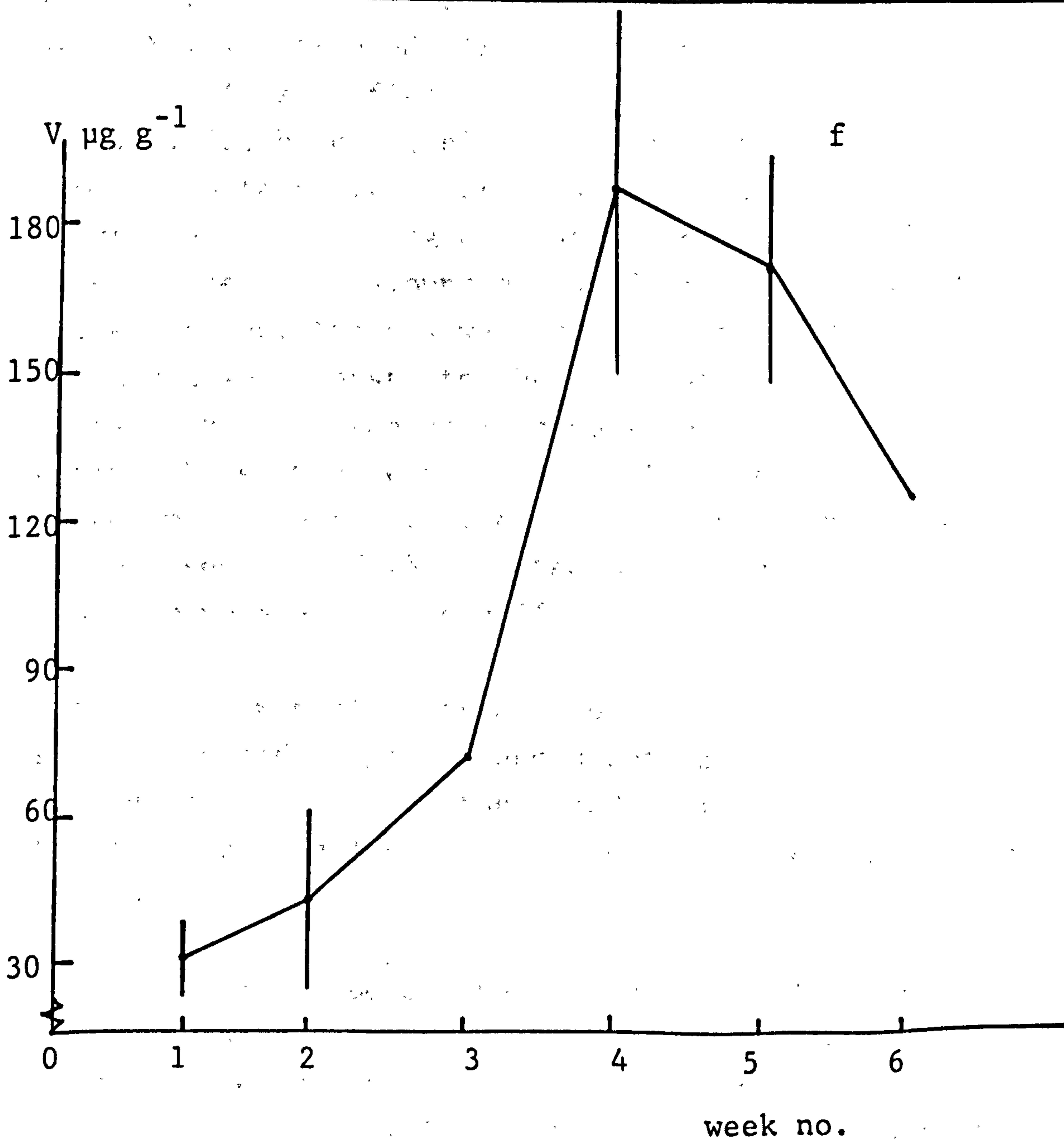
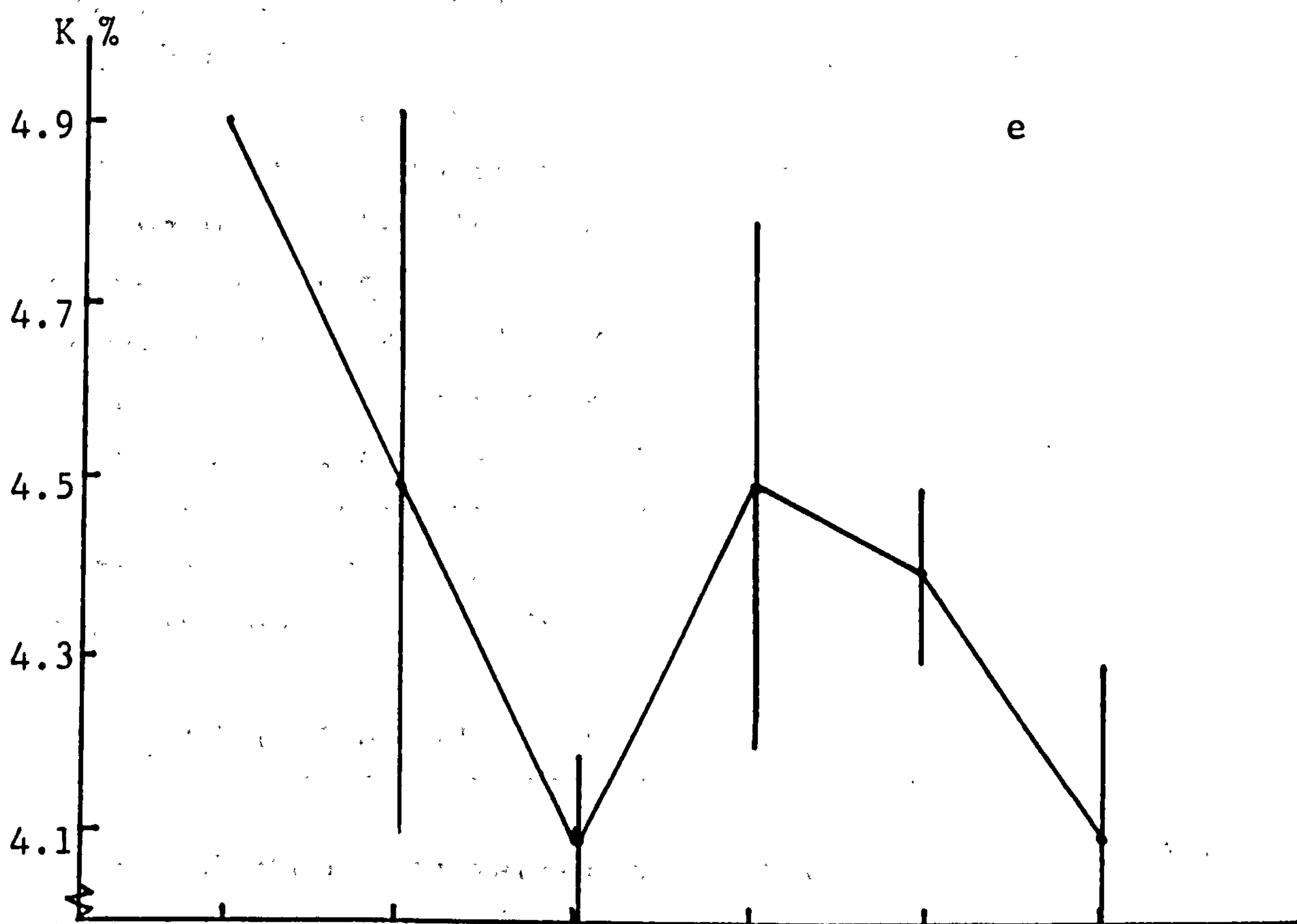
fig. 5.15 Ion levels in shoots of NH_4VO_3 treated plants over a six week period.

- (a) iron
- (b) zinc
- (c) manganese
- (d) calcium
- (e) potassium
- (f) vanadium

fig. 5.15



week no.



significantly alter with time although they are highly variable (fig. 5.15 d + e). The vanadium level of shoot tissue increases up to the fourth week and then declines (fig. 5.15f) although the total amount in the tissue continues rising until the sixth week (fig. 5.17a). Root iron levels do not vary significantly over the period (fig. 5.16a); however levels of all the other ions can be seen to significantly change over the course of the six week period (fig. 5.16 b, c, d + f). Root vanadium levels vary tremendously over the six week period (fig. 5.16e) although total root level can be seen to increase continually over the six week period (fig. 5.17b).

VOSO₄ (V+4) Treated plants.

Root and shoot dry matter production increase over the six week growth period (fig. 5.12c). Shoot ion levels vary as with V(+5) treated plants i.e. iron, zinc and manganese show significant effects calcium and potassium do not (fig. 5.18a, b, c, d, e). Shoot vanadium also shows a similar pattern i.e. increasing concentration in tissues weeks 1 - 4, declining concentration weeks 5 and 6 (fig. 5.18f). However the total amount of vanadium in the shoot tissue increases over the six week period (fig. 5.17a). Root tissues also demonstrate the same pattern as V(+5) treated roots with only iron showing no significant differences during the growth period (fig. 5.19 a, b, c, e + f). Vanadium levels (fig. 5.18d) also show a similar variability to that seen for V(+5) treated plants and once again total vanadium uptake increases (fig. 5.17b).

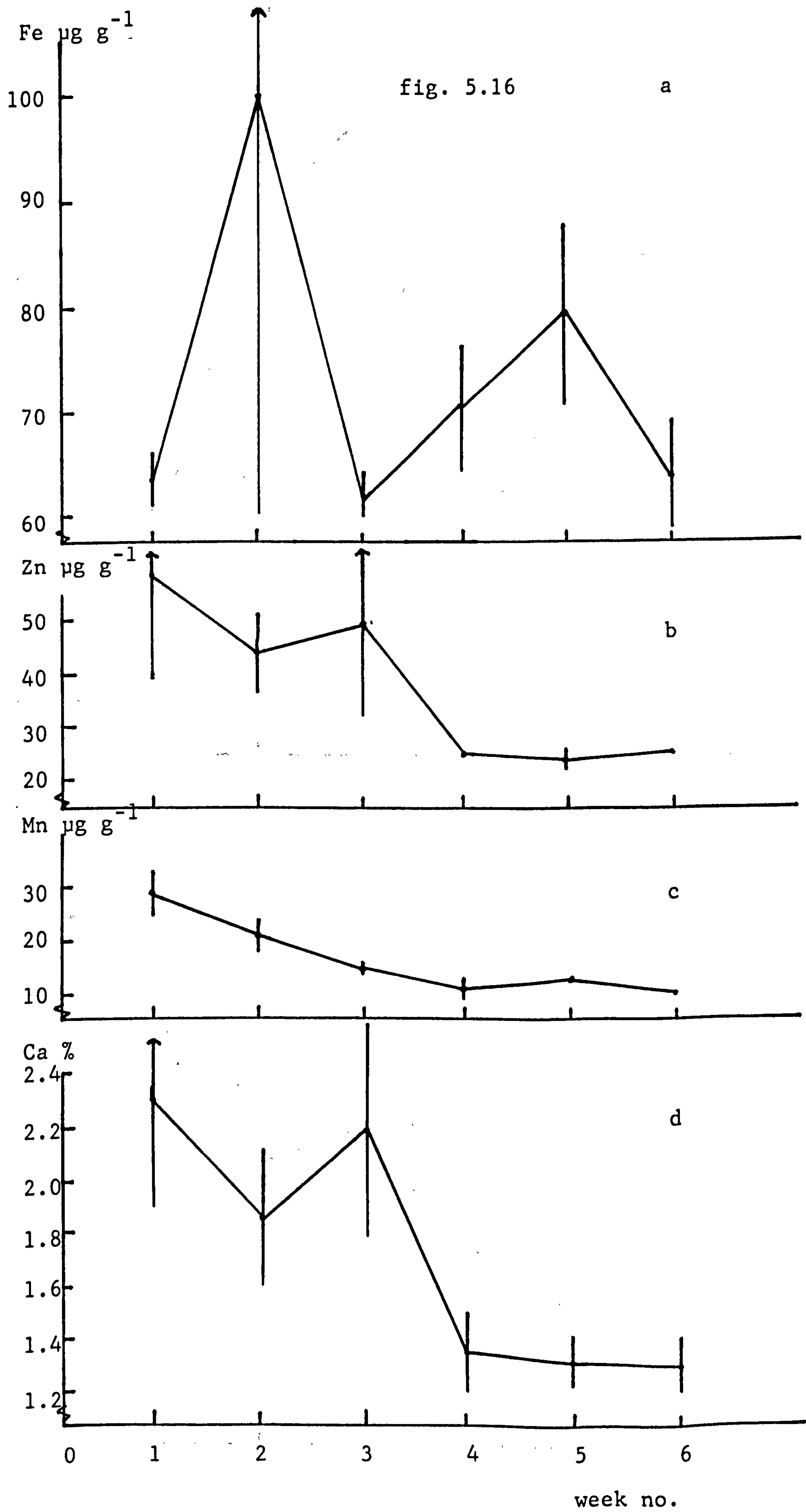
Significant negative correlations ($P < 0.05$) exist between shoot zinc and manganese and shoot vanadium irrespective of the applied form of the vanadium (fig. 5.20 a + b). No other correlations were found either in root or shoot levels.

Cannon (1963) proposed that vanadium levels in plants may be affected by calcium levels. The suggested mechanism involves precipitation in the root of the sparingly soluble calcium vanadate. This hypothesis has received very little

fig. 5.16 Ion levels in roots of NH_4VO_3 treated plants over a six week period.

- (a) iron
- (b) zinc
- (c) manganese
- (d) calcium
- (e) vanadium
- (f) potassium

fig. 5.16



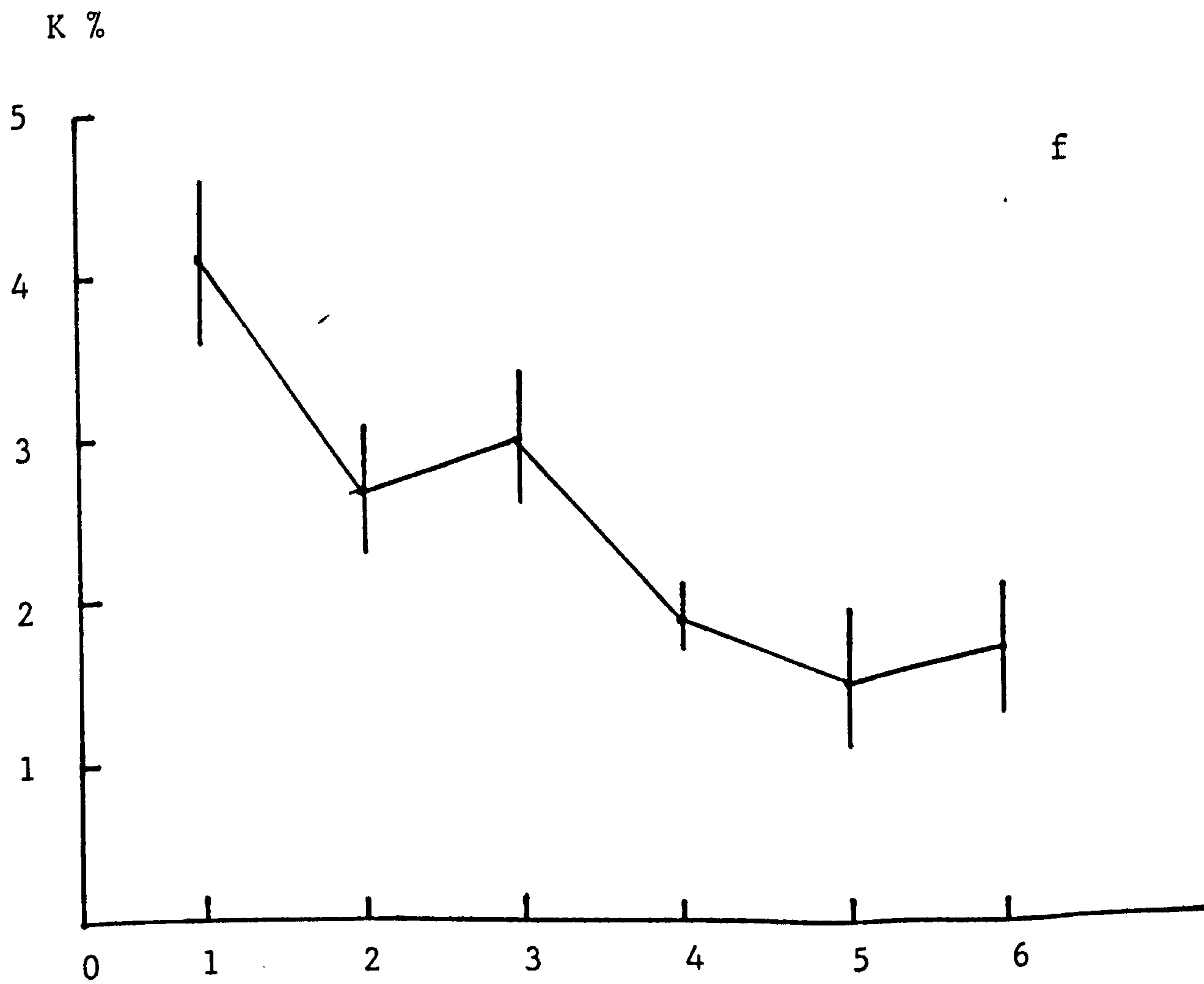
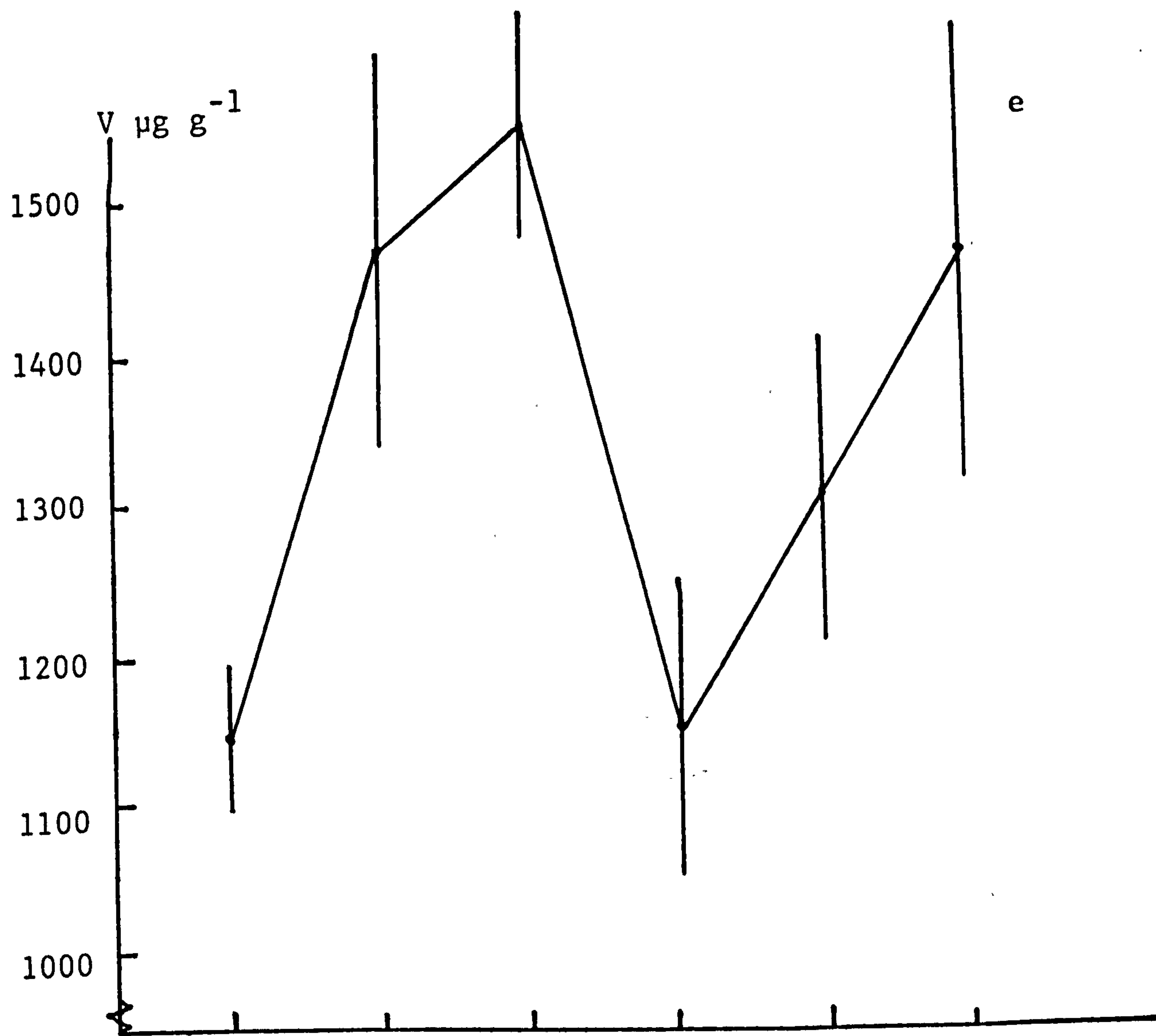


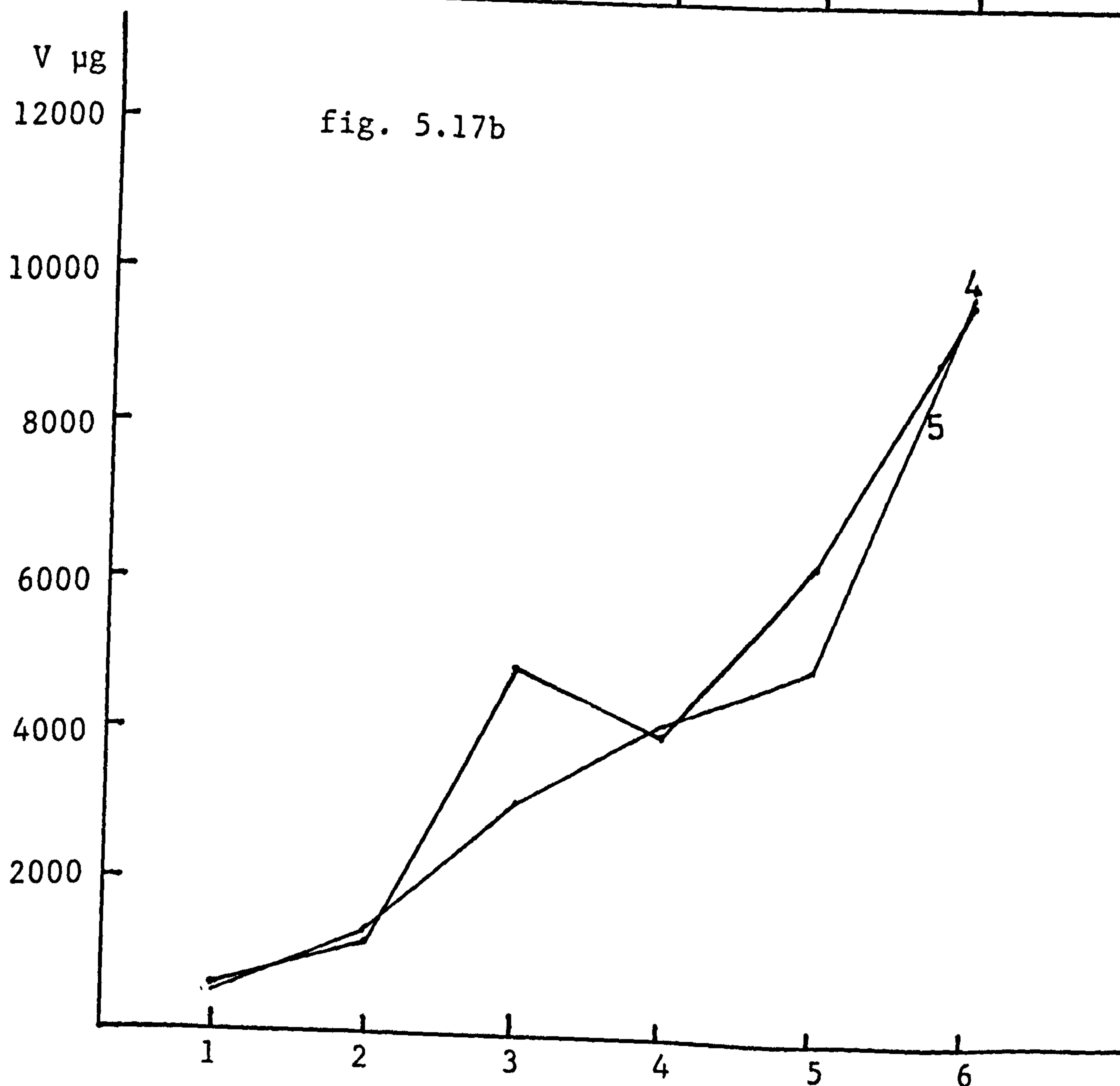
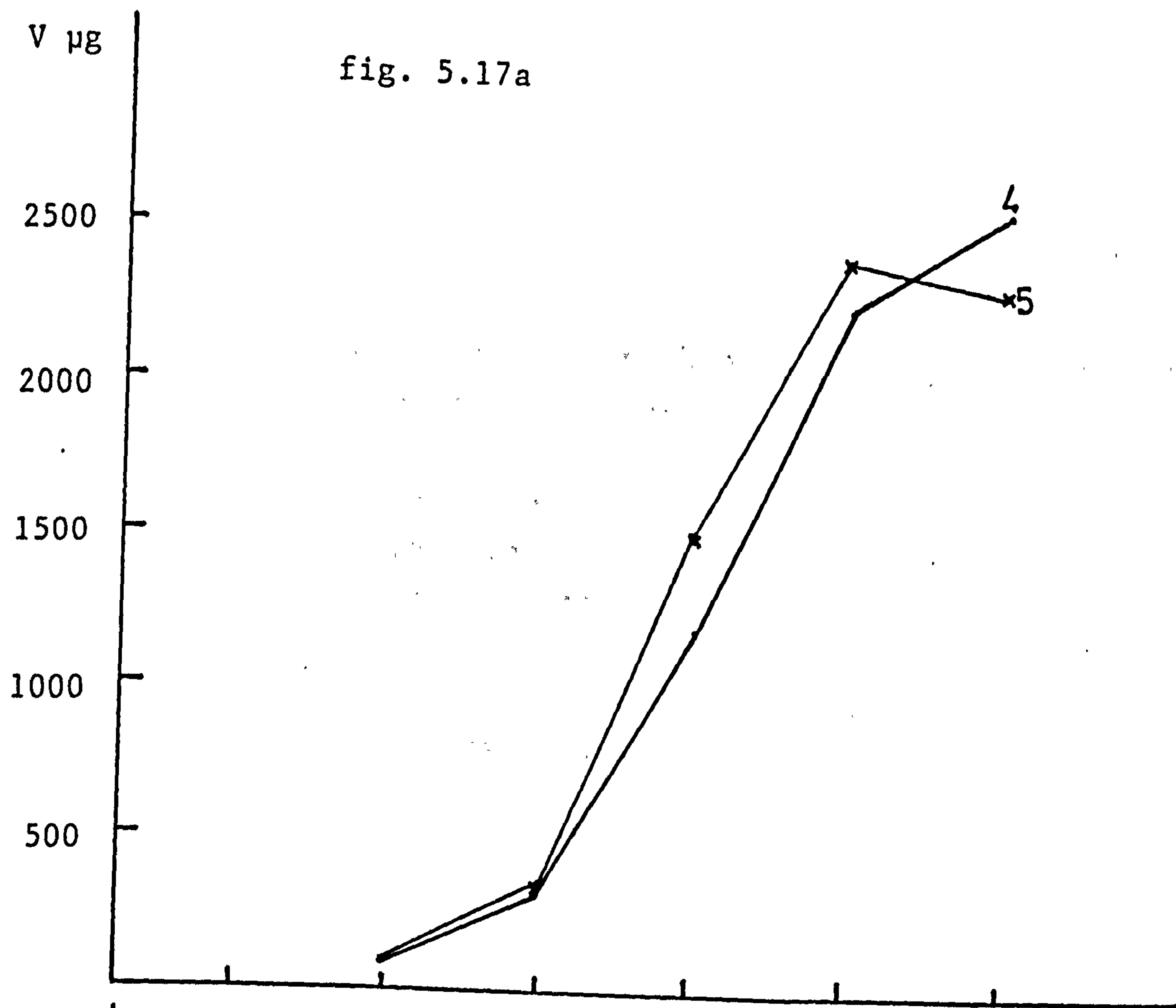
fig. 5.17 Total vanadium in plant tissues over a six week period.

(a) shoots

(b) roots

4 = VOSO_4 treated plants

5 = NH_4VO_3 treated plants

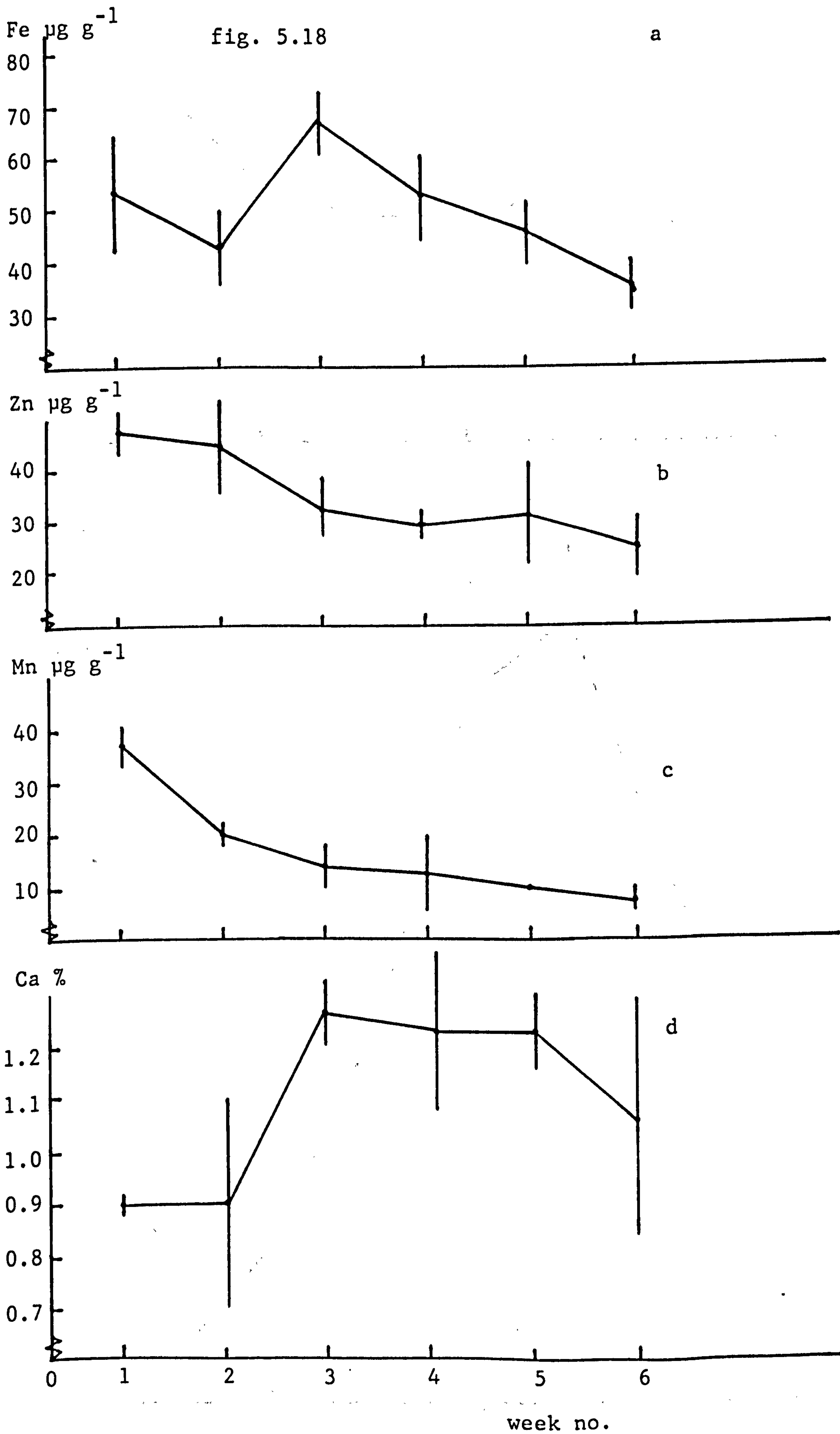


week no.

fig. 5.18 Ion levels in shoots of VOSO_4 treated plants over a six week period.

- (a) iron
- (b) zinc
- (c) manganese
- (d) calcium
- (e) potassium
- (f) vanadium

fig. 5.18



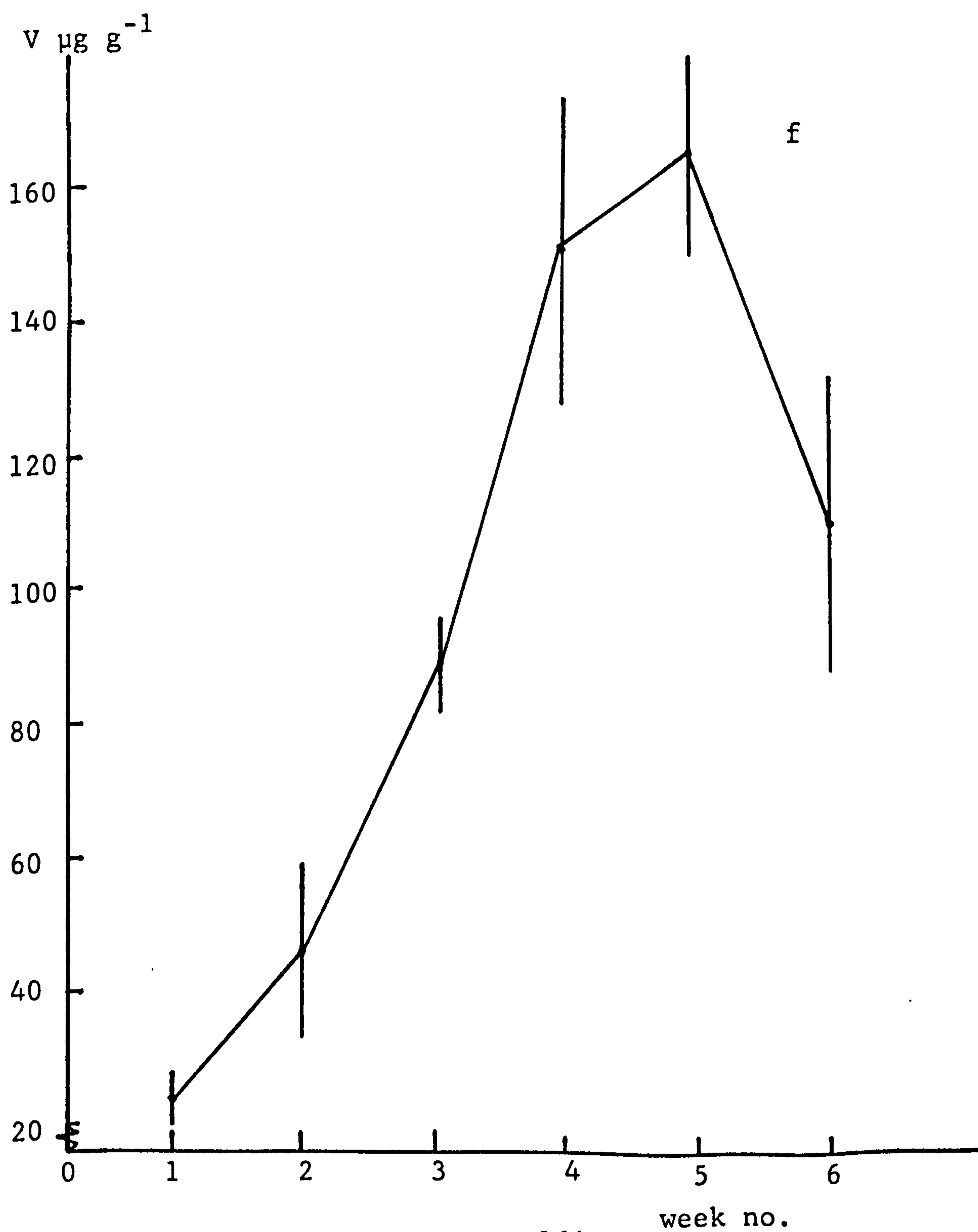
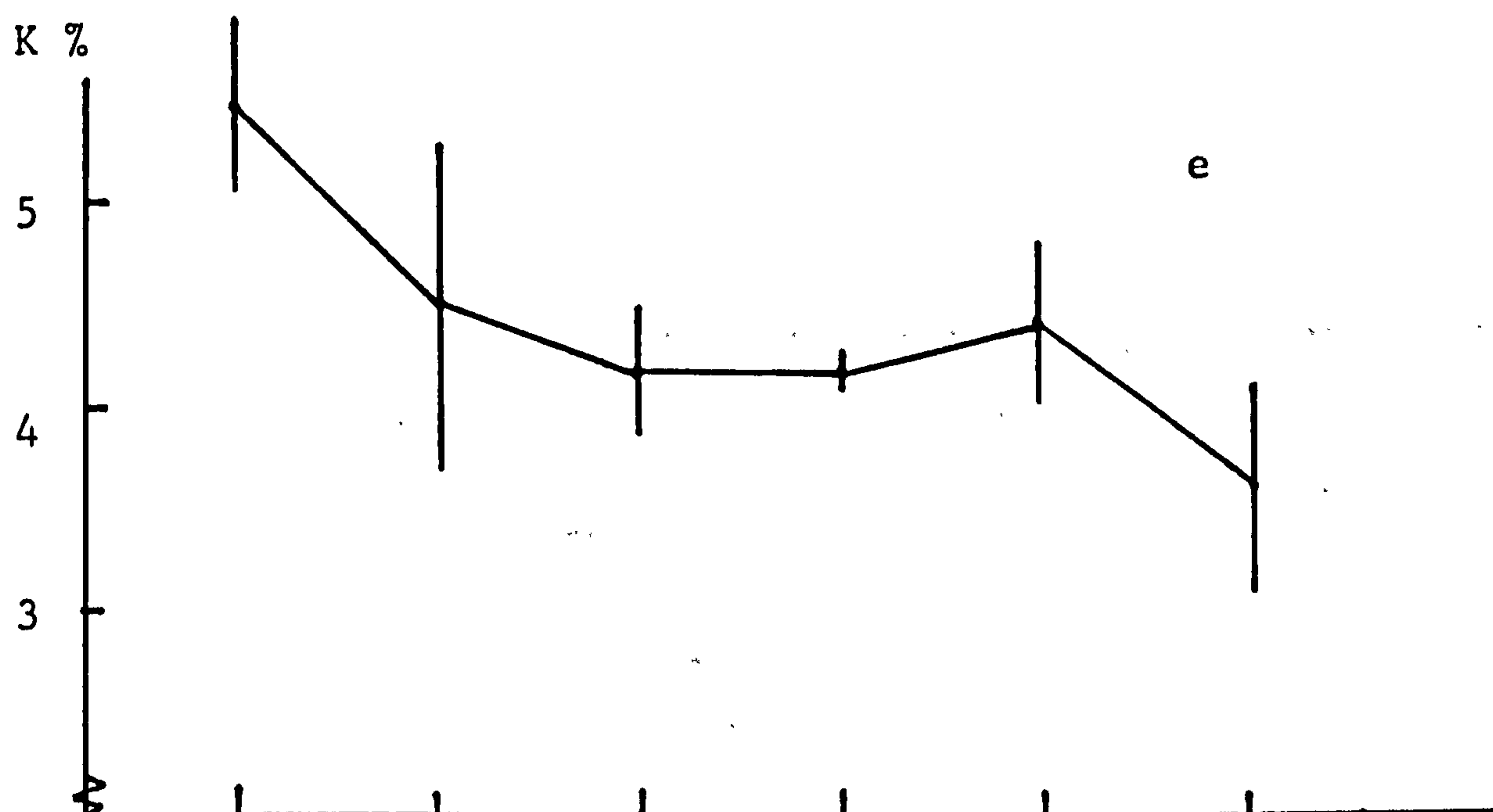
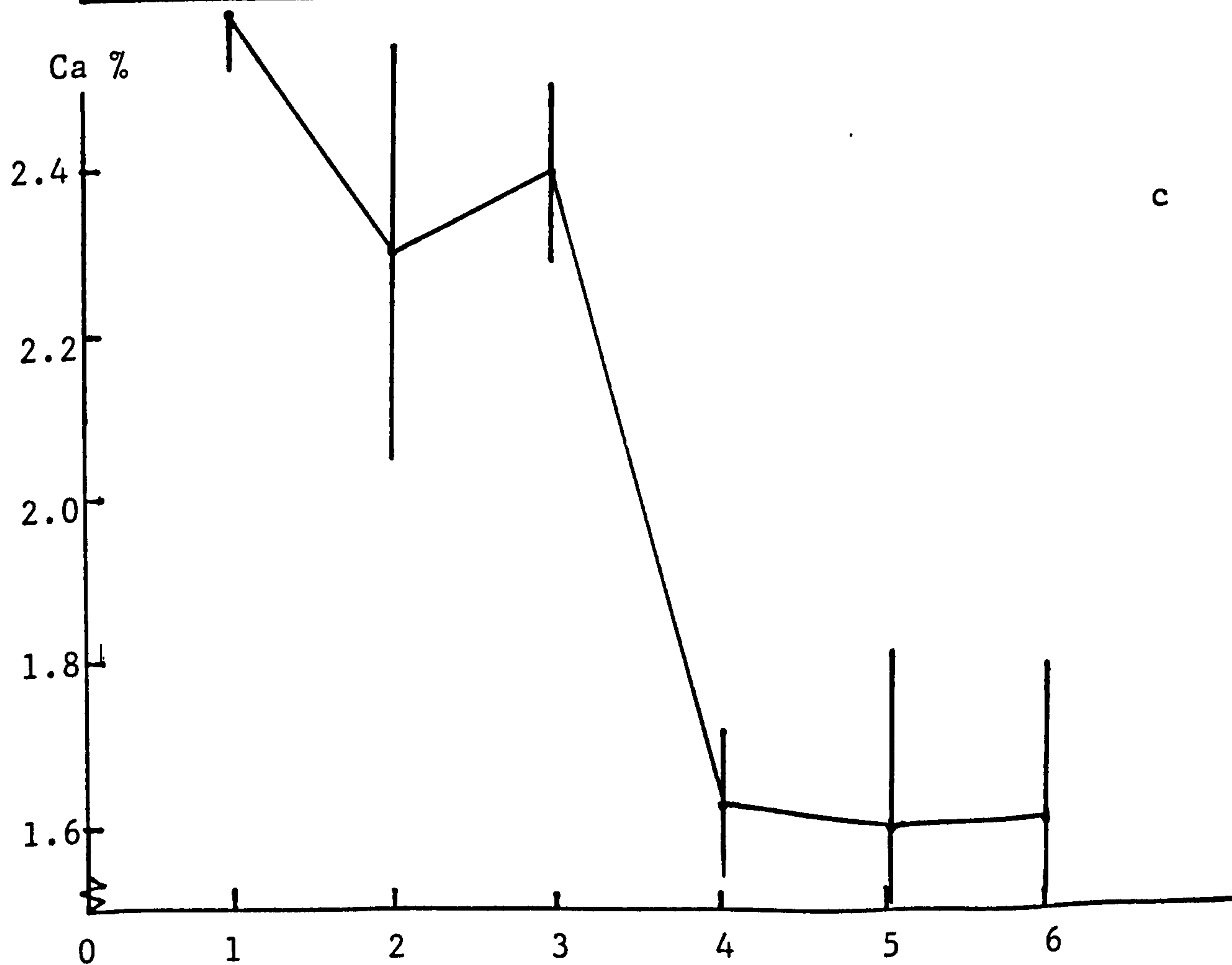
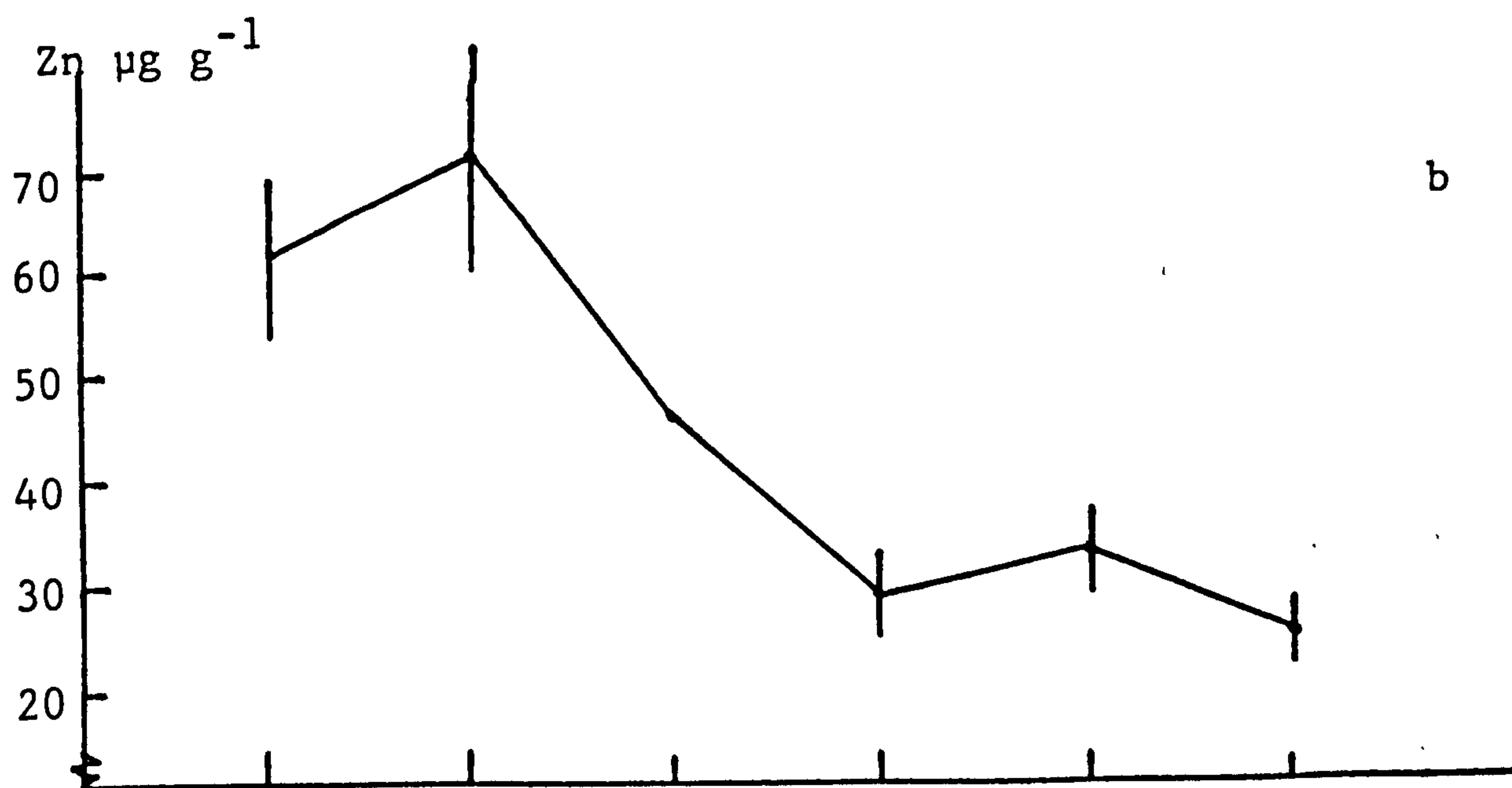
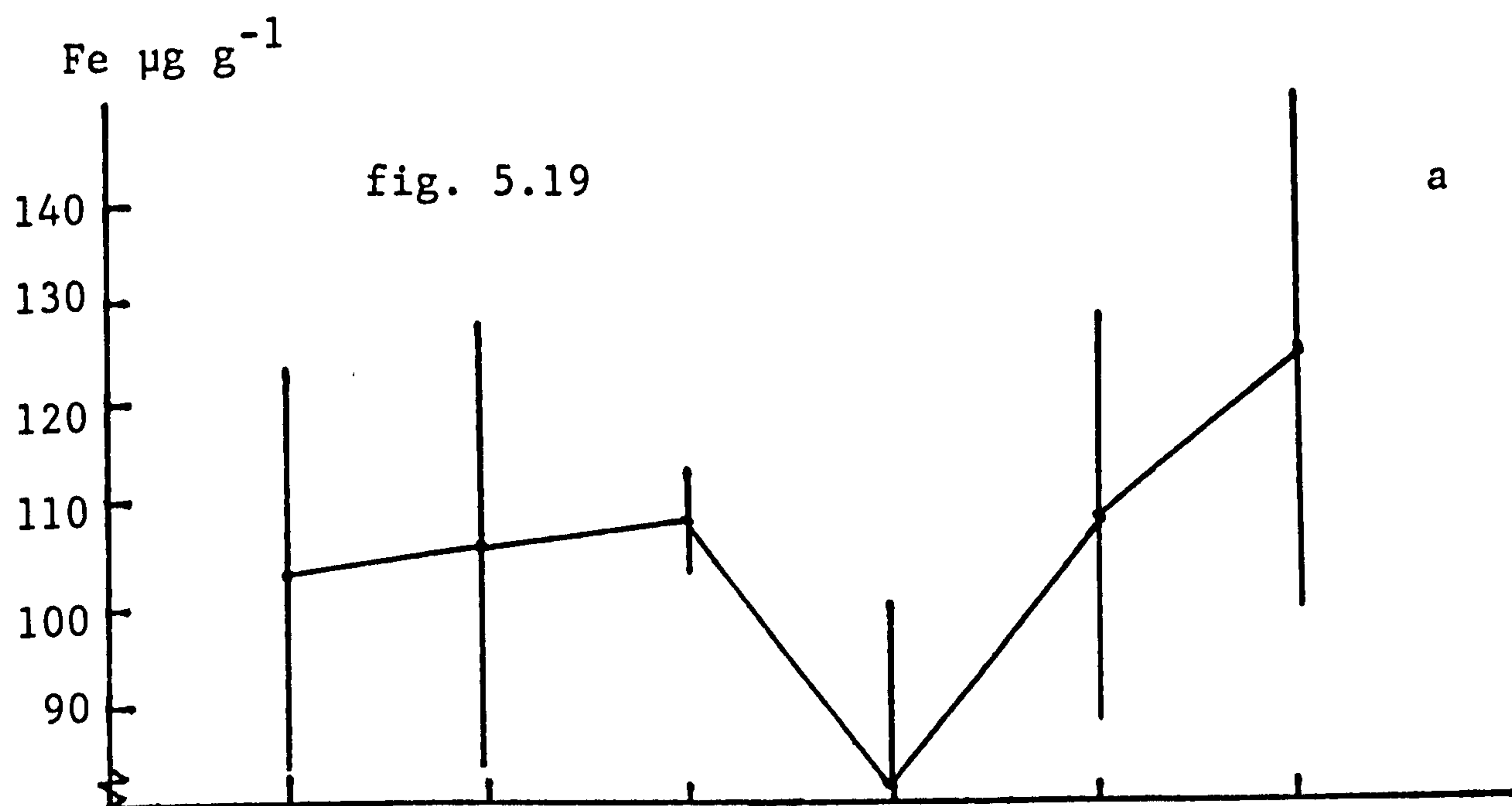


fig. 5.19 Ion levels in roots of VOSO_4 treated plants
over a six week period.

- (a) iron
- (b) zinc
- (c) calcium
- (d) vanadium
- (e) manganese
- (f) potassium

fig. 5.19



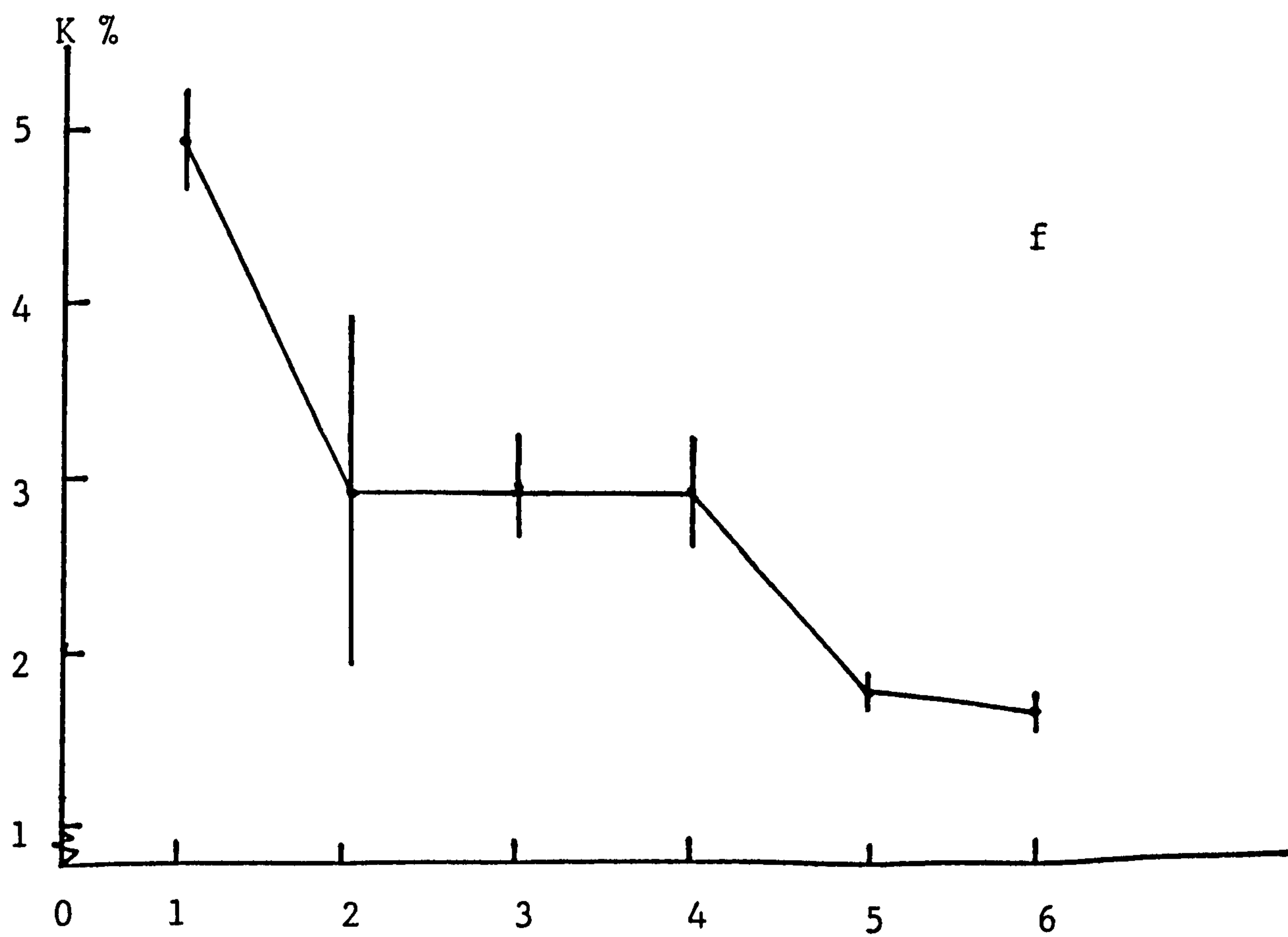
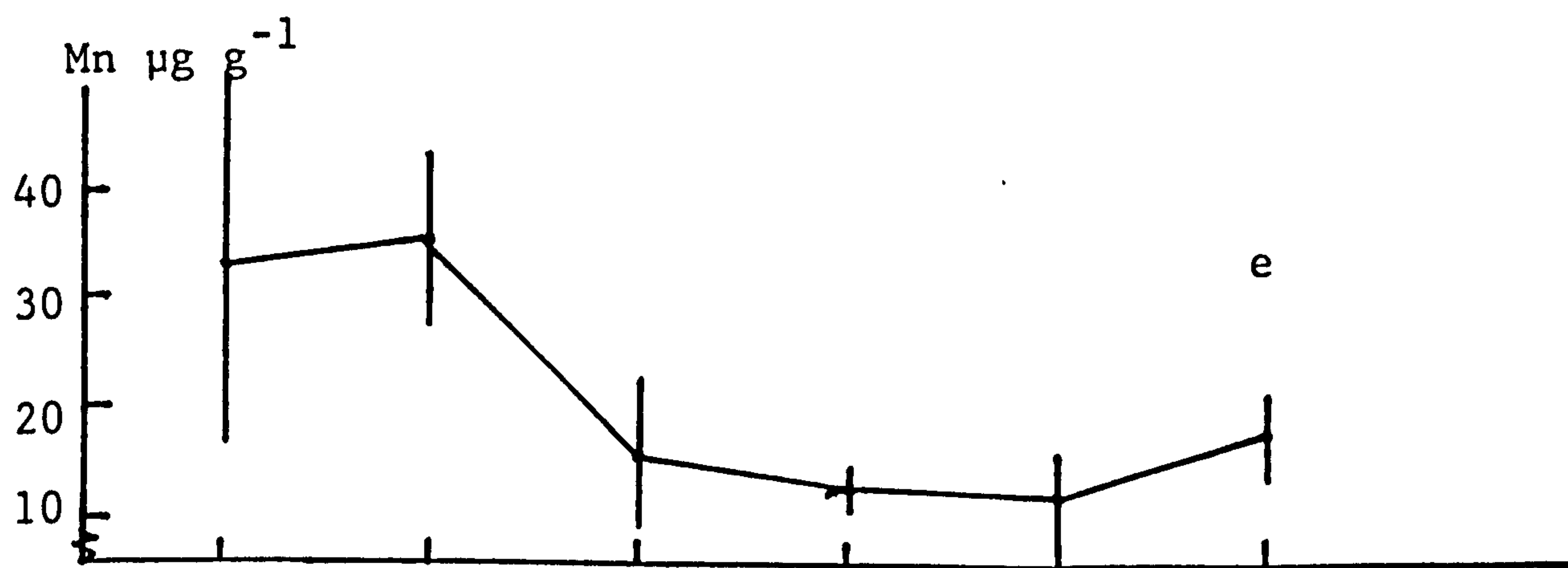
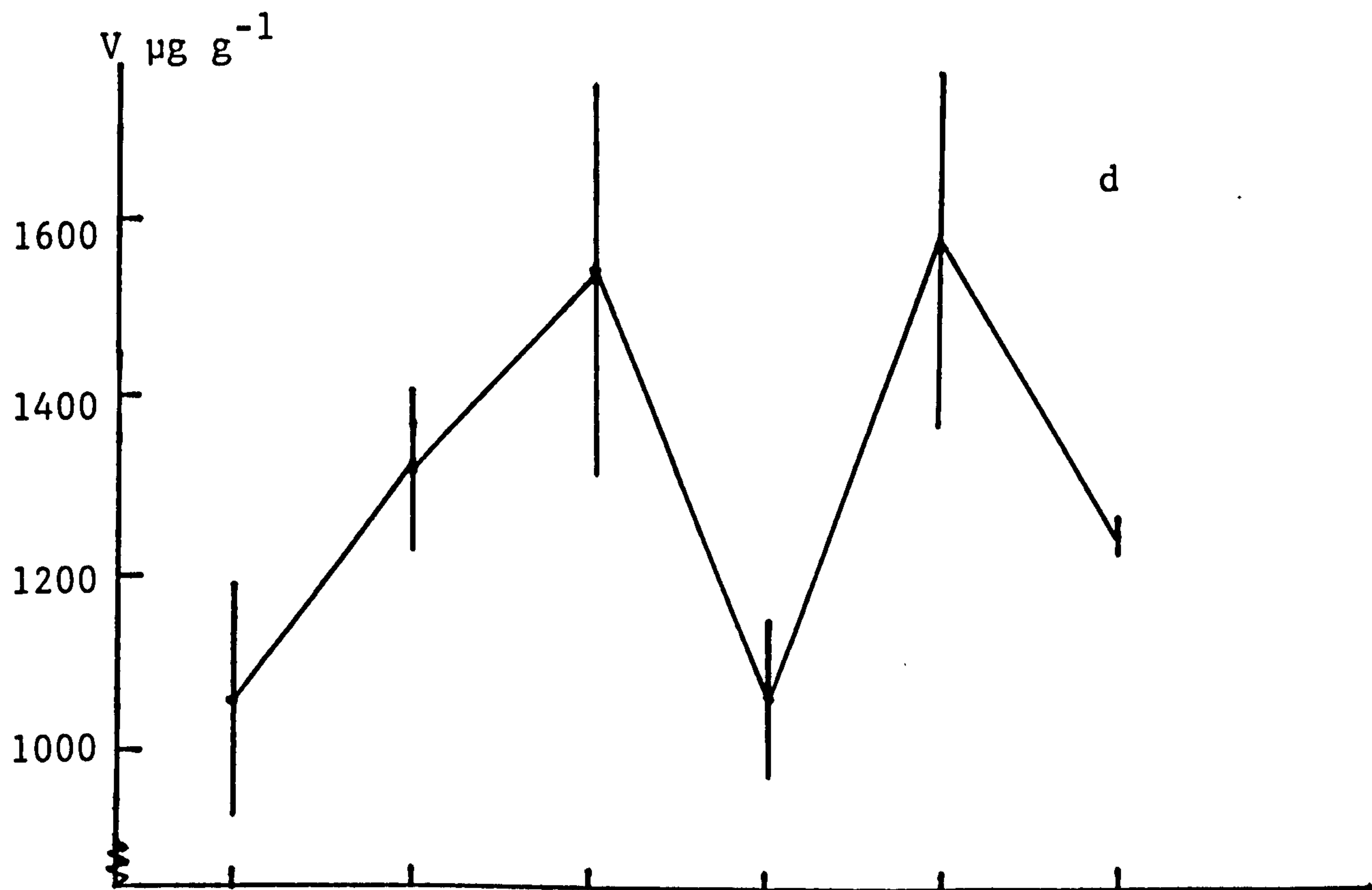
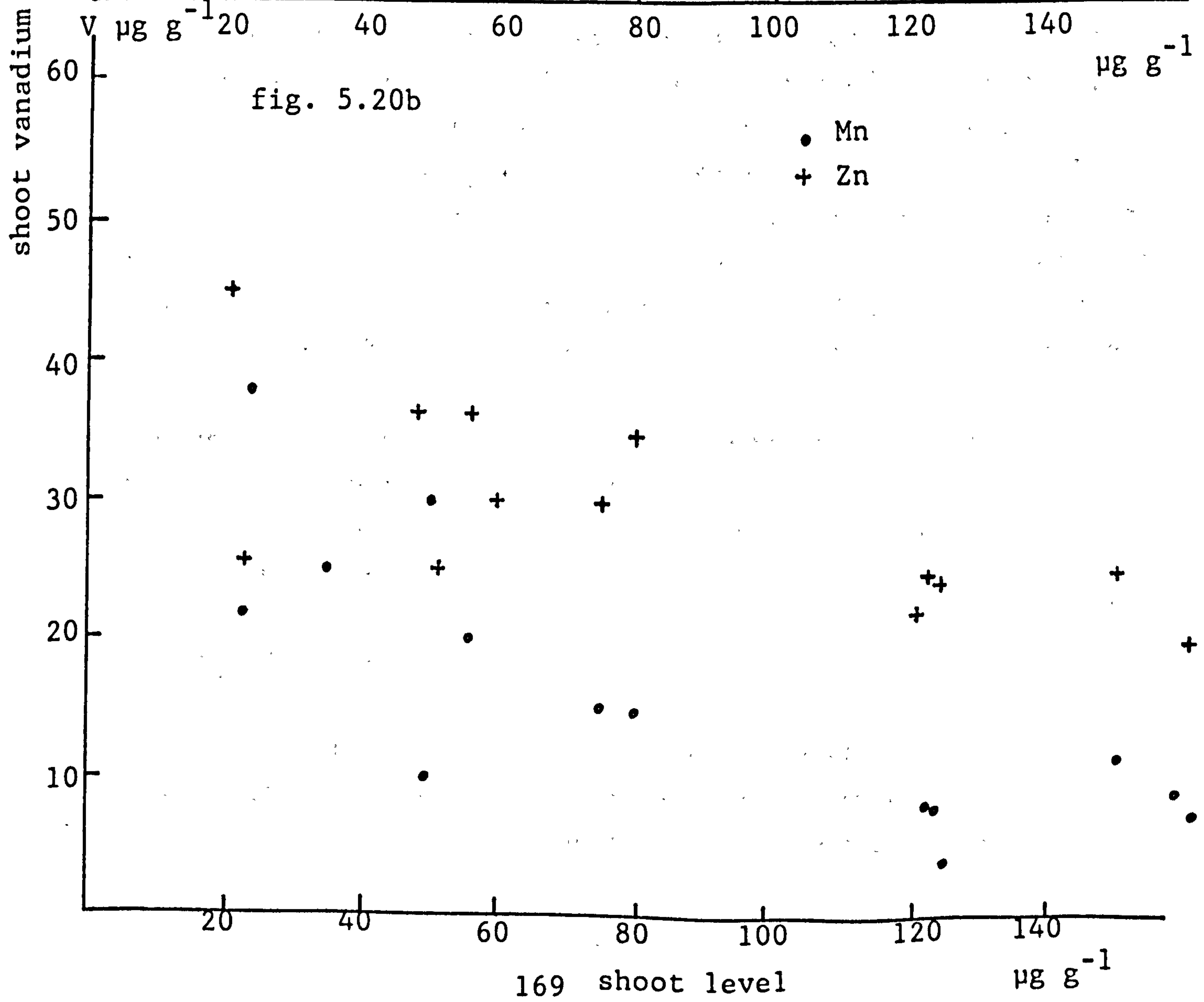
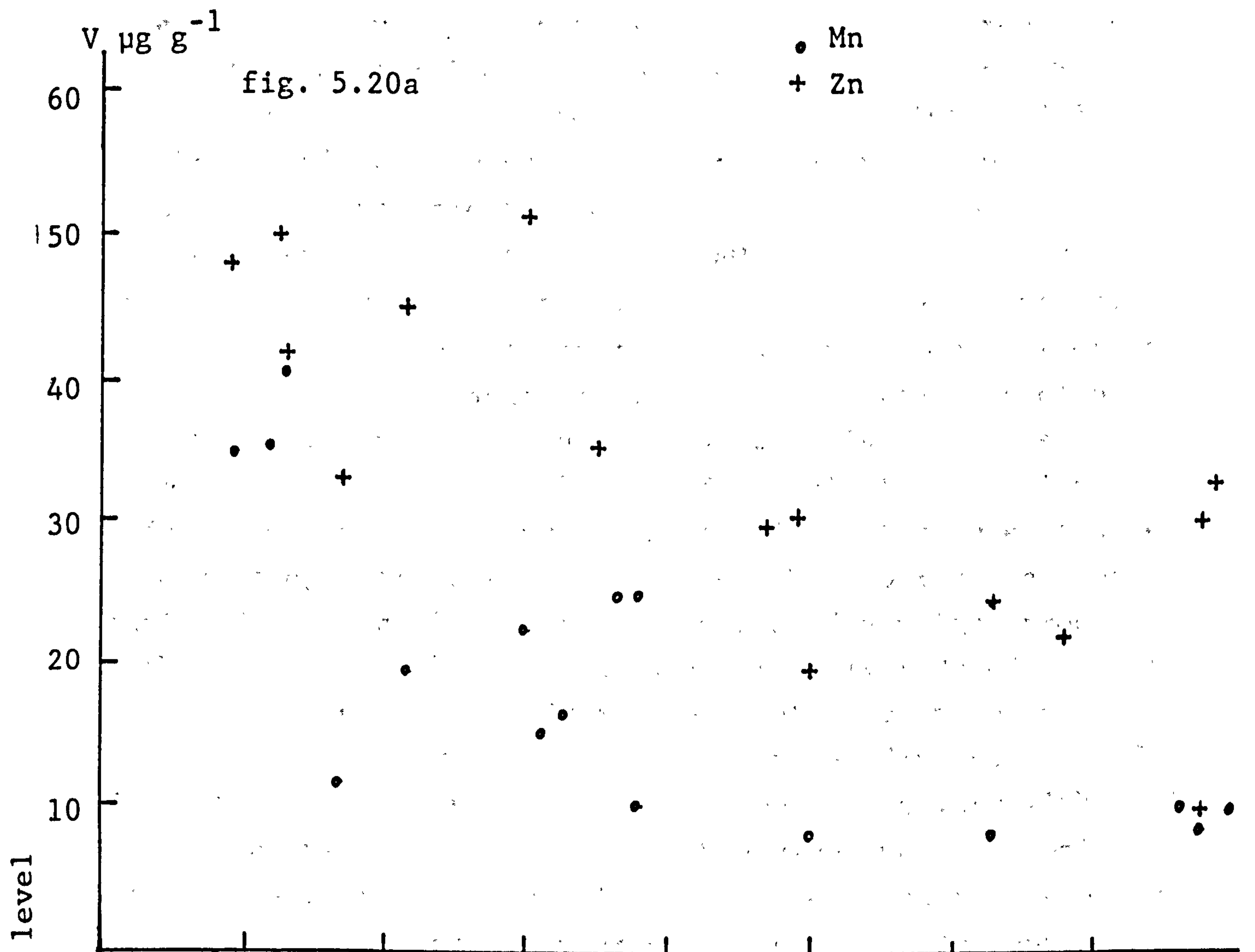


fig. 5.20 Relationship between shoot vanadium, zinc
and manganese.

(a) vanadium applied as VOSO_4

(b) vanadium applied as NH_4VO_3



attention. To produce data relating to this hypothesis the uptake of vanadium was examined in the presence of varying levels of calcium. Two nutrient solutions were prepared; one contained normal calcium levels (appendix 1) and the second had the calcium salt omitted (although this solution did have low levels of calcium present due to impurities in the other salts used to prepare the solution). The pH of both solutions was adjusted to 6.5 with HCl and NaOH. Plants were grown for 28 days then harvested and analysed for several elements. Table 5.5 summarizes the results obtained for this experiment. Calcium levels in plants grown with the 'normal' level of calcium in the nutrient solution showed tissue levels appropriate to maize (Jones and Lunt 1967). Those plants receiving a reduced supply of calcium showed significantly lower tissue levels than controls ($P < 0.05$). Over the experimental period employed this had little effect on root dry weight production although shoot dry weight was significantly reduced ($P < 0.05$). In plants receiving a reduced supply of calcium there was a significant positive increase in shoot vanadium content ($P < 0.05$). The effect on root vanadium levels appears to vary with ionic species of vanadium presented. When vanadium was supplied as the vanadate anion and calcium levels were low root vanadium levels decreased when compared to controls. In contrast when vanadium was supplied as the vanadyl cation and the calcium levels were low root vanadium levels appeared to increase, although none of these results is significant. These results are similar to those presented in Morrell et al (1983) although in that instance no significant effects were detected when the vanadium was presented as the vanadyl cation. Low calcium grown plants tend to show a higher degree of variability in tissue levels, although this is not evident for other ions examined.

Iron and zinc levels vary in the manner expected on the basis of previous experiments i.e. no significant effects on iron tissue levels or root zinc, but a significant decrease in zinc shoot levels when vanadium is applied.

5.3 Broad Beans.

Broad bean (*Vicia faba* L. cv Aquadulce claudia) were employed in several experiments similar to those carried out with maize in an attempt to produce data of a comparable nature.

Table 5.5 Ion levels in maize tissue as affected by calcium levels in the nutrient solution.

Table 5.5

Treatment		Ca %	Ion			Organ
			Fe $\mu\text{g}\cdot\text{g}^{-1}$	Zn $\mu\text{g}\cdot\text{g}^{-1}$	V $\mu\text{g}\cdot\text{g}^{-1}$	
LC Control	\bar{x}	0.074	63.8	96.4	ND	S
	sd	0.019	7.5	9.7	-	
LC Vanadate	\bar{x}	0.109	68.6	55.04	51.2	S
	sd	0.017	7.1	11.1	13.4	
LC Vanadyl	\bar{x}	0.091	73.8	63.9	77.8	S
	sd	0.039	17.2	9.5	31.0	
NC Control	\bar{x}	0.579	101.9	107.8	ND	S
	sd	0.025	52.3	26.3	-	
NC Vanadate	\bar{x}	0.514	65.5	44.6	24.5	S
	sd	0.073	6.2	8.7	3.3	
NC Vanadyl	\bar{x}	0.555	71.7	49.2	39.1	S
	sd	0.072	6.9	9.7	8.6	
LC Control	\bar{x}	0.11	99.2	37.1	ND	R
	sd	0.03	14.6	9.9	-	
LC Vanadate	\bar{x}	0.13	80.4	39.6	1497.7	R
	sd	0.03	11.9	10.5	385.1	
LC Vanadyl	\bar{x}	0.14	108.8	30.4	2680.6	R
	sd	0.04	37.3	7.1	533.5	
NC Control	\bar{x}	0.71	106.9	42.2	ND	R
	sd	0.27	13.7	10.5	-	
NC Vanadate	\bar{x}	0.47	74.5	29.6	1868.1	R
	sd	0.04	7.7	18.9	837.9	
NC Vanadyl	\bar{x}	0.53	77.4	28.5	1839.8	R
	sd	0.06	15.4	7.1	340.5	

LC = low calcium level

NC = normal calcium level

ND = not detectable

S = shoot

R = root

The effects of applied concentration and form on dry matter production can be seen in figs 5.21 a and b for vanadium applied as NH_4VO_3 and VOSO_4 respectively. Although dry matter production can be seen to decline as the applied concentration of vanadium increases; analysis of variance for each subset revealed no significant differences. This is probably due to the large standard deviations associated with each treatment which arose from differential growth under similar conditions. In later experiments, plants were selected for uniform growth prior to treatment (see chapter 3). In these later experiments significant reductions in shoot, leaf and root dry matter production were observed at concentrations greater than 100 μM irrespective of the applied form of vanadium.

Plant vanadium levels are shown in fig. 5.22 (leaf and stem) and fig. 5.23 (root). Tissue vanadium levels show no significant differences in respect of applied form therefore results descriptions will be made in terms of vanadium rather than referring to the particular ionic species applied.

Vanadium in these plants increases with increased applied vanadium. The bulk of the vanadium remained within the root tissues and most of the vanadium in the aerial parts was to be found in the leaves. In all cases stem levels were lower than levels in the leaves (fig. 5.22 a + b).

Results from two experiments were pooled and regressions calculated between vanadium levels in different plant parts. Root-leaf, root-stem and stem-leaf all show significant linear relationships (fig. 5.24 a, b + c). These relationships are similar, irrespective of the applied form of the vanadium (table 5.6).

The effect of vanadium on tissue ion levels showed some dependence on the species of vanadium. Results are presented in tables 5.7 (vanadium as NH_4VO_3) and 5.8 (vanadium VOSO_4). Most of the ion/tissue relationships were not significantly affected by applied vanadium. Irrespective of the applied form however zinc in stem and roots showed significant level changes. This was particularly true for root tissue where

fig. 5.21 Bean dry matter production as affected
by applied vanadium.

(a) vanadium applied as NH_4VO_3

(b) vanadium applied as VOSO_4

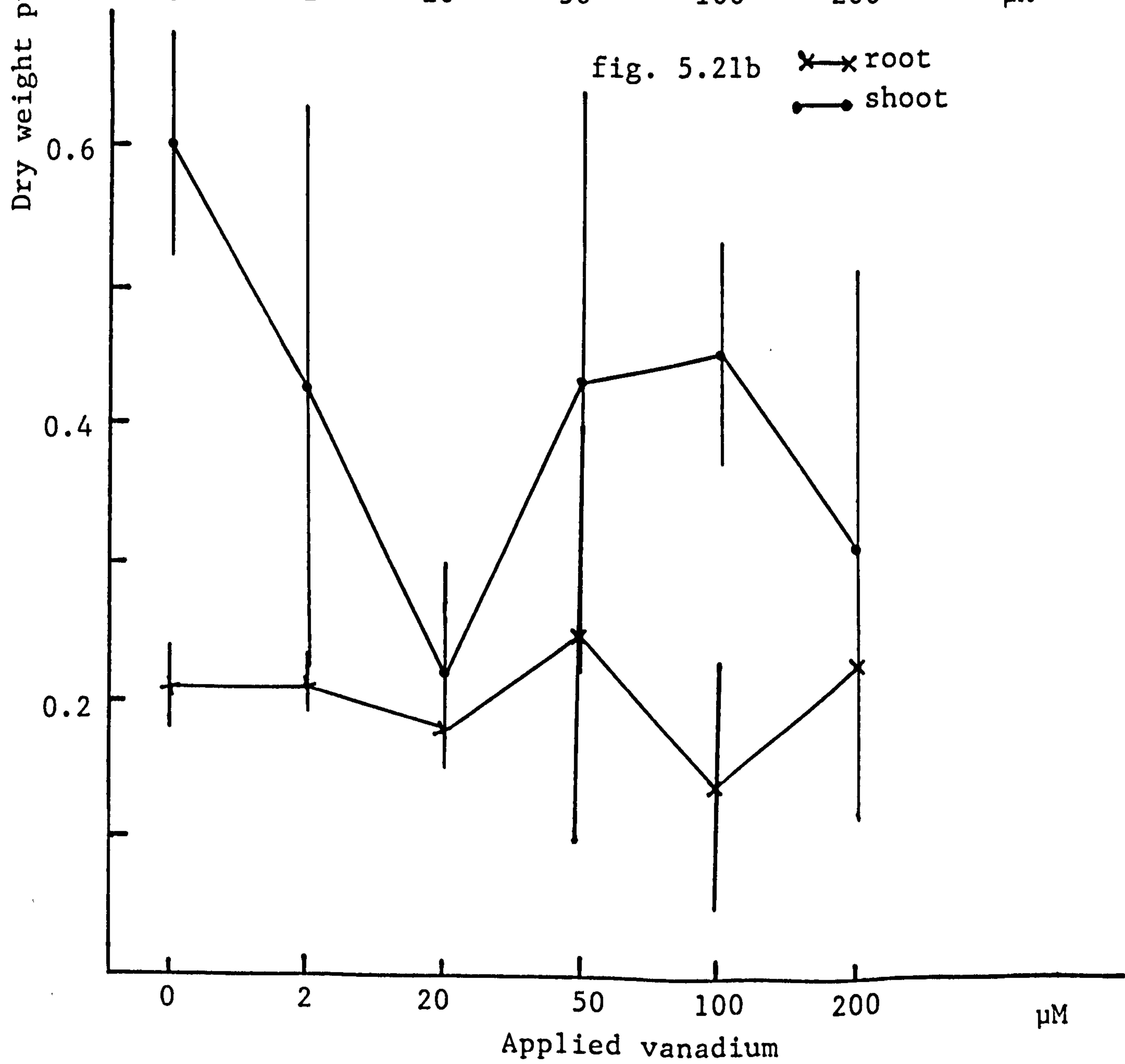
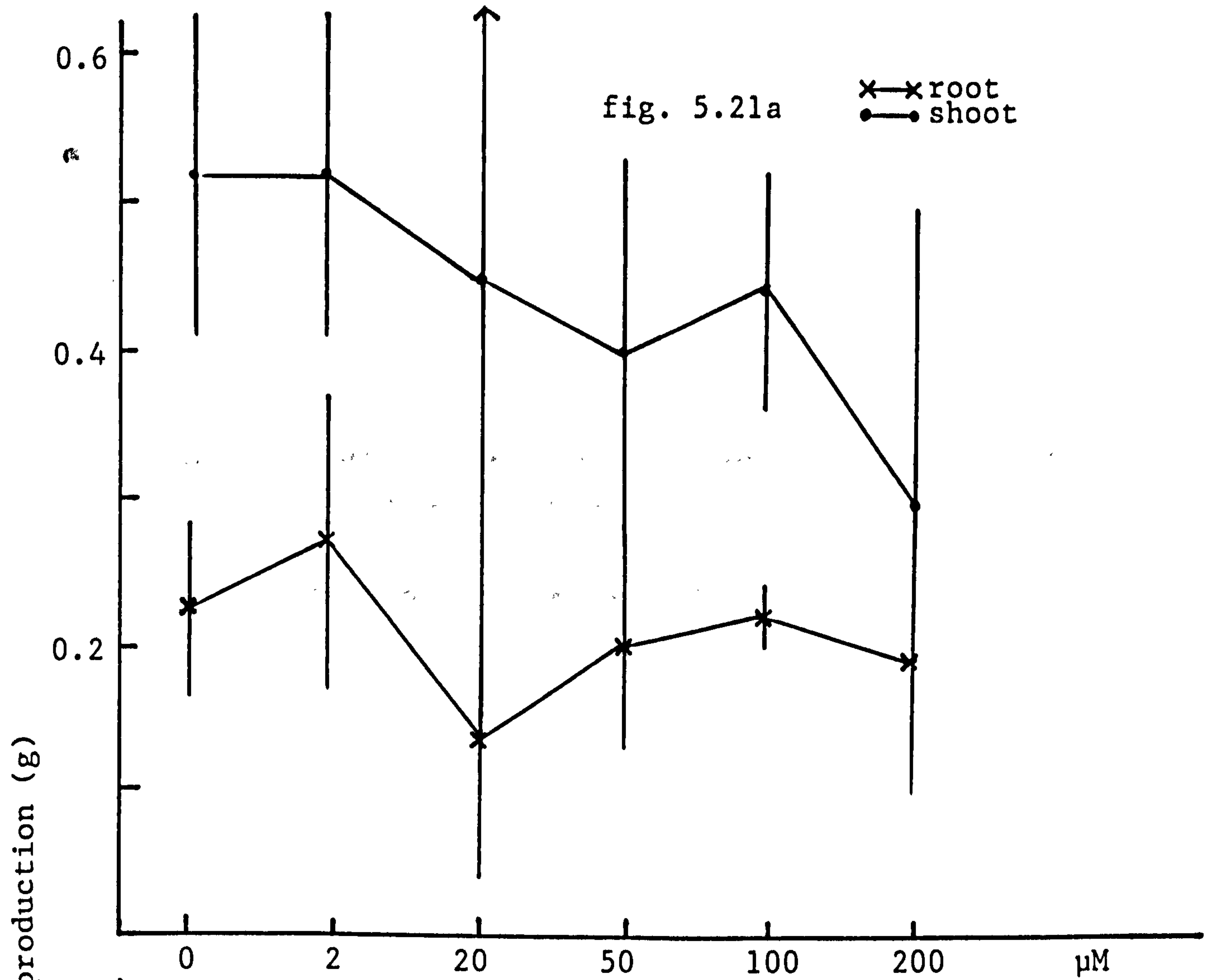

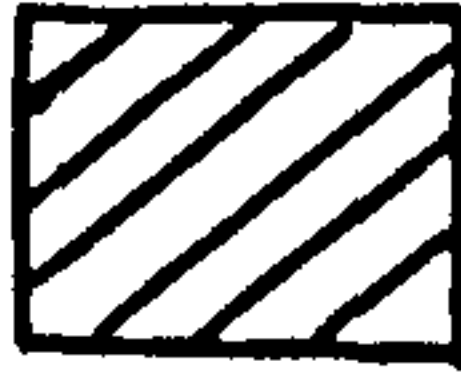


fig. 5.22 Bean leaf and stem tissue vanadium levels as affected by applied vanadium.

- (a) vanadium supplied as NH_4VO_3
- (b) vanadium supplied as VOSO_4

fig. 5.22

 leaf tissue
 stem tissue

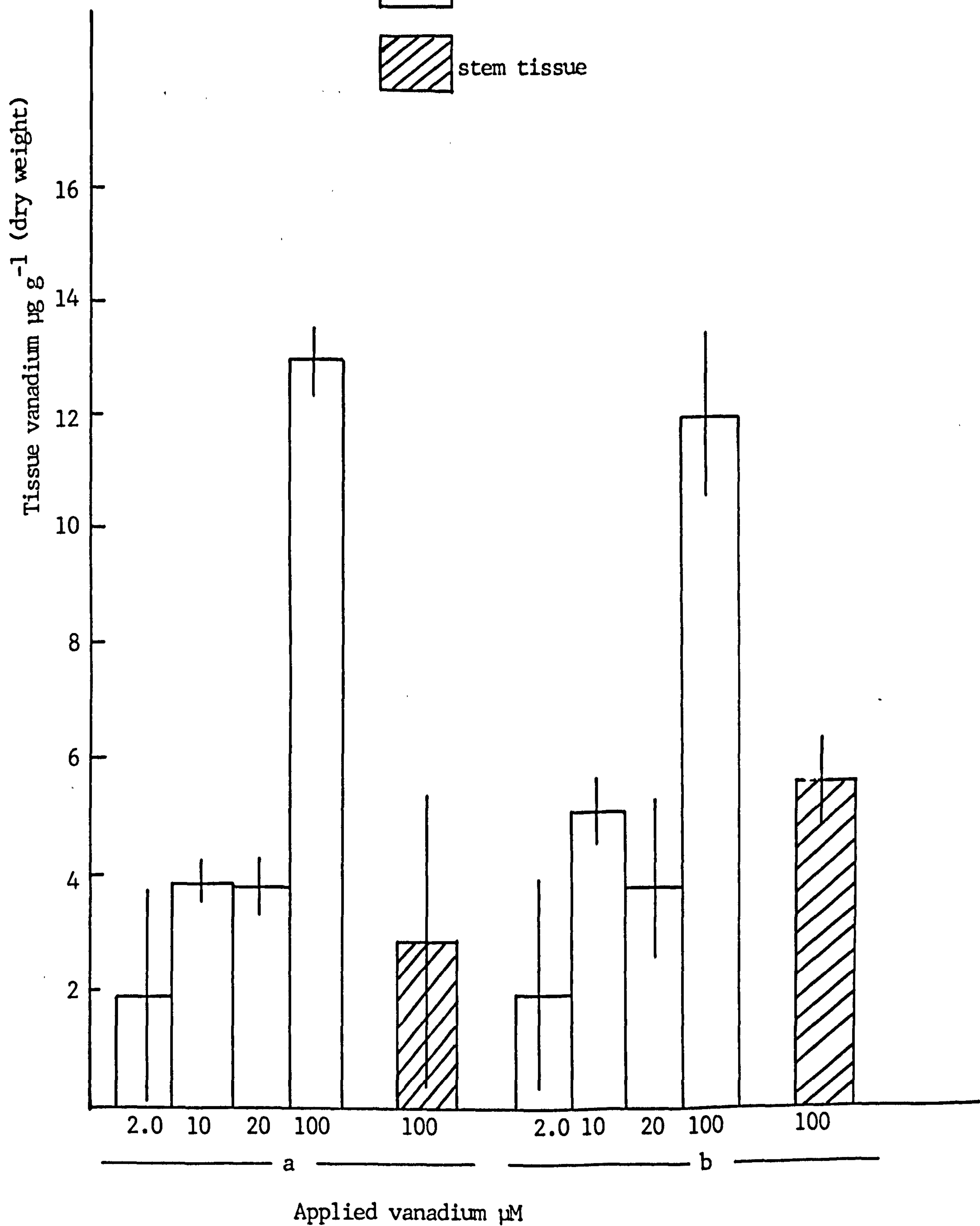


fig. 5.23 Bean root vanadium levels as affected by applied vanadium.

(a) vanadium supplied as NH_4VO_3

(b) vanadium supplied as VOSO_4

fig. 5.23

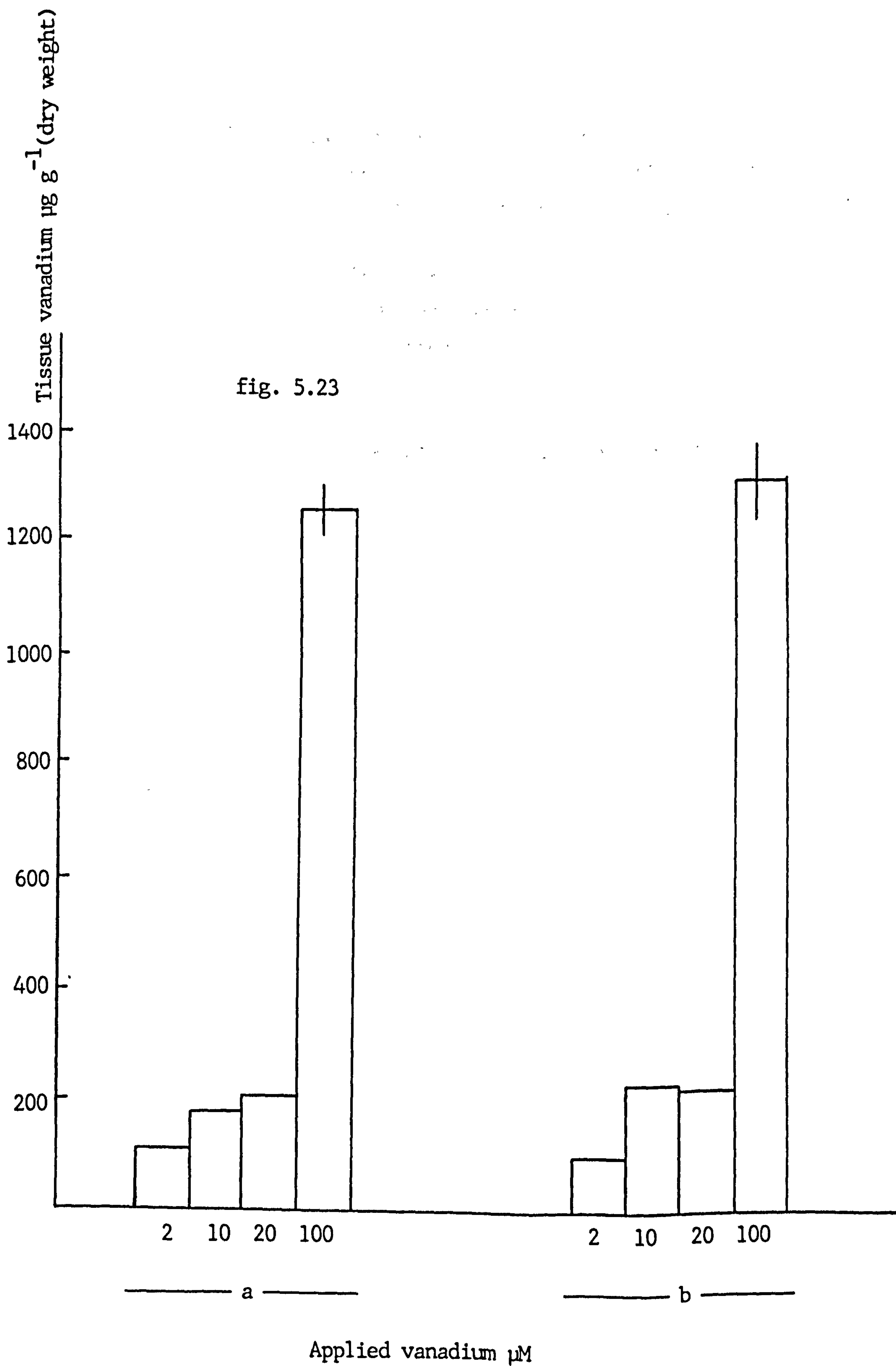


fig. 5.24 Linear regressions to illustrate the relationship between the vanadium content of various bean tissues.

(a) root/leaf

(b) root/stem

(c) stem/leaf

4 = vanadium applied as VOSO_4

5 = vanadium applied as NH_4VO_3

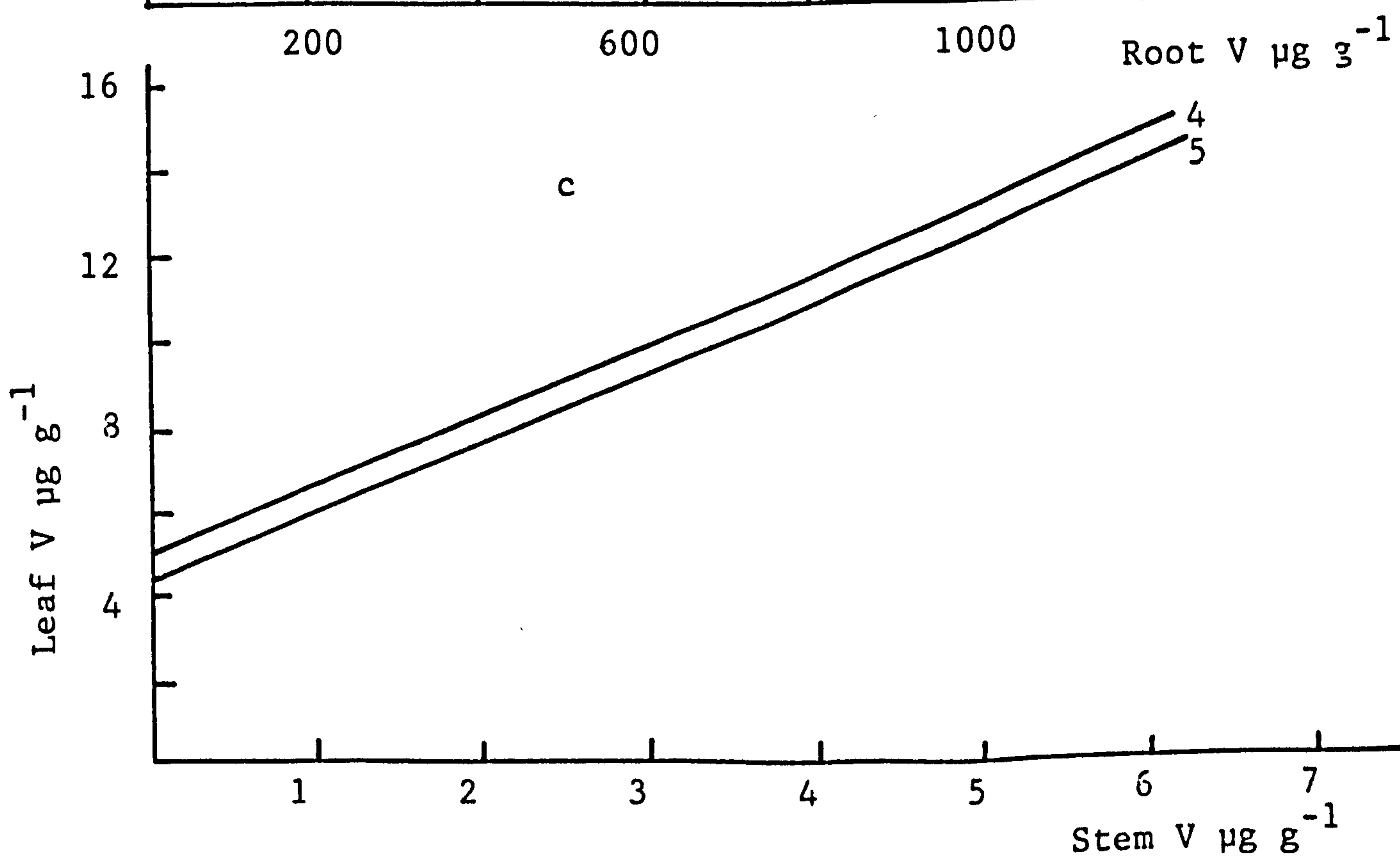
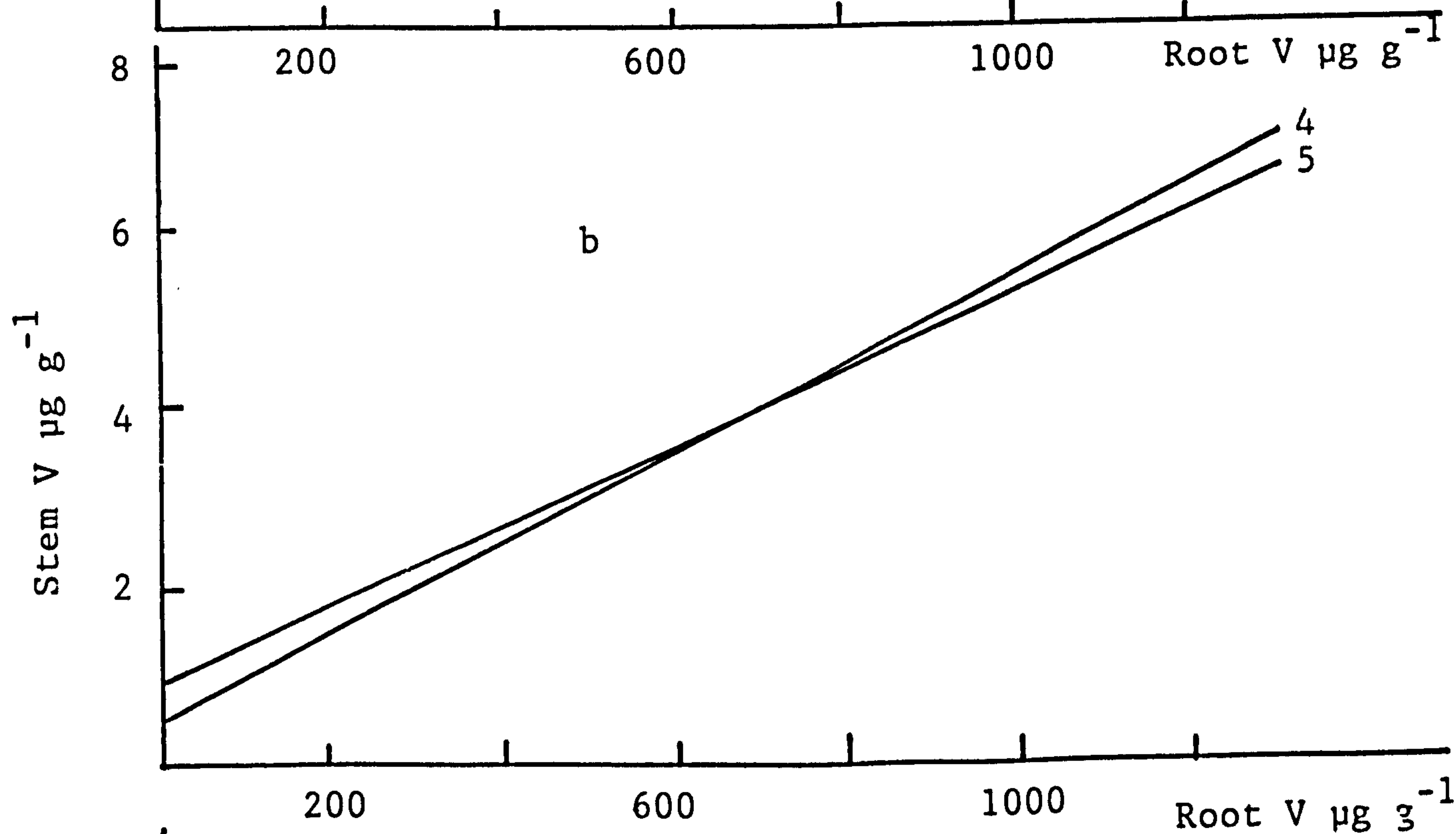
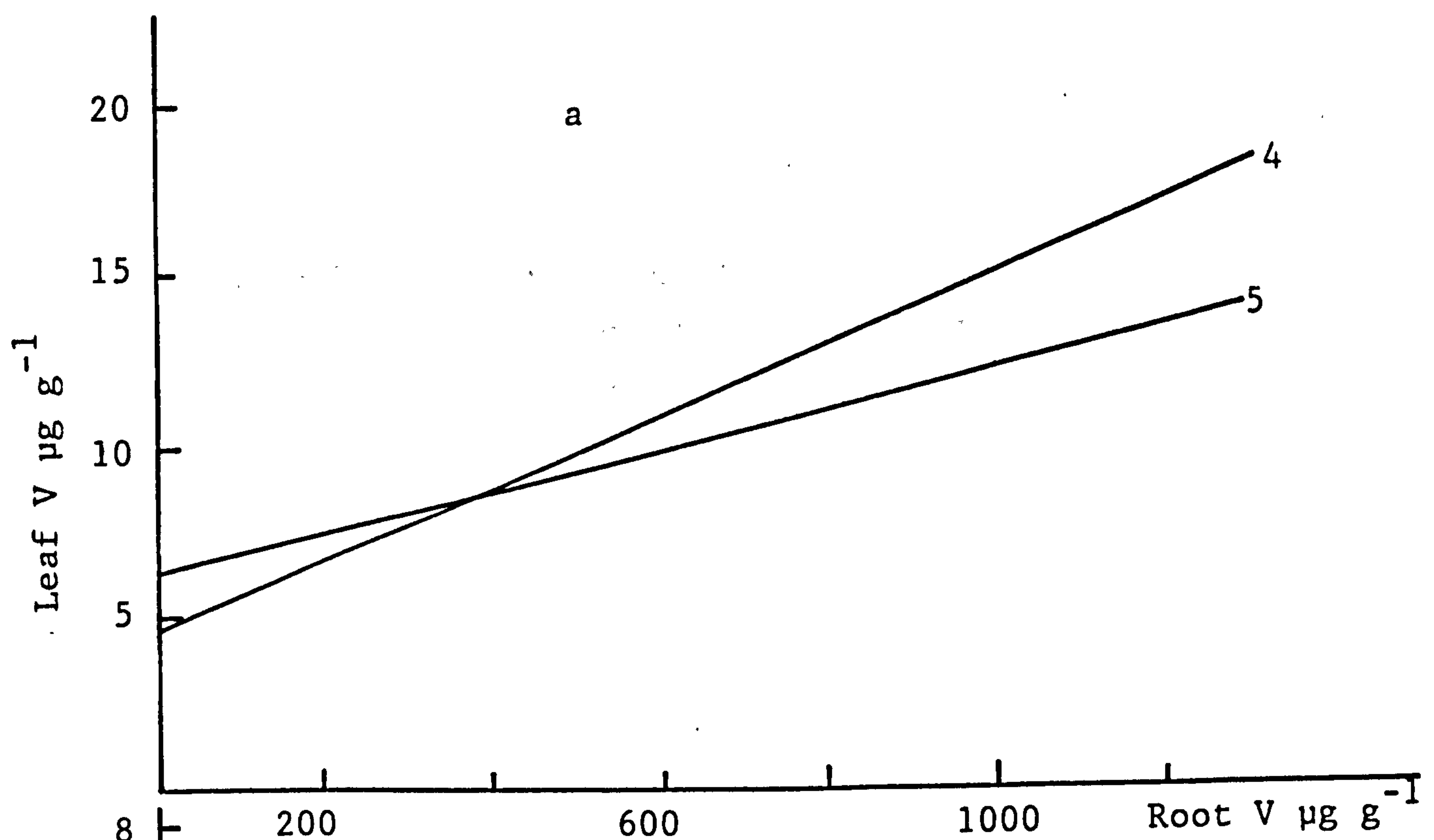


Table 5.6 Data to show figures involved in the
comparison of regression coefficients of the
lines illustrated in fig. 5.23

Table 5.6

Root-leaf regressions.

	VO^{2+}	VO_3^-
Sum $(x-\bar{x})^2$	8.02×10^6	7.09×10^6
Variance	19.15	18.25
n	27	27
B	0.0062	0.0099
F = 1.049	NS	Variances are equal
d = 1.672	NS	No differences in B values

Root-stem regressions.

	VO^{2+}	VO_3^-
Sum $(x-\bar{x})^2$	8.021×10^6	7.092×10^6
Variance	2.65	4.51
n	27	27
B	0.0045	0.0049
F = 1.700	NS	Variances are equal
d = 0.453	NS	No difference in B values

Stem-leaf regressions.

	VO^{2+}	VO_3^-
Sum $(x-\bar{x})^2$	230.2×7	287.33
Variance	8.008	27.8
n	27	27
B	1.592	1.606

Variance comparison $F = 3.48$ variances significantly different.
As the regression lines have unequal variances, a modified number of df must be calculated, in this instance $df = 41$,
 $d = 0.04$ no difference in B values.

Table 5.7 Ion levels in various bean tissues as affected by vanadium applied as NH_4VO_3 at various concentrations.

* significant difference $P < 0.05$

** significant difference $P < 0.01$

Table 5.7

		Ion. ($\mu\text{g}\cdot\text{g}^{-1}$ d.wt.)			
Applied V (μM)		V	Fe	Zn	Mn
Leaf	0.0 \bar{X}	ND	123.5	189.4	88.2
	sd	-	22.5	26.5	5.7
	2.0 \bar{X}	4.9	112.8	171.3	97.4
	sd	0.5	19.3	38.3	6.7
	10.0 \bar{X}	8.7	107.5	177.5	78.9
	sd	0.2	12.3	16.1	8.2
	20.0 \bar{X}	13.9	135.2	232.3	66.9
	sd	1.8	33.0	42.6	5.6
	100.0 \bar{X}	19.1	114.5	196.9	58.9
	sd	2.0	4.1	8.1	2.6
		**	NS	NS	**
	0.0 \bar{X}	ND	51.5	78.1	25.4
Stem	sd	-	5.6	6.5	4.5
	2.0 \bar{X}	1.52	54.7	76.6	19.6
	sd	1.36	3.3	9.9	2.8
	10.0 \bar{X}	2.67	37.9	67.6	22.0
	sd	0.36	6.0	5.9	1.6
	20.0 \bar{X}	1.10	41.2	75.4	18.9
	sd	1.15	3.1	7.5	3.2
	100.0 \bar{X}	8.71	34.6	55.7	22.4
	sd	1.14	1.3	5.2	3.4
		**	**	*	NS
	0.0 \bar{X}	ND	127.6	225.4	180.8
Root	sd	-	20.3	8.5	21.2
	2.0 \bar{X}	29.5	95.1	159.5	183.0
	sd	3.2	33.9	8.2	9.5
	10.0 \bar{X}	150.4	85.1	153.2	176.8
	sd	20.4	9.9	5.3	16.5
	20.0 \bar{X}	227.9	86.4	212.5	170.1
	sd	6.9	6.1	12.1	45.1
	100.0 \bar{X}	1348.3	96.5	101.7	177.1
	sd	280.1	7.7	2.2	10.9
		**	NS	**	NS

Table 5.8 Ion levels in various bean tissues as affected by vanadium applied as VOSO_4 at various concentrations.

* significant difference $P < 0.05$

** significant difference $P < 0.01$

Table 5.8

		Ion level ($\mu\text{g}\cdot\text{g}^{-1}$ d.wt.)			
Applied V (μM)		V	Fe	Zn	Mn
Leaf	0.0 \bar{X}	ND	130.9	196.6	93.7
	sd	-	17.1	17.9	11.4
	2.0 \bar{X}	5.8	129.6	226.7	99.3
	sd	0.3	10.0	13.5	6.9
	10.0 \bar{X}	9.42	131.3	214.9	93.2
	sd	3.6	22.7	4.5	8.7
	20.0 \bar{X}	13.9	141.6	212.5	90.6
	sd	0.8	8.3	19.8	4.6
	100.0 \bar{X}	15.4	105.1	227.5	82.1
	sd	2.9	13.2	115.3	4.6
		**	NS	NS	NS
	0.0 \bar{X}	ND	38.8	61.7	23.6
Stem	sd	-	3.9	3.1	4.63
	2.0 \bar{X}	ND	36.7	63.9	23.8
	sd	-	7.5	7.9	5.7
	10.0 \bar{X}	3.4	34.1	59.2	17.2
	sd	2.1	3.7	5.5	2.6
	20.0 \bar{X}	3.7	43.5	71.0	17.3
	sd	0.9	3.9	6.1	0.3
	100.0 \bar{X}	8.6	35.9	51.3	19.7
	sd	1.9	4.3	9.5	1.7
		**	NS	*	NS
	0.0 \bar{X}	ND	105.3	213.9	197.4
Root	sd	-	2.6	5.8	
	2.0 \bar{X}	55.7	103.4	184.5	200.1
	sd	7.8	3.1	2.8	11.9
	10.0 \bar{X}	209.5	101.5	186.8	186.6
	sd	1.9	2.80	12.2	7.1
	20.0 \bar{X}	277.2	89.2	175.9	185.2
	sd	26.9	18.0	14.2	8.8
	100.0 \bar{X}	1453.4	95.6	126.7	192.4
	sd	647.3	13.2	6.9	7.8
		**	NS	**	NS

zinc levels declined as vanadium levels increased regardless of the ionic form of the applied vanadium.

The results presented here compare well with those reported for related species. Wallace et al (1977) using *Phaseolus vulgaris* found similar tissue levels when vanadium was applied as the vanadate anion (fig. 5.25). This would seem to support the trends reported here i.e. the bulk of absorbed vanadium retained within root tissues and the larger part of aerial vanadium in the leaves. Wallace et al (1977) also reported significant decreases in leaf iron although this was not found here. Wallace et al (1977) also found decreased shoot manganese and increased root manganese levels although once again this was not evident in the present study.

5.4 Tomato.

Limited results are available on the effects of vanadium on the growth of tomato, *Lycopersicon esculentum* Mill. Welch and Huffman (1973) tested the hypothesis that vanadium is an essential element for higher plants using tomato. No differences in yield (dry weight) were recorded between plants grown in purified nutrient solution (0.04 ng ml^{-1}) and plants grown in nutrient solution with added vanadium (50 ng ml^{-1} as NH_4VO_3). The tops of plants grown in nutrient solution containing added vanadium contained between 0.117 and $0.418 \text{ } \mu\text{g g}^{-1}$ on a dry weight basis.

The effect of vanadium applications on growth of tomato plants was dramatic. A highly significant ($P < 0.01$) decrease in dry weight production occurred over the range of concentrations tested. This decrease in growth occurred irrespective of the applied form of the vanadium (fig. 5.26a and 5.26b). Plates 5.1 and 5.2 show plants grown in these experiments; the particularly severe retardation of root growth at higher applied vanadium concentrations can clearly be seen. There was also evidence of apical chlorosis in the plants which received the higher applied levels. Plants grown with a low level of vanadium supplement appeared perfectly healthy.

fig. 5.25 Comparison of bean uptake results with those of
Wallace et al (1977)

Data from this study



Data from Wallace et al
(1977)



- (a) Root tissue
- (b) Leaf tissue
- (c) Stem tissue

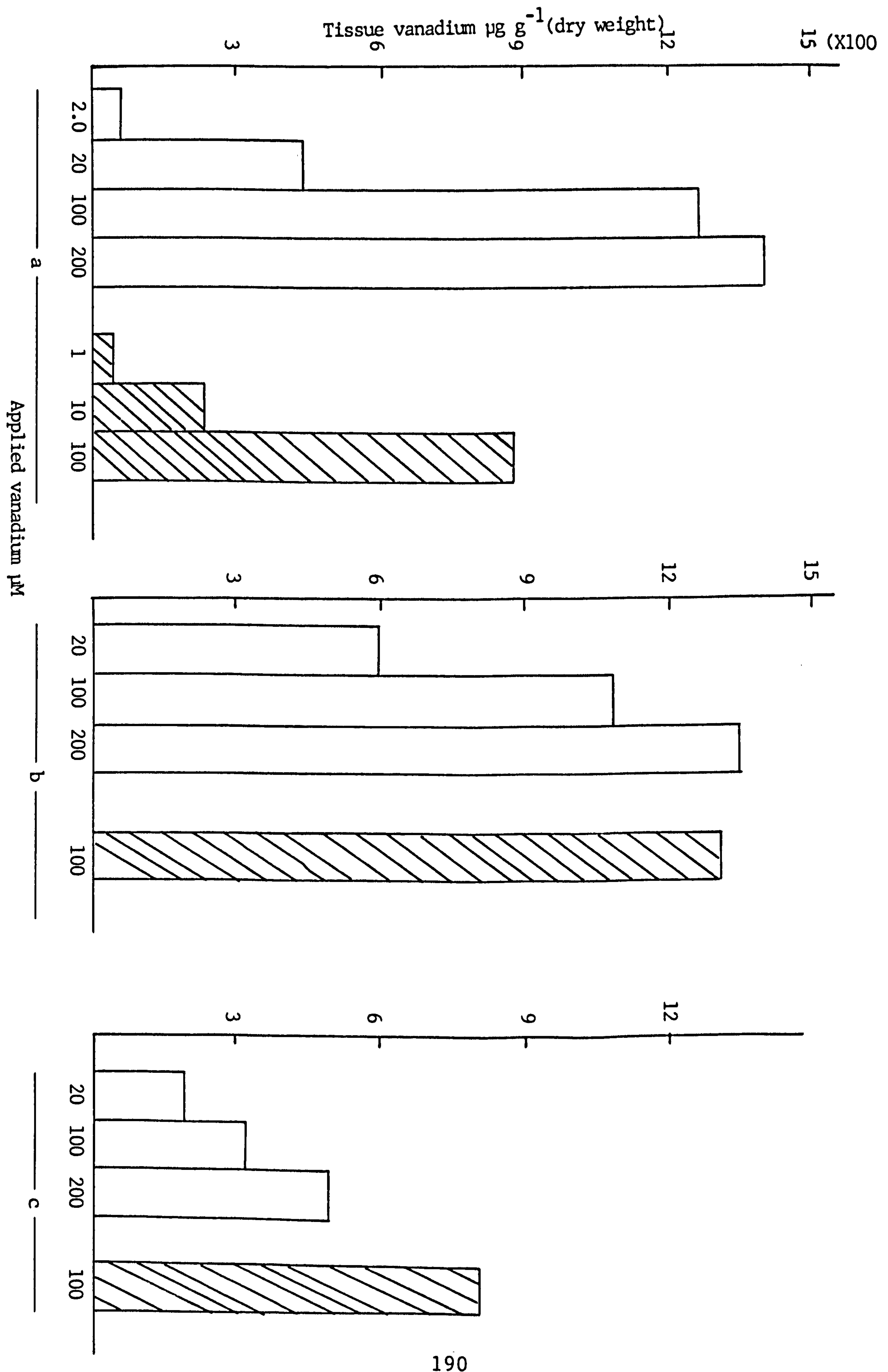


fig. 5.26 Tomato dry matter production as affected by
applied vanadium at various levels.

(a) vanadium applied as NH_4VO_3

(b) vanadium applied as VOSO_4

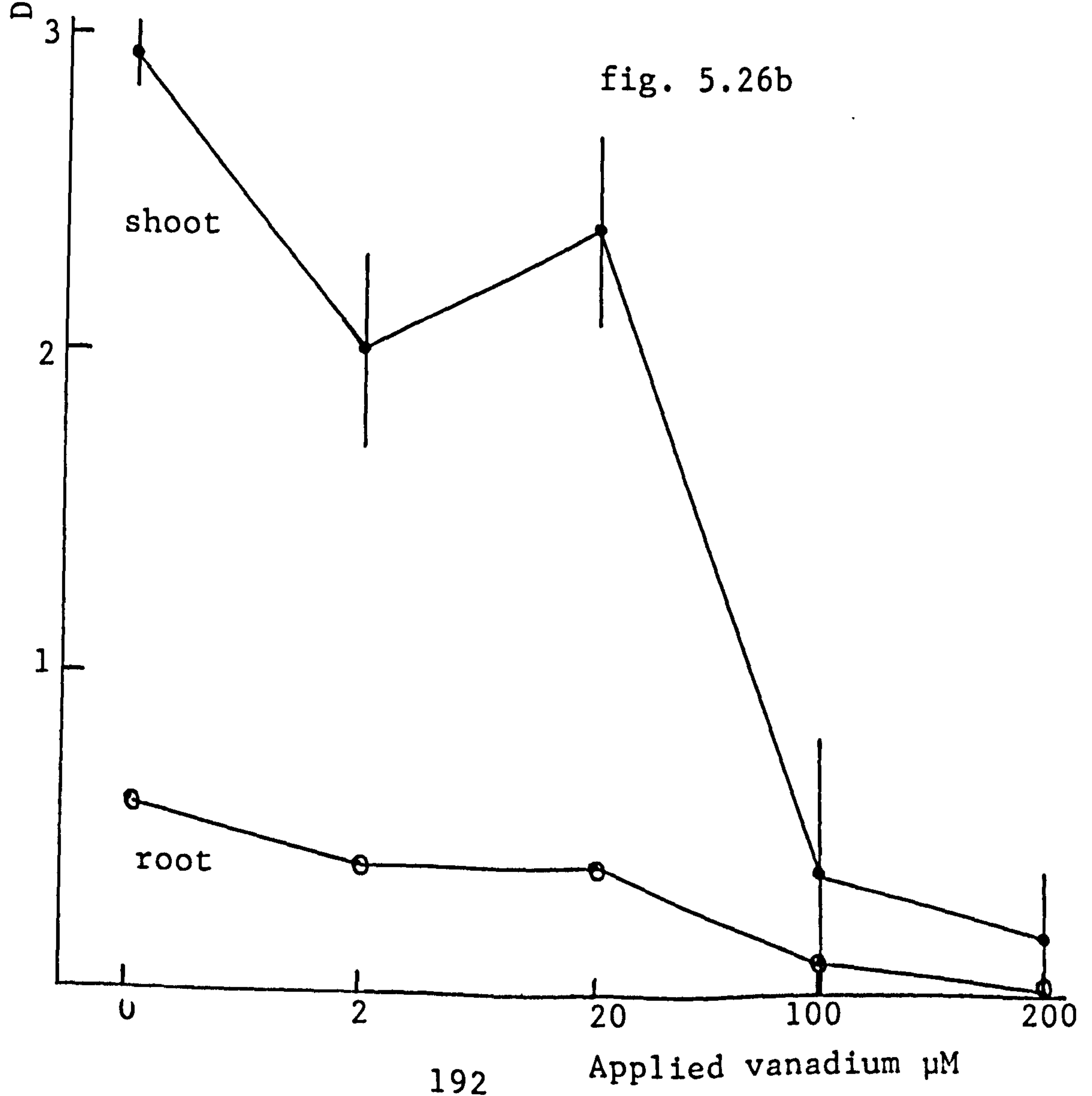
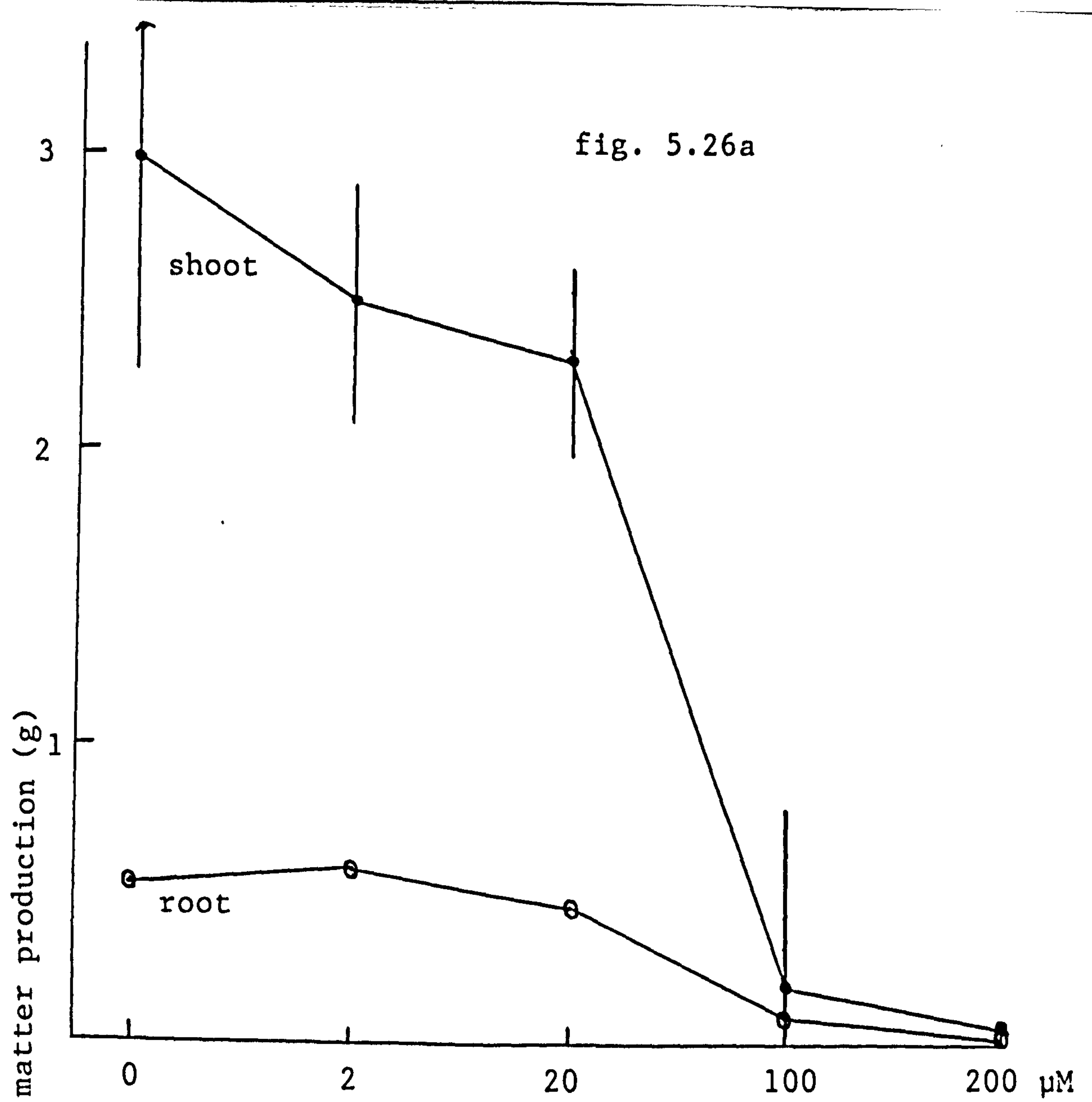


Plate 5.1 Tomato plants as affected by vanadium applied
as VOSO_4 at various concentrations.

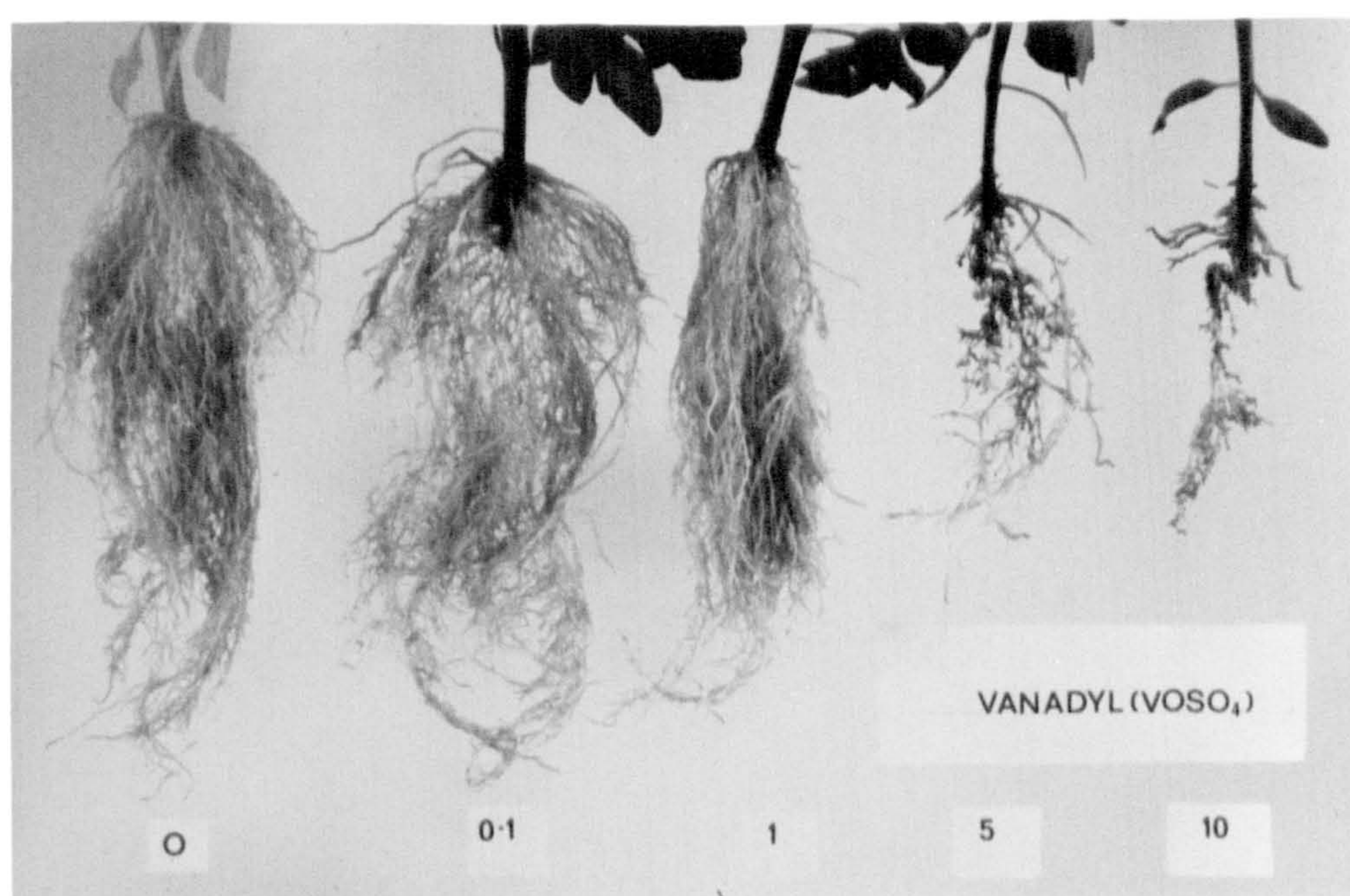
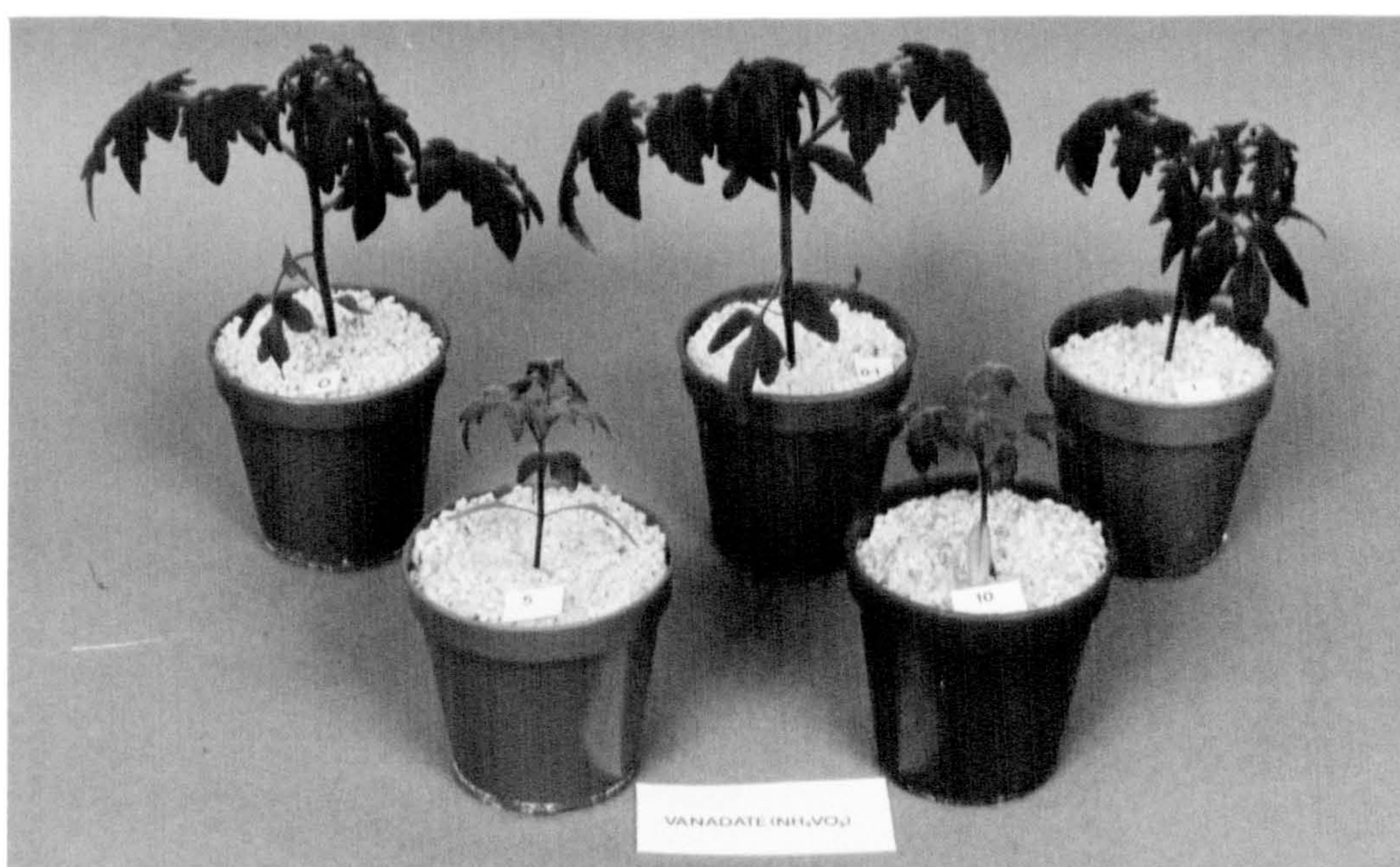


Plate 5.1

Plate 5.2 Tomato plants as affected by vanadium applied
as NH_4VO_3 at various concentrations.

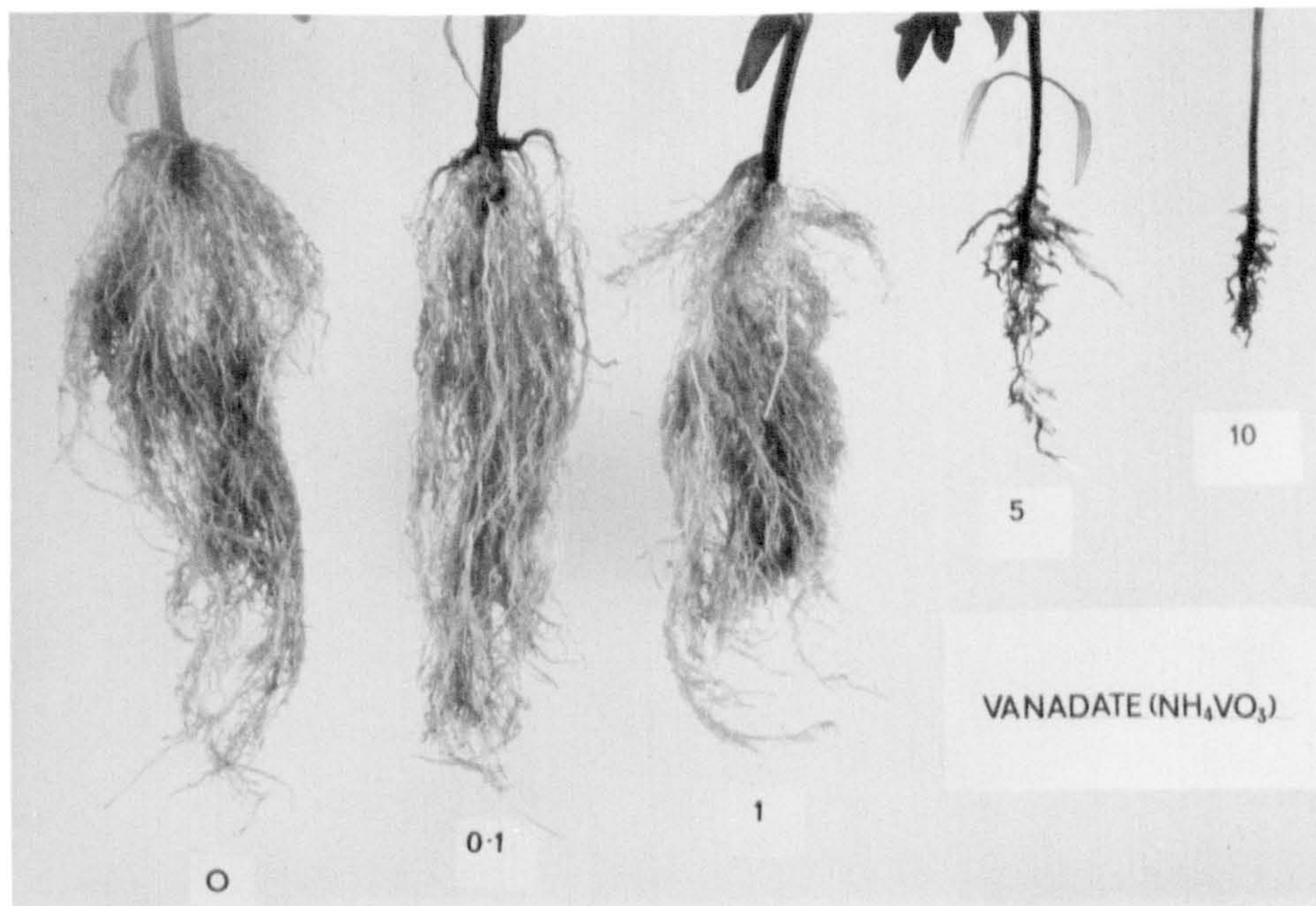
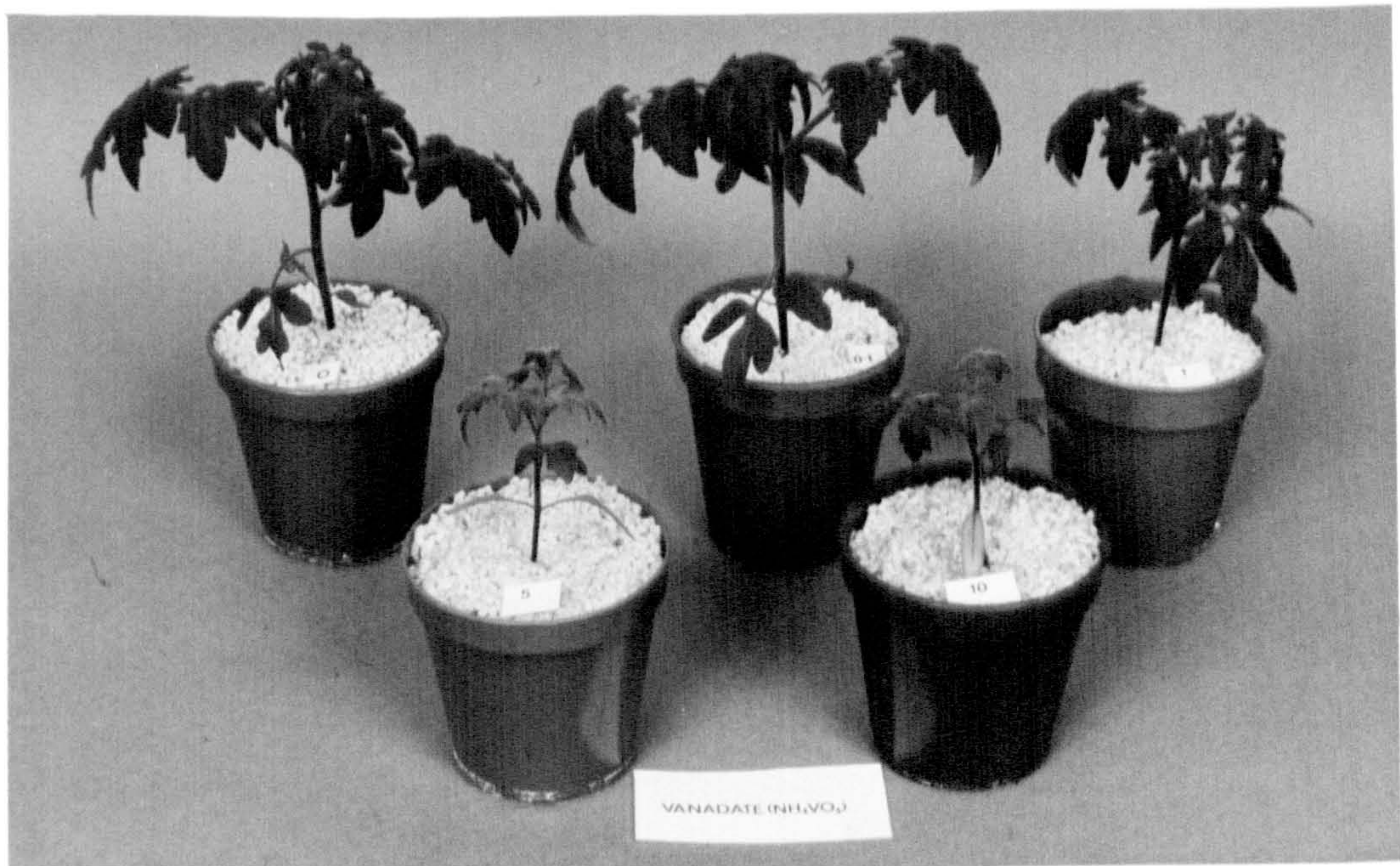


plate 5.2

The vanadium levels in the tissues of tomato are shown in fig. 5.27 (root) and fig. 5.28 (shoot). No differences in levels taken up from solutions of different ionic form were apparent. Partitioning within the plant tissues is similar to that observed for other plant species i.e. retention within root tissues. The results are highly variable and some high vanadium shoot levels occur. These can to some degree be attributed to growth effects rather than high levels of uptake.

No root-shoot correlations or regressions have been attempted. The levels of other ions within the tissues has not been examined.

5.5 Discussion.

Of the three plant species examined in this study maize and beans were tolerant of moderately elevated levels of vanadium in the nutrient solution but tomato exhibited reduced growth when the vanadium level in the nutrient solution was only slightly elevated. The basis for these differential growth responses is at present unclear, although it seems unlikely to be based upon differential uptake since all three species absorb approximately equivalent amounts. It would seem at present that the difference arises from differential susceptibility to vanadium in the tissues.

Uptake of the two different forms showed no differences in most cases, although in one instance there was significantly greater uptake of the vanadyl cation than the vanadate anion. This difference may have been attributable to differences in the pH of the nutrient solutions since this was an experiment where pH was not adjusted prior to the beginning of the experiment. In all subsequent experiments the pH of nutrient solution was balanced before use.

Once absorbed the two vanadium ions showed remarkably similar patterns of partitioning within plant tissues. In all experiments the bulk of the absorbed vanadium (usually more than 90%) was retained within the root tissues. This

fig. 5.2 7 Tomato root vanadium levels as affected by applied vanadium.

- (a) vanadium applied as NH_4VO_3
- (b) vanadium applied as VOSO_4

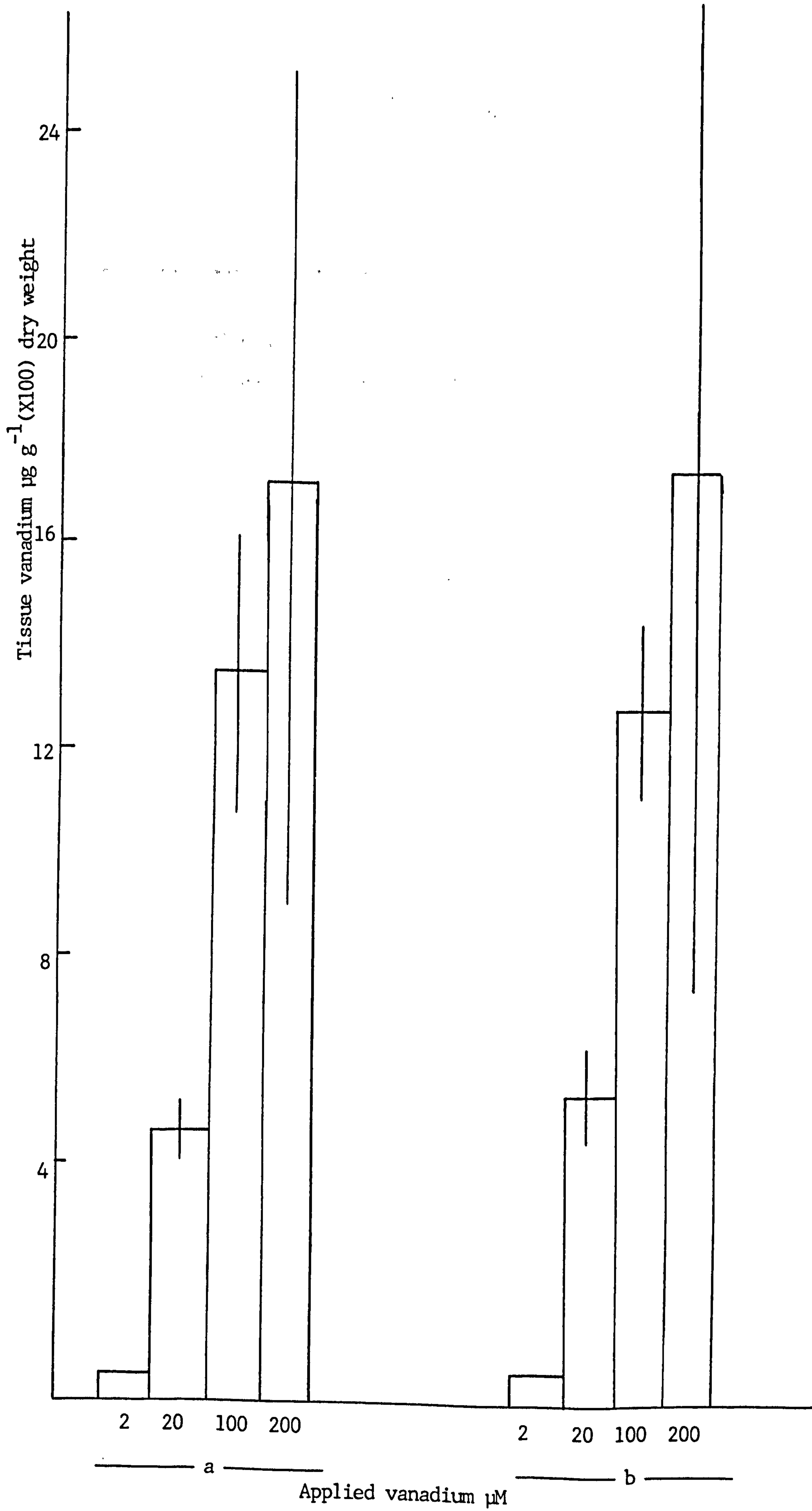
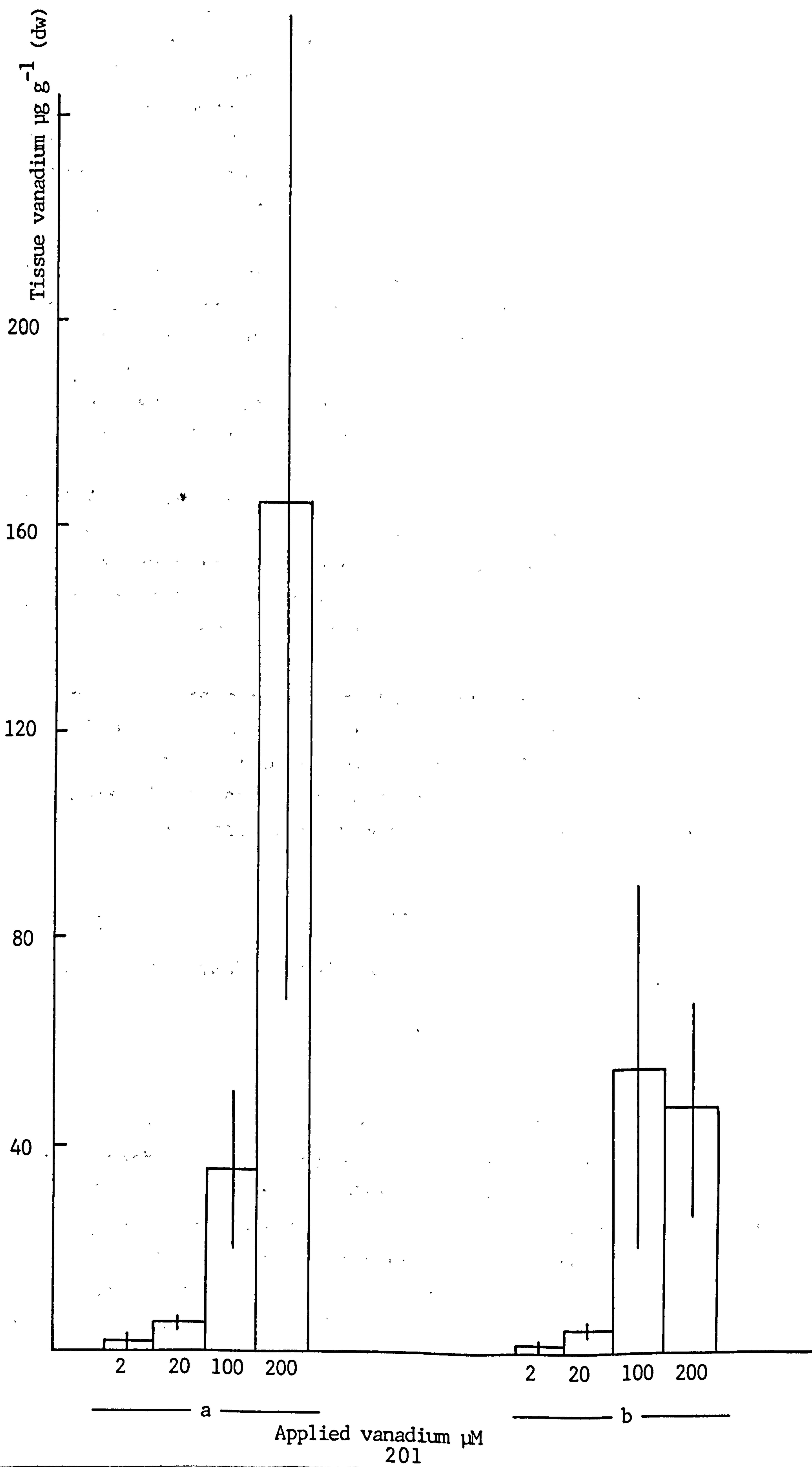


fig. 5.2.8 Tomato shoot levels as affected by applied vanadium.

(a) vanadium applied as NH_4VO_3

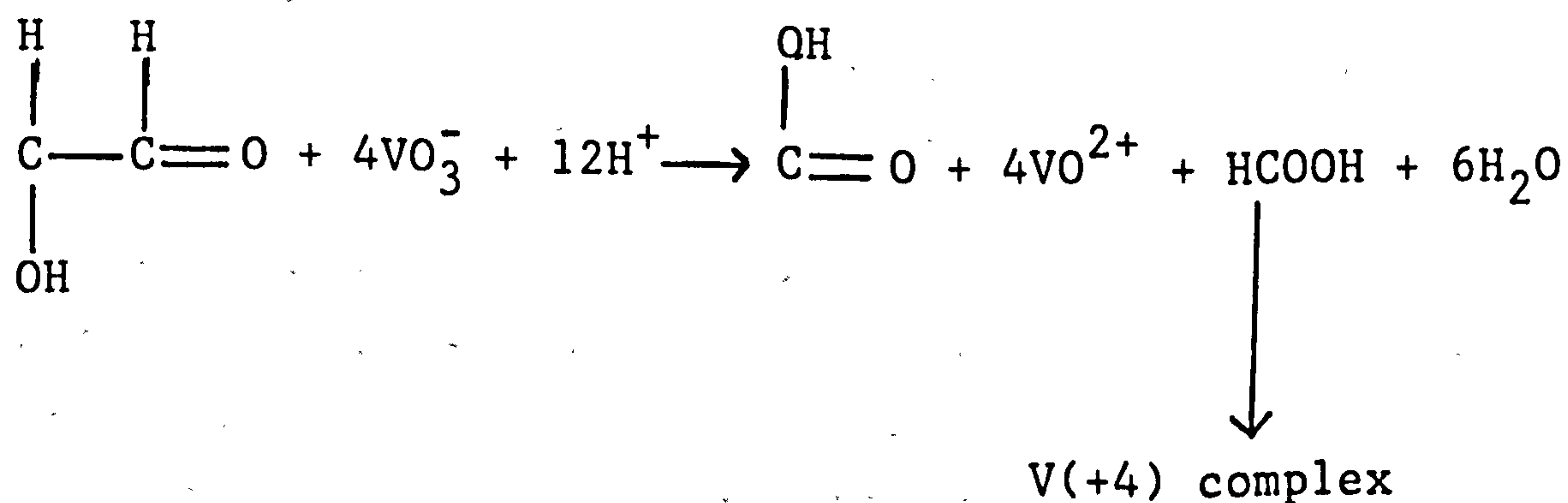
(b) vanadium applied as VOSO_4



pattern is similar to that observed for other metal ions noticeably cadmium and lead where 57 - 80% of the metal was retained within the root tissues (Jarvis and Jones 1978; Jones et al 1973). As with these metals the mechanism of uptake and binding of vanadium has not yet been resolved although suggestions have been proposed (*Vide infra*).

Transport ratios within the various plant tissues have been examined and are similar for both ions. Indeed, when the relationships between root-shoot vanadium have been characterized using linear regressions no differences can be discerned between the slopes of lines for the different ionic forms of vanadium. An hypothesis which explains this distinct similarity in uptake and transport of distinctly dissimilar ions is that some form of interconversion of the ions may take place in the root tissues. The biotransformation need not be complete, although it is probable that one form will dominate, particularly in transport to aerial plant tissues.

One possible mechanism for this biotransformation which may also help explain the retention of vanadium within root tissues is that proposed by Deiana et al (1983). This mechanism involves the reduction of V(+5) at the cell wall soil solution interface in a manner similar to that shown below;



Such a mechanism is feasible over a wide pH range since V(+5) it is a powerful oxidant. Even at pH 7 it will readily oxidize aldehydes, ketones, catechols and sulfhydryls (Macara 1980) all commonly found in plant cell walls. The corollary to this conclusion is that effects on plants will be identical regardless of the applied form.

In complex systems individual interactions between elements are difficult to detect. However, on the basis of the data presented here it would appear that vanadium, regardless of applied ionic form, does interact with zinc and manganese particularly in shoot tissues i.e. increased vanadium transport to the shoot decreases the transport of these ions. The experiments using beans also suggest that some interaction between vanadium and root zinc may be occurring. Where data is available it tends to agree with these observations. Warrington (1951) observed that increased vanadium levels reduced chlorosis caused by excess manganese presumably through reduction of manganese in plant tissue although no plant levels were specified. Wallace et al (1977) also showed reduced manganese in aerial tissue when plant vanadium levels were elevated. These workers also noted increased root manganese levels with increased root vanadium although no evidence of this was found in this study. Similarly some authors (Basiouny 1984; Wallace et al 1977) have reported vanadium effects on iron levels although none could be detected in this study.

The effect of calcium levels on vanadium mobility within the plant has been speculated upon in the past (Cannon 1963) although no experimental evidence has been presented to support these speculations. Cannon (1963) states:

"Plant species that absorb large amounts of calcium are most tolerant of high vanadium soils, as the vanadium is precipitated in the root".

There is however no evidence to support this supposition. The fact that plant species which contain large amounts of calcium are tolerant of high levels of vanadium is not surprising given calcium's recognized ability to ameliorate the adverse effects of high levels of heavy metals (Jones and Lunt 1967; Garland and Wilkins 1981).

Results presented here are inconclusive although they tend not to support this hypothesis. Whilst significant increases in shoot levels occur no significant effects on root levels can be detected when calcium levels were altered.

Although the data is highly variable trends between experiments can be seen and it does seem likely that increased shoot vanadium is a 'real' effect. At present however it would seem difficult to explain this phenomenon, and further study is required to clarify the problem.

CHAPTER SIX

ESR INVESTIGATIONS

Electron spin resonance investigations

6.1 Background

Electron spin resonance (ESR), alternatively known as electron paramagnetic resonance (EPR), is a technique which allows the detection and, in favourable circumstances, characterization of ions, radicals or molecules containing unpaired electrons. Biologically important species such as free radicals, transition metal ions and rare earth ions will contain unpaired electrons and are thus susceptible to analysis by this technique.

Although a rigorous description of the electron spin resonance phenomenon can only come from quantum mechanics, a brief summary of the events involved and conditions required is given below. It is hoped that this will both 'set the scene' and indicate some of the pitfalls involved in ESR work.

The electron spin of the title crudely refers to the rotation of the electron about its own axis. Quantum restrictions dictate that no two electrons can have identical states; since there are only two possible spin states electrons in close proximity must spin in opposite directions. This is termed an electron pair. Most organic and important biological molecules contain an even number of electrons which are invariably paired. If however a molecule contains an odd number of electrons one electron is unsatisfied because it has no partner. Such a molecule is paramagnetic.

When electrons are paired in chemical bonds or elsewhere their spins are opposed and any magnetic moments generated by these spinning charges are effectively cancelled out. For this reason most chemical and biochemical substances which contain only paired electrons have no net electron magnetic moments. However paramagnetic molecules have an uncanceled electron spin magnetic moment because of their unpaired electron. ESR spectroscopy takes advantage of this and the fact that only the paramagnetic molecules in a sample can be made to interact with a suitable external magnetic field. Hence a small number of paramagnetic molecules can

be detected and examined in a matrix composed predominantly of other substances provided they themselves are not paramagnetic.

The manner in which the ESR signal is obtained for a paramagnetic species containing a single unpaired electron can briefly be described thus;

If an external magnetic field is applied to a sample all the unpaired electrons in that sample (which are in effect tiny spinning magnets) will become aligned with that field. Laws of space quantization demand one of two possible alignments, either directed parallel to the external field or opposed to it (antiparallel - which is a slightly higher energy and hence less stable condition). Thus the applied magnetic field segregates the population of equally energetic paramagnets into two groups with a very small energy difference between them. Energy transitions are now possible in which the orientation of the electron spins will alter. Such transitions will occur if the spins lined up by the applied magnetic field are also subjected to electromagnetic radiation of an appropriate frequency. To be of an appropriate energy the quantum energy of the field must correspond exactly to the energy difference between the two spin states.

The condition in which the second electromagnetic field frequency brings about this energy exchange is a resonance condition. In simple terms this means that spinning electrons can both take energy from the field and give energy to it. Under normal circumstances however, due to the distribution of spin states, there is a net absorption of energy from the field. It is this net absorption of energy that is detected and amplified to provide the sample signal in ESR spectroscopy.

The simplest type of ESR spectra consist of a single line which can be characterized by its shape, width, intensity and g-factor*. Unfortunately these properties of single line ESR spectra do not vary much between paramagnetic molecules. Indeed if all paramagnetic species produced

* g-factors are determined by the ratio of field and frequency at the centre of the resonance line.

only single line spectra ESR spectroscopy would be of little value. Fortunately however many ESR signals are multi lined and can be identified by their detailed pattern using particularly a feature known as the Hyperfine structure.

Hyperfine structure arise because of interactions between the spinning electrons and nuclei which have non-zero magnetic moments. The effect of these electron - nucleus interactions is to split the absorption spectra into $2I + 1$ components, where I is the nuclear spin. For vanadium $I = 7/2$ which produces the prominent octet hyperfine splitting.

Correct interpretation of ESR spectra depend upon at least a basic knowledge of the principles involved. ESR differs significantly from most other spectroscopies in that the nature of the spectra obtained are highly dependent upon the choice of instrumental settings and subtleties of sample conditions. Selection of operating conditions and the properties of different samples critically affect the spectra obtained and may even result in the lack of detection of some strong components, inappropriate emphasis of minor components and serious distortion of signal shape.

In view of these comments experimental conditions were kept as simple as possible, to exclude all other molecules which might interfere with the ESR spectra.

When using ESR for detection and characterization of transition metal ions certain 'new' features of the spectra have to be considered;

1. Spin orbit coupling is strong and exhibits strong anisotropy* so that g-factors vary over a wide range.
2. The features of the ESR spectrum depend strongly upon the environment of the ion.
3. Low temperatures are often required if the resonance condition is to be observed.
4. For ions with an even number of unpaired electrons it may be impossible to observe any spectrum at all.

* anisotropy refers to the orientation dependence of the hyperfine interaction.

However not all these features apply to the VO^{2+} ion, for example it is possible to observe spectra with rather narrow hyperfine structure even at room temperature. In general terms the VO^{2+} ion, with its unpaired electron occupying the d_{xy} orbital is well suited to study by ESR spectroscopy.

ESR spectroscopy has been usefully employed by many workers concerned with the role of vanadium in biological systems. Chasteen (1983) has reported on some of these investigations including those leading to the suggestion that *in vivo* reduction of vanadium occurs within living tissue. Goodman and Cheshire (1975) demonstrated the reduction of vanadium by soil organic matter. This aspect of soil-vanadium interactions was further studied by Cheshire et al (1977) who used ESR to study the distribution of vanadium between humic and fulvic acid fractions of a soil. McBride (1980), using ESR, was able to suggest a role for carboxylated polysaccharides in vanadium binding in natural soils.

Only 2 studies of vanadium in plant tissues, using ESR, have been reported. Gilliard and Lancashire (1983) demonstrated that the same VO^{2+} complex was present throughout the entire structure of the mushroom *A. muscaria*. The ESR spectra obtained were very similar to those obtained for alpha amino acid complexes containing terdentate species such as L-cysteine or L-serine. The only study utilizing ESR spectroscopy for the study of the uptake of the vanadyl ion by higher plants is that of Mcphail et al (1982). From their results they were able to draw very specific conclusions as to the events resulting from the uptake of the vanadyl ion by plants.

The aim of the present study is to demonstrate that the vanadate anion could be reduced by plant root tissue *in vivo*. Goodman and Cheshire (1975) have already demonstrated the reduction of the vanadate ion by soil organic matter but this in no way precludes the possibility of plant roots coming into contact with vanadate ions in the soil solution.

6.2 Materials and Methods.

Barley seeds (*Hordeum vulgare* L. cv Maris Mink) were germinated and after two days growth seedlings were transferred to culture solutions. Plants were exposed to two sets of conditions; (A) Grown in 0.5mM CaCl_2 for five days and then transferred to 0.5mM CaCl_2 + 0.1mM V (as either VOSO_4 or NH_4VO_3) for two days. (B) Grown in 0.5mM CaCl_2 + 0.1mM V (as either VOSO_4 or NH_4VO_3) from the start of the experiment.

All plants were harvested after nine days growth, washed in distilled water, and freeze dried to a constant weight. Small subsamples of tissue were threaded into glass ESR tubes and analysed at ambient temperature using a Bruker EPR spectrophotometer operating at X-band frequencies.

6.3 Results and Discussion.

The spectra obtained when the tissue was analysed are shown in figs. 6.1a, 6.1b, 6.2a and 6.2b. All spectra represent the first derivative of absorption. All spectra are similar and exhibit the hyperfine octet characteristic of the VO^{2+} entity. (fig. 6.1a was obtained at reduced gain setting in order to produce a spectra comparable to the others).

It is felt that these results clearly demonstrate the ability of barley roots to reduce pentavalent vanadium to the tetravalent form. It is unlikely that this reduction occurred in the solution given its constant aeration and relatively high pH (4.0 - 5.7). Whilst the results clearly demonstrate the presence of V(+4) they do not necessarily suggest the complete absence of V(+5).

Reduction of metal ions by the roots of higher plants is a well documented phenomenon (Chaney et al 1972, Uren 1981, 1982, Julian et al 1983 and Rohmeld and Marschner 1983). Uren (1981) has shown reduction of insoluble manganese oxides by the roots of sunflower seedlings. The reduction of Fe^{3+} to Fe^{2+} is a well documented event although the actual mechanism of reduction is at present unclear. Julian et al (1983) have proposed that secreted reductants (e.g. caffeic

fig. 6.1a ESR spectra for roots exposed to 0.5mM CaCl_2
+ 0.1mM VOSO_4 from the beginning of the
experiment.

fig. 6.1b ESR spectra for roots exposed to 0.5mM CaCl_2
+ 0.1mM NH_4VO_3 from the beginning of the
experiment.

Fig. 6.1a

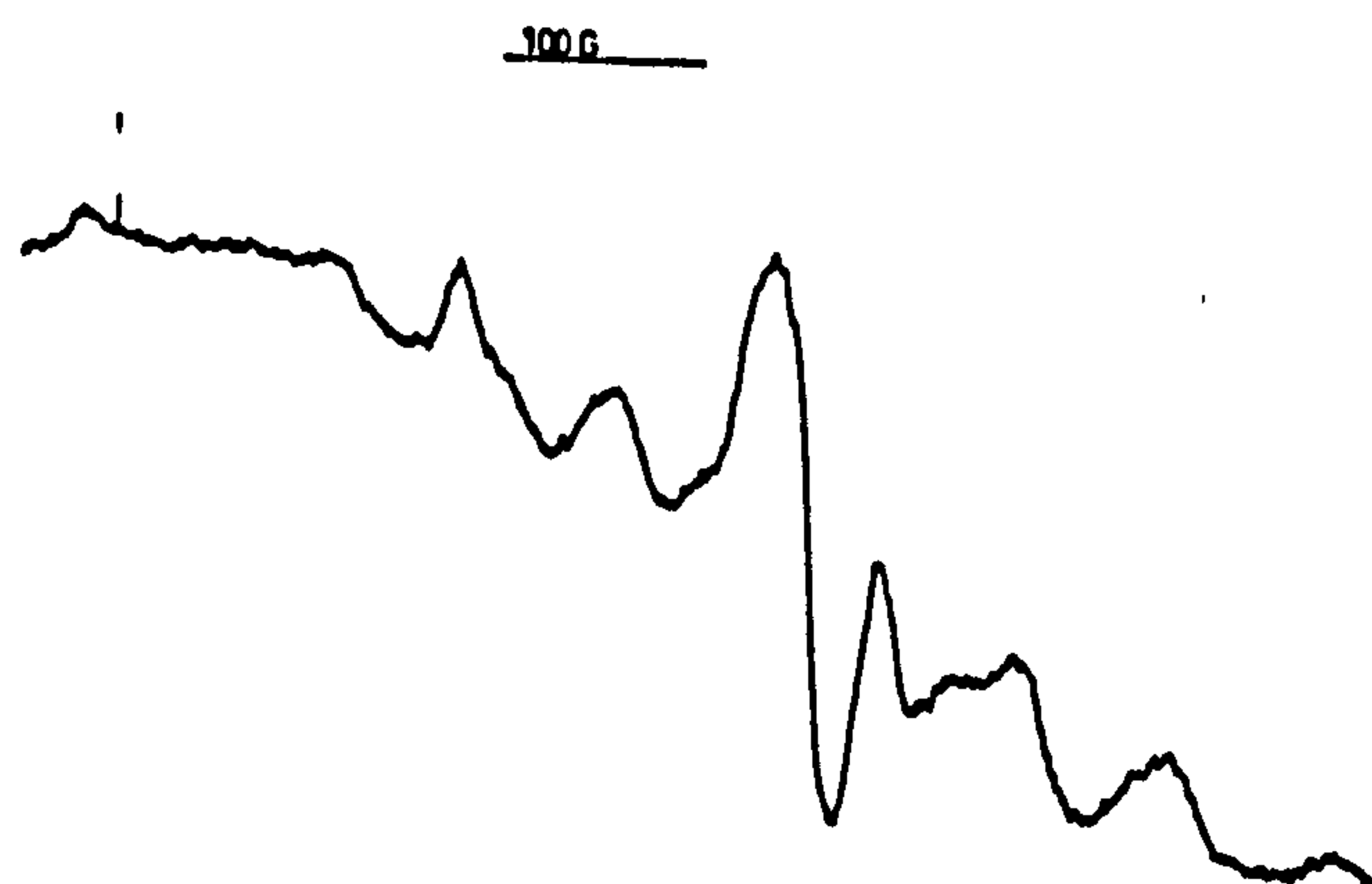


Fig. 6.1b

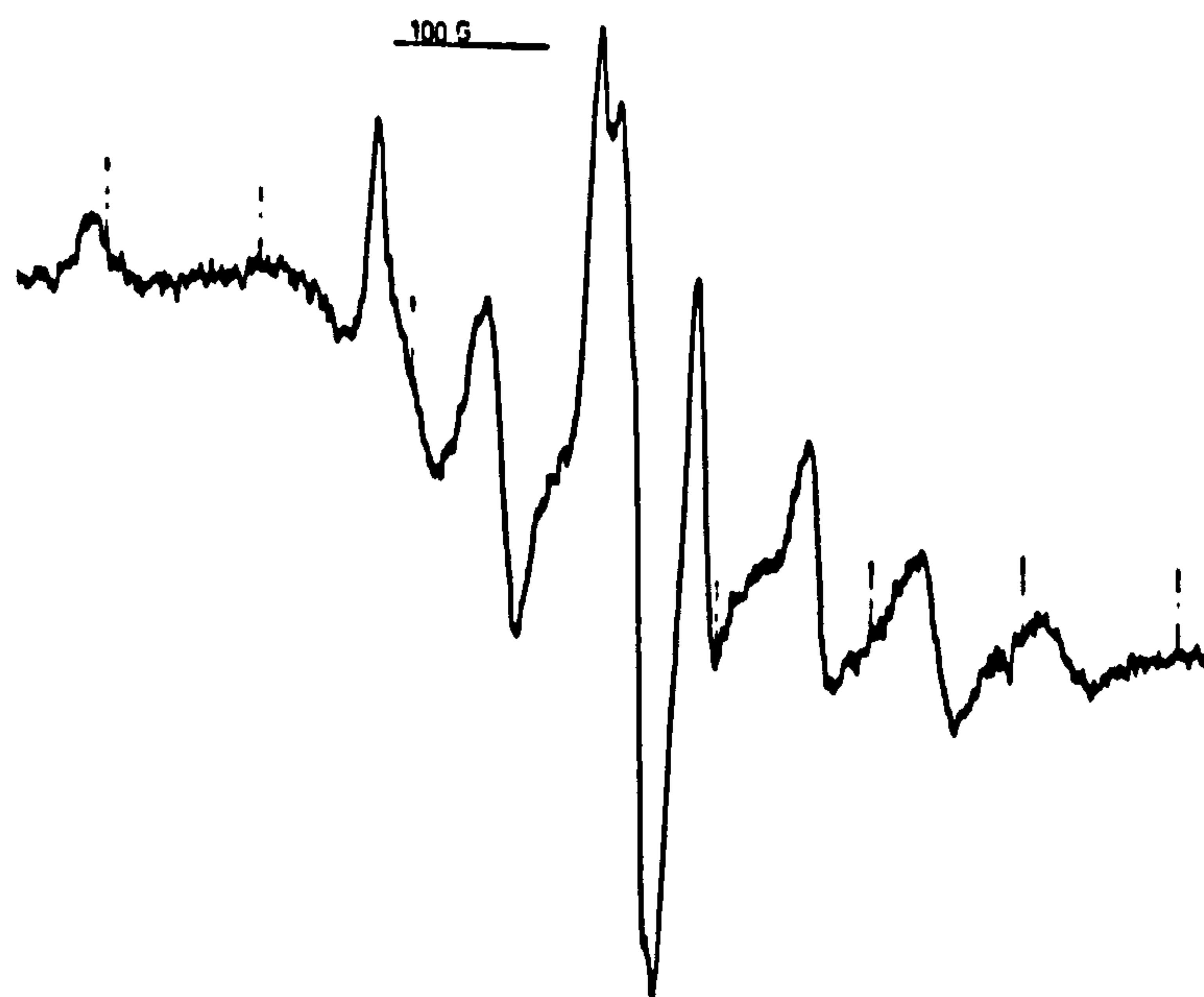


fig. 6.2a ESR spectra for roots exposed to 0.5mM CaCl_2
for 5 days prior to exposure to 0.5mM CaCl_2
+ 0.1mM VOSO_4 for 2 days.

fig. 6.2b ESR spectra for roots exposed to 0.5mM CaCl_2
for 5 days prior to exposure to 0.5mM CaCl_2
+ 0.1mM NH_4VO_3 for 2 days.

fig. 6.2a

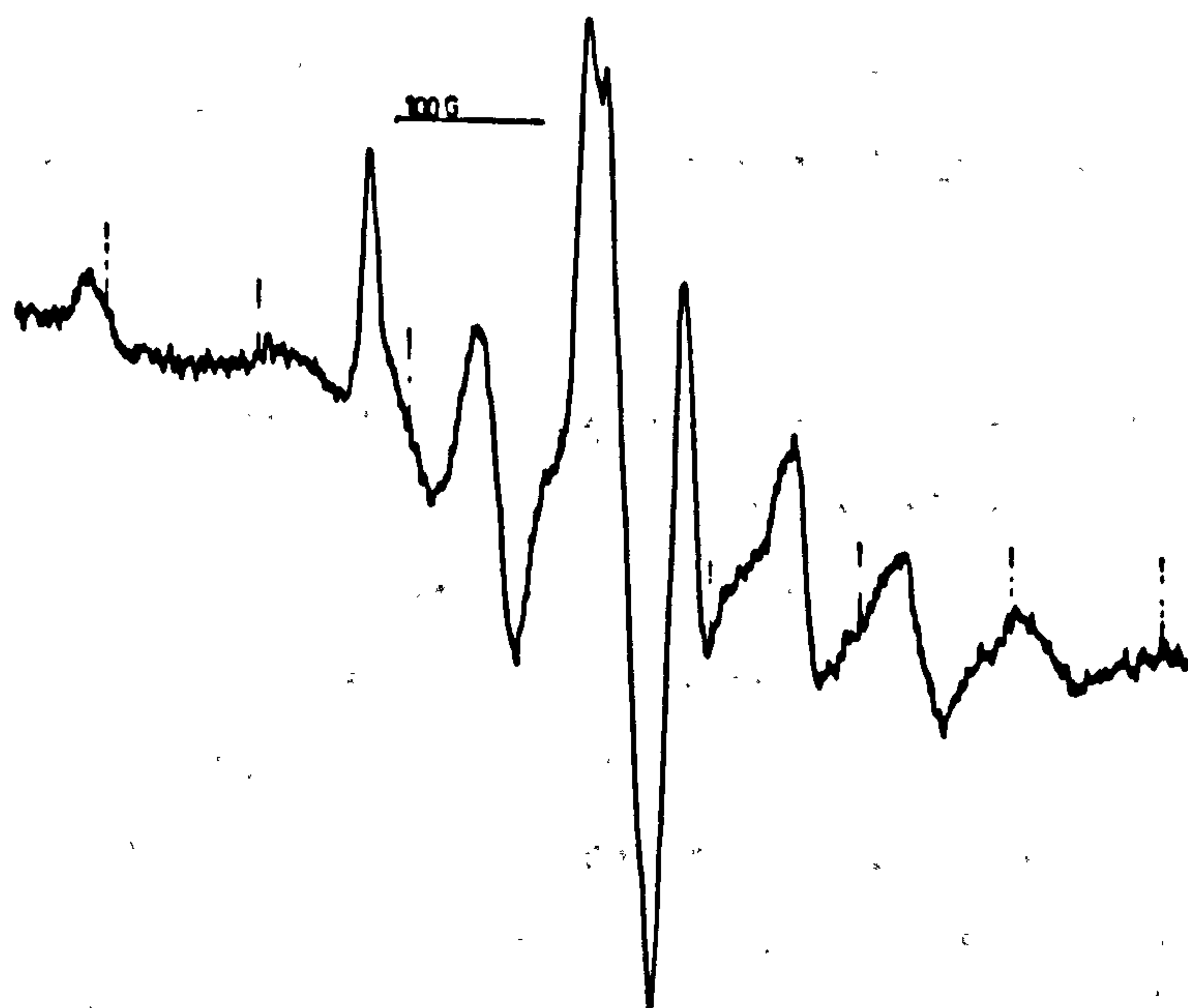
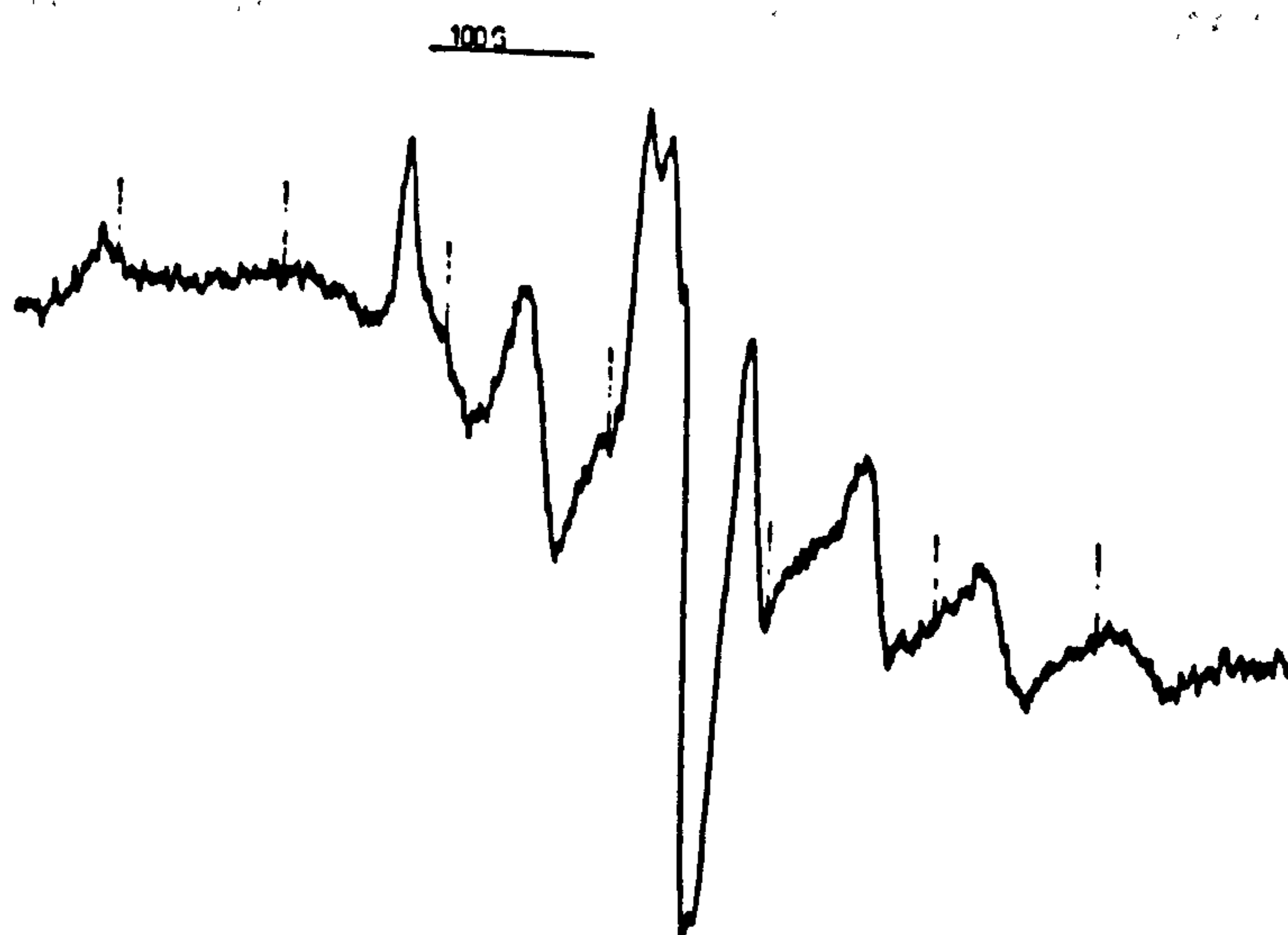


fig. 6.2b



acid) reduced Fe^{3+} in intracellular spaces from where it is absorbed. Chaney et al (1972) favour reduction of Fe^{3+} at the plasmalemma by the uptake sites. Rohmelt and Marschner (1983) point to the myriad advantages of such a system but also point out that such a system would require at least some soluble Fe^{3+} in the rooting medium and apparent free space.

The mechanism of vanadium reduction in plant roots may be different from above possibly involving cell wall polyuronates. Polygalacturonic acid will react with soil mineral species such as V(+5) because of the reducing properties of the polysaccharide end units. Deiana et al (1983) have proposed a mechanism for the reduction and subsequent complexation of V(+5), (see previous chapter). These authors pointed to the significance of these processes in maintaining a micronutrient supply for higher plant roots, especially in the case of Fe which can only be absorbed in the reduced form. In the case of vanadium however it is probably the reverse in that reduction and subsequent complexation of vanadium probably reduces its availability. This suggestion agrees in a limited way with the findings of Mcphail et al (1982) who also showed initial immobilization of vanadium upon root cell walls.

The use of ESR spectroscopy has great potential however, as the initial discussion of ESR principles showed, great attention will have to be given to experimental conditions.

CHAPTER SEVEN

DISCUSSION

7.1 Discussion.

For over three decades vanadium has been known to be an essential element for certain algae (Arnon and Wessel 1953). There has also been a growing recognition of the essential role of this element in the nutrition of higher animals (Macara 1980), but to date there is no unequivocal statement relating to the role of vanadium in the nutrition of higher plants.

Trace amounts of vanadium have been reported as favourable to plant growth (Singh 1971; Basiouny 1984) but higher levels have been reported to produce a detrimental effect on plant growth (Hara et al 1976; Wallace et al 1977). Attempts to produce a clear demonstration of an essential requirement for vanadium in the nutrition of higher plants have proved unsuccessful. It is now recognized that if vanadium is required by higher plants, only minute quantities are necessary (Welch and Huffman 1973; Hewitt pers. comm.). Despite this, vanadium has been detected in the tissues of most plants which have been examined for its presence.

Our present knowledge of all aspects of the interaction of vanadium with higher plants is totally inadequate. The experiments reported above represent an attempt to begin to rectify this situation. The problem has been approached in several ways; however these should not be considered as disparate and unco-ordinated, as each shares a common objective. The following discussion should serve to emphasize this interrelationship, as the data is drawn together to produce a more integrated statement relating to the uptake of vanadium by higher plants.

Initial experiments in this study involved examining the uptake of vanadium by excised *Hordeum* roots. These studies had three clear objectives:

- (a) To evaluate the characteristics of uptake of different ionic species of vanadium.
- (b) To attempt to characterize the mechanism of vanadium uptake.

- (c) To produce data which could be compared with that already available for vanadium and other chemically similar elements.

The first positive observations however related to experimental technique. Initial results were erratic and inconsistent. These inconsistencies were attributed to the fact that uptake solutions were freshly prepared and equilibria between the various ionic species had not become established. These erratic results were found in experiments relating to both V(+4) and V(+5). Welch (1973) reported similar difficulties in obtaining consistent results and suggested techniques which would allow these difficulties to be overcome. As a result all experimental solutions used in uptake studies were prepared a minimum of three days prior to use to allow stabilization of the ionic species present.

This slowness of equilibration is an important factor which all experimenters should be aware of when examining the effect of vanadium on biological systems.

Initial experiments were aimed at establishing the extent and nature of free space accumulation of vanadium. Knowledge of this component of total uptake is essential if meaningful attempts are to be made at a mechanistic examination of uptake. Clear differences between free space accumulation of the two ionic species examined would be expected because of the charges which the ions bear. A monovalent anion such as vanadate would be expected to show limited free space accumulation because of the small finite number of anion exchange sites in the root tissue. Furthermore it might be expected that this limited uptake would be readily reversed by a brief wash in distilled water as has been demonstrated for other ions (Epstein 1955). In contrast to this the free space accumulation of a divalent cation, such as vanadyl ion, would be large because of the profusion of fixed negative charges on the cell wall. Such binding as does occur with the vanadyl cation is likely to be strong given its position at the head of an Irving - Williams type series (Trujillo and Brito 1957).

The patterns of free space uptake for both the vanadyl cation and vanadate anion were very similar. Both ionic species exhibited free space accumulation which was complete in approximately thirty minutes after the beginning of the experiment. Effective removal of this free space accumulation could only be brought about through a post uptake treatment in a solution of the chromate ion. Evidence has been put forward to suggest that this removal of free space vanadium by the chromate ion is a genuine exchange process rather than the result of cellular disruption, although at present the nature of this exchange process is unclear.

The accumulation of vanadium in the apoplast of the root cortex cannot be described purely in terms of Donnan free space phenomenon^a. At present the evidence seems to support the interpretation that vanadium (+4) complexation with cell wall polysaccharides is involved. When vanadium in the free space is in the pentavalent form this would also involve a reduction step. Such a reduction is not unlikely given the tendency of vanadium (+5) to act as an oxidant, especially in acidic conditions. Even at neutral pH vanadium (+5) will readily oxidize aldehydes, ketones, catechols, olefins and sulphhydryls (Macara 1980). Deiana et al (1983) have proposed a mechanism for the reduction of vanadium (+5) involving end units of polysaccharide chains. Such reducing end units would be readily available amongst the shorter chain hemicelluloses and pectic substances which form the bulk of the cell wall matrix. Reduced vanadium would readily and rapidly complex with available compounds such as proteins, polysaccharides or smaller ligands such as catecholamines, organic acids and free amino acids (Chasteen 1983).

This type of free space complexation can be considered analagous to that which has been described for borate (Bowen and Nissen 1976). Boron is known to combine readily with compounds containing cis-diol functions. Such functional groups occur frequently in the highly polymerized root polysaccharides, and it is thought that complexation between boron and these polysaccharides explains the free space-bound component of boron uptake (Bowen and Nissen 1976). In general, boron-polysaccharide complexes have lower stability constants than boron-monomer complexes, and as a

result a post uptake treatment in suitable monomers (e.g. lactic acid, gluconate and salicylic acid) was effective in removing free space accumulations of boron. Comparison of the present work with that of Bowen and Nissen (1976) suggests that similar complexing monomers may be useful and effective tools for the removal of extracellular vanadium although at present no data is available.

The mechanism by which the chromate anion causes the release of extracellular vanadium is at present obscure.

There are however reported similarities in the behaviour of the chromate anion and vanadate anion which may serve to indicate how such a process could arise. Bartlett and Kimble (1976) have reported the reduction of hexavalent chromium by soil organic matter. This can be likened to the reduction of pentavalent vanadium by organic matter which has been reported by several workers (Goodman and Cheshire 1975; Szalay and Szilagy 1967). Chromate ions have also been shown to be very similar to vanadate ions in their behaviour in plant tissues. Zaccheo et al (1982) demonstrated the ability of chromate ions to inhibit the acidification of root incubation medium and reduce potassium uptake by roots without any alteration of the root respiration rate. An identical pattern of effects was reported for the vanadate anion by Cocucci et al (1980).

A complete analysis of the mechanisms involved in the uptake of vanadium using the approaches of other investigators (Nissen 1974; Epstein 1973) was considered inappropriate. These workers, and others, critically examined the changing pattern of ion uptake over a wide concentration range of supplied ion. In the case of vanadium, altered concentration may lead to alteration of the ionic species present in solution. This is particularly the case over a wide concentration range where monomers would exist at low concentration and polymers would exist at high concentrations. As a result only a limited examination of concentration dependent uptake was attempted, more emphasis being given to other methods of characterization uptake.

Using the chromate desorption technique, the uptake of vanadium

as a function of time is almost linear, regardless of the form in which it is initially supplied. This is similar to the pattern of uptake usually exhibited by anions and cations when the free space compartment of uptake has been removed. For example, a linear time course has been shown for zinc (Schmid et al 1965), strontium (Epstein and Leggett 1954) and rubidium (Epstein et al 1963). Such linear time courses are considered to be the result of carrier mediated uptake.

The pattern of vanadium uptake in response to altered concentration also indicates the involvement of a carrier in the uptake process. However as has already been mentioned interpretation of these patterns is exceedingly difficult since the ionic species present will vary with altered concentration. At concentrations where saturation of the carrier appears it is almost certain that polynuclear vanadium species will be present, and it is possible that these may account for the reduced rates of uptake witnessed at these high concentrations.

Altered temperature has little effect on the uptake of vanadium by root tissues. Typically a Q_{10} of around 1.0 was detected, indicating a non metabolically mediated process of entry. This compared with a Q_{10} of 1.2 for Beryllium uptake (Holst et al 1980) which was considered to suggest that uptake was a physical process. Colclasure and Schmid (1974) calculated a Q_{10} of 2.2 for cobalt uptake and went on to suggest that the uptake of this element was an active process.

It is these three lines of evidence which are considered most crucial in terms of interpretation of the mechanism of vanadium uptake into the symplast. Passive movement involving a carrier is also supported by the data obtained using metabolic inhibitors although interpretation of this data is hindered by the multitude of effects which inhibitors may have on metabolic systems.

This description of vanadium uptake is consistent with the previous examination of uptake by excised root tissue

(Welch 1973) although the conclusions reached in that study differ from those presented here. Saturation kinetics were not observed in the study by Welch largely because the concentration of available ion never exceeded 100 μ M. In this study saturation was not observed until the concentration of the supplied ion was circa 1mM. It may be argued that this saturation is due to polymerization of the metavanadate ion which would occur at this concentration. The extent of this polymerization at a concentration of 1mM is probably only slight, as indicated by Pope and Dale (1968).

Welch (1973) suggests that passive diffusion of vanadate into the cells of higher plants is not a tenable hypothesis given the known concentration ratios (i.e. vanadium is found within plant cells at concentrations greater than twice those of the bathing solution). However carrier mediated passive transport, or facilitated diffusion as it is sometimes termed, can produce accumulation ratios greater than one (see Hodges 1973).

Significantly, Welch (1973) does suggest that alteration of the chemical form of vanadium may provide the means necessary to maintain a gradient for the accumulation of vanadium by plant cells. This could occur by a mechanism similar to that proposed by Macara et al (1979) for entry of vanadium into the vacuoles of vanadocytes in Tunicates. The process involved relied upon the presence of a reductant, Tunichrome. However reduction of vanadate (+5) to vanadyl (+4) occurs in the presence of a number of reducing agents common to cells. These include ascorbic acid, glutathione, norepinephrine, cysteine and possibly NADH (Chasteen 1983). Such a mechanism could explain accumulation of vanadium by plant tissues and would exhibit similar kinetics to those observed in this study provided the reductant was in limited supply.

In summary the following can be stated about vanadium uptake by excised roots: uptake of vanadium demonstrates the biphasic pattern exhibited by numerous elements. The first phase which is rapid, and complete within 30 minutes, represents movement into the free space of the tissue. Such binding of vanadium as occurs within this space cannot be

considered simply in terms of electrostatic forces. The second phase of uptake is irreversible and occurs at a constant rate. It is thought to represent accumulation of vanadium within the symplast of the root. These uptake patterns are not dependent on the applied ionic form of vanadium which has led to the suggestion that biotransformation of vanadium to a common form occurs within root tissues.

Whilst studies of relatively simple systems such as excised roots provide useful information for plant physiologists they are of limited interest to those with a more pragmatic approach to plant nutrition. Researchers in this field adopt a broader approach with interests in many areas including growth affects, tissue ion levels and their effects upon metabolism and interaction between mineral elements. Attempts to obtain information relating to these aspects of the role of vanadium in plant mineral nutrition were made using pot experiments with a variety of plant species.

Although not immediately apparent from the experimental results, vanadium at only moderately elevated levels is injurious to plant growth. Most of the plants grown in these experiments were exposed to vanadium for six to eight weeks only, during which time growth reductions were only observed at higher applied concentrations. There were however qualitative differences in the plants exposed to moderately elevated levels of vanadium. These differences were most apparent in the root systems taking the form of root discolouration and absence of new vigorous growth.

Marked differences were noted in the response of different species to vanadium applications. Tomato appeared particularly sensitive to applied vanadium, which caused drastic reductions in root and shoot growth at levels (100 μM) which barely affected the other plant species tested. With regard to this it is interesting to note that Basiouny (1984) reported that low levels of vanadium (250 ng ml^{-1} as NH_4VO_3) produced enhanced growth of tomato plant shoots but retarded root growth. No cogent explanation of how this paradoxical situation may have arisen is offered but the information serves to reinforce the idea that vanadium affects tomato plants in a peculiar fashion.

Plant response to applied vanadium was not dependent upon the ionic form presented. This is true both in terms of plant growth and the uptake of the element. Once again the patterns of uptake are suggestive of a biotransformation to a common form although the location of this transformation mechanism or its nature are uncertain.

The single most notable feature of vanadium uptake by higher plants is the acquisition and retention of this element by root tissues. In all plant species examined, the bulk of vanadium absorbed from the growing medium remained within the root, presumably in some physiologically immobile form. Whether this immobilization occurs extracellularly on cell wall polysaccharides or intracellularly in vacuoles is at present unclear.

These results are consistent with those of other workers who have studied vanadium uptake. Hara et al (1976) reported that 95-98% of the vanadium absorbed by cabbage roots was retained within the root tissues. This high degree of retention in root tissues persisted even at excessively high root vanadium concentrations (i.e. 2000 ppm). Wallace et al (1977) similarly reported 97-100% retention of absorbed vanadium by the roots of bush beans grown in nutrient solution. Warrington (1956) reported root and shoot levels of vanadium for both peas and soybean grown with variable iron supply. Essentially the shoot/root balance did not alter in response to altered iron nutrition. Soybean roots containing 170 ppm vanadium (dry weight basis) had only 2.3 ppm vanadium in their shoots. Similarly peas with 510 ppm vanadium (dry weight) in their roots contained only 17 ppm vanadium in their shoots.

Basiouny (1984) reported a more even distribution of vanadium in plant tissues when levels were low (i.e. less than one ppm in the tissue on a dry weight basis) but as tissue levels rose the characteristic pattern of retention of vanadium within root tissues became apparent.

Such restricted transport between root and shoot has been reported for other elements. Chromium, lead cadmium and copper all show preferential root retention (Welch and Cary 1975, Peterson and Girling 1981, Jones et al 1973, Jarvis

et al 1976 and Lepp 1981). Whilst this phenomenon has been well documented no thorough mechanistic interpretations have been attempted. Some workers have attempted basic desorption studies similar to those employed with excised roots (e.g. Jones et al 1973 attempted to remove extracellular lead with a wash in a barium solution but this proved unsuccessful) but little worthwhile data has been obtained.

Present observations are consistent with the concept that roots provide a barrier which restricts the movement of vanadium, and other selected elements, through the plant. The exact mechanism of immobilization is uncertain, although there does seem at least circumstantial evidence to suggest that this is an extracellular phenomenon. The capacity of this mechanism to reduce transport is massive and witnessed by the small quantities of ions which reach aerial tissues even when root levels are extraordinarily high.

The concept of such a barrier has been based on studies with actively growing plants. Mitchell and Reith (1966) examined lead uptake and found that when active growth ceased during winter the concentration of lead in shoots of field grown grass increased substantially. It was considered probable that this affect was due to either continued uptake from the soil or increased transport from the roots; the possibility of increased aerial contamination having been ruled out. This type of evidence emphasizes the need for further information regarding this root/shoot barrier.

A cursory examination of interactions between vanadium and other nutrient elements has been attempted for both maize and bean plants. Interactions with manganese and zinc are those most commonly occurring. No attempts have been made to assess the exact nature or extent of these interactions which would require a different approach to that employed here. No significant interactions with iron were observed in the present study, although Basiouny (1984) has suggested that vanadium may play an important role in the metabolism of iron in higher plants.

The long standing but somewhat enigmatic association between

vanadium and calcium in plant tissues has been given some consideration in these studies. Cannon (1963) originally suggested that plants which absorb large amounts of calcium were most tolerant of large amounts of vanadium in their tissues, because the vanadium was precipitated in the root as the sparingly soluble calcium vanadate. Results presented here provide general support for this notion. Simply stated, the idea is that the more calcium present in the vicinity of the root the less vanadium reaches the shoot. However this does not necessarily imply that the vanadium is precipitated in the root. There are numerous ways in which the observed effect could be produced, particularly when the essential role of calcium in maintaining the structural and functional integrity of cell membranes is considered. Since no direct evidence of precipitation of vanadium in root tissues has been presented, it is considered more probable that this effect can be described more accurately in terms of the general regulatory role of calcium in plant tissues.

Excised root experiments and whole plant studies seem to support one another in the contention that vanadium within plant tissues adopts a common form. It has been speculated that this biotransformation involves reduction of pentavalent vanadium by cell wall polysaccharides and subsequent complexation of reduced vanadium. Direct evidence of reduction of pentavalent vanadium has come from studies utilizing ESR. Normal healthy roots incubated in an aerated solution of pentavalent vanadium produced a spectra characteristic of reduced tetravalent vanadium when examined using ESR. Whilst none of these ESR parameters were determined it cannot be doubted that these spectra are attributable to the vanadyl ion.

These results do not fully substantiate the proposed mechanisms. They provide no indication of the likely timing, extent, location and mechanism of reduction. They do however clearly demonstrate the ability of root tissue to reduce pentavalent vanadium and thus lend credibility to the proposed mechanism.

Whilst the present work has clearly demonstrated the presence

of tetravalent vanadium in root tissues, it does not preclude the presence of pentavalent vanadium. The extent to which any given species is present will depend not only upon the stability of its own complexes but also upon the stability of complexes of other ionic species of vanadium present. Given the known high stability of vanadium (+4) complexes it would be imagined that these dominate in plant tissues but at present this lacks experimental support.

How far does present work allow a description of vanadium uptake from natural soils? Before such a question can be answered a description of the likely forms of vanadium in the soil is necessary. Fig. 7.1 provides a simplified description of the forms in which vanadium may be encountered by plant roots. The existence of free ions in the soil solution is open to doubt given the high number of potential donor ligands which may be encountered in the soil solution. Certainly free vanadyl cations would not be encountered. Movement in the vicinity of the root is likely to be dominated by complexes, although no empirical evidence for this is available.

Once within the free space of root tissue vanadium is probably immobilized upon cell walls. If the vanadium entered the root as the pentavalent form this would also involve a reduction step. Extracellular vanadium accumulated in this fashion probably represents the bulk of vanadium within plant tissues.

Clearly some vanadium does enter the symplast of growing plants although how it reaches the plasmalemma and is transported across is uncertain. The formation of soluble complexes (either $V(+4)$ or $V(+5)$) would provide one method for the release of trapped vanadium. Soluble complexes would allow movement of vanadium to the plasmalemma. Transport across the plasmalemma would probably involve a carrier. If the soluble vanadium complex were pentavalent vanadium a reduction step would also be involved. Postulating transport of vanadium as a cation obviates the requirement to invoke a driving force for transport. Fig. 7.2 represents an attempted description of one such mechanism. If the complex involved tetravalent

Fig. 7.1 A tentative description of the events likely to be involved in transport of vanadium to the roots of higher plants.

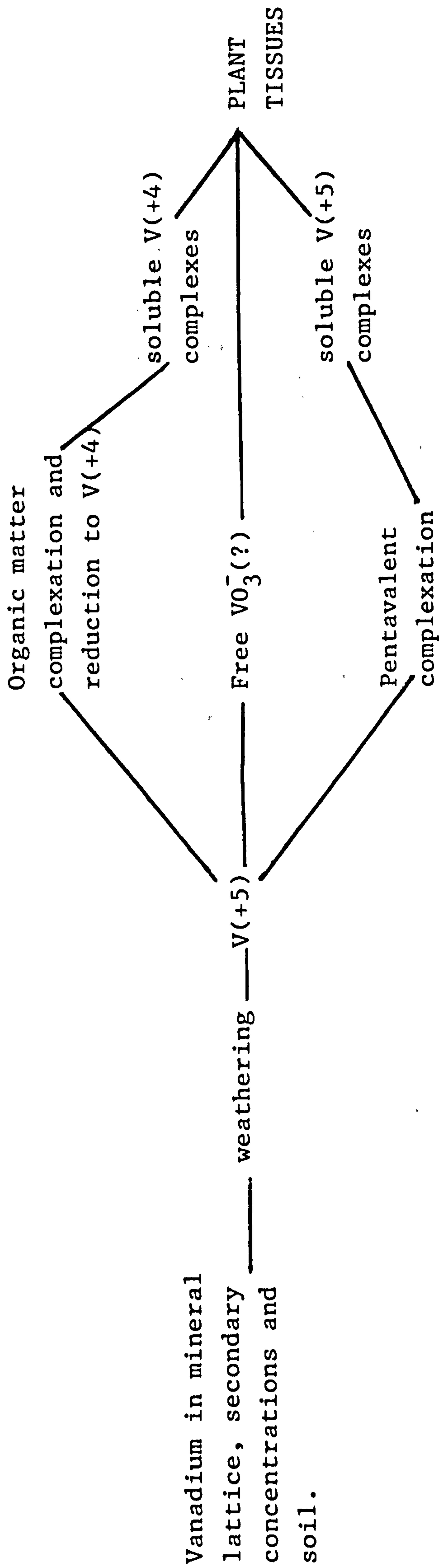


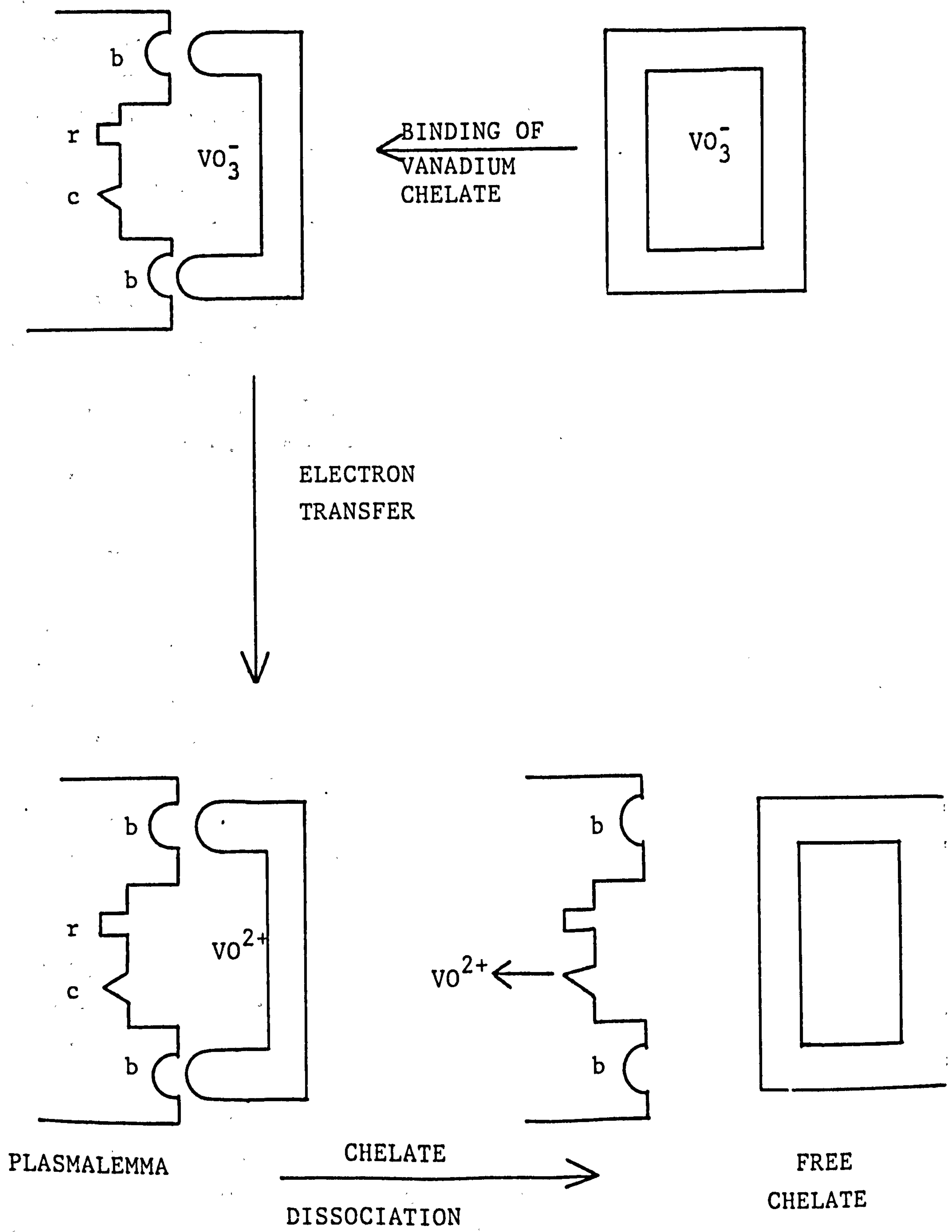
Fig. 7.2 Speculative scheme for uptake of vanadium based on a model proposed for iron uptake by Rohmald and Marschner(1983).

b = specific binding site

r = reducing enzyme

c = carrier

An alternative mechanism may involve the vanadium reaching the plasmalemma in the reduced form which would obviate the need for a reduction step at this membrane.



vanadium no reduction prior to uptake would occur.

If pentavalent vanadium were transported across the plasmalemma it would undoubtedly be reduced as soon as it entered the cytoplasm. The reducing environment and abundance of free ligands would ensure reduction to tetravalent vanadium. Such reduction may be viewed as a detoxification mechanism since free vanadate ions in the cytoplasm would have the potential to greatly disrupt numerous enzyme systems. Mcphail et al (1982) further suggest that accumulated vanadium would be transported into the vacuole.

Only limited transport of vanadium to the aerial parts of plants has been observed. It is probable that this is due to immobilization by cell wall polysaccharides. The route via which the limited transport of vanadium occurs is unclear; its nature (i.e. symplastic or apoplastic) has yet to be clarified.

7.2 Future research requirements.

The questions raised by current research are the key to increased understanding of problem areas in the future. This present work has raised numerous important questions relating to problems in such diverse fields as soil science, plant physiology and biochemistry. Selection of problems most urgently warranting attention is difficult, however certain problem areas seem to have precedent because of their general applicability. Some of these are:

(1) The speciation of vanadium (and other ions) within soil systems. To date no comprehensive study of the factors which govern the valency of vanadium within soil systems has been attempted. Equally unclear are the factors controlling the availability of soil vanadium. Organic material has been shown able to sequester vanadium from the soil solution but its ultimate fate as the material decomposes is unknown. Plant roots have been shown able to liberate bound manganese and iron from the mineral lattice (Uren 1981, 1982) whether or not such an event is possible when vanadium is being considered is unknown.

(2) Resolution of the barrier between the root-shoot interface particularly with reference its location and mechanism. Whilst at present it is felt that this barrier is primarily extracellular its exact nature is unclear. Use of electron microscopic techniques, particularly EDAX analysis, may provide a clearer insight into this problem.

(3) The nature of the limited transport of vanadium which is known to occur in plants is at present totally unclear. Vanadium has been detected in phloem tissue but its appearance in other tissues has not been demonstrated. Equally unclear is the form of vanadium in aerial parts of higher plants. Although reduction of vanadium is known to occur in root tissues there is no evidence to suggest that the transported vanadium is in the reduced form. Clearly the application of electrophoretic- isotope experiments to this area of study could yield useful results.

(4) The role of vanadium in lower plants is an area of particular interest. Essentiality of vanadium for algae has been clearly shown, and numerous effects on algal metabolism have been reported but at present the basis of these effects is unclear. The function of vanadium in the fungus *Amanita muscaria* is equally unclear. Whilst numerous reports exist relating to the abnormally high levels of vanadium found within the tissues of this fungus no real attempts have been made at providing a functional description of vanadium's role. Vanadium-lower plant interactions may prove to be a fruitful area of study in the future, yielding results of some relevance to higher plants.

There are of course numerous other areas of interest which require attention if our knowledge is to progress. An awareness of this need for further basic research into vanadium-plant interactions will become apparent as more workers recognise vanadium's multifarious roles in biological systems. Current concern over vanadium's effect on human metabolism suggest its status as a "low profile" element may soon be reviewed.

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APPENDIX 1

NUTRIENT SOLUTION

COMPOUND	STOCK SOLUTION CONCENTRATION g l^{-1}	VOLUME OF STOCK ¹ SOLUTION USED PER LITRE
KNO_3	101.10	6.0 ml
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	236.16	4.0
$\text{NH}_4\text{H}_2\text{PO}_4$	115.08	2.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.49	1.0
KCl	3.728	
H_3BO_3	1.546	
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.338	1.0
		2
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.575	
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ³	0.125	
H_2MoO_4	0.081	
Fe-EDTA	6.922	1.0

1 These values are those recommended in Epstein (1972) and employed throughout this study.

2 All these micronutrients may be included in one solution.

APPENDIX 2

PUBLICATIONS

PUBLICATIONS

Morrell B.G., Lepp N.W. and Phipps D.A. (1983) Vanadium uptake by plants. J.Sci. Food Agric. 34 pp 58-59.

Morrell B.G., Lepp N.W. and Phipps D.A. (1983) Vanadium uptake and translocation in higher plants. Conference Proceedings Heavy Metals in the Environment Heidelberg Sept 1983 1 pp 638-641.

Morrell B.G., Lepp N.W. and Phipps D.A. (1983) Vanadium uptake by plants; the uptake of vanadium by excised barley roots (Hordeum vulgare L. cv Maris Mink) Inorganica Chimica Acta 79 B7 pp 228-229.

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Morrell B.G., Lepp N.W. and Phipps D.A. (1986) Vanadium uptake by higher plants: some recent developments.
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Lepp N.W., Harrison S.C.S. and Morrell B.G. (1985) The ecological significance of cadmium and vanadium accumulation in the sporophore of the fungus Amanita muscaria L.
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containing less than 0.04 ng ml^{-1} of vanadium with no adverse effects. Hewitt (1966) grew sugar beet, tomato, lettuce, alfalfa, white clover and alsike clover in purified nutrient solutions containing less than $2 - 3 \text{ ng ml}^{-1}$ of vanadium. These plants showed no response to supplements of vanadium. Levels in the plants were however detectable - Hewitt attributed this to contamination from greenhouse dust. Thus if vanadium is an element required by higher plants it is one that is required in extremely small quantities. Welch and Cary (1975) carried out a survey of vegetable oils and wheat seed to establish the range of levels of vanadium and also to decide if vanadium has an affinity for natural biogenic fats as reported earlier (Schroeder et al 1963). Levels of vanadium in commercial vegetable oils gave no indication that vanadium has an affinity for biogenic fats. The survey of the wheat seed showed very low levels in general; many of the variations which occurred could be attributed to soil type on which the wheat was grown. These workers also carried out a number of preliminary experiments on the uptake of vanadium from nutrient solution by lettuce and tomato. When vanadium was added to the nutrient solution as a supplement levels in the upper parts of the plants, including seeds, increased substantially. Hara et al (1976) examined the growth response of cabbage to vanadium. Levels of vanadium increased in all parts of the plant with an increase in the supply of the element. Most of the vanadium was retained within the root tissues; only a small proportion being transported to the shoots. Accompanying these increased tissue levels was a decrease in dry matter production by the cabbage plants. This pattern of vanadium retention in root tissues was one which had been reported before (Notton and Hewitt 1972). Lepp (1977) reported the effect of vanadium on germination and subsequent seedling growth of lettuce. No effects on germination were observed but effects on subsequent seedling growth were dramatic. At all applied concentrations vanadium reduced root and shoot growth. Welch (1973) performed a study investigating the effect

GROUP	V $\mu\text{g g}^{-1}$ (d.w.)	REFERENCE
Coelenterates	2.3	N.A.S. 1974
Annelids	1.2	" "
Molluscs	0.7	" "
Echinoderms	1.9	" "
Crustaceans	0.4	" "
Porifera	1.7	Bengtsson & Tyler 1976
Tunicata		" " " "
Ascididae	740 - 6520	" " " "
Polyclinidae	0.0 - 8.3	" " " "
Pyuridae	0.0 - 7.1	" " " "
Cionidae	166 - 1300	" " " "

Table 1.2

ANIMAL	TISSUE	LEVEL	REFERENCE
Calf	Liver	0.51 ppm (A.W.)	Soremark (1967)
Rat	Liver	0.39 ppm (W.W.)	Schroeder et al (1963)
Deer	"	0.07 ppm (W.W.)	"
Rabbit	"	0.94 ppm (W.W.)	"
Horse	Blood	N.D.	"
Human	Urine		Byrne & Kosta (1978)
Human	Blood	0.2-0.5 ppm (D.W.)	" " " "
Human	Bile	0.5-1.85 ppm (D.W.)	" " " "
Human	Teeth	2.0-5.1 ppm (D.W.)	" " " "

Table 1.3

