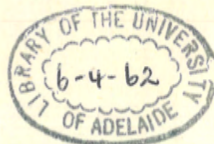


NITROGEN NUTRITION OF THE TOMATO PLANT

by

John S. Hawker, B.Sc. (Hons) ADEL

Botany Department

The University of Adelaide

Thesis submitted to The University of Adelaide
for the Degree of Doctor of Philosophy.

August, 1961.

FOREWORD

This is to certify that material contained in this thesis is the work of the author, except where acknowledged otherwise.

. .

TABLE OF CONTENTS

		Page No.
PART I	INTRODUCTION	1
PART II	METHODS	14
	1 Water culture technique	14
	2 Determinations of nitrate and ammonium nitrogen in culture solutions	19
	3 Chemical analyses of plant material	19
	(a) Nitrogenous fractions	19
	(b) Inorganic elements	21
	(c) Organic acids	22
	(d) Carbohydrates	23
	4 Determinations of amino acids and amides in plant material	23
	(a) Extraction from plant material	23
	(b) Determinations by paper chromatography	24
	5 Amino acid composition of leaf proteins	31
	(a) Extraction of protein from tomato leaves	31
	(b) Determination of amino acid composition of proteins by ion-exchange column chromatography	32
	6 Determination of incorporation of ^{15}N into leaf proteins	41
	(a) Isolation of subcellular fractions from tomato leaves and extraction of proteins from subcellular fractions	41
	(b) Estimation of ^{15}N abundance in proteins	44
	7 Determination of ribonucleic acid and deoxyribonucleic acid in tomato root tips	48
	8 Determination of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate in tomato leaves	52

Page No.

PART III THE EFFECT OF NITRATE AND AMMONIUM IONS ON THE GROWTH OF TOMATO PLANTS 60

1 The growth of tomato plants on either nitrate, ammonium or nitrate and ammonium nitrogen in water culture at pH 6.0 - 6.5 60

2 The effect of pH between pH 3.5 and 8.5 on the growth of tomato plants in water culture supplied with either nitrate or ammonium nitrogen 66

3 The growth of tomato plants supplied with different forms of nitrogen in otherwise similar solutions 70

4 The growth of tomato plants on nitrate nitrogen and on three concentrations of ammonium nitrogen 76

5 The effect of different proportions of nitrate and ammonium nitrogen in culture solutions on the growth of tomato plants 81

6 The effect on the growth of tomato plants of adding small amounts of nitrate to ammonia culture solutions 84

PART IV THE UPTAKE AND ASSIMILATION OF NITROGEN BY TOMATO PLANTS 91

1 The uptake of nitrogen by tomato plants from culture solutions containing either nitrate, ammonium or nitrate and ammonium nitrogen 93

2 Ammonia, amide and protein levels in tomato plants grown on either nitrate, ammonium or nitrate and ammonium nitrogen 103

3 The effect of different sources of nitrogen on root hair formation 114

4 The concentrations of elements in tomato plants grown on either nitrate, ammonium, or nitrate and ammonium nitrogen 118

Page No.

PART V PROTEIN SYNTHESIS IN TOMATO PLANTS GROWN ON EITHER NITRATE NITROGEN, AMMONIUM NITROGEN OR NITRATE AND AMMONIUM NITROGEN 124

1 Amino acids and amides 124

2 Amino acid composition of proteins of leaves 136

3 The incorporation of ^{15}N into proteins of subcellular fractions of leaves of tomato plants supplied with ^{15}N labelled potassium nitrate or ammonium chloride 141

PART VI LEVELS OF ORGANIC ACIDS, CARBOHYDRATES, RIBONUCLEIC ACID, DEOXYRIBONUCLEIC ACID, NICOTINAMIDE ADENINE DINUCLEOTIDE, AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE IN TOMATO PLANTS GROWN ON EITHER NITRATE, AMMONIUM OR NITRATE AND AMMONIUM NITROGEN. 148

1 Organic acids 148

2 Carbohydrates 151

3 Ribonucleic acid and deoxyribonucleic acid in root tips 154

4 Nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate in leaves 157

PART VII THE GROWTH OF _HORDEUM VULGARE_ L., _PISUM SATIVUM_ L., AND _ATRIPLEX HORTENSIS_ L. ON NITRATE CULTURE SOLUTION AND AMMONIA CULTURE SOLUTION 162

PART VIII GENERAL DISCUSSION 167

PART IX SUMMARY 176

PART X ACKNOWLEDGEMENTS 177

PART XI BIBLIOGRAPHY 178

LIST OF ABBREVIATIONS

The following abbreviations have been used:-

DNA	Deoxyribonucleic acid
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
TCA	Trichloroacetic acid

LIBRARY OF THE UNIVERSITY OF ADELAIDE

PART I

INTRODUCTION

Ammonia or ammonium salts are usually found to be satisfactory fertilizers for plants in the field (Pardo, 1935; Nightingale, 1937, 1948; Reuther et al., 1958). Exceptions to this finding can usually be attributed to the effects of low pH in acid soils, to mineral deficiencies or to poor soil aeration. Although ammonium nitrogen is applied to the soil, the plant does not necessarily absorb nitrogen only in this form.

Ammonia or the ammonium ion is usually rapidly oxidised to nitrate by nitrifying bacteria in the soil except in poorly buffered soils of extremely high or low pH value (Burris, 1959) or in cases where soil fumigants have been used (Nightingale, 1948). However even in the latter cases it is probable that at least some nitrate is present in the soil together with the added ammonium ions. Consequently plants growing under field conditions rarely have ammonium nitrogen as their only source of nitrogen. Nitrate, or mixtures of nitrate and ammonium ions, or of nitrate, ammonium ions and urea are present in the soil depending on the form of fertilizer added.

In recent years foliar application of mineral nutrients has become popular and work in this field has been reviewed by Wittwer and Teubner (1959). These authors discuss the absorption and hydrolysis of urea by plants; even when a single nitrogen containing

substance like urea is applied as a foliar spray it is rarely the case that it is the only form of nitrogen available to the plant. Other forms of nitrogen already present in the soil, and nitrate produced by the oxidation of the ammonia liberated by hydrolysis of the urea which has run off the leaves, supplies the plants with a mixture of nitrogen sources.

Comparison of the growth of plants grown on nitrate nitrogen or ammonium nitrogen in sand and water culture has been extensively studied. This work has been reviewed by Pardo (1935), Nightingale (1937, 1948), McKee (1949) and Burris (1959). Pardo (1935) listed numerous species of plants and described their relative growth on nitrate nitrogen or ammonium nitrogen. These reports indicated that some plants produce more growth on nitrate nitrogen, others on ammonium nitrogen, whereas others produce similar growth on either ammonium or nitrate nitrogen. In the later reviews the growth rates of various plants on different forms of nitrogen are discussed in relation both to the composition of the culture solutions and to plant metabolism. Differences in the pH of culture solutions and in the metabolism of plants are observed when plants are grown on ammonium nitrogen rather than nitrate nitrogen. Unless the pH of culture solutions is controlled, plants grown on solutions containing only ammonium nitrogen or on solutions containing an ammonium:nitrate ratio greater than 3:17, the value recommended by Trelease and Trelease (1933) for a "physiologically balanced" culture solution, show reduced growth or eventual death. This is due to the

drop in pH of the culture solution brought about by rapid uptake of ammonium ions and consequent increase in hydrogen ion concentration (Trelease and Trelease, 1935; Wander and Sites, 1956; Woolhouse, 1959). Arnon and Johnson (1942) examined the growth of tomato plants in water culture solutions over a range of pH values between 3 and 9. Optimum growth was found at pH 6 - 7 and no growth was observed at pH 3.0. These results would explain the lower growth rates of plants on ammonia culture solutions in which the pH was not controlled. Therefore unless the pH of culture solutions is controlled the growth of plants on different sources of nitrogen cannot be compared. Even in sand cultures in which the pH of the culture solution is carefully controlled, Nightingale (1937) has shown that the pH of culture solution close to the roots can be as much as 1.5 of a pH unit below the average pH of the solution emerging from the sand. Consequently differences observed in plants grown in sand cultures and supplied with ammonium nitrogen at controlled pH, have to be interpreted both in terms of the lower pH in the vicinity of the roots as well as of the direct effect of the ammonium ion itself. In well stirred water cultures it was found that differences in pH in the vicinity of the roots were less serious.

The optimum growth of tomato plants at pH 4.0 when supplied with nitrate nitrogen in sand culture as found by Tiedgens (1934) is contradictory to the results of Arnon and Johnson (1942). However Tiedgens supplied iron to culture solutions only at the onset of iron deficiency symptoms in the plants, and he does not indicate to which plants he

supplied iron. Traces of iron present in the sand and culture solutions used, would probably have been more readily available to the plants at pH 4.0 than at pH 6.0 or 7.0 (Sideris et al., 1943). These authors grew pineapple plants on solutions containing either nitrate or ammonium nitrogen without added iron. The only iron available to these plants was that present as impurities in the other salts supplied. The culture solutions were changed at two weekly intervals. Between changes, the pH of the ammonia culture solution dropped from 6.6 to 4.2 and the pH of the nitrate culture solution increased from 4.4 to 6.8. Under these conditions more dry weight was produced on the ammonia culture solution than on the nitrate culture solution. This was attributed to the greater availability to the plants of iron at the lower pH values compared to the higher pH values where precipitation of iron occurred. Similar increases in the solubility of iron were obtained by maintaining the pH of nitrate culture solution at a pH of approximately 4.0. If Tiedgens (1934) had supplied sufficient iron for maximum growth at all pH values it is possible that optimum growth of the tomato plants would have occurred at pH 6.0 rather than at pH 4.0.

Nightingale (1937) discussed the work of Clark (1936) who grew tomato plants in sand culture supplied with either nitrate or ammonium nitrogen. He observed that plants grown on nitrate nitrogen produced more growth than plants grown on ammonium nitrogen. Clark suggested that the lower growth rate was caused by the lower ash content and lower organic acid levels which he observed in plants supplied with ammonium

nitrogen. Nightingale suggested that it was due to the low light conditions causing a lack of carbohydrates in the plants grown on ammonium nitrogen. Clark did not analyse the tissues for carbohydrates. Neither author suggested the possibility of the pH of the ammonia culture solution in the sand falling below the optimum for growth. The pH of the solution emerging from the sand did fall to 5.4 in some cases and the pH of the solution was probably lower in the vicinity of the roots.

Recently Wander and Sites (1956) have shown that the dry weight of leaves of rough lemon seedlings grown in water culture was lower when the plants were supplied with ammonium nitrogen rather than a mixture of nitrate and ammonium nitrogen or nitrate nitrogen alone. However the pH of the ammonia culture solution dropped to 3.5 in some cases. Wallace and Ashcroft (1956) grew beans and rough lemon seedlings in sand culture at pH 4.5 - 5.5, and found reduced growth with ammonium nitrogen compared with nitrate nitrogen or urea. However the pH of the solution containing only ammonium nitrogen was probably well below 4.0 in the vicinity of the roots.

Smith (1957) examined the growth of Pineapple sweet orange seedlings in water culture and carefully controlled the pH of all solutions. At pH 6.0 total dry weights of plants were similar on all solutions even when nitrate was excluded from ammonia culture solutions.

Woolhouse (1959) grew tomato plants in water culture and observed less growth on ammonium nitrogen than on nitrate nitrogen or on a

mixture of nitrate and ammonium nitrogen. He gave data on pH values of culture solutions during the initial period of the experiment. However no data was given for the 8 days prior to harvesting when the greatest changes in pH would be expected. It is possible that low pH values were causing the results he observed.

Nightingale (1937, 1948) suggested that in many experiments low light intensities limited the synthesis of carbohydrates in plants. These plants, when grown on ammonium nitrogen, had lower concentrations of starch in their tissues than when grown on nitrate nitrogen (Nightingale et al., 1928; Tiedgens and Robbins, 1931; Tiedgens, 1934; Burstrom, 1943; Sideris and Young, 1944; Vladimirov, 1945). If sufficient carbohydrates are not available for detoxification of ammonium nitrogen by the formation of amides, then ammonium nitrogen may accumulate in tissues causing injury to the plants (Nightingale, 1937). However in tomato plants grown in bright sunshine where light was unlikely to be limiting, there was still a suggestion of lower concentrations of starch in plants supplied with ammonium nitrogen than in plants supplied with nitrate nitrogen (Woolhouse, 1959). It is possible that ammonium nitrogen supplied to plants causes a decrease in the rate of photosynthesis and consequently lower levels of carbohydrates within the tissues. Krogmann et al.(1959) have shown that ammonium nitrogen uncouples photosynthetic phosphorylation in isolated chloroplasts. The lower levels of carbohydrates observed in plants grown on ammonium nitrogen could result either from the utilization of

carbohydrates for amide synthesis or from lower rates of carbohydrate synthesis in these plants.

Investigations have been made on salt uptake in the presence of ammonium nitrogen (Nightingale, 1937, 1948). Recent studies (Fawzy et al., 1954; Wallace and Ashcroft, 1956; Wander and Sites, 1956; Smith, 1957; Jackson and Coleman, 1959) have confirmed the observations of earlier workers that the presence of ammonium ions in the culture solution reduces the uptake of other cations. Moreover the effect was found irrespective of the presence or absence of nitrate (Wallace and Ashcroft, 1956; Smith, 1957; Jackson and Coleman, 1959).

In those investigations where the pH of the ammonia culture solution was not rigidly controlled (Wallace and Ashcroft, 1956; Wander and Sites, 1956), the decrease in cation concentration could be attributed to the increase in hydrogen ion concentration (Arnon et al., 1942; Arnon and Johnson, 1942; Fawzy et al., 1954; Jacobson et al., 1957; Jackson and Coleman, 1959). However the results of experiments where pH was controlled (Fawzy et al., 1954; Smith, 1957; Jackson and Coleman, 1959) indicate that the ammonium ion antagonizes the uptake of other cations. Although there is a decrease in the cation concentration in plants supplied with a mixture of nitrate and ammonium nitrogen as compared to those supplied with nitrate alone (Wallace and Ashcroft, 1956; Smith, 1957) the plant growth on both culture media is the same (Smith, 1957; Woolhouse, 1959).

It was demonstrated that, in some cases, to obtain maximum growth of plants it was necessary to add more calcium to culture solutions containing ammonium nitrogen rather than nitrate nitrogen (Nightingale, 1937). In contrast to these findings, Skok (1941), under conditions where calcium was limiting, observed greater growth of bean plants supplied with urea than with nitrate nitrogen. However no data was given on the final pH of the culture solutions or on levels of calcium within the plant tissues.

It has been demonstrated that the assimilation of ammonium nitrogen is increased by aeration of the culture solutions (Arnon, 1937; Haas, 1937; Hoagland and Arnon, 1941; Shive, 1941; Gilbert and Shive, 1942). In view of this it is rather surprising that ammonium salts are more readily utilized than nitrates by young rice plants as these plants usually grow with their roots completely submerged in soil water (Nagoaka, 1904; Kelley, 1911; Espino, 1920; Trelease and Paulino, 1920; Thelin and Beaumont, 1934; Pardo, 1935; Bonner, 1946; Wahhab and Bhatti, 1957). The apparent inability of rice plants to use nitrate is unlikely to be due to the absence of nitrate reductase because Tang and Wu (1957) have recently shown it to be adaptively formed in rice seedlings in the presence of nitrate. Indeed the inability of rice plants to utilise nitrate is questionable since Malavolta (1954) showed greater growth of rice plants grown on unaerated solutions containing nitrate nitrogen than on solutions containing ammonium nitrogen whether aerated or not. He suggests that the reduced growth of plants grown on ammonium nitrogen was caused by toxic levels of ammonium nitrogen in these tissues.

Nightingale (1937) discussed the role of amides as a form of storage of ammonium nitrogen. It is clearly evident from the numerous reports which he quoted that high amide concentrations occur in plants when they are supplied with ammonium nitrogen rather than nitrate nitrogen. Weismann (1951, 1959) has also shown high levels of amides in young wheat seedlings grown on ammonium nitrogen. In tomato plants glutamine is usually present in greater amounts than asparagine (Clark, 1936; Margolis, 1960). It is now clear that glutamine is an important intermediate in the assimilation of inorganic nitrogen into plant compounds (Yemm and Folkes, 1958). Differences in free amino acid levels between plants grown on nitrate nitrogen or ammonium nitrogen have also been reported (Weismann, 1959; Margolis, 1960; Reisner et al., 1960). The amino acid composition of protein of young wheat seedlings was slightly different in plants grown on nitrate nitrogen to that in plants grown on ammonium nitrogen (Weismann, 1959). The significance of the differences in free amino acid levels and amino acid compositions of proteins in plants grown on either nitrate or ammonium nitrogen is not known.

The concentration of organic acids is lower in plants grown on ammonium nitrogen as compared to those grown on nitrate nitrogen (Clark, 1936; Wadleigh and Shive, 1939; Gilbert et al., 1951; Bershtein and Okanenko, 1953; Skvortsova, 1955). This is probably due, in part, to the lower levels of cations present in ammonia grown plants because it

has been shown that a greater uptake of cations than anions is accompanied by increases in organic acid levels in excised roots (Ulrich, 1941; Jacobson and Ordin, 1954; Jackson and Coleman, 1959). Also high amide levels in plants grown on ammonium nitrogen would probably result in lower levels of organic acids due to utilization of α-keto acids in amide synthesis. Vines and Wedding (1960) have reported lower rates of respiration in several plant tissues and isolated mitochondria in the presence of ammonium nitrogen and this would also result in decreased organic acid levels.

Woolhouse (1959) grew tomato plants in water culture and observed less growth on ammonium nitrogen than on nitrate nitrogen or a mixture of nitrate and ammonium nitrogen. He concluded that tomato plants require nitrate nitrogen for optimum growth whether ammonium nitrogen is present or not. However he did not measure the uptake of ammonium nitrogen from solutions containing only ammonium nitrogen or a mixture of nitrate and ammonium nitrogen. It seems unlikely that the reduced growth he observed was due to an initial lack of carbohydrates because several of his growth experiments, although conducted in a glasshouse, were carried out during periods of bright sunshine. However either the presence of the ammonium ion in the absence of nitrate ions, or the absence of nitrate ions may have affected carbohydrate production in plants grown on ammonia culture solution. This would be an effect resulting from the form of nitrogen supplied rather than from a limiting

supply of light. He did observe lower organic phosphate concentrations in plants grown on ammonium nitrogen alone. He suggested that the increased concentrations of organic phosphate which resulted from the addition of nitrate to ammonia culture solution was due to the possible induced synthesis of an enzyme.

Nightingale (1948) suggested that ammonium nitrogen is more rapidly utilised than nitrate providing (1) the cultures are well aerated (2) there is adequate absorption of other essential elements, and (3) that carbohydrates are not limiting in the plants. McKee (1949) came to the conclusion that plant species differ in their requirements for source of nitrogen. Burris (1959) however stated that "experimental data indicate that under proper conditions plants usually assimilate ammonia more readily than nitrate". The "proper conditions" were not defined. In support of this statement he referred to the work of Colgrove and Roberts (1956) and Tamm (1956). Their results do show increased growth in the presence of ammonium nitrogen under the experimental conditions used, but they do not necessarily indicate a more rapid utilization of ammonium nitrogen than of nitrate nitrogen. The increased growth of Azaleas on ammonium nitrogen was attributed to a lowering of the pH and activation of iron in the tissues of the plants (Colgrove and Roberts, 1956). Epilobium angustifolium has been shown to utilize ammonium nitrogen quite satisfactorily by Tamm (1956). Although this plant produced more dry weight when grown on ammonium nitrogen than on nitrate nitrogen in water

culture under conditions in which the supply of nitrogen was limiting, there is no evidence that better growth would take place on ammonium nitrogen under conditions where ample nitrogen was available.

From the experimental evidence reported in the literature on the relative effects of ammonium and nitrate nitrogen on plant growth and metabolism, it is apparent that in many instances the results have to be qualified in terms of the experimental conditions used. It is generally accepted that nitrate is first reduced to ammonia before being incorporated into organic compounds and that the organic reductive route is unimportant in the assimilation of nitrogen (Yemm and Folkes, 1958; Burris, 1959; Folkes, 1959). Therefore ammonium nitrogen would provide plants with nitrogen in a form suitable for immediate incorporation without prior reduction, and reports of increased growth in the presence of ammonium nitrogen as compared to nitrate nitrogen under carefully controlled experimental conditions (Bonner, 1946) may be due to limited nitrate reduction.

Previous work on tomato plants (Clark, 1936; Woolhouse, 1959) showed that nitrate reduction is not limiting the growth on nitrate nitrogen as plants grown on nitrate produce more dry weight than plants grown on ammonium nitrogen.

It was considered important to investigate further the growth of tomato plants on different forms of nitrogen and consequently it was necessary to check the previous observations using more rigidly controlled

conditions. Tiedgens (1934) did not supply iron in all cases and neither Clark (1936) nor Woolhouse (1959) maintained uniform pH conditions throughout each experiment.

Investigations were made to determine whether the differences in growth of tomato plants grown on different forms of nitrogen as reported by Woolhouse (1959) were caused by other variations in composition of the culture media used. In addition, experiments were carried out to determine the relative uptake of nitrate and ammonium nitrogen from culture solutions containing various concentrations of these forms of nitrogen. In view of the previous reports on differences in chemical composition of plants depending on the source of nitrogen, analyses were carried out on a wide range of compounds in an attempt to correlate different growth rates with chemical composition.

PART II

METHODS

1 WATER CULTURE TECHNIQUE

Culture Technique

Seeds of *Lycopersicum esculentum* Mill., (variety Bonny Best), were surface sterilised by wetting with 95 per cent ethyl alcohol, washing with water and followed by soaking in one per cent formalin for 30 minutes. The seeds, after thorough washing with distilled water, were placed on moist filter paper in petri dishes in an incubator at 25°C. When the radicles had emerged, the seeds were transferred to waxed gauze supported in a pyrex dish. The dish, which contained distilled water, was painted black on the outside and covered with a clear lid. The dish was placed in the glasshouse, in shade to prevent overheating, and the water was continuously aerated. After about 7 days when the hypocotyls had elongated and most of the testas had been shed, the lid was removed and the distilled water was replaced with culture solution. The dish was placed in full sunlight. About fifteen days after germination the seedlings were planted out into 2 litre beakers containing culture solution. Each seedling was supported in non-absorbent cotton wool between the halves of a waxed cork. Four corks were supported in a waxed masonite lid covering the top of each beaker. To reduce the growth of algae in the culture solutions, the sides and bottoms of the

beakers were covered with opaque paper, black on the inside and white on the outside. A glass tube dipping into the solution through a hole in each lid and connected to an aeration system, maintained continuous mixing and aeration of each solution.

Glasshouses.

Initial growth experiments were carried out in a wooden framed glasshouse. The air temperature was maintained above 55°F by a thermostatically controlled heating system but no cooling system was available. Later experiments were done in an aluminium framed glasshouse which had facilities for heating and cooling. The temperature varied from 55°F to 100°F. Differences were observed in the growth rate of plants depending on the glasshouse used and the season of the year, the rate of growth being reduced during the colder winter months. In the wooden framed glasshouse light was also limiting during the winter months. In all the experiments described culture vessels were placed in a statistically randomised arrangement on the greenhouse benches to avoid any systematic variations of environment within the glasshouses.

Culture Solutions.

The culture solutions used were those described by Woolhouse (1959) and details of their compositions are given in Table 1. The complete culture solution was essentially that of Arnon and Hoagland (1940) but contained sodium chloride and was one fifth the concentration used by

TABLE 1

COMPOSITION OF CULTURE SOLUTIONS

Culture solution.	Complete	Nitrate	Ammonia
	Concentration of salt ($M \times 10^{-4}$)		
Salt			
KCl	-	-	20
KNO_3	20	20	-
$CaCl_2$	-	-	6
$Ca(NO_3)_2$	6	6	-
$MgSO_4$	4	4	4
$NH_4H_2PO_4$	4	-	4
NaH_2PO_4	-	4	-
NH_4Cl	-	-	36
$NaCl$	16	16	-
$NaNO_3$	-	4	-

MICRO-ELEMENTS

The following concentrations were used in all solutions

B 0.1 p.p.m.
Mn 0.1 p.p.m.
Zn 0.01 p.p.m.
Cu 0.004 p.p.m.
Mo 0.004 p.p.m.
Fe 5.0 p.p.m.

these authors. For the ammonia culture solution potassium nitrate and calcium nitrate were replaced by potassium chloride and calcium chloride and ammonium chloride was added. Ammonium di-hydrogen phosphate was replaced by sodium di-hydrogen phosphate in the nitrate culture solution. Distilled water was used to make up all culture solutions. Iron was supplied in the form of ferric potassium ethylene-diamine tetra-acetate (Jacobson, 1951). The concentrations of potassium, calcium, magnesium, nitrogen, sulphur, phosphorus, iron and the micro-elements were the same in each solution but the concentrations of sodium and chlorine present were different. The pH of each solution was measured at a glass electrode and adjusted to the required pH with either 0.02M sodium hydroxide or 0.02M hydrochloric acid. These culture solutions were used throughout the investigation except in those experiments where plant growth in modified solutions was studied. These modifications are described under the appropriate sections.

Harvesting.

In the following growth experiments, day numbers given refer to the number of days after transferring the seedlings from the pyrex dishes to the culture beakers.

By the time the plants growing on ammonia culture solution had four or five leaves it became difficult to maintain the pH of this culture solution within a certain range owing to the rapid uptake of ammonium

nitrogen by plants of this size. Therefore the plants were usually harvested at this stage, about the 20th day, so that the pH of the culture solution could be maintained within the required range. Consequently it was necessary to bulk material from several plants in order to have sufficient material for certain analyses. At the time of harvest, cultures were carried into the laboratory. Plants from these cultures were separated into fractions which were quickly weighed. In the experiments in which chemical analyses of root material were carried out the roots were washed with distilled water. These washings were added to the culture solutions if these solutions were to be analysed for remaining nutrients. Adhering water was removed from the roots by gently pressing them between paper tissues before weighing. After drying for 48 hours in a well ventilated oven at 90°C, the plants were cooled in a desiccator to room temperature and their dry weights determined.

Statistical Analyses.

Analyses of variance were carried out on data obtained. Either the standard errors and number of degrees of freedom on which the error mean squares were based or the lowest differences for significance at the 5% level are given in the tables of results.

2 DETERMINATION OF NITRATE AND AMMONIUM NITROGEN IN CULTURE SOLUTIONS

(a) Estimation of Nitrate in Culture Solutions.

The phenol-disulphonic acid method (Anon, 1954) proved satisfactory for estimating nitrate in culture solutions. Samples of 5 ml of culture solution were used. After the reaction with phenol-disulphonic acid the solutions were made alkaline with ammonium hydroxide and made up to 100 ml. Optical densities of these solutions were determined at 400 mμ.

(b) Estimation of Ammonium Nitrogen in Culture Solutions.

Nessler's reagent, prepared by the method of King (1951), was used to estimate ammonium nitrogen in culture solutions. This reagent (3 ml) was added to a mixture of one ml of culture solution and 4 ml of water. The optical density at 480 mμ was immediately determined. Unreliable results were obtained if the solution was allowed to stand for more than 15 minutes due to the formation of a precipitate.

3 CHEMICAL DETERMINATIONS OF PLANT MATERIAL

(a) Nitrogenous Fractions.

Digestions.

In preliminary investigations the method described by Ma and Zuazaga (1942) was employed. Experiments were then carried out to check the possibility of omitting the steam distillation step. After digestion

of plant material with sulphuric acid containing catalyst, Nessler's reagent (prepared above) was added to samples of the diluted digest. When a catalyst containing mercuric oxide was used in the digestion, a red precipitate was formed on the addition of the Nessler's reagent. Omitting the mercuric oxide produced solutions which did not immediately form a precipitate when Nessler's reagent was added. However, after about 30 minutes a fine precipitate appeared. Therefore optical densities at 480 mμ of solutions were determined immediately after the addition of Nessler's reagent. Standard solutions of ammonium sulphate made up in acid with and without catalyst gave similar readings when Nessler's reagent was added. Samples of a solution of 23 amino acids were digested and heated for 2, 3 and 4 hours after clearing. The nitrogen values obtained were similar after 2 and 3 hours but slightly lower after 4 hours.

The following procedure was finally adopted. Two drops of concentrated sulphuric acid and two glass beads were added to solutions containing combined nitrogen in Kjeldahl flasks and the solutions were boiled nearly to dryness. Concentrated sulphuric acid was added to these concentrated solutions and also to fresh or dry plant material with a mixture of 350 g of potassium sulphate and 7.5 g of copper sulphate added at the rate of 0.33 g per ml of acid. The digestion mixtures were heated for a further two hours after clearing. After the digests had been made up to suitable volumes, Nessler's solution was added and the optical densities were immediately determined at 480 mμ. Blank values were determined by omitting plant material from digests treated similarly.

Extraction and Estimation of Ammonium, Amide, Soluble and Insoluble Nitrogen in Tomato Plants.

Yemm and Willis (1956) showed that much of the ammonium and amide nitrogen was lost from plant tissues if dried at 80°C. They also demonstrated that cold 0.01 N hydrochloric acid was a suitable solution for extracting plant tissues. Therefore in the following work fresh plant material was weighed and immediately ground in a chilled glass mortar and pestle in 5 volumes of 0.01 N hydrochloric acid at 2°C. After 30 minutes the residues were removed by centrifugation and washed twice with cold acid. The extract and washings were combined and made up to volume. Ammonia and amide nitrogen were estimated in these solutions by the method of Pucher *et al.*(1935). Total nitrogen in the extracts and residues was determined after digestion as already described (Methods, 3a).

(b) Inorganic Elements.

Samples of 0.5 - 1.0 gram of dry plant material were digested with a mixture of 10 ml concentrated nitric acid and 2 ml 60 per cent perchloric acid. After digestion the samples were diluted to suitable volumes for phosphate, sodium, potassium, calcium and magnesium estimations. Phosphate was estimated by the method of Strickland *et al.*(1956). Sodium and potassium were determined using an "Eel" flame photometer. Calcium was determined using the flame photometer after lanthanum nitrate had been added so that the ratio of lanthanum to phosphorus in the solution was

10:1 on a molarity basis to reduce the interference due to phosphate in this estimation (Williams, 1960). Total calcium and magnesium in the solutions was determined by a compleximetric titration using sodium ethylene diamine tetracetate, solochrome black and an ammonia-ammonium chloride buffer at pH 10 (Hutton, 1954). Magnesium values were calculated as the difference between the total calcium and magnesium estimated in this way and the calcium estimated with the flame photometer. Chlorine was determined in hot water extracts of dried plant material by the method of Best (1950). Iron, manganese, copper and molybdenum were estimated by spectrographic analysis on dried plant material.

Values for each element were expressed as the number of grams of the element in 100 grams of dry plant material.

(c) Organic Acids.

Total organic acids were estimated in 0.5 gram samples of dried plant material using the technique of Pucher et al.(1941). The corrections for oxalic acid and sulphuric acid were not made. However, any slight error caused by the sulphuric acid should have been the same in all cases as similar weights of plant material and volumes of sulphuric acid were used for each extraction of organic acids. The error caused by only determining 50 per cent of the oxalic acid, depended on the amount of oxalic acid present in the tissues.

(d) Carbohydrates.

Hydrolysis of total carbohydrates in dried tomato plant material was carried out using the method of Weinmann (1947) and the resulting total sugars were estimated by the method of Somogyi (1952) using the modifications suggested by May and Davidson (1958).

4 DETERMINATION OF AMINO ACIDS AND AMIDES IN PLANT MATERIAL

(a) Extraction from Plant Material.

Amino acids and amides were extracted from fresh plant material by the method of Possingham (1954) except that a glass mortar and pestle were used instead of a "homogeniser". Briefly the method consisted of grinding fresh plant material in cold 80 per cent ethanol and allowing to stand overnight at 2°C followed by removal of the residues by centrifugation. The alcohol extract was evaporated to dryness in a rotary evaporator below 40°C and the amino acids and amides were dissolved in water and poured onto a "Zeokarb 225" resin column. Sugars and salts were washed through with water and the amino acids and amides were eluted with 1.5N ammonium hydroxide. The ammonia extracts were evaporated almost to dryness in a rotary evaporator maintained below 40°C and then completely to dryness in a vacuum desiccator over phosphorus pentoxide. The dried extracts were stored in a deep freeze until required for analysis when they were dissolved in a known volume of 10 per cent aqueous

isopropanol. If the resin column step was omitted it was found, in agreement with Possingham (1954), that the extracts when run on chromatograms produced considerable streaking.

(b) Determinations by Paper Chromatography.

Qualitative.

Amino acids and amides in the extracts were separated using either one or two directional descending paper chromatography.

Several solutions, each containing one known amino acid or amide, were separately spotted two inches apart on a line 7 cm from one edge of sheets of Whatman No.1 filter paper (24" x 24") and dried with a current of warm air. These sheets of paper were then placed in wooden chromatography boxes coated on the inside with paraffin wax and provided with a glass window on one side. Each box was fitted with four stainless steel troughs enabling 8 papers to be run simultaneously. Each paper was supported by two glass rods with one edge of the paper dipping over the side of one trough. The chromatograms were run in a constant temperature room at 25 $\pm$ 3°C. Two solvents were used;

1) butanol : acetic acid : water (52:13:35 by volume) and
2) phenol saturated with water.

The phenol was purified by redistillation (Partridge, 1948). To each trough 120 ml of the appropriate solvent were added. The chromatograms were allowed to run until the solvent fronts were near the lower edges

of the papers. The papers were then placed in a drying chamber and dried in an air stream at 40°C. The positions of the amino acid spots were determined by spraying the papers with a solution of 0.1% ninhydrin in absolute ethanol containing 0.1% pyridine and heating for 10 minutes at 95°C (Possingham, 1954).

When butanol : acetic acid : water was used as the solvent discreet amino acid spots were obtained. However, phenol : water produced some streaking. This streaking was reduced by washing the papers with 2 N acetic acid followed by distilled water and then drying before use. Therefore only washed papers were used for subsequent chromatograms when phenol : water was used as a solvent.

Two dimensional chromatograms were obtained by spotting a mixture of known amino acids and amides, developing with butanol : acetic acid : water, drying the paper, rotating the paper 90°, and developing with phenol: water. After drying the papers, the amino acid and amide spots were developed with the ninhydrin spray reagent and their identity determined by comparing their Rf values with those of amino acids and amides run on separate sheets in each solvent. Plant extracts were then spotted and chromatograms were run either in one dimension in butanol : acetic acid : water or in two dimensions in butanol : acetic acid : water followed by phenol : water. The identification of the spots was made by comparing their Rf values with those of known amino acids run under similar conditions.

Quantitative Paper Chromatography.

Two general methods are available for determining quantitatively the amount of amino acids present on chromatograms. The position of amino acid spots can be determined, these are cut out and coloured compounds then developed in tubes. Alternatively the coloured compounds can be developed on the chromatograms directly and are measured either on the paper or are eluted and measured in tubes.

Fowden's method (1951) was first tried using the fluorescence developed under ultra violet light by amino acids on paper when heated at 100°C for 15 minutes to locate the amino acids on the paper. Using this method it was possible to detect the amino acids at the origin prior to chromatography and also after running in butanol : acetic acid : water. However with phenol:water the spots spread and it was difficult to determine the outer limits of each spot. This method was not suitable for two dimensional chromatograms where several spots were in close proximity.

The method described by Thompson et al.(1951) was tried. In this procedure the ninhydrin amino acid complexes are developed directly on the paper in an atmosphere of carbon dioxide at 60°C saturated with ethanol vapour. A perspex developing chamber which was slightly larger than that suggested by Thompson et al.(1951) was used to allow for the larger papers. This chamber was placed in a wooden box in which the temperature was maintained at 60°C by circulating air over thermostatically controlled electric strip heaters. Three different concentrations

of ninhydrin (0.5, 1.0, and 2.0%) in the spray reagent were tested. Maximum amino acid colour development was found with the two higher concentrations. Alternatively spraying twice with the 0.5 per cent solution produced similar results.

In extracts from tomato plant material the amino acids present at relatively high concentrations and easily detected on paper chromatograms were aspartic acid, glutamic acid, serine, glycine, alanine, γ-amino butyric acid, asparagine and glutamine. A standard solution of these amino acids and amides was prepared using 10% aqueous isopropanol and appropriate volumes were spotted onto papers to give different loadings of amino nitrogen. Duplicate papers were prepared loaded with the following amounts of amino nitrogen - 2.24, 4.48, 6.16, and 7.84 µg amino nitrogen of each amino acid or amide. The eight papers were run in both solvents and the chromatograms were developed by the method of Thompson et al.(1951) with the following slight modifications. The spots were cut out, the paper eluted with 7.5 ml of 50 per cent aqueous ethanol and the optical densities of the solutions were read on a Coleman Spectrophotometer Model 14 at 570 mµ. 7.5 ml of aqueous ethanol was used rather than 10 ml as used by Thompson et al. because this smaller volume was found to be sufficient for elution and gave a more concentrated solution. The yellow brown coloured solution produced by asparagine was read at 360 mµ. In some cases dilution of the solution was necessary. In all cases the optical density values were expressed on a

basis of a total volume of 7.5 ml for determinations of colour values. The colour value of each amino acid is the quantity of amino nitrogen in micrograms divided by the corrected optical density, i.e. optical density reading corrected for blank and expressed on a 7.5 ml basis. These values and their deviations (expressed as a percentage of the mean) are shown in Table 2. The results showed considerably greater variation than those reported by Thompson and Steward (1951).

The procedure was repeated using one dimensional chromatograms with butanol : acetic acid : water as the solvent. Except for asparagine and leucine there was less variation present in these standard values. (Table 2). However one-dimensional chromatography failed to separate glutamine and serine, and glutamic acid and threonine. As the colour values ($\frac{\mu g\ N}{O.D.}$) for glutamic acid and threonine were approximately the same (Table 2) the optical density readings for the two amino acids could be multiplied by 5.0 and the results quoted as glutamic acid and threonine.

Owing to the large difference in the colour values for glutamine and serine, it was not possible to quote these values in a similar way. Instead the results for glutamine and serine had to be expressed as the optical density produced by the glutamine and serine present in one gram fresh weight of plant material instead of µg amino nitrogen per gram fresh weight as for all other determinations.

The possibility of employing one dimensional paper chromatography was examined since this had been shown to yield less variable results (Table 2), and in addition ten extracts could be analysed simultaneously

TABLE 2

QUANTITATIVE DETERMINATION OF AMINO ACIDS SEPARATED BY PARTITION CHROMATOGRAPHY*

Amino acid or amide.	Two dimensional chromatograms.		One dimensional chromatograms.	
	µg amino N / OD	Standard deviation expressed as percentage of mean.	µg amino N / OD	Standard deviation expressed as percentage of mean.
Aspartic acid	10.5	4.63	-	
Asparagine	41.3	7.23	17.8	8.29
Serine	5.6	8.19	5.1	2.86
Glycine	6.9	9.26	6.2	2.65
Glutamic acid	5.4	5.71	5.0	2.56
Threonine	6.2	4.36	5.8	3.05
Glutamine	32.1	10.02	29.1	2.98
Alanine	5.5	5.29	5.1	2.77
γ-amino butyric acid	6.9	5.29	6.2	2.54
Valine			5.5	2.89
Phenylalanine			20.8	2.18
Leucine			6.1	5.83

* The amino acids, on paper chromatograms, were converted to the ninhydrin complexes by reaction with ninhydrin in an atmosphere of carbon dioxide saturated with ethanol vapour at 60°C for 30 minutes. Each value is the mean of 8.

Values are $\frac{\mu g\ amino\ N}{OD}$

Where

µg.N. = The amount of amino nitrogen spotted on the papers (2.24-7.84 µg).

O D = Optical density (after subtraction of blank value) of the amino acid - ninhydrin coloured compound dissolved in 7.5 ml of 50 per cent aqueous ethanol. All optical densities were measured on a Coleman Spectrophotometer Model 14 against 50% aqueous ethanol as a reference at a wave length of 570 mµ except for the yellow brown solution produced by asparagine which was measured at 360 mµ.

on one chromatogram as compared to a single extract by two-dimensional chromatography. However one dimensional chromatograms do not yield satisfactory separations of all the amino acids and amides present in plant extracts, and consequently it was necessary to use them in conjunction with two-dimensional chromatograms for qualitative and quantitative estimations.

The details of the method deemed most satisfactory for the quantitative estimation of amino acids separated by paper chromatography are as follows. Suitable samples of amino acid and amide extracts from plant material were spotted onto sheets of washed filter paper (Whatman No.1) and run either in one dimension in butanol:acetic acid:water or in two dimensions in butanol:acetic acid : water followed by phenol:water. After the chromatograms were dried, colour was developed by the method of Thompson et al.(1951) using one per cent ninhydrin solution. The coloured complex was eluted with 7.5 ml 50 per cent aqueous ethanol, and the optical density at 570 mμ was determined. In the estimation of asparagine, the optical density at 360 mμ was recorded. Although the accuracy obtained in the present investigation was not as great as that reported by Thompson and Steward (1951) it was still considered sufficiently accurate for preliminary studies in the amino acid and amide levels in plants grown on different forms of nitrogen.

Quantitative estimations of amino acids and amides in extracts from plant material were also made by the method of ion exchange chromatography using Dowex 50 resin as described in the following section.

Apart from serine, the qualitative and quantitative results for amino acids by the two methods were in good agreement. Although separation on Dowex 50 failed to give a satisfactory quantitative estimation of the amides present, it was found that the height of the amide plus serine peak eluted from Dowex 50 corresponded to the amide levels as determined by paper chromatography.

5 AMINO ACID COMPOSITION OF LEAF PROTEINS

(a) Extraction of protein from tomato leaves.

When discussing the amino acid compositions of proteins from leaves, Pirie (1959) comments "we can have trustworthy analyses on uncertain starting material or uncertain analyses on a definite starting material". It was decided in this work to extract proteins from tomato leaves and to free them from other compounds before hydrolysis rather than to hydrolyse impure protein samples. The method of Lugg and Weller (1944) was used with slight modifications. Fresh tomato leaf laminae (3 to 10 g) were ground with a mortar and pestle in 2.5 volumes of borate buffer at pH 9.2 at room temperature. Two volumes of buffer were used to wash the slurry into a beaker and the pH of the slurry was then adjusted to 9.2 with sodium hydroxide solution. Four volumes of ethanol-ether mixture (4:1) were added with vigorous stirring. After 15 minutes the solid residues were removed by filtering through muslin followed by centrifugation. The green opalescent liquid was adjusted to pH 5.2 with acetic acid and heated to 70°C. The resulting

precipitate of protein was washed successively with 5×10^{-4}M hydrochloric acid, boiling 98 per cent. ethanol, boiling 5×10^{-4}M citric acid, twice with boiling 98 per cent. ethanol and once with warm ether. After being dried in a vacuum desiccator, the white protein samples were stored at -15°C.

Protein nitrogen determinations on the original leaf material and on extracted protein showed that 70 per cent. of the protein of the tomato leaves was extracted by this method. Lugg and Weller (1944) have shown that protein extracted by this method gives a representative sample of the whole protein of leaves. Protein extracted and washed in this way appeared to be free from non-nitrogenous compounds as it contained over 16 per cent. nitrogen. (Table 35).

(b) Determination of amino acid composition of proteins by ion-exchange column chromatography.

(i) Separation by ion-exchange chromatography and estimation of amino acids.

For the quantitative analysis of the amino acid composition of protein hydrolysates, the method of Moore and Stein (1954) was tried. The apparatus and technique used were essentially those of the above authors. Minor modifications included a fraction cutter as used by Weller (1957), a wooden turntable, a copper tank with thermostatically controlled heating elements from which water was circulated through the heating jacket of the column by a bronze centrifugal pump with a stainless steel shaft. Unfortunately a dust-free atmosphere could not be

maintained in this laboratory and although every possible precaution was taken against contamination of the fractions by dust and slight traces of external ammonia, undoubtedly some contamination did take place. Distilled water and citric acid solution were treated with Dowex 50 to remove traces of ammonia. No detergent was used in the buffers.

Analyses of fractions for amino nitrogen were carried out by the method of Yemm and Cocking (1954) after adjusting the pH of each fraction to 5.0. It was not necessary to prepare hydrindantin and the ninhydrin reagent did not need to be stored under nitrogen in this method. The optical densities were measured in 1 cm cuvettes in a Coleman Model 14 spectrophotometer. A sample of leucine (99 per cent. pure) was used as the standard for determinations. Reproducible results were obtained, and the amount of colour produced was strictly proportional to the concentration of leucine. Proline also gave similar results when readings were taken at 440 mμ. Tyrosine and phenylalanine readings were corrected for the lower colour yield given by these acids (Yemm and Cocking, 1954) when calculating the amounts of amino acids in the peaks. It was found that the potassium cyanide-methyl Cellosolve-ninhydrin solution was stable for at least 10 days. Some batches of methyl Cellosolve produced high blank values when used in the reagent. Distillation of the methyl Cellosolve failed to reduce the blank values. However, after heating the methyl Cellosolve with acid washed Norite (20 grams per litre of methyl Cellosolve), filtering and distilling, the resulting methyl Cellosolve, when used in the ninhydrin reagent, gave reasonable blanks.

A standard solution of a mixture of the 17 amino acids normally found in leaf proteins was run on the column and percentage recoveries of the known amino acids were determined. In four runs the percentage recoveries varied from 80-100 per cent. Separation of the individual amino acids was not as good as that reported by Moore and Stein (1954). The use of Dowex 50 x 5 did not alter the relative positions of lysine and histidine. Subsequently it was found that the internal diameter of the glass tube containing the resin was less than 9 mm in diameter and this probably caused poor separations of some amino acids. However, recoveries of amino acids which did separate completely still showed a great deal of variation. It had been found difficult using this tehnique to keep the nitrogen pressure above the reservoirs constant throughout a run. Consequently the flow rate varied and this may have had an effect on the results.

In view of the technical difficulties described above and the publication of an improved method (Moore et al., 1958) it was decided to try this method using Dowex 50 x 8 and a 150 cm column of exactly 9 mm diameter. The Dowex 50 x 8 had been separated by the hydraulic method of Hamilton (1958) and particles in the size range 35-70 microns were used in the preparation of the resin columns*. The length of the small column was increased from 15 cm to 20 cm to improve the separation of the basic amino acids.

---

* (The Dowex 50 x 8 in the separated form was kindly supplied by Dr. D. H. Simmonds, Agricultural Chemistry Department, Waite Agricultural Research Institute.)

It was found that buffer at pH 3.22 followed by buffer at pH 4.25 gave the most satisfactory separations of the acidic and neutral amino acids which were as good as those reported by Moore et al. (1958). Proline was determined using the method of Chinard (1952) on the first few fractions collected from the 20 cm column. The acidic and neutral amino acids present in these fractions did not interfere with the reaction of the ninhydrin with proline in acid solution.

γ-amino butyric acid appeared as a peak in the same position as the tyrosine-phenylalanine peak from the 20 cm column. Consequently values for phenylalanine plus tyrosine plus γ-amino butyric acid were determined on extracts of amino acids from plants. As tyrosine could not be detected by paper chromatography of plant extracts, these peaks would have consisted mainly of phenyl-alanine and γ-amino butyric acid.

Even with the better separation of peaks and a constant flow rate the percentage recoveries of known amino acids were still too variable.

Before adding the ninhydrin reagent and buffer to each fraction for the determination of amino nitrogen present, it was necessary to adjust the pH of the fractions to pH 4.9 - 5.0 with sodium hydroxide solution or hydrochloric acid. As it was impracticable to check that the pH of each fraction was between 4.9 and 5.0, fractions between peaks were adjusted and checked and then similar volumes (number of drops of 1 N acid or 1 N alkali) were added to the other fractions. This procedure may have been a source of error as the drop size may have varied causing the fractions to have varying pH values and causing conditions which were

not optimum for colour development.

To avoid the necessity of adjusting the pH of each fraction, the method of Rosen (1957) was tested with standard leucine solution. The buffer used in this procedure is very concentrated and no adjustment of pH is necessary. Reproducible straight lines were obtained by plotting the optical density against concentration of leucine solutions analysed by this procedure and threonine, valine and aspartic acid were found to give similar colour yields to leucine. However it was noted that some of the blank values were much higher than the others. This was eventually found to be due to the type of test tube used. Six by three quarter inch rimless pyrex test tubes were used throughout, but tubes of 3 or 4 different makes were present. It was found that blank values were different if determined using different makes of tubes. If similar tubes were used to determine blank values, these values were constant. Temperatures of the solutions in different types of tubes during the heating and cooling steps in the development of colour were tested at minute intervals. No differences in temperature were observed between solutions in the different types of test tubes. The reason for higher blanks in some types of tubes remained unexplained.

Following this finding, similar types of tubes were used to collect the fractions constituting peaks or groups of peaks and several tubes on each side of the peak so that errors would not be caused by the higher readings produced in some types of tubes. Recoveries of known amino acids separated on 150 and 20 cm columns of Dowex 50 x 8 and analysed by

the method of Rosen (1957) using similar types of tubes were found to vary between 95 and 105 per cent. Values for recovery of ammonium chloride separated from a mixture of basic amino acids and ammonium chloride varied between 80 and 88 per cent. when compared with standard leucine solutions.

(ii) Hydrolysis of protein and analysis of protein hydrolysates.

Samples of protein* (20 mg) from leaves of *Medicago sativa* L. which had been extracted by the method of Lugg and Weller (1944) were hydrolysed in sealed tubes with 10 ml of twice glass distilled constant-boiling hydrochloric acid for 20 hours at 110°C in a pressure cooker with a thermostatically controlled heating element. The tubes were cooled, cut open and the hydrolysate was filtered through Whatman No.42 filter paper. The hydrochloric acid was evaporated off under reduced pressure and the resulting amino acids were dissolved in water to make a volume of 20 ml. Samples (1 ml) for the 150 cm column and 2 ml samples for the 20 cm column were placed in small tubes and evaporated to dryness in a vacuum desiccator over phosphorus pentoxide. The amino acids were then dissolved in citrate buffer either at pH 3.22 or at pH 5.25 for addition to the 150 cm column or 20 cm column. Samples of the diluted hydrolysate (4 ml) and 6 mg samples of the untreated protein were digested and total nitrogen values were determined (Methods, 3a).

---

* (The protein was kindly supplied by Mr. R. A. Weller, C.S.I.R.O. Adelaide.)

When samples were added to the 150 cm resin column and the amino acids were separated and estimated, no methionine was recovered. As methionine is normally present in leaf proteins, it seemed likely that methionine had been oxidized during the hydrolysis with air present. Therefore hydrolyses were carried out as above but the tubes were evacuated before sealing. Under these conditions methionine was found to be present in the hydrolysate. Results of amino acid analyses on hydrolysates after hydrolyses in the presence and absence of air are shown in Table 3. When these analyses were carried out the ninhydrin reagent of Yemm and Cocking (1954) was still being used so that the values are subject to some variation. Most of the amino acids showed similar values in hydrolysates prepared by either method with the exception of methionine. All further hydrolyses were therefore carried out in evacuated sealed tubes.

In the later work with tomato leaf proteins in which reliable results were obtained, values for amino acids were not corrected for losses during hydrolysis. Thus serine, threonine and methionine values were probably lower than the amounts present in the proteins (Schram et al., 1953). However as the aim was to compare proteins of differently treated plants rather than to determine accurate amino acid compositions of protein, the uncorrected values were adequate for this comparison.

The nitrogen of each amino acid was expressed as a percentage of the nitrogen present in the filtered hydrolysate. The total nitrogen recovered was about 90 per cent. of that present in the hydrolysate.

TABLE 3

AMINO ACID COMPOSITIONS OF LEAF PROTEINS OF MEDICAGO SATIVA L. AS DETERMINED BY ANALYSIS OF ACID HYDROLYSATES PREPARED IN THE PRESENCE OR ABSENCE OF AIR

Each value is the amino acid nitrogen as a percentage of the total nitrogen of the filtered hydrolysate. Each value is the mean of results from three estimations.

Amino Acid	Hydrolysis in Sealed Tubes	Hydrolysis in Evacuated Sealed Tubes
Aspartic acid	5.6	5.8
Threonine	3.7	3.5
Serine	3.2	3.2
Glutamic acid	5.9	5.9
Glycine	6.1	5.6
Alanine	5.6	5.9
Valine	4.3	4.6
Methionine	0	0.9
Isoleucine	2.8	3.5
Leucine	5.9	6.5
Tyrosine	2.3	2.3
Phenylalanine	3.6	3.3
Proline	4.5	4.5
Lysine	8.1	7.7
Histidine	4.7	3.7
Arginine	12.1	11.6
Ammonia	13.4	7.7
N accounted for	91.8	86.2
% N in protein sample	15.2	

Comparison of data from the columns and from ninhydrin analyses on hydrolysates not separated on the columns, showed that 98-100% of the nitrogen which reacted with ninhydrin in unseparated hydrolysates was recovered after separation on the columns. The other 10 per cent. of the nitrogen present in the hydrolysates may have been in the form of fine humin or other nitrogenous compounds. Cystine and tryptophane are both not present in acid hydrolysates and these amino acids were not determined.

As a result of these investigations, the method adopted for the determination of the amino acid compositions of leaf proteins was as follows: 20 mg samples of protein were hydrolysed with 10 ml of twice glass distilled constant-boiling hydrochloric acid in evacuated sealed glass tubes for 20 hours at 110°C. The hydrolysate was filtered through Whatman No.42 filter paper and evaporated to dryness under reduced pressure. The amino acids were dissolved in 20 ml of water. Samples (1 and 2 ml) were evaporated to dryness and then dissolved in citrate buffer for addition to the 150 and 20 cm columns of Dowex 50 x 8 respectively. Total nitrogen was determined on 4 ml samples of the hydrolysate. Amino acids were separated on the resin columns by the method of Moore et al. (1958) and fractions were analysed for amino nitrogen by the method of Rosen (1957) using similar types of test tubes for fractions containing peaks or groups of peaks.

Proline was determined by the method of Chinard (1952) using the first few fractions from the 20 cm column. Amino acid nitrogen of each

amino acid was expressed as a percentage of the total nitrogen in the filtered hydrolysate. No corrections were made for any losses which may have occurred during hydrolysis and cystine and tryptophane were not analysed.

6 DETERMINATION OF INCORPORATION OF ^{15}N INTO LEAF PROTEINS

(a) Isolation of subcellular fractions from tomato leaves and extraction of proteins from subcellular fractions.

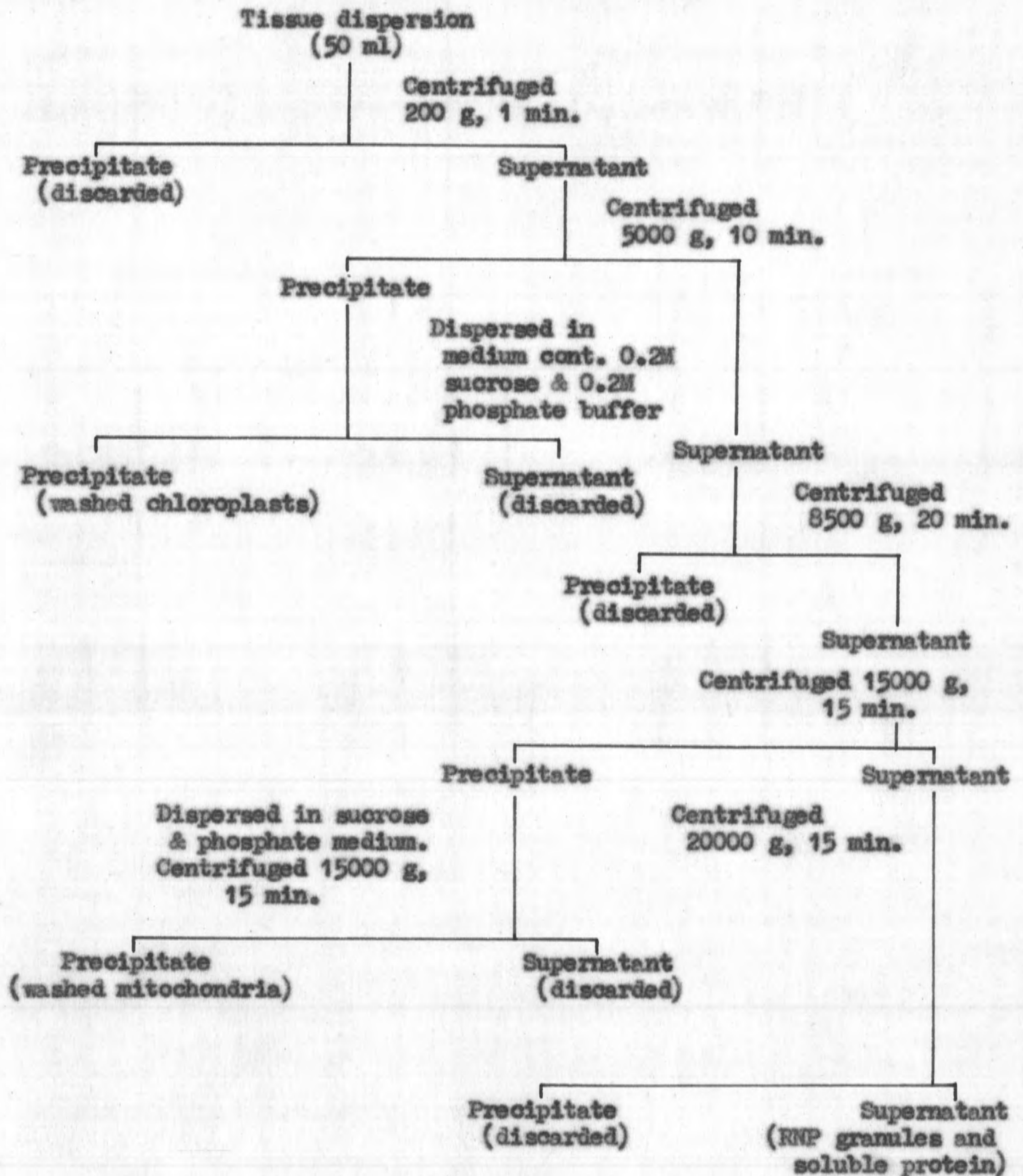
(i) Isolation of fractions.

Subcellular fractions were isolated from tomato leaf laminae by a combination of the methods of Gorham (1955) and Martin and Morton (1956a). Gorham (1955) describes a technique for the isolation of chloroplasts. As Martin and Morton (1956) extracted cytoplasmic particles from white petioles of silver beet they did not obtain a chloroplast fraction.

A schematic representation of the method used for the isolation of chloroplasts and mitochondria from tomato leaf laminae is shown in Figure 1.

Approximately 20 g of tomato leaf laminae were ground in a cold glass mortar and pestle with 1½ volumes of medium containing 0.2M sucrose and 0.2M potassium phosphate, adjusted to pH 7.4. The slurry was strained through 4 thicknesses of muslin cheese cloth. The filtrate was centrifuged at 200 xg for 1 minute to remove cell debris and extraneous

FIGURE 1. FRACTIONATION OF TOMATO LEAF DISPERSION.



particles. All centrifugations were carried out at 0°C using an International refrigerated centrifuge. The supernatant was centrifuged at 5,000 xg for 10 minutes and the resulting precipitate was resuspended in fresh medium and centrifuged a further 10 minutes at 5,000 xg. The precipitate was designated as washed chloroplasts. The original supernatant from the centrifugation at 5,000 xg was centrifuged at 8,500 xg for 20 minutes to remove further chloroplasts and larger chloroplast fragments. This precipitate was discarded. The supernatant was centrifuged at 15,000 xg for 15 minutes and the resulting precipitate was resuspended in fresh medium and reprecipitated at 15,000 xg for 15 minutes. This precipitate was designated as washed mitochondria. The original supernatant from the mitochondria was centrifuged at 20,000 xg for 15 minutes to remove further mitochondria. This precipitate was discarded. The supernatant contained mainly endoplasmic reticulum and soluble protein.

(ii) Extraction of protein from fractions.

The washed chloroplasts and washed mitochondria were suspended in water. These suspensions and the final supernatant were adjusted to pH 4.5 with trichloracetic acid and boiled for one minute. The resulting protein suspension was precipitated by centrifugation and washed once with 5×10^{-4}N hydrochloric acid and three times with boiling ethanol. The protein was dried in a vacuum desiccator.

Two samples of similar leaf tissue were taken and filtered tissue

dispersions prepared. Assays of the total nitrogen of the leaves and of that present in the dispersions showed a 60 per cent. recovery (Table 4). This incomplete recovery indicates that the method of extraction leaves many of the cells unbroken.

The fractionation procedure and protein assays described above were carried out on two similar samples of leaf tissue. In addition estimations were made of soluble nitrogen and the nitrogen of the fractions normally discarded. As is shown in Table 5, 71 per cent. of the total dispersion nitrogen was recovered. The 29 per cent. discrepancy was probably due to the fact that solutions used to wash the chloroplasts and mitochondrial fractions were not analysed. Moreover the nitrogen removed when precipitating and washing the protein of each fraction was not determined. It is interesting to note that the value for mitochondrial protein is similar to that determined for the mitochondrial fraction of beet petiole dispersion by Martin and Morton (1956). In the present work the washed mitochondrial fraction was heterogeneous as washing with alcohol extracted some chlorophyll. Apparently fragments of chloroplasts were associated with the mitochondrial fraction. The protein from the final supernatant contained no chlorophyll indicating no contamination by smaller chloroplast fragments.

(b) Estimation of ^{15}N abundance in proteins.

Protein samples from plants supplied with ^{15}N labelled potassium nitrate or ammonium chloride (see Part V, 3 for details of supplying ^{15}N)

TABLE 4

NITROGEN IN TOMATO LEAVES AND TISSUE DISPERSIONS

Each value is the mean from two leaf samples.

	Nitrogen (mg/100g fresh weight)	% of total nitrogen
Total N of leaf tissue	607	100
Dispersion (after filtering through muslin)	388	64

TABLE 5

DISTRIBUTION OF NITROGEN OR PROTEIN NITROGEN AMONG SUBCELLULAR FRACTIONS FROM TOMATO LEAF DISPERSIONS

Each value is the mean from two leaf samples.

Fraction	Isolation procedure	Fraction resuspended and washed	N (mg/100g fr. wt.)	N as % of dispersion-N
Dispersion	Leaves ground and slurry filtered	-	388	100
Unbroken cells, etc.	200g, 1 min	-	46.2	11.9
Protein from chloroplasts	5000g, 10 min	+	32.4	8.3
Chloroplasts and chloroplast fragments	8500g, 20 min	-	19.0	4.9
Protein from mitochondria	15000g, 15 min	+	2.95	0.76
Mitochondria	20000g, 15 min	-	9.47	2.4
Protein from supernatant	Protein ppt. and washed	-	124.8	32.2
Soluble nitrogen		-	40.0	10.3
Total			274.82	71.7

were digested in microkjeldahl flasks as described above (Methods 3, a). The ammonia was distilled off in a Pregle steam distillation apparatus and collected in 5.0 ml of 0.7N sulphuric acid. After dilution, the amount of ammonium nitrogen was determined according to King (1951). Ammonium sulphate was added to the mitochondrial nitrogen to bring the total amount of nitrogen present to 1 mg. These samples and samples of the other solutions containing about 1 mg of nitrogen were concentrated to a volume of 2 to 3 ml under an infra-red lamp. Each sample was washed into one limb of a Rittenberg tube (Sprinson and Rittenberg, 1949) and 2 ml of sodium hypobromite, prepared by the method of Rittenberg <u>et al</u>. (1939) with the modification of Simms and Cocking (1958), were added to the other limb of the tube. The tubes at 50°C were partially evacuated with a water pump. They were then placed in a mixture of ethanol and solid carbon dioxide and with a mercury diffusion pump evacuated to a pressure of 5×10^{-6} mm of mercury. The nitrogen liberated by the action of the sodium hypobromite on the ammonium sulphate was introduced into a mass spectrometer. One mg of nitrogen was needed for accurate determinations. The nitrogen was tested for the presence of oxygen by measuring the peak at mass 32. If there were no leaks in the system the percentage of oxygen in the sample of gas was about 0.05 per cent. This value rose to above 10 per cent. when a leak did occasionally occur.

Mass 28 and 29 peaks were measured and the abundance of ^{15}N calculated by the method of Rittenberg <u>et al</u>. (1939). After subtraction of

the natural abundance the results were expressed as atom per cent. excess of ^{15}N. In cases where extra nitrogen had been added to the solutions corrections were made to give atom per cent. excess of ^{15}N of the original nitrogen.

7 DETERMINATION OF RIBONUCLEIC ACID AND DEOXYRIBONUCLEIC ACID IN TOMATO ROOT TIPS

A method was designed for a comparative study of the concentrations of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in root tips and of RNA in cytoplasmic particles in root tips of tomato plants grown on different sources of nitrogen. Differences in the optical density readings at 260 mμ and 290 mμ and at 268 mμ and 290 mμ were taken as a measure of the concentrations of RNA and DNA respectively. Comparative readings for plants grown on different sources of nitrogen were made on a constant volume of solution prepared either from equal fresh weights of root tip material or alternatively from samples of root tip material containing equivalent amounts of protein nitrogen.

Preparation of tissue brei.

The terminal 0.5 cm or 1.0 cm segments of root tips of tomato plants were cut off and placed in culture solution at 2°C. The root tips were blotted to remove excess solution. The fresh weights of these tips were then measured. Samples of 0.5-0.7 g of 0.5 cm root

tips and 1.0-1.4 g of 1.0 cm root tips were used in the analyses. The root tips were ground in 15 ml of 0.5 M sucrose solution at 0°C in a top drive homogeniser. The slurry was made up to 20 ml.

Determination of total RNA and DNA.

The method used in the present work was based on that of Schmidt and Thanhauser (1945) and Ogur and Rosen (1950).

Perchloric acid (0.6 M) was added to 8 ml of the slurry to make the concentration of perchloric acid 0.2 M. The slurry was centrifuged in the cold room and the precipitate was washed with 0.2 M perchloric acid. The precipitate was then washed three times with ethanol-ether (3:1) at 0°C. The precipitate was thoroughly dispersed in 1.0 N potassium hydroxide and kept at 37°C overnight. The potassium hydroxide was neutralised with 6 N perchloric acid at 0°C and 1 ml of 0.6 M perchloric acid was added for each 2 ml of suspension. The suspension was centrifuged in the cold room and the supernatant (A) containing RNA breakdown products, was decanted. The precipitate was washed with 0.2 M perchloric acid and then dispersed in 0.6 M perchloric acid and heated at 90°C for 15 minutes. After centrifugation, the supernatant (B) containing DNA breakdown products, was decanted. The precipitate was washed with 0.6 M perchloric acid. In each case the perchloric acid used for washing was combined with the supernatant and made up to volume. The optical densities at 260 and 290 mμ of A and the optical densities at 268 and 290 mμ of B were determined. In view of the limitations of the method, such as the

interference of other substances extracted into 1.0 N potassium hydroxide which also absorb at 260 mμ (Martin and Morton, 1956b), results were expressed only as differences in optical densities and not as μg of phosphorus or nucleic acid. In order to compare curves in the present work with those of Ogur and Rosen (1950), readings were in some cases taken between 220 and 300 mμ on the two solutions (A and B). Typical curves obtained are shown in Figure 2. The curve for RNA is similar to those obtained by Ogur and Rosen (1950) for RNA from corn root tips. These workers reported values of 8 μg of RNA per g fresh weight in the terminal 3 mm segment of corn roots. Applying their curves and values, the results in the present investigation yielded a value of 4 μg RNA per g fresh weight in the terminal 5 mm segments of tomato roots.

Separation of cytoplasmic particles and determination of RNA.

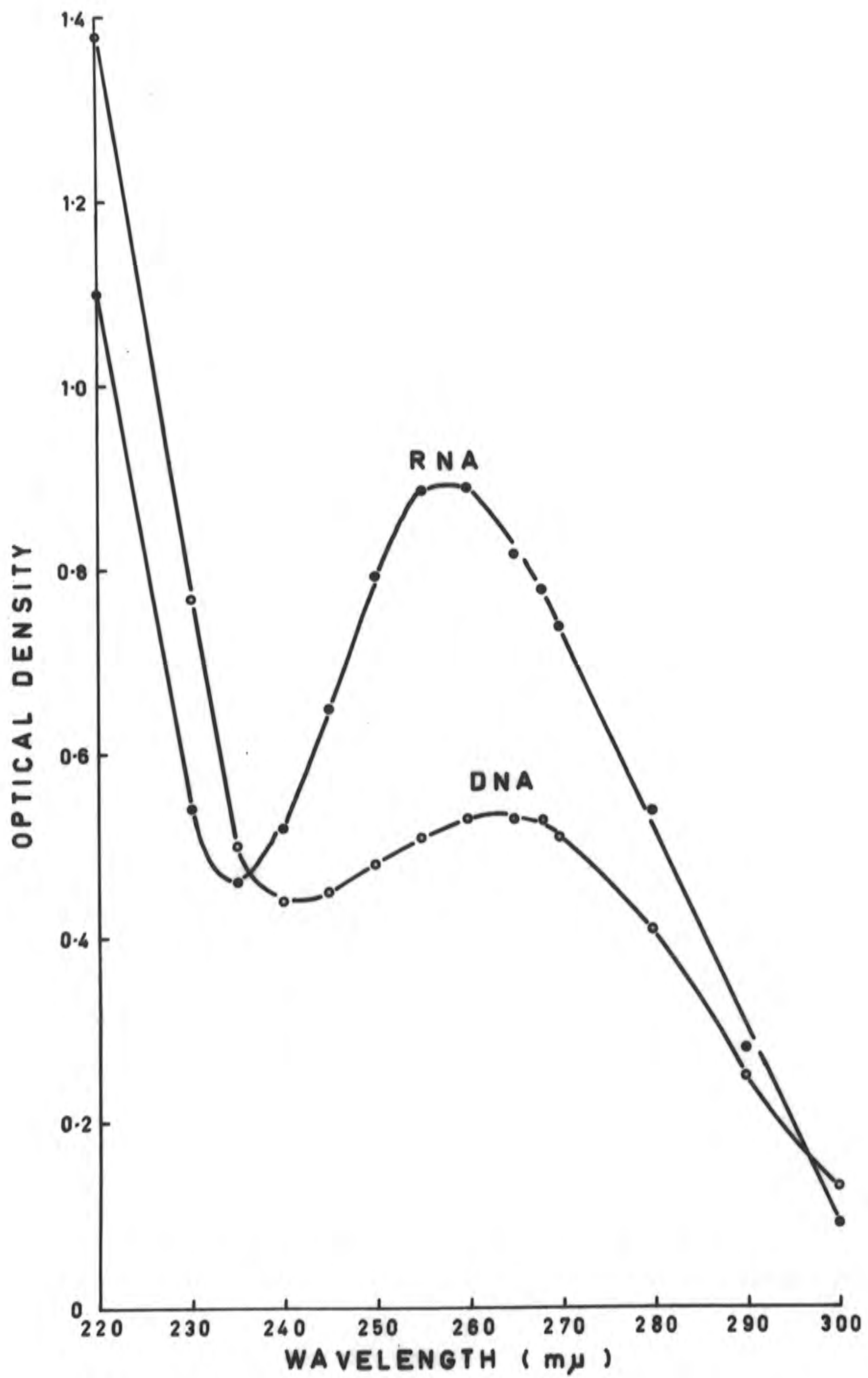
Separation of cytoplasmic particles from root tips and the estimation of their relative RNA concentrations was based on the method used by West *et al*. (1960).

The slurry (8 ml) was centrifuged at low speed to remove cell debris and nuclei. Mitochondria were precipitated by centrifugation at 20,000 x g for 15 minutes. The supernatant was centrifuged at 100,000 x g for 90 minutes to isolate the ribonucleoprotein (RNP) granules of the endoplasmic reticulum. Perchloric acid (0.6 M) was added to a final concentration of 0.2 M in the supernatant. The pellets of mitochondria and RNP granules were transferred to conical centrifuge tubes with 0.2 M perchloric acid at

FIGURE 2. RNA and DNA from terminal segments (0.5 cm) of tomato roots.

● RNA, in 50 ml 0.2M $HClO_4$, from 0.28 g root tips.

○ DNA, in 10 ml 0.2M $HClO_4$, from 0.25 g root tips.



1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
OPTICAL DENSITY
RNA
DNA
220
230
240
250
260
270
280
290
300
WAVELENGTH (mμ)

0°C. The resulting suspensions were precipitated by centrifugation at 2000 x g in a bench model centrifuge at 2°C. The precipitates were resuspended in 0.2 M perchloric acid and reprecipitated as above. The precipitates were washed three times with ethanol-ether (3:1). Perchloric acid (5 ml 0.6 M) was added to the precipitates and, after stirring, these suspensions were heated at 80°C for 25 minutes. After centrifugation the optical density of each solution, diluted if necessary, was measured at 260 and 290 mμ.

Determination of protein nitrogen of root tips.

The remaining 4 ml of slurry were used to determine the protein nitrogen content of the root tips. The pH of the slurry was adjusted to pH 4.5 with acetic acid and boiled for 1 minute. The suspension was centrifuged and the precipitate was washed three times with boiling ethanol and once with water. The final precipitate was transferred to a kjeldahl flask and digested, and the nitrogen estimated (Methods, 3 a).

Results.

The results for RNA and DNA were expressed as differences in optical densities between 260 and 290 mμ and 268 and 290 mμ respectively of 5 ml solutions containing breakdown products of RNA and DNA from 1 g fresh weight of root tip, or from the amount of root tips containing 1 mg of protein nitrogen. In many cases considerable dilution was necessary before spectrophotometric readings could be made. However, for the

purpose of comparison all readings were expressed as optical densities of solutions made up to a constant volume of 5 ml.

8 DETERMINATION OF NICOTINAMIDE ADENINE DINUCLEOTIDE AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE IN TOMATO LEAVES

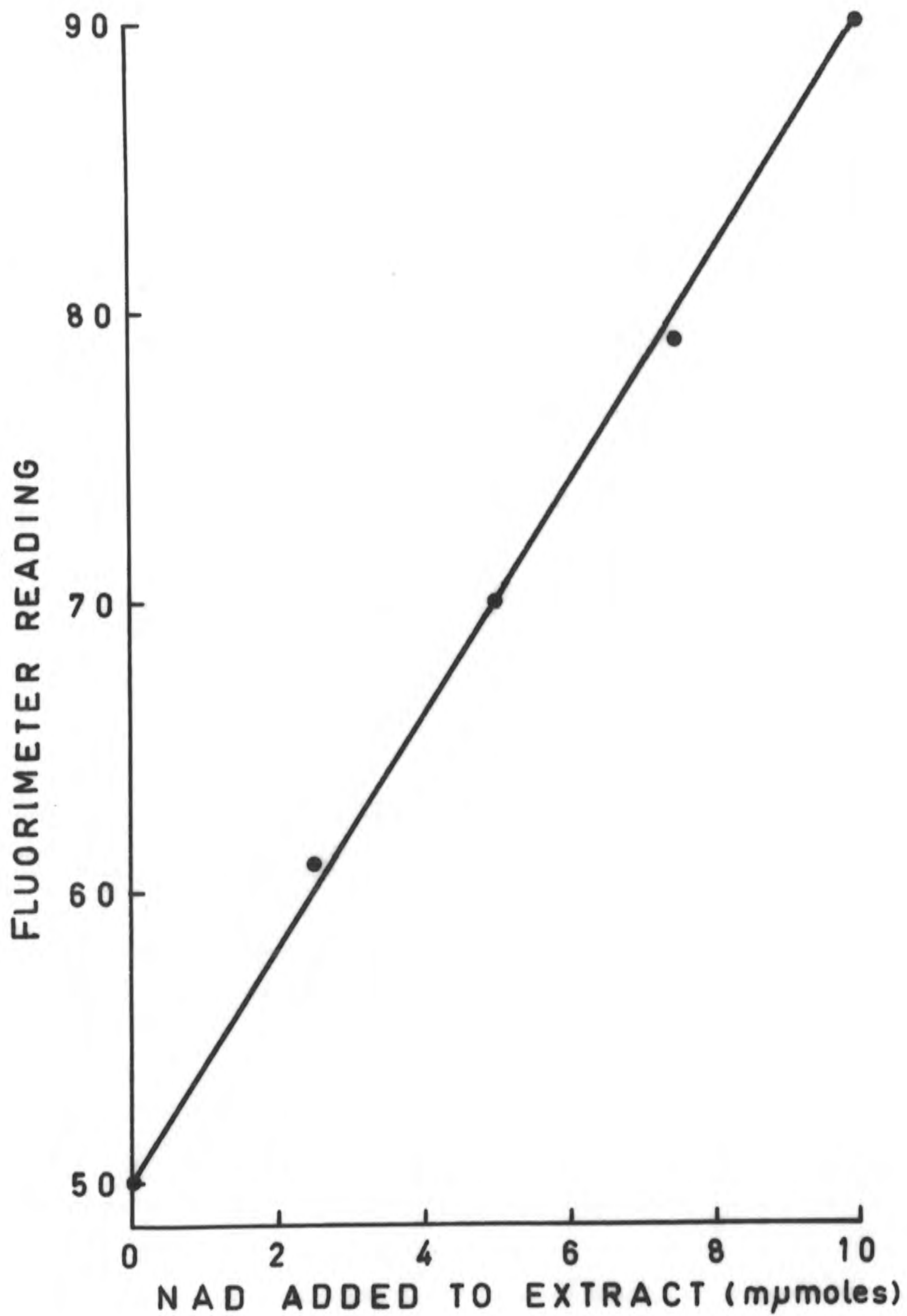
The method of Anderson and Vennesland (1954) for the estimation of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NAD and NADP) in leaves was examined for use in the present investigations. Hydrogen peroxide has been used to oxidise reduced NAD and NADP in tissues to prevent their destruction in acid extraction media (Fiegelson et al., 1950). Anderson and Vennesland (1954) used leaves which had been stored in the dark for at least 12 hours and this may have explained the finding that no increase in values for NAD and NADP occurred when hydrogen peroxide was used in the extraction medium. Any reduced NAD or NADP present in the leaves at the time of harvest was probably oxidised during the dark period. In the present work leaves were homogenised immediately after harvesting the plants from the glasshouse. Consequently hydrogen peroxide was added to the trichloroacetic acid as reccomended by Fiegelson et al. (1950). Tomato leaf laminae (50 g) were homogenised in a top drive homogeniser in a mixture of 125 ml 4.8 per cent. trichloroacetic acid (TCA) and 25 ml of 30 per cent. hydrogen peroxide. Apart from this modification the procedure of Anderson and Vennesland (1954)

was followed. In agreement with these workers it was found necessary to purify further the extracts after elution from the Nuchar C column and acetone precipitation as the optical density of the solution at 340 mμ was still too high for accurate spectrophotometric assays. The extract was absorbed onto a 5 x 1 cm column of Dowex 1 x 4, 200-400 mesh in the formate form (Hurlbert et al., 1954). NAD and NADP were eluted with 150 ml of 3N formic acid. Even then the optical density at 340 mμ of a suitable aliquot of the extract treated with alcohol dehydrogenase only changed from 0.78 to 0.80. This represented a value of about 2 mμ moles of NAD per gram fresh weight of leaf tissue. This procedure was not considered suitable for NAD and NADP analyses in tomato leaf extracts for two reasons. Firstly the change in optical density was too small for accurate determinations and secondly the values for NAD and NADP were very small. As Anderson and Vennesland (1954) pointed out it seemed more likely that nucleotides were being lost in the purification procedure than that tomato leaves contained less nucleotides than other leaves. Consequently it was necessary to find a method for determining NAD and NADP in extracts of tomato leaves without extensive purification of the extracts. The method of Basham et al. (1959), in which the fluorescent compounds produced by the action of strong sodium hydroxide and hydrogen peroxide on NAD and NADP were measured in a fluorimeter, was examined using tomato leaf extracts which had been prepared as above but without purification on a Dowex column. To reduce the breakdown of the fluorescent products by

ultra violet light (Ciotti and Kaplan, 1957), the samples were diluted so that the final concentration of sodium hydroxide was 1N before reading. Using this method higher values for NAD and NADP were obtained. However, after the reduced NAD and NADP had been decomposed by the action of HCl, the extract still fluoresced considerably. Also the readings obtained using this procedure showed some variation. Another disadvantage of the method was the lengthy procedure necessary before readings could be made.

A modification of this procedure (Ciotti and Kaplan, 1957) using methyl ethyl ketone, eliminates some intermediate steps and has the further advantage of reducing the heating period from 1 hour at 38°C to 5 min at 100°C. The fluorescence produced by standard NAD and NADP solutions with methyl ethyl ketone was measured in an "Eel" fluorimeter, and was found to be proportional to the concentration of nucleotide. When standard NAD and NADP solutions were added to extracts of tomato leaves and treated with methyl ethyl ketone some quenching of fluorescence took place. However this decreased fluorescence was still proportional to the concentration of nucleotides (Figure 3). After reduction of NAD with alcohol dehydrogenase and NADP with isocitric dehydrogenase the fluorescence produced with methyl ethyl ketone was only slightly more than that of the "no NaOH" blank. This "no NaOH" blank produced virtually no fluorescence and therefore water was used as a blank in further analyses. Details of the reaction mixtures and procedures used are given in Figure 4. They are essentially those of Ciotti and Kaplan (1957). The readings

FIGURE 3. Fluorescence of methyl ethyl ketone derivative of NAD in the presence of tomato leaf extract.



FLUORIMETER READING
90
80
70
60
50
0
2
4
6
8
10
NAD ADDED TO EXTRACT (mμmoles)

FIGURE 4. REACTION MIXTURES AND PROCEDURES USED FOR ANALYSIS OF NAD AND NADP IN EXTRACTS OF TOMATO LEAVES.

Reduction of NAD

Reaction mixture.

0.1 ml extract
0.075 ml twice glass distilled ethanol
0.8 ml 0.1M sodium pyrophosphate
0.025 ml alcohol dehydrogenase
Shake and allow to stand 5 min
(SOLUTION A)

Methyl ethyl ketone procedure
0.1 ml extract plus 0.9 ml H_2O
or SOLUTION A
or SOLUTION B
or 0.1 ml extract plus 5 $m\mu$moles DPN
or 0.1 ml extract plus 10 $m\mu$moles DPN
0.2 ml 0.2M $MnCl_2$ in methyl ethyl ketone
0.6 ml 3.5N NaOH
Shake and allow to stand 5 min
Add 6.2 ml 0.4N HCl
Shake and then heat in boiling H_2O bath 5 min
Cool and read in fluorimeter

Reduction of NADP

Reaction mixture.

0.1 ml extract
0.1 ml 0.01M magnesium chloride
0.1 ml 0.05M sodium isocitrate
0.675 ml 0.1M potassium phosphate buffer, pH 7.5
0.025 ml isocitric dehydrogenase
Shake and allow to stand 5 min
(SOLUTION B)

"No NaOH" blank
0.1 ml extract plus 0.9 ml H_2O
0.1 ml 0.2M $MnCl_2$ in methyl ethyl ketone
Shake and allow to stand 5 min
Add 1 ml 0.4N HCl and 5.8 ml H_2O
Shake and heat in boiling H_2O bath 5 min
Cool and read in fluorimeter

For analysis of NAD and NADP in each extract readings of the following solutions were made in an "EEL" fluorimeter.

Fluorescence was produced in the following solutions with methyl ethyl ketone as described above.

2 samples of extract
2 " " " plus 5 $m\mu$moles NAD
2 " " " " 10 $m\mu$moles NAD
2 " " " after reaction with alcohol dehydrogenase
2 " " " after reaction with isocitric dehydrogenase

Frequently but not in every case 2 samples of extract plus 10 $m\mu$moles NAD after reaction with alcohol dehydrogenase
2 " " " plus 10 $m\mu$moles NADP after reaction with isocitric dehydrogenase

obtained after the action of alcohol dehydrogenase on extracts with and without added NAD and subsequent reaction with methyl ethyl ketone were similar showing that all the NAD was being reduced and broken down in this procedure. Similar results were obtained with NADP using isocitric dehydrogenase. Because of the quenching of the fluorescence in extracts, internal standards were used with each extract. NAD (5 and 10 mµ moles) was added to aliquots of the extract being analysed and the amounts of NAD and NADP present in the extract were determined by measuring the decrease in fluorescence when separate aliquots of extract were first treated with alcohol dehydrogenase or isocitric dehydrogenase. All analyses were done in duplicate. When differences occurred between duplicates the whole estimation was repeated. Frequent checks were made on the activity of the alcohol dehydrogenase and isocitric dehydrogenase by comparing readings obtained after the action of alcohol dehydrogenase and isocitric dehydrogenase on extracts with and without added NAD and NADP. Results of NAD and NADP determinations were only considered valid if these values were similar. Using this procedure it was possible to measure concentrations of NAD and NADP of 1×10^{-7}M.

Extraction of NAD and NADP from tomato leaves.

Because there are present in plant tissues, few compounds other than pyridine nucleotides which react with methyl ethyl ketone to produce fluorescent compounds, it was considered probable that the extraction procedure described by Anderson and Vennesland (1954) could be simplified.

Leaf laminae were homogenised at 2°C in a mixture of 4.8 per cent. TCA and 30 per cent. hydrogen peroxide. After centrifugation the TCA was removed by extracting the supernatant with redistilled ether. The pH of the resulting solution was adjusted to pH 3.5 with 5% NaOH and the solution freeze dried. Samples of this extract were analysed for NAD and NADP as described above. However, it was found that only 60% of NAD added to samples of the extract was being reduced by alcohol dehydrogenase. The same solution of alcohol dehydrogenase completely reduced NAD in the absence of extract. Thus it appeared that an inhibitor was present in this extract which was not allowing the complete reduction of NAD. After adsorption of the NAD and NADP onto Nuchar C, elution with 10% aqueous pyridine, and removal of the pyridine with chloroform, no inhibition was found.

The procedure finally adopted was as follows. All manipulations prior to freeze drying were carried out at 2°C. Tomato leaf laminae (5-10 g) were homogenised in a top drive homogeniser in a mixture of 25 ml of 4.8 per cent. TCA and 5 ml of 30 per cent. H_2O_2. The suspension was centrifuged at 12,000 xg for 15 minutes and the precipitate was re-extracted twice with 15 ml of TCA and H_2O_2 mixture. The combined supernatants were filtered through muslin and centrifuged at 15,000 xg for 30 minutes. The supernatant was poured onto a 10 x 1.5 cm column of Nuchar C prepared by the method of LePage and Mueller (1949) and washed with 100 ml of 4 per cent.TCA. After adsorption, the column was washed with 75 ml 4 per cent. TCA followed by 75 ml of water. Elution was performed with

40 ml of 10 per cent. pyridine. The pyridine was removed by extracting the eluate three times with 40 ml of chloroform. The pH of the solution was adjusted to 3.5 with 5 per cent. NaOH and the solution was then freeze dried. The solid residue was dissolved in water and made up to a volume of 5 ml. Samples of 0.1 ml of this extract were used for analysis of NAD and NADP as described above.

Recovery experiment.

Tomato leaf laminae (60 g) were homogenised in 180 ml of TCA:H_2O_2 mixture and the suspension was divided into six equal parts. Five or ten ml of solution containing known amounts of NAD or NADP were added to some of this suspension as shown in Table 6. The samples were then centrifuged, re-extracted, and extracts prepared as described above. The extracts were analysed for NAD and NADP and tested for the presence of inhibitors. No inhibitors of alcohol dehydrogenase or isocitric dehydrogenase were present. Percentage recoveries of added NAD and NADP ranged from 85 to 110 per cent. This variation is in part due to unequal division of the suspension. It was difficult to divide the thick viscous suspension accurately. The higher percentage recoveries obtained by using this procedure, as compared to the recoveries reported by Anderson and Vennesland (1954), were probably due to the omission of the acetone precipitation step.

TABLE 6

RECOVERY OF NAD AND NADP ADDED TO TOMATO LEAF HOMOGENATES

Sixty g of leaf material were homogenised and divided into six equal portions. NAD and NADP were added as shown. Samples were adsorbed onto Nuchar C columns, washed and eluted with aqueous pyridine.

Weight of leaf material	Addition (μmoles)		Analyses (μmoles)		% recovery	
	NAD	NADP	NAD	NADP	NAD	NADP
10 g	0	0	0.317	0.433		
10 g	0.5	0	0.853	0.441	107	
10 g	1.0	0	1.415	0.390	110	
10 g	0	0.435	0.353	0.912		110
10 g	0	0.87	0.267	1.330		103
10 g	0.5	0.435	0.741	0.879	85	103

PART III

THE EFFECT OF NITRATE AND AMMONIUM IONS ON THE GROWTH OF TOMATO PLANTS

1 THE GROWTH OF TOMATO PLANTS ON EITHER NITRATE, AMMONIUM OR NITRATE AND AMMONIUM NITROGEN IN WATER CULTURE AT pH 6.0-6.5

Introduction.

Tiedgens (1934) found that tomato plants grown in sand culture grew equally well on either nitrate or ammonium nitrogen provided that the solutions containing the nitrogen were at optimal pH values for growth. With ammonium nitrogen this value was 6.0 and with nitrate nitrogen it was 4.0. As discussed earlier (Part I), iron was not supplied in all solutions and this may possibly explain these optimum pH values. However Clark (1936), using sand cultures at pH 6.7, recorded greater growth of tomato plants on nitrate nitrogen than on ammonium nitrogen. In addition, Woolhouse (1959) reported that tomato plants grown in water culture at pH 6.5 produced more growth if supplied with nitrate nitrogen than if supplied with ammonium nitrogen. These observations (Clark, 1936; Woolhouse, 1959) apparently conflict with those of Tiedgens (1934). It was decided, therefore, to repeat the growth experiment of Woolhouse and in addition to determine the amount of protein synthesis by these plants grown on different forms of nitrogen.

Materials and Methods.

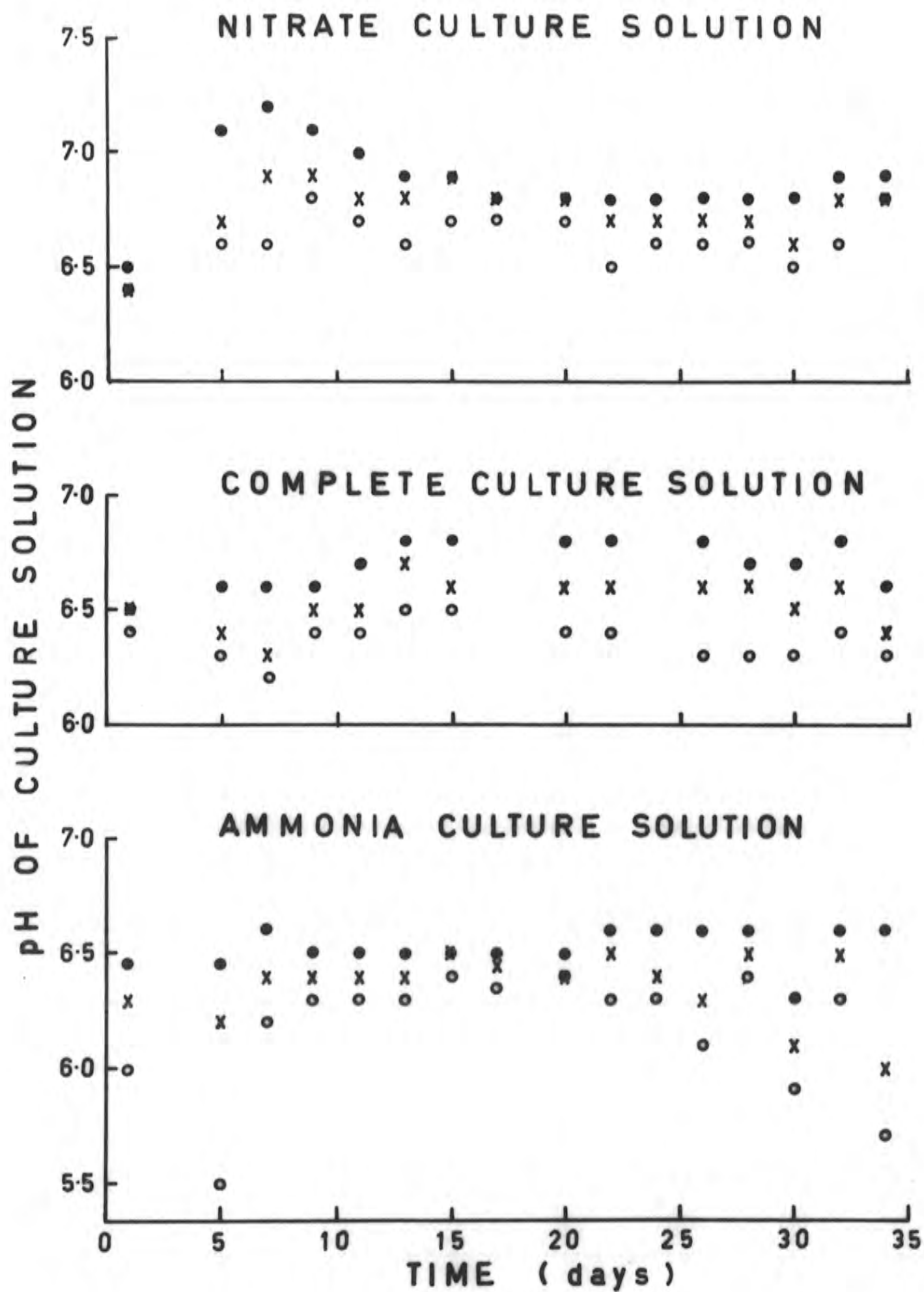
Tomato plants were grown on solutions containing nitrogen as ammonium nitrogen, nitrate nitrogen or a mixture of the two ("Complete" medium of Table 1). The pH of each solution was adjusted to pH 6.5 periodically (Figure 5). The pH values of the ammonia culture solutions were maintained within the range 6.0 - 6.5. According to Tiedgen's data, this would give maximum growth on ammonia culture solution and Woolhouse (1959) had also used this pH range in his experiment. Eight cultures were used for each treatment. During the experiment a few of the plants died possibly due to injury at the time of planting out. Plants were harvested on the 34th day and fresh weights obtained. Leaf laminae of some of the plants were removed for preliminary examination of the leaf proteins (Part V). After drying, the rest of the material was separated into leaf laminae, stems plus petioles, and roots, and dry weights were determined. In order to give enough material for protein nitrogen estimations, material from several plants growing on similar culture solutions was combined. Total protein nitrogen was obtained from each sample by the method of Wood and Sibly (1952), followed by digestion and estimation by the method of Ma and Zuazaga (1942).

Results and Discussion.

Decreases in the pH of the ammonia culture solutions and increases in the pH of the nitrate culture solutions are apparent from Figure 5. The pH of the complete culture solutions changed only slightly during the

FIGURE 5. pH values of culture solutions supplied to tomato plants.

● highest pH value, ○ lowest pH value, x mean pH value of 6 similar culture solutions. Immediately after these determinations were made solutions were adjusted to pH 6.5 with either 0.02M NaOH or 0.02M HCl.



NITRATE CULTURE SOLUTION
COMPLETE CULTURE SOLUTION
AMMONIA CULTURE SOLUTION
pH OF CULTURE SOLUTION
TIME (days)
7·5
7·0
6·5
6·0
5·5
0
5
10
15
20
25
30
35

experiment. Each solution was adjusted to pH 6.5 with 0.02 M sodium hydroxide or 0.02 M hydrochloric acid after these measurements had been made.

From the 20th day onwards the leaves of plants growing on nitrate culture solution could be distinguished from the leaves of plants growing on ammonia culture solution by the darker green colour of the latter. Plants growing on ammonia culture solution produced significantly less fresh and dry material than plants growing on either nitrate or complete culture solution (Table 7). Growth on nitrate culture solution and complete culture solution was not significantly different.

The protein content of the plants can be expressed in two ways; when the results are expressed in terms of concentration (mg protein nitrogen per g dry weight) the protein in the leaf laminae was similar in all plants. However the overall concentration of protein nitrogen was higher in plants grown on ammonia culture solution, as the levels for 1) stem and petioles, and 2) roots were increased in the ammonia grown plants (Table 7).

However when the results are expressed on a per plant basis there was less protein in the ammonia grown plants as these plants were smaller. Comparing the different portions of the plants the results only gave a statistically significant difference for the lamina portions (Table 7).

The smaller plants grown on ammonia culture solution may have contained a lower proportion of lignified cells than the larger plants

TABLE 7

FRESH WEIGHTS, DRY WEIGHTS AND PROTEIN NITROGEN OF TOMATO PLANTS GROWN ON COMPLETE, NITRATE, AND AMMONIA CULTURE SOLUTION

Plants were grown at pH 6.5 and harvested on the 34th day.

Culture Solution	Complete	Nitrate	Ammonia	Degrees of freedom of error mean square
Fresh weight (g/whole plant)	1.96 ± 0.14 (31)	1.57 ± 0.14 (32)	1.10 ± 0.17 (22)	82
Dry Weight				187
Laminae (mg/plant)	50 ± 5 (7)	54 ± 4 (8)	36 ± 5 (7)	
Stems and petioles (mg/plant)	24 ± 2 (31)	21 ± 2 (32)	12 ± 3 (22)	
Roots (mg/plant)	26 ± 2 (31)	24 ± 2 (32)	18 ± 3 (22)	
Protein Nitrogen				16
Laminae (mg N/g dry wt.)	45.9 ± 1.0 (2)	45.9 ± 1.0 (2)	45.2 ± 1.0 (2)	
Laminae (mg N/plant)	2.27 ± 0.16 (2)	2.49 ± 0.16 (2)	1.63 ± 0.16 (2)	
Stems and petioles (mg/g dry wt.)	23.5 ± 0.7 (3)	22.6 ± 0.7 (3)	28.8 ± 1.0 (2)	
Stems and petioles (mg N/plant)	0.60 ± 0.11 (3)	0.49 ± 0.11 (3)	0.34 ± 0.16 (2)	
Roots (mg N/g dry wt.)	28.8 ± 0.7 (3)	25.7 ± 1.0 (2)	31.8 ± 1.0 (2)	
Roots (mg N/plant)	0.78 ± 0.11 (3)	0.58 ± 0.16 (2)	0.60 ± 0.16 (2)	

Figures in brackets beneath the values are the numbers of individual readings on which the means are based.

grown on nitrate or complete culture solution. Support to this suggestion is given by the work of Woolhouse (1959), who found a lower percentage of lignin in shoots and roots of plants grown on ammonia culture solution than in plants grown on nitrate culture solution. This increased concentration of lignin and the subsequent removal of protoplasmic protein from the lignified cells in the plants grown on nitrate culture solution could have decreased the concentration of protein nitrogen within these plants. Thus the finding that there is a higher concentration of protein nitrogen in (1) stems and petioles and (2) roots of plants grown on ammonia culture solution than in the plants grown on the other two solutions may have a similar explanation. Although there was only a small number of analyses made and there was considerable variation in the results, a later experiment, in which plants were grown on nitrate and ammonia culture solutions, showed similar results for stems plus petioles and leaf laminae (Table 8). In this experiment the protein content of the roots was not determined.

The reduced growth of tomato plants grown on ammonia culture solution compared with the growth on nitrate or complete culture solution agrees with results obtained by Woolhouse (1959). In addition it was found in this experiment that the total protein nitrogen per plant was less in plants grown on ammonia culture solution than in plants grown on nitrate or complete culture solution.

TABLE 8

DRY WEIGHTS AND PROTEIN NITROGEN CONTENT OF TOMATO PLANTS GROWN ON NITRATE OR AMMONIUM NITROGEN AT pH 6.5

Each value is the mean of 6.

Culture Solution	Nitrate	Ammonia	L.S.D. at 5% level
Dry weights			
Stems and petioles (mg/plant)	270	130	110
Leaves (mg/plant)	660	400	230
Protein nitrogen			
Stems and petioles (mg N/plant)	4.55	2.77	N.S.
Stems and petioles (mg N/g dry wt.)	17.22	21.40	2.08
Laminae (mg N/plant)	27.52	16.56	9.91
Laminae (mg N/g dry wt.)	41.29	41.14	N.S.

2 THE EFFECT OF pH BETWEEN pH 3.5 AND 8.5 ON THE GROWTH OF TOMATO PLANTS IN WATER CULTURE SUPPLIED WITH EITHER NITRATE OR AMMONIUM NITROGEN

Introduction.

In the previous experiment plants were grown at only one pH range. Pirschle (1931) and Tiedgens (1934) showed that different species of plants have different pH optima when growing on culture solutions in which the nitrogen is supplied, either as the ammonium ion or the nitrate ion. Tomato plants grown in sand cultures in which the pH of the culture solution dripping through did not change by more than 0.2 pH units from that initially supplied, produced maximum growth on ammonium nitrogen at pH 6.0 and on nitrate nitrogen at pH 4.0, the growth being the same in each case (Tiedgens, 1934).

Woolhouse (1959) grew tomato plants on ammonia culture solutions at pH 6,7 and 8 and periodically adjusted the pH of each solution with 0.02 M sodium hydroxide. No data are reported on the changes in pH occurring in the culture solutions during growth, although in an earlier experiment, when pH adjustments were frequently made, he observed a drop in pH from 6.5 to 4.5 on the 12th day. No pH values are recorded for the period from the 12th day to the time of harvest (20th day).

It is possible that, in the experiment in which the initial pH values of the ammonia culture solutions were adjusted to 6, 7 and 8, the pH values may have dropped considerably before being adjusted with sodium

hydroxide. In the following experiment tomato plants were grown on culture solutions at carefully controlled pH values to check the effect of pH on the growth of tomato plants supplied with either nitrate nitrogen or ammonium nitrogen.

Materials and Methods.

Tomato plants were grown on culture solutions containing either nitrate or ammonium nitrogen (Methods, 1). For each culture solution five ranges of pH were used, viz. 3.5-4.5, 4.5-5.5, 5.5-6.5, 6.5-7.5, 7.5-8.5. Ranges of pH rather than specific pH values were used because of the extreme difficulty of keeping a culture solution at any one pH. Three replicate cultures were used for each treatment. pH measurements of the culture solutions were made daily at a glass electrode and adjusted with 0.02 M hydrochloric acid or 0.02 M sodium hydroxide. On the 19th day, the plants were harvested and separated into roots and shoots. The total fresh weight yields of roots and shoots from the four plants in each culture were determined and total dry weights of the four plants in each culture in the pH ranges 5.5-8.5 were obtained.

Results.

pH measurements of the culture solutions are shown in Figures 6 and 7. It is evident that the pH values of the culture solutions remained close to their specific ranges apart from a rise in the ammonia culture solutions (pH 3.5-4.5) from the 5th day to the 9th day owing to a rapid

FIGURE 6. pH values of ammonia culture solutions at 5 pH ranges supplied to tomato plants. ●, ○, X ; pH of solutions in culture vessels 1, 2 and 3 respectively at each pH range. Immediately after these determinations were made the pH of each solution, where necessary, was adjusted with either 0.02M NaOH or 0.02M HCl to maintain the solution within the specified pH range.

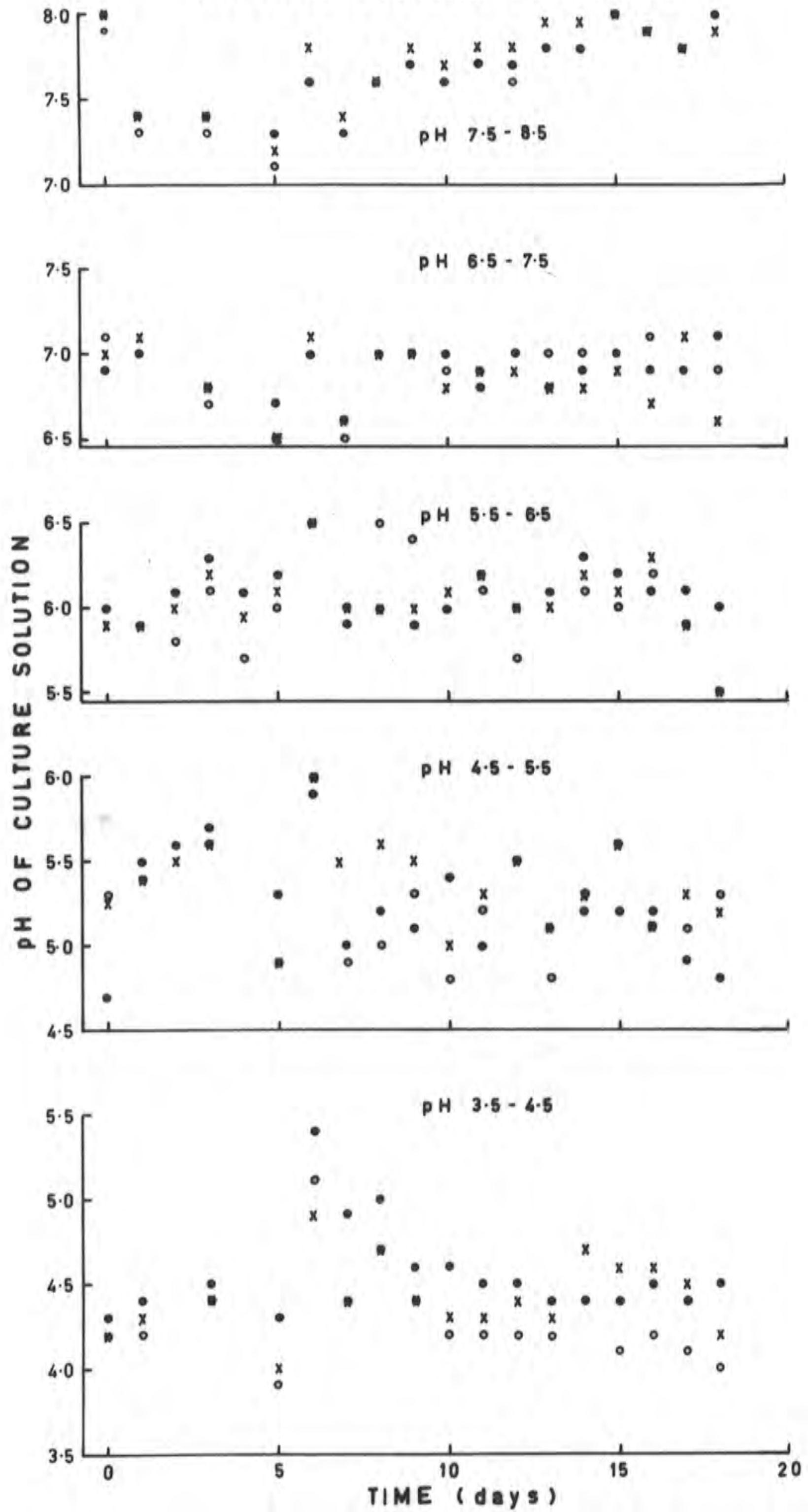
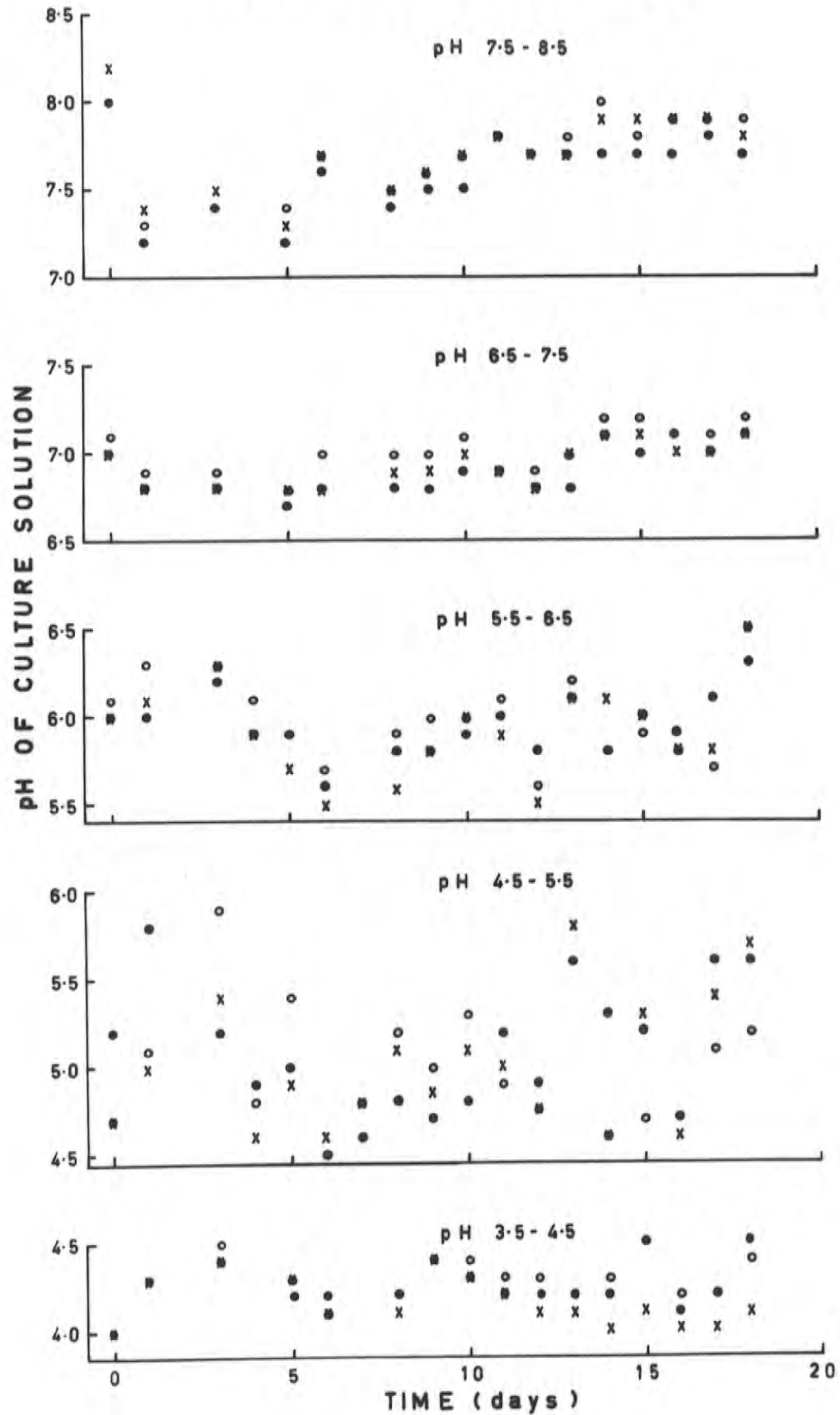

AMMONIA CULTURE SOLUTIONS
pH 7·5 - 8·5
pH 6·5 - 7·5
pH 5·5 - 6·5
pH 4·5 - 5·5
pH 3·5 - 4·5
pH OF CULTURE SOLUTION
TIME (days)
8·0
7·5
7·0
6·5
6·0
5·5
5·0
4·5
4·0
3·5
0
5
10
15
20

FIGURE 7. pH values of nitrate culture solutions at 5 pH ranges supplied to tomato plants. ●, ○, X ; pH of solutions in culture vessels 1, 2 and 3 respectively at each pH range. Immediately after these determinations were made the pH of each solution, where necessary, was adjusted with either 0.02M NaOH or 0.02M HCl to maintain the solution within the specified pH range.


NITRATE CULTURE SOLUTIONS
pH 7·5 - 8·5
pH 6·5 - 7·5
pH 5·5 - 6·5
pH 4·5 - 5·5
pH 3·5 - 4·5
pH OF CULTURE SOLUTION
TIME (days)
8·5
8·0
7·5
7·0
6·5
6·0
5·5
5·0
4·5
4·0
0
5
10
15
20

rise in pH on the addition of 0.02 M sodium hydroxide at this stage (Figure 6).

Mean fresh and dry weight yields from each culture are given in Table 9. Dry weights of plants grown at pH values between 3.5 and 5.5 were not determined. It can be seen that maximum growth on both nitrate and ammonia culture solutions occurred in the pH range 5.5-8.5. It can also be seen that, in no instance, within the pH range tested, did the maximum yield of plants grown on ammonia culture solution equal that of plants grown on nitrate culture solution.

Discussion.

Due to the variation in plant growth within the pH range 3.5-5.5 it was not possible to show a statistical difference between yields of plants grown on different nitrogen sources in this pH range. However the results indicate that under these experimental conditions a nitrate culture solution always gives a higher yield of plant growth than does an ammonia culture solution. The results of this experiment are very different from those of Tiedgens (1934), particularly in relation to the growth on nitrate nitrogen, but these results for the growth on nitrate nitrogen are similar to those reported by Arnon and Johnson (1942). The better growth on nitrate at pH 4.0 obtained by Tiedgens was probably due to greater availability of iron at this pH.

From the results of this experiment it seems likely that the difference in growth rate between plants grown on different solutions is not due to unfavourable pH values of the culture solutions.

TABLE 9

THE EFFECT OF pH AND SOURCE OF NITROGEN ON THE GROWTH OF TOMATO PLANTS

Plants were harvested on the 19th day. Each value is the mean yield of 3 cultures. Each culture contained 4 plants which were weighed together.

Fresh Weights (g / 4 plants)

Culture Solution pH range	Shoots		Roots	
	Nitrate	Ammonia	Nitrate	Ammonia
3.5 - 4.5	2.91	1.12*	1.75	.80*
4.5 - 5.5	5.45*	1.76	3.22*	1.41
5.5 - 6.5	7.02	3.85	3.74	2.56
6.5 - 7.5	8.68	4.68	3.64	2.55
7.5 - 8.5	8.40	3.46	3.54	1.98
L.S.D. at 5% level	1.80		0.69	

Dry Weights (g / 4 plants)
Whole Plants

Culture Solution pH range	Nitrate	Ammonia
5.5 - 6.5	0.58	0.37
6.5 - 7.5	0.68	0.42
7.5 - 8.5	0.66	0.35
L.S.D. at 5% level	0.20	

* These values were omitted from the analysis of variance owing to marked variation between the three cultures.

3 THE GROWTH OF TOMATO PLANTS SUPPLIED WITH DIFFERENT FORMS OF NITROGEN IN OTHERWISE SIMILAR SOLUTIONS

Introduction.

Although the solutions used in the previous experiments had equal concentrations of most ions, chloride and sodium ions were present in strikingly different concentrations. Takahashi and Yoshida (1957b) report that high levels of chloride in the presence of ammonium nitrogen cause malnutrition of tobacco plants in sand culture. Also Harward et al. (1956) found a high chloride to sulphate ratio detrimental to the growth of potato plants especially in the presence of ammonium ions. During the latter days of their growth experiment, the pH of the culture solution containing ammonium nitrogen, which was dripping through the sand cultures, had dropped to 3.9. This low pH may have contributed to their results.

A new culture solution which provided equal concentrations of chloride ions when supplying different forms of nitrogen, was made up as shown in Table 10. Nitrogen was supplied either as ammonium hydroxide or sodium nitrate. The ratio of chloride to sulphate in this solution is approximately equal whereas in the solutions used previously it had been 17:1 in the ammonia culture solution and 4:1 in the nitrate culture solution.

Theoretically it would have been desirable to add the nitrate as nitric acid but this proved impossible in practice because it lowered the

TABLE 10

CULTURE SOLUTION WITHOUT NITROGEN

Nitrogen was supplied either as sodium nitrate or ammonium hydroxide. Micronutrients were supplied at the same concentrations as described above (see TABLE 1)

Solution A

	($M \times 10^{-4}$)
K_2SO_4	10
$Ca\ Cl_2$	6
$Mg\ SO_4$	4
NaH_2PO_4	4

pH of the culture solution, originally pH 5.5, to about pH 4.5. There have been no reports in the literature of any beneficial effect on the growth of plants when the sodium level in culture solutions was increased above the concentrations present in the above solutions. Therefore it would seem very unlikely that the higher level of sodium in the solution containing sodium nitrate would allow plants to produce more fresh and dry weight than plants grown on solutions containing ammonium hydroxide.

Materials and Methods.

Sixteen cultures were set up. Four cultures were supplied with nitrate culture solution and four with ammonia culture solution. The pH of each of these solutions was maintained in the pH range 6.0-7.5 with sodium hydroxide or hydrochloric acid. The remaining eight cultures contained the solution given in Table 10. Four of these received nitrogen as sodium nitrate and four as ammonium hydroxide. It was found that 2 ml of 0.2 M ammonium hydroxide, containing 5.6 mg of nitrogen, added to two litres of culture solution raised the pH of this solution to about 7.5. The pH of each of these four solutions was checked daily and 2 ml of ammonium hydroxide were added on the 1st, 3rd, 6th, 9th, 12th, 14th, 16th, 18th and 19th days and 4 ml on the 17th day to keep the solutions within the pH range 6-7.5. On the 16th day it was found necessary to add 4 ml of ammonium hydroxide to one culture as the plants in this culture were slightly larger than those on the other three cultures.

At the same time, after adjusting the other four culture solutions to pH 6.5 with sodium hydroxide, 0.2 M sodium nitrate was added equivalent

to the additions of ammonium hydroxide except that on the 16th day 4 ml of sodium nitrate were added to each solution. This extra nitrogen was supplied to these plants because by this time they were larger than the plants supplied with ammonium hydroxide. A total of 61.6 mg of nitrogen as ammonium hydroxide was added to each of three of the cultures, 67.2 mg as ammonium hydroxide to one culture and 67.2 mg of nitrogen as sodium nitrate to each of the other four cultures. On the 20th day all plants from the 16 cultures were harvested and the four plants from each culture were weighed together.

Results and Discussion.

Mean fresh weight yields, which are given in Table 11, show that tomato plants grown on both sources of ammonium nitrogen produced less growth than plants grown on both sources of nitrate nitrogen. Altering the chloride to sulphate ratio had no effect on the growth rate of plants on either nitrate nitrogen or ammonium nitrogen. On the 14th day, plants growing on ammonium nitrogen were visibly smaller than plants growing on nitrate nitrogen. Because of this difference in growth rate 5.6 mg more nitrogen was supplied as sodium nitrate than as ammonium hydroxide on the 16th day.

It is most unlikely that nitrogen was limiting in the solutions supplied with ammonium hydroxide for the following reasons. Firstly, the pH of the solution before addition of ammonium hydroxide was about 5.5. Ammonium hydroxide was added to bring the pH to 7.5 and from then on the

TABLE 11

FRESH WEIGHTS OF TOMATO PLANTS GROWN ON DIFFERENT NITROGEN SOURCES IN THE pH RANGE 6.0-7.5

Plants were harvested on the 20th day and each value is the mean of 4 cultures.

Culture Solution	Nitrate	A + $NaNO_3$	Ammonia	A + NH_4OH
Fresh Wt. (g / 4 pl.)	16.28	15.45	12.13	11.35
L.S.D. at 5% level	2.92			

pH was not allowed to fall below 6.0. It is unlikely that the pH of the solution would have risen from 5.5 to 6.0 for any other reason than the presence of the ammonium ions during the experiment. Thus there was probably always some ammonium nitrogen present. This was confirmed by testing the solution with Nesslers' solution when the pH had dropped to pH 6.0. Ammonium nitrogen was found to be present.

Secondly preliminary investigations showed that the amount of nitrogen present in a whole young tomato plant is less than 0.4 per cent. of the fresh weight. Thus 61.6 mg of nitrogen, as supplied in this experiment, would allow the growth of at least 15 g fresh weight of this type of plant per culture. However the mean fresh weight of plants supplied with ammonium hydroxide was only about 12 g.

The 67.2 mg of nitrate nitrogen supplied would have allowed the growth of 17 g fresh weight of plant material. The actual mean fresh weight was less than 16 g.

In Part III, 2, it was shown that tomato plants grow equally well on ammonium culture solution at pH ranges between pH 5.5 and 8.5. Therefore maintaining the pH of the culture solutions in the present experiment within the range pH 6.0-7.5 provided the plants with conditions most favourable for growth.

It would appear from this experiment that the reduced growth rate of tomato plants on the ammonia culture solution compared with the growth rate on nitrate culture solution is due to the presence of the nitrogen as

the ammonium ion rather than to any difference in the concentrations of the other ions present in the culture solutions.

4 THE GROWTH OF TOMATO PLANTS ON NITRATE NITROGEN AND ON THREE CONCENTRATIONS OF AMMONIUM NITROGEN

Introduction.

In the earlier experiments, the concentration of ammonium nitrogen supplied in the culture solutions was initially 3.6×10^{-3}M except in Part III, 3, where the concentration was 2×10^{-4}M. Tiedgens (1934) supplied tomato plants with culture solution containing ammonium nitrogen at a concentration of 2.8×10^{-3}M and Clark (1936) used ammonium nitrogen concentrations of 8.2×10^{-3}M and 2.8×10^{-3}M. Woolhouse (1959) grew tomato plants at three concentrations of ammonium nitrogen (4×10^{-3}M, 4×10^{-4}M and 4×10^{-5}M). From his data it can be calculated that the maximum amount of nitrogen supplied to the plants, which were growing on the lowest concentration of nitrogen (4×10^{-5}M), would have been 13 mg. Normally young tomato plants contain at least 3 per cent. of nitrogen by dry weight when growing on nitrate and more than this when growing on ammonium nitrogen. However from the amount of nitrogen supplied and the amount of growth produced, the plants would have only contained about 2 per cent. nitrogen. Consequently under these conditions the reduced growth he observed compared to nitrate nitrogen grown plants could have

arisen from a nitrogen deficiency rather than any specific effect of the ammonium nitrogen supply. Also the phosphate content of the culture solution containing the highest concentration of ammonium nitrogen (4×10^{-3}M) was ten times higher than that of all the other solutions.

For these two reasons it was decided to study the effect of varying the concentration of ammonium nitrogen in culture solutions in which ample nitrogen was supplied and macroelement concentrations were kept similar in all solutions.

Materials and Methods.

Tomato plants were grown in culture solutions containing ammonium nitrogen at three levels (3.6×10^{-3}, 7.2×10^{-4}, and 4.0×10^{-4}M) and on culture solutions containing nitrate nitrogen (3.6×10^{-3}M). The composition of the culture solutions is shown in Table 12. There were six cultures in each treatment. The nitrate culture solutions were changed on the 4th, 8th, 12th and 16th days and the ammonia culture solutions changed on the 4th, 8th, 11th, 13th, 15th and 17th days. The pH of each solution was adjusted to pH 6.5 with sodium hydroxide. Plants were harvested on the 18th day and the four plants from each culture were weighed together. Plants from three of the cultures from each treatment were dried and reweighed. Protein nitrogen estimations were carried out on the leaf laminae by the method described in Part III, 1. The method and results of determinations of amino acids from the plants of the other cultures is described elsewhere (see Part II and Part V).

TABLE 12

COMPOSITION OF CULTURE SOLUTIONS

The pH of each solution was adjusted to pH 6.5 with NaOH. Microelements supplied as described above (see TABLE 1).

Nitrate Culture Solution
($M \times 10^{-4}$)

KNO_3	20
$Ca(NO_3)_2$	6
$Mg\ SO_4$	4
NaH_2PO_4	4
NaCl	16
$NaNO_3$	4

Ammonia culture solutions
($M \times 10^{-4}$)

KCl	20	20	20
$CaCl_2$	6	6	6
$MgSO_4$	4	4	4
$NH_4H_2PO_4$	4	4	4
NaCl	-	28.8	32
NH_4Cl	32	3.2	-

Results and Discussion.

On the 10th day the plants growing on all concentrations of ammonium nitrogen were visibly smaller than plants growing on nitrate culture solution. When harvested the fresh and dry weight yields of plants grown on all concentrations of ammonium nitrogen were statistically less than the yields of plants grown on nitrate nitrogen (Table 13). As in Part III (Tables 7 and 8) protein nitrogen values for leaf laminae were lower in plants grown on ammonium nitrogen than in plants growing on nitrate nitrogen. Expressed on a dry weight basis the protein nitrogen of plants grown on the two lower levels of ammonium nitrogen was just statistically lower than in plants grown on nitrate or 3.6×10^{-3}M ammonium nitrogen. No explanation can be offered for this finding.

As each beaker contained approximately 2.3 litres of culture solution, the amount of nitrogen supplied to the plants growing on the lowest concentration of ammonium nitrogen was about 90 mg over the period of the experiment. As preliminary investigations showed that the nitrogen in similar plants made up to 4 to 5 per cent. of the dry weight, 90 mg of nitrogen would be sufficient for the production of about 2 g dry weight of plant material. Although all the nitrogen may not have been utilized between changes of culture solutions, it is unlikely that nitrogen was limiting as the maximum amount of dry weight produced on nitrate nitrogen was only one gram.

The results of this experiment and the experiment described in Part III, 3, suggest that the growth of tomato plants supplied with ammonium

TABLE 13

THE EFFECT OF AMMONIUM ION CONCENTRATION IN CULTURE SOLUTIONS ON THE GROWTH OF TOMATO PLANTS

Fresh and dry weight yields of plants and protein N of leaf laminae on the 18th day. Each weight is the mean weight of plants from 6 cultures for fresh weight and 3 cultures for dry weight. Each protein nitrogen value is the mean of 3.

Culture Solution	Nitrate	Ammonia			L.S.D. at 5% level
	3.6×10^{-3}M	3.6×10^{-3}M	7.2×10^{-4}M	4×10^{-4}M	
Fresh Weights (g/4 plants)	15.19	8.42	8.55	9.55	3.00
Dry Weights (g/4 plants)	1.01	0.63	0.59	0.74	0.23
Protein Nitrogen of leaf laminae					
(mg N/4 plants)	26.26	16.08	14.48	17.99	6.26
(mg N/g dry wt.)	49.4	49.2	45.5	45.5	3.3

nitrogen is not affected by the concentration of ammonium nitrogen between concentrations of 3.6×10^{-3}M and 2×10^{-4}M. These concentrations of ammonium nitrogen may still be too high for maximum growth of tomato plants but if the concentrations were to be lowered still further the problem of an insufficient nitrogen supply would be encountered.

5 THE EFFECT OF DIFFERENT PROPORTIONS OF NITRATE AND AMMONIUM NITROGEN IN CULTURE SOLUTIONS ON THE GROWTH OF TOMATO PLANTS

Introduction.

In Part III, 1, tomato plants grown on complete culture solution containing both nitrate and ammonium nitrogen, produced as much dry weight as plants grown on culture solution containing nitrate nitrogen alone (Table 7). In Part III, 4, plants grown on the same concentration of ammonium nitrogen as in the complete solution in Part III, 1, but without nitrate present did not produce as much dry weight as plants grown on nitrate alone (Table 13). However in Part III, 1, the plants may have taken up the ammonium nitrogen in the first few days and from then on the plants would have been growing on a solution containing only nitrate nitrogen. Woolhouse (1959) added ammonium chloride to the complete culture solution at two day intervals to maintain the supply of ammonium nitrogen to plants growing on it. These plants still produced as much growth as plants growing on nitrate nitrogen alone.

It was decided to determine whether similar results to those of Woolhouse could be obtained by growing tomato plants on complete culture solution and changing the solution at frequent intervals to ensure a continuous supply of ammonium nitrogen rather than by adding ammonium chloride. In addition the effect on the growth of tomato plants of increasing the concentration of ammonium nitrogen, in the presence of nitrate nitrogen, above the level present in the complete culture solution was examined.

Materials and Method.

Tomato seedlings were planted out on ammonia and complete culture solutions. Some seedlings were supplied with a new solution which contained equal quantities of nitrate and ammonium ions (Table 14). Half of the cultures containing complete culture solution were changed on the 5th, 9th, 12th and 14th days. It was necessary to change the solutions in the other complete solution cultures on the 14th day, as the nutrients were being taken up so rapidly by the plants. This experiment was carried out during the summer months when the growth of the plants was very rapid. The solutions containing equal concentrations of nitrate and ammonium nitrogen were changed on the 12th day after tests with Nessler's solution had shown that only a low level of ammonium nitrogen remained. The pH values of all solutions were checked twice daily and adjusted with 0.02 M sodium hydroxide to maintain a pH between 6 and 7. There were 3 cultures in each treatment. Plants were harvested on the 16th day and the four plants from each culture were weighed together.

TABLE 14

COMPOSITION OF CULTURE SOLUTION IN WHICH NITRATE AND AMMONIUM NITROGEN ARE PRESENT IN EQUAL CONCENTRATIONS

Microelements supplied as in Table 1

	($M \times 10^{-4}$)
KNO_3	20
$CaCl_2$	6
$MgSO_4$	4
$NH_4H_2PO_4$	4
NH_4Cl	16

Results and Discussion.

In the later days of the experiment, some of the culture solutions, especially those containing only ammonium nitrogen, dropped from pH 7 to a pH slightly below 6 during the 15 hours between additions of sodium hydroxide. From the results in Table 9 it seems unlikely that this drop in pH would have had any depressing effect on the growth of the plants.

From Table 15 it can be seen that plants grown on all solutions in which nitrate was supplied were larger than plants grown on solutions in which only ammonium nitrogen was supplied. These results are in agreement with Woolhouse (1959). Plants which were grown on culture solution containing 2×10^{-3}M ammonium nitrogen in the presence of nitrate still produced more fresh weight than plants growing on ammonium nitrogen alone. Thus the growth rate of tomato plants is not decreased by the presence of ammonium nitrogen but rather by the absence of nitrate nitrogen in the culture solution.

6 THE EFFECT ON THE GROWTH OF TOMATO PLANTS OF ADDING SMALL AMOUNTS OF NITRATE TO AMMONIA CULTURE SOLUTIONS

Introduction.

The previous experiments have not established the minimum concentration of nitrate in the presence of ammonium nitrogen needed to produce growth equivalent to that observed on nitrate alone. In all cases so far

TABLE 15

THE GROWTH OF TOMATO PLANTS ON CULTURE SOLUTIONS CONTAINING AMMONIUM NITROGEN OR MIXTURES OF NITRATE AND AMMONIUM NITROGEN

Fresh weights of plants harvested on the 15th day. Each value is the mean of 3.

Culture solution	Ammonia	Complete	Complete (changed)	$\frac{1}{2}NO_3:\frac{1}{2}NH_3$
Fresh weight (g/4 plants)	11.00	21.84	21.92	20.60
L.S.D. at 5% level		8.44		

described, nitrate has been present at relatively high concentrations. There is a possibility that nitrate may cause the synthesis of an enzyme or enzymes which allows maximum growth of plants supplied with nitrate. Evans and Nason (1953) detected no difference in nitrate reductase activity in soy-bean plants grown on nitrate or ammonium nitrogen. Candela _et al._ (1957) however found less nitrate reductase activity in cauliflower plants growing on ammonium nitrogen than on nitrate nitrogen. Also this enzyme has been shown to be present only when nitrate has been supplied to rice seedlings (Tang and Wu, 1957) and to excised embryos of _Capsella_ and wheat (Rijven, 1958). Nason and Evans (1953) convincingly demonstrated the adaptive formation of nitrate reductase in _Neurospora crassa_ in the presence of nitrate and nitrite. This was truly adaptive and not caused by inhibition by ammonium nitrogen as ammonium nitrate produced the same effect as nitrate. The lowest concentration of nitrate present was probably about 0.01 M. Kinskey and McElroy (1958) found not only the complete absence of nitrate reductase in ammonia grown felts of _Neurospora crassa_ but also that the TPNH - cytochrome C reductase activity was only 20 per cent. of that developed in nitrate grown felts. It was decided to test the effect on the growth of tomato plants of small amounts of nitrate in culture solutions containing mainly ammonium nitrogen. Concentrations of nitrate much lower than those supplied by Nason and Evans to _Neurospora crassa_ had to be used so that the plants would still be assimilating mainly ammonium nitrogen.

Materials and Methods.

Tomato plants were grown on solutions containing nitrate nitrogen, and on solutions containing ammonium nitrogen at two concentrations, viz. 3.6×10^{-3}M and 7.2×10^{-4}M. The solutions used were those described in Table 12. All solutions were changed on the 6th, 11th, 15th and 19th days so that nitrogen would not be limiting in the solutions containing 7.2×10^{-4}M ammonium nitrogen. On the 1st, 4th, 6th, 8th, 11th, 15th, 18th, 19th and 20th days 9.2 ml of one millimolar sodium nitrate were added to 2 sets of 3 cultures containing ammonium nitrogen at concentrations of 3.6×10^{-3}M and 7.2×10^{-4}M and 18.4 ml of one millimolar sodium nitrate were added to 2 similar sets of cultures. A further 3 cultures contained only nitrate nitrogen, 3 cultures contained 3.6×10^{-3}M ammonium nitrogen and 3 cultures contained 7.2×10^{-4}M ammonium nitrogen, the ammonium solutions containing no nitrate. The solutions containing ammonium nitrogen were adjusted daily to pH 6.5 with 0.02 M sodium hydroxide. Plants were harvested on the 20th day, and when dried, the 4 plants from each culture were weighed together.

Results and Discussion.

The results for the dry weight determinations given in Table 16 show that plants grown on nitrate nitrogen yielded much higher values than those grown on ammonia nitrogen. Plants which were supplied with small amounts of nitrate in the presence of ammonium nitrogen did not grow as well as plants grown on nitrate alone. The lower dry weight values for plants

TABLE 16

THE GROWTH OF TOMATO PLANTS ON AMMONIA CULTURE SOLUTION CONTAINING SMALL AMOUNTS OF SODIUM NITRATE

Plants were harvested on the 20th day. Each value is the mean of 3.

Culture Solution.	Nitrate nitrogen added	Dry weight (g/4 plants)
Nitrate		2.70
3.6×10^{-3}M Ammonium N	-	0.57
3.6×10^{-3}M Ammonium N	1.29 mg	0.72
3.6×10^{-3}M Ammonium N	2.58 mg	0.84
7.2×10^{-4}M Ammonium N	-	0.81
7.2×10^{-4}M Ammonium N	1.29 mg	0.78
7.2×10^{-4}M Ammonium N	2.58 mg	0.83
L.S.D. at 5% level		0.24

grown on 3.6×10^{-3}M ammonium nitrogen without nitrate cannot be explained. There was no increase in the dry weights of plants grown on ammonium nitrogen and supplied with nitrate nitrogen over those found for plants grown on 7.2×10^{-4}M ammonium nitrogen without nitrate nitrogen added. The amounts of nitrate added in this experiment may have been too small to stimulate the synthesis of enzymes. However, if larger amounts had been added and had increased growth, the effect could have been due to the plants using the nitrate nitrogen rather than ammonium nitrogen for the synthesis of nitrogen compounds within the plant. It seemed more profitable at this stage of the investigation to study the uptake of ammonium nitrogen and its incorporation into organic compounds rather than continue indirect studies on adaptive enzyme formation.

Summary.

The experiments so far described have established that, in agreement with Woolhouse (1959), tomato plants grown in water culture at pH 6.5 and supplied with nitrogen in the form of nitrate show more growth than if, under the same conditions of pH, nitrogen is supplied as the ammonium ion. This difference in growth is expressed in fresh and dry weights of the plants and in the total protein of each plant. Further, as is shown by Part III, 2, this difference in the growth pattern is not due to unfavourable pH values of the culture solutions. The results of Part III, 3, show that this decreased growth with ammonium nitrogen was not caused by differences in concentrations of elements other than nitrogen in the

culture solutions. Neither lowering the concentration of ammonium nitrogen in the ammonia culture solution in Part III, 4, nor adding traces of nitrate to the ammonia culture solution in Part III, 6, enhanced the growth of tomato plants on these solutions. However, plants grown on culture solutions containing equal concentrations of nitrate and ammonium nitrogen (2×10^{-3}M) in Part III, 5, produced as much growth as plants grown on nitrate culture solution, even though the ammonium nitrogen concentration in this case was greater than had been used in some of the solutions in Part III, 4.

These experiments provide strong support for the statement (Woolhouse, 1959) that it is the absence of nitrate from the culture media rather than the presence of ammonium ions which affects the normal growth pattern of tomato plants.

PART IV

THE UPTAKE AND ASSIMILATION OF NITROGEN BY TOMATO PLANTS

Introduction.

The observation that the presence of nitrate in culture solutions containing ammonium nitrogen increases the growth rate above that of plants grown on culture solutions containing only ammonium nitrogen, may be explained in at least three ways. Firstly, it has been shown that ammonium ions in the absence of nitrate ions depress the uptake of potassium by excised roots (Jackson and Coleman, 1959) and the uptake of other cations by whole plants (Sideris et al., 1943; Sideris and Young, 1946; Wander and Sites, 1956; Wallace and Ashcroft, 1956; Smith, 1957). In the presence of nitrate ions, Wallace and Ashcroft (1956) and Smith (1957) found that the depressing effect of the ammonium ions was of the same magnitude as that when nitrate ions were absent, whereas Wander and Sites (1956) found it to be less. The greatest concentrations of cations in the plants were obtained when only nitrate nitrogen was supplied to the plants. Clark (1936) found a reduction in other cations in tomato plants growing on culture solutions containing ammonium ions without nitrate present as compared with that of plants growing on only nitrate nitrogen. It may be that growth of tomato plants in ammonium nitrogen culture solutions is restricted by a limited supply within the plants of one or more cations essential for normal plant growth. The bulk of the evidence

suggests that the concentrations of cations would be low in the presence or absence of nitrate as long as the ammonium nitrogen concentrations were equal in each case. However, plants grown on a mixture of nitrate and ammonium nitrogen produce as much dry weight as plants grown on nitrate alone (Tables 7 and 15). Unless the presence of nitrate reduced the antagonising effect of the ammonium ion on the uptake of other cations, the level of cations within the plant tissues should be the same in the presence of ammonium ions irrespective of the presence or absence of nitrate, and consequently it seems unlikely that the reduced plant growth occurring on ammonium nitrogen cultures results from a deficiency of another cation.

Secondly, although it has been shown in Part III that it is the absence of nitrate rather than the presence of ammonium nitrogen in the culture solution which restricts the growth of tomato plants grown on ammonium nitrogen, there is no evidence to show that it is the absence of nitrate within the plant tissues which limits growth. The uptake of ammonium nitrogen and the levels of ammonium nitrogen present in tissues of plants grown on ammonia culture solution or nitrate plus ammonia culture solution were not determined. If ammonium nitrogen is taken up in large quantities it may be toxic to the plants. Weismann(1950) has shown that four day old wheat seedlings grown in the dark take up less ammonium nitrogen from solutions containing nitrate and ammonium nitrogen than from solutions containing only ammonium nitrogen. Tomato plants may take up less ammonium nitrogen from a culture solution containing

nitrate and ammonium nitrogen than from a solution containing only ammonium nitrogen, even though the concentration of ammonium nitrogen is the same in each case. If this is so, then the smaller amounts of ammonium nitrogen taken up by the plants may not be toxic at these levels and thus allow comparable growth to plants growing without ammonium nitrogen present.

Thirdly, if ammonium nitrogen, or compounds into which ammonium nitrogen is incorporated in the roots, cannot be translocated as rapidly as nitrate nitrogen then it may be that a deficiency of nitrogen is limiting growth in the shoots.

The experiments described below were carried out to examine these three different hypotheses.

1 THE UPTAKE OF NITROGEN BY TOMATO PLANTS FROM CULTURE SOLUTIONS CONTAINING EITHER NITRATE, AMMONIUM OR NITRATE AND AMMONIUM NITROGEN

Introduction.

It was decided to examine the uptake of ammonium nitrogen in the presence and absence of nitrate.

There are three ways in which the uptake of nitrogen by plants from a culture solution can be measured; firstly by analysing the plants for nitrogen, secondly by the use of isotopic nitrogen, and thirdly by

analysing the culture solution and subtracting the residual nitrogen from that originally present. The first method would give total nitrogen taken up by the plants but not the relative assimilation of nitrate and ammonium nitrogen from a mixed culture solution. By supplying the plants with either ^{15}N labelled nitrate or ammonium nitrogen and analysing the plant material for isotopic nitrogen, it would be possible to calculate the amount of either nitrate or ammonium nitrogen taken up by the plants from a solution containing both nitrate and ammonium nitrogen. Using the third method it would be possible to determine the amounts of both nitrate and ammonium nitrogen taken up from a mixed solution. This method, employed by Clark and Shive (1934), Weismann(1950) and Wander and Sites (1956), seemed most convenient for this experiment. However Woolhouse (1959) reported the loss of up to 70 per cent. of the ammonium nitrogen from a culture solution at pH 6.5 aerated in a glasshouse for 48 hours. Therefore the possible loss of ammonium nitrogen from culture solutions under the present experimental conditions was checked. Two culture vessels containing ammonia culture solution at pH 6.5, and two containing solution at pH 6.5 with equal concentrations of nitrate and ammonium nitrogen, were placed in the glasshouse and aerated for 9 days. Over this period there was no measurable loss of ammonium nitrogen and the pH of the solutions remained in the pH range 6.45-6.55. The results of this test demonstrated that analysis of culture solutions would give reliable values for the uptake of ammonium nitrogen.

Materials and Methods.

Tomato plants were grown in 30 culture vessels containing nitrate culture solution (Methods, 1). Treatments were not applied immediately as the low uptake of ammonium nitrogen by plants of this size caused no measurable change in the concentration of nitrogen in the culture solution. On the 10th day, 12 of the culture solutions were replaced with 3.6×10^{-3}M ammonia culture solution, 6 with 1.8×10^{-3}M ammonia culture solution, 6 with culture solution containing equal concentrations (1.8×10^{-3}M) of nitrate and ammonium nitrogen, and the remaining 6 with fresh nitrate culture solution (Table 17). At the same time 6 control culture vessels without plants were set up. Three contained 3.6×10^{-3}M ammonia culture solution and three contained nitrate culture solution. These control solutions were adjusted to pH 6.5 with 0.02M sodium hydroxide and continuously aerated. On the 14th day the culture solutions were renewed in all cultures except that 6 of the 12 3.6×10^{-3}M ammonia culture solutions were replaced with culture solution containing equal concentrations (1.8×10^{-3}M) of nitrate and ammonium nitrogen. The culture solutions were maintained in the pH range 5.5-6.5 by the daily additions, when necessary, of 0.02M sodium hydroxide or 0.02M hydrochloric acid. Plants were harvested on the 17th day, divided into roots and shoots, and the total fresh and dry weights of roots and shoots of the 4 plants from each culture were determined.

After the harvest the ammonia culture solutions and the nitrate culture solutions on which the plants had been growing were adjusted

TABLE 17

COMPOSITION OF CULTURE SOLUTIONS

Each solution was adjusted to pH 6.5 with 0.02M NaOH. Microelements were supplied as above (TABLE 1).

Culture Solution	Nitrate	Ammonia		Nitrate plus Ammonia
		3.6×10^{-3}M	1.8×10^{-3}M	
Salt	Concentration of salt ($M \times 10^{-4}$)			
KNO_3	20	-	-	18
KCl	-	20	20	2
$Ca(NO_3)_2$	6	-	-	-
$CaCl_2$	-	6	6	6
$MgSO_4$	4	4	4	4
$NH_4H_2PO_4$	-	4	4	4
NaH_2PO_4	4	-	-	-
$NaNO_3$	4	-	-	-
NaCl	16	-	-	-
NH_4Cl	-	32	14	14

accurately to pH 6.5. These solutions and the control solutions were aerated for a further seven days.

Before setting up the experiment, marks were made on all the culture vessels to measure 2.2 litres volume of solution. Before taking samples for estimations of ammonium nitrogen the culture solution was brought to this level with water to compensate for the water taken up by the plants. The error incurred in this method of measuring volume was found to be $\pm$ 1 per cent. Samples (1 ml) were taken from each solution at intervals during the experiment and analysed for ammonium nitrogen (Methods, 2). On the 17th day nitrate nitrogen was estimated in the solutions (Methods, 2).

Results.

The concentration of ammonium nitrogen in the control solutions remained constant throughout the 14 days. The solutions on which plants had been growing did not lose any ammonium nitrogen once the plants had been removed. No ammonium nitrogen could be found in the control nitrate culture solutions and the concentrations of nitrate nitrogen remained constant throughout. No change in pH of the control solutions was found during the experiment.

The fresh and dry weights of plants grown on nitrate culture solution and on culture solution containing equal quantities of nitrate and ammonium nitrogen were significantly higher than those of plants grown on both concentrations of ammonia culture solution. The weights of plants grown on both concentrations of ammonium nitrogen were similar. Plants grown on

ammonia culture solution and then transferred to nitrate plus ammonia culture solution were slightly but not significantly larger than plants grown on ammonia culture solution (Table 18).

Figure 8 and Table 19 give the uptake of ammonia nitrogen by four plants from each culture solution. After 44 hours the uptake by plants of ammonium nitrogen from culture solutions containing equal concentrations of nitrate and ammonium nitrogen was significantly less than that of plants growing on ammonia culture solution. This difference remained from this time on and was large at the time of harvest in spite of the fact that plants grown on ammonia culture solution were smaller than those grown on the nitrate plus ammonia culture solution.

The differences between the values for uptake of ammonium nitrogen by plants from the solutions containing only ammonium nitrogen at concentrations of $1.8 \times 10^{-3}M$ or $3.6 \times 10^{-3}M$ were not significant.

The uptake of ammonium nitrogen by plants originally on $3.6 \times 10^{-3}M$ ammonia culture solution and then transferred to a culture solution containing both nitrate and ammonium nitrogen, rapidly fell below that of plants remaining on $3.6 \times 10^{-3}M$ ammonia culture solution.

Table 20 shows the uptake of nitrogen by the plants during the last 66 hours of the experiment. Results for nitrogen uptake when expressed on a dry weight basis show that the uptake was determined mainly by the size of the plants.

TABLE 18

FRESH AND DRY WEIGHTS OF TOMATO PLANTS GROWN ON DIFFERENT SOURCES OF NITROGEN

Plants were grown at pH 6.5 and harvested on the 17th day. Each value is the mean of 6.

Culture solution	Fresh Weight (g/4 plants)		Dry Weight (g/4 plants)	
	Shoots	Roots	Shoots	Roots
Nitrate (3.6×10^{-3}M)	25.98	12.95	1.79	0.50
Ammonia (3.6×10^{-3}M)	16.12	6.52	1.29	0.34
Ammonia (1.8×10^{-3}M)	17.28	6.75	1.35	0.39
Ammonia then nitrate plus ammonia	21.05	9.55	1.42	0.39
Nitrate plus ammonia	28.00	12.38	1.84	0.49
L.S.D. at 5% level	3.10	1.52	0.21	0.05

TABLE 19

UPTAKE OF AMMONIUM NITROGEN BY TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Figures are the mean values in mg for the decrease in ammonium nitrogen from 6 culture solutions.

Culture solution	Ammonia		Ammonia then	Nitrate plus	L.S.D. at 5%
	3.6×10^{-3}M	1.8×10^{-3}M	NO_3 plus NH_3	Ammonia	level
Time (hr)					
27	3.5	5.1	1.7	2.6	N.S.
44	9.1	7.7	6.1	2.6	2.4
51	11.5	12.6	8.0	7.1	2.4
68	15.4	18.1	11.0	9.8	2.4
75	18.5	21.8	16.0	13.2	3.2
94	30.3	31.4	26.0	20.4	5.3
Solutions changed					
117	48.1	53.5	40.2	36.9	6.9
123	54.9	58.5	42.8	39.2	5.7
141	66.2	70.6	48.1	48.8	5.7
164	77.0	82.6	53.8	56.8	6.4

FIGURE 8. Uptake of ammonium nitrogen by young tomato plants from

● culture solution containing 3.6×10^{-3}M NH_3-N

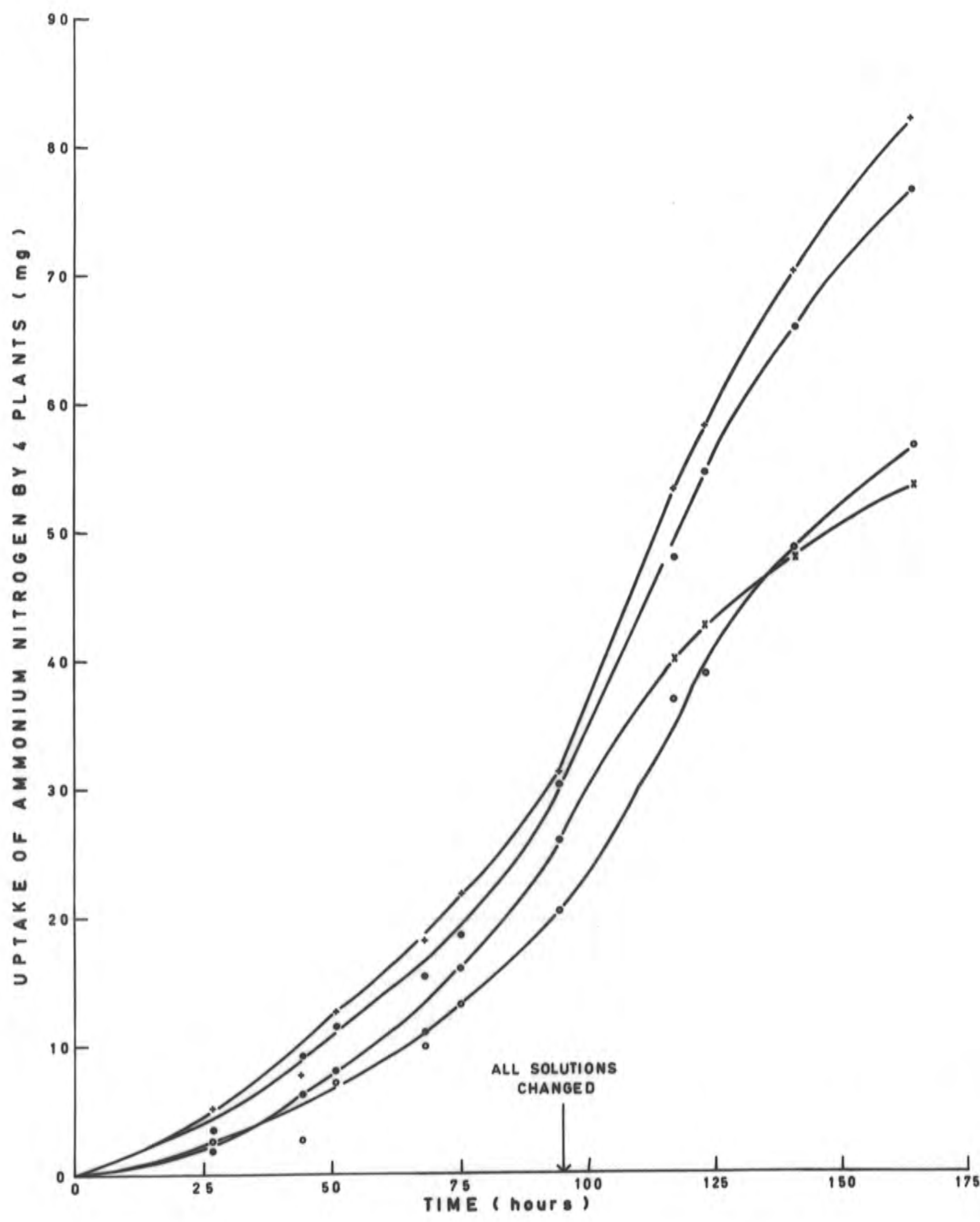
+ culture solution containing 1.8×10^{-3}M NH_3-N

o culture solution containing 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N

●-X culture solution containing 3.6×10^{-3}M NH_3-N initially followed by 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N

All plants had previously been growing on nitrate culture solution.

Each value is the mean of determinations on 6 culture solutions.


UPTAKE OF AMMONIUM NITROGEN BY 4 PLANTS (mg)
TIME (hours)
ALL SOLUTIONS CHANGED
0
10
20
30
40
50
60
70
80
90
25
50
75
100
125
150
175

LIBRARY OF THE UNIVERSITY

TABLE 20

UPTAKE OF NITROGEN FROM CULTURE SOLUTIONS FOR 66 HOURS

Each value is the mean of 6.

Culture solution	Nitrate	Ammonia $3.6x10^{-3}M$	Ammonia $1.8x10^{-3}M$	Nitrate plus Ammonia	L.S.D. at 5% level
Form of nitrogen taken up (mg/4 plants)					
(1) Nitrate	60.2	-	-	40.3	
(2) Ammonia	-	46.7	51.2	36.4	
Total nitrogen	60.2	46.7	51.2	76.7	10.2
Form of nitrogen taken up (mg/g dry weight)					
(1) Nitrate	26.2	-	-	17.3	
(2) Ammonia	-	28.6	29.5	15.6	
Total nitrogen	26.2	28.6	29.5	32.9	3.1

On the 15th day some of the plants supplied with only ammonium nitrogen showed white patches on the older leaves. These remained and were still evident at the time of harvest. The younger leaves of these plants and the leaves of plants supplied with nitrate nitrogen or nitrate plus ammonium nitrogen were not affected. In another experiment plants were transferred from nitrate culture solution to solutions containing either 3.6×10^{-3}M nitrate nitrogen, 3.6×10^{-3}M ammonium nitrogen or 1.8×10^{-3}M nitrate plus 1.8×10^{-3}M ammonium nitrogen on the 10th day. Each solution was maintained in the pH range 6.0-7.0. Plants grown on nitrate plus ammonium nitrogen were similar to those grown on nitrate nitrogen and showed no abnormalities. By the 16th day white patches were visible on the older leaves of the 24 plants grown on ammonium nitrogen. The patches were most visible on the 17th day. Figures 9 and 10 show plants which were photographed on the 18th day. The younger leaves and the developing leaves showed no visible symptoms. By the 21st day the areas of white leaf tissue had diminished in size considerably and none of the newly developed leaves showed any signs of abnormalities. This effect was not observed in experiments described in Part III where seedlings were planted immediately onto ammonia culture solution.

Discussion.

The results from the control culture solutions showed that the decrease in nitrogen concentrations in culture solutions was due solely to the uptake of nitrogen by the plants growing on them. This experiment

FIGURE 9. Tomato plants supplied with 3.6×10^{-3}M NH_3-N at pH 6.0 - 7.0.

These plants had been grown on nitrate culture solution until the 10th day when they were transferred to ammonia culture solution. On the 16th day white patches appeared on the older leaves and were still clearly visible on the 18th day when these plants were photographed. The young leaves and developing leaves showed no abnormalities.

FIGURE 10. Tomato plants supplied with $3.6 \times 10^{-3}M$ NH_3-N (left) and $3.6 \times 10^{-3}M$ NO_3-N at pH 6.0 - 7.0 (right).

These plants had been grown on nitrate culture solution until the 10th day when they were transferred to ammonia culture solution or fresh nitrate culture solution. On the 16th day white patches appeared on the older leaves of the plants supplied with ammonium nitrogen and were still clearly visible on the 18th day when these plants were photographed. The young leaves and developing leaves of the plants grown on ammonia culture solution and all the leaves of plants grown on nitrate culture solution showed no abnormalities.

shows that the uptake of ammonium nitrogen from culture solutions is greatly reduced when nitrate is present. However the total uptake of nitrogen from a solution containing nitrate and ammonium nitrogen is slightly greater than from a solution containing only one form of nitrogen. Weismann (1950) observed similar results with wheat seedlings; moreover he also observed that about equal amounts of nitrate and ammonium nitrogen were taken up from a culture solution containing equal concentrations of the two forms of nitrogen. Wallace and Mueller (1957) found a higher uptake of ammonium nitrogen over nitrate nitrogen in lemon cuttings in sand cultures. Experiments with tomato plants in culture solutions have shown an equal uptake of both forms of nitrogen, both in the present investigation and also in 41 day old tomato plants (Clark and Shive, 1934). Consequently the amount of ammonium nitrogen entering the roots is considerably lowered in the presence of nitrate as compared to plants grown on an ammonia culture solution. In view of these findings it was of interest to determine whether the levels of nitrogenous compounds in the tomato plants were affected by the addition of nitrate to the ammonia culture solution.

2 AMMONIA, AMIDE AND PROTEIN LEVELS IN TOMATO PLANTS GROWN ON EITHER NITRATE, AMMONIUM OR NITRATE AND AMMONIUM NITROGEN

Introduction.

Weismann (1951) found a high amide concentration in 4 day old wheat seedlings supplied with ammonium nitrogen. Amides, especially glutamine,

are very quickly synthesised when plants are supplied with inorganic nitrogen (Yemm and Folkes, 1958). It was decided to determine the levels of ammonium and amide nitrogen in tomato plants supplied with different forms of nitrogen because, if the form of inorganic nitrogen supply results in changes in the concentrations of nitrogen compounds within the plants, this may well be reflected in the ammonia and amide levels. Total insoluble nitrogen was also determined. In the previous experiments the uptake of ammonium nitrogen from the two concentrations of ammonia culture solutions used was almost equal. Consequently in this experiment it was decided to supply 3.6×10^{-3}M ammonium nitrogen in the ammonia culture solution so that the total nitrogen concentrations in all the solutions were the same.

Materials and Methods.

Tomato plants were grown in 39 culture vessels on nitrate culture solution (Methods, 1). On the 10th day plants from 3 cultures were harvested. Twelve of the remaining culture solutions were replaced with 3.6×10^{-3}M ammonia culture solution, 12 with 1.8×10^{-3}M nitrate plus 1.8×10^{-3}M ammonia culture solution (Table 17) and 12 with fresh 3.6×10^{-3}M nitrate culture solution. All solutions were maintained in the pH range 5.5-6.5 with 0.02M sodium hydroxide or 0.02M hydrochloric acid. Plants from 3 cultures in each treatment were harvested on the 12th, 14th, 17th and 20th days. The solutions in the three remaining cultures in each treatment were renewed on the 17th day. At each harvest the fresh weights

of roots and shoots of the four plants from each culture were determined. Ammonium, amide and insoluble nitrogen were determined (Methods, 2). The amount of ammonium nitrogen taken up by the 4 plants was determined as described above (see Part IV, 1).

Results.

As is shown in Table 21 and Figure 11, lower uptake of ammonium nitrogen from solutions containing nitrate and ammonium nitrogen as compared to the uptake from solutions containing only ammonium nitrogen was again observed. At the final harvest, the fresh weights of roots and shoots of the plants supplied with ammonia culture solution were less than those of plants grown on nitrate or a mixture of nitrate and ammonium nitrogen (Table 22 and Figure 12).

Ammonium nitrogen concentrations in the roots and shoots increased rapidly when plants were supplied with ammonia culture solution. In the roots of plants supplied with nitrate and ammonium nitrogen, the ammonium nitrogen concentration was significantly greater than in plants supplied with nitrate culture solution but significantly less than in plants supplied with ammonia culture solution. This was true at all stages except the final harvest. The concentration of ammonium nitrogen in shoots was higher in plants grown on ammonia culture solution than in the plants on the other two treatments. When nitrate and ammonium nitrogen was supplied, the ammonium nitrogen concentration was not significantly higher in the shoots than in plants supplied with nitrate culture solution (Table 23 and Figure 13).

TABLE 21

UPTAKE OF AMMONIUM NITROGEN BY TOMATO PLANTS GROWN ON AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Each value is the mean uptake of ammonium nitrogen from 3 culture solutions.

Culture solution	Ammonia	Nitrate plus ammonia	L.S.D. at 5% level
	Ammonium nitrogen (mg)		
Time (hr)			
49	9.4	1.7	2.8
96	18.1	5.1	3.6
165	36.1	18.2	8.2
239	73.0	42.2	12.2

FIGURE 11. Uptake of ammonium nitrogen by young tomato plants from

● culture solution containing $3.6 \times 10^{-3}M$ NH_3-N

○ culture solution containing $1.8 \times 10^{-3}M$ NH_3-N plus $1.8 \times 10^{-3}M$ NO_3-N

All plants had previously been growing on nitrate culture solution.

Each value is the mean of determinations on 3 culture solutions.

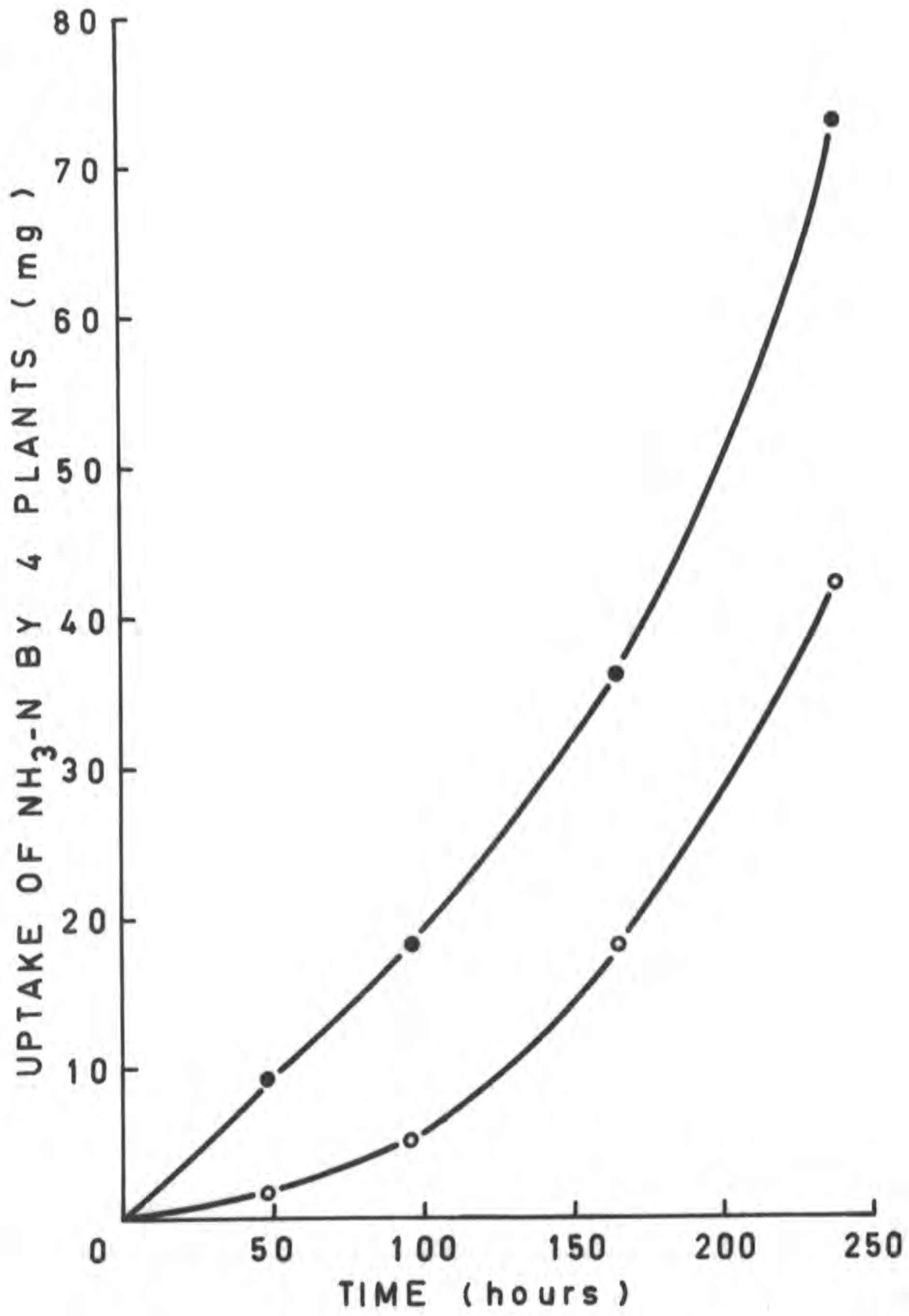

UPTAKE OF NH_3-N BY 4 PLANTS (mg)
80
70
60
50
40
30
20
10
0
50
100
150
200
250
TIME (hours)

TABLE 22

FRESH WEIGHTS OF SHOOTS AND ROOTS OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Each value is the mean from 3 cultures.

Culture solution	Nitrate	Ammonia	Nitrate plus Ammonia	L.S.D. at 5% level
	Fresh weight of shoots (g/4 plants)			
Time (hr) from start of treatment				
0	1.52			
49	2.92	2.54	2.77	N.S.
96	4.86	4.39	4.47	N.S.
165	8.68	6.41	8.75	N.S.
239	16.49	11.14	18.04	3.50
	Fresh weight of roots (g/4 plants)			
0	0.82			
49	1.44	1.35	1.35	N.S.
96	2.23	2.33	2.15	N.S.
165	3.53	3.33	4.12	N.S.
239	7.45	5.84	7.52	1.28

FIGURE 12. Increase of fresh weight with time of roots and shoots of young tomato plants supplied with

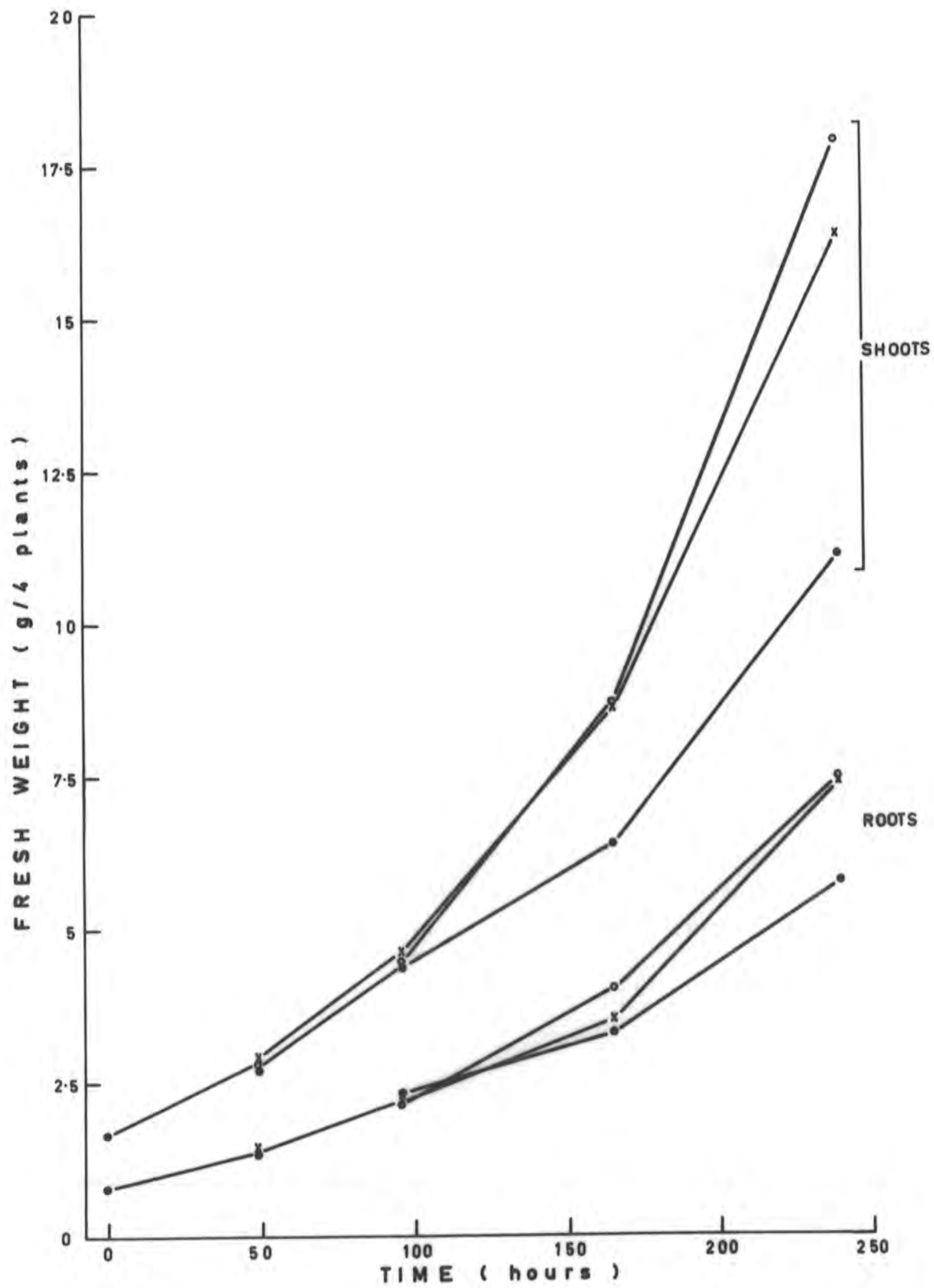
× culture solution containing 3.6×10^{-3}M NO_3-N

● culture solution containing 3.6×10^{-3}M NH_3-N

○ culture solution containing 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N

All plants had previously been growing on nitrate culture solution.

Each value is the mean of 3.



FRESH WEIGHT (g/4 plants)
20
17·5
15
12·5
10
7·5
5
2·5
0
TIME (hours)
0
50
100
150
200
250
SHOOTS
ROOTS

TABLE 23

CONCENTRATION OF AMMONIUM NITROGEN IN SHOOTS AND ROOTS OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Each value is the mean from 3 cultures.

Culture solution	Nitrate	Ammonia	Nitrate plus Ammonia	L.S.D. at 5% level
	Ammonium nitrogen in shoots (µg/g fresh weight)			
Time (hr) from start of treatment				
0	20.4			
49	21.4	60.4	26.2	8.5
96	20.5	66.9	29.6	28.0
165	27.3	56.5	38.4	14.8
239	15.0	61.7	22.5	6.5
	Ammonium nitrogen in roots (µg/g fresh weight)			
Time (hr) from start of treatment				
0	24.1			
49	20.8	127.6	57.5	15.9
96	16.2	137.0	70.6	41.5
165	22.0	95.5	60.2	21.9
239	15.5	70.6	47.0	24.5

FIGURE 13. Concentration of ammonium nitrogen in roots and shoots of young tomato plants supplied with

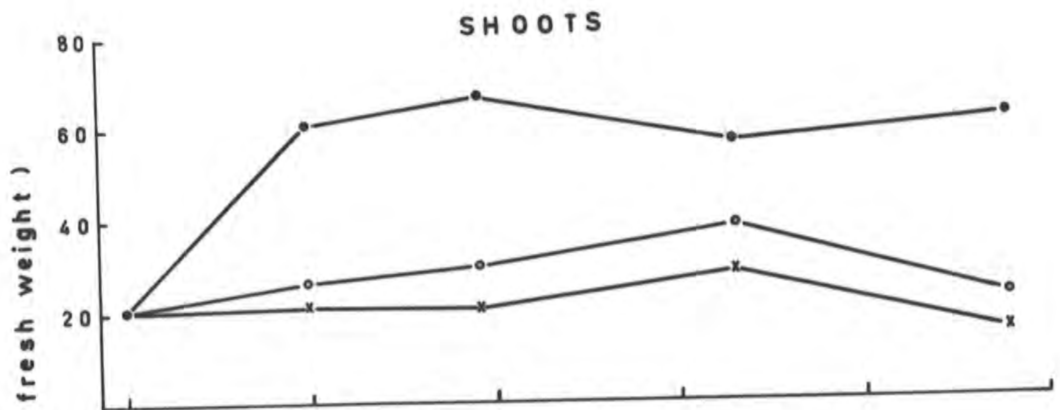
x culture solution containing 3.6×10^{-3}M NO_3-N

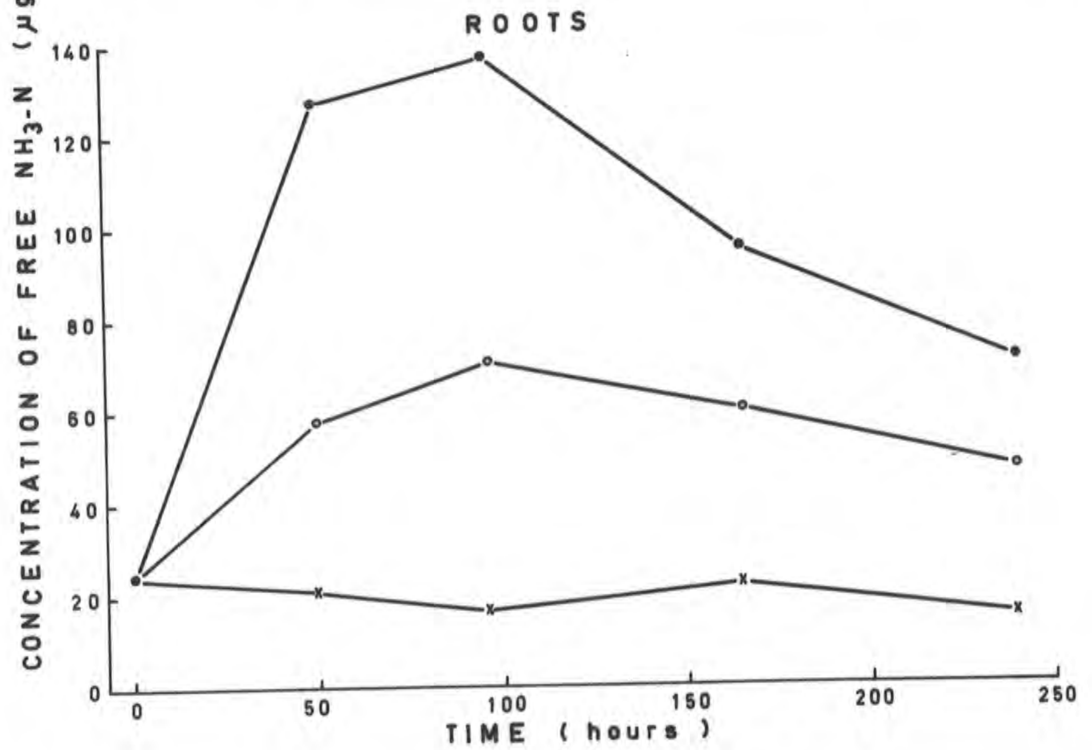
● culture solution containing 3.6×10^{-3}M NH_3-N

o culture solution containing 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N

All plants had previously been growing on nitrate culture solution.

Each value is the mean of 3.


SHOOTS
80
60
40
20
CONCENTRATION OF FREE NH3-N (μg/g fresh weight)


ROOTS
140
120
100
80
60
40
20
0
0
50
100
150
200
250
TIME (hours)

Amide nitrogen concentrations in the roots and shoots of plants grown on ammonia culture solution increased rapidly for about 165 hours. Whereas the ammonium nitrogen concentration was at its maximum after 96 hours, the amide nitrogen concentration was still increasing at this time and after 165 hours was still increasing. The concentrations of amide in the roots and shoots of plants given the other two treatments were the same in each case and much lower than those in plants grown on ammonia culture solution (Table 24 and Figure 14).

Insoluble nitrogen values for roots and shoots are shown in Table 25 and Figure 15. At the final harvest, the shoots and roots of the plants grown on nitrate culture solution contained lower concentrations of protein nitrogen (mg protein nitrogen per 10 grams fresh weight) than plants grown on ammonia culture solution. Values for protein concentrations in plants grown on nitrate plus ammonia culture solution were between those of the other plants. When the results were expressed on a per plant basis, the plants grown on nitrate plus ammonia culture solution showed the highest values and plants grown on ammonia culture solution the lowest values.

Discussion.

Although the roots were washed with distilled water, the high ammonium nitrogen figures in the roots of plants supplied with ammonium nitrogen may have been caused partly by traces of culture solution adhering to the roots and by ammonium nitrogen in the free spaces of the roots. Contamination from these sources could not occur in shoots. As the amide

TABLE 24

CONCENTRATION OF AMIDE NITROGEN IN SHOOTS AND ROOTS OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Each value is the mean from 3 cultures.

Culture solution	Nitrate	Ammonia	Nitrate plus Ammonia	L.S.D. at 5% level
		Amide nitrogen in shoots (µg/g fresh weight)		
Time (hr) from start of treatment				
0	93.3			
49	48.3	109.0	82.0	17.6
96	65.5	157.0	88.5	36.7
165	38.0	297.0	84.0	53.1
239	36.8	324	40.5	43.1
		Amide nitrogen in roots (µg/g fresh weight)		
Time (hr) from start of treatment				
0	60.0			
49	29.3	76.6	40.2	22.9
96	25.0	115.9	55.6	32.3
165	16.3	203.0	39.4	35.4
239	21.6	200	34.5	56.0

FIGURE 14. Concentration of amide nitrogen in roots and shoots of young tomato plants supplied with

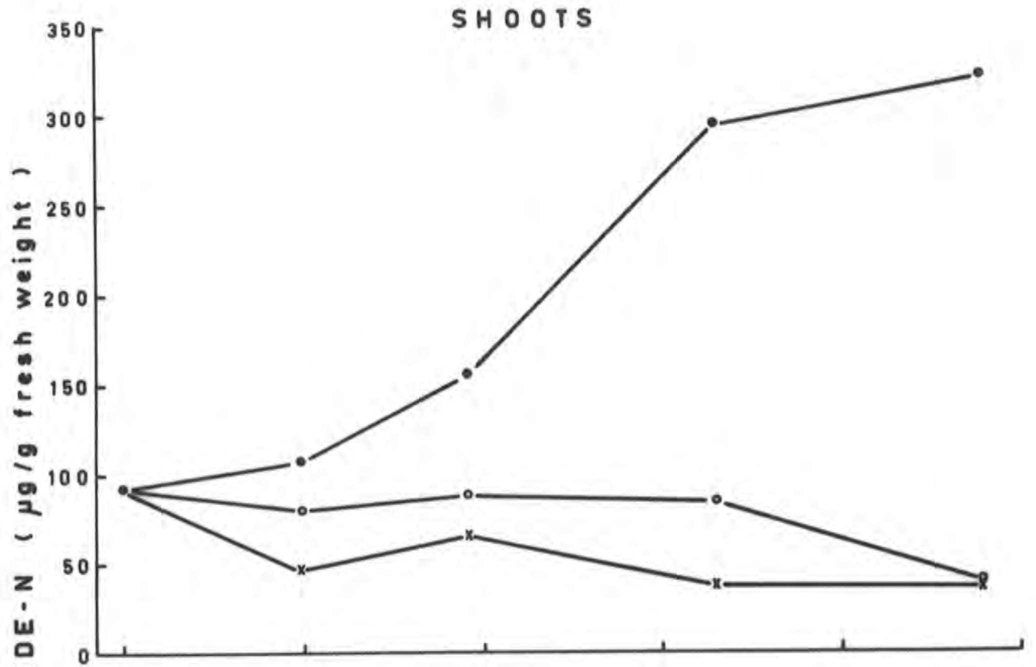
x culture solution containing 3.6×10^{-3}M NO_3-N

● culture solution containing 3.6×10^{-3}M NH_3-N

o culture solution containing 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N

All plants had previously been growing on nitrate culture solution.

Each value is the mean of 3.


SHOOTS
350
300
250
200
150
100
50
0
CONCENTRATION OF AMIDE-N (µg/g fresh weight)

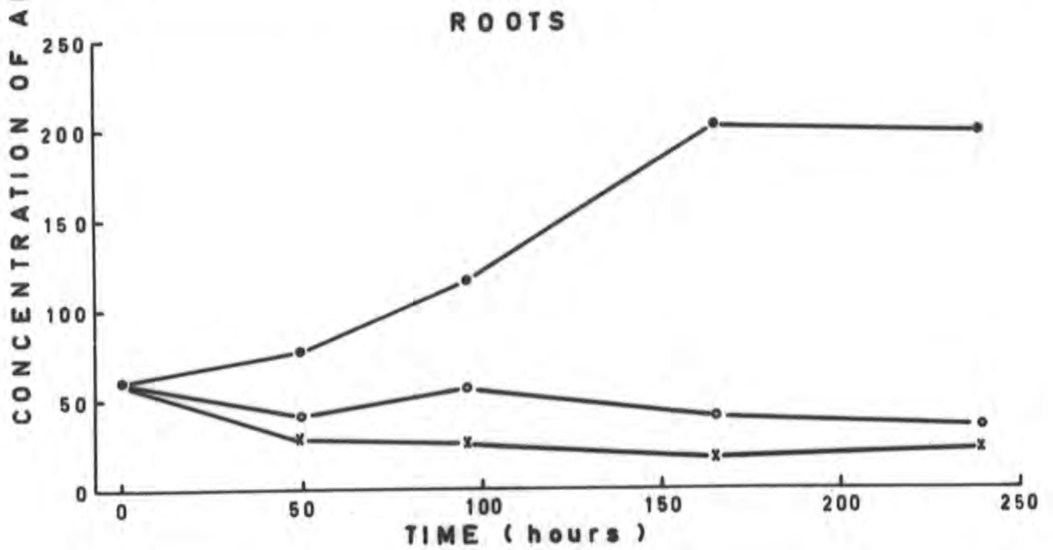

ROOTS
250
200
150
100
50
0
0
50
100
150
200
250
TIME (hours)

TABLE 25

INSOLUBLE NITROGEN IN SHOOTS AND ROOTS OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Each value is the mean from 3 cultures.

Culture solution	Nitrate	Ammonia	Nitrate plus ammonia	L.S.D. at 5% level
	Insoluble nitrogen in shoots (mg/4 shoots)			
Time (hr) from start of treatment				
0	3.66			
49	9.72	8.44	9.34	N.S.
96	15.58	15.05	16.66	N.S.
165	24.49	21.18	27.04	N.S.
239	46.1	37.6	55.8	9.55
	Insoluble nitrogen in roots (mg/4 roots)			
0	0.75			
49	1.78	1.64	2.16	N.S.
96	2.76	3.01	3.15	N.S.
165	4.66	4.46	5.82	N.S.
239	8.60	7.80	9.90	1.39
	Insoluble nitrogen (mg/10g fresh weight)			
239 Shoots	28.13	33.69	30.93	2.96
239 Roots	11.71	13.38	13.14	1.09

FIGURE 15. Insoluble nitrogen in roots and shoots of young tomato plants supplied with

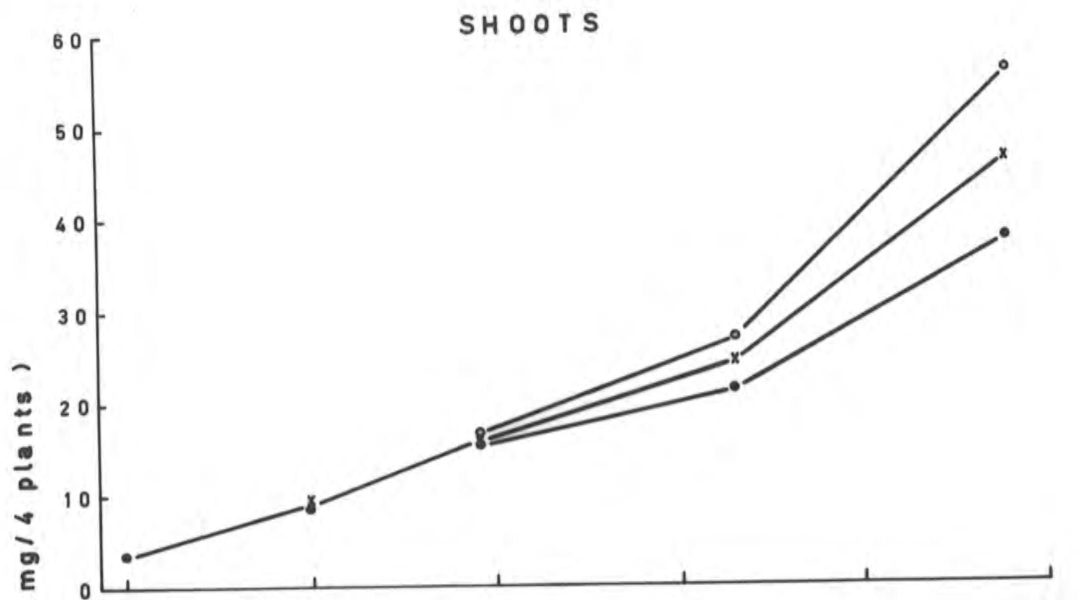
x culture solution containing 3.6×10^{-3}M NO_3-N

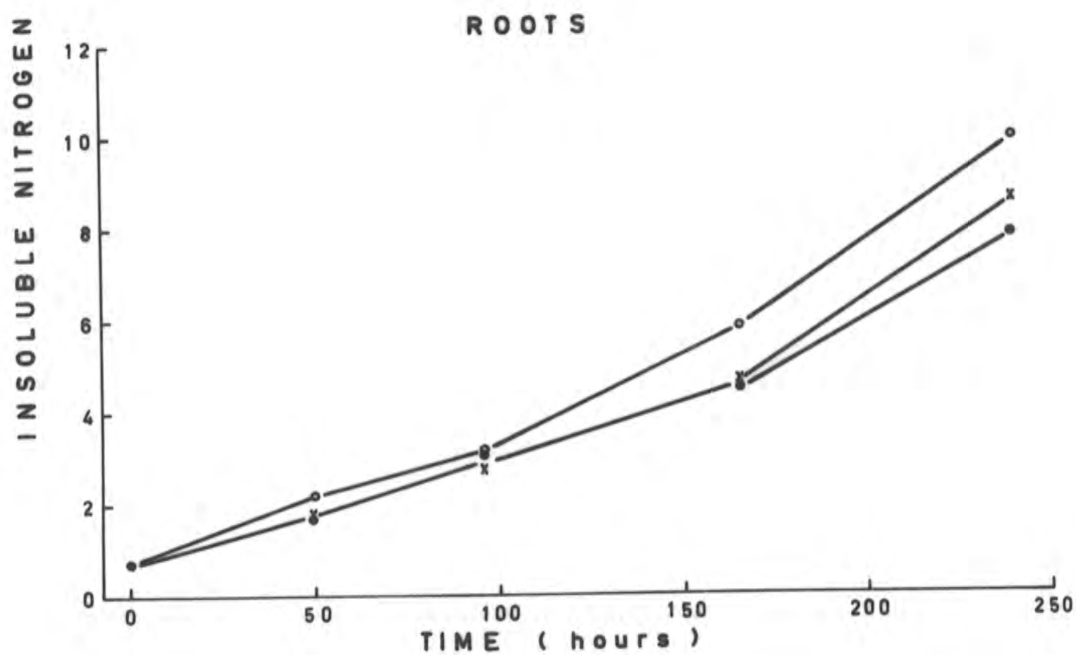
● culture solution containing 3.6×10^{-3}M NH_3-N

o culture solution containing 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N

All plants had previously been growing on nitrate culture solution.

Each value is the mean of 3.


SHOOTS
60
50
40
30
20
10
0
INSOLUBLE NITROGEN (mg/4 plants)


ROOTS
12
10
8
6
4
2
0
0
50
100
150
200
250
TIME (hours)

concentrations were highest in roots and shoots of plants grown on ammonium nitrogen alone, it appears likely that the high ammonia values for the roots were a measure of the ammonium nitrogen concentration in the root tissues and not due to external ammonium nitrogen contamination.

It would seem that increases in amide concentration followed increased ammonium nitrogen levels in the tissues of plants grown on ammonia culture solution.

With nitrate and ammonium nitrogen in the culture solutions the level of ammonium nitrogen in the tissues was much lower than in plants grown on only ammonium nitrogen. The low level of amide nitrogen in plants grown on nitrate and ammonium nitrogen followed the low concentration of ammonium nitrogen. It is apparent from these results that the presence of nitrate in a culture solution containing ammonium nitrogen prevents the formation of high levels of ammonium nitrogen in the tissues. When plants were transferred from nitrate culture solution to ammonia or to ammonia plus nitrate culture solutions, higher levels of ammonium nitrogen were detected within 49 hours in the first group. It seems difficult to discuss this problem in terms of competition for sites. According to our current concepts of salt absorption, it is hard to understand why ammonium chloride and ammonium nitrate would act so differently. Because the high ammonium nitrogen levels, which precede increased amide synthesis, do not occur when nitrate is present as well as ammonium nitrogen in the culture solution, it does not seem that competition for carbon skeletons (e.g. α-keto glutaric acid) was significant. The "block" appeared earlier than this.

As was shown in Part IV, 1, the high rate of uptake of ammonium nitrogen by plants grown on ammonia culture solution decreased within 29 hours of transferring these plants to a solution containing nitrate and ammonium nitrogen. Therefore the ammonia culture solution had no permanent effect on the rate of uptake of ammonium nitrogen by these roots. Whether nitrate affected existing root tissue or affected the synthesis of new tissue during the 29 hour period is not known.

The white patches on the older leaves of plants supplied with ammonium nitrogen as reported in Part IV, 1, may have been caused by high concentrations of ammonium ions in these plants. However it is hard to see why only the older leaves were affected and why the effect diminished after the 17th day.

The high concentrations of ammonium nitrogen in the shoots of plants grown on ammonia culture solution suggest that ammonium nitrogen was rapidly translocated from the roots to the shoots. The high amide levels could have arisen by synthesis in the shoots, from carbon skeletons and ammonium nitrogen, or by translocation of amide synthesised in the roots. Therefore it was unlikely that a low rate of translocation of nitrogen or compounds of nitrogen was limiting the supply of nitrogen to the shoots. Thus it would seem that the reduced growth of plants grown on ammonia culture solution as compared to the growth of plants on nitrate culture solution was not due to a deficiency of nitrogen in the shoots.

3 THE EFFECT OF DIFFERENT SOURCES OF NITROGEN ON ROOT HAIR FORMATION

Introduction.

In view of results of experiments described in Part IV, 1 and 2, it is of interest to note a morphological difference which was observed in plants grown in the presence of ammonium or nitrate nitrogen. Tomato plants grown on nitrate form approximately 7 times as many root hairs on the terminal 5 cm of each root as do plants grown on ammonium nitrogen (Woolhouse, 1959). It was decided to determine whether plants grown on nitrate and ammonium nitrogen show similar numbers of root hairs as plants grown on nitrate. Observations were also made on the time taken for changes in root hair numbers to occur when plants are transferred from nitrate culture solution to ammonia culture solution.

Materials and Methods.

Tomato plants were grown in 24 culture vessels on nitrate culture solution (Methods, 1). On the 12th day 8 of the culture solutions were replaced with 3.6×10^{-3}M ammonia culture solution, 8 with 1.8×10^{-3}M nitrate plus 1.8×10^{-3}M ammonia culture solution (Table 17), and 8 with fresh 3.6×10^{-3}M nitrate culture solution. All solutions were maintained in the pH range 6.0-7.0 with 0.02M sodium hydroxide or 0.02M hydrochloric acid. Plants from 2 cultures in each treatment were harvested 12, 24, 36 and 48 hours after applying the treatments. Samples of the roots were removed and placed in 70 per cent. aqueous ethyl alcohol. Ammonium nitrogen was determined in the remaining roots and shoots (Methods, 2).

Also tomato seedlings were planted out into 9 culture vessels containing either 3.6×10^{-3}M nitrate culture solution, 3.6×10^{-3}M ammonia culture solution or 1.8×10^{-3}M nitrate plus 1.8×10^{-3}M ammonia culture solution. The pH of each culture solution was maintained in the range 6.0-7.0. On the 19th day root samples were taken and placed in 70 per cent. aqueous ethanol.

Results.

As can be seen from Table 26 the concentration of ammonium nitrogen in the roots and shoots of plants supplied with both ammonia culture solution and nitrate plus ammonia culture solution rapidly increased above that in plants supplied with nitrate culture solution. As was observed previously (Table 23) concentrations of ammonium nitrogen in the shoots of plants grown on ammonium nitrogen alone were higher than those in plants grown on nitrate plus ammonium nitrogen. The fact that the levels of ammonium nitrogen in the roots of plants grown on ammonium nitrogen and on nitrate plus ammonium nitrogen in this experiment were not significantly different was probably due to the large amount of variation and small number of samples analysed. Within 12 hours of transferring plants to culture solutions containing ammonium nitrogen the levels of ammonium nitrogen in the roots of these plants had risen almost to the maximum value obtained after 48 hours. However in the shoots, after 12 hours, the level was only half the maximum value reached. Within 24 hours the level of ammonium nitrogen in the shoots had risen almost to the maximum value.

TABLE 26

CONCENTRATIONS OF AMMONIUM NITROGEN IN ROOTS AND SHOOTS OF TOMATO PLANTS SUPPLIED WITH NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Plants were grown on nitrate nitrogen and then transferred to culture solutions in the pH range 6.0-7.0, containing nitrate, ammonium or nitrate plus ammonium nitrogen. Values are µg ammonium N / gram fresh weight.

Each value is the mean of analyses on two samples of plants.

Culture Solution		Nitrate	Ammonia	Nitrate + Ammonia	L.S.D. at 5% level
			Roots		
Time (hr)					
12	9 p.m.	13.6	177.8	145.6	62.5
24	9 a.m.	5.9	155.5	130.6	34.4
36	9 p.m.	47.6	246.5	215.2	100
48	9 a.m.	5.7	235.0	196.2	88
			Shoots		
12	9 p.m.	7.2	28.4	21.8	13.2
24	9 a.m.	13.6	61.3	33.2	26.5
36	9 p.m.	11.3	51.9	26.5	17.1
48	9 a.m.	35.1	73.7	55.6	17.0

The terminal segments of the roots supplied with nitrate nitrogen and with nitrate plus ammonium nitrogen were similar at all harvests. However within 12 hours the roots of plants supplied with ammonium nitrogen alone had formed swollen areas about 1 cm from the root tip and there was a large number of root hairs at these swollen regions (Figure 16). At later harvests the roots had increased in length resulting in the swollen regions being further from the root tips. At the final harvest it was also observed that there were fewer root hairs on the terminal segments of the roots beyond the swollen regions in the ammonia grown plants than on the corresponding parts of the roots of the other plants.

Plants grown for 19 days on different sources of nitrogen showed differences in root hair formation. Those grown on ammonia nitrogen had fewer root hairs than plants grown on nitrate nitrogen (Figure 17), as was also observed by Woolhouse (1959). Although root hair formation in plants grown on nitrate plus ammonium nitrogen was slightly reduced as compared to plants grown on nitrate nitrogen, the number of root hairs was very much greater than in plants supplied with ammonium nitrogen alone (Figure 17).

Discussion.

Plants supplied with ammonium nitrogen alone showed a rapid increase in ammonium ion concentration within the plants and morphological changes were also apparent within 12 hours.

FIGURE 16. Terminal segments of roots of tomato plants supplied with different forms of nitrogen (X5). Plants were grown on nitrate culture solution and then transferred to solutions containing either 3.6×10^{-3}M NO_3-N, 3.6×10^{-3}M NH_3-N, or 1.8×10^{-3} NH_3-N plus 1.8×10^{-3}M NO_3-N.

The roots shown are (from left to right)

a. root from plant harvested 12 hours after transferring to nitrate culture solution.

b. root from plant harvested 12 hours after transferring to nitrate plus ammonia culture solution.

c, d, e, and f. roots from plants harvested 12, 24, 36, and 48 hours respectively after transferring to ammonia culture solution.

g. root from plant harvested 48 hours after transferring to nitrate plus ammonia culture solution.

h. root from plant harvested 48 hours after transferring to nitrate culture solution.

Note the swollen regions on the roots of plants supplied with ammonium nitrogen (c, d, e, f).

There are large numbers of root hairs on these swollen regions.

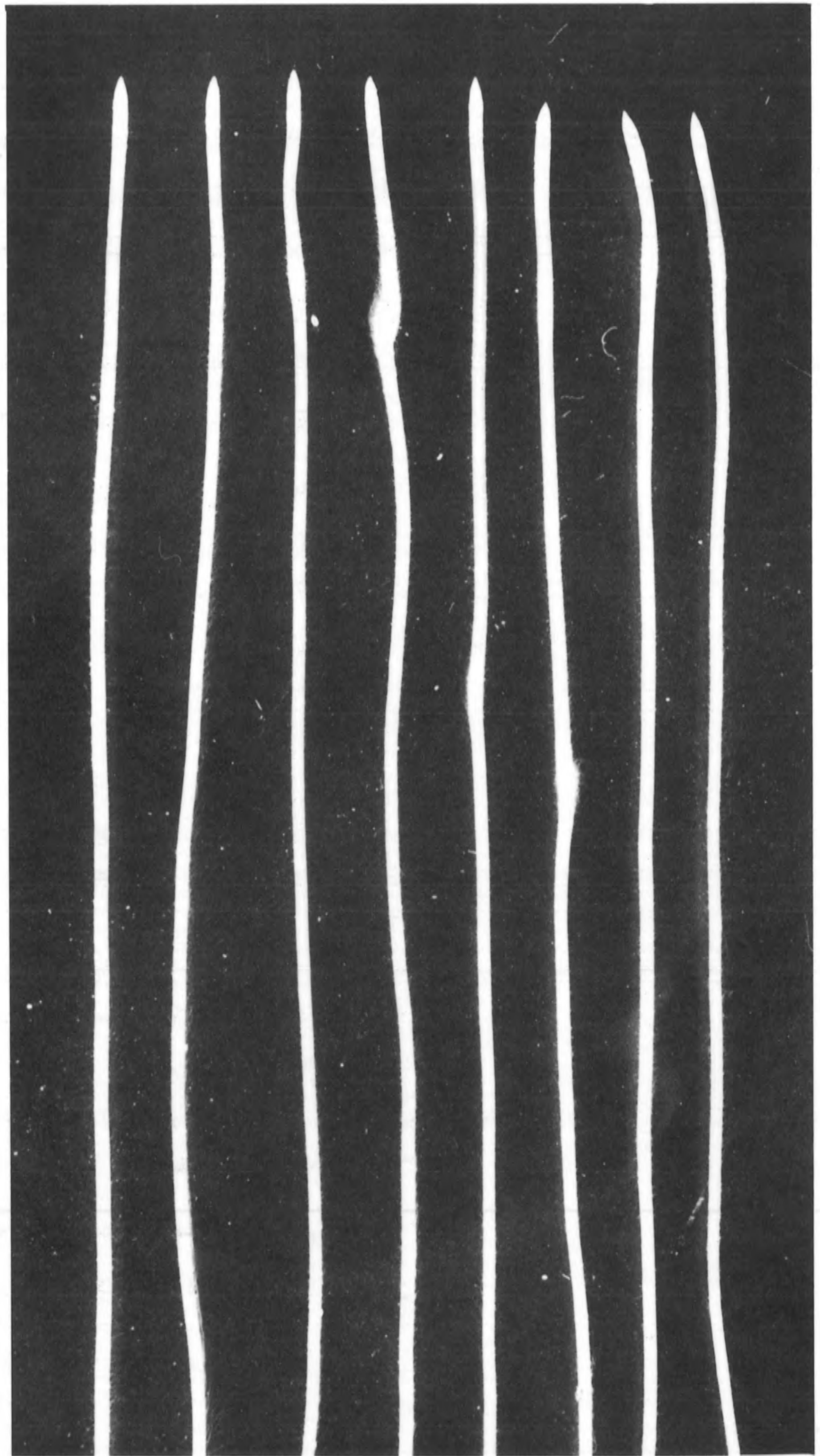


FIGURE 17. Terminal segments of roots of tomato plants grown for 19 days on different forms of nitrogen (X5).

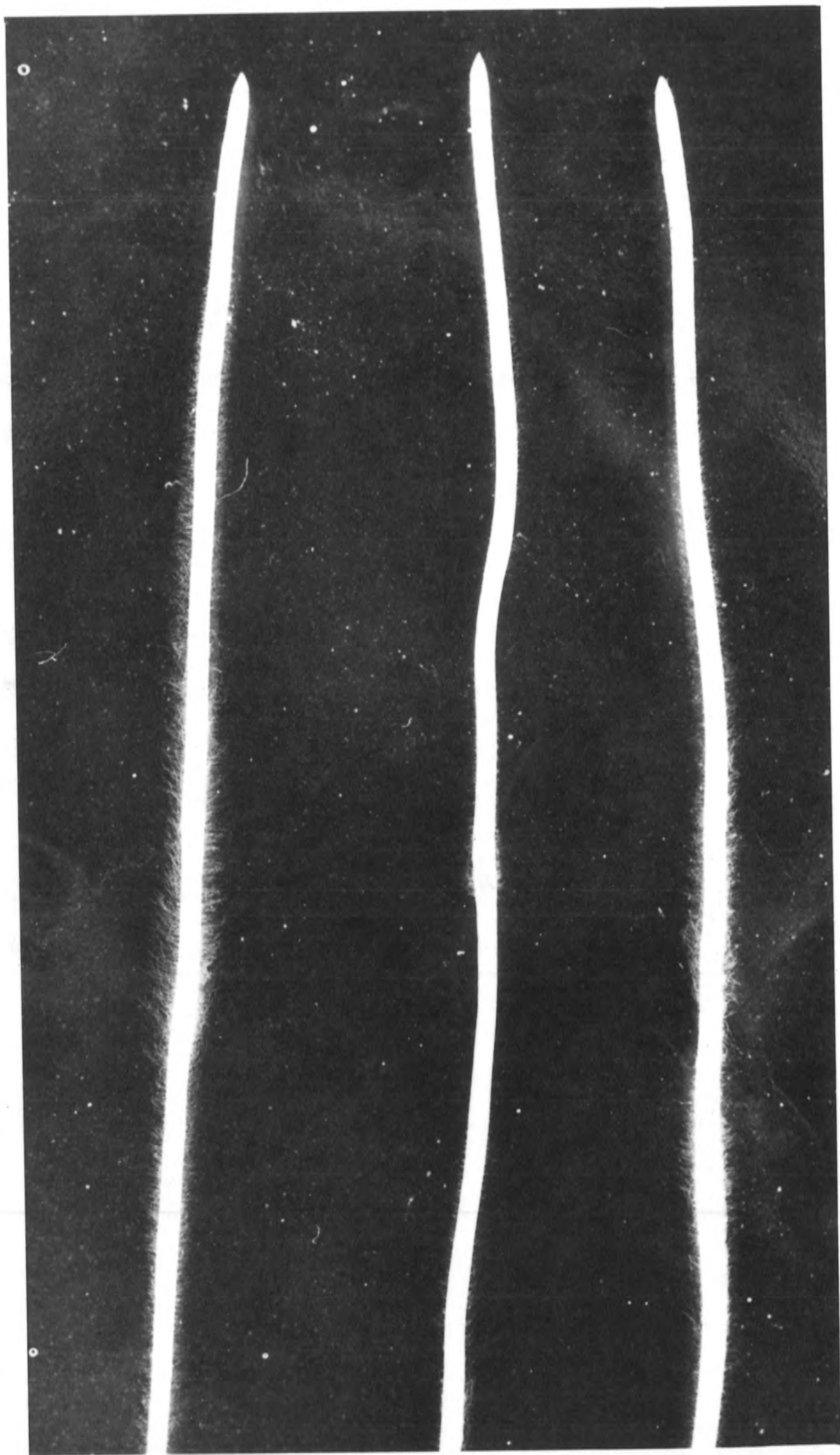
The roots shown are (from left to right)

a. root from plant grown on 3.6×10^{-3}M NO_3-N

b. root from plant grown on 3.6×10^{-3}M NH_3-N

c. root from plant grown on 1.8×10^{-3}M NH_3-N plus 1.8×10^{-3}M NO_3-N.

Note the reduced number of root hairs on the root of the plant grown on ammonium nitrogen.



An increase in ammonium ion concentration was also found in plants supplied with nitrate plus ammonium nitrogen, but in these plants no morphological changes were observed. Consequently there was no obvious explanation for the development of the swellings in the roots of plants supplied with ammonium nitrogen alone. There are no results for the ammonium ion concentrations in the terminal root segments as analyses were made on the complete root systems. There is still a possibility that high ammonium ion concentrations developed rapidly in the region of active ion absorption after transfer to ammonium nitrogen alone. This may have caused the swellings.

Although there was a marked decrease in root hair formation in plants transferred to ammonium nitrogen, the results for ammonium nitrogen analyses showed that there was still a rapid absorption of ammonium ions.

4 THE CONCENTRATIONS OF ELEMENTS IN TOMATO PLANTS GROWN ON EITHER NITRATE, AMMONIUM OR NITRATE AND AMMONIUM NITROGEN

Introduction.

In the presence of nitrate the rate of uptake of ammonium nitrogen was reduced (Part IV, 1 and 2). Also there were greater numbers of root hairs present in plants grown on these solutions than in plants grown on ammonium nitrogen alone (Part IV, 3). These facts may have had some effect on the uptake of other salts. To examine this, dried material

from plants grown on nitrate, ammonia or nitrate plus ammonia culture solutions was assayed for sodium, potassium, calcium, magnesium, chlorine, phosphorus, iron, manganese, copper and molybdenum.

Materials and Methods.

Roots and shoots from material grown at pH ranges from 5.5-8.5 (described in Part III, 2) and roots and shoots from other material grown on different sources of nitrogen (described in Part IV, 1) were analysed for sodium, potassium, calcium, magnesium, chlorine, phosphorus, iron, manganese, copper and molybdenum (Methods, 3).

Results and Discussion.

The mean concentrations in dry material of each element estimated are given in Table 27. There was sufficient plant material for three replicate analyses of plants grown on ammonia culture solution, two of plants grown on nitrate culture solution and one of plants grown on nitrate and ammonium nitrogen. The concentrations of sodium and calcium were higher and the concentration of chlorine was lower in both shoots and roots of plants grown on nitrate nitrogen as compared to roots and shoots of plants grown on ammonium nitrogen. Phosphorus concentrations in the shoots were lower and magnesium and manganese concentrations in the roots were higher in plants grown on nitrate nitrogen than in plants grown on ammonium nitrogen. The concentrations of all these elements in plants grown in cultures containing both nitrate and ammonium nitrogen were

TABLE 27

CHEMICAL COMPOSITION OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Each value is the number of grams of element present in 100 grams dry weight of plant material.

Culture solution	Nitrate	Ammonia	L.S.D. between NO_3 and NH_3	Nitrate plus Ammonia	L.S.D. between NH_3 and NO_3 + NH_3
Number of samples analysed	Two	Three		One	
Element			Shoots		
Phosphorus	0.66	0.90	0.19	0.99	0.24
Sodium	0.35	0.14	0.05	0.13	0.06
Potassium	5.68	5.57	N.S.	6.83	
Calcium	2.81	1.32	0.48	1.88	0.60
Magnesium	0.56	0.46	N.S.	0.43	
Chlorine	0.94	2.47	0.22	2.81	0.28
Iron	0.01	0.014	N.S.	0.013	
Manganese	0.012	0.013	N.S.	0.01	
Copper	0.006	0.006	N.S.	0.005	
Molybdenum	0.0008	0.0007	N.S.	0.0008	
			Roots		
Phosphorus	0.94	0.91	N.S.	1.0	
Sodium	0.44	0.20	0.07	0.12	0.09
Potassium	6.30	5.46	N.S.	8.41	
Calcium	0.79	0.55	0.21	0.64	0.27
Magnesium	0.59	0.19	0.26	0.23	0.35
Chlorine	1.18	2.60	0.87	2.59	1.12
Iron	0.32	0.21	N.S.	0.20	
Manganese	0.099	0.036	0.03	0.066	0.03
Copper	0.018	0.013	N.S.	0.010	
Molybdenum	0.0007	0.0015	N.S.	0.0009	

similar to the concentrations of these elements in plants grown on ammonium nitrogen alone. The higher concentrations of cations and lower concentrations of anions in plants grown on nitrate culture solution as compared to plants grown on ammonia culture solution agrees with reports of other workers (Clark, 1936; Sideris and Young, 1946; Wallace and Ashcroft, 1956; Wander and Sites, 1956; Smith, 1957). Wallace and Ashcroft (1956) and Smith (1957) reported similar concentrations of cations in plants grown on either ammonium nitrogen or nitrate and ammonium nitrogen. Differences in cation concentrations in plants grown on these two types of solution reported by Wander and Sites (1956) may have been due to different pH values of the solutions rather than the presence of nitrate because Jackson and Coleman (1959) showed that the uptake of potassium by excised roots was greater from solutions at pH values above 7 than from solutions below pH 7. The better growth of plants grown on ammonia culture solution containing low concentrations of iron than on nitrate culture solutions containing similar low concentrations of iron was due to the greater availability of iron at pH 4.2 of the ammonia culture solution than at pH 6.8 of the nitrate culture solution (Sideris _et al._, 1943). Colgrove and Roberts (1956) reported that azalea plants developed iron chlorosis if grown on nitrate nitrogen. The pH of leaf sap (the pH of a slurry prepared by grinding leaves in 5 volumes of water) was 5 or higher. When only ammonium nitrogen was supplied the leaf sap pH was about 4 and the plants were not chlorotic. Similar pH measurements on tomato leaves showed that leaf sap of plants grown on

nitrate culture solution had similar pH values to leaf sap of plants grown on ammonia culture solution. In each case the pH value was 6.0 ± 0.1. Also in the present experiment iron values were similar in plants grown on all of the sources of nitrogen.

There were differences in concentrations of some elements in plants grown on either nitrate nitrogen or a mixture of nitrate and ammonium nitrogen, but these differences had no effect on the amount of dry weight produced. The quantities of the elements assayed were essentially the same in plants grown on culture solutions containing ammonium nitrogen or ammonium plus nitrate nitrogen. Therefore it seems unlikely that the reduced growth of plants grown on ammonia culture solution was caused by limiting or toxic concentrations of any element within the plant tissues. Owing to the small number of samples analysed it was difficult to obtain significant differences when these differences were small. However, from this preliminary survey, the levels of sodium, potassium, calcium, magnesium, chlorine, phosphorus, iron, manganese, copper and molybdenum in those plants grown on ammonium nitrogen alone would appear quite favourable for optimum growth by comparison with data given by Goodall and Gregory (1947).

Summary.

It has been shown (Part III) that the decreased growth of tomato plants grown on ammonia culture solution was due to the absence of nitrate rather than the presence of ammonium nitrogen in the culture solution.

The experiments described in this Section have shown that the addition of nitrate to ammonia culture solution reduced the uptake of ammonium nitrogen by tomato plants. Although under these conditions the total nitrogen absorbed by the plants was slightly greater than the nitrogen absorbed from ammonia culture solution, the concentrations of ammonium nitrogen and amide within the tissues were much lower than in plants grown on ammonia culture solution. White patches appeared on the older leaves of plants grown on ammonium nitrogen whereas no abnormalities were observed on plants grown on either nitrate nitrogen or nitrate plus ammonium nitrogen. Root hair formation was markedly reduced in plants grown on ammonium nitrogen as compared to plants grown on nitrate plus ammonium nitrogen or nitrate nitrogen. The concentrations of the essential elements in the plant tissues were similar in plants grown on either ammonia or nitrate plus ammonia culture solutions. The concentrations of some cations were higher and the concentrations of some anions were lower in plants grown on nitrate culture solution than in plants grown on the other solutions.

PART V

PROTEIN SYNTHESIS IN TOMATO PLANTS GROWN ON EITHER NITRATE NITROGEN, AMMONIUM NITROGEN, OR NITRATE AND AMMONIUM NITROGEN

1 AMINO ACIDS AND AMIDES

Introduction.

Vlasyuk *et al.* (1957) found similar concentrations of free amino acids in sugar beet plants supplied with nitrate nitrogen or ammonium nitrogen in the field. This is not surprising as probably nitrate was already present in the soil or nitrification could have taken place. Weismann (1959) using wheat seedlings, Steward *et al.* (1959) and Margolis (1960) using tomato plants, and Reisner *et al.* (1960) using Chlorella, showed differences in the concentrations of amides and some amino acids when these plants were supplied with either nitrate or ammonium nitrogen. Steward *et al.* (1959) also found that the levels of amino acids and amides in mint depended on day length and night temperature.

It has been shown (Part IV, 2) that the amide level in tomato plants grown on ammonia culture solution was high. However, the individual amides making up this fraction were not identified, and it was decided to determine the nature and relative concentrations of free amides present and also to determine the concentrations of some of the free amino acids in tomato plants supplied with either nitrate or ammonium nitrogen.

Apart from seasonal variations in these experiments in the day length and night temperatures during the growth periods, within one experiment, plants receiving either nitrate or ammonium nitrogen or mixtures of nitrate and ammonium nitrogen were grown under identical conditions. Therefore any differences in amide or amino acid concentrations in plants grown in one experiment would be due to the different type of culture solution supplied rather than to any variations in day length or night temperatures. Any differences observed in plants grown on the same culture solution but in separate experiments may well have been due to variations in environment.

Materials and Methods.

Amino acids and amides were extracted from plant material and analysed on paper chromatograms (Methods, 4). The source of plants, the parts of plants analysed and the type of paper chromatography used for the estimations are shown in Table 28. The culture solutions referred to in c) and d) of Table 28 are those described above (Methods, 1).

The identity and concentrations of some of the amino acids of certain extracts were checked on Dowex 50 columns (Methods, 5).

Results.

Using plants grown on nitrate culture solution and on culture solutions containing one of three different levels of ammonium nitrogen, the relative concentrations of amino acids and amides of shoots were determined (Table 29) by two-dimensional paper chromatography.

TABLE 28

PLANT MATERIAL ANALYSED FOR AMINO ACIDS AND AMIDES

Source of plants	Parts of plants used	Type of paper chromatography
a) Growth experiment (Part III, 4)	Shoots	Two-dimensional
b) Growth experiment (Part III, 5)	Shoots; roots	One-dimensional
Plants grown only for amino acid and amide analyses		
c) Plants grown on nitrate or ammonia culture solution	Leaves; stems; roots	One-dimensional
d) Plants grown on nitrate, complete, or ammonia culture solution.	Shoots; roots	One-dimensional

TABLE 29

AMINO ACIDS AND AMIDES OF SHOOTS OF TOMATO PLANTS ANALYSED BY TWO-DIMENSIONAL PAPER CHROMATOGRAPHY

Values are expressed as micrograms of amino nitrogen per gram fresh weight and each value is the mean of two analyses on each of three samples.

Culture solution	Nitrate	Ammonia			L.S.D.
	3.6×10^{-3}M	3.6×10^{-3}M	7.2×10^{-4}M	4×10^{-4}M	at 5% level
Amino acid or amide					
Aspartic acid	5.0	4.8	2.8	4.6	N.S.
Glutamic acid	24.1	35.9	36.6	37.8	6.8
Serine	8.8	23.1	20.3	19.6	6.1
Glycine	4.8	5.3	3.8	5.2	N.S.
Threonine	2.8	3.3	1.7	2.8	N.S.
Alanine	9.8	9.7	7.0	9.1	N.S.
γ- amino butyric acid	3.7	10.6	6.9	13.1	3.6
Asparagine	2.2	46.0	40.0	36.6	8.8
Glutamine	36	577	451	465	80

Glutamine was present in far greater amounts than asparagine in all plants and the concentrations of both amides in the shoots of plants grown on all concentrations of ammonium nitrogen were higher than in the plants grown on nitrate nitrogen. Glutamic acid, serine and γ- amino butyric acid concentrations were also higher in plants supplied with ammonium nitrogen. Samples of the extracts from plants grown on 4×10^{-4}M ammonium nitrogen and from plants grown on 3.6×10^{-3}M nitrate nitrogen were analysed for amino acids and amides using 150 cm and 20 cm resin columns (Methods, 5). In general, the amino acids present and their concentrations agreed (Table 30) with the results from paper chromatography. The higher values obtained for aspartic acid and glutamic acid using this technique rather than paper chromatography on extracts from ammonium grown plants were probably due to breakdown of asparagine and glutamine on the column releasing the two acids. Proline, which was not measured on paper chromatograms, was also determined on the 20 cm column.

Although only two samples were analysed, the concentration of proline in shoots of plants grown on ammonium nitrogen was nearly five times greater than in plants grown on nitrate nitrogen.

Results of analyses of amino acids in shoots and roots of tomato plants from the experiment described in Part III, 5, are shown in Table 31. Glutamine plus serine values were high in plants grown on ammonium nitrogen alone. From the values for serine and glutamine in tomato plants shown in Table 29 it is likely that much of this was glutamine. Asparagine values are also higher in plants supplied with ammonia culture solution as compared

TABLE 30

AMINO ACIDS AND AMIDES OF SHOOTS OF TOMATO PLANTS ANALYSED BY ION EXCHANGE CHROMATOGRAPHY

Values are expressed as micrograms of amino nitrogen per gram fresh weight and each value is the result of one analysis on one extract.

Culture solution	Nitrate 3.6×10^{-3}M	Ammonia 4.0×10^{-4}M
Amino acid or amide		
Aspartic acid	6.9	6.0
Glutamic acid	23.9	47.8
Glycine	3.4	4.2
Threonine	3.4	3.9
Alanine	9.6	13.0
γ-amino butyric acid + phenylalanine + tyrosine	3.3	13.9
Amides + serine	low	high
Proline	1.2	5.8

TABLE 31

AMINO ACIDS AND AMIDES OF SHOOTS AND ROOTS OF TOMATO PLANTS GROWN ON DIFFERENT SOURCES OF NITROGEN

Values are expressed as micrograms of amino nitrogen per gram fresh weight. Values for shoots are the means of analyses on 2 samples and for roots on 1 sample.

Culture solution	Ammonia $3.6x10^{-3}M$	Complete	Complete changed	Nitrate + Ammonia $2x10^{-3}$ + $2x10^{-3}M$	L.S.D. at 5%
			Shoots		
Amino acid or Amide.					
Glutamic acid + threonine	55.4	45.1	38.7	48.3	N.S.
Alanine	24.4	20.4	15.5	23.5	N.S.
Valine	6.7	7.1	6.8	7.0	N.S.
γ - amino butyric acid	14.1	5.0	6.0	6.9	3.2
Leucine	3.3	2.3	2.2	2.6	N.S.
Glutamine + serine *	51.3	13.3	6.7	16.7	12.9
Asparagine	42.3	10.6	8.2	14.9	19.4
			Roots		
Glutamic acid + threonine	28.1	14	36.4	27.4	
Alanine	3.4	4.9	4.7	4.0	
Valine	6.2	6.9	8.2	8.2	
γ - amino butyric acid	4.5	4.1	4.1	4.0	
Leucine	4.5	2.5	1.8	2.3	
Glutamine + serine *	19.2	5.0	12.2	5.9	
Asparagine	48.0	Trace	Trace	22.2	

* These values are expressed as optical density readings produced by the amount of amino nitrogen from one gram of fresh plant material (See Methods, 4).

to plants supplied with nitrate or a mixture of nitrate and ammonium nitrogen.

Glutamine plus serine values are again higher in roots, stems and leaves of plants supplied with ammonium nitrogen than in plants supplied with nitrate nitrogen (Tables 32 and 33). In these plants the amino acids are generally present at higher concentrations in the plants grown on ammonium nitrogen than in plants supplied with nitrate nitrogen although in the roots from one set of plants the reverse is the case (Table 33).

Discussion.

The high levels of amide in the tomato plants grown on ammonia culture solution described above is not surprising in view of the findings of other workers (Clark, 1936; Steward et al., 1959; Woolhouse, 1959; Margolis, 1960). The increased concentrations of glutamic acid in plants grown on ammonium culture solution as compared to plants grown on nitrate culture solution are similar to those found by Margolis (1960) and these are not surprising when one considers the important position of glutamic acid in the incorporation of inorganic nitrogen into plant compounds (Yemm and Folkes, 1958). Regarding the interconversion of glutamic acid, there was a suggestion that higher proline concentrations accompanied high glutamic acid concentrations in the tomato plants grown on ammonia culture solution. The level of free arginine was too low to be detected either by paper chromatographic or ion exchange methods.

TABLE 32

AMINO ACIDS AND AMIDES OF ROOTS, STEMS AND LEAVES OF TOMATO PLANTS GROWN ON NITRATE OR AMMONIA CULTURE SOLUTION

Values are expressed as micrograms of amino nitrogen per gram fresh weight. Each value is the mean of analyses on two samples.

Culture solution	Nitrate	Ammonia		Nitrate	Ammonia	
	Roots			Stems		
Amino acid or amide			L.S.D. 5% level			L.S.D. 5% level
Glutamic acid + threonine	10.0	11.9	N.S.	12.1	31.7	N.S.
Alanine	1.5	2.5	N.S.	3.6	6.0	N.S.
Valine	2.1	2.6	N.S.	-	-	
γ - amino butyric acid	2.9	4.6	N.S.	3.6	5.6	N.S.
Leucine	0.6	0.9	N.S.	-	-	
Glutamine + serine*	1.9	18.9	10.2	5.0	16.2	6.9

	Leaves		
	Nitrate	Ammonia	L.S.D. 5% level
Glutamic acid + threonine	40.6	92.7	N.S.
Alanine	21	39.2	N.S.
Valine	4.3	3.5	N.S.
γ - amino butyric acid	4.8	15.7	5.4
Leucine	1.7	4.1	N.S.
Glutamine + serine*	6.4	87.8	43

* These values are expressed as optical density readings produced by the amount of amino nitrogen from one gram of fresh plant material (See Methods, 4).

TABLE 33

AMINO ACIDS AND AMIDES OF ROOTS AND SHOOTS OF TOMATO PLANTS GROWN ON NITRATE, COMPLETE OR AMMONIA CULTURE SOLUTIONS

Values are expressed as micrograms of amino nitrogen per gram fresh weight. Each value is the mean of analyses on 2 samples.

Culture solution	Nitrate	Complete	Ammonia	L.S.D. at 5% level.
		Shoots		
Amino acid or amide.				
Glutamic acid + threonine	11.4	8.6	20	2.2
Alanine	1.7	1.4	5.0	0.2
Valine	2.3	1.5	2.8	N.S.
γ - amino butyric acid	1.3	1.3	3.0	0.7
Phenylalanine	1.2	1.0	4.8	1.8
Leucine	0.4	0.3	1.4	0.5
Glutamine + serine *	0.7	1.0	17.9	0.5
Asparagine	Trace	Trace	36.0	
		Roots		
Glutamic acid + threonine	21.0	6.8	9.9	0.7
Alanine	3.2	0.9	1.0	1.5
Valine	5.8	2.9	2.3	1.4
γ - amino butyric acid	3.9	1.1	1.4	1.2
Phenylalanine	4.4	2.4	3.5	N.S.
Leucine	1.4	0.9	1.1	N.S.
Glutamine + serine *	1.9	0.8	4.7	1.3
Asparagine	Trace	Trace	11.0	

* These values are expressed as optical density readings produced by the amount of amino nitrogen from one gram of fresh plant material (See Methods, 4).

The higher concentrations of γ- amino butyric acid found in tomato plants grown on ammonium nitrogen are interesting. Yemm and Folkes (1958) and Burris (1959) point out that evidence suggests that this acid is produced by decarboxylation of glutamic acid in some plants and Yemm and Folkes (1958) indicate that this occurs particularly under conditions of partial anaerobiosis. Vines and Wedding (1960) showed that the oxidation of reduced diphosphopyridine nucleotide was inhibited by ammonia. It is possible that the high levels of ammonium nitrogen reported in Part IV, 2, are producing the same effect as lack of oxygen and thus allowing increased decarboxylation of glutamic acid to form γ- amino butyric acid.

As there is a high soluble nitrogen to protein nitrogen ratio in plants grown on ammonium nitrogen perhaps the synthesis of protein in these plants is not as rapid as in plants grown on nitrate nitrogen. However, the levels of protein nitrogen, expressed on a dry weight basis, are as high or higher in plants grown on ammonium nitrogen than in plants grown on nitrate nitrogen (Tables 7 and 8). Expressed on a per plant basis, protein nitrogen became higher in plants growing on nitrate nitrogen at the same time as the differences in fresh weights between the two groups became apparent (Table 25). It is not known therefore whether lower protein values for a whole plant grown on ammonia culture solution are merely the result of these plants being smaller than plants grown on nitrate culture solution or whether a lower protein synthesis causes the plants to be smaller.

The amino acids and amides detected by paper chromatography and ion exchange chromatography in tomato plants grown on ammonium nitrogen were in most cases present at higher or similar concentrations to those in plants grown on nitrate nitrogen. There seemed to be ample precursors for protein synthesis unless one or more of the amino acids necessary for protein synthesis, but not detected by chromatography, were limiting. This would be possible if the amino acids participate directly in the synthesis of proteins as suggested by Folkes (1959), and current theories and evidence on protein synthesis largely substantiate this view (Lipmann, 1958).

Nicholas (1959) discussing evidence available on the reduction of nitrate in plant tissues comes to the conclusion that "the inorganic reductive route is physiologically important in plants" and that there is no evidence for an organic reductive route. Therefore it seems most unlikely that supplying ammonium nitrogen rather than nitrate nitrogen to tomato plants would provide a form of nitrogen unsuitable for the formation of any amino acids necessary for protein synthesis. The effect of the high ammonium nitrogen levels in tomato plants grown on ammonia culture solution on the enzymes involved in the synthesis of individual amino acids is not known.

From the above data, there is no evidence to suggest that low concentrations of one or more amino acids in tomato plants grown on ammonium nitrogen were limiting the growth of these plants and causing the differences in dry weight between plants grown on either nitrate or ammonium nitrogen.

2 AMINO ACID COMPOSITION OF PROTEINS OF LEAVES

Introduction.

Amino acid compositions of proteins of leaves have been determined by various workers (Smith and Agiza, 1951; Kelley and Baum, 1953; Yemm and Folkes, 1953; Bryant and Fowden, 1959). Small differences in amino acid composition between proteins of leaves of different ages were found in some cases. Schutte and Schendel (1958) have reported differences in amino acid composition of entire plant protein from bean plants grown on solutions deficient in micronutrient elements. Weismann(1959) determined the amino acid composition of proteins of 4 day old wheat seedlings grown on either 0.01M nitrate or 0.01M ammonium or 0.005M nitrate plus 0.005M ammonium nitrogen in the dark. The protein of plants grown on nitrate and on nitrate plus ammonium nitrogen contained a higher percentage of arginine and a lower percentage of lysine. Weismann suggested that perhaps "nitrate stimulated the formation of an enzyme, possibly a nitrate reducing system, with high arginine and low lysine content".

It was decided to extract proteins from leaves of plants grown on either nitrate or ammonia culture solution and determine the amino acid composition of these proteins.

Materials and Methods.

Tomato plants were grown in 18 culture vessels containing nitrate and in 18 culture vessels containing ammonia culture solution. The solutions were maintained at pH 6.0-7.0 by additions of 0.02M sodium

hydroxide or 0.02M hydrochloric acid when necessary. On the 23rd day the plants were harvested, and fresh weights were determined. Twenty four plants from each treatment were harvested and laminae of the second and third leaves (in order of development) of each plant were removed and bulked to give three samples from each treatment. Proteins were immediately extracted from the samples, and, after hydrolysis of the proteins, their amino acid compositions were determined using ion exchange columns. (Methods, 5).

Results and Discussion.

Total fresh weights of the four plants in each culture and the mean fresh weights of the leaf laminae from which protein was extracted are given in Table 34. Plants grown on nitrate culture solution again showed greater fresh weights than plants grown on ammonia culture solution.

When the plants which were grown on nitrate culture solution were harvested, the fifth leaf was developing rapidly. In plants grown on ammonia culture solution this leaf was only just visible. The 2nd and 3rd leaves were chosen for extraction of protein because they were more comparable in size and stage of development in plants grown on different forms of nitrogen than the 4th or 5th leaves. The first leaf was not used as this leaf had appeared before the plants were planted out onto the different solutions. The fresh weights of the leaf laminae used for protein extraction of plants grown on nitrate nitrogen were still higher than those of plants grown on ammonia culture solution (Table 34).

TABLE 34

FRESH WEIGHTS OF TOMATO PLANTS AND THE 2ND AND 3RD LEAF LAMINAE OF PLANTS GROWN ON NITRATE OR AMMONIA CULTURE SOLUTION AT pH 6.0-7.0

Plants were harvested on the 23rd day. Each plant value is the mean yield of 18 cultures. Each culture contained 4 plants which were weighed together. The 2nd and 3rd leaf laminae from 3 sets of 24 plants were bulked in each treatment and each fresh weight value is therefore the mean of 3.

Culture solution	Nitrate	Ammonia	L.S.D. at 5% level
Whole plants. (Fresh weight in g/4 plants).	5.31	3.22	0.47
2nd and 3rd leaf laminae. (Fresh weight in g/24 plants).	4.58	3.32	0.17

The amino acid composition of the protein extracted from the leaves ven in Table 35. The values for each amino acid are of the same r of magnitude as those of other leaf proteins quoted by other workers ibnall, 1939; Weller, 1957; Bryant and Fowden, 1959). The differences in glycine and methionine content of proteins from plants grown on different nitrogen sources were barely significant. The difference in the percentage of ammonia nitrogen between the two treatments was not accompanied by differences in glutamic or aspartic acid content of the protein.

It would seem that the amino acid compositions of the mixtures of proteins extracted from leaves of plants grown on either nitrate or ammonium culture solution were almost identical. Of course any difference in concentration of a protein present in the leaves in small amounts would not necessarily cause a detectable change in the amino acid composition of the mixture of proteins in the leaves. Also different proteins can have similar amino acid compositions so that changes in proportions of these would not be reflected in the amino acid compositions of mixtures of these proteins.

From these results it is obvious that the proportions of amino acids (except cystine and tryptophane which were not determined) incorporated into protein during protein synthesis, are similar in leaves of plants grown on nitrate culture solution or ammonia culture solution. If low concentrations of an amino acid are limiting protein synthesis, although this seems unlikely from the previous experiment, it can be seen that it is not reflected in the amino acid composition of the proteins of the leaves.

TABLE 35

AMINO ACID COMPOSITION OF PROTEINS OF THE 2ND AND 3RD LEAF LAMINAE OF TOMATO PLANTS GROWN ON EITHER NITRATE OR AMMONIA CULTURE SOLUTION AT pH 6.0-7.0

Values are expressed as grams of amino acid nitrogen per 100 grams total protein hydrolysate nitrogen. Each value is the mean of duplicate analyses on each of three samples.

Culture solution.	Nitrate	Ammonia	L.S.D. at 5% level
Amino acid.			
Aspartic acid	5.75	5.78	N.S.
Threonine	3.20	3.33	N.S.
Serine	3.52	3.46	N.S.
Glutamic acid	6.58	6.41	N.S.
Glycine	6.11	5.80	0.25
Alanine	6.11	5.88	N.S.
Valine	4.45	4.12	N.S.
Methionine	0.95	1.13	0.10
Isoleucine	3.28	3.40	N.S.
Leucine	6.15	6.10	N.S.
Tyrosine	2.16	2.20	N.S.
Phenylalanine	2.84	3.06	N.S.
Proline	4.38	4.35	N.S.
Lysine	7.69	7.58	N.S.
Histidine	3.39	3.19	N.S.
Ammonia	11.44	9.32	1.43
Arginine	12.69	12.63	N.S.
N accounted for	90.86	87.74	
% N in isolated protein sample	16.8	16.6	N.S.

3 THE INCORPORATION OF ^{15}N INTO PROTEINS OF SUBCELLULAR FRACTIONS OF LEAVES OF TOMATO PLANTS SUPPLIED WITH ^{15}N LABELLED POTASSIUM NITRATE OR AMMONIUM CHLORIDE

Introduction.

Although the bulk proteins of leaves of plants grown on either nitrate or ammonium nitrogen showed almost similar amino acid compositions it is still possible that the synthesis of one or more proteins in the plants grown on ammonium nitrogen was limited. This could, of course, be any one of a number of proteins. To determine whether any protein or group of proteins associated with a particular fraction within leaf cells were synthesised at a different rate in plants grown on ammonia culture solution than in plants grown on nitrate culture solution, the incorporation of ^{15}N into proteins of the cell fractions from either labelled ammonium chloride or labelled potassium nitrate was determined.

Previous work has been concerned with incorporation of ^{15}N into various nitrogen fractions of plants rather than into subcellular fractions. (Vickery *et al.*, 1940; MacVicar and Burris, 1948; Mendel and Visser, 1951; Delwiche, 1951; Cocking, 1956; Yemm and Willis, 1956).

Although proteins can become labelled without net synthesis (Yemm and Folkes, 1958), protein synthesis in rapidly growing tomato plants would be expected to be active and much of the incorporation of ^{15}N into the leaf proteins could be due to the synthesis of new protein.

Materials and Methods.

Tomato plants were grown in sixteen culture vessels containing complete culture solution (Methods, 1). On the twenty-fourth day the solutions in eight of the culture vessels were replaced by culture solution containing ^{15}N labelled ammonium chloride and in the other eight by culture solution containing ^{15}N labelled potassium nitrate (Table 36). The ammonium chloride contained 99 atom per cent. excess ^{15}N and the potassium nitrate contained 96.2 atom per cent. excess ^{15}N. The solutions were maintained in the pH range 5.5-6.5 by additions of 0.02M sodium hydroxide or 0.02M hydrochloric acid twice daily. Forty eight and 96 hours after supplying the ^{15}N, plants were harvested and, with a mass spectrometer (Methods, 6), the ^{15}N abundance was determined in the proteins of subcellular fractions separated out from leaf laminae. Leaf material from eight plants was bulked in each case giving two replicate samples in each treatment at each harvest.

Results and Discussion.

The mean fresh weights of leaf laminae obtained from plants on each treatment at the two harvests are given in Table 37. The ^{15}N abundance in the proteins precipitated out from the subcellular fractions of the leaves is shown in Table 38 and in all cases the isotopic abundance was slightly higher in the extracted proteins from plants grown on nitrate nitrogen as compared to proteins extracted from plants grown on ammonium nitrogen. The values for soluble nitrogen, which included inorganic nitrogen, amide nitrogen, amino nitrogen, peptide nitrogen, and any other nitrogen compounds

TABLE 36

COMPOSITION OF CULTURE SOLUTIONS CONTAINING ^{15}N LABELLED POTASSIUM NITRATE OR AMMONIUM CHLORIDE

The concentrations of microelements were as described above (TABLE 1).

Culture solution	Nitrate	Ammonia
	Concentration of salt ($M \times 10^{-4}$)	
Salt		
KCl	4.5	20
$K^{15}NO_3$	15.5	-
$CaCl_2$	6	6
$MgSO_4$	4	4
NaH_2PO_4	4	4
$^{15}NH_4Cl$	-	15.5

TABLE 37

MEAN FRESH WEIGHTS OF LEAF LAMINAE FROM EIGHT PLANTS FED EITHER LABELLED AMMONIUM CHLORIDE OR POTASSIUM NITRATE

Each value is the mean of two.

Culture solution.	Ammonia	Nitrate
Harvest 1 (48 hours)	19.53 g	19.25 g
Harvest 2 (96 hours)	25.95 g	23.45 g

TABLE 38

ATOM PER CENT. EXCESS ^{15}N OF PROTEINS SEPARATED FROM SUBCELLULAR FRACTIONS OF LEAVES OF TOMATO PLANTS SUPPLIED WITH ^{15}N LABELLED POTASSIUM NITRATE OR AMMONIUM CHLORIDE

Each value is the mean of one analysis on each of two samples.

	48 hours			96 hours		
	$^{15}NH_4Cl$ fed plants	$K^{15}NO_3$ fed plants	L.S.D. at 5% level	$^{15}NH_4Cl$ fed plants	$K^{15}NO_3$ fed plants	L.S.D. at 5% level
Mitochondria	12.56	15.07	N.S.	23.61	28.19	N.S.
Chloroplasts	11.08	14.24	N.S.	22.33	28.51	3.37
Soluble Proteins (i.e. proteins not ppt at 20,000 g)	10.69	12.48	N.S.	20.26	27.14	2.85
Soluble Nitrogen	19.59	33.93	8.78	36.43	30.36	N.S.

not precipitated by heating at pH 4.5, were high in the leaves of plants grown on nitrate nitrogen after 48 hours. After 96 hours the isotopic abundance in the soluble nitrogen of leaves of plants grown on ammonium nitrogen was greater than that of leaves of plants grown on nitrate nitrogen. The ^{15}N abundance in the soluble nitrogen of leaves of plants grown on ammonium nitrogen may not have been a true estimate of ^{15}N due to a possible loss of ammonium nitrogen from the extracting medium, pH 7.4.

These results suggest that the rate of synthesis of protein in leaves of plants supplied with ammonium nitrogen was slower than in plants supplied with nitrate nitrogen. However the relative rates of incorporation of ^{15}N into chloroplasts, mitochondria, and the remaining proteins seemed to be similar whether plants were supplied with ammonium nitrogen or nitrate nitrogen. Therefore it seems unlikely that the synthesis of one major protein or one major group of proteins was being particularly affected by the type of culture solution supplied to the plants. If any significant effect was present in plants supplied with ammonium nitrogen it was an effect on the protein in all the fractions tested rather than on any one particular fraction.

Summary.

Concentrations of individual amino acids and amides which were present in quantities measurable by the techniques used were generally higher in plants grown on ammonia culture solution than in plants grown on

nitrate culture solution or nitrate plus ammonia culture solution. Amino acid compositions of leaf proteins of plants grown on either ammonia culture solution or nitrate culture solution were almost identical. Studies on the incorporation of ^{15}N from ^{15}N labelled ammonium chloride or ^{15}N labelled potassium nitrate into protein of subcellular fractions of leaves suggested that the synthesis of proteins was generally lower in plants grown on ammonia culture solution than in plants grown on nitrate culture solution. From these observations, it seemed unlikely that the synthesis of any major protein was limited in plants grown on ammonia culture solution by insufficient concentrations of one or more amino acids.

PART VI

LEVELS OF ORGANIC ACIDS, CARBOHYDRATES, RIBONUCLEIC ACID, DEOXYRIBONUCLEIC ACID, NICOTINAMIDE ADENINE DINUCLEOTIDE, AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE IN TOMATO PLANTS GROWN ON EITHER NITRATE, AMMONIUM OR NITRATE AND AMMONIUM NITROGEN

1 ORGANIC ACIDS

Clark (1936) found that the concentration of organic acids in tomato plants grown on ammonium nitrogen was less than in plants grown on nitrate nitrogen. The concentration of oxalic acid was lower in leaves of tung plants grown on nitrate plus ammonium nitrogen than in plants grown on nitrate alone (Gilbert *et al*., 1951). Wadleigh and Shive (1939), Bershtein and Okanenko (1953), and Skvortsova (1955), found lower levels of organic acids in plants grown in the presence of ammonium nitrogen than in plants grown on nitrate nitrogen. It was decided to determine the level of total organic acids in shoots of tomato plants grown on either nitrate, ammonium or nitrate plus ammonium nitrogen.

Materials and Methods.

Dried plant material from the shoots of plants grown on different sources of nitrogen (described in Part IV, 1) and from the shoots of plants grown in the pH range 5.5-8.5 (described in Part III, 2) were analysed for total organic acids (Methods, 3).

Results and Discussion.

The results are shown in Table 39. Higher values of organic acids were present in the plants grown on nitrate culture solution than in the plants grown on ammonia culture solution or on nitrate plus ammonia culture solution. The lower concentrations of cations and higher concentrations of anions in plants grown on ammonium nitrogen or nitrate plus ammonium nitrogen than in plants grown on nitrate nitrogen as shown in Table 27 would possibly account for part of this difference.

Ulrich (1941), Jacobson and Ordin (1954), and Jackson and Coleman (1959) have shown that a greater uptake of cations than anions is accompanied by increases in organic acid levels in excised roots. Moreover the high amide levels in plants supplied with ammonium nitrogen (see Parts IV and V) would probably have caused a reduction in the level of organic acid due to utilization of the α-keto acids in amide synthesis.

It has been shown (Table 23) that ammonium nitrogen levels were higher in plants grown on ammonia culture solution than in plants grown on nitrate culture solution or nitrate plus ammonia culture solution. Vines and Wedding (1960) have demonstrated that in the presence of ammonium nitrogen respiration is inhibited in a number of plant tissues and in isolated beet mitochondria. If, in ammonia grown plants, the concentration of ammonium nitrogen decreases the rate of respiration, this may, by decreasing tricarboxylic acid cycle activity, reduce the supply of organic acids from this source.

However the lower levels of organic acids also found in plants grown on nitrate and ammonium nitrogen, where the level of ammonium nitrogen in

TABLE 39

TOTAL ORGANIC ACIDS OF SHOOTS OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM, OR NITRATE PLUS AMMONIUM NITROGEN

Values are expressed as milliequivalents of organic acid per gram dry weight of plant material. Each value is the mean of 3 determinations.

Culture solution.	Nitrate	Ammonia	Nitrate plus ammonia	L.S.D. at 5% level
Total organic acids (m-equiv./g dry wt. of shoot).	1.54	1.00	0.81	0.42

the plant tissues was low, would suggest that the level of organic acids was largely influenced by the presence of ammonium nitrogen in the external solution. Moreover the fact that these plants grown on nitrate plus ammonium nitrogen, although possessing a lowered organic acid content, still produce as much dry weight as those plants grown on nitrate nitrogen alone, indicates that the decreased levels of organic acids does not directly influence the reduced growth of plants grown on ammonium nitrogen.

2 CARBOHYDRATES

Introduction.

Tiedgens (1934) found lower amounts of starch in tomato plants and Sideris et al.(1938) found lower concentrations of sugars in pineapple plants grown on ammonium nitrogen than in plants grown on nitrate nitrogen. Sideris and Young (1944) showed a lower concentration of starch in pineapple plants grown on ammonium nitrogen as compared to plants grown on nitrate nitrogen. Takahashi and Yoshida (1957a) report that carbohydrate synthesis was reduced in tobacco plants grown on ammonium nitrogen. Also Woolhouse (1959) observed a prominent white layer of starch in precipitates after centrifugation of homogenates of leaves from plants grown on nitrate nitrogen. No starch was observed from leaves of plants grown on ammonium nitrogen. Krogmann et al. (1959) showed that ammonium chloride uncoupled photosynthetic phosphorylation in isolated spinach leaf chloroplasts. It

was shown (Table 23) that the level of ammonium nitrogen in shoots of plants grown on ammonia culture solution was higher than in plants grown on nitrate culture solution or culture solution containing nitrate and ammonium nitrogen. The higher levels of ammonium nitrogen may have had an effect on the rate of photosynthesis in plants grown on ammonia culture solution. Therefore it was decided to determine the levels of total carbohydrates in shoots and roots of tomato plants grown on different sources of nitrogen.

Materials and Methods.

Samples of dried plant material (0.5 g) from roots and shoots of plants grown on different sources of nitrogen (described in Part IV, 1) were used for total carbohydrate analyses (Methods, 3).

Results and Discussion.

The results of the analyses are shown in Table 40. No differences in concentrations of total carbohydrates were evident. When centrifuging leaf homogenates a layer of starch, similar to that reported by Woolhouse (1959), was observed in all precipitates of leaf homogenates irrespective of whether the homogenates were obtained from plants grown on nitrate culture solution or from plants grown on ammonia culture solution. From the results of this preliminary study it seems unlikely that a lack of carbohydrate was limiting the growth of plants grown on ammonia culture solution.

TABLE 40

TOTAL CARBOHYDRATES OF ROOTS AND SHOOTS OF TOMATO PLANTS GROWN ON NITRATE, AMMONIUM, OR NITRATE PLUS AMMONIUM NITROGEN

Values are expressed as mg of carbohydrate per g of dried plant material. Each value is the mean of 3 determinations.

Culture solution.	Nitrate	Ammonia	Nitrate plus ammonia.
	Shoots		
Total carbohydrate (mg/g dry wt. of plant material)	22.1	22.1	21.0
	Roots		
Total carbohydrate (mg/g dry wt. of plant material)	10.3	13.4	9.9

3 RIBONUCLEIC ACID AND DEOXYRIBONUCLEIC ACID IN ROOT TIPS

Introduction.

The work of Ts'o (1958), Hanson (1960) and West and Hanson (1960) has suggested that the ratio of divalent to monovalent cations is important in the control of the degradation of the ribonucleoprotein (RNP) granules of the endoplasmic reticulum. In Part IV, 3, it was observed that the ratio (Ca^{++} + Mg^{++}) : K^{+} was lower in the roots of plants grown on ammonium nitrogen (0.14) than in plants grown on nitrate nitrogen (0.22). In roots of plants grown on complete culture solution this ratio (0.11) was similar to that in plants grown on ammonia culture solution; however, the higher concentration of the monovalent cation, NH_4^+, in ammonia grown plants would decrease the divalent : monovalent cation ratio. This lower ratio may have led to the breakdown of the RNP granules of the roots. RNP granules are involved in protein synthesis (Bonner, 1959). It was therefore decided to determine the levels of RNA in RNP granules of roots of plants grown on either nitrate, nitrate plus ammonia, or ammonia culture solution. The tips of tomato roots were chosen for a study of the RNA levels in the RNP granules as root tips generally contain high levels of RNA (Ogur and Rosen, 1950). RNA and DNA in other subcellular fractions of the root tips were also determined.

Materials and Methods.

Tomato plants were grown in 9 litre plastic buckets containing either 3.6×10^{-3}M nitrate culture solution or 3.6×10^{-3}M ammonia culture solution.

Sixteen plants were grown in each bucket. Each solution was maintained at pH 6.0-7.5 by the addition of sodium hydroxide or hydrochloric acid when necessary. In a later experiment plants were grown similarly except that complete culture solution was also used. On about the 20th day terminal segments (1.0 cm) were cut from the larger roots. Root tips from 32 plants were combined giving one sample from each treatment. RNA was determined in the RNP granules, mitochondria, and supernatant. Total protein nitrogen was determined (Methods, 7). In the later experiment this procedure was repeated except that 0.5 cm terminal segments were used and the total RNA and DNA concentration in each sample was determined. Owing to the time required to prepare samples containing a sufficient number of terminal root tip segments, it was not practicable to run replicates.

Results and Discussion.

The results of the two experiments are shown in Table 41 and are expressed as RNA and DNA per g fresh weight or per mg total protein nitrogen of the root tips. Contrary to expectation the RNP granules of root tips from plants grown on ammonia culture solution contained slightly higher concentrations of RNA than plants grown on the other sources of nitrogen. As would be expected, the terminal 0.5 cm segments contained higher concentrations of RNA than the terminal 1.0 cm segments. Total RNA values were higher than the sum of the RNA from the RNP granules + mitochondria and soluble RNA. No determinations were made on the low speed precipitate.

TABLE 41

RIBONUCLEIC ACID AND DEOXYRIBONUCLEIC ACID IN ROOT TIPS AND SUBCELLULAR PARTICLES OF ROOT TIPS FROM TOMATO PLANTS GROWN ON NITRATE, AMMONIUM OR NITRATE PLUS AMMONIUM NITROGEN

Values are differences between the absorption at 260 and 290 mμ for RNA and 268 and 290 mμ for DNA of 5 ml of solution expressed per gram fresh weight or per milligram of protein nitrogen.

	Protein N (mg/g fresh wt.)	RNA $D_{260} - D_{290}$ of 5 ml of solution		DNA $D_{260} - D_{290}$ of 5 ml of solution	
		Per g fresh wt.	Per mg protein N.	Per g fresh wt.	Per mg protein N.
July 1960 Terminal cm					
Nitrate					
Root tip	1.44				
Mitochondria		1.22	0.85		
RNP granules		1.52	1.06		
Supernatant		0.40	0.28		
Ammonia					
Root tip	1.24				
Mitochondria		1.42	1.14		
RNP granules		2.62	2.12		
Supernatant		0.36	0.29		
September 1960 Terminal 0.5 cm					
Nitrate					
Root tip	3.49	19.10	5.47	2.24	0.64
Mitochondria		2.65	0.76		
RNP granules		7.04	2.02		
Supernatant		3.43	0.98		
Ammonia					
Root tip	3.16	18.11	5.72	1.89	0.60
Mitochondria		3.67	1.16		
RNP granules		8.75	2.77		
Supernatant		-	-		
Nitrate plus ammonia					
Root tip	3.33	21.79	6.55	2.24	0.67
Mitochondria		2.43	0.73		
RNP granules		7.07	2.13		
Supernatant		3.07	0.92		

Total RNA and DNA concentrations were of the same order irrespective of the source of nitrogen supplied to the plants. It is clear from these results that the lower divalent : monovalent cation ratio in ammonia grown plants was not affecting the degradation of the RNP granules in a way similar to that reported by Ts'o (1958), Hanson (1960) and West and Hanson (1960). West and Hanson (1960) reported a reduction by at least one third in the amount of RNA in isolated RNP granules in the presence of 0.1 M KCl.

4 NICOTINAMIDE ADENINE DINUCLEOTIDE AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE IN LEAVES

The results of Preiss and Handler (1958) and of Sundaram *et al.* (1960) show that the nicotinic acid pathway is normally responsible for the synthesis of NAD in yeast. In *Leuconostoc mesenteroides* the nicotinic acid pathway was found to be the only one operating in the synthesis of NAD as this organism could not utilize nicotinamide (Sundaram *et al.*, 1960). In the nicotinic acid pathway Preiss and Handler (1958) showed that nicotinic acid adenine dinucleotide is amidated by glutamine in the presence of adenosine triphosphate. If this pathway also operates in higher plants then the high levels of glutamine observed in tomato leaves (Part V, 1) may have stimulated the synthesis of NAD in these leaves.

Morton (1958) has discussed the role of NAD in the control of cell growth pointing out that low levels of NAD generally lead to increased

mitotic activity. Oide (1958) demonstrated that intrasplenic implantation of nicotinamide increased the levels of pyridine nucleotides and decreased the number of mitoses taking place in regenerating liver. Fujii and Mizuno (1958) showed that nicotinamide inhibited the growth of chick heart fibroblasts *in vitro*, and also inhibited cleavage of fertilized sea-urchin eggs. It was further shown that nicotinamide, when injected subcutaneously into methylcholanthrene-treated mouse skin, inhibited the epidermal hyperplasia which is so characteristic of the carcinogen-treated skin when no nicotinamide is injected. The authors concluded that "nicotinamide acts directly to inhibit mitosis, presumably by increasing the concentration of diphosphopyridine nucleotide in the cell".

If high NAD levels have the same effect on mitosis in tomato plants and if high glutamine levels lead to high levels of NAD in tomato plants, then it is possible that the reduced growth of tomato plants grown on ammonia culture solution as compared to the growth on nitrate culture solution could be due to a decrease in the rate of mitosis in these plants. As the increase in glutamine concentration resulting from growing tomato plants on ammonia culture solution as compared to nitrate culture solution was most marked in the leaves (Table 32), it was decided to determine whether the high concentration of glutamine in the leaves was also accompanied by increased concentrations of NAD and NADP.

Materials and Methods.

Tomato plants were grown on ammonia culture solution and nitrate culture solution in plastic buckets as described in the previous experiment.

Each solution was maintained in the pH range 6.0-7.5. On the 20th day plants were harvested, leaf laminae weighed and then quickly homogenised in cold TCA:H_2O_2 mixture. The extracts were purified and the concentrations of NAD and NADP determined (Methods, 8). Three separate samples of leaf material from each treatment were analysed.

Results and Discussion.

Values for NAD and NADP, expressed as mμmoles per gram fresh weight of leaf material, are shown in Table 42. There were no differences in concentrations of NAD or NADP in leaves of plants grown on nitrate culture solution or ammonia culture solution. Thus it appears that the high levels of glutamine in leaves of plants grown on ammonia culture solution were not affecting the levels of NAD in the leaves.

The concentrations of NAD and NADP determined in this experiment are twice as great as those reported by Anderson and Vennesland (1954) for spinach leaves and eight times greater than those reported by the same authors for tomato leaves. In agreement with these authors it can be seen that the concentration of NAD in the leaves was about the same as the concentration of NADP. In a previous determination using older tomato leaves the concentration of NADP was found to be slightly higher than the concentration of NAD (Table 6). However the ratio of NAD to NADP from the results of these experiments is still much higher than the earlier values given by Whatley (1951).

TABLE 42

NAD AND NADP CONTENT OF LEAVES OF TOMATO PLANTS GROWN ON EITHER NITRATE CULTURE SOLUTION OR AMMONIA CULTURE SOLUTION AT pH 6.0-7.5

Values are mμmoles of NAD or NADP per gram fresh weight. Each value is the mean of analyses on 3 separate leaf samples.

Culture solution	Nitrate	Ammonia	Significance at 5% level
NAD	32.7	29.3	N.S.
NADP	35.8	33.6	N.S.

Summary.

The concentrations of total carbohydrates in roots and shoots, total organic acids in shoots, and RNA and DNA in root tips of tomato plants grown on either nitrate culture solution, ammonia culture solution, or ammonia plus nitrate culture solution were determined. In addition the concentrations of NAD and NADP in leaves of plants grown on either nitrate culture solution or ammonia culture solution were determined. Carbohydrate and NAD and NADP concentrations were similar in plants grown on all types of solution. Organic acid concentrations were lower in plants grown on ammonia culture solution and ammonia plus nitrate culture solution than in plants grown on nitrate culture solution. RNA of the RNP granules was slightly higher in root tips from plants grown on ammonia culture solution than in root tips from plants grown on the other two types of solution.

PART VII

THE GROWTH OF HORDEUM VULGARE L., PISUM SATIVUM L., AND ATRIPLEX HORTENSIS L. ON NITRATE CULTURE SOLUTION AND AMMONIA CULTURE SOLUTION

Introduction

During the experiments carried out with tomato plants the growth of three other species viz. *Hordeum vulgare* L. (variety Prior), *Pisum sativum* L. (variety Greenfeast), and *Atriplex hortensis* L. (red foliage variety) was examined on nitrate culture solution and ammonia culture solution.

Materials and Methods.

Seeds of the above species were germinated in a similar way to the seeds of tomato (Methods, 1). The seedlings were planted out in 2 l. beakers as described for tomatoes on 3.6×10^{-3}M nitrate culture solution and 3.6×10^{-3}M ammonia culture solution. Four barley plants were grown in each beaker. One culture vessel of plants was grown at one of each of five pH ranges between pH 3.5 and 8.5 as in Part III, 2. The solutions were maintained at the required pH by additions of 0.02M hydrochloric acid or 0.02M sodium hydroxide. These plants were harvested on the 17th day and fresh weights of roots and shoots were determined.

The pea and Atriplex plants were grown three in each beaker on ammonia culture solution or nitrate culture solution which was maintained

in the pH range 6.0-7.5. Twelve Atriplex plants and nine pea plants were grown on each type of solution. The plants were harvested on the 25th day and fresh and dry weights of roots and shoots were determined.

Results and Discussion.

Fresh weights of the barley plants are shown in Table 43. Although there are insufficient data for any definite conclusions to be reached it would appear that growth was similar on both solutions at all pH ranges except on ammonia culture solution in the pH range 3.5-4.5 where the growth of barley plants was slightly lower. When these results are compared with the growth of tomato plants (Table 9) it can be seen that barley plants are not as sensitive to low pH as tomato plants and that the form of nitrogen supplied has little effect, if any, on growth.

The fresh and dry weights of roots and shoots of pea and Atriplex plants are shown in Table 44. Shoots of pea plants showed slightly greater growth on nitrate culture solution than on ammonia culture solution. The roots however were similar on each type of solution. Although only one pH range was tested it is likely, from the results of Pirschle (1931), that it was optimum for plants grown on ammonia culture solution. Pirschle also observed slightly greater growth of pea plants on nitrate culture solution than on ammonia culture solution.

The growth of Atriplex plants on ammonia culture solution was greatly reduced especially the growth of the roots. Here again only one pH range was tested.

TABLE 43

FRESH WEIGHTS OF BARLEY PLANTS GROWN ON NITRATE OR AMMONIA CULTURE SOLUTIONS AT pH RANGES BETWEEN 3.5 and 8.5

Each value is the mean weight in grams of four plants from one culture vessel when harvested on the 17th day.

	Nitrate $3.6x10^{-3}M$		Ammonia $3.6x10^{-3}M$	
pH Range	Shoot	Root	Shoot	Root
3.5-4.5	0.80	0.92	0.59	0.48
4.5-5.5	1.38	1.11	1.14	0.80
5.5-6.5	1.21	1.02	1.46	0.88
6.5-7.5	1.43	1.07	1.35	0.75
7.5-8.5	1.42	1.04	1.26	0.82

TABLE 44

FRESH AND DRY WEIGHTS OF *Pisum sativum* L. AND *Atriplex hortensis* L. GROWN ON NITRATE OR AMMONIA CULTURE SOLUTIONS IN THE pH RANGE 6.0-7.5

Values are expressed as mean weights in grams of plants harvested on the 25th day.

Species	No. of plants in each treat.		Shoot			Root		
			NO_3	NH_3	L.S.D. at 5% level	NO_3	NH_3	L.S.D. at 5% level
Pisum sativum	9	Fresh Wt.	10.23	7.41	2.67	9.33	10.31	N.S.
		Dry Wt.	1.13	0.81	0.22	0.50	0.50	N.S.
Atriplex hortensis	12	Fresh Wt.	6.78	3.35	1.23	4.58	0.77	0.34
		Dry Wt.	0.69	0.27	0.12	0.40	0.07	0.02

It is clear from these results that, under the conditions in these experiments, the influence of the form of nitrogen supplied to plants varies with the species. The growth of barley is not affected by the type of nitrogen supplied, pea plants grow slightly better on nitrate culture solution, and Atriplex plants produce much more growth on nitrate culture solution than on ammonia culture solution. It was unlikely that carbohydrates were limiting when the experiments were set up as the seedlings had been growing in bright light in the glasshouse. No further analyses were carried out on these plants so that data is not available on levels of elements or compounds within the tissues.

PART VIII

GENERAL DISCUSSION

It is apparent that tomato plants, growing in water culture, produce more dry weight when supplied with nitrate nitrogen than when supplied with only ammonium nitrogen. An examination (Part III) of a range of concentrations of ammonium nitrogen, of pH values and of chemical composition of culture solutions showed no situation in which tomato plants grew as rapidly as on nitrate nitrogen. In addition, tomato plants which were supplied with a mixture of nitrate and ammonium nitrogen produced as much dry weight as those supplied with nitrate nitrogen alone.

As the chemical composition of the plant is a reflection of metabolism, a comparative study was made of the concentrations of a number of elements and compounds in plants grown on ammonium nitrogen or on nitrate nitrogen. In this way, differences peculiar to plants grown on ammonium nitrogen might become apparent. Such differences would only be significant if they could not be demonstrated in plants grown on ammonium plus nitrate nitrogen, since the latter situation afforded maximal tomato plant growth.

Nightingale (1948) suggested that the lower growth rates of plants grown on ammonium nitrogen could be explained by low light intensities

and consequent low carbohydrate levels in the plants. However, many of the experiments described in this thesis were carried out in bright sunlight in a glasshouse where light was definitely not limiting growth. Further, analyses of carbohydrate content of ammonium and of nitrate grown plants (Part VI) showed no significant differences.

It seemed possible that low levels of nutrients in the culture solutions or in the plant tissues could contribute to the lower growth rate of plants grown on only ammonium nitrogen as the sole nitrogen source. However, alteration of the composition of the culture medium did not reduce depression of growth. Estimation of elements present in the two types of tissues did show that certain cations, sodium, calcium, magnesium and manganese, were lower in plants grown on ammonium nitrogen, but the same elements were present in similarly low concentrations in plants grown on ammonium plus nitrate nitrogen.

Since tomato plants achieve equal growth on nitrate nitrogen or on mixtures of nitrate and ammonium nitrogen, Woolhouse (1959) suggested that ammonium ions are not toxic to these plants. He postulated that it is the absence of nitrate nitrogen rather than the presence of ammonium ions within the tissues that limits the growth of tomato plants on ammonia culture solution. The results of experiments described in Parts III and IV show that, in agreement with Woolhouse, the lower growth of plants on ammonia culture solution is caused by the absence of nitrate from the culture medium. Plants grown on solutions containing similar

amounts of ammonium nitrogen as in the ammonia culture solution but with nitrate added produce more dry weight than plants grown on ammonium nitrogen alone. Morphological changes, depression of root hair formation (Figures 16 and 17) and the appearance of white patches on mature leaves (Figures 9 and 10), were observed only in plants grown with ammonia as the sole nitrogen source.

Thus the presence of ammonium nitrogen in culture solutions has no deleterious effect on growth if nitrate nitrogen is also present in the growth medium. The conditions in the external solution are, however, not necessarily a measure of the situation within the plant. Woolhouse did not measure the absorption of nitrate or ammonium nitrogen from culture solutions because he recorded that ammonium nitrogen was lost to the atmosphere from these solutions. However, in the present work, no such loss of ammonium nitrogen was detected from aerated culture solutions even over periods of two weeks. In addition, culture solutions in which plants were growing ceased to lose ammonium nitrogen as soon as the plants were removed. Thus it was possible, by analysing the culture solutions at various times during the experiment, to follow the absorption of nitrogen by the plants. Using this technique, it was shown (Part IV) that, in the presence of nitrate nitrogen, the uptake of ammonium nitrogen was depressed. Furthermore, it was shown that the levels of ammonium ions and of amides in plants supplied with only ammonium nitrogen were far greater than in plants supplied with a mixture of nitrate and ammonium nitrogen or with nitrate nitrogen alone. Thus the possibility still

exists that the higher levels of ammonium ions in plants grown on ammonium nitrogen are deleterious and cause reduced growth. This effect would be evident only in plants grown with ammonium ions as the sole nitrogen source and this is in agreement with the results of all growth experiments described.

Woolhouse (1959) suggested also that the concept of non-toxicity of ammonium ions was supported by the observation that plants grown on a low concentration of ammonium nitrogen (4.0×10^{-4}M) produced no more dry weight than did plants grown on 3.6×10^{-3}M ammonia culture solution. However, it has been shown (Table 19) that the uptake of ammonium nitrogen from culture solution containing 3.6×10^{-3}M or 1.8×10^{-3}M ammonium nitrogen was similar. Furthermore the levels of amides in tomato plants grown on solutions containing 3.6×10^{-3}M, 7.2×10^{-4}M or 4.0×10^{-4}M ammonium nitrogen were quite similar (Table 29). It was shown (Tables 23 and 24) that high levels of ammonium ions preceded the formation of large amounts of amide in tomato plants grown on 3.6×10^{-3}M ammonia culture solution. It is reasonable then to consider that, in plants grown on lower concentrations of ammonium nitrogen, since the amide levels were high, the ammonium ion concentrations were also high. Thus the evidence suggests that even if low concentrations of ammonium nitrogen are present in ammonia culture solutions, tomato plants absorb ammonium nitrogen and high levels of ammonium ions build up within the tissues. It is possible, then, that the reduced growth of tomato plants on ammonia culture solutions is caused by deleterious effects of ammonium ions within these plants.

Vines and Wedding (1960) have shown that ammonium ions inhibit respiration in a number of plant tissues, and in isolated beet mitochondria. As illustrated in Table 23, it is quite possible that the levels of ammonium ions present in tomato plants grown on ammonia culture solution were as high as those found by Vines and Wedding to cause such inhibition. The concentration of ammonium ions at specific sites within the cells of these tomato plants is, of course, unknown. If respiration is inhibited in plants grown on ammonia culture solution, the supply of organic acids might well be limited as a result of reduced tricarboxylic acid cycle activity. In such plants the lower concentrations of cations (Table 27) and the higher concentrations of amides (Table 24) could also result in decreased organic acid levels compared with those of plants grown on nitrate or on ammonia plus nitrate. Organic acid analyses (Table 39) showed that the levels of organic acids were lower in plants grown on ammonia culture solution than in plants grown on nitrate culture solution. However, similar low levels were found in plants grown on nitrate plus ammonia culture solution. It therefore seemed unlikely that differences in concentrations of total organic acids were affecting the growth rates of tomato plants on various forms of nitrogen. Quantitative analyses of the components of the organic acid fraction may reveal differences in the amounts of individual acids in plants grown on ammonia culture solution and in plants grown on nitrate plus ammonia culture medium. This has not been attempted in the present work but Clark (1936) recorded large differences in several of the organic acids (particularly citric, oxalic and

malic acids) in tomato plants grown on nitrate or ammonia culture solutions. Clark did not examine plants grown on nitrate plus ammonia culture solutions.

Krogmann *et al.* (1959) reported a further effect of ammonium ions on the metabolism of plants. Ammonium ions were found to uncouple photosynthetic phosphorylation in chloroplasts isolated from spinach leaves. This inhibition was reversible since, after the chloroplasts were washed, photosynthetic phosphorylation was restored almost to the control rate. These results may have some bearing on observations (Woolhouse, 1959) that the levels of organic phosphate in tomato plants, especially in young leaves, were lower in plants grown on ammonia culture solution than in plants grown on nitrate culture solution. It was suggested that the presence of nitrate possibly induced synthesis of an enzyme. However, the observed results could have been due to uncoupling of photosynthetic phosphorylation by high levels of ammonium ions in the tissues of plants grown on ammonium nitrogen. When nitrate nitrogen was fed to ammonia grown plants Woolhouse observed an increase in organic phosphate synthesis within 24 hours. It has been shown (Table 19) that, within a short time of transferring plants from ammonia culture to nitrate plus ammonia culture solution, the rate of uptake of ammonium nitrogen by the plants fell far below that of plants remaining in ammonia culture solution. It may be that in Woolhouse's experiment when nitrate was supplied to the ammonia culture solution, ammonium ion levels in the

tissues fell allowing photosynthetic phosphorylation to proceed at a greater rate than in plants not supplied with nitrate. When nitrate was supplied, the increased rate of organic phosphate synthesis was more pronounced in young leaves than in older leaves. Possibly the high levels of ammonium ions had a permanent effect on the older chloroplasts and did not permit maximum photosynthetic phosphorylation to occur even when the levels of ammonium ions fell. This type of effect may also explain the observation (Woolhouse, 1959) that the isolated chloroplasts from ammonia grown plants also showed a lower rate of photosynthetic phosphorylation. A possible correlation, within tomato plants, of high ammonium ion concentration and decreased efficiency of photosynthetic phosphorylation is purely a hypothesis. There seems to be no more evidence for an induced enzyme synthesis as suggested by Woolhouse (1959) than there is for an uncoupling of photosynthetic phosphorylation by high levels of ammonium ions within the tissues.

Traces of nitrate (Table 16) did not enhance growth of plants growing on ammonia culture solution but the levels supplied may have been insufficient. Considerably more evidence is needed before any conclusion can be drawn that the presence of ammonium ions or the absence of nitrate causes a reduction in the synthesis of organic phosphate.

Woolhouse further suggested that this difference in levels of organic phosphate reduced the levels of starch in plants grown on ammonium nitrogen. His evidence on starch concentrations was very limited and it

has been shown (Table 40) that the total non-cellulosic carbohydrates were similar in tomato plants grown on nitrate, nitrate plus ammonia, and ammonia culture solutions.

Determinations (Part V) of free amino acid and amide concentrations and of the amino acid composition of proteins suggest no reasons for the lower growth of plants on ammonia culture solution. There seems to be no imbalance of proteins in plants grown on such solution. As a percentage of the dry weight, ammonia grown plants contain as much, perhaps more, protein than plants grown on nitrate culture solution. On a per plant basis, plants grown on nitrate culture solution produce more protein than plants grown on ammonia culture solution (Tables 7 and 8). This may simply be an expression of the smaller size of the plants grown on ammonia culture solution and not due to an inhibition of synthesis of these compounds. Some of the precursors necessary for protein synthesis (amino acids and amides) were found to be present within the tissues of plants grown on ammonia culture solution at concentrations as high, or higher, than in plants grown on nitrate culture solution. The levels of free amino acids necessary for protein synthesis, but not detected by the chromatographic procedures employed in Part V, are unknown but there is no evidence to suggest that any one of them was limiting. Studies of the amino acid composition of leaf proteins and of incorporation of ^{15}N into subcellular fractions of leaves failed to show any striking differences between plants grown on different sources of nitrogen except an indication of a slightly lower rate of protein synthesis in plants supplied with ammonium nitrogen.

Analyses of RNA in root tips (Table 41) provided no evidence for differences in growth as observed on different forms of nitrogen.

The levels of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate in leaves of tomato plants (Table 42) are interesting in relation to previously reported values (Anderson and Vennesland, 1954). These authors reported similar levels in leaves of 5 species but considerably lower values (approximately 4 mμmoles) for tomato leaves. Using an improved technique (Methods, 8), these higher values (Table 42) of NAD and NADP were demonstrated. However, the concentrations were not affected by the type of nitrogen supplied to the plants.

The growth of *Hordeum vulgare* L., *Pisum sativum* L., and *Atriplex hortensis* L. on nitrate culture solution or ammonia culture solution varied with the species (Part VII). Although the growth of these plants on different solutions was not tested as extensively as was the growth of tomato plants, it seems probable that species other than tomato produce more dry weight on nitrate nitrogen than on ammonium nitrogen. As discussed earlier (Part I), when plants are supplied with ammonium nitrogen, high amide levels, and presumably high ammonium ion concentrations, usually result. Whether a similar effect, whatever it may be, is operating in all species which show reduced growth rates on ammonium nitrogen is not known.

PART IX

SUMMARY

Modifications were made to culture solutions to determine whether equal growth of tomato plants could be obtained on solutions containing either nitrate nitrogen alone or ammonium nitrogen alone. However, in all cases higher growth rates were found when nitrate nitrogen was supplied.

Studies on the uptake and incorporation of nitrogen from culture solutions containing either nitrate nitrogen, nitrate plus ammonium nitrogen, or ammonium nitrogen showed that the uptake of ammonium nitrogen and levels of free ammonium ions and amides in tissues were highest in plants supplied with only ammonium nitrogen. White patches appeared on the older leaves of these plants. In addition root hair formation was reduced in the roots of these plants as compared to plants supplied with nitrate nitrogen or nitrate plus ammonium nitrogen. Possible deleterious effects of high levels of ammonium ions in tissues are discussed.

Determinations were made of ammonium ions, free amino acids, amides, proteins, amino acid composition of leaf protein, RNA and DNA, NAD and NADP, carbohydrates, and organic acids within the tissues of tomato plants grown on the different sources of nitrogen. The results of these determinations, and of studies on the incorporation of ^{15}N into the proteins of subcellular fractions of leaves, were not correlated with the reduced growth of plants grown on ammonium nitrogen.

The effect of the source of nitrogen on the growth of three other plant species was determined.

PART X

ACKNOWLEDGEMENTS

I wish to express my gratitude for help received from the late Professor J. G. Wood, of the Department of Botany, University of Adelaide, Professor R. K. Morton, Drs. Janet Graham and Adele Millerd, of the Department of Agricultural Chemistry, University of Adelaide, and Dr. J.P.R. Riches, of the Department of Botany, University of Adelaide.

I would also like to thank the many other people who have generously given help and suggestions during this work.

I am indebted to the University of Adelaide and to the Commonwealth Government of Australia for grants received without which this work would not have been possible.

PART XI

BIBLIOGRAPHY

Anderson, D. G., and Vennesland, B. (1954). - The occurrence of di- and triphosphopyridine nucleotides in green leaves. J. Biol. Chem. 207: 613-20.

Anon (1954). - Diagnosis and improvement of saline and alkali soils. Agricultural Handbook No.60. U.S. Department of Agriculture, 100.

Arnon, D. I. (1937). - Ammonium and nitrate nutrition of barley at different seasons in relation to hydrogen concentration, manganese, copper, and oxygen supply. Soil Sci. 44: 91-113.

Arnon, D. I., and Hoagland, D. R. (1940). - Crop production in artificial culture solutions and in soils with special reference for factors influencing yields and absorption of inorganic nutrients. Soil Sci. 63: 159-82.

Arnon, D. I., and Johnson, C. M. (1942). - Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. Plant Physiol. 17: 525-39.

Arnon, D. I., Fratzke, W. E., and Johnson, C. M. (1942). - Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. 17: 515-24.

Basham, J. A., Birt, L. M., Hems, R., and Loening, U. E. (1959). - Determination of the reduced and oxidised pyridine nucleotides in animal tissues. Biochem. J. 73: 491-9.

Bershtein, B. I., and Okanenko, A. S. (1953). - The effect of nitrogen sources on metabolism of Taraxacum Kok-saghyz. Voprosy Biokhim. Azot. i Mineral. Pitaniya Rastenii. Akad. Nauk Ukr. S.S.R., 62-72; Chem. Abstr. 52: 3048. (1958).

Best, R. J. (1950). - A rapid and accurate electrotitrimetric method for determining the chloride content of soils, water and plant materials. Trans. Fourth Int. Congr. Soil Sci. 3: 162-5.

Bonner, J. (1946). - The role of organic matter, especially manure, in the nutrition of rice. Bot. Gaz. 108: 267-79.

Bonner, J. (1959). - Protein synthesis and the control of plant processes. Amer. J. Bot. 46: 58-62.

Bryant, M., and Fowden, L. (1959). - Protein composition in relation to age of daffodil leaves. Ann. Bot., Lond. 23: 65-74.

Burris, R. H. (1959). - Nitrogen nutrition. Ann. Rev. Pl. Physiol. 10: 301-28.

Burstrom, H. (1943). - Photosynthesis and assimilation of nitrate by wheat leaves. Ann. Agr. coll. Sweden 11: 1-50.

Candela, M. I., Fisher, E. G., and Hewitt, E. J. (1957). - Molybdenum as a plant nutrient. X. Some factors affecting the activity of nitrate reductase in cauliflower plants grown with different nitrogen sources and molybdenum levels in sand culture. Plant Physiol. 32: 280-8.

Chibnall, A. C. (1939). - Protein Metabolism in the Plant. (Yale University Press).

Chinard, F. P. (1952). - Photometric estimation of proline and ornithine. J. Biol. Chem. 199: 91-5.

Ciotti, M. M., and Kaplan, N. O. (1957). - Procedures for determination of pyridine nucleotides. Methods in Enzymology. III: 890-9.

Clark, H. E. (1936). - Effect of ammonium and nitrate nitrogen on the composition of the tomato plant. Plant Physiol. 11: 5-24.

Clark, H. E., and Shive, J. W. (1934). - The influence of the pH of a culture solution on the rates of absorption of ammonium and nitrate nitrogen by the tomato plant. Soil Sci. 37: 203-25.

Cocking, E. C. (1956). - Synthesis of amino acids and proteins in barley. Doctoral Thesis, University of Bristol, Bristol, England.

Colgrove, M. S. Jr., and Roberts, A. N. (1956). - Growth of the Azalea as influenced by ammonium and nitrate nitrogen. Proc. Amer. Soc. Hort. Sci. 68: 522-36.

Delwiche, C. C. (1951). - The assimilation of ammonium and nitrate ions by tobacco plants. J. Biol. Chem. 189: 167-75.

Espino, R. (1920). - Some aspects of the salt requirements of young rice plants. Philipp. J. Sci. 16: 455-525.

Evans, H. J., and Nason, A. (1953). - Pyridine nucleotide-nitrate reductase from extracts of higher plants. Plant Physiol. 28: 233-54.

Fawzy, H., Overstreet, R., and Jacobson, L. (1954). - The influence of hydrogen ion concentration on cation absorption by barley roots. Plant Physiol. **29**: 234-7.

Feigelson, P., Williams, J. N. Jr., and Elvehjem, C. A. (1950). - Spectrophotometric estimation of pyridine nucleotides in animal tissues. J. Biol. Chem. **185**: 741-7.

Folkes, B. F. (1959). - The position of amino acids in the assimilation of nitrogen and the synthesis of proteins in plants. Society for Experimental Biology (Symposia) Number XIII. 126-47.

Fowden, L. (1951). - The quantitative recovery and colorimetric estimation of amino acids separated by paper chromatography. Biochem. J. **48**: 327-33.

Fujii, T., and Mizuno, T. (1958). - Inhibition of mitosis by nicotinamide as seen in tissue culture and methylcholanthrene-treated mouse skin. J. Fac. Sci. Tokyo Univ. Sect. IV. 8 (2): 199-210.

Gilbert, S. G., and Shive, J. W. (1942). - The significance of oxygen in nutrient substrates for plants. I. The oxygen requirement. Soil Sci. **53**: 143-52.

Gilbert, S. G., Shear, C. B., and Gropp, C. M. (1951). - The effects of the form of nitrogen and the amount of base supply on the organic acids of tung leaves. Plant Physiol. **26**: 750-6.

Goodall, D. W., and Gregory, F. G. (1947). - Chemical composition of plants as an index of their nutritional status. Imperial Bureau of Horticultural and Plantation Crops. Technical Communication No.17. Published in East Malling, Kent, England.

Gorham, P. R. (1955). - Preparation of chloroplasts and disintegrated chloroplasts. Methods in Enzymology 1: 22-5.

Haas, A.R.C. (1937). - Nitrogen fertilisation and root aeration (of citrus trees). Calif. Citogr. 22: 286, 332-3.

Hamilton, P. B. (1958). - Ion exchange chromatography of amino acids. Effect of resin particle size on column performance. Analyt. Chem. 30: 914-9.

Hanson, J. B. (1960). - Impairment of respiration ion accumulation and ion retention in root tissue treated with ribonuclease and ethylenediamine tetraacetic acid. Plant Physiol. 35: 372-9.

Harvard, M. E., Jackson, W. A., Piland, J. R., and Mason, D. D. (1956). - The relationship of chloride and sulphate ions to form of nitrogen in the nutrition of Irish potatoes. Proc. Soil Sci. Soc. Amer. 20: 231-6.

Hoagland, D. R., and Arnon, D. I. (1941). - Physiological aspects of availability of nutrients for plant growth. Soil Sci. 51: 431-44.

Hurlbert, R. B., Schmitz, H., Brumm, A. F., and Potter, V. R. (1954). - Nucleotide Metabolism. II. Chromatographic separation of acid soluble nucleotides. J. Biol. Chem. 209: 23-39.

Hutton, J. T. (1954) - The titration of calcium and magnesium by E.D.T.A. Part C. The method recommended for calcium plus magnesium. C.S.I.R.O. Divisional Report 9/54.

Jackson, W. A., and Coleman, N. T. (1959). Ion absorption by bean roots and organic acid changes brought through carbon dioxide fixation. Soil Sci. 87: 311-9.

Jacobson, L. (1951). - Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylene-diamine tetra-acetate. Plant Physiol. 26: 411-3.

Jacobson, L., and Ordin, L. (1954). - Organic acid metabolism and ion absorption in roots. Plant Physiol. 29: 70-5.

Jacobson, L., Overstreet, R., Carlson, R. M., and Chastain, J. A. (1957). - The effect of pH and temperature on the absorption of potassium and bromide by barley roots. Plant Physiol. 32: 658-62.

Kelley, W. P. (1911). - The assimilation of nitrogen by rice. Hawaii Sta. Bull. 24.

Kelley, E. G., and Baum, R. R. (1953). - Protein amino acids: Contents of vegetable leaf proteins. J. Agric. Fd. Chem. 1: 680-3.

King, E. J. (1951). - Micro-analysis in medical biochemistry. J. & A. Churchill Ltd. London, p.10.

Kinskey, S. C., and McElroy, W. S. (1958). - Neurospora nitrate reductase: The role of phosphate, flavine and cytochrome c reductase. Arch. Biochem. 73: 466-83.

Krogmann, D. W., Jagendorf, A. T., and Avron, N. (1959). - Uncouplers of spinach photosynthetic phosphorylation. Plant Physiol. 34: 272-7.

Le Page, G. A., and Mueller, G. C. (1949). - Preparation of triphosphopyridine nucleotide. J. Biol. Chem. 180: 975-84.

Lipmann, F. (1958). - Symposium on amino acid activation. Proc. nat. Acad. Sci., Wash. 44: 67-104.

Lugg, J.W.H., and Weller, R. A. (1944). - Large-scale extraction of protein samples reasonably representative of the whole proteins in the leaves of some plants: the amide, tyrosine, tryptophane, cystine (plus cysteine) and methionine contents of the preparations. Biochem. J. 38: 408-11.

Ma, T. S., and Zuazaga, G. (1942). - Micro-kjeldahl determination of nitrogen. A new indicator and improved rapid method. Industr. Engng. Chem. (Anal.) 14: 280-2.

MacVicar, R., and Burris, R. H. (1948). - Studies on nitrogen metabolism in tomato with use of isotopically labelled ammonium sulphate. J. Biol. Chem. 176: 511-6.

Malavolta, E. (1954). - Studies on the nitrogenous nutrition of rice. Plant Physiol. 29: 98-9.

Margolis, D. (1960). - The range of free amino acids and amides in tomato plants and the effects of nitrate or ammonium as nutrients. Contr. Boyce Thompson Inst. 20: 425-36.

Martin, E. M., and Morton, R. K. (1956a). - Enzymic properties of microsomes and mitochondria from silver beet. Biochem. J. 62: 696-704.

Martin, E. M., and Morton, R. K. (1956b). - The chemical composition of microsomes and mitochondria from silver beet. Biochem. J. 64: 221-35.

May, L. H., and Davidson, J. L. (1958). - The role of carbohydrate reserves in regeneration of plants. Aust. J. Agric. Res. 9: 767-77.

McKee, H. S. (1949). - Review of recent work on nitrogen metabolism. New Phytol. 48: 1-83.

Mendel, J. L., and Visser, D. W. (1951). - Studies on nitrate reduction in higher plants. Arch. Biochem. 32: 158-69.

Moore, S., and Stein, W. H. (1954). - Chromatographic determination of amino acids on four per cent. cross-linked sulfonated polystyrene resins. J. Biol. Chem. 211: 894-902.

Moore, S., Spackman, D. H., and Stein, W. H. (1958). - Chromatography of amino acids on sulfonated polystyrene resins. An improved system. Analyt. Chem. 30: 1185-90.

Morton, R. K. (1958). - Enzymic synthesis of coenzyme I in relation to chemical control of cell growth. Nature, Lond. 181: 540-2.

Nagaoka, M. (1904). - On the behaviour of the rice plant to nitrates and ammonium salts. Bull. Coll. Agric. Tokyo. 6: 285-334.

Nason, A., and Evans, H. J. (1953). - Triphosphopyridine nucleotide-nitrate reductase in Neurospora. J. Biol. Chem. 202: 655-73.

Nicholas, D.J.D. (1959). - Metallo-enzymes in nitrate assimilation of plants, with special reference to micro-organisms. Society for Experimental Biology (Symposia). Number XIII. 1-23.

Nightingale, G. T., Schermerhorn, L. G., and Robbins, W. R. (1928). - The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. N.J. Agric. Expt. Sta. Bull. 461.

Nightingale, G. T. (1937). - The nitrogen nutrition of green plants. Bot. Rev. 3: 85-174.

Nightingale, G. T. (1948). - The nitrogen nutrition of green plants. II. Bot. Rev. 14: 185-221.

Ogur, M., and Rosen, G. (1950). - The nucleic acids of plant tissues. I. Arch. Biochem. 25: 262-76.

Oide, H. (1958). - The influence of the intrasplenic implantation of nicotinamide on the mitotic activity and the phosphopyridine nucleotide content of regenerating liver. Gann. 49: 49-56.

Partridge, S. M. (1948). - Filter-paper partition chromatography of sugars. Biochem. J. 42: 238-50.

Pardo, J. H. (1935). - Ammonium in the nutrition of higher green plants. Quart. Rev. Biol. 10: 1-31.

Pirie, N. W. (1959). - Leaf proteins. Ann. Rev. Pl. Physiol. 10: 33-52.

Pirschle, K. (1931). - Nitrate und ammonsalze als stickstoffquellen fur hohere pflanzen bei konstanter wasserstoffionkonzentration III. Planta. 14: 583-676.

Possingham, J. V. (1954). - The variations in the free amino acids and amide levels of tomato plants grown under differing nutritional conditions. M.Sc. Thesis, University of Adelaide.

Preiss, J., and Handler, P. (1958). - Biosynthesis of diphosphopyridine nucleotide. II. Enzymatic aspects. J. Biol. Chem. 233: 493-500.

Pucher, G. W., Vickery, H. B., and Leavenworth, C. S. (1935). - Determination of ammonia and of amide nitrogen in plant tissue. Industr. Engng. Chem. (Anal.) 7: 152-6.

Pucher, G. W., Wakeman, A. J., and Vickery, H. B. (1941). - Organic acids in plant tissues. Industr. Engng. Chem. (Anal.) 13: 244-6.

Reisner, G. S., Gering, R. K., and Thompson, J. F. (1960). - The metabolism of nitrate and ammonia by Chlorella. Plant Physiol. 35: 48-52.

Reuther, W., Embleton, T. W., and Jones, W. W. (1958). - Mineral nutrition of tree crops. Annu. Rev. Pl. Physiol. 9: 175-206.

Rijven, A.H.G.C. (1958). - Effects of some inorganic nitrogenous substances on growth and nitrogen assimilation of young plant embryos in vitro. Aust. J. Sci. Res. (B) 11: 142-54.

Rittenberg, D., Keston, A. S., Rosebury, F., and Schoenheimer, R. (1939). - Studies in protein metabolism. II. The determination of nitrogen isotopes in organic compounds. J. Biol. Chem. 127: 291-9.

Rosen, H. (1957). - A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. 67: 10-5.

Schmidt, G., and Thannhauser, S. J. (1945). - A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem. 161: 83-9.

Schram, E., Dustin, J. P., Moore, S., and Bigwood, E. J. (1953). - Application de la chromatographie sur echangeur d'ions a l'etude de la compositions des aliments en acides amines. Analyt. chim. acta. 9: 149-62.

Schutte, K. H., and Schendel, H. E. (1958). - Influence of trace elements upon plant protein composition. Nature. Lond. 182: 958.

Shive, J. W. (1941). - The balance of ions and oxygen tension in nutrient substrates for plants. Soil Sci. 51: 445-59.

Sideris, C. P., Krauss, B. H., and Young, H. Y. (1938). - Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. 13: 489-527.

Sideris, C. P., Young, H. Y., and Krauss, B. H. (1943). - Effects of iron on the growth and ash constituents of Ananas comosus (L.) Merr. Plant Physiol. 18: 608-32.

Sideris, C. P., and Young, H. Y. (1944). - Effects of iron on chlorophyllous pigments, ascorbic acid, acidity and carbohydrates of Ananas comosus (L.) Merr., supplied with nitrate or ammonium salts. Plant Physiol. 19: 52-75.

Sideris, C. P., and Young, H. Y. (1946). - Effects of nitrogen on growth and ash constituents of Ananas comosus (L.) Merr. Plant Physiol. 21: 247-70.

Simms, A. P., and Cocking, E. C. (1958). - Assay of isotopic nitrogen by mass spectrometer. Nature, Lond. 181: 474.

Skok, J. (1941). - Effect of the form of the available nitrogen on the calcium deficiency symptoms in the bean plant. Plant Physiol. 32: 11-5.

Skvortsova, L. A., O. Pitanii Rasenii (Moscow: Gosudarst. Izdatel. Kolkhoz i Sovkhoz. Lit.) Sbornik, 131-9 (1955). - The effect of calcium and magnesium on the organic acid metabolism of the leaves of Nicotiana rustica in nitrate and ammonia types of nutrition. Chem. Abstr., 52: 3932 (1958).

Smith, P. F. (1957). - Studies on the growth of Citrus seedlings with different forms of nitrogen in solution cultures. Plant Physiol. 32: 11-5.

Smith, A. M., and Agiza, A. H. (1951). - The amino-acids of several grassland species, cereals and bracken. J. Sci. Food Agric. 2: 503-20.

Somogyi, M. (1952). - Notes on sugar determination. J. Biol. Chem. 195: 19-23.

Sprinson, D. B., and Rittenberg, D. (1949). - The rate of utilization of ammonia for protein synthesis. J. Biol. Chem. 180: 707-26.

Steward, F. C., Crane, F., Millar, K., Zacharius, R. M., Rabson, R., and Margolis, D. (1959). - Nutritional and environmental effects on the nitrogen metabolism of plants. Society for Experimental Biology (Symposia) Number XIII. 148-76.

Strickland, K. P., Thompson, R.H.S., and Webster, G. R. (1956). - The action of the phosphatases of human brain on lipid phosphate esters. J. Neurol Psychiat. 19: 12-6.

Sundaram, T. K., Rajagopalan, K. V., Pichappa, C. V., and Sarma, P. S. (1960). - Studies on pyridine nucleotide biosynthesis. Biochem. J. 77: 145-9.

Takahashi, T., and Yoshida, D. (1957a). - Studies on interrelation of various ions on absorption by tobacco plant. V. Relation between nitrogen form and chloride levels in culture solution. Nippin Dojo-hiryogaku Zasshi 28: 176-80. Chem. Abstr. (1958) 52: 20832.

Takahashi, T., and Yoshida, D. (1957b). - Studies on interrelation of various ions in absorption by tobacco plant. VI. Relation between nitrogen form and both chloride and sulphate in culture solution. Nippon Dojo-hiryogaku Zasshi 28: 181-4. Chem. Abstr. (1959) 53: 1472.

Tamm, T. O. (1956). - The response of Chameenerion angustifolium (L.) Scop. to different nitrogen sources in water culture. Physiol. Plant 9: 331-7.

Tang, P., and Wu, H. (1957). - Adaptive formation of nitrate reductase in rice seedlings. Nature. Lond. 179: 1355-6.

Thelin, G., and Beaumont, A. B. (1934). - The effect of some forms of nitrogen on the growth and nitrogen content of wheat and rice plants. J. Amer. Soc. Agron. 26: 1012-7.

Thompson, J. F., Zacharius, R. M., and Steward, F. C. (1951). - Investigations on nitrogen compounds and nitrogen metabolism in plants. I. The reaction of nitrogen compounds with ninhydrin on paper: a quantitative procedure. Plant Physiol. 26: 375-97.

Thompson, J. F., and Steward, F. C. (1951). - Investigations on nitrogen compounds and nitrogen metabolism in plants. II. Variables in two directional paper chromatography of nitrogen compounds: a quantitative procedure. Plant Physiol. 26: 421-40.

Tiedgens, V. A. (1934). - Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple. Plant Physiol. 9: 31-57.

Tiedgens, V. A., and Robbins, W. R. (1931). - The use of ammonia and nitrate by certain crop plants. N.J. Agric. Exp. Sta. Bull. 526.

Trelease, S. F., and Paulino, P. (1920). - The effect on the growth of rice of the addition of ammonium and nitrate salts to soil cultures. Philipp. J. Agric. 8: 293-313.

Trelease, S. F., and Trelease, Helen M. (1933). - Physiologically balanced culture solutions with stable hydrogen-ion concentration. Science 78: 438-9.

Trelease, S. F., and Trelease, Helen M. (1935). - Changes in hydrogen-ion concentration of culture solutions containing nitrate and ammonium nitrogen. Amer. J. Bot. 22: 520-42.

Ts'o, P.O.P., in "Microsomal Particles and Protein Synthesis (R.B. Roberts, Ed.) pp. 156-68. Permagon, New York. 1958.

Ulrich, A. (1941). - Metabolism of non volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Amer. J. Bot. 28: 526-37.

Vickery, W. B., Pucher, G. W., Wakeman, A. S., and Leavenworth, C. S. (1940).- Chemical investigations of the tobacco plant VIII. The effect on composition of the plant of the form in which nitrogen is supplied. Conn. Agr. Exp. Sta. Bull. 442.

Vines, H. M., and Wedding, R. T. (1960). - Some effects of ammonia on plant metabolism and a possible mechanism for ammonia toxicity. Plant Physiol. 35: 820-5.

Vladimirov, A. V. (1945). - Influence of nitrogen sources in the formation of oxidised and reduced organic compounds in plants. Soil Sci. 60: 265-75.

Vlasyuk, P. A., Kosmatyi, E. S., and Klimovitskaya, Z. M. Fiziol. Rastenii. 4: 432-8 (1957). Chem. Abstr. 52: 4755 (1958).

Wadleigh, C. H., and Shive, J. W. (1939). - Organic acid content of corn plants as influenced by pH of substrate and form of nitrogen supplied. Amer. J. Bot. 26: 244-8.

Wahhab, A., and Bhatti, H. M. (1957). - Effect of various sources of nitrogen on rice paddy yield. Agronomy J. 49: 114-6.

Wallace, A., and Ashcroft, R. T. (1956). - Preliminary comparisons of the effects of urea and other nitrogen sources on the mineral composition of rough lemon and bean plants. Proc. Amer. Soc. Hort. Sci. 68: 227-33.

Wallace, A., and Mueller, R. T. (1957). - Ammonium and nitrate nitrogen absorption from sand culture by rough lemon cuttings. Proc. Amer. Soc. Hort. Sci. 69: 183-8.

Wander, I. W., and Sites, J. W. (1956). - The effect of ammonium and nitrate nitrogen with and without pH control on the growth of rough lemon seedlings. Proc. Amer. Soc. Hort. Sci. 68: 211-26.

Weinmann, H. (1947). - Determination of total carbohydrates in plants. Plant Physiol. 22: 279-90.

Weissman, G. S. (1950). - Growth and nitrogen absorption of wheat seedlings as influenced by the ammonium : nitrate ratio and the hydrogen ion concentration. Amer. J. Bot. 37: 725-38.

Weissman, G. S. (1951). - Nitrogen metabolism of wheat seedlings as influenced by the ammonium : nitrate ratio and the hydrogen ion concentration. Amer. J. Bot. 38: 162-74.

Weissman, G. S. (1959). - Influence of ammonium and nitrate on the protein and free amino acids in shoots of wheat seedlings. Amer. J. Bot. 46: 339-46.

Weller, R. A. (1957). - The amino acid composition of hydrolysates of microbial preparations from the rumen of sheep.. Aust. J. Biol. Sci. 10: 384-9.

West, S. H., and Hanson, J. B. (1960). - The degradation of isolated microsomes from cucumber and corn seedlings. Weeds 8: 341-8.

West, S. H., Hanson, J. B., and Key, J. L. (1960). - Effect of 2,4-dichlorophenoxyacetic acid on the nucleic acid and protein content of seedlings tissues. Weeds. 8: 333-40.

Whatley, F. R. (1951). - Coenzymes in plants. New Phytol. 50: 244-57.

Williams, C. H. (1960). - The use of lantharum chloride to prevent interferences in the flame photometric determination of exchangeable calcium in soils. Analyt. chim. acta. 22: 163-71.

Wittwer, S. H., and Teubner, F. G. (1959). - Foliar absorption of mineral nutrients. Annu. Rev. Pl. Physiol. 10: 13-32.

Wood, J. G., and Sibly, P. M. (1952). - Carbonic anhydrase activity in plants in relation to zinc content. Aust. J. Sci. Res. (B) 5: 244-55.

Woolhouse, H. W. (1959). - The effect of nitrate and ammonium ions on the metabolism and growth of the tomato plant, Lycopersicum esculentum Mill. Ph.D. Thesis. University of Adelaide.

Yemm, E. W., and Folkes, B. F. (1953). - The amino acids of cytoplasmic and chloroplastic proteins of barley. Biochem. J. 55: 700-6.

Yemm, E. W., and Cocking, E. C. (1954). - The determination of amino-acids with ninhydrin. Analyst. 80: 209-13.

Yemm, E. W., and Willis, A. J. (1956). - The respiration of barley plants. IX. The metabolism of roots during the assimilation of nitrogen. New Phytol. 55: 229-52.

Yemm, E. W., and Folkes, B. F. (1958). - The metabolism of amino acids and proteins in plants. Annu. Rev. Pl. Physiol. 9: 245-80.