TRACE ELEMENTS
IN
PLANTS AND ANIMALS

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by

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To

EDWARD JAMES SALISBURY

Director of the Royal Botanic Gardens, Kew
Chairman of the Mineral Deficiencies Conference of the Agricultural Research Council

IN FRIENDSHIP

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PREFACE

TRACE elements, micro-nutrients and minor elements are terms applied to a number of chemical elements which are essential for the lives of plants and animals, but which are required in extremely small quantity. The development of our knowledge of the part played by the trace elements in the life of plants and animals is very recent, nearly all our present information on the subject having been acquired during little more than the last twenty years. Nevertheless, in the course of that time a great many observations have been made in the field, and much experimental work carried out in both field and laboratory, while many hundreds of publications, some of slight value, others of considerable scientific and economic importance, dealing with trace elements in living plants and animals have appeared in the scientific press. The time thus seems ripe for the presentation of a digest of this information, so that the salient facts may be available in a convenient form, and the present position of our knowledge of trace elements in living organisms made plain. It is with this intention that the present book has been written.

The role of the trace elements in organisms is, in the first place, a matter for the plant or animal physiologist, but as deficiency or excess in the supply of the various trace elements may lead to a diseased condition of the plant or animal with serious economic consequences, the trace elements are also of interest to the plant pathologist, the veterinary surgeon, the agriculturist and horticulturist. Indeed, as regards plants at any rate, much more is definitely known of the plant pathological aspect of the trace elements than of their physiological functions.

I would take this opportunity of expressing my thanks to Mr W. Morley Davies of Harper Adams Agricultural College, who first introduced to me the effects of trace-element deficiencies in the field, and who has generously and constantly put at my disposal both his knowledge of the pathological effects of trace-element deficiency, and the pathological material itself.

I also have pleasure in thanking Dr C. S. Piper of the Waite Agricultural Research Institute, University of Adelaide, for permission to use the photographs reproduced in Figs. 4 and 12, and my colleagues Dr K. W. Dent and Mr A. E. Roberts for the preparation of the rest of the photographs in the book.

Throughout the book the following contractions have been used:

ml. (millilitre)	= one-thousandth of a litre.
μg. (microgram)	= one-millionth of a gram.
A. (Ångström unit)	= one ten-thousandth of a millimetre.
p.p.m.	= parts per million.

WALTER STILES

BIRMINGHAM
28 *October* 1944

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CHAPTER I

HISTORICAL INTRODUCTION

In the year 1699 Woodward published the results of experiments in which cuttings of plants were grown, not in soil, but in rain water. More than a century and a half later Julius Sachs (1860) and W. Knop (1860) independently developed this method of water culture by growing plants of several different species in a dilute aqueous solution of various salts. In this way the materials available for absorption by the roots of the growing plants were controlled, and Sachs and Knop concluded from their experiments that so long as the culture solution contained salts involving the elements nitrogen, sulphur, phosphorus, potassium, calcium, magnesium and iron, a perfectly healthy plant would result. The elements occur in soil as constituents of compounds present in, or derived from, the minerals of the underlying rock, and are therefore generally known as mineral nutrients. Experiments of this kind have been repeatedly carried out by subsequent investigators and many formulae for water-culture solutions have been used and recommended. The water-culture method has also been extensively used for research in plant nutrition. Some of the best known and most widely used water-culture solutions are those of Sachs and Knop themselves and the later ones of Pfeffer and Von der Crone. Plants of a great number of species have been successfully grown in water culture, and in recent years the method has been advocated, perhaps too optimistically (cf. Hoagland and Arnon, 1938), as a means of cultivating certain crop plants on a commercial or semi-commercial scale. The compositions of some of the best known and most widely used water cultures are given below.

Sachs's nutrient solution

Potassium nitrate	1·0 g.
Sodium chloride	0·5 g.
Calcium sulphate	0·5 g.
Magnesium sulphate	0·5 g.
Calcium phosphate	0·5 g.
Water	1 l.

A few drops of a solution of ferric chloride or ferrous sulphate.

Pfeffer's stronger nutrient solution

Calcium nitrate	4·0 g.
Potassium nitrate	1·0 g.
Potassium dihydrogen phosphate	1·0 g.
Potassium chloride	0·5 g.
Water	3 l.
Ferric chloride medicinal solution	3 to 6 drops

Pfeffer's weaker solution is similar, except that the same quantity of these salts is dissolved in 7 l. of water.

Von der Crone's nutrient solution

Potassium nitrate	1·0 g.
Calcium sulphate	0·5 g.
Magnesium sulphate	0·5 g.
Calcium phosphate	0·25 g.
Ferrous phosphate	0·25 g.
Water	1 l.

It will be noticed that, in addition to the elements given above, Pfeffer's solution also contains chlorine, while Sachs's solution contains not only chlorine, but sodium. While the general opinion of plant physiologists for many years was that most plants required neither of these elements, it was rather vaguely held that some plants required sodium or chlorine in addition to those regarded as always necessary. Thus Pfeffer (1900) remarked that probably no plant had been grown in complete absence of chlorine, and he considered it uncertain whether a minimum amount was essential or not. Miller (1931) considered that experimental results had shown that under certain conditions sodium had a definite effect on the growth of plants of some species.

So opinion on the mineral nutrition of plants remained during the latter half of the nineteenth century. It was well established that many elements, apart from those regarded as necessary, were present in plants, but their presence was regarded as incidental, due to their presence in the soil in which the plants grew. But in 1897 G. Bertrand claimed that manganese was constantly associated in the plant with an oxidizing enzyme, laccase, and he came to regard manganese as an essential constituent of the oxidase system. In 1905 he claimed that an insufficient supply of manganese to plants brought about diminution or cessation of growth, and that manganese was to be regarded as an essential element. Much subsequent experi-

mental work and many field observations have gone to confirm Bertrand's view.

In 1914, by the use of water-culture experiments with carefully prepared culture vessels and nutrient salts, Mazé showed that not only manganese but zinc also was necessary for the growth of maize. As a result of later work on similar lines he claimed that aluminium, boron, chlorine and silicon were all essential in small amounts for the healthy growth of plants. Of these various elements, boron had already been found by Agulhon (1910) to induce an increased production of dry matter in wheat, oats and radish grown in sand cultures and in maize, colza and turnip grown in field-plot experiments. It is, however, scarcely correct, as has sometimes been done, to quote Agulhon as the first worker to point out the *essential* nature of boron for higher plants. As Dennis and O'Brien (1937) have pointed out, Agulhon provided no proof that normal growth was not possible without boron, and the credit for first calling attention to the necessity of this element for any plant is due to Mazé. The importance of boron as an essential plant nutrient was brought into prominence in 1923 by Miss Warington, who showed by means of water cultures that while a concentration of boric acid as low as 1 in $12{\cdot}5 \times 10^6$ was sufficient to allow the normal growth of the broad bean (*Vicia faba*), in complete absence of boron death of the plant supervened after the development of quite characteristic pathological symptoms. A few years later Sommer and Lipman (1926) added cotton, castor oil, buckwheat, flax, mustard and barley to the list of plants for the growth of which a supply of boron is essential. Since then the list of such species has been further extended.

Confirmation of Mazé's finding that zinc is necessary for the healthy development of maize has been provided by more recent observations of Barnette and Warner (1935), Mowry and Camp (1934) and others, who have shown that the curious chlorotic condition of this plant known as 'white-bud' can be cured by application of zinc sulphate. Sommer and Lipman (1926) also found zinc essential for the growth of sunflower and barley, and later Sommer (1928) reported that buckwheat and beans could only undergo normal development in presence of zinc. In beans, for example, abscission of leaves and flower buds

occurred in cultures deprived of zinc, whereas in controls adequately supplied with this element flowering took place and seeds were produced. A number of trees have been shown to require a supply of this element.

As regards the remaining elements that Mazé concluded were necessary for the growth of maize, reference has already been made to the doubt which has existed for many years about the necessity of chlorine for plant development. As long ago as 1862, Nobbe and Siegert concluded that chlorine was essential for the normal development of buckwheat, but many subsequent experiments carried out to check this conclusion yielded conflicting results. But in 1938 the much-debated question of the effect of chlorine on the growth of buckwheat was examined by Lipman, and it was found that plants grown with 5 p.p.m. of chlorine added as potassium chloride produced markedly more dry matter and seeds than plants grown without added chlorine. Moreover, the seeds from plants grown with added chlorine gave a higher percentage of germination than the seeds from plants without added chlorine, and of the seedlings produced from the latter little more than half developed to any extent. A second series of cultures was made from the seed obtained in the first series, the seedlings from seeds obtained from the cultures with added chlorine being again supplied with this element, and those from seeds grown in cultures without added chlorine being again deprived of it. The superiority of the plants supplied with chlorine was emphasized, and Lipman concluded that if chlorine is not absolutely essential to buckwheat it certainly greatly influences its growth and seed production. A like conclusion was drawn from water-culture experiments with peas.

As regards aluminium, Stoklasa in 1922 grew a number of hydrophytes in water cultures and silica gel cultures with and without aluminium, and found growth was considerably improved in presence of aluminium. Indeed, silica gel cultures of *Glyceria aquatica* without aluminium died in 22 days and aluminium-free water cultures of *Juncus effusus* died in from 56 to 69 days. Addition of aluminium also improved the growth of wheat, barley and oats, but no beneficial effect of this element was observed with plants of a number of other species, including

Polygonatum officinale and *Iris bohemica*. More critical water-culture experiments to test the essentiality of aluminium were carried out by Sommer (1926) with peas and millet. Specially purified nutrient salts were used in making up the culture solutions, and in those containing aluminium this element was present to the extent of 5·5 or 1 p.p.m. of the solution. The presence of 1 p.p.m. brought about a slight increase in the dry weight of peas, but with millet the presence of aluminium brought about a definite increase in dry weight and a very pronounced increase in the weight of seeds produced, the respective weight of seeds produced with and without aluminium being 0·23 and 4·98 g.

In 1938, Lipman obtained similar results with sunflowers and maize, the effect being particularly noticeable with the latter, where the addition of aluminium sulphate to the extent of 1 p.p.m. of aluminium (renewed from time to time) resulted in an increase in dry weight of the vegetative parts of about 20 per cent and an increase in the dry weight of the ears of about 155 per cent.

Sommer and Lipman also investigated the essentiality of silicon. Sommer in 1926 found that the presence of silicon, added as silica gel, increased the dry weight of rice plants from 4·4 to 7 g., and she concluded that this increase was big enough to indicate that silicon is essential for the growth of rice. Marked increases in seed production as a result of the presence of silicon in the culture solution were observed with millet. Lipman in 1938 found that sunflowers and barley grown in water culture produced more dry matter in a given time with colloidal silica added to the solution than without such addition. With barley the addition of silica also resulted in a greater yield of grain.

Since Lipman could not be sure that in his control cultures without added silicon, aluminium or chlorine respectively there was not a small quantity of the particular element present, he did not go further than emphasize the importance of silicon, aluminium and chlorine for the plants used and probably for higher plants in general. However, he regarded the probability of the indispensability of these elements as very great indeed.

In recent years evidence has been accumulating that copper

is essential for the normal growth of a number of plants. Thus in 1931 Sommer found that the addition of small quantities of copper induced a considerable increase in growth of sunflowers, flax and tomatoes, while in the same year Lipman and Mackinney found that flax and barley grown in water culture failed to produce seed in complete absence of copper. Evidence has also been presented by Anderssen (1932) in South Africa and by Oserkowsky and Thomas (1933) in America indicating that chlorosis and dying back of the branches of various fruit trees result from a deficiency of copper. Further, a pathological condition of cereals and some other plants known as 'reclamation disease', occurring in plants growing on reclaimed heath land in Holland and elsewhere, was attributed by Sjollema in 1933 to copper deficiency. In cereals exhibiting this condition the tips of the leaves turn yellow or white while seed fails to form.

In 1939, Arnon and Stout obtained evidence that molybdenum may be an essential element for higher plants. They found that tomato plants exhibited pathological symptoms when grown in a culture solution containing all the ordinary nutrient elements along with manganese, boron, zinc and copper but which was freed from all trace of molybdenum. The lower leaves of the plants first developed a mottling; then dying of the marginal cells followed while the flowers fell without setting fruit. The condition was prevented by the addition of one part of molybdenum as molybdic acid in 100,000,000 to the culture solution, while the pathological symptoms were removed in the molybdenum-deficient plants by spraying the leaves with a very dilute solution of molybdic acid. More recently, Steinberg (1941) concluded that both molybdenum and gallium are essential for the growth of *Lemna*.

So far only higher plants have been considered, but it is quite clear that the same trace elements may be equally essential for the growth of lower plants, at any rate of fungi. Indeed, the necessity of an element for the growth of a fungus has in more than one instance been demonstrated before its essentiality for a higher plant has been claimed.

The favourite species among the fungi for studies on mineral nutrition has been *Aspergillus niger*. In addition to the generally acknowledged mineral nutritive elements, with the

exception of calcium,[1] but definitely including iron,[2] it has been shown that this fungus definitely requires zinc, manganese, copper, molybdenum and gallium. Indeed, as long ago as 1869, Raulin showed that for *A. niger* zinc is necessary, although the significance of this finding was rather obscured subsequently by the attempt to explain the increased growth resulting from the presence of a small quantity of zinc as a stimulation effect on normal development due to the action of a poison in low concentration. While the possibility of such a stimulation effect in general is not to be ruled out without proof, such proof is forthcoming when it has been shown that with rigid exclusion of any substance normal development is prevented. For zinc this proof was definitely provided by Steinberg (1919), who, by means of a special technique, succeeded in removing all but the minutest traces of zinc from the culture medium, and then showed that the cultures of *A. niger* provided with zinc produced more than 2000 times as much dry matter as the controls deprived of all but the last traces of that element. Steinberg's result was later confirmed by Bortels (1927), Roberg (1928, 1931) and Gollmick (1936).

The need for manganese for the normal development of *A. niger* was claimed by Bertrand and Javillier (1911*a*), and this was confirmed by Steinberg (1935*a*). That copper is essential for the growth of this fungus was shown by Bortels (1927) and by Wolff and Emmerie (1930). In 1928, Davis, Marloth and Bishop reported that the yield of a species of *Dothiorella* grown on an artificial medium was reduced to half by the removal of traces of boron from the nutrient salts used, while in 1933 Lockwood found that the growth of *Penicillium Javanicum* was increased by additions of columbium, molybdenum and tungsten. The rigid proof of the need of molybdenum for the growth

[1] There exists considerable doubt about the necessity or otherwise of calcium for the growth of fungi. The general opinion at present appears to be that this element is necessary for the growth of some species but not of others. Much experimental work is necessary to place our knowledge of this question on a reliable basis. According to Davis, Marloth and Bishop (1928) calcium is necessary for the development of *A. niger*. Mann (1932), on the other hand, came to the opposite conclusion.

[2] Iron is placed in the category of micro-nutrients by some workers on fungus nutrition, e.g. Steinberg (1939) and Foster (1939).

of *Aspergillus niger* was given by Steinberg in 1937, while in the following year the same worker added gallium to the list of elements essential for this fungus.

While the mineral nutrition of no other fungus has received so much attention as that of *Aspergillus niger*, sufficient information has now accumulated to justify the conclusion that for the fungi in general a supply of trace elements is necessary. Thus McHargue and Calfee (1931) concluded that zinc, manganese and copper are necessary for the growth of *A. flavus* and *Rhizopus nigricans*, and the same three elements were considered necessary for *Ceratostomella Ulmi* by Ledeboer (1934), for *Trichophyton interdigitale* by Mosher, Saunders, Kingery and Williams (1936), and for *Phymatotrichum omnivorum* by Rogers (1938). Foster and Waksman (1939) reported that *Rhizopus nigricans* fails to produce zygospores in absence of zinc, while the favourable effect on development produced by zinc and copper on a number of fungi belonging to different families has been reported by Metz (1930) and for a number of species of *Aspergillus* by Roberg (1928, 1931).

Thus, as far as our information goes at present, both higher plants and fungi, or some of them, require a supply of manganese, zinc, copper and molybdenum. Many higher plants have been shown to need boron, and it is rather surprising that this element, one of the most definitely established micro-nutrients of higher plants, has received scarcely any attention from workers on fungus nutrition. On the other hand, the need for columbium and tungsten has so far not been claimed for any higher plant.

Very little information is available about the need for trace elements of plants other than angiosperms and fungi, although a few observations dealing with algae are on record. Thus manganese has been shown to be essential for the unicellular green alga *Chlorella* by Hopkins (1930, 1934) and for the diatom *Ditylum brightwelli* by Harvey (1939). Roberg (1932) has reported increased growth of two unicellular green algae *Coccomyxa simplex* and *Chlorella vulgaris* as a result of small additions of salts of iron, zinc and copper to the normal nutrient solution. From his work, however, it is not made clear that zinc and copper are actually essential for the growth of these

plants, and Roberg himself says that these elements are to be considered as acting as stimulants. However, having regard to work with plants of other groups, it may well be that these elements will prove to be actually essential for the growth of these and other algae.

From what has so far been discovered regarding the essentiality of trace elements, two questions arise which only further research can answer. The first is how far the necessity for these elements is general throughout the plant kingdom, and the second is whether we now know the complete list of these elements. As regards the first question, it would seem probable rather than merely possible that when plants differing as widely, both taxonomically and anatomically, as well as physiologically, as angiosperms and fungi, both exhibit these requirements, we are dealing with something very fundamental in plant nutrition, and we are justified in concluding that the best established of the trace elements, manganese, boron, zinc and copper, are likely to be found essential for the nutrition of plants in general. In regard to the second question, Steinberg (1938*c*) has contributed an interesting discussion on the relations between essentiality of elements and their atomic structure, and he draws the conclusion from such considerations that scandium may be an essential element for plant nutrition. In this connexion some experiments carried out by Arnon are of interest. In 1937 this worker described the results of water-culture experiments in which the growth of barley plants was improved by the addition of small quantities of molybdenum, chromium and nickel. In the following year further experiments were described in which plants of asparagus and lettuce were grown in four different culture solutions. The first of these contained the ordinary nutrient elements. The second contained these together with the four well-established micro-nutrients, manganese, boron, zinc and copper (designated A 4) and some chlorine. The third contained all the elements present in the second solution together with another seven (designated B 7); these were molybdenum, titanium, vanadium, chromium, tungsten, cobalt and nickel. The fourth solution contained all the elements present in the third solution together with thirteen other elements, namely, aluminium, arsenic, cadmium, strontium, mercury, lead, lithium,

rubidium, bromine, iodine, fluorine, selenium and beryllium. This group was designated C13. The fourth solution also contained sodium. All the elements of the A4, B7 and C13 groups were present in very low concentration, that is, as traces. The four solutions, which may be denoted by I, II, III and IV thus contained respectively the following mineral elements:

I: N, S, P, K, Ca, Mg, Fe.
II: elements of I + B, Zn, Mn, Cu, + Cl.
III: elements of II + Mo, Ti, V, Cr, W, Co, Ni.
IV: elements of III + Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be, + Na.

The fresh weights in grams of the plants grown in these different solutions are shown in Table I. Thus the great effect of the four well-established micro-nutrients is well demonstrated, but the seven additional elements of solution III produced a further increase in growth of asparagus, while for lettuce their effect was most striking, the yield being increased about ten times as a result of their addition. The subsequent demonstration by Arnon and Stout that molybdenum is necessary for the tomato has been mentioned earlier, and this leaves

TABLE I. Growth of plants of asparagus and lettuce in four different culture solutions. (Data from Arnon)

Culture solution	Fresh weight in g.			
	Asparagus		Lettuce	
	Shoots	Roots	Shoots	Roots
I	16·2	12·8	71·4	14·5
II	88·2	38·2	105·7	22·0
III	118·1	81·3	1068·3	188·6
IV	121·7	74·5	984·4	196·2

in doubt whether the effectiveness of the seven elements of solution III not included in solution II is due solely to the molybdenum or whether some or all of the others are also in part responsible for this. That no further increase in yield results from the addition of the extra thirteen elements of solution IV at first sight suggests that none of these is essential for the growth of asparagus and lettuce, but it may be that one or more of these is actually necessary but exists in sufficient quantity as impurity of one of the salts used in making up solution III.

Using Arnon's technique, Twyman (1943) obtained a similar result with oats. Four weeks after germination of the grains the average dry weights of plants supplied with the A4, B7 and A4+B7 groups of trace elements were respectively 0·101, 0·130 and 0·238 g. This again shows the necessity of one or more of the elements of Arnon's group B7.

The question arises why for so many years the necessity for the trace elements in plant nutrition was not recognized. The answer given to this question by Mazé was no doubt the correct one. Mazé considered that the importance of the micro-nutrients had been overlooked because in water-culture experiments a sufficient quantity of these was introduced into the cultures from (1) the seeds used, (2) impurities in the salts used in preparing the culture solutions, and (3) solution from the vessels containing the culture solutions. Indeed, knowledge of the existence of the various trace elements has only been obtained through the purification of the water and nutrient salts used, and the choice of suitable culture vessels.

The securing of an adequate degree of purity of the materials used is of the utmost importance in experimental work designed to examine the indispensability or otherwise of particular substances. The methods that have been developed to this end, along with other experimental methods of value in work on micro-nutrients, form the subject of the next chapter.

Although addition of a compound of a particular element to the nutrient medium in which plants are growing may bring about increase in rate of growth of the plants, it does not follow that the element is essential for the growth of plants. Indeed, increases in growth rate as a result of the addition of compounds of a number of different elements have been recorded from time to time. Among recent observations of this kind particular mention may be made of those of R. S. Young (1935), who examined the effect on the growth of timothy (*Phleum pratense*) of thirty-five of the rarer elements when added in five different concentrations (2000, 500, 100, 10 and 0·1 p.p.m.) to a sandy loam. Beneficial effects were observed with molybdenum, supplied in 2000 p.p.m., and with antimony, barium, bismuth, bromine, cerium, manganese, strontium, tungsten, uranium and yttrium, supplied at the rate of 500 p.p.m.

Aluminium, cadmium, copper, fluorine, lanthanum, lead, mercury, tin and zinc gave an increased growth when supplied at the rate of 100 p.p.m., while with 10 p.p.m. arsenic, beryllium, chromium, iodine, lithium, selenium, thorium, titanium, vanadium and zirconium were beneficial. At a concentration of 0·1 p.p.m., boron, nickel and thallium brought about an increase in growth. Of the thirty-five elements, the effects of which were tested, only cobalt appeared to be slightly toxic at this lowest concentration employed, while silver at this concentration appeared to have no effect. At higher concentrations than those stated for the respective elements the action of these was depressing on growth.

Experiments with cultures of two green algae, species of *Chlorella* and *Crucigina* respectively, led Young to conclude that on the whole any element will stimulate the growth of algae at a definite concentration which depends on the element and the species.

We certainly cannot conclude that an element which stimulates growth is necessarily essential for growth, although if increased growth is observed to result from the presence of a particular element there is always the possibility that that element may be an essential one. Whether it is so or not can only be proved by growing the plant in carefully controlled cultures, in which every care is taken rigidly to exclude from the culture medium the element under examination.

To conclude this introduction to our subject here is appended a list of those elements which, it has been claimed, constitute micro-nutrients of plants. Under the name of each of these elements are listed those species for which it has been claimed that the element either is essential or induces an increase in rate of growth or in yield. In the latter circumstance it does not necessarily follow that the element is essential although the evidence of increased growth indicates that this may be so.

In the following list the name of the first worker to call definite attention to the favourable effect of each particular element on the growth of the species concerned is given, whether that worker regarded the element as essential for the species or not. Thus Nakamura in 1903 reported increased growth of peas and spinach as a result of adding boron to the soil, but it

was not until 1915 that Maze claimed the essential quality of boron for plant growth. For a few the claim is very tentative and in others the justification for the claim rather tenuous, but for most the evidence is definite enough to be accepted.

Manganese

Rumex Acetosa (sorrel) Boullanger, 1912
Fagopyrum esculentum (buckwheat) Meyer, 1931
Beta maritima (mangold) Gilbert and McLean, 1928
B. maritima (beet) Gregoire, Hendrick and Carpiaux, 1907
Spinacia oleracea (spinach) Tacheuchi, 1909
Bougainvillea sp. Dickey and Reuther, 1938
Cochlearia Armoracia (horse-radish) Picado and Vicente, 1923
Sinapis alba (white mustard) Clausen, 1913
Brassica campestris (turnip) Chittenden, 1915
Raphanus sativus (radish) McHargue, 1923
Fragaria vesca (strawberry) Hoagland and Synder, 1933
Prunus Persica (peach) Weinberger and Cullinan, 1937
Lupinus spp. (lupins) Montemartini, 1911; blue lupin, Scholz, 1934
Medicago sativa (lucerne) D'Ippolito, 1914
M. denticulata (toothed bur clover) Samuel and Piper, 1929
Trifolium subterraneum (subterranean clover) Samuel and Piper, 1929
Vicia Faba (broad bean) Bonomi, 1908; Tacheuchi, 1909; Montemartini, 1911
Pisum arvense (field pea) McHargue, 1923
P. sativum (garden pea) Kakahi and Baba, 1907
Lathyrus tuberosus (earth-nut pea) Zlartarov, 1934
Cicer arietinum (chick pea) Zlartarov, 1934
Phaseolus communis (kidney bean) Andouard and Andouard 1911; Stoklasa, 1911
Glycine hispida (soya bean) McHargue, 1923
Vigna sinensis (cow pea) McHargue, 1923
Linum usitatissimum (flax) Takeuchi, 1909
Citrus sinensis (orange) Haas, 1932
C. limonia (lemon) Haas, 1932
C. limonia (rough lemon) Haas, 1932
Aleurites fordii (tung-oil tree) Reuther and Dickey, 1937
A. montana (mu-oil tree) Reuther and Dickey, 1937
Vitis vinifera (vine) Montemartini, 1911
Lagerstroemia indica (crape myrtle) Dickey and Reuther, 1938
Psidium cattleianum (cattley guava) Dickey and Reuther, 1938
Apium graveolens (celery) Boullanger, 1912
Daucus Carota (carrot) Boullanger, 1912
Vaccinium corymbosum (blueberry) Shive, 1933
Allamanda cathartica Dickey and Reuther, 1938
Solanum tuberosum (potato) Gregoire, Hendrick and Carpiaux, 1907
Lycopersicum esculentum (tomato) Schreiner and Dawson, 1927

Bignonia venusta (flame vine) Dickey and Reuther, 1938
Thunbergia grandiflora Dickey and Reuther, 1938
Lactuca sativa (lettuce) Gilbert and McLean, 1928

Zea mais (maize) Suthert and Ingle, 1908
Saccharum officinarum (sugar cane) Lee and McHargue, 1928
Oryza sativa (rice) Aso, 1904
Phalaris bulbosa (Toowoomba canary grass) Samuel and Piper, 1929
Phleum pratense (timothy) Gram, 1936
Avena sativa (oat) Bertrand, 1905
Arrhenatherum avenaceum (tall oat grass) Gram, 1936
Danthonia penicillata (slender Wallaby grass) Samuel and Piper, 1929
Bromus uniloides (prairie grass) Samuel and Piper, 1929
Triticum vulgare (wheat) Nazari, 1910; Andouard and Andouard, 1911
Secale cereale (rye) Scharrer and Schropp, 1934
Hordeum distichum (barley) Katayama, 1906
Lolium subulatum (Wimmera rye-grass) Samuel and Piper, 1929
Lemna minor (duckweed) Hopkins, 1931
Allium Cepa (onion) Gilbert and McLean, 1928

Chlorella Hopkins, 1930
Ditylum brightwelli Harvey, 1939

Rhizopus nigricans McHargue and Calfee, 1931
Saccharomyces cerevisiae (yeast) McHargue and Calfee, 1931
S. apiculatus (yeast) Seiss, 1908
S. ellipsoideus (yeast) Seiss, 1908
Aspergillus niger Bertrand and Javillier, 1911
A. flavus McHargue and Calfee, 1931
Ceratostomella Ulmi Ledeboer, 1934
Trichophyton interdigitale Mosher, Saunders, Kingery and Williams, 1936
Phymatotrichum omnivorum Rogers, 1938

Streptococcus lactis Zlataroff and Kaltschewa, 1936
Lactobacillus casei Woolley, 1941 (increased rate, but not extent, of growth)

Zinc

Carya olivaeformis (pecan) Alben, Cole and Lewis, 1932
Juglans regia (walnut) Chandler, Hoagland and Hibbard, 1932
J. Hindsii (black walnut) Chandler, Hoagland and Hibbard, 1935
Populus sp. (Carolina poplar) Chandler, Hoagland and Hibbard, 1935
Ficus Carica (fig) Chandler, Hoagland and Hibbard, 1935
Fagopyrum esculentum (buckwheat) Sommer, 1928
Sinapis nigra (black mustard) Hoagland, Chandler and Hibbard, 1936

Pyrus Malus (apple) Chandler, Hoagland and Hibbard, 1932
P. communis (pear) Chandler, Hoagland and Hibbard, 1932
Fragaria vesca (strawberry) Hoagland and Snyder, 1933
Prunus Armeniaca (apricot) Chandler, Hoagland and Hibbard, 1934
P. domestica (plum) Chandler, Hoagland and Hibbard, 1932
P. cerasus (cherry) Chandler, Hoagland and Hibbard, 1932
P. Persica (peach) Chandler, Hoagland and Hibbard, 1932
Vicia Faba (broad bean) Sommer, 1928
Pisum sativum (garden pea) Scharrer and Schropp, 1934
Phaseolus vulgaris (red kidney bean) Sommer, 1928
Citrus sinensis (orange) Johnston, 1933
C. limonia (lemon) Haas, 1932
C. grandis (grape-fruit) Parker, 1936
Melia sp. Chandler, Hoagland and Hibbard, 1935
Aleurites fordii (tung-oil tree) Mowry and Camp, 1934
A. montana (mu-oil tree) Mowry and Camp, 1934
Vitis vinifera (grape) Chandler, Hoagland and Hibbard, 1932
Gossypium sp. (cotton) Hoagland, Chandler and Hibbard, 1936
Ligustrum sp. Chandler, Hoagland and Hibbard, 1935
Nicotiana tabacum (tobacco) Hoagland, Chandler and Hibbard, 1936
Lycopersicum esculentum (tomato) Hoagland, Chandler and Hibbard, 1936
Cucurbita maxima (squash) Hoagland, Chandler and Hibbard, 1936
Helianthus annuus (sunflower) Sommer and Lipman, 1926
Zea mais (maize) Mazé, 1914
Triticum vulgare (wheat) Scharrer and Schropp, 1934
Secale cereale (rye) Scharrer and Schropp, 1934
Hordeum distichum (barley) Sommer and Lipman, 1926
Lemna minor (duckweed) Steinberg, 1941
Pinus radiata Kessell and Stoate, 1938

Coccomyxa simplex Roberg, 1932
Chlorella vulgaris Roberg, 1932

Rhizopus nigricans McHargue and Calfee, 1931
R. suinus Nielsen and Hartelius, 1933
Aspergillus niger Raulin, 1869
A. flavus Metz, 1930
A. fumigatus Roberg, 1928
A. oryzae Roberg, 1928
A. ficuum Roberg, 1928
A. cinnamomeus Roberg, 1928
A. fuscus Roberg, 1928
Ceratostomella Ulmi Ledeboer, 1934
Trichophyton interdigitale Mosher, Saunders, Kingery and Williams 1936
Phymatotrichum omnivorum Rogers, 1938
Fusarium oxysporum Niethammer, 1938

Acaulium nigrum Niethammer, 1938
Penicillium sulfureum Metz, 1930
P. luteum Metz, 1930
Macrosporium sp. Metz, 1930
Phoma betae Metz, 1930
Ovularia sp. Metz, 1930
Botrytis cinerea Metz, 1930

Boron[1]

Carya olivaeformis (pecan) Blackmon and Camp, 1932
Cannabis sativa (hemp) Skolnik, 1935
Fagopyrum esculentum (buckwheat) Sommer and Lipman, 1926
Beta vulgaris (sugar beet) Brandenburg, 1921
Spinacia oleracea (spinach) Nakamura, 1903
Persea gratissima (avocado) Haas, 1939
Papaver officinale (poppy) Brandenburg, 1942
Iberis umbellata (candytuft) Löhnis, 1937
Sinapis alba (white mustard) Sommer and Lipman, 1926
S. nigra (black mustard) Chandler, 1941
Brassica campestris (turnip) Scharrer and Schropp, 1934
B. campestris (swede) Jamalainen, 1935
B. campestris (rutabaga) Muhr, 1942
B. oleracea (cabbage) Walker, Jolivette and McLean, 1939
B. oleracea var. *gemmifora* (Brussels sprout) Chandler, 1940
B. oleracea (kale) Chandler, Chucka and Mason, 1938
B. oleracea var. *italica* (sprouting broccoli) Chandler, 1940
B. oleracea (cauliflower) Ferguson, 1938
B. pekinensis (Chinese cabbage) Chandler, 1940
B. caulorupa (kohlrabi) Chandler, Chucka and Mason, 1938
B. Napus (rape) Schropp and Areuz, 1938
Raphanus sativus (radish) Agulhon, 1910
Camelina sativa (gold of pleasure) Schropp and Arenz, 1938
Ribes rubrum (red currant) Löhnis, 1937
Pyrus Malus (apple) McLarty, 1928
Fragaria vesca (strawberry) Hoagland and Snyder, 1933
Rosa sp. (rose) Davidson and Biekert, 1939
Prunus Persica (peach) Weinberger and Cullinor, 1937
P. Armeniaca (apricot) Hoagland, Chandler and Hibbard, 1936
Lupinus albus (white lupin) van Gennep, 1936
L. hispidus (blue sweet lupin) Schropp and Arenz. 1942
L. luteus (yellow lupin) van Gennep, 1936
Medicago sativa (lucerne) Brenchley and Warington, 1927
Melilotus sp. (melilot) Cook, 1938
Trifolium incarnatum (scarlet clover) Warington, 1923
T. pratense (red clover) Brenchley and Warington, 1927

[1] A number of the later entries under this head are given on the authority of Dennis and Dennis (1941, 1943).

Trifolium repens (white clover) Brenchley and Warington, 1927
T. minus (lesser clover) Brenchley and Warington, 1927
T. hybridum (Alsike clover) Cook, 1938
Onobrychis sativa (sainfoin) Mevius, 1928
Ornithopus sativus (serradella) Kedrov-Sichman and Dankova-Anochina, 1940.
Arachis hypogea (pea-nut) Burkhart and Collins, 1942
Vicia Faba (broad bean) Warington, 1923
V. sativa (common vetch) Sommer, 1927
V. villosa (hairy vetch) Löhnis, 1937
Pisum sativum (garden pea) Nakamura, 1903
Lathyrus odoratus (sweet pea) Laurie and Wagner, 1940
Lens esculenta (lentil) Kalantyr, 1939
Phaseolus multiflorus (scarlet runner) Warington, 1923
P. vulgaris (dwarf bean) Brenchley and Warington, 1927
Glycine hispida (soya bean) Brenchley and Warington, 1927
Linum usitatissimum (flax) Sommer and Lipman, 1926
Citrus sinensis (orange) Haas, 1929
C. limonia (lemon) Haas, 1929
Ricinus communis (castor oil) Sommer and Lipman, 1926
Euphorbia pulcherrima (poinsettia) Laurie and Wagner, 1940
Impatiens balsamina (balsam) Rehm, 1937
Vitis vinifera (vine) Maier, 1937
Malva verticillata (forage mallow) Schropp and Arenz, 1940
Gossypium sp. (cotton) Sommer and Lipman, 1926
Begonia semperflorens Laurie and Wagner, 1940
Apium graveolens (celery) Purvis and Ruprecht, 1935
Daucus Carota (carrot) Warington, 1940
Vaccinium corymbosum (blueberry) Shive, 1935
Ipomoea Batatas (sweet potato) Cooper, 1938
I. purpurea (morning glory) Ark and Thomas, 1940
Phacelia sp. Schropp, 1941
Salvia occidentalis s'Jacob, 1936
Perilla ocymoides Skolnik, 1935
Solanum tuberosum (potato) Breckenridge, 1921
Lycopersicum esculentum (tomato) Johnson and Dore, 1928
Nicotiana tabacum (tobacco) Swanback, 1927
Calceolaria herbeohybrida (calceolaria) Ark and Tompkins, 1941
Sinningia speciosa (gloxinia) Ark and Tompkins, 1941
Gardenia Veitchii Laurie and Wagner, 1940
Coffea arabica (coffee) s'Jacob, 1936
Cucumis Melo (melon) Brenchley, 1926
Cucurbita Pepo (pumpkin) Sommer, 1927
C. maxima (squash) Purvis and Hanna, 1940
C. Melo var. *cantalupensis* (cantaloupe) Stier, 1942
Helianthus annuus (sunflower) Sommer and Lipman, 1926
Dahlia variabilis Sommer, 1927
Chrysanthemum indicum Ferguson and Wright, 1940
Carthamnus tinctorius (safflower) Schropp, 1941

Crepis biennis (hawk's beard) Herzinger, 1940
Lactuca sativa (lettuce) McHargue and Calfee, 1933
Sonchus oleraceus (sow thistle) Herzinger, 1940
Cichorium Intybus (chicory) Muhr, 1942
Taraxacum dens-leonis (dandelion) Muhr, 1942
Zea mais (maize) Mazé, 1919
Saccharum officinarum (sugar cane) Martin, 1924
Sorghum vulgare Sommer, 1927
S. sudanense Skolnik, 1935
Panicum miliaceum (millet) Sommer, 1927
Phalaris canariensis (canary grass) Schropp, 1940
Avena sativa (oat) Agulhon, 1910
Triticum vulgare (wheat) Agulhon, 1910
Secale cereale (rye) Löhnis, 1937
Hordeum distichum (barley) Sommer and Lipman, 1926
Lemna minor (duckweed) Steinberg, 1941
L. polyrrhiza (small duckweed) Geigel, 1935
Tradescantia albiflora Meier, 1938
Allium Cepa (onion) Löhnis, 1937

Chlorella sp. Geigel, 1935
Ulothrix tenerrima Herzinger, 1940

Dothiorella sp. Davis, Marloth and Bishop, 1928
Azotobacter chroococcum Herzinger, 1940

Silicon

Beta vulgaris (red beet) Raleigh, 1939
Helianthus annuus (sunflower) Lipman, 1938
Zea mais (maize) Mazé, 1919
Oryza sativa (rice) Ishibashi, 1937
Hordeum distichum (barley) Lipman, 1938

Aluminium

Pisum sativum (garden pea) Sommer, 1926
Helianthus annuus (sunflower) Lipman, 1933
Zea mais (maize) Mazé, 1919
Panicum miliaceum (?) (millet) Sommer, 1926
Avena sativa (oat) Stoklasa, 1922
Glyceria aquatica (reed meadow grass) Stoklasa, 1922
Triticum vulgare (wheat) Stoklasa, 1922
Hordeum distichum (barley) Stoklasa, 1922
Juncus effusus (rush) Stoklasa, 1922

Chlorine

Fagopyrum esculentum (buckwheat) Nobbe and Siegert, 1862
Pisum sativum (garden pea) Lipman, 1938
Zea mais (maize) Mazé, 1919

Copper

Brassica oleracea (cabbage) Russell and Manns, 1933
Linum usitatissimum (flax) Sommer, 1931; Lipman and Mackinney, 1931
Pyrus Malus (apple) Anderssen, 1932
P. communis (pear) Anderssen, 1932
Prunus domestica (plum) Anderssen, 1932
P. Persica (peach) Anderssen, 1932
P. Armeniaca (apricot) Anderssen, 1932
Trifolium subterraneum (subterranean clover) Piper, 1942
Medicago sativa (lucerne) Piper, 1942
Pisum sativum (garden pea) Piper, 1942
Vicia Faba (broad bean) Russell and Manns, 1933
Glycine hispida (soya bean) Russell and Manns, 1933
Ipomoea Batatas (sweet potato) Russell and Manns, 1933
Solanum tuberosum (potato) Russell and Manns, 1933
Lycopersicum esculentum (tomato) Skinner and Ruprecht, 1930
Helianthus annuus (sunflower) Sommer, 1931
Zea mais (maize) Russell and Manns, 1933
Saccharum officinale (sugar cane) Allison, 1930
Oryza sativa (rice) Harrison and Subrahmanya Azya, 1917
Phalaris tuberosa (a canary grass) Piper, 1942
Avena sativa (oat) McHargue and Shedd, 1930
Triticum vulgare (wheat) Russell and Manns, 1933
Hordeum distichum (barley) Lipman and Mackinney, 1931
Lolium subulatum (Wimmera rye-grass) Piper, 1942

Rhizopus nigricans McHargue and Calfee, 1931
Aspergillus niger Bortels, 1927
A. fumigatus Roberg, 1928
A. oryzae Roberg, 1928
A. ficuum Roberg, 1928
A. cinnamomeus Roberg, 1928
A. fuscus Roberg, 1928
A. flavus McHargue and Calfee, 1931
Ceratostomella Ulmi Ledeboer, 1934
Trichophyton interdigitale Mosher, Saunders, Kingery and Williams, 1936
Phymatotrichum omnivorum Rogers, 1938

Bacillus fluorescens Russell and Manns, 1933

Columbium

Penicillium Javanicum Lockwood, 1933

Molybdenum

Prunus cerasifolia (myrobalan plum) Hoagland, 1941
Lycopersicum esculentum (tomato) Arnon and Stout, 1939

Avena sativa (oat) Piper, 1940
Hordeum distichum (barley) Arnon, 1937
Lemna minor (duckweed) Steinberg, 1941

Penicillium Javanicum Lockwood, 1933
Aspergillus niger Steinberg, 1936

Tungsten

Penicillium Javanicum Lockwood, 1933

Gallium

Lemna minor (duckweed) Steinberg, 1941

Aspergillus niger Steinberg, 1938

In addition to the elements included in the above list, claims have been made that others, for example, tin and uranium, 'stimulate' plant growth. Until further information accumulates it would be wiser to defer judgement on the significance of these claims. It should also be noted that some of the elements in the list are scarcely generally accepted at present as micro-nutrients. In this category are columbium and tungsten. The elements most definitely accepted as micro-nutrients are manganese, zinc, boron, copper and molybdenum, but the evidence for aluminium, silicon, chlorine and gallium for the plants cited in the list is very strong.

CHAPTER II

METHODS OF INVESTIGATING MICRO-NUTRIENT PROBLEMS

1. The Purification of Materials used in Culture Experiments

The existence of the micro-nutrients raises a number of problems which can only be solved after the development of methods designed specially to deal with them. In the first place the question inevitably arises as to how we can be certain that any particular element is really essential or not. This is obviously a problem of mineral nutrition requiring immediate solution; it is clearly an essential preliminary for all investigations on the micro-nutrients that we should know what these are.

It has already been pointed out that the essentiality of the micro-nutrients for plant development was overlooked for half a century simply because an adequate supply of them was introduced into the culture (1) from the seed from which the plant developed, (2) from impurities present in the water and salts used in preparing the culture solutions, and (3) by solution from the vessels used to hold the culture solutions. Hence the problem of determining the micro-nutrients clearly resolves itself into devising means for preventing the introduction of micro-nutrients from these three sources. If this can be done the effect of the absence or presence of any particular element in the culture medium can then be determined for a wide range of plant species.

The prevention of the introduction of any particular elements from the seed used for the cultures is perhaps scarcely possible. But when the seed is small the amount of any micro-nutrient present in it is likely to be negligible, and when the seed is large the amount introduced can often be considerably reduced by removal of the cotyledons from the seedling as soon as the latter is established. Reference has already been made to Lipman's method of meeting the difficulty in his experiments with buckwheat, in which seed was used from plants grown in culture

solutions devoid of chlorine. It appears clear that by this procedure seed was obtained containing a negligibly small quantity of this element.

The problem of obtaining water and salts free from the trace elements for work on the nutrition of fungi has been dealt with by a number of workers, notably Steinberg (1919, 1935*b*). It may be stated at once that pure salts sold as analytical reagents may contain a sufficiency of trace elements present as impurities to allow the growth of plants, nor does recrystallization necessarily afford an adequate means for the removal of these contaminants.

Steinberg's original precedure for obtaining a nutrient solution free from so-called heavy metal contaminants consisted in heating the complete nutrient solution with pure calcium carbonate under pressure. The nutrient solution used was one due to Pfeffer and was made up as follows:

Sucrose	50 g.
Ammonium nitrate	10 g.
Potassium dihydrogen phosphate	5 g.
Magnesium sulphate	2·5 g.
Ferrous sulphate	Trace

Since Steinberg's treatment of the solution leads to the removal of iron it is obvious that the trace of ferrous sulphate may be omitted.

To a litre of this solution 15 g. of pure calcium carbonate were added and the mixture heated in an autoclave for 20 min. under a pressure so as to give a temperature of 120·5° C. The mixture was then allowed to stand overnight and the clear solution then decanted from the sediment. Subsequently, Steinberg recommended filtering the solution from the sediment immediately after autoclaving.

The principle involved in this method of purification consists in increasing the alkalinity of the solution so that the traces of heavy metals are precipitated as carbonates, hydroxides and phosphates which are adsorbed on calcium salts precipitated in some bulk. In this way traces of iron, manganese, zinc and copper are removed from the solution.

Various modifications of this procedure have been proposed. Steinberg himself points out that the substitution of basic magnesium carbonate for calcium carbonate has certain advan-

tages where work on fungi is concerned. It does not involve the introduction into the solution of calcium, an element of which the necessity for fungus nutrition is, as we have already seen, doubtful. Indeed, it appears to effect a pretty complete removal of any calcium present as impurity in the nutrient solution. Also the use of an autoclave is unnecessary, heating for 20 min. at 100° C. being sufficient to precipitate the heavy metals. Care has, however, to be taken to avoid excess of the basic magnesium carbonate, as otherwise more or less complete removal of phosphate may result.

Bortels (1927) similarly purified the nutrient solution by precipitating the traces of the heavy metal contaminants with a small quantity of ammonium sulphide and adsorbing the precipitate on charcoal. Actually the ammonium sulphide appears to be unnecessary, according to Roberg (1928), who also purified the charcoal from mineral ash constituents by a preliminary treatment with acid. However, Steinberg pointed out that removal of the ash constituents appears to reduce the adsorbing power of charcoal, and he concluded that the use of charcoal is only advisable when for some reason it is essential to avoid the use of an alkaline earth compound.

In 1927, Hopkins and Wann made use of the adsorptive property of calcium phosphate for the removal of iron from culture solutions for the green alga *Chlorella* and later, for work with green algae and *Lemna*, Hopkins (1934) again employed calcium phosphate as an adsorbent for removal of traces of manganese from the nutrient solution. Sakamura, who had previously (1933, 1934) used charcoal for removal of traces of heavy metals from the nutrient medium of *Aspergillus* spp., concluded (1936), by polarographic examination[1] of nutrient solutions after treatment with charcoal and calcium phosphate respectively, that the latter effected a much more complete removal of the heavy metal contaminants, a conclusion which was confirmed by growth experiments. His procedure was as follows. Calcium phosphate was first purified by washing with water distilled in a glass still, a suspension of 50 g. calcium phosphate in a litre of distilled water being shaken for 5 hr., during which time the water was

[1] See this chapter, p. 27.

changed four times. The calcium phosphate was then filtered off with the use of ash-free filter paper and dried. Calcium phosphate so purified was then added to the culture solution so that it was contained in the latter to the extent of 0·5 per cent and the whole was brought to a *p*H of 5·5 by means of sodium hydroxide. The solution was then shaken for 2 hr. and twice filtered. The final filtrate comprised the working culture solution.

The preparation of culture solutions free from trace elements for work with higher plants presents a somewhat different problem from the preparation of nutrient solutions for the growth of fungi. It will be appreciated that it is impracticable to purify the complete culture solution for higher plants owing to the large quantities of solution required, and it is thus necessary to remove the trace elements from the water and nutrient salts separately. The means by which this may be done have been described in detail by Stout and Arnon (1939). As regards the water used, it is necessary to avoid the use of distillation apparatus made of or containing copper, silver, tin or other metal. Although the actual content of contaminants in distilled water from a metal still may be very small, yet the quantity of water used, particularly in the culture of higher plants, is very considerable, so that the absolute amount of contaminant presented to the plant may be far from negligible. Stout and Arnon found that ordinary distilled water contained from 0·1 to 0·01 p.p.m. of metal contaminants. They recommended, therefore, that water should be redistilled, using a trap and condenser of pyrex glass and distilling at a rate slow enough to give a cool distillate. Water is obtained in this way free from metal impurities. It should be noted that the use of Jena glass is to be avoided, since this contains zinc which may appear in the distilled water.

The mineral salts used by Stout and Arnon were calcium nitrate, potassium nitrate, magnesium sulphate, diammonium phosphate, dipotassium phosphate and ammonium sulphate. Molar solutions of each of these salts were prepared and purified separately, 5 l. at a time, in 6 l. pyrex flasks provided with a plug of cotton-wool. The principle involved in the purification of the solutions was the same as that employed by Steinberg, but the details of the purification varied somewhat for different

salts. In all cases 65 g. calcium carbonate and a small quantity of a solution of some other salt were added to 5 l. of the solution to be purified. For calcium nitrate and potassium nitrate the solution added along with the calcium carbonate was 50 ml. of molar dipotassium phosphate; with dipotassium phosphate and diammonium phosphate the added solution consisted of 25 ml. of molar calcium nitrate, and with magnesium sulphate and ammonium sulphate the added solution was 50 ml. of molar calcium nitrate + 50 ml. of molar dipotassium phosphate. The purification of all the solutions except that of ammonium sulphate was then effected by autoclaving the mixture for an hour at 20 lb. pressure, allowing the solution to stand overnight and then filtering. The ammonium sulphate was treated similarly except that the solution was heated for 45 min. in a steamer instead of in an autoclave. The final filtrates of the dipotassium and diammonium phosphate were acidified to *p*H 5·5 with pure sulphuric or nitric acid.

For testing the purity of the solutions so prepared Stout and Arnon made use of dithizone (diphenylthiocarbazone). The testing reagent is prepared by dissolving 0·1 g. of purified dithizone in 100 ml. of redistilled chloroform. This reagent gives a red or purple colour in the chloroform layer when it is added to a solution containing zinc, copper, lead, nickel, cobalt, cadmium, thallium, mercury or bismuth. By comparing the colour produced by standard solutions with that produced by the purified nutrient solutions it was found that the latter usually contained less than one part of metal contaminants in 10^8 parts of solution, a degree of purity which was deemed sufficient for culture work on micro-nutrients. Although manganese does not give the colour reaction with dithizone it was concluded that if the other metal contaminants which do produce the colour with dithizone are removed, the manganese will have been removed also.

The salts so purified did not include iron. This was provided as a solution containing 0·5 per cent ferrous sulphate + 0·5 per cent tartaric acid which was added twice weekly to the extent of 0·5 ml. per litre of culture solution.

That the method of purification used by Stout and Arnon was justified is clear from the fact that they obtained definite effects

which were reproducible in the growth of plants by adding 1 part of zinc in 2×10^8 parts of culture solution.

There remains the question of the vessels used to contain the culture solutions. There appears to be a general agreement that containers made of pyrex glass form suitable culture vessels for work on micro-nutrients.

2. The Estimation of Micro-nutrient Elements in Plant Material

In order to investigate the part played by micro-nutrients in plants it is a prerequisite that methods should be available for the quantitative determination of each of them in plant tissues, for without quantitative data little advance in knowledge of any value is likely to accrue. In general, however, the quantities of these elements present in the tissues are so small that the ordinary methods of quantitative chemical analysis are useless for the purpose, and methods have to be found by which very small quantities of the elements concerned can be determined with a reasonable degree of accuracy. Indeed, the advance of plant physiology in general has been retarded very considerably by the lack of methods for measuring many substances in very small quantity, and it is certain that increase of knowledge of the physiology of plants waits in large measure on the development of such micro-methods.

During the last decade a number of physical instruments have been developed which can be employed by the plant physiologist for the measurement of small quantities of material, and it is now possible with their aid to determine with sufficient accuracy all the known micro-nutrients in plants. These instruments are the absorptiometer, the polarograph and the spectrograph. The absorptiometer is an adaptation of the colorimeter in which the depth of colour of a solution is measured by matching it against that of a standard solution, the matching being made, not by the eye, but by a photoelectric cell. The use of this instrument for the determination of small quantities of phosphorus has been described by Berenblum and Chain (1938), who have shown that quantities as small as 0·1μg. can be measured with it. Not only have A. D. Skelding and I used the

instrument for this purpose, but it has also been used successfully in my laboratory to determine small quantities of magnesium, iron, aluminium and manganese in the course of work not yet published. The smallest quantities of each of these elements which have so far been determined in this manner are noted later.[1]

The polarograph is an instrument in which a solution of an electrolyte in presence of another electrolyte in much higher concentration (known as the ground substance or supporting electrolyte) is subjected to a gradually increasing difference of potential between two electrodes, one of which consists of a series of small drops of mercury delivered from the end of a capillary tube, while the other consists of a still mass of mercury with a comparatively large surface. In these circumstances, when certain experimental conditions are fulfilled a current (the so-called "wave") flows through the solution when the potential difference reaches a certain value determined by the nature of the cation, or in certain circumstances by the anion, present, while the magnitude of the current is determined by the concentration of these cations (or anions). By means of this instrument it is possible to measure quantities of a number of cations and anions of the order of 1μg. or less. Among the ions which have so far been determined in this way in my laboratory in the course of the last five years are potassium, copper, manganese, aluminium, iron, zinc, barium, chloride, sulphate and nitrate, though in general it should be noted that it is not possible with the polarograph to determine one alkali metal in presence of another.

The polarograph was developed by Jaroslav Heyrovsky and appears to have been first described by Heyrovsky and Shikata in 1925, but although much of the pioneer work with the instrument was described in English in the *Collection of Czechoslovak Chemical Communications*, it appears until recently to have been little used in this country. There can, however, be no doubt whatever that this instrument is a most valuable tool for the student of plant nutrition, and the recent publication in English of a book on polarographic analysis by Kolthoff and Lingane (1941) may render an appreciation of the value of the polarograph more widespread.

[1] Since this was written there has appeared an authoritative work on the absorptiometer by F. W. Haywood and A. A. Wood (1944).

Undoubtedly the use of the spectrograph affords the most sensitive method of measuring small quantities of a large number of elements.

Under certain conditions a substance can be made to emit radiation, this radiation being limited to certain wave-lengths which are characteristic of the elements in the substance and of the conditions used to excite the radiation. If the radiation passes through a prism or diffraction grating the radiations of different wave-lengths are separated and a spectrum results, the radiation possessing the longest wave-lengths being at one end of the spectrum and that possessing the shortest wave-lengths at the other. In the spectrograph such a spectrum is made to fall on a photographic plate, and the plate, on development, constitutes a photographic negative of the spectrum. This usually consists of a number of lines, each line corresponding to radiation of a definite wave-length and possessing an intensity of blackness depending, within the limits of under-exposure and over-exposure of the plate, on the intensity of radiation of that particular wave-length. Determination of the intensity of blackening of a line characteristic of a particular element should therefore afford a means of estimating the quantity of that element in a sample of material, for example, plant ash, which has been subjected to the necessary conditions for inducing an emission of radiation from it.

Of the various ways in which radiation suitable for spectrographic examination may be produced three have been developed to a considerable extent; these are by means of a flame, by the use of the electric arc and by the use of an electric spark. The spectra produced in these ways are known as flame, arc and spark spectra respectively. Each method has its own particular advantages and disadvantages. Perhaps with workers on plant material the flame method has so far proved the most popular, but this is by no means so with workers in other scientific fields.

Flame spectra, as the name suggests, are produced when a substance is heated in a flame. Although spectra are produced when the flame is that of a Bunsen burner, for analytical purposes a flame much hotter than this is generally required, and air-acetylene, oxy-coal gas, oxy-hydrogen and oxy-acetylene

flames have all been employed with more or less success. Two distinct procedures have been employed for introducing the substance into the flame. In one a small quantity of the substance is contained on or in a piece of filter paper and the latter then introduced into the flame. In the other a solution of the substance is sprayed into the flame through a very fine nozzle. These two methods are largely associated with the names of Ramage and Lundegårdh respectively, but they have both been used, with a variety of modifications in detail, by other workers as well. By maintaining the conditions of experimentation constant the same density of spectral line can be obtained from the same quantity of material, so if calibration is made by the use of a number of samples of known composition it is possible to determine the amount of the element in a sample under examination by measurement of the density of the line and reference to a calibration graph.

In exciting spectra by means of the arc, electrodes in the form of rods, usually of graphite, but sometimes of copper, nickel, iron or other metal, and of as great a purity as possible, are used. The electrodes are in a vertical line and the lower contains at its upper end a cavity in which the substance under examination is held. After bringing the electrodes into contact they are separated to a standard distance apart so that the conditions of the arc are kept as constant as possible. Even so the arc is so variable that it has so far been found impossible to devise an arrangement such that the same amount of material subjected to excitation will produce the same intensity of spectral lines. Hence in quantitative estimation of any element by means of arc spectra it is necessary to have recourse to the device of the 'internal standard'. This is achieved by introducing along with the substance to be examined a known amount of some other substance involving an element which yields a spectral line in the near neighbourhood in the spectrum of the line to be measured. This same consideration holds when the spark discharge is used for exciting spectra. Thus Foster and Horton, in determining boron in plant material by the spark method, added a known quantity of a gold salt to the material they were examining. Within limits the ratio of the intensities of the lines of the element to be measured and of the internal standard is

proportional to the ratio of the quantities of the two elements present, so that by measuring the intensities of both lines and reference to a calibration graph obtained by the use of known mixtures in which the quantities of the two elements are varied the desired determination can be made.

For measuring the intensities of the spectral lines various methods have been devised, but this is now generally effected by means of the microphotometer. In this instrument a narrow beam of light passes through the photographic negative of the spectrum, and then falls on a photoelectric cell, with the result that a current is induced which is measured by a galvanometer. By means of a rack and pinion the plate is moved very slowly over the beam of light so that this passes in turn through the clear plate and the spectral line. The difference in the galvanometer deflexion obtained for the clear plate and the spectral line gives a measure of the intensity of the line and hence of the amount of material. The principles of spectrographic analysis have, of course, been given here in the broadest and simplest terms. Actually such analysis is full of difficulties and many precautions have to be taken to ensure reliable results. A description of these details is outside the scope of this book, and those interested should consult works on spectrographic analysis, particularly the publications by F. Twyman (1935, 1938*a*, 1938*b*), published by Adam Hilger, Ltd., which aim at keeping information on this subject up to date, and the four major works by Lundegårdh (1929, 1934, 1936, 1938), which are of particular value to workers with biological material. The same remarks apply to the use of the microphotometer and the method of calculating results.

It is to be noted that in the method developed by Lundegårdh solutions are analysed, whereas in most other procedures solid samples are used.

It has already been stated that the spectrograph affords the most sensitive method of measuring small quantities of many elements. This is undoubtedly the case when an arc or spark is used, but results obtained with the flame method indicate that the latter, as used up to now, is capable, broadly speaking, of yielding about the same degree of sensitivity as the polarograph and absorptiometer.

But from Lundegardh's experience (1929, 1934) it would appear that the flame method is sufficiently sensitive for the quantitative determination of only three of the micro-nutrient elements listed in Chapter I, namely, manganese, copper and gallium. The most dilute solutions of manganese and copper that can be used are those of a concentration of 5×10^{-6} *M*. As a quantity of 5–15 ml. of solution is required this would mean that the smallest quantity of these elements determinable by the flame method as Lundegårdh used it would be of the order of 1·4–4μg. With a procedure more recently described by Griggs, Johnstin and Elledge (1941), the minimum concentration of manganese usable is given as $1{\cdot}125 \times 10^{-5}$ *M*, while the use of as little as 2 ml. of solution is possible. This would mean that the smallest quantity of manganese measurable would be about 1·37μg.

Data for gallium are not at present available, but a flame spectrum of this element reproduced by Lundegårdh suggests that the sensitivity is certainly high for this metal.

Using the arc the author has found it possible to measure quantities of manganese as small as 0·05μg., and no doubt even smaller quantities than this could be measured with a suitable choice of experimental conditions.

It must be borne in mind that for the determination of any particular element one or other of the methods that have been here indicated may be inapplicable. Thus so far it has not been found possible to determine either boron or magnesium, both important plant nutrients, by means of the polarograph. The presence of magnesium as an impurity in graphite may render the use of a graphite arc impracticable for the determination of that element spectrographically. A method, while usable for the determination of larger quantities, may not be sufficiently sensitive for measuring the small amounts of micro-nutrients present in available samples of plant material. Many preliminary trials of the different methods may thus be necessary before the investigator can decide what method to use for the estimation of any particular micro-nutrient.

The degree of accuracy generally obtainable by what may be called micro-methods is much less than that obtainable in most macro-chemical determinations, but is generally sufficient for

the kind of problem which faces the investigator of plant nutritional and pathological problems. Broadly speaking it may be said that the results of a single determination obtained by the methods that have been outlined here are correct within about 5–10 per cent. A higher degree of accuracy is no doubt often possible, and with the absorptiometer, polarograph and spectrograph it has been claimed in certain circumstances that the error of a single determination does not exceed 2 per cent. By making a number of replicate determinations and taking the mean value a considerable increase in the accuracy of an estimation may be achieved, but the limited amount of material available may often render this procedure impossible.

We may next turn to a consideration of the methods suitable for the estimation of the individual micro-nutrients in plant material. They are usually determined in plant ash. This should be prepared by first drying the material in an oven at 70–100° C., grinding it to a powder and then incinerating it in a furnace at a temperature of from 450 to 600° C. Burning in a crucible over a Bunsen burner or blowpipe is not to be recommended, as this leads to an intense local heat causing partial volatilization of some of the mineral matter. The ash, after cooling, is dissolved in a small quantity of mineral acid and the resulting solution evaporated to dryness on a water-bath. The residue is then dissolved in a definite volume of water or dilute acid. Insoluble silica may be removed by filtering.

The preparation of ash in this way is not always to be recommended. For example, Reed and Cummings (1941) found that there was a considerable loss of copper, amounting to 50 per cent or more, involved in this method of ashing, even when the temperature of ignition was as low as 450° C. For the estimation of copper in plant material they therefore recommend a procedure in which from 0·5 to 2 g. of the plant material are heated with 5 ml. of concentrated nitric acid until brown fumes are evolved, when 1 ml. of concentrated sulphuric acid is added and heating continued until charring begins and all nitric acid is given off. After addition of 1–2 ml. of 60 per cent perchloric acid, heating is resumed until the solution is colourless or pale yellow and excess of perchloric acid also given off. The resulting solution should then contain all the copper originally present in the sample used.

It is also possible that such a 'wet ashing' method may be preferable to dry ashing when micro-nutrients other than copper are to be estimated. In this connexion it may be mentioned that Griggs, Johnstin and Elledge (1941) found that dry ashing at 400° C. resulted in a loss of 30 per cent of the total potassium and 10 per cent of the total calcium. They themselves recommend the extraction of the mineral elements with nitric acid and perhydrol. Nitric acid is added to a weighed quantity of the plant material in a 70 ml. pyrex test-tube and the mixture heated on a sulphuric acid bath at a temperature between 120 and 140° C. until the solution is clear. Perhydrol is then added and the tube carefully heated over a micro-burner. If necessary, small additions of nitric acid and perhydrol may be made to effect complete decoloration of the solution.

The best established of the trace elements are, as we have seen, manganese, zinc, copper and boron. The determination of each of these in plant material will now be considered.

Manganese. A considerable number of methods of reasonable accuracy are available for the determination of manganese in small quantities. The spectrograph, polarograph and absorptiometer can all be employed successfully for this purpose. With regard to the spectrograph, the flame, arc and spark methods can all be used, but, as has already been indicated, the sensitivity of the flame method is less than that of the arc. For the determination of manganese in plant material the flame method has been used and described by Lundegårdh (1929, 1934) and more recently by Griggs, Johnstin and Elledge (1941). As already mentioned, by its means about 1·4μg. of manganese can be determined. Lundegårdh claims that the probable error of a single determination made by the flame method is about 1–2 per cent, but the accuracy is no doubt less than this as the amounts determined approach the lower limit of measurable quantities. The line used for the measurement is 4031 A. (Lundegårdh, 1929). Considerably smaller quantities of manganese can be determined by the arc and spark, but, although they have been used quite extensively in metallurgical work, they have received relatively little attention from the point of view of the determination of manganese in plants.

However, Melvin and O'Connor (1941) have used the arc

method for the simultaneous determination of manganese, boron and copper in fertilizers, and their method would appear to be suitable for the determination of these same trace elements in plant material. The lines used were the manganese 2605·7 A., boron 2497·7 A. and copper 3247·5 A., with the beryllium line 3130 A. as internal standard. An accuracy of about ± 5 per cent is claimed for the estimations. Analyses published by the advocates of the method indicate that quantities as small as, or smaller than, 0·1 μg. can be determined by means of their procedure.

Manganese is readily determined with the polarograph, a good, well-defined wave being obtained when a chloride of an alkali or alkaline earth is present in considerable excess. It is usual to employ a few ml. of solution, and as concentrations of from M/250,000 to M/300,000 are measurable, with the use of, say, 5 ml. of solution 1 μg. of manganese is determinable. With the use of special micro-cells taking smaller quantities of solution, very much smaller quantities of manganese can be measured.

But although the polarograph would at first sight appear to offer an ideal way for determining manganese in plants, actually the polarographic determination of manganese in plant ash is not straightforward, and in general is not to be recommended. This is particularly so where ash or extract contains a considerable quantity of phosphate. Plant ash consists chiefly of oxides, phosphates and sulphates of potassium, calcium, magnesium and other metals and is only soluble in acid. Since hydrogen-ion gives a wave in solutions of alkali or alkaline earth chlorides very near to that of manganese, an ash solution cannot be polarographed for manganese directly because the waves for manganese and hydrogen tend to coalesce. On neutralizing the solution the manganese precipitates as phosphate, and in consequence no wave for manganese is then given. To deal with this situation the following procedure has been found by the writer to give in some cases fairly reasonable results. After removal of sulphate by barium chloride the phosphate is removed from the ash solution by the addition of barium carbonate in excess and filtering. This also removes ferric iron and aluminium, the wave for the latter of which is sufficiently close to that of manganese

to render the end-point of the wave for the latter rather indeterminate, even if the two waves do not coalesce. The large quantity of potassium, calcium and magnesium chlorides present in the solution acts as ground substance and the filtered solution can be polarographed directly. Where the quantities of sulphate and phosphate in the ash are relatively small, results obtained by this treatment have been in reasonable agreement with results obtained by other methods, but where much sulphate and phosphate are present results are not reliable, owing probably to adsorption of manganese by barium sulphate or phosphate. For each determination two solutions are taken, one consisting of ash solution, the other consisting of ash solution containing the same concentration of ash but with a known amount of added manganese. The difference in the heights of wave given by the two solutions is then attributable to the added manganese, to which the height of the wave given by the pure ash solution is referred.

Since a number of metals are reduced at more positive potentials than manganese there is the possibility that they might interfere with the wave for this ion. The principal elements concerned are copper, cadmium, lead, chromium, molybdenum, cobalt, nickel, iron and zinc. It is extremely unlikely that any of these, with the exception of the last two, are likely to occur in sufficient quantity in plant material to disturb the polarographic determination of manganese. As regards iron this is practically all removed by the treatment of the ash solution outlined above, and, as a matter of fact, no wave for iron appears in the polarograms of solutions so treated. The possible influence of zinc on the manganese wave was examined by the writer, who found that no effect was produced on the wave of $M/10{,}000$ manganese by zinc in concentrations up to five times that amount. No ash examined by the writer has been found to contain a proportion of zinc to manganese approaching that value.

A number of methods for determining manganese with the use of the absorptiometer are available. Most of these depend on the oxidation of the manganese salt with the production of permanganate, the intensity of the colour of which is determined with the absorptiometer. The methods are known as the periodate, persulphate and bismuthate methods, according to the

reagent used for effecting the oxidation. Trials carried out by my colleagues, Dr K. W. Dent and Dr E. S. Twyman, indicate that the periodate method is the most satisfactory of these, as regards both simplicity of procedure and accuracy of the results obtained. This experience appears to be fairly general if we are to judge from the fact that this method is the one most commonly used by recent workers. A number of accounts of the procedure employed in using the method have been published; two of the more recent are those by Coleman and Gilbert (1939) and Cook (1941). Coleman and Gilbert found that the same values for manganese content of tea and coffee were obtained with a wet ashing process and with incineration in a muffle, so it is concluded that no loss of manganese occurs with either ashing process. When dry ashing is employed 1 g. of material is moistened with 1–2 ml. of concentrated sulphuric acid and ashed at a dull red heat in a muffle. The ash is dissolved in 15 per cent sulphuric acid and filtered. The filter paper is ignited and any residue from this dissolved in about 10 ml. of dilute sulphuric acid and filtered into the main filtrate. To the combined filtrate 0·1 g. of potassium iodate is added and the mixture boiled for a few minutes and then kept hot for 30 min. for the development of the pink colour. The solution is made up to standard volume and the intensity of the colour determined.

Amounts of manganese down to about 12μg. can be estimated in this way.

For a description of the bismuthate method, which is particularly recommended for the determination of manganese in soils, reference may be made to a paper by Dean and Truog (1935) and for the persulphate method to papers by Majdel (1930) and Olsen (1934).

A method for the estimation of small quantities of manganese which has been described by Sideris (1940) depends on the colour produced when formaldoxime is added to a solution of a manganese salt. The formaldoxime reagent is prepared by dissolving (by boiling) 20 g. of trioxymethylene + 47 g. of hydroxylamine sulphate in 100 ml. of distilled water. Ten ml. of the hydrochloric acid solution of the ash are neutralized with sodium hydroxide and then acidified with 2 ml. of a 20 per cent solution of acetic acid. Excess phosphate is then removed by adding

0·5 ml. of a 5 per cent solution of lead acetate, the mixture shaken, allowed to stand for 10 min. and then treated with 1 ml. of a 20 per cent solution of sodium sulphate to remove excess lead. After 30 min. the precipitate is removed by filtration or centrifuging and the clear solution neutralized with 40 per cent sodium hydroxide. Three or four drops of formaldoxime reagent are added to the liquid and then more 40 per cent sodium hydroxide until a wine-red colour develops, the intensity of which is said to be directly proportional to the concentration of manganese. The liquid is made up to a standard volume and the intensity of the colour determined. According to Sideris it would appear that quantities of manganese of the order of 5μg. can be determined in the presence of 10–100μg. of phosphate with an error not exceeding 4 per cent. Under more favourable conditions quantities of manganese down to 0·25μg. would appear to be determinable.

Yet another method for the determination of small amounts of manganese has been described by Wiese and Johnson (1939). The nitric acid solution of the ash containing from 1 to 10μg. of manganese is first rendered free from chlorides by three times evaporating it to dryness and redissolving in nitric acid + 10 ml. of distilled water. About 0·2 g. of sodium bismuthate is added and the mixture boiled for 2–3 min. On cooling to below 30° C. 0·2–0·3 g. of sodium bismuthate is added, and after mixing the sample thoroughly and allowing it to stand for a few minutes the excess of sodium bismuthate is filtered off through a Gooch crucible. The solution filters directly into the absorptiometer cell containing two drops of a solution of benzidine (1 per cent in 5 per cent acetic acid) in 3 ml. of distilled water. The solution is made up to standard volume and the intensity of the yellow-green colour which develops is estimated after 5 minutes.

Zinc. For the determination of zinc in plant material by means of the spectrograph, the flame method is not sufficiently sensitive. Several workers, however, have described procedures for the spectrographic determination of zinc in such material by using the arc. Thus Rogers (1935) advocated using the zinc line 2138·5A. but found it was necessary to sensitize the photographic plate by spreading mineral oil over the emulsion, or, alternatively, using a special plate (Eastman spectroscopic

plate type III-0) with ultra-violet sensitization. The tellurium line 2143·0A. was used as internal standard. Vanselow and Laurance (1936), on the other hand, recommended the use of the zinc line 3345·0A. with cadmium line 3252·5A. as internal standard. To a hydrochloric-acid solution of plant ash a known amount of cadmium sulphate was added and the zinc and cadmium then precipitated as sulphides by a special technique: the sulphides were then spectrographed. Rogers and Gall (1937) reported unfavourably on the procedure of Vanselow and Laurance and suggested that zinc in plant ash is not completely extracted by hydrochloric acid. O'Connor (1941), in developing spectrochemical methods for the determination of trace elements in fertilizers, like Rogers used the zinc line 2138·5A. for the determination of this element, but employed the beryllium line 2348·6A. as internal standard. The spectrograms were obtained on photographic plates with ultra-violet sensitization (Eastman I-0 spectroscopic ultra-violet sensitive plate). O'Connor claims that in this way zinc can be determined within the limits of 2 p.p.m. to 1 per cent with an accuracy within ± 5 per cent. As a 20 mg. sample is used for a determination this means that a quantity of zinc as small as 0·04μg. can be determined.

Lundegårdh (1934) recommends the use of the line 3345·0A. for the determination of zinc by the spark method.

Methods for the polarographic determination of zinc in plant materials have been elaborated by Stout, Levy and Williams (1938), by Reed and Cummings (1940) and by Walkley (1942).

In the method of Stout, Levy and Williams the hydrochloric-acid ash solution (about 100 ml.) from 1 to 2 g. of dried plant material is treated with 5 ml. of N ammonium citrate and then rendered slightly alkaline by the addition of ammonium hydroxide. The resulting solution is then shaken with 10 ml. of a solution of 1–3 mg. of dithizone in chloroform. The resulting chloroform layer, which then contains the zinc, nickel, cadmium, lead and copper, is separated from the aqueous layer containing iron and manganese. The zinc, and accompanying metals, are then removed from the chloroform by two extractions with 10 ml. of 0·5 N hydrochloric acid. The hydrochloric-acid extracts are evaporated to dryness and the residue dissolved in a solution of 0·1 N ammonium acetate $+0{\cdot}025$ N potassium thiocyanate.

On polarographing this solution well-defined and well-separated waves of lead, cadmium, nickel and zinc are obtained.

Reed and Cummings experienced some trouble with the method of Stout, Levy and Williams, particularly when large quantities of zinc or of cadmium, lead, copper, nickel or cobalt were present, and they found that to effect a quantitative separation of the zinc from aluminium, iron and alkali metals five or six extractions, instead of only one, were necessary. They therefore devised a different procedure in which the ash solution in hydrochloric acid was brought to a *p*H of between 4 and 5 by addition of dilute ammonium hydroxide. This precipitates practically all the aluminium and ferric iron which are filtered off. The filtrate is evaporated to dryness on a steam bath and the residue dissolved in a solution of 0·1 *N* ammonium acetate of *p*H 4·6 and containing also potassium thiocyanate of concentration 0·025 *N*, and the resulting solution polarographed. It is stated that no interference with the height of the zinc wave then results from the presence of chloride, sulphate, phosphate, carbonate, sodium, potassium, calcium, magnesium or manganese, and no interference results from lead, cadmium or nickel up to concentrations ten times that of the zinc. Copper interferes if it is present in concentrations ten times or more that of zinc, while the cobalt and zinc waves tend to coalesce if the former is present in relatively high concentration. Actually Reed and Cummings found that if cobalt occurs in concentrations greater than 1×10^{-5} g. per ml. it only interferes with determinations of zinc when the ratio of cobalt to zinc exceeds 2. It would, as a matter of fact, be a very exceptional plant ash in which any of the ions noted occurred in sufficient amount to interfere with the polarographic determination of zinc in this way.

The lowest measurable concentration of zinc is stated by Reed and Cummings to be 0·2μg. of Zn per ml., that is, about *M*/300,000, so that, using 5 ml. of solution, 1μg. of zinc should be determinable.

In Walkley's procedure the dried plant material is first subjected to wet ashing, about a gram of the material being digested with a mixture of 10 ml. of nitric acid, 1 ml. of sulphuric acid and 1 ml. of perchloric acid. Frothing is prevented by addition of a drop of kerosene. The cooled digest is taken up in 15 ml. of

water and boiled, and on cooling 25 ml. of an ammonium citrate buffer are added. This buffer is prepared by dissolving 5 g. of citric acid in 50 ml. of water and 200 ml. of 4 *N* ammonium hydroxide, and then extracting impurities by three successive shakings with 10 ml. of a solution of dithizone in chloroform (1 g. of dithizone in 100 ml. of chloroform), and running off the chloroform layers. The final purified buffer solution contains dithizone in solution.

The zinc is separated from the digest after treatment with the ammonium citrate buffer by three extractions each with 5 ml. of chloroform. The chloroform extracts are evaporated to dryness, then treated with a mixture of 2·5 ml. of nitric acid, 0·5 ml. of perchloric acid, and two drops of sulphuric acid and again evaporated to dryness, boiling being avoided. The residue is then dissolved in 1 ml. of a ground liquid of 0·1 *N* ammonium chloride + 0·02 *N* potassium thiocyanate containing 0·0002 per cent of methyl red and polarographed in a small electrolysis vessel. Practical details for carrying out Walkley's method will be found in Piper's book on *Soil and Plant Analysis* (1942).

For the absorptiometric determination of zinc the most promising method appears to be that based on the coloration given by zinc salts with dithizone (diphenylthiocarbazone). Reference has already been made in this chapter to the fact that this reagent gives a red or purple colour when added to solutions of compounds of a number of metals, including zinc (see p. 25). The resulting coloured compound is quantitatively extracted with chloroform or carbon tetrachloride, and according to R. H. Caughley (see Holland and Ritchie, 1939) sodium diethyldithiocarbamate in 0·02 *N* ammonium hydroxide solution inhibits the reaction of dithizone with all metals except zinc. Cowling and Miller (1941) have made use of this very useful fact to work out an absorptiometric method for the quantitative estimation of zinc in plant materials. Unfortunately, the addition of the sodium diethyldithiocarbamate (generally denoted by 'carbamate' for the sake of brevity) renders the extraction of zinc by dithizone incomplete. By carefully standardizing the technique, however, Cowling and Miller claim that this drawback can be overcome and the quantity of zinc in plant ash determined with reasonable accuracy.

After ashing a 5 g. sample of the dried plant material at 500–550° C., the ash is dissolved in hydrochloric acid, insoluble matter being removed by filtration. The metals which form complexes with dithizone are then extracted from the solution by repeated treatment with excess of a solution of dithizone in carbon tetrachloride at a *p*H of 8·5–9 in presence of ammonium citrate; the latter prevents the precipitation of iron and aluminium. The dithizone extract is then treated with 0·02 *N* hydrochloric acid; copper and the excess of dithizone remain in the carbon tetrachloride phase while the zinc and other metals pass into the aqueous phase. The *p*H of the latter is then adjusted to between 8·5 and 9 by the addition of ammonia-ammonium citrate buffer containing carbamate and the zinc extracted with dithizone in carbon tetrachloride. The resulting extract is used for the determination of the zinc, the reading obtained being compared with those given by solutions of known zinc content which are plotted to give a standard curve. It is essential that the same conditions should be rigidly adhered to both in obtaining the standard curve and in the analysis of samples of plant material; that is, the same *p*H should be used in the extraction, the volumes of phases, the amount of dithizone and the amount of carbamate used should be the same. Tests made by the authors show that if this is done the method is highly reliable. The presence of other metals does not interfere significantly with the determination, a good degree of accuracy was obtained in the reovery of zinc added to plant material, while good agreement was obtained between determinations of zinc in duplicate samples of the same material. The method would appear to be capable of determining quantities of zinc as small as 2μg. or even less.

Copper. The spectrographic determination of copper in plant material can be carried out by both the flame and arc methods. With the use of the Lundegårdh flame method the sensitivity, according to the recent experience of Griggs, Johnstin and Elledge (1941), is about half that found for manganese, these workers giving the minimum concentration of copper usable as 0·000025 *M*. As the minimum quantity of solution which can be employed in their arrangement is 2 ml. this would give the lowest measurable amount of copper to be 3·15μg. The line used for the measurements is 3247·5 A. (Lundegardh, 1929).

It has already been mentioned (p. 33) that with the use of the arc Melvin and O'Connor (1941) have achieved the simultaneous determination of boron, manganese and copper. The copper line used for the determinations was 3248A., and the beryllium line 3130A. was used to provide the internal standard. From the analyses published by Melvin and O'Connor it would appear that the copper forming 0·001 per cent of a 10 mg. sample of material can be measured, from which it would seem that quantities of copper of the order of 0·1μg. can be estimated.

For the spectrographic determination of copper in grasses Rusoff, Rogers and Gaddum (1937), employing the arc, used cadmium as internal standard.

A procedure for the estimation of copper in plant materials by means of the polarograph has been described by Reed and Cummings (1941). The solution obtained by wet ashing of from 0·5 to 2 g. of plant material (see p. 32) is diluted to 15–20 ml., heated to boiling and then rendered alkaline by the addition of a slight excess of concentrated ammonium hydroxide. The solution is then boiled for a minute and filtered, the residue being washed with slightly ammoniacal water and the washings added to the filtrate. The latter is evaporated to dryness and the resulting residue dissolved in 10 ml. of a ground liquid consisting of 4·5 ml. of 0·5 *M* sodium hydroxide + 4·5 ml. of 0·5 *M* citric acid + 1 ml. of 0·05 per cent of acid fuchsin. This liquid, when polarographed in absence of oxygen, gives a well-defined wave for copper. In this way, according to Reed and Cummings, concentrations of copper down to about 3×10^{-6} *M* can be determined, corresponding to about 2 p.p.m. of copper in the plant. Published data indicate that in normal plants the amount of copper is usually well above this value, but in plants showing symptoms of copper deficiency it may be lower than this. In such cases it would be necessary either to use larger quantities of plant material for the determination or to use a method other than a polarographic one.

The absorptiometric method generally used for the determination of copper in plant material depends on the intense colour produced by copper salts with diethyldithiocarbamate (Callan and Henderson, 1929), the coloured complex being extracted with amyl alcohol. Procedures particularly applicable

to biological material have been described by Eisler, Rosdahl and Theorell (1936), by Eden and Green (1940), and by Piper (1942). In the procedure of the first group of workers the dried material is heated on a sand-bath with sulphuric acid and perchloric acid until the mixture is colourless or pale yellow. The resulting liquid is cooled under the tap and then rendered slightly alkaline to litmus by the addition of 8–9 per cent ammonium hydroxide. Iron is precipitated by treating the alkaline solution with a few ml. of a 4 per cent solution of sodium pyrophosphate, then heating at 80° C. for 30 min. and cooling to room temperature. If a crystalline precipitate is present at this stage it must be dissolved by the addition of water. Now 2 ml. of water and 5 ml. of amyl alcohol are added and then immediately 0·5 ml. of a 2 per cent solution of sodium diethyldithiocarbamate.

The mixture is now strongly shaken and centrifuged. The amyl alcohol layer is separated and its light absorption in light of wave-length 4400A. measured. The depth of colour of the amyl alcohol layer is dependent not only on the concentration of copper, but also on the salt concentration of the aqueous phase from which the amyl alcohol layer is separated, the salt concentration depending on the amount of sulphuric acid used. Hence it is necessary to use a constant technique in order to obtain reliable results.

Eden and Green also used a wet ashing method, the material e.g. 5 ml. of blood, 1–5 g. of fresh tissue or 1 g. of dried material, being digested with a mixture of sulphuric and perchloric acids to which nitric acid is added later. After dilution with water the digest is treated with 2 ml. of 50 per cent ammonium citrate and 5 ml. of ammonia (sp. gr. 0·880). The ammonium citrate effects the deionization of the iron and prevents the precipitation of calcium phosphate. The solution is then made up to 25 ml. with water. If the material is relatively low in calcium and phosphorus but high in iron it is preferable to use 10 ml. of 4 per cent hydrated sodium pyrophosphate instead of the ammonium citrate, as the resulting solution is colourless. whereas with citrate the solution is slightly coloured.

Two ml. of a recently filtered 0·5 per cent solution of sodium diethyldithiocarbamate are now added to the solution, kept constantly shaken, then 5 ml. of amyl alcohol added, and the

mixture vigorously shaken for 30 sec. The amyl alcohol extract, which contains the coloured copper diethyldithiocarbamate, is centrifuged or filtered through acid-extracted filter paper to remove any water in suspension and the depth of colour determined with a colorimeter or absorptiometer by comparison with a standard solution of copper diethyldithiocarbamate prepared in the same way as that from the tissue digests. With adequate precautions it would appear that quantities of copper as small as 0·3 g. are determinable by the procedure of Eden and Green.

In the method as used by Piper a wet ashing process is also used, and after diluting the digest with water and treating it with ammonium citrate solution to dissolve any hydrolysed manganese and to prevent precipitation of phosphates, the resulting solution is brought to *p*H 3 and the copper extracted with dithizone in carbon tetrachloride. The carbon tetrachloride is then removed by evaporation and the dithizone by heating with a little sulphuric acid containing a drop or two of perchloric acid. After dilution with water and rendering the solution alkaline with ammonium hydroxide, the copper is precipitated by the addition of a few drops of a 3 per cent aqueous solution of sodium diethyldithiocarbamate. The copper diethyldithiocarbamate is then extracted with amyl alcohol and the intensity of colour of the extract compared with that of a standard. For full details of the experimental procedure reference should be made to Piper's book.

Boron. As regards the determination of boron by means of the spectrograph, the usual flame method is useless. Even with a 0·1 *M* solution of boric acid no boron line was obtained by Lundegårdh in a flame spectrum. Boron in plant material has, however, been determined by means of the spark method by Foster and Horton (1937). The plant material was used fresh, being neither dried nor ashed, but crushed and disintegrated into a fine pulp in a small copper mortar. The samples used consisted of 100 mg. of fresh material. Measurements were made on the boron line 2497·7 A., and the gold line 2427·9 A. was used as internal standard. Six replicate determinations of the boron in turnip leaves gave values varying from 3·7 to 4·3μg. of boron per g. of fresh tissue, with an average value of 4·1μg. Foster and Horton conclude that the error of a single deter-

mination is not in excess of 10 per cent. The limit of sensitivity of the method is not clearly stated, but it would seem probable that quantities of boron as small as 0·1μg. might be measurable in this way. Lundegårdh (1929) suggested the use of the cadmium line 2573A. as internal standard in the determination of boron by the spark method.

As mentioned earlier (see p. 33), Melvin and O'Connor (1941) have used the arc method for the simultaneous determination of boron, manganese and copper in fertilizers, using beryllium as internal standard. The method would seem to be applicable to the determination of boron in plant material, and the accuracy and sensitivity would appear to be of the same order as in the spark method of Foster and Horton.

Although the flame method is not suitable for the spectrographic determination of boron, McHargue and Calfee (1932) have successfully made use of flame spectra for the optical spectroscopic determination of boron. In their earlier procedure the boron was first converted into methyl borate, then volatilized with methyl alcohol and burnt in an atmosphere of oxygen in front of a cell containing a solution of potassium permanganate. The concentration of this solution was adjusted so that the bright lines of the boron spectrum were just obscured by it. By previous standardization of solutions of potassium permanganate against standard boron solutions the concentration of the experimental boron solution was obtained.

Later (Calfee and McHargue, 1937) a different procedure was devised. The spectrum was excited in an oxygen-methane flame, methane saturated with a solution of methyl borate in methyl alcohol being ignited in an oxygen blast. The light emitted from this was polarized and by an optical arrangement the spectrum was brought into juxtaposition with a second spectrum similarly produced by the burning of a standard boron solution. By rotation of an analysing plate the intensity of the spectrum of the standard boron solution could be varied and so matched with that of the solution the boron concentration of which it was desired to measure. For a quantitative determination in this way the boron content of a sample should lie between 25 and 50μg. Agreement between the two procedures was good, but the second method was found to be more exact.

So far it has not been found possible to estimate boron polarographically.

A colorimetric method for the determination of small quantities of boron which has been applied to the estimation of boron in plant material depends on the colour change of quinalizarin effected by boric acid (see e.g. Smith, 1935). To 1 ml. of solution containing from 1 to 40μg. of boric acid 9 ml. of concentrated sulphuric acid are added followed by 0·5 ml. of a 0·01 per cent solution of quinalizarin in 93 per cent sulphuric acid. A colour change from reddish violet to blue results, the complete process taking about 5 min. Nitrate, dichromate and fluoride must not be present, but the common metals do not interfere with the reaction.

While this method appears to be quite satisfactory for the estimation of boron in plant material, the necessity of using concentrated sulphuric acid is something of a drawback. Another colorimetric method for the determination of small quantities of boron, which does not involve the use of this reagent, has more recently been described by Naftel (1939). This method depends on the colour produced when a solution of boric acid is treated with oxalic acid and either curcumin or an extract of turmeric and the mixture evaporated to dryness. According to the procedure recommended by Naftel, the soil or plant-ash extract containing from 0·5 to 8μg. of boron is first rendered alkaline by the addition of 5 ml. or more of 0·1 N calcium hydroxide and then evaporated to dryness on a water-bath. After cooling there are added to the residue 1 ml. of a freshly prepared solution of oxalic and hydrochloric acids (made by adding 80 ml. of a saturated solution of oxalic acid to 20 ml. of concentrated hydrochloric acid) and 2 ml. of a 0·1 per cent solution of curcumin or a 1 per cent freshly prepared extract of turmeric in 95 per cent ethyl alcohol. The mixture is evaporated to dryness on a water-bath at 55° C., heated for a further 30 min. at this temperature and then cooled, extracted with 95 per cent ethyl alcohol and the colour of the clear solution obtained after filtering or centrifuging compared with that of standard solutions prepared in the same manner. Quantities of boron down to 0·5μg. can be determined by this method. In soils other elements present do not appear to interfere with the

estimation of boron, but if large quantities of other substances present are found to interfere with the determination of boron, the latter may first be separated by volatilization with methyl alcohol.

An electrometric titration method has been specially devised by Wilcox (1940) for the determination of boron in plant material. A quantity of the dried and powdered plant material (say, from 5 to 25 g.) containing not more than 2 mg. of boron is mixed with one-tenth of its weight of calcium oxide and ignited in a furnace at a low red heat. The resulting ash after cooling is moistened with water and taken up in 15–20 ml. of 6 *N* hydrochloric acid and then heated on a steam-bath for 30 min. Phosphate is removed from the resulting solution by the addition of *N* lead nitrate solution to the extent of 1 ml. for each gram of plant material used, followed by sodium bicarbonate until a precipitate is produced, when the mixture is heated on a steam-bath and more sodium bicarbonate added until the solution is neutral to brom-thymol-blue (about *p*H 7). The mixture is then made up to 250 ml. and filtered through a dry filter paper. Carbon dioxide is removed by acidifying with 6 *N* hydrochloric acid and heating to boiling, then making alkaline with 0·5 *N* sodium hydroxide and reacidifying with 2 *N* hydrochloric acid until 5–10 drops in excess have been added. On making up to 300 ml. the solution is boiled for a few minutes. It is then ready for electrometric titration. For this the quinhydrone electrode may be used in conjunction with a 0·7 *N* calomel electrode, these giving a null point at approximately *p*H 7. The electrodes having been introduced into the solution, 0·5 *N* sodium hydroxide is added until the solution is neutral to brom-thymol-blue, when the galvanometer should register approximately zero; if it does not the null point is obtained by the addition to the solution of either 0·0231 *N* sodium hydroxide or hydrochloric acid. Five grams of mannitol are then added, and if boric acid is present a galvanometer deflexion results. Standard 0·0231 *N* sodium hydroxide is then added until the null point is again reached. The volume of sodium hydroxide gives a measure of the amount of boron present, 1 ml. 0·0231 *N* sodium hydroxide being equivalent to 0·25 mg. boron. This method is claimed by Wilcox to be specially suitable for deter-

mination of boron in tissues where this element is present in quantity less than 50 p.p.m.

Molybdenum. Molybdenum in plant material is generally determined with the colorimeter or absorptiometer as molybdenum thiocyanate by the method described by Marmoy (1939). A 50 ml. sample of the hydrochloric acid solution of the ash containing not more than 20μg. of molybdenum and having an acid concentration of 14 per cent by volume is treated first with 3 ml. of potassium thiocyanate and then with 3 ml. of stannous chloride. The molybdenum thiocyanate produced is then extracted with ether, the extractions being repeated until the ether layer is colourless, and the depth of colour of the combined ether extract is compared with that of a standard solution in ether of molybdenum thiocyanate prepared from ammonium molybdate in the same way as that from the ash.

Aluminium. Aluminium is present in quantity in many soils and is generally present in plants, but so far its essentiality has been indicated for only a few species. Perhaps for this reason not so much attention has been given to the determination of aluminium in plant material during recent years as to the better established micro-nutrients.

For the measurement of small quantities of aluminium, such as might be expected in samples of plant material, direct measurement of the intensity of the aluminium line in the air-acetylene flame spectrum is not suitable, for Lundegårdh (1929) found that no line was obtained with even a 0·1 *M* solution of aluminium chloride. Mitchell and Robertson (1936) have, however, described a means by which aluminium in concentrations ranging from about 2 to 10 mg. per litre can be determined by the Lundegårdh method. It depends on the fact that the presence of aluminium brings about a lessening of the intensity of the calcium and strontium lines of the flame spectrum, the decrease in intensity varying with the amount of aluminium present and also with the relative amounts of calcium and strontium present. Hence with careful control of the conditions the depression in the intensity of these lines can be used to determine the aluminium content of solutions of the concentrations indicated above. Using 15 ml. of solution for a determination, this means that quantities down to 30μg. of aluminium can be measured in this way.

With the spark the best aluminium line for measurement is, according to Lundegårdh (1934), 3961·5A., but if the sample under examination has a high calcium content, which may frequently be so with plant material, the calcium line 3968·5A. may interfere with the aluminium line.

Aluminium may be determined polarographically with the use of lithium chloride, barium chloride or magnesium chloride as supporting electrolyte, but, owing to the fact that the aluminium wave occurs at a rather high negative potential as well as to difficulties resulting from the presence of phosphates, it is unlikely that the polarograph will afford a simple means for the determination of aluminium in plant material.

Two colorimetric methods, suitable for use with the absorptiometer, depend on the formation of lakes, fairly stable in the presence of acetic acid, when alizarin and the ammonium salt of aurin tricarboxylic acid, respectively, are added to a solution of an aluminium salt. Both appear to be adaptable to the determination of aluminium in plant tissues. The first method appears to have been described first by Atack (1915). The modification of it described by Underhill and Peterman (1929) has been used in my laboratory on fairly pure solutions with marked success. The second method was described by Hammett and Sottery (1925) and was adapted for the determination of aluminium in animal tissues by Myers, Mull and Morrison (1928). Amounts of aluminium of the order of 5μg. can be measured by both methods.

Cobalt and Nickel. Although evidence has occasionally been adduced to indicate that small quantities of cobalt and nickel may bring about an increase in the rate of growth of plants, there has up to now been no definite proof provided that either of these elements is essential for the growth of any plant. There is, however, very definite evidence that cobalt is essential for sheep and cattle, and as the deficiency of this element in the animal must arise from the low content of cobalt in the plants on which the animal feeds, the determination of small quantities of cobalt, at any rate, in plants may be necessary for investigations on cobalt deficiency in animals. There does not seem so far to be any very definite indication that nickel is essential either for any plant or any animal, but since the determination of

nickel can be made in the same way as that of cobalt it is as convenient to consider the two elements as cobalt only.

Although these elements can be determined spectrographically, the flame method scarcely has sufficient sensitivity for their ready estimation in plant material, for it would appear from Lundegårdh's data that the smallest amount of either metal measurable in this way is of the order of 100μg., which means that decidedly large samples of material would generally have to be used.

For the semi-quantitative determination of cobalt in soils, Mitchell (1940), who has made a special study of the estimation of trace elements in soils, recommends the use of the cathode layer arc. The method is based on the fact that in the region of the arc adjoining the cathode (the cathode layer), the emission of spectral energy from cations may be up to 100 times as intense as that from the column of the arc. By means of a spherocylindrical quartz lens the image of the arc is focused sharply on the slit, so that the image of the cathode appears as a horizontal line across the top of the slit while the image of the anode falls well below the bottom of the slit. This arrangement results in greatly increased sensitivity as compared with the ordinary arc method, and 2 p.p.m. of cobalt and 1 p.p.m. of nickel can be detected by its use. The spectral lines used for the determination of these two elements are 3453·5 and 3414·8A. respectively.

Cobalt and nickel can be readily determined simultaneously with the polarograph by the procedure described by Lingane and Kerlinger (1941), the essential feature of which is the use of pyridine as supporting electrolyte. Using a normal solution of potassium chloride containing from 0·05 *M* to *M* pyridine and 0·05 per cent gelatin the waves of nickel and cobalt are well separated and defined, while manganese does not interfere with them. Ferric iron, if present in large excess, interferes with the determination of nickel and cobalt, but its effect can be eliminated by the use of a supporting electrolyte with *p*H of about 5·4 containing equal concentrations of pyridine and pyridium chloride in which the ferric iron is precipitated as hydrous ferric oxide. Small amounts of copper do not interfere with the determination of nickel and cobalt, but if present in considerable

excess the bulk of it must be removed before polarographing for nickel and cobalt. The author is not aware of the polarographic method having been used for the determination of nickel or cobalt in plant material, and Piper (1942) points out that the nearness of the deposition potential of zinc to that of cobalt, and the small amount of the latter relative to that of zinc, usually present in plant material, renders the polarographic determination of cobalt in plant ash uncertain.

The estimation of cobalt in plant material and in soil is usually effected by colorimetric or absorptiometric means depending on the intense coloration produced by cobalt compounds on treatment with the sodium salt of 1-nitroso-2-naphthol-3:6-disulphonic acid, generally known as nitroso-*R*-salt. Procedures have been described by Kidson, Askew and Dixon (1936) and by Davidson and Mitchell (1940) for the determination of cobalt in soils in this way and by McNaught (1938), Kidson and Askew (1940) and Marston and Dewey (1940) for the similar estimation of this element in plant material. It would appear that quantities of cobalt down to about 0·5μg. are determinable in this way.

3. The Diagnosis of Mineral Deficiencies of Plants

It is obvious that a ready means of diagnosing deficiencies of the various mineral constituents of plants is likely to have great economic value, particularly where crop plants are concerned. Where the deficiency of a particular element is great the plant generally displays symptoms which are readily recognizable by an observer with experience of the effects on the species in question of deficiency in that element. These symptoms are often so definite that the resulting condition has a descriptive name, and a number of well-defined deficiency diseases of crop plants are known to agriculturists and horticulturists: the most important of these are described in a later chapter.[1]

[1] See particularly Wallace (1943, 1944) for diagnoses and coloured illustrations of crop plants of Britain affected by deficiency diseases, and *Hunger Signs in Crops* by a number of authors (Washington, 1941) for an account, with coloured illustrations, of deficiency diseases of crop plants in the United States.

But by the time unmistakable symptoms of deficiency have shown themselves it may be late, and, perhaps, too late, to effect a cure of the condition by the application of the deficient mineral; this is likely to be so with annuals such as cereals and leguminous crop plants with a short life period rather than with perennials such as fruit and other trees where the longer life of the plant may provide adequate time for recovery. But even for the latter early diagnosis is obviously desirable in order to avoid a period of feeble growth or poor fruit yield. Also it may be possible that the deficiency of a particular element is insufficient for actual symptoms of a deficiency disease to develop and yet sufficient to bring about a reduction in the rate of growth and finally in crop yield.

A second way of determining micro-nutrient deficiency is provided by analysis of plant material by the methods described earlier in this chapter. This might provide very definite evidence of the adequacy or otherwise of the quantity of the various nutrients in the plant, although it would be necessary first to establish the minimum quantities of the respective mineral elements which must be expected in the different organs of the plants of each species at different stages of development. While a certain amount of such information is available it must be admitted that it is far from complete for any one species. The acquisition of the requisite data takes time, but there can be no doubt that the necessary information will ultimately be obtained.

A third method of diagnosing mineral deficiencies, and one which should enable this to be made early, consists in introducing a solution of the salt of the element in question, or even the solid salt, into the plant and observing the reaction. The introduction of the salt into the plant is generally spoken of as 'injection'. The generally accepted meaning of this word is the forcing of material into the organism under pressure, whereas in practice the plant is generally allowed to absorb the solution through a cut surface, or even through an intact leaf, without the application of pressure. However, there is no other simple term to denote this process, and we may follow Roach, who has developed this method for diagnosis of trace-element deficiencies, in extending the use of the term 'injection' to include 'the

introduction by various methods of liquids and solutions into plant organs, whether under pressure or not, and their spread therein'.

In a long discussion on injection of plants as a physiological method, Roach (1939) describes no less than ten ways in which injection can be carried out; each of these has its own particular value. The ten injection methods are these:

1. Intervenal leaf injection.
2. Leaf-tip injection.
3. Leaf immersion (Anderssen).
4. Leaf-stalk injection.
5. Shoot-tip injection.
6. Branch-tip injection.
7. Shoot injection (Leach) and branch injection (Collison, Harlan and Sweeney).
8. Injection of individual branches.
9. Injection of individual branches together with their roots.
10. Injection of whole trees.

Not all these methods of introducing material into plants have been designed for the purpose of diagnosing mineral deficiencies, nor are all of them equally valuable for this purpose, although any one of them could no doubt serve to demonstrate the existence of such deficiency. But on the whole the methods in which leaves or young shoots are injected are those which are most useful for diagnostic purposes, while those in which larger branches or a whole tree are used are more generally useful for some other purpose, as, for example, the cure of a deficiency.

The principle underlying injection methods of diagnosis is that the introduction into a leaf of a salt of an element in which the plant is deficient will produce a definite response which is in the direction of a cure of the deficiency. The most usual response is a colour change in the leaf which generally becomes greener; sometimes increased rate of growth of a leaf occurs. These responses are best observed when leaf areas permeated by the nutrient are in close juxtaposition to control non-permeated areas; a difference in colour between permeated and control

areas is then most easily recognizable, while if one simple leaf or leaflet contains both permeated and control areas a difference in the rate of growth of the two parts of the leaf will result in a puckering of the leaf which is readily observed.[1]

These conditions are fulfilled when leaves of certain species, as, for example, apple, pear, plum, strawberry and broad bean, are subjected to *intervenal injection*. For this treatment a small incision is made near the midrib of the leaf between two major secondary veins. A dilute solution of the salt of the element of which a deficiency is suspected is contained in a small tube, and a wick made of filter paper, or, for small leaves, of darning cotton, passes from the solution through the incision into the leaf. By the use of dyes it is shown that the solute diffuses through the whole area between the two secondary veins and the leaf margin before diffusing into neighbouring intervenal areas. The length of time for which injection is allowed to proceed should be such as to give a long boundary between the injected and neighbouring control area, but not so long that the solute diffuses into neighbouring areas. The best time must be found by preliminary trials, for it varies with the species and climatic conditions. However, for apple, pear, strawberry and Shasta daisy, Roach suggests a period of from 7 to 12 hr. The leaves which give the best response are those about half-grown. The maximum response is generally given in about 10 days, but a response has been observed in as short a time as 2 days. This was recorded by Roach as having been observed by Lal as a result of intervenal injection of soya-bean leaves with a 0·025 per cent solution of ferrous sulphate.

In *leaf-tip injection* the tip of a leaf or leaflet is cut off at right angles to the midrib and the cut edge of the leaf immersed in a solution of the substance to be injected. This method can be used for any type of leaf but is particularly suitable for long narrow leaves. The greater the proportion of the leaf removed, the greater is the penetration of the solute into the rest of the leaf. For example, it was found that if the removed tip con-

[1] It should, perhaps, be pointed out that these responses should only be regarded as indicative of deficiencies when they have been correlated with successful curative treatment, since an improved appearance of the leaf might result from injection without there being a deficiency.

tained one-tenth of the midrib, half the rest of the leaf was permeated, but if more than one-fifth of the midrib was contained in the part removed, the whole of the remainder of the leaf and parts of neighbouring ones became permeated. This should be avoided since neighbouring leaves can serve as a control. With compound leaves, such as those of the strawberry, injection can be so contrived that one leaflet becomes permeated and another unaffected, while the third is partially permeated. Roach found that with leaves of apple and pear injection should proceed for about 10 hr.; a response is apparent in from 7 to 10 days.

With *leaf-stalk injection* the whole, instead of part only, of the lamina is removed, and the leaf stalk left attached to the plant is connected with narrow rubber tubing (such as tyre valve tubing) to a reservoir of the solution. As a result certain leaves of the plant become completely permeated, others partially and yet others not at all; the greater the angular distance of any leaf from the injected leaf stalk the less the permeation. In partially permeated leaves the permeated and non-permeated areas are, at any rate in the case of apple, sharply delimited, and such leaves are considered by Roach to be almost ideal for showing differences in colour and rate of growth of affected and control areas. The method would appear to be applicable to a wide range of species.

For *shoot-tip injection* the tip of a shoot is removed and either a small glass tube of solution is attached to the cut end of the shoot with fine rubber tubing if the shoot is rigid enough to support it, or the cut end of the shoot is bent over into a reservoir of the solution. As a result one or more of the leaves on the shoot become permeated.

The remaining methods of injection listed by Roach are of less interest from the point of view of their value for diagnostic purposes, but reference may be made to the methods used by Anderssen and by Storey and Leach, since these were both devised in connexion with work on mineral deficiency of plants. Anderssen's method consisted in bending over the leaf and immersing it in a weak solution of copper sulphate containing 0·3 p.p.m. of copper. By this treatment chlorotic leaves of plum recovered their normal green colour in 2 weeks, a result which afforded confirmatory evidence that the pathological condition of the

trees bearing the leaves was due to a deficiency of copper. Anderssen's work is referred to in more detail in the next chapter.

Storey and Leach (1933) were interested in a disease of the tea plant known as 'yellows', which, as the name implies, involves a chlorosis of the leaves. They traced this to a deficiency of sulphur. Among other pieces of evidence which led them to this conclusion was the effect of introducing various salts into plants growing in the field. The injection of any particular salt was effected by cutting a small side shoot under water and immersing the cut end of the shoot in a solution of the salt. The quantity of solution was maintained by daily additions, and every fourth day the immersed shoots were cut farther back to give a fresh absorbing surface of unchoked wood. It was found that when a 0·5 per cent solution of sodium sulphate, potassium sulphate or magnesium sulphate was used the normal green colour was regained by the leaves on the branch beyond the cut shoot, but that no such recovery resulted when other salts, such as chloride or nitrate, of these metals were used.

CHAPTER III

TRACE ELEMENT DEFICIENCY DISEASES OF PLANTS

At the end of Chapter I a list was given of species which have been shown to be dependent for growth on one or other of the micro-nutrients, or which at least have benefited by treatment with a micro-nutrient. It has been established that certain well-recognized pathological conditions met with in the field are associated with deficiency of a micro-nutrient, and some of these are so widespread or of sufficient economic importance to be designated by common names. Such, for example, are the grey speck disease of oats, and heart-rot of sugar beet. In this country diseases due to a deficiency of manganese and boron are both widespread and of economic importance; elsewhere shortages of zinc and copper have been shown to be responsible for diseases causing considerable damage to fruit crops. So far no well-defined diseases attributable to lack of molybdenum or other trace elements have been recognized.

The more important of the deficiency diseases attributable to shortage of trace elements are described in this chapter.

1. Diseases Attributable to a Deficiency of Manganese

The most general effect of manganese deficiency appears to be in the first place the development of small chlorotic patches localized in intervenal areas of the leaves. The form these patches take in different species is no doubt largely dependent on the anatomy of the leaf, so that in grasses with their parallel venation they tend to take an elongated form, producing 'stripes' or 'streaks', while in reticulate-veined dicotyledons they produce a spotted, speckled or mottled effect, as in potatoes and sugar beet. Other symptoms may follow, including reduction or cessation of growth and the development of necrotic areas which may not be limited to the affected regions of the leaf,

but which may even affect the seeds, as in the case of the garden pea.

Grey Speck of Oats. The disease of oats most usually known as grey speck, but also sometimes called grey stripe, grey spot, or dry spot, is characterized by the appearance in the leaves of spots of a greyish colour, small chlorotic areas, chiefly in the lower half of the leaf, which tend to coalesce and form elongated streaks which finally turn brown. The first sign of the disease often occurs in young plants in the third or fourth leaf. Very characteristically a line of withering and weakness develops transversely across the leaf blade so that the distal portion of the leaf hangs down (see Figs. 1, 2). In the young leaves this line of weakness is often about 1 or 2 in. from the base of the leaf lamina, but correspondingly higher up in older and longer leaves. The leaves may eventually turn completely brown and die. Colour photographs of oats badly affected by grey speck are given by Wallace (1943, p. 95, pl. 77; 1944, p. 37, pl. 188).

Badly affected plants may be stunted and die early; in less severe cases flowers may be produced but little grain is formed. Root development tends to be poor, so that affected plants are much more readily pulled out of the soil than healthy ones.

Grey speck appears to be widely distributed. It occurs in different parts of Europe, including Britain, and in America and Australia. It appears to be most liable to occur on certain soils with an alkaline reaction, especially if they contain much humus, and in such conditions the disease may be so serious as to lead to the complete failure of the crop.

It has been recognized for many years that grey speck disease could be controlled by treatment with a soluble manganese salt, either as a soil dressing or by spraying the foliage, but the proof that grey speck was actually related to manganese deficiency was provided by Samuel and Piper (1928, 1929). They grew Algerian oats in carefully controlled water cultures, using carefully purified materials, and with various amounts of manganese sulphate added to the culture solutions containing the usual major nutrients. The initial concentrations of manganese in the different cultures were 0, 1 in 50×10^6, 1 in 10×10^6, 1 in 5×10^6 and 1 in 1×10^6. Cultures grown in solutions free from manganese developed the symptoms of grey speck in about 4 weeks,

and this occurred whether the solutions also contained some other trace element such as boron, zinc, cobalt, copper, etc., or not. With culture solutions containing 1 part of manganese in 50×10^6, the symptoms developed suddenly in about 8 weeks. On renewing the culture solution, including the manganese supply, new healthy growth took place, but the symptoms of deficiency again appeared after about 4 weeks. Recovery again took place after a second renewal of the solution. With culture solutions containing 1 part or more of manganese in 10×10^6 no symptoms of grey speck appeared, the solutions being renewed after 10 weeks.

Reference has been made above to the fact that grey speck tends to occur on plants growing on alkaline soils containing much humus. This has led to suggestions that the disease might be associated with excess of calcium ions or with certain organic compounds in the soil. Water-culture experiments carried out by Samuel and Piper to test these possibilities yielded no support for such views. Culture solutions containing calcium ions in various degrees of excess, or various organic substances (humus, sucrose, glucose, starch, cellulose), in no case induced symptoms of manganese deficiency in oats growing in them provided manganese sulphate had been added to the solutions.

That grey speck disease is the direct effect of manganese deficiency has been disputed by Gerretsen (1937). He points out that Lundegårdh (1932) had recorded that the manganese content of affected plants might be higher than that of healthy ones; indeed, he gave values up to 420 p.p.m. of manganese in affected plants and down to 1 p.p.m. in healthy plants. This is certainly contrary to general experience, and Samuel and Piper found that about 14 p.p.m. was the minimum amount of manganese likely to be present in healthy Algerian oats at the flowering stage.

Gerretsen states that when a soil which had borne a crop showing symptoms of grey speck was sterilized with formalin, oat plants subsequently grown on it were free from grey speck, although the manganese content was the same as before and there had been no increase in either water-soluble or exchangeable manganese. On reinfecting such sterilized soil with 10 per cent of the original soil grey speck again appeared on oats grown on

it and the dry weight of the plants was reduced to 59 per cent of that of plants grown on the sterilized soil, while the manganese content of the affected plants was reduced from 51·5 to 19·3 p.p.m. Some very striking results were given by sand-culture experiments. Plants were grown in sterile sand and in the same sand infected with 5 per cent of so-called 'diseased' soil. The plants in the sterile sand were all healthy, had a mean dry weight of 436 mg. and a manganese content of 15·0 p.p.m., whereas those in the infected sand all showed typical symptoms of grey speck, a mean dry weight of only 216 mg., but a manganese content of 26·6 p.p.m.

Oats grown in sterile water-culture solutions containing very small quantities of manganese showed no symptoms of grey speck, although the plants were stunted and might contain less than 10 p.p.m. of manganese. But when the solutions were inoculated with a root tip from an affected plant or with bacteria isolated from affected roots, the symptoms of grey speck developed strongly. Again, the addition of 0·001–0·002 per cent of Germisan, a germicide, to the culture solution of non-sterile plants kept the plants healthy even when the manganese content was low, whereas without the addition of the Germisan the plants were badly affected.

These facts are held to indicate that grey speck is related to the presence of micro-organisms. According to Gerretsen the roots of affected plants always show signs of microbiological disintegration. It is suggested that alkaline products are produced in the roots by the infecting micro-organisms and that these products are carried in the transpiration stream to the leaves, where they produce the grey spots.

Gerretsen therefore concludes that it is necessary to distinguish between the direct physiological effect of manganese deficiency which is a retardation of growth, and the symptoms of grey speck disease which are related to the infection of the roots by micro-organisms. The capacity of the root to resist parasitic attack by micro-organisms is indeed held to depend on the manganese content, but if the roots are maintained sterile healthy plants are produced in presence of a very small supply of manganese so that the manganese in the plant is only from 5 to 35 p.p.m.

Fig. 1. Oat seedling affected with grey speck. Note the grey areas and the characteristic line of weakness in affected leaves.

Fig. 2. Oat leaf exhibiting the characteristic symptoms of grey speck.

Fig. 3. Sugar beet suffering from manganese deficiency. Note the speckling of the leaves, appearing in the photograph as a mottling.

By courtesy of Dr. C. S. Piper

Fig. 4. Peas suffering from marsh spot.

It must be admitted that Gerretsen has made a strong case for the view that grey speck disease is not the result of manganese deficiency only. The problem is clearly deserving of further study.

In wheat, Gallagher and Walsh (1943) observed that the first sign of manganese deficiency usually appears with the development of the third or fourth leaf. Frequently there is a general similarity with grey speck of oats, but the transverse line of withering tends to occur nearer the tip of the leaf than in oats, later extending to the lower regions of the leaf. The withering may develop as in oats by the coalescence of small grey elongated areas or it may begin at the leaf margin. As soon as the line of withering reaches right across the leaf the upper part of the leaf soon loses its green colour. Sometimes, instead of the development of the line of withering across the leaf as in oats, small grey oblong areas appear scattered parallel with the veins. The whole plant becomes chlorotic and the leaves wither, beginning at the tip.

In barley, Gallagher and Walsh record the first symptom of manganese deficiency to be a localized paling of the leaf, followed by the development in a few days of small grey oblong spots with brownish margins. These enlarge and coalesce to form stripes parallel with the veins. Sometimes the spotting is most marked near the tip, which finally withers. The plant as a whole becomes somewhat chlorotic.

Rye appears to be affected by manganese deficiency in much the same way as oats, the spots that develop on the leaves being described by Gallagher and Walsh as whitish. The leaves bend over in the same manner as those of oats affected by grey speck. Neither barley nor rye, however, appears to be so badly affected by manganese deficiency as oats.

From the work of Pettinger, Henderson and Wingard (1932), manganese deficiency appears to produce a chlorotic condition in maize very similar to grey stripe. In sand-culture experiments with maize they met with three types of chlorosis which they attributed respectively to deficiency of magnesium (type A), excess of sodium (type B) and deficiency of manganese (type C). In type A the chlorotic areas take the form of long narrow streaks more or less continuous from the base to the

apex of the leaves, but with very irregular margins. In type B the streaks are also continuous throughout the leaf but have very regular margins and occupy the whole intervenal region. In the type attributable to manganese deficiency, on the other hand, the chlorotic areas form discontinuous spots or stripes. White or chlorotic spots first appeared in the cultures when these were about 3 weeks old. As the leaves grew the spots also increased in area, and as the affection became more severe the spots tended to coalesce into elongated chlorotic streaks. The tissue in the middle of the chlorotic areas then turned brown, broke down and was dead. Sometimes the dead tissue fell out of the leaf, leaving a number of holes. The similarity of the condition to grey stripe of oats is striking, and a photograph of maize leaves affected by their type C chlorosis published by Pettinger, Henderson and Wingard bears a close resemblance to that of oat leaves affected by grey stripe reproduced in Fig. 2.

Pahala Blight of Sugar Cane. The disease of sugar cane named Pahala blight, after a small town in Hawaii where it was first observed, is characterized by a partial chlorosis of the leaves, the chlorotic areas taking the form of long white streaks. These are limited to the leaf blades and do not occur on the leaf sheaths. The third, fourth and fifth youngest leaves are generally those most affected. As the chlorotic cells die red spots appear, and as these increase in number neighbouring spots may coalesce so that continuous red streaks result, and there may then follow splitting of the leaf along the line of the streak. By the time red spots appear the plant is generally very much stunted. A fungus, *Mycosphaerella striatiformans*, frequently appears on the red spots, and when the disease was first described in 1906 it was attributed to the attack of this fungus, but in 1928 Lee and McHargue produced evidence that the Pahala blight results from a deficiency of manganese, the fungal attack being secondary to this. This conclusion is based on three lines of evidence derived respectively from the results of the application of solutions, and particularly powders, containing manganese sulphate to the leaves, from chemical analyses of normal and affected leaves, and from sand-culture experiments.

As regards the effect of applying manganese sulphate to the leaves it was found that this salt, generally applied as a dust with

dusting sulphur as a carrier, brought about good recovery of affected plants so that new leaves were healthy and of a dark green colour. No recovery resulted from similar treatment with ferrous sulphate, which indeed had the effect of 'burning' the leaves.

Chemical analyses of normal, semi-chlorotic and chlorotic leaves showed no very marked difference in the content of any of the mineral constituents except manganese. The difference in this element, however, between normal and affected leaves is marked, the percentages of manganese in normal, semi-chlorotic and chlorotic leaves being respectively 0·003, 0·0005 and a trace.

The sand-culture experiments were carried out with cuttings of a variety of sugar cane very susceptible to Pahala blight. Manganese-free sand was used. Ten cultures were supplied with a culture solution free from manganese while another ten, supplied with a solution containing manganese, served as controls. These latter plants grew normally, but the plants grown without manganese gradually developed Pahala blight, and by the end of six months the affection was quite severe.

It may be noted that Lee and McHargue report that Pahala blight only appears to occur on plants growing in alkaline or neutral soils. Addition of substances such as sulphur and superphosphates which increase the hydrogen-ion concentration of the soil and so make the manganese in the soil more readily available for absorption will tend to diminish the incidence of Pahala blight.

Speckled Yellows of Sugar Beet. The disease of sugar beet known as speckled yellows also involves an intervenal chlorosis in the leaves, the general appearance of the plant resulting from the presence of the yellow chlorotic areas being indicated by its name. As the disease progresses the margins of the affected leaves curl upwards and over the upper surfaces of the leaves. Other cultivated varieties of *Beta maritima*, namely, mangold, red beet, and spinach beet, may also show the same condition, although in red beet the characteristic speckled yellow effect is masked by the red pigment present in the sap of the leaf cells. Spinach (*Spinacia oleracea*), which belongs to the same family as beet (Chenopodiaceae), may be similarly affected.

Some good colour photographs of both sugar beet and red beet affected with the disease are given by Wallace (1943, pp. 97–9, plates 81–5).

The attribution of speckled yellows to a deficiency of manganese is chiefly based on the fact that the disease is cured by applications of a soluble manganese salt. Analyses carried out by the writer and Dr K. W. Dent, however, show a very striking difference in the manganese content in normal and affected plants of sugar beet. The affected plants examined were grown by Mr W. Morley Davies on soil which had been heavily limed in order to induce manganese deficiency. The normal plants were grown on an adjoining plot not subjected to heavy liming. The differences in manganese content of healthy and affected plants are clearly shown by the data in Table II. The values marked *p* were obtained by the polarograph, those marked *a* by the absorptiometer.

TABLE II. Manganese content of normal and speckled sugar beet

		Manganese content in p.p.m. dry matter			
		Normal		Speckled	
Date of collection of material	Plant organ	*p*	*a*	*p*	*a*
10 July 1942	Leaf	181	183	13	10
	Petiole	—	7	—	Not recognizable
	Root	—	6	—	Not recognizable
10 August 1942	Leaf	551	—	51	54
	Root	32	36	18	14·5

Although the determinations by the two methods show in some cases a little divergence, they make it clear that the plants affected with speckled yellows contain considerably less manganese than normal plants. This is particularly so in the leaves, where the manganese content of the speckled plants is of the order of one-tenth that of healthy plants. The data afford supporting evidence for the view that speckled yellows is a manganese deficiency disease.

Marsh Spot of Peas. The symptom of the disease of peas known as marsh spot is the occurrence on the seeds in the pod of brown or black spots or cavities on the internal surface of the

cotyledons. These necrotic spots generally only affect cotyledonary tissue, although occasionally the plumule may be affected. Pods containing exclusively healthy seed, and pods containing only diseased seed may occur on the same plant, and pods are even found containing both healthy and diseased seed. Externally the plant may appear quite normal, although sometimes mild chlorosis or mottling of the younger leaves may be present. In this country it occurs particularly in Romney Marsh where it appears to be limited to alkaline soils (Heintze, 1938). In Holland, Ovinge (1935) generally found it on alkaline and relatively new polder soils.

The fact that he found peas affected with marsh spot growing near oats which had developed grey speck led Pethybridge (1936) to suspect that marsh spot might be attributable to the same condition as grey speck, that is, to manganese deficiency. This view was supported by the finding of Löhnis (1936) that peas affected by marsh spot contained somewhat less manganese than healthy peas, and by the experiences of Ovinge (1938) in Holland and of Lewis (1939) in this country who found that the application of soluble manganese salt either as a soil dressing or a spray was effective in reducing the incidence of marsh spot. Also Heintze found that among the Romney Marsh soils those on which marsh spot occurred contained less salt-soluble manganese (manganese extracted by a normal solution of magnesium nitrate or calcium nitrate) than those on which peas were free from the disease, the difference in the extractable manganese being related not to the total manganese content, but to the acidity or alkalinity of the soil.

Determinations of the manganese content of different parts of healthy peas and of peas affected by marsh spot made by Glasscock and Wain (1940) show a considerably lower manganese content in the diseased seed. The peas examined were of the variety Harrison's Glory, the diseased sample having been obtained from Romney Marsh and the healthy peas from Folkingham in Lincolnshire. The results are summarized in Table III.

TABLE III. Manganese content of healthy and marsh spotted peas. (Data from Glasscock and Wain)

Part of seed	Manganese content in p.p.m.	
	Healthy seed	Diseased seed
Germ	15	3
Cotyledon outer tissue	11	5
Cotyledon centre tissue	6	< 2
Seed coat	4	2

The relationship of marsh spot to manganese deficiency was definitely established by Piper (1941) by means of carefully controlled water-culture experiments in which specially purified media and carefully regulated amounts of manganese were used. In addition to the ordinary major mineral nutrients the culture solution contained small amounts of boron, copper, zinc and molybdenum as well as sodium chloride. One series of peas was grown in this culture solution without manganese, to four other series 5, 10, 20 and 500μg. manganese per litre were respectively added. For the first 39 days after the seeds were put to germinate no differences were observable in the various cultures, but then in the manganese-free cultures there appeared mottling of the younger leaves and brown lesions on the internodes and tendrils. In a further 2–3 weeks growth stopped.

In the cultures supplied with 5μg. of manganese per litre these same symptoms appeared, but not until 8 weeks from the beginning of germination. On renewal of the solution, including the manganese, healthy new growth was resumed, but in a fortnight the same pathological symptoms again appeared. The cultures produced a few flowers, but no fruits formed.

The cultures supplied with 10μg. of manganese per litre showed no unfavourable symptoms after 8 weeks, apart from slight mottling of the upper leaves, and after renewal of the culture solution growth was vigorous and moderate flowering took place. After another 3–4 weeks, however, the symptoms of manganese deficiency appeared, and although some fruits formed only a few ripened and these were small and imperfectly developed, while the seeds they contained were all badly affected with marsh spot.

The cultures supplied with 20μg. of manganese per litre grew normally, flowered freely and produced numerous fruits with a

good yield of ripe seeds. But although the vegetative parts of the plant were free from symptoms of manganese deficiency, 33 per cent of the seeds were severely affected with marsh spot, 24 per cent were slightly affected, while 43 per cent were normal.

The cultures supplied with 500μg. of manganese per litre grew normally and vigorously and showed no symptoms of marsh spot.

The necessity for manganese was also shown by the yield of the cultures; Piper's results are given in Table IV.

TABLE IV. Yield of peas in water cultures supplied with different quantities of manganese. (Data from Piper)

Conc. of Mn in μg. per litre	Yield in g. per plant				No. of seeds per plant	Incidence of marsh spot per cent
	Shoots	Roots	Seeds	Total		
0	4·3	1·3	0	5·6	0	—
5	12·5	4·0	0	16·5	0	—
10	18·2	3·7	0·4	22·3	6	100
20	21·9	3·2	10·2	35·3	68	57
500	30·7	3·5	13·5	47·7	88	0

These results demonstrate very clearly that marsh spot arises from a partial deficiency of manganese.[1]

Frenching of Tung Trees. *Aleurites*, a genus of the Euphorbiaceae, contains five species which are of economic importance on account of the oil yielded by their fruits. They are all trees growing to a height of 25 to 40 ft. The species *A. fordii*, the tung tree or tung-oil tree is the source of tung oil, a drying oil used in the manufacture of paints, varnishes and linoleum and for waterproofing.

In plantations of tung trees in Florida, Reuther and Dickey (1937) reported a rather widely distributed affection which they described as 'frenching'. It is possible that this disease was previously unnoticed because it was masked by another, known as bronzing, attributed to a deficiency of zinc and described later. In trees affected with frenching, chlorotic areas develop between the veins of the leaves, and as the disease advances the tissue in the chlorotic areas dies and necrotic spots arise. Premature abscission of leaves may follow.

[1] After this was written E. J. Hewitt (1945) described experiments in which he succeeded in inducing symptoms of marsh spot in broad beans (*Vicia Faba*) and runner beans (*Phaseolus multiflorus*) grown in manganese-deficient sand cultures.

Frenching may also occur in another species of *Aleurites*, *A. montana*, the mu-oil tree.

It was found that frenching was not limited to alkaline soils but was related to a low value of exchangeable manganese in the soil.

In the earlier stages of chlorosis recovery could be brought about in from 3 to 6 weeks by dipping the shoots in a 1 per cent solution of manganese sulphate containing 1 per cent calcium hydroxide and 1 per cent calcium caseinate spreader.

Determinations by Reuther and Burrows (1942) of the photosynthetic activity of affected leaves and leaves which had regained their normal colour by treatment with manganese sulphate did not indicate any very significant increase in photosynthetic activity as a result of treatment, and it is suggested that an environmental condition such as high leaf temperature, solarization or stomatal closure might limit the rate of photosynthesis under field conditions in Florida. Reuther and Burrows also point out that trees severely affected by frenching tend to produce small leaves, so that the total photosynthetic activity of the tree and consequently its production of new material may be reduced by frenching.

2. Diseases Attributable to a Deficiency of Zinc

So far there have been no records from Britain of plant diseases attributable to a deficiency of zinc, but in America such diseases may be serious. They largely affect fruit trees, but have also been recorded as occurring in maize and some other herbaceous plants.

As with manganese deficiency, the first sign of zinc deficiency is usually an intervenal chlorosis, but in trees this is generally followed by very characteristic symptoms of abnormal growth known as rosetting. In spring, instead of the development of elongated shoots with normal-sized leaves distributed along the length of the shoot, there develops a rosette of small stiff leaves. According to the species affected, the disease is variously known as rosette, little-leaf, mottle-leaf or yellows. Chandler (1937) prefers to class all these conditions, including those of zinc deficiency in maize, as one disease, which he calls zinc-deficiency disease, although he points out that the evidence may not yet

be sufficient to justify the conclusion that the disease is due only to a shortage of zinc. This attitude is no doubt logically sound, but as the symptoms in different species may vary, and as the investigations of the diseases in the various species or groups of species have been to a large extent carried out independently, it has been considered more satisfactory here to describe the disease in these various groups separately.

Knowledge of the internal symptoms of zinc deficiency is due to the work of Reed and his co-workers. In 1935, Reed and Dufrénoy described the result of a microscopical examination of mottled leaves of *Citrus*, which, as we shall see later, may suffer from a deficiency of zinc. In such leaves the palisade cells are broader than in normal leaves, being often transversely divided so that the cells are rhomboidal rather than columnar in shape, while the contents show various abnormalities. Thus chloroplasts are few, their stromata are often rich in fat, and the starch grains within them are generally thin and elongated. The vacuoles of the cell contain phenolic material and little spheres of phytosterol or lecithin. These substances are absent from normal leaves and tend to disappear when zinc is applied to plants affected by mottle leaf.

Later the cytology of the leaves of a number of other species suffering from zinc deficiency was examined by Reed (1938). These included apricot, peach, tomato, maize, squash, mustard and buckwheat. The general effect of zinc deficiency on the growth of the leaves appears to be retarded differentiation, the palisade cells appearing rhomboidal in shape rather than columnar, while the mesophyll is markedly compact, owing to a great reduction in intercellular spaces. Hypertrophy of cells may also occur, and it may be said that zinc deficiency promotes enlargement of the palisade cells rather than their multiplication and differentiation, while in tomato actual atrophy of mesophyll was observed.

In very young apricot and peach leaves the protoplast shows an abnormally great affinity for dyes. This character disappears later, at any rate in apricot, but proteolysis of the cytoplasm may reduce this to an almost invisible layer.

The chloroplasts are particularly affected by zinc deficiency, for this may result in inhibition of their development or in

their destruction, injury being greatest in cells receiving the strongest illumination. Plastid injury may be very localized, affected and normal cells being found in juxtaposition.

The phenolic substances, which were noted by Reed and Dufrénoy as occurring in zinc-deficient *Citrus* leaves, were also observed in zinc-deficient leaves of apricot, peach and buckwheat, but were absent from similarly affected leaves of mustard and maize. Since some phenolic material is present in the normal healthy leaves of some species such as apricot, Reed concludes that the differences in the content of phenolic substances in healthy and affected leaves may be one of degree. No toxic effect appears to be involved.

Reed (1939) also examined the structure of zinc-deficient leaves of tomato grown in water culture. Such leaves exhibited dwarfing, paleness, downward curvature of the leaflets, incurved laminae and necrotic spots on the midrib and laminae. The palisade cells were longer and the spongy tissue more compact than in normal leaves. The chloroplasts of the palisade cells of affected leaves were small and tended to aggregate at the lower end of the cell and, owing to degeneration of some of them, the number of plastids was abnormally low. Degeneration was even more conspicuous in the spongy tissue, the signs of it being increase in the amount of calcium oxalate, shrinkage, the formation of a melanotic substance, and reduction in size and number of plastids.

Reed has also examined the cytological effects of zinc deficiency in the apical buds of apricot and peach trees suffering from little leaf. In apricot some of the meristematic cells in such buds exhibit strong staining with haematoxylin and methyl green; this is followed by premature vacuolization and polarization. A similar state of affairs was observed in zinc-deficient peach buds except that the strong affinity for dyes was not evident. In the apricot nuclei may become masked by densely stained masses of cytoplasm, and phenolic materials arise from altered cell constituents. These changes were observed while the buds were still in the resting stage. During the early spring tannins, which are present in normal cells, become replaced by phloroglucinol in affected cells, especially in the more active of these. As growth and differentiation proceed

tannin compounds reappear, their accumulation being associated with enlargement of the cells and inhibition of cell division. At the same time the amount of phenolic compounds diminishes, reaching a minimum in early summer, after which their quantity increases until it reaches a maximum at the onset of the resting stage. The accumulation of these phenolic compounds in the vacuoles results in an increase of cell size but does not appear to be connected with necrosis of the cells.

Reference has already been made (p. 3) to the observation of Sommer on the necessity of zinc for the completion of the normal life cycle of beans and buckwheat. More recently, by means of carefully controlled water cultures of garden pea, wax bean and milo (*Andropogon sorghum*), Reed (1942) has shown that a supply of zinc is necessary for seed production in these plants. Zinc was supplied in a range of concentrations, namely, 0·0, 0·005, 0·02, 0·10 and 0·20 p.p.m. In garden peas no seed was produced when the concentration of zinc was 0·005 p.p.m. or less, but with zinc concentrations of 0·02, 0·10 and 0·20 p.p.m. seeds were produced, the numbers forming increasing with the concentration of zinc supplied. Results with beans and milo were similar except that in these the minimum concentration necessary for seed formation was 0·10 p.p.m.

Pecan Rosette. The pecan (*Carya olivaeformis*), a member of the Juglandaceae, is not cultivated in Britain, but its fruit, resembling a small walnut, was becoming familiar to people in this country in the years immediately before 1939. The tree is largely cultivated in the United States where the disease known as pecan rosette is widely spread, and where, according to Finch and Kinnison (1933), it was recognized by growers as long ago as 1900.

The first symptom of the disease is a yellow mottling of the leaves at the tip of a branch, the chlorosis being often evident as the leaves unfold. The leaves at the top of a tree are generally those first affected. As the disease proceeds the affected leaves remain small and are usually crinkled, brittle and misshapen, while the veins tend to stand out prominently. The chlorotic areas of the leaves are abnormally thin and frequently become dark reddish brown in colour and die. Sometimes the intervenal tissue fails to develop at all, with the result that smooth-

margined holes are scattered over the leaf. Internode development is poor and finally the branches die back. The development of lateral buds below the dead region results in the rosette appearance from which the disease takes its name. A morphological examination by Finch and Kinnison of the roots of affected trees revealed no indication of an abnormal condition, either externally or internally.

Death of the tree rarely, if ever, results from rosette, but fruit production may be so poor that the cultivation of the trees becomes unprofitable, and in 1932 Alben, Cole and Lewis stated that in some south-eastern states hundreds of acres of pecan orchards had been abandoned on account of rosette, while in south-western states where plantations were more recent, as many as 95 per cent of the trees were roseting in some places. There can thus be no doubt of the economic importance of pecan rosette.

Researches by Orton and Rand (1914) showed fairly conclusively that the disease is not due to the attack of any micro-organism, nor did it appear to be limited to any type of soil.

At first it appeared that the disease might be related to iron deficiency, for Alben, Cole and Lewis (1932*a*) found that some improvement in rosetted leaves was brought about by dipping them in, or spraying them with, a 0·6–1 per cent solution of ferric chloride or ferric sulphate.

A little later, however (1932*b*), they found that favourable results by such treatment were obtained only when galvanized iron containers were used for the solutions. This suggested the possibility that the effect at first attributed to iron might be due to zinc salts present as impurities in the iron salts used, and accordingly treatment with solutions of zinc chloride and zinc sulphate was tried. It was found that an immersion of the terminal branches of trees exhibiting rosette in a solution of an iron salt produced no improvement in the condition of the leaves, but that with a solution of a zinc salt young leaves were restored to their normal condition. Similar favourable results were obtained by the use of zinc-lime and zinc-sulphate sprays. Alben, Cole and Lewis concluded from their experiments that zinc is essential for the healthy growth of the pecan tree.

Similar conclusions with regard to the cause of pecan rosette

were reached by Finch and Kinnison (1933). Among other aspects of the problem they examined soils on which rosette appeared, but could find no relation between any soil factor and the incidence of rosette. The effects of a number of substances on affected trees were examined. These substances included salts of iron, magnesium, manganese and zinc. Three treatments were employed: (1) placing the dry material in holes bored in the trunk of the tree, (2) spraying leaves with solutions of the substances or dipping the leaves in the solutions, and (3) injecting the solutions into the tree trunks. With zinc salts a great improvement in the condition of the trees was effected by all three methods of treatment. In some cases, but by no means in all, some improvement was observed with the use of iron salts, a result which could be attributed to the presence of zinc as an impurity in the iron salts, especially as no improvement resulted with the use of purer iron salts. No benefit occurred as the result of treatment with either magnesium or manganese salts.

Determinations of the zinc content in different parts of the terminal 6 in. of some shoots taken from the top of pecan trees were made; the results are summarized in Table V. They show that the shoots of the tree affected with rosette contained very much less zinc than those of healthy trees, while in a rosetted tree treated with zinc chloride by solid injection and in which recovery from rosetting had taken place there was already after 8 weeks a very considerable increase in zinc content.

TABLE V. Zinc content of leaflets, petioles and stems of pecan (*Carya olivaeformis*). The quantities are given as p.p.m. of dry matter. (Data from Finch and Kinnison)

Condition of tree	Leaflets	Petioles	Stems
Healthy	16·7	11·0	7·9
Healthy	10·0	Trace	10·3
Rosetted	3·5	Trace	Trace
Rosetted; then treated with 6 g. zinc chloride injected in trunk (55 days after treatment)	3·9	8·6	15·3

Finch and Kinnison also published data of the zinc content of irrigation waters used in different districts of Arizona for supplying pecan plantations. Five out of six of these waters

contained no measurable amount of zinc, and in all cases rosette was severe or common in the districts supplied. In the sixth case the water contained a measurable amount of zinc (0·14 p.p.m.) and the district was essentially free from rosette.

The evidence presented by Finch and Kinnison thus supports the view that zinc is an essential element for the growth of the pecan, and that when there is a deficiency of it the condition known as rosette results. The favourable effect of zinc in controlling pecan rosette was also recorded by Demaree, Fowler and Crane (1933) working in Georgia.

Another member of the Juglandaceae, the walnut, may also exhibit the effects of zinc deficiency, but according to Chandler (1937) these do not include rosetting, although the leaves are mottled, crinkled and rather small.

Little Leaf or Rosette of Deciduous Fruit Trees. Similar to pecan rosette is the disease of deciduous fruit trees known in California as little leaf. The most characteristic symptom is the development in the spring of rosettes of very small leaves which, according to Chandler, Hoagland and Hibbard (1932), who have made a special study of the disease, generally possess less than 5 per cent of the area of normal leaves. The affected leaves generally exhibit a chlorotic mottling. Shoots bearing normal leaves may develop later in the season below the little-leaf rosettes, but as the season proceeds the new leaves are progressively smaller and mottled and may be abnormal in shape. Sometimes after one or two years, in other trees after a much longer period, the branches begin to die back. Fruit generally fails to set on badly affected branches and any which does is small and malformed. Stone fruits tend to have brown areas in the flesh (Chandler, 1937). Chandler, Hoagland and Hibbard mention apple, pear, plum, cherry, peach, apricot, almond and grape as all liable to the affection.

Concluding that little leaf was not the result of attack by micro-organisms, Chandler, Hoagland and Hibbard sought for its cause in the soil. As a result of various fertilizer trials they found that affected trees responded to the application of ferrous sulphate, but only when this contained zinc as an impurity. Further trials showed that it was the zinc that was actually responsible for the improvement. Solid injection of zinc sulphate

into the trunks of the trees appears to be the most effective treatment, but favourable results are also obtained by the use of zinc sulphate as a soil dressing or as a spray in winter. No improvement could be detected as a result of treatment with salts of silver, nickel, cobalt, tin, cadmium, mercury, iron, copper, chromium, manganese, aluminium, molybdenum, selenium, zirconium, uranium, strontium, tungsten and titanium (Chandler, Hoagland and Hibbard, 1934, 1935). A number of organic compounds gave equally negative results.

Determinations of the zinc content of stems and leaves of various fruit trees affected by, and free from, little leaf published by Chandler, Hoagland and Hibbard suggest that the zinc content of shoots from trees in orchards free from little leaf is on the whole higher than in those of trees affected by little leaf. Hoagland, Chandler and Hibbard (1936) have been able to induce the symptoms of little leaf in young apricot trees grown in water cultures from which care was taken to exclude zinc, as far as practicable.

Chandler, Hoagland and Hibbard were at first very reluctant to attribute little leaf to actual zinc deficiency. Their reasons for this reluctance were the suddenness with which healthy trees might begin to die from little leaf, the recovery of some trees without any obvious improvement in the zinc supply, and the fact that, whereas trees are susceptible to little leaf, annual plants growing on the same soils are apparently free from any symptoms of zinc deficiency.

That simple zinc deficiency alone may not afford a complete explanation of the cause of little leaf is suggested by the work of Ark (1937). This investigator sterilized, by means of steam, soil from orchards showing little leaf and found this treatment very beneficial to maize and tomato. Also sand cultures of maize were treated respectively with little-leaf soil and sterilized soil. In the former the plants soon showed symptoms of zinc deficiency (white bud, see p. 79), while the plants receiving sterilized soil, or in sand without any soil, remained normal. Further, from little-leaf soils he isolated two strains of bacteria which when added to the artificial culture medium in which maize seedlings were growing induced symptoms of white bud which were removed by raising the content of zinc in the

medium or by the injection of a zinc salt into the stems. Addition of one of the bacteria to cultures of peach and walnut also resulted in symptoms very similar to little leaf, symptoms which were prevented by the presence of zinc. Altogether Ark's observations are very reminiscent of those of Gerretsen on the relation of micro-organisms to the grey-speck disease of oats.

Chandler (1937) suggests that if little leaf is the result of a simple zinc deficiency this might be brought about by the production in certain soils of a flourishing micro-flora that absorbs zinc in large quantities. The effect of sterilization, by killing this flora, would thus be to leave more zinc available for higher plants rooted in the soil. Alternatively, Chandler suggests that soil organisms might excrete, or yield on dying, substances which combine with zinc to form insoluble zinc compounds and so render it non-available. In these circumstances the effect of the sterilization treatment might be to break up the insoluble zinc compounds and release the zinc in a soluble form.

Mottle Leaf (Little Leaf Type) or Frenching of *Citrus*. The disease of *Citrus* trees known as mottle leaf in California has been described in detail by Johnston (1933). The name is derived from the fact that yellow areas arise between the veins of the leaves giving these a mottled appearance. These chlorotic areas enlarge, and as fresh leaves develop these are progressively smaller until in severe cases they may be only an inch long, with chlorophyll developing only at the basal end of the midrib. In extreme cases dieback may follow, ultimately resulting in the death of the tree. The root system is also affected, the smaller rootlets first and the larger ones later, until in severe examples only large roots with a few rootlets remain functional. All species and varieties of *Citrus* can be affected by the disease. The severity of the affection is increased by extremes of high or low temperature.

Since mottle leaf of *Citrus* and little leaf of deciduous trees often occur in the same orchard, and since Chandler, Hoagland and Hibbard (1932, 1933) had found that application of zinc sulphate was a cure for little leaf, Johnston tried the same treatment for mottle leaf of *Citrus* and found that this resulted in restoring the normal green condition to mottled trees. The zinc sulphate was applied as a circle of salt on the soil round the tree,

by injection of crystals of the salt in holes bored in the trunk and then sealed, and by spraying the foliage with various sprays containing zinc sulphate. In all cases a favourable result was obtained. Johnston is of opinion that there are probably several kinds of mottle leaf affecting *Citrus* which are due to different causes, and he proposes to designate the type which responds to treatment with zinc as little leaf, thus bringing the terminology into line with that used for the similar condition found in deciduous trees.[1]

In Florida the chlorotic condition of *Citrus* trees is known as frenching. This is presumably the same disease as the mottle leaf described by Johnston, and affected trees respond favourably to the application of zinc sulphate. Thus Satsuma orange trees showing symptoms of frenching were treated by Mowry and Camp (1934) with a soil dressing of zinc sulphate, and as a result showed signs of complete recovery.

Whether mottle leaf or frenching is a zinc-deficiency disease in the strict sense is not clear. Johnston, indeed, stated that mottle leaf does not appear to be a case of soil deficiency and suggests that the zinc may act as an antitoxin.

Bronzing of Tung Trees. The condition of tung trees (see p. 67) known as bronzing was first noted in 1930 by Newell, Mowry and Barnette as occurring in trees in Florida growing on soils containing large amounts of phosphate. Later observation has shown that bronzing is not confined to such soils.

The disease usually appears first in the late spring or early summer or even later. The first symptoms are the appearance of a bronze colour in a number of leaves, together with a deformation of the terminal leaves of the shoots. With the development of the dark bronze colour in the leaves necrotic spots develop and parts of the leaves die so that these appear ragged. Ultimately, a twig may lose many or all of its leaves. After the first appearance of the disease in a tree the severity of attack generally increases rapidly. New leaves are successively smaller and become more deformed, the internodes fail to develop

[1] Already in the earlier of the papers by Chandler, Hoagland and Hibbard cited above they had included *Citrus* fruits and walnut along with deciduous fruit trees as liable to little leaf. They also showed (1934) the favourable effect of zinc on orange trees affected in this way.

normally, resulting in a bunched appearance of the foliage, the twigs remain thin and adventitious buds sprout on the older wood. Although the affection may be at first local, finally the whole of the tree may be involved. Affected trees are unduly subject to injury by the low temperatures of winter, and a bronzed tree may come into activity in the following spring smaller in size. By the third season after the first appearance of bronzing a tree may be reduced greatly in size and, with its almost bare branches, appear half dead.

Mowry and Camp (1934) found that bronzing could be cured by the application of about 1/4–1/2 lb. of zinc sulphate per tree to the soil or by spraying the foliage with a spray containing 6 lb. of hydrated lime and 3 lb. of 89 per cent zinc sulphate in 50 gal. of water containing some calcium caseinate spreader. While not definitely committing themselves to the view that bronzing of tung trees is a deficiency disease they conclude that it should be considered the result of zinc deficiency until proved otherwise. As a result of this work by Mowry and Camp on the control of bronzing by the application of zinc sulphate Reuther and Dickey were able to report in 1937 that comparatively little bronzing was then to be found in properly conducted tung plantations.

***Pinus radiata* Rosette.** Trees of *Pinus radiata* growing in plantations on poor soil in Western Australia are liable to exhibit a condition of rosetting reminiscent of that occurring on so many species of trees in America already described. Field trials of the effect of spraying with zinc chloride or zinc sulphate described by Kessell and Stoate (1936, 1938) showed that, as with other species affected by rosetting, treatment with zinc was a cure for the disease. The presumption that rosette of *P. radiata* is brought about by a deficiency of zinc was shown to be correct by carefully controlled water cultures carried out by Smith and Bayliss (1942). Removal of zinc, copper and lead from the stock solutions was effected by the use of dithiocarbazone. Boron, manganese and copper were added to the culture solutions containing the usual major nutrients. Zinc was added to the controls only. The method of purifying the solutions did not remove molybdenum and it was not necessary to add it.

The first symptom of zinc deficiency was a decreased rate of growth which began to be noticeable after about 3 months. This was soon followed by the shoot apices acquiring a flat-topped appearance due to the lower activity of the apical meristem. The apical buds appeared bunched together while secondary needles stopped growing and so appeared short, thick and stiff and the bundles did not spread open. Chlorotic symptoms did not appear until the lapse of another 5 weeks. Then small yellow dots appeared near the tip of the needles followed by browning, soon giving the tops of the branches a bronzed appearance. With the appearance of the first signs of chlorosis in the leaves conical swellings sometimes arose on the root apices. As far at least as the shoots are concerned the effect of zinc deficiency on *P. radiata* thus resembles the effects produced on other trees.

It should be noted that the cultures grown by Smith and Bayliss were non-mycorrhizal.

White Bud of Maize. As stated early in this book (p. 3), as long ago as 1914 Mazé demonstrated the essentiality of zinc for the growth of *Zea mais*. Mazé reported that maize plants grown in water culture deficient in zinc at first developed normally, but that quite suddenly the leaves darkened and developed a metallic sheen while nocturnal exudation became very abundant, a deposit of soluble salts being left on the leaves. Death of the plants followed in 3–5 days.

According to the investigations of Barnette and Warner (1935) it would appear that the disease of maize known in Florida as white bud is due to zinc deficiency. They report the disease as occurring chiefly on land which has been under cultivation for a number of years and also on poorer land recently brought into cultivation. The disease may sometimes be serious enough for the crop to fail completely.

The first symptoms of the disease may appear within a week of the emergence of the seedlings from the soil. The first sign of the trouble is the appearance of light yellow streaks between the veins of the older leaves followed by the rapid development of white necrotic spots. As the newer leaves unfold they are often pale yellow to white in colour, a symptom which gives its name to the disease. The older leaves develop light slate to dark brown

necrotic areas which increase in size and merge with one another until the whole leaf is dead. Meanwhile the younger chlorotic leaves continue to unroll and as they expand develop typical yellow intervenal striping. Internodes fail to develop properly so that the whole plant is stunted. The root system, however, appears to be normal.

The effect of various fertilizer treatments on maize plants affected with white bud was examined by Barnette and Warner. They found that a dressing which included 20 lb. of zinc sulphate per acre induced a very much higher yield of grain than any inorganic dressing which did not include zinc. With the application of zinc sulphate the chlorotic plants recovered a normal green colour, made healthy growth and produced grain. The same effect was produced by the application of stable manure or leaf mould, while chicken manure and alkaline peat produced some improvement in the condition of affected plants. Spectroscopic examination of the ash of these various organic manures revealed the presence of zinc in all of them. The work of Barnette and Warner, although not definitely establishing white bud of maize as a disease due to deficiency of zinc, indicates the probability of this being the case.

The possible relationship of bacteria to white bud has already been mentioned in the discussion of little leaf of deciduous fruit trees (p. 75).

3. Diseases Attributable to a Deficiency of Boron

The effects of boron deficiency in plants have been well dealt with by Brenchley and Warington (1927) and more recently by R. W. G. and A. C. Dennis (1937, 1939, 1941, 1942). From the accounts of these and other authors it appears that the first external symptom of boron deficiency is generally the death of the apical growing point of the main stem. This is followed by growth of lateral buds into side shoots, the apices of which then die. Further symptoms are slight thickening of the leaves, a tendency for these to curl, and sometimes a slight chlorosis. The petioles, and even the leaves, often become brittle, flowers may not form, or if they do fruit may not set. Stunted root growth is general.

Quite a number of investigations have been made on the histology of boron-deficient plants. These include broad bean (Warington, 1926), tomato (Johnston and Dore, 1929; Fisher, 1935; Van Schreven, 1935), *Citrus* (Haas and Klotz, 1931), tobacco (Van Schreven, 1934), sugar beet (Jamalainen, 1935), maize (Eltinge, 1936), potato (Van Schreven, 1939), apple (MacArthur, 1940), carrot (Warington, 1940), *Brassica* spp. (Chandler, 1941), radish (Skok, 1941), squash (Alexander, 1942), sunflower (Lowenhaupt, 1942), garden beet and cabbage (Jolivette and Walker, 1943).

In *Vicia Faba* Warington found that the chief internal symptoms of boron deficiency were frequent hypertrophy of the cells of the cambium and subsequent discoloration and degeneration of the cells; disintegration of the cells occurred, however, whether there was previous enlargement or not. Disintegration of phloem and ground tissue was also frequent, while xylem development was poor and the cells might also ultimately disintegrate. Brenchley and Thornton (1925) had previously found that development of the xylem of the root nodules either failed altogether or was very poor.

In sugar beet and *Citrus* hypertrophy of the cambium, followed by its disintegration and that of the phloem, is also symptomatic of boron deficiency. In *Citrus* boron was found to be essential for meristematic and cambial activity. In tobacco Van Schreven found that the first symptoms appeared in the cells of the root apex and later in the stem apex. In these regions cells became brown and degenerated. The symptoms then extended backwards from the apices, the vascular tissue being particularly affected. Proliferation of the cells of the phloem occurred resulting in the individual elements being compressed and distorted, while the xylem development was poor. Cells of the ground tissue might also undergo disintegration. The same worker recorded similar effects in tomato where thin-walled cells, such as those of cambium, phloem and ground tissue, undergo degeneration. The degeneration of the phloem in tomato was earlier recorded by Johnston and Dore and at about the same time by Fisher.

A number of species of *Brassica* were examined by Chandler. In broccoli and Brussels sprouts boron deficiency was found to

bring about cessation of cell division in the root apex and degeneration of the root cap. In rutabaga the thin-walled cells of the meristematic tissue of the stem apex and of the root cortex became crushed, while cells near the cambium became elongated and the cork cambium failed to produce cork. In cabbage Jolivette and Walker recorded considerable proliferation of cells in the cambial region resulting in a zone of meristematic tissue between xylem and phloem several times the usual width of this band, while there was a corresponding reduction in the amount of differentiated xylem and phloem. In radish Skok found that vascular tissue near the axis which might have been produced while boron from the seed was still available, or from boron present as impurity in the substrate or chemicals used, was usual, but that there was a complete absence of normally developed and lignified xylem vessels in the region between this inner tissue and the cambium, while the later formed phloem disintegrated. Xylem parenchyma cells, although smaller than normal, appeared uninjured. Owing to the failure of the development of normal vascular tissue the roots cracked and then the cambium and phloem mostly disintegrated. In swede, according to Jamalainen, the first symptom of boron deficiency is enlargement of xylem parenchyma.

In the squash Alexander noticed that boron deficiency brought about hypertrophy and collapse of the meristematic cells and of more mature cortical cells of the apical region of the stem, while, towards the apex of the root, cells of the central cylinder were similarly affected. In older regions of the stem parenchyma of the ground tissue, xylem, and the region between xylem and internal phloem showed abnormal enlargement. Cells of the cambium were also enlarged. Enlargement of thin-walled cells also occurred in the leaves.

In garden beet the first internal symptom, according to Jolivette and Walker, appears in the phloem where certain cells resembling companion cells become filled with a densely staining substance. Occasional hypertrophy of cambial cells was also noted. Later degeneration of xylem tissue occurs.

The first internal deficiency symptom of maize was found by Eltinge to be a disintegration of some of the leaf cells. Later, entire cross-sections of a leaf might collapse, and in other

parts of such leaves cells might fail to differentiate, and in yet other parts hypertrophy of cells, particularly those of the lower epidermis, might occur. Later, disintegration of cells of the stem apex took place.

Thus, although the internal symptoms vary somewhat from species to species, it may be said that in general boron deficiency leads to degeneration of the meristematic tissues, including the cambium, to breakdown of the walls of parenchyma cells and to feeble development of the vascular tissues. Of these the phloem appears to be most affected, but imperfect development of xylem is also a common feature. Hypertrophy of thin-walled cells and then discoloration are frequent precursors of their disintegration. Sometimes this latter is preceded by abnormally active cell division.

Heart Rot of Sugar Beet and Mangold. The disease of sugar beet and mangold known as heart rot, crown rot or dry rot, is widely distributed through Britain, Europe and America. It is generally most severe on alkaline soils and in dry years. As the names given to it imply, the most prominent symptom is a necrosis of tissues of the crown and interior of the root. The first symptoms of the disease, however, appear in the youngest (inner) leaves (cf. Fig. 5). These are stunted, become markedly curled, and the petioles develop a brown to black colour which may extend into the veins of the lower part of the laminae. As the plants grow older the leaves become affected, the veins becoming yellowish and the petioles brittle. Next, the inner leaves become brown or black and die, the main growing point dies and the outer leaves turn yellow, wilt, wither and finally also die. New shoots now develop in the axils of the dead leaves (cf. Fig. 6), but these become affected in the same way as the earlier formed leaves.

With the death of the first crop of leaves the tissue of the crown begins to rot. First necrosis occurs at a number of spots on the crown and these develop inwards into the root, increasing in size until in severe cases the greater part of the tissues of the root may be destroyed. Invasion of the affected parts by *Phoma betae* usually follows. The percentage of sugar in even the healthy parts of affected beets is less than that of healthy roots, so that from an economic point of view heart rot can be of very serious consequence.

The connexion of heart rot with boron deficiency was shown by Brandenburg by means of controlled water cultures, first of mangolds (1931) and later of sugar beet (1932). Similar results were obtained by Bobko and Belvoussov (1933) and by Rowe (1936). In the experiments of Bobko and Belvoussov, seedlings provided with no boron developed symptoms of heart rot after about a week. Renewed root development of boron-starved cultures resulted on the addition of boric acid to the culture solutions. Too high a concentration proved toxic, the most satisfactory range of boron concentrations being from 0·5 to 5 mg. of boric acid per litre. These findings were confirmed with sand cultures and field trials carried out by Brandenburg and others.

Canker and Internal Black Spot of Red or Garden Beet. Pathological conditions of garden beet attributed to boron deficiency have been described both here and in the United States. A full description of the disease as it occurs in the United States has been given by Walker (1939), who terms it internal black spot in reference to its most prominent symptom, the presence of internal hard black necrotic masses which render the beet unfit for canning. These masses, irregular in size and shape, are not characteristic of any particular part of the root, for although they may be confined either to the central region or the peripheral region, they may also be scattered throughout the root. When the black spot occurs near the periphery a rift in the surface tissues may occur, soil micro-organisms may enter and attack the root and a surface canker may result. But when the necrotic areas are well inside the root there may be no external symptom of the disease.

In this country the lesions on boron-deficient garden beet appear always to be superficial and are known as canker. Thus Wallace (1943) states that 'rotting occurs on the sides of the roots and may not penetrate into the more central tissues', and he gives some good colour photographs of cankered garden beet (op. cit. plates 106, 107, pp. 109, 110). The absence of the internal necrosis in red beet grown in this country is not explained, but it may be related in some way to varietal differences in growth. Walker states that the necrotic areas are most obvious between the prominent rings marked by the thick-

Fig. 5. Sugar beet suffering from boron deficiency. Note the stunted and curled appearance of the young inner leaves.

Fig. 6. Mangold suffering from boron deficiency. Note the dying or dead inner leaves and the small fresh leaves which are developing on the crown.

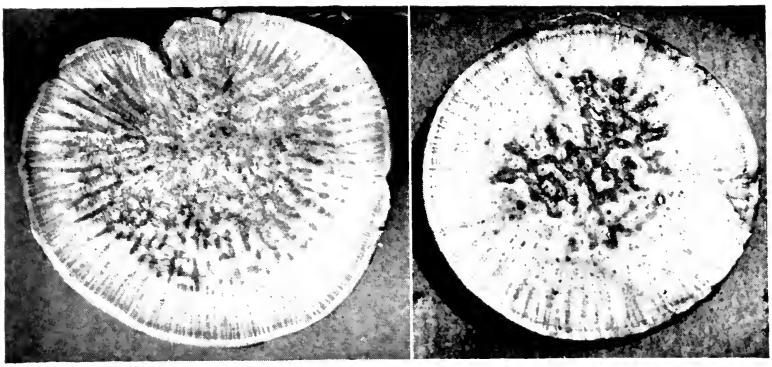


Fig. 7. Transverse section through swedes showing characteristic appearance of brown heart.

Fig. 8. Longitudinal section through swede affected with brown heart.

walled vessels formed by the activity of the secondary cambium zones in the pericycle.

The symptoms of boron deficiency exhibited by the shoot of garden beet are similar to those of sugar beet and mangold but generally less conspicuous. The older leaves are generally normal, but the younger leaves towards the middle of the crown become malformed, abnormally small, and rich in anthocyanin. The malformed leaves tend to die early and a rosette of dead stunted leaves may result. This condition is generally followed by the sprouting of dormant buds at the base of the dead leaves and the consequent development of new leaves, which, however, generally develop the characteristic symptoms indicative of boron deficiency.

Brown Heart or Raan of Swede and Turnip. The disease of swedes generally known as brown heart in England and raan in Scotland has a wide distribution, having been recorded in all parts of Britain, different countries of northern Europe, Iceland, Canada, Newfoundland and the United States, Australia and New Zealand. A good account of it has been published by Dennis and O'Brien (1937).

No external symptoms of brown heart appear during the growing season, and the root appears normal externally. On cutting an affected root across, however, the middle region presents a mottled appearance owing to a discoloration of patches of tissue within the xylem (see Figs. 7, 8). According to Dennis and O'Brien the affected tissue is limited to that within the cambium. Usually it is the outer region of the xylem which is affected so that the middle of the root has a normal healthy appearance, but in severe cases the whole of the region within the cambium may exhibit mottling, and in very severe cases the central tissue may break down and the root become hollow (Wallace, 1943, and see Fig. 9). The colour of the affected patches shows some variation. Sometimes they have a greyish, slightly brownish or 'water-soaked' appearance (Dennis and O'Brien, 1937; Wallace, 1943), the coloration being partly due to reduction in the intercellular spaces owing to slight swelling of the affected cells, partly to the development of a brown pigment apparently connected with slight swelling of the cell walls. Dennis and O'Brien state that bacteria can always be isolated

from the affected tissue, and they suggest that the coloration may be partly related to increased activity of bacteria normally present in the intercellular spaces.

Swedes affected with brown heart are unfit for human consumption, as the discoloured parts remain hard when the root is cooked, while affected roots contain less sugar than healthy roots and have a bitter taste.

In 1934, Güssow reported that application of boron as sodium tetraborate resulted in a large measure of control over brown heart, whereas other elements were not effective, and in the following year a number of investigators working independently in different countries published the results of field experiments on the control of brown heart by the application of borax or boric acid. These workers included O'Brien and Dennis in Scotland, Whitehead in Wales, Jamalainen in Finland and Hurst and Macleod in Canada.

The proof of the essentiality of boron for swedes was, however, provided by the sand-culture experiments of Hill and Grant (1935) and Jamalainen (1935) and the water-culture experiments of Dennis and O'Brien (1937), which clearly indicate that without a supply of boron swedes fail to grow beyond a very young stage.

The effect of boron deficiency in turnips is, as might be expected, similar to that in swedes.

Browning of Cauliflower. The most noticeable symptom of boron deficiency in cauliflower is the formation of brown patches in the heads. The disease has been investigated by Dearborn *et al.* (1936, 1937, 1942) by means of field trials, pot cultures and histological examination. The first external sign of the disease is the appearance of 'water-soaked' patches on the developing head (see Fig. 10). These patches soon turn brown and hard and in wet weather may rot. A certain amount of chlorosis may become apparent in the leaves, particularly at the apices of the older ones, which may become thicker, brittle and liable to curl downwards. Blistering may occur on the petiole and along the midrib. The root system is poorly developed. The internal symptoms resemble those found in other cases of boron deficiency (see p. 81). Thin walled parenchyma cells of pith and cortex appear to be the first affected, individual

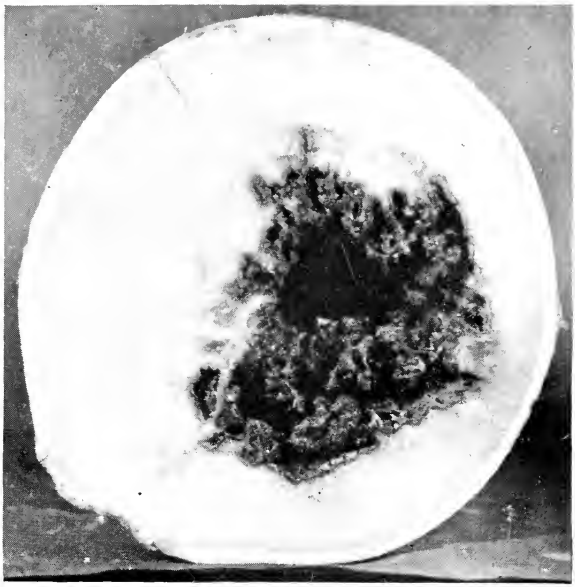


Fig. 9. Late stage in boron-deficient swede.

Fig. 10. Boron-deficient cauliflower. Note patches of discoloration of head.

Fig. 11. Stem of cauliflower suffering from boron deficiency and showing very typical breakdown of central tissue.

cells undergoing enlargement while the intercellular spaces appear to become filled with mucilage, giving the tissues first a water-soaked appearance, followed by a gradual darkening to a deep brown colour. The brown patches on the head appear to arise in the same way. Very characteristic is the breakdown of the pith to form an elongated cavity (Fig. 11).

In field experiments it was found that browning of cauliflower was completely eliminated by a dressing of 10 lb. of borax per acre.

Cracked Stem of Celery. A disease of celery, known as cracked stem, is widely distributed in the United States and Canada. It was first recorded in Florida and described by Purvis and Ruprecht (1935, 1937). The first external symptoms of the disease are a brownish mottling of the leaf, first in the marginal region, and brittleness of the stem. Next, cracks develop transversely across the leaf stalks which then curl outwards and turn brown. The roots suffer the same discoloration and finally die. In extreme cases the terminal bud may suffer the same fate. Where the disease is prevalent it may be very serious, and bring about a loss of half the crop in bad cases. Investigations by Purvis and Ruprecht, including the use of water cultures and sand cultures, as well as field trials, indicate that cracked stem results from boron deficiency and can be controlled in the field by the application of borax either as a soil dressing or as a spray.

As far as I am aware cracked stem of celery has not been recorded in Britain, although I have seen examples of it induced artificially by Mr Morley Davies through heavy liming of the soil.

Lucerne Yellows or Yellow Top. The effects of boron deficiency on lucerne (alfalfa) were described by McLarty, Wilcox and Woodbridge (1937) as a uniform yellowing of the terminal leaves, or a bronzing of the intervenal areas, poor development of internodes and death of the growing points. It appears that the symptoms may be confused with those resulting from attacks by the potato leafhopper (*Empoasca fabae*), and recently Colwell and Lincoln (1942) have published a comparative account of the symptoms produced in lucerne by boron deficiency and by the leafhopper, their account being the result of work carried out both in the greenhouse and under field

conditions. From this it would appear that when due to boron deficiency yellowing or reddening is always confined to the terminal leaves, including those of lateral branches, whereas when due to the leafhopper, yellowing or reddening may occur at various levels on the shoots. In boron-deficient plants the terminal internode is always abnormally short, whereas with leafhopper injury this is not so, although the plant may be generally stunted. With boron deficiency the terminal bud is always abnormal, and death of the growing points results. Colour photographs of boron-deficient lucerne are published in the paper by Colwell and Lincoln referred to above.

Several workers have reported increased growth and flowering, and increased yield of seed of lucerne as a result of application of boron to the soil (e.g. Grizzard and Mathews, 1942), although apparently the non-treated plants did not suffer from yellowing. This suggests that boron deficiency may be met with which is insufficient to induce yellowing but which is yet sufficient to bring about a decrease in growth, flowering and fruiting.

Top Sickness of Tobacco. The effect of boron deficiency on tobacco growing in the field in Sumatra was described by Kuyper (1930) who called the disease 'top sickness'. In America the effect of boron deficiency on tobacco growing in water cultures, pot cultures and on field plots has been described by McMurtrey (1929, 1933, 1935). The first external symptoms appear in the terminal bud, the leaves of which have a pale green colour, the bases of the leaves being paler than the tips. At the same time the leaves have stopped growing. The tissue at the base of the young leaves of the bud now breaks down and the bud dies. Next the older leaves become thicker and increase in area and later become brittle. The midrib may break and the vascular tissue then becomes discoloured. The upper leaves tend to take a drooping position. If the disease is not too severe and lateral buds develop in the axils of the leaves, they generally degenerate in the same way as the terminal bud. If there is partial recovery from the disease the younger leaves and the upper part of the stem as they develop may appear twisted to one side owing to growth round the damaged tissue.

Internal Cork of Apples. Disease of apples as a result of boron deficiency occurs in Europe, Canada, the United States,

Australia and New Zealand. According to Dennis (1937) the disease was recorded in Australia as long ago as 1892, when it was, however, confused with bitter-pit. The symptoms of the disease are rather varied, and partly as a result of this a number of names have been given to it including internal cork, drought or drouth spot, corky pit, corky core, brown heart, poverty pit, die-back and rosette. Internal cork refers to lesions which first appear as clear or slightly greenish rounded regions, generally not exceeding 1 cm. in diameter, and which may arise anywhere in the flesh of the fruit. The patches become dry and darken, and finally are dark brown in colour and of a corky or spongy consistency according as the lesions appear earlier or later in the developing fruit (Carne and Martin, 1937; Burrell, 1937). In apples affected with drought or drouth spot the lesions are in the form of large superficial patches, while in the case of corky core it is the middle region including the core which is affected. According to Burrell a greater deficiency of boron is required to produce rosetting and die-back than to induce internal cork in the fruit. Many investigators have demonstrated that this disease can be controlled by the application of borax or boric acid to the soil (see, e.g., Askew, Chittenden and Thomson, 1936). Internal cork does not appear so far to have been recorded in Britain.

There is evidence that boron deficiency may also induce the formation of internal cork in the fruit and die-back of the shoots in pears.

Brown-spotting of Apricots. On certain light-textured soils in New Zealand apricots tend to develop brown spots in the flesh, especially near the stem end, and a dry, spongy condition round the stone. This condition is attributed by Askew and Williams (1939) to boron deficiency. The condition is controlled by the application of $\frac{1}{2}$ lb. of hydrated borax per tree to the soil, or as a 0·1 per cent spray. The increase in the boron content of the leaves and fruit resulting from these treatments was accompanied by freedom from brown-spotting.

4. Diseases Attributable to a Deficiency of Copper

Although copper has now been shown to be essential for the growth of a number of plants, diseases attributable to a shortage of this element are rarely met with in the field and none are known to occur in Britain. The two well-recognized diseases which appear to be related to copper shortage are an affection of fruit trees known as exanthema, die-back or chlorosis, and a disease of various herbaceous plants known as reclamation disease. These are described later.

The general effects of copper deficiency on the leaves of tomato plants have been described by Reed (1939). The leaves exhibit restricted growth, but although they are considerably smaller than normal leaves the number of leaflets and lobes of the leaflets is not reduced. The laminae of the leaflets are incurved and they develop a bluish green colour with a distinct sheen. Later necrotic areas appear.

Microscopic examination showed that in the early stages of development the palisade cells of affected leaves contained many large hyperchromatic plastids. These plastids ultimately degenerate, at the same time tending to form aggregations at one end of the cell. Cavities tend to form below stomata by the separation of the upper ends of adjacent palisade cells. The cavity may lengthen so that ultimately it extends the whole length of the cells and at the same time broadens owing to the shrinkage of the cells, this being followed by a disappearance of the cells owing to lysis of their contents. These processes appear to lead to the production of the necrotic areas already mentioned.

Exanthema or Die-back of Fruit Trees. A pathological condition known as exanthema affects various fruit trees including both *Citrus* species and rosaceous trees as well as the olive. The disease was recorded in *Citrus* in Florida in 1875, but it was not described for other trees until 1928, when Smith and Thomas recorded that for a long time it had been known in California as affecting other fruit trees. They reported it as occurring in French prune, Japanese plum, apple, pear and olive trees.

Exanthema in *Citrus* is of widespread occurrence throughout the world. The symptoms of it in *Citrus* trees in Western Australia have been described by Pittman (1936) as follows. In the orange strong water shoots tend to bear abnormally large leaves while the shoots themselves, instead of growing straight, form an S-shaped curve. Small blister-like swellings containing gum develop on the young shoots. Later these swellings develop into longitudinal ruptures bordered with brown or reddish brown ridges from within which the yellow or reddish gum exudes in wet weather. Shoots so affected lose their leaves and die back, and lateral shoots developing at the base of affected twigs produce a typically bunchy appearance. A condition of 'multiple-bud' development, resulting from the development of a cluster of buds instead of two, is frequent, thus emphasizing the bunchy habit of the tree. Die-back is typical of badly affected trees. The fruit is small and frequently marked with irregular-shaped brown spots or blotches where finally the skin, after becoming dry, splits open.

In the lemon, gum formation on the branches is rare, but gum pockets may develop on the skin of the fruit.

Haas and Quayle (1935) state that leaves of *Citrus* in the early stages of exanthema may be unusually green, but may become mottled or chlorotic in later stages of the disease.

According to Smith and Thomas (1928), in French prune trees (*Prunus domestica*) affected with exanthema there is vigorous growth of new shoots each spring, but in June the terminal buds wither and the terminal leaves develop a chlorosis. There is a similar development of lateral shoots and of multiple buds as in *Citrus* as well as the production of eruptions of the bark. Apples, pears and Japanese plums can develop similar symptoms. Pittman describes exanthema of Japanese plums in Western Australia as involving the development of cracks in the bark which in some varieties may reach the cambium. Later, exudation of gum takes place through the ruptures. Die-back is here also very characteristic of the condition.

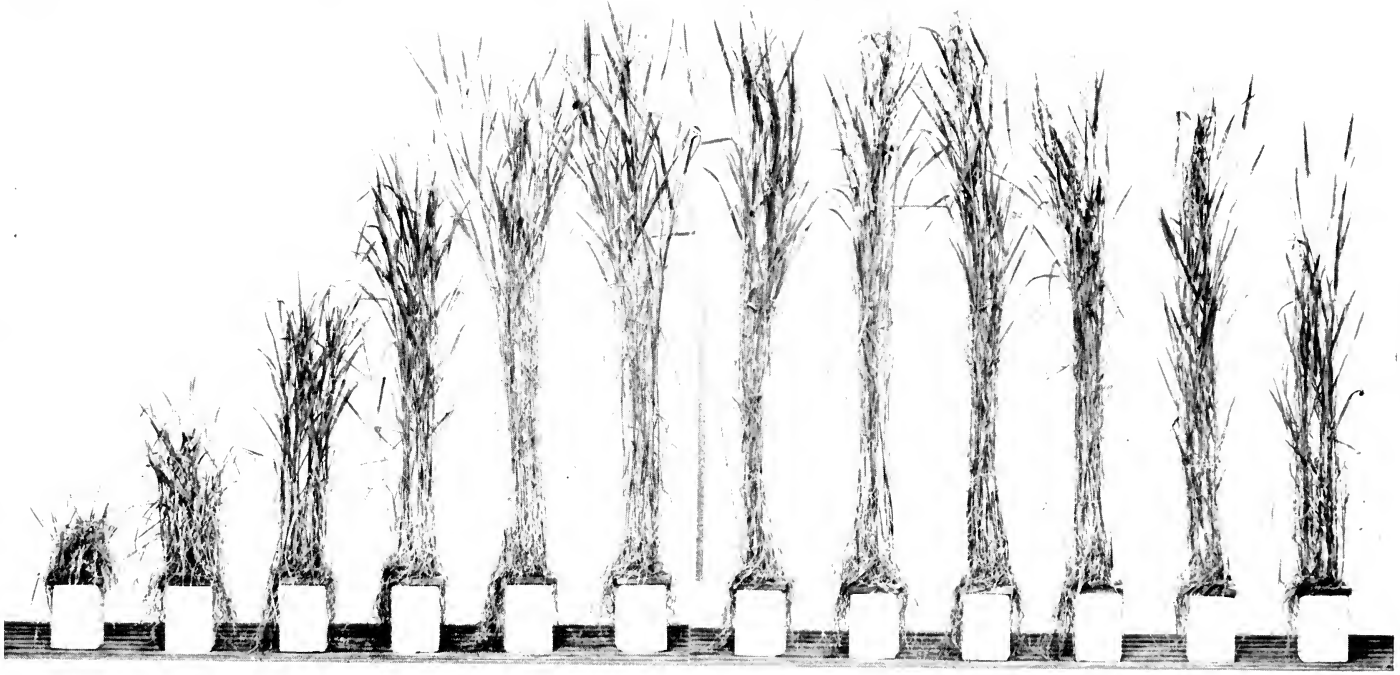
As long ago as 1917 Floyd reported the beneficial effect of copper sulphate on *Citrus* trees in Florida affected with this disease, and confirmation of this was forthcoming from Wickens (1925) in the treatment of affected orange trees in Western

Australia, from Smith and Thomas (1928) in regard to plum, apple, pear and olive in California and from Pittman (1936) for *Citrus*, plum and apple in Western Australia.

Oserkowsky and Thomas (1933) showed that the condition of the leaves of Bartlett pear trees in relation to this disease corresponded to their copper content. Thus, leaves affected with exanthema contained 3·1–5·1 p.p.m. of the dry weight, while normal leaves from localities free from the disease contained 11–20 p.p.m. of the dry weight. While these workers considered that these analyses afforded strong evidence that exanthema resulted from a deficiency of copper, they pointed out that there was no evidence to decide whether the disease was due directly to copper deficiency or whether the effect was an indirect one such as might be brought about, for example, if the action of copper resulted from its neutralizing the effect of toxins absorbed by the plant from the soil.

More definite evidence that exanthema in *Citrus* is due to a deficiency of copper was provided by a culture experiment described by Haas and Quayle (1935). Valencia orange trees grafted on sour-orange stocks were grown in twelve large tanks containing pure sand. A nutrient solution containing all the known mineral nutrients except copper was supplied to the trees. Although for some years the trees grew vigorously, after seven years they displayed typical and pronounced symptoms of exanthema including the S-shaped growing shoots, exudation of gum, blisters on the surface of the shoot and dying back of many shoots. The leaves became covered on the ventral side by a resinous stain and many leaves developed chlorosis.

The effect of copper deficiency on deciduous fruit trees in South Africa has been described by Anderssen (1932). Apple, pear, plum, peach and apricot trees are all affected to different degrees. Thus plums, peaches and apricots exhibit a very decided chlorosis in the areas of the leaves between the veins. This is accompanied by very marked rosetting of the leaves, cessation of apical growth followed by the dying back of the branches from their apices. Apples, on the other hand, exhibit chlorosis much less frequently, but rosetting is severe and the long shoots die back. Pears similarly do not show chlorosis very often, but unlike apples they do not develop rosetting; however,



By courtesy of Dr. C. S. Piper

Fig. 12. Effect of concentration of copper on the growth of oats.

the shoot apices and the youngest leaves become browned and finally die back.

Anderssen makes no reference to the surface eruptions on the shoot which are so characteristic of copper-deficient *Citrus* trees as to give the name exanthema to the disease, and which are recorded both by Thomas and his collaborators and by Pittman as occurring in deciduous fruit trees.

Analyses of leaves of affected and normal plum trees showed that of the mineral constituents determined (Cl, N, SO_4, PO_4, Ca, Mg, K, Fe, Mn, Cu) the only ones which were present in lower amount in chlorotic than in normal plants were manganese and copper. Some of Anderssen's determinations are shown in Table VI. No effect was, however, produced by applying manganese to the chlorotic trees, but dipping chlorotic leaves in a solution of copper sulphate (0·3 p.p.m.) cured the chlorosis, and treatment of the soil with copper sulphate to the extent of 0·25–2 lb. of copper sulphate per tree brought about recovery from the diseased condition. Isaac (1934) also reported the cure of chlorosis in young peach trees in South Africa by the application of copper sulphate to the soil, while manganese sulphate effected no improvement.

TABLE VI. Ash, manganese and copper content of leaves from chlorotic and normal Kelsey plum trees. (Data from Anderssen)

Sample	Ash per cent of dry weight	Manganese p.p.m. dry weight	Copper p.p.m. dry weight
Top leaves chlorotic	10·56	10·0	3·2
Top leaves normal	8·85	17·1	7·3
Middle leaves chlorotic	12·34	14·7	2·9
Middle leaves normal	9·15	16·6	4·6
Lower leaves chlorotic	14·28	25·5	3·5
Lower leaves normal	19·83	19·5	6·9

Die-back of apple trees ('wither tip' or 'summer die-back') as a result of copper deficiency has also been reported as occurring in Western Australia by Dunne (1938), but the earlier symptoms of the disease do not seem to be quite the same as those described by Anderssen. Thus Dunne makes no reference to rosetting, but states that first brown spots and then small necrotic areas appear on the terminal leaves. Eventually the leaves wither and fall, and then follows the dying back of the shoot. Application of

copper sulphate, either to the soil or by injection, was effective in bringing about arrest of the disease and recovery of the trees. The copper content of leaves from healthy trees varied from 5·5 to 12 p.p.m. of the dry weight, whereas in leaves from affected shoots the content varied from 1 to 3·6 p.p.m. of the dry weight. These values agree well with those found by Anderssen for the leaves of Kelsey plum trees, and by Oserkowsky and Thomas for the leaves of Bartlett pear.

However, Haas and Quayle made many determinations of the copper content of orange and lemon leaves and fruits, including samples from normal and exanthematic trees and from affected trees that had recovered as a result of the application of copper sulphate, and concluded that so much variation exists in the copper content of the leaves and fruits of trees from different localities and sites that it is not possible to decide from a knowledge of the copper content whether trees are suffering from a deficiency of that element or not. It may be said, however, that the values they obtained are of the same order as those already noted here. Thus the copper content of mature healthy orange leaves from untreated trees varied from about 7 to 15 p.p.m. of the dry weight, the corresponding values for the leaves of lemon being between 4 and 13 p.p.m. For two grapefruit leaves the values were 6·4 and 7·38 p.p.m. The fruit appears to contain much less copper calculated on a dry-weight basis, the content of the element in oranges and lemons ranging respectively from roughly 2 to 4 and 3 to 5 p.p.m. in these fruits.

Reclamation Disease. A disease attributed to copper deficiency, which affects oats and other cereals, beet and leguminous crop plants, occurs on reclaimed heath and moorland soils in Denmark, Holland and other parts of Europe. In affected plants the tips of the leaves become chlorotic, and in cereals this is followed by a failure of the plants to set seed. This disease, known as reclamation disease or yellow-tip, was originally attributed to the toxic action of a constituent of peat, but Sjollema (1933) in Holland showed that the disease could be cured by the addition of copper sulphate to the soil, and that the content of copper in wheat, rye and hay grasses was raised by this soil treatment. Later, Gram (1936) obtained similar results with barley and oats in Denmark, and Undenäs (1937) with oats in Sweden, while in 1938 Piper found the disease affecting

cereals in South Australia and also found that it could be controlled by the application of copper sulphate to the soil.

Oats were grown by Brandenberg (1933, 1934) in water culture in which the amount of copper present in the culture solution was carefully controlled. When copper was excluded, growth was poor and the plants developed the symptoms of reclamation disease. With small amounts of copper added to the solution vegetative growth was more normal, but fruiting was not unless the concentration of copper in the solution was at least 0·5 mg. per litre. More recently, Piper (1942) has carried out water-culture experiments with specially purified reagents in which the initial copper concentration of the culture solutions varied from 0 to 3 mg. per litre. In addition to the ordinary major mineral nutrients the solutions contained small quantities of boron, zinc, manganese and molybdenum as well as sodium chloride. The main results were as follows. In the cultures without copper, 27 days after setting the seeds to germinate, growth and tillering were noticeably less than in cultures provided with copper. During the next fortnight these symptoms became more pronounced, the leaves were a paler green, the tips of the younger leaves appearing definitely chlorotic, withering and dying without unrolling, while the base of the leaf continued to emerge and grow. In subsequently emerging leaves these symptoms were more pronounced until growth of the tiller ceased and its death ensued.

In cultures provided with 3μg. of copper per litre definite symptoms of copper deficiency were not observed until 71 days after setting the seeds to germinate, although the plants made less growth than those with more copper. Slightly older leaves developed a marginal chlorosis. Ultimately the main tillers ceased growth and died, and although secondary tillers were produced these also developed the symptoms already described and ultimately stopped growing.

When cultures were initially provided with 6μg. of copper per litre more growth took place before the symptoms of deficiency developed in the youngest leaves. In these cultures the slightly older leaves exhibited a loss of turgor so that they appeared limp and drooping. Secondary tillers developed as before, but all stopped growing before ear production.

The plants in a culture solution containing initially 10μg. of

copper per litre grew normally for 19 weeks before the symptoms of copper deficiency were evident. Flowering took place but grain was not formed. When the concentration of copper in the culture solution was 20μg. per litre some grain was produced but a proportion of the spikelets were sterile. In cultures grown in solutions containing from 50 to 500μg. of copper per litre growth was normal. If the concentration of copper was higher than this, a toxic effect was observed, growth and tillering being depressed while the leaves became stiff and erect. Although the leaves were first of a particularly deep green colour the younger leaves developed a strong chlorosis, which was more marked the higher the copper concentration of the culture solution.

The effect of copper concentration on the growth of oats is very clearly demonstrated by Piper's photograph of his cultures reproduced in Fig. 12.

Piper's work has confirmed the view that reclamation disease in oats is the result of copper deficiency. Similar experiments by Piper on other members of the Gramineae, namely, wheat, *Lolium subulatum* and *Phalaris tuberosa*, showed that these also are affected in the same way by shortage of copper.

5. The Symptoms of Molybdenum Deficiency

The symptoms ascribable to molybdenum deficiency displayed by tomato plants in water culture as described by Arnon and Stout (1939) are these. First the lower leaves develop a very characteristic mottling. This is followed later by necrosis at the leaf margins along with a characteristic curving over of the marginal regions of the leaf. Abscission of flowers takes place so that no fruit is produced.

The symptoms of molybdenum deficiency of oats grown in water culture are described by Piper (1940). About the time of emergence of the panicles necrotic areas appear about midway along the lamina of the upper leaves, and these areas frequently extend right across the leaf. With a line of weakness so produced the leaf bends back sharply; the band is first smooth, but finally a kink develops and the middle necrotic region of the leaf dries out a light reddish brown. Although the inflorescence develops normally the grain consists entirely of empty husks.

So far there is no record of molybdenum deficiency occurring in plants in the field.

CHAPTER IV

THE FUNCTIONS OF TRACE ELEMENTS IN PLANTS

Some years ago R. W. Thatcher (1934) published a short paper in which he put forward a classification of the chemical elements based on their functions in plant nutrition. In the first place he pointed out that nearly all the elements which are known or have been suggested to have a function in plant nutrition are included in the first four periods or orbits of the periodic classification. These first four periods, with the atomic numbers[1] of the elements, are shown in Table VII. Of the

Table VII. The first four periods of the periodic classification

	Group										
Period	I	II	III	IV	V	VI	VII	VIII			O
1	H	—	—	—	—	—	—	—			He
	1										2
2	Li	Be	B	C	N	O	F	—			Ne
	3	4	5	6	7	8	9				10
3	Na	Mg	Al	Si	P	S	Cl	—			A
	11	12	13	14	15	16	17				18
4	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	
	19	20	21	22	23	24	25	26	27	28	
	Cu	Zn	Ga	Ge	As	Se	Br	—	—	—	Kr
	29	30	31	32	33	34	35				36

elements shown in this table we may dismiss the inert gases of group O. In the other groups there are a number of elements which so far have not been found essential for plants, but all the elements known to be necessary for plants are included in these first four periods with the one exception of molybdenum (period 5, group VI, atomic number 42). The heavier metals do not figure among elements necessary for plants.

Thatcher's classification of the elements in relation to their functions in the plant was based on the conception that 'green plants are the energy-absorbing and energy-storing agents of

[1] The atomic number is the number of free positive charges in the nucleus of the atom. Except for hydrogen it is very roughly half the atomic weight.

the cycle of life'. From this point of view he divided the elements into eight groups as shown in Table VIII.

TABLE VIII. Thatcher's classification of elements in plants

Group	Type	Function	Elements
I		Energy exchange	H, O
II	Anion (or acid) group formers	Energy storers	C, N, S, P
III	Cation (or base) formers with fixed valency	Translocation regulators	Na, K, Ca, Mg
IV	Cation (or base) formers with varying valency	Oxidation-reduction regulators	Mn, Fe, (Co, Ni), Cu, Zn
V	Ampholytes with varying valency	Functions unknown	B, Al, Si, As, Se
VI	Anion (or acid) formers with fixed valency	Functions unknown	Cl, F, (Br, I)
VII	Cation (or base) formers with varying valency	Perhaps those of group IV	Co, Ni
VIII	Ampholytes	Functions unknown	Ge, Ga and other rare elements

It is not within the scope of the present discussion to consider the value of this scheme of classification, but we may note that three of the well-established trace elements, manganese, copper and zinc, are placed, along with iron, itself often classed as a trace element, in one group, that of oxidation-reduction regulators. The fourth, boron, is placed in another group, one with unknown function, that of ampholytes of varying valency. At the time of publication of Thatcher's paper molybdenum had not been recognized as a plant trace element; had it been, it might have been included in the same group as boron. Further, it is not clear why the ampholytes of Thatcher's group VIII, which included gallium, were separated from those of his group V, since in both the functions are listed as unknown. However this may be, Thatcher's classification divides the trace elements into two groups, the oxidation-reduction regulators including manganese, copper and zinc, and also iron, the other including boron of unknown function. With regard to the oxidation-reduction regulators Thatcher pointed out that if nickel and cobalt are included the group contains six elements

with consecutive atomic numbers, namely, 25–30. It is interesting to note that gallium, only recently shown to be essential for any plant, is the next element in the series with an atomic number of 31. This means that each successive member of the series differs as regards atomic structure from the element before it only by the addition of one electron to the proton nucleus. Whether this close connexion between these elements really has any significance in respect of their physiological function it would be premature to say, but it is a fact that several of them have rather similar chemical properties. Thatcher expressed the opinion that there was sufficient evidence to justify the opinion that manganese and iron on the one hand, and copper and zinc on the other, were pairs of 'mutually co-ordinating catalysts for oxidation-reduction reactions', the former pair for reactions in which the addition or removal of oxygen is involved, the latter for reactions which concern the transference of hydrogen. As we shall see in the sequel there is a certain amount of experimental evidence in favour of at least one of these hypotheses.

Frey-Wyssling (1935) has also attempted to find a relation between essentiality and position of elements in the periodic table. He uses a table in which group O appears both on the extreme left and the extreme right, but with each element of the group one period higher in the right than in the left column. If a line is then drawn through the table from argon on the left to carbon and then on to argon on the right, this line passes through or near the positions in the table of all the essential elements with the exception of hydrogen. This line Frey-Wyssling calls the nutrient-line. This relationship expresses with greater precision the fact pointed out by Thatcher, that all the essential elements occur in the first four periods of the periodic table. The further the position of an element is from the nutrient-line the more toxic it is in general. It will be observed that essential elements occur in all groups I–VIII.

A very interesting discussion on the relation between the biological essentiality of the elements and their atomic structure has been contributed by Steinberg (1938*c*). When considered from the point of view of their position in the periodic table Steinberg concludes that three and no more than three essential elements are to be found in each group. Where less than three

essential elements are known to exist in a group there is a presumption that the missing ones are yet to be found. However, Steinberg considers that correlations between essentiality and atomic structure can best be shown by tabulating the elements on the basis of their transition subshell, those in which the electron numbers have undergone a regular change in the formation of the elements and which largely determine the chemical properties of the latter. From such considerations Steinberg makes certain deductions regarding the relationship between essentiality and atomic structure and of the possible essential elements not yet recognized as such. Thus the non-essentiality of silicon is indicated, while the possibility of columbium as an essential element is suggested. Steinberg's conclusions only claim to be very tentative.

That the trace elements are required by plants in such small quantities strongly suggests that they all function as catalysts or are at least closely linked up with some catalytic process. Miss Warington (1923) thought that the function of boron in the broad bean was nutritive rather than catalytic, and it is certainly true that the results of boron deficiency are generally of a distinctly different kind from those resulting from deficiency of manganese, zinc and copper. With boron deficiency, as we have seen, the most characteristic effect is a breakdown of thin-walled tissues, especially those of the meristematic regions, followed by degeneration of the vascular tissues, whereas with the other three well-established trace elements early external symptoms are generally localized chloroses, although these may precede more serious disturbances in growth such as those which lead, in the case of zinc and copper, to die-back of the terminal buds of the shoots. Nevertheless, chlorosis may also be a symptom of boron deficiency, as, for example, in tobacco (Van Schreven, 1934) and in sugar cane (Martin, 1934), while plants do not appear to require more boron than manganese. It is not very clear why a catalyst essential for the maintenance of normal metabolism and growth should not be regarded as fulfilling a nutritive function.

Manganese, zinc and copper are generally regarded, like iron, as playing a part in vital oxidations and reductions, and some writers consider boron to play a similar role. It must be

emphasized, however, that if the parts the various elements play are similar, they are not identical, for they cannot replace one another in the organism. Reference has already been made to Thatcher's view that manganese and iron form a pair of mutually coordinating catalysts for oxidation-reduction processes involving the addition and removal of oxygen, while zinc and copper form another pair concerned in similar processes involving the addition and removal of hydrogen.

Since deficiencies of manganese, zinc and copper characteristically induce chlorosis, it is understandable that they have been held to be concerned in chlorophyll formation (cf. McHargue, 1923, 1926*a*; Bishop, 1928). They have also been thought to act as catalytic agents in photosynthesis (see e.g. Stoklasa, 1911; McHargue, 1923; Dufrénoy and Reed, 1934). Recent support for this view is forthcoming from work by Emerson and Lewis (1939), who found that when the trace elements of Arnon's groups A 4 and B 7 (see p. 9) were added to the culture medium of *Chlorella pyrenoidosa* not only was the rate of growth of the alga increased but the amount of photosynthesis per unit quantity of light absorbed was also increased. The trace elements were added in two groups, those of Arnon's A 4 group + molybdenum, now denoted by the symbol A 5, and those of Arnon's B 7 group without molybdenum, this group now being denoted B 6. The addition of the A 5 group was more effective than the B 6 group, and the addition of A 5 + B 6 more effective than either alone. It would thus appear that the trace elements play some part in the photosynthetic process, but what this is can at present be only a matter of conjecture.

Some observations of Steinberg (1942) may be mentioned here. Starting from the observation that when nitrogen is supplied only as nitrate the growth of the fungus *Aspergillus niger* is lessened in air deprived of carbon dioxide, he examined the effect of removal of carbon dioxide on the growth of this fungus in absence of various micro-nutrients. Under these conditions omission of zinc, copper or manganese from the culture medium reduced the growth of the fungus proportionately more when carbon dioxide was absent than when it was present. Steinberg concludes that the trace elements probably play a specific part in the utilization of carbon dioxide by *A. niger*, and compares

this with the conclusion of Emerson and Lewis on the part they play in the utilization of carbon dioxide in green plants. Incidentally, Steinberg calls attention to the significance of this similarity in regard to the question of the validity of the suggestion of Ruben and Kamen that carbon dioxide utilization by micro-organisms is essentially the same process as the dark or Blackman reaction in photosynthesis.

So far the functions of the trace elements in general have been considered. With regard to the specific functions of the individual trace elements little, if anything, of any definite value can be said regarding copper, molybdenum and the less well-established trace elements, but contributions of considerable interest have been made regarding the functions of manganese, zinc and boron. A consideration of these follows.

Manganese. The view has been very generally held that manganese is related to oxidation in the plant. Bertrand (1897), as we have seen, first called attention to the importance of manganese when he found it essential for the action of the oxidizing enzyme laccase. Later he maintained that it was essential for the action of oxidizing enzymes in general. In recent years Lundegårdh (1939) has produced more direct evidence that manganese is concerned in respiration. Thus he found that the oxygen intake by wheat roots was increased by 155 to 470 per cent by the addition of 0·00005 *M* manganese chloride. Contrasted with this, addition of ferric chloride or ferric citrate generally brought about a decrease in oxygen intake, the average reduction with 0·00005 *M* ferric citrate being about 21 per cent. From these observations it is concluded that manganese, but not iron, catalyses aerobic respiration.

About the same time Burström (1939) examined the assimilation of nitrate both by whole wheat roots and by wheat-root pulp in presence of small quantities of iron and manganese, and came to the conclusion that, whereas without iron and manganese nitrogen assimilation does not take place, in presence of a small concentration of manganese assimilation takes place both with whole roots and root pulp, the optimum effect being produced with about 4 mg. manganese per litre with whole roots and about 12·3 mg. per litre with pulp. Without added manganese the addition of iron brought about feeble nitrogen

assimilation of whole roots but not of pulp, and even this assimilation by whole roots is ascribed by Burström to its effect on respiration and ion uptake. His general conclusion is therefore that manganese, and not iron, directly catalyses nitrate assimilation.

It will be observed that the observations of both Lundegardh and Burström emphasize the contrasting effects of manganese and iron. A number of other workers have called attention to this, and indeed there appears to be considerable reason to suppose that the function of manganese is to be found in its relation to the oxidation and reduction effected by iron salts. Thus Hopkins (1930), from observations on the growth of the unicellular green alga *Chlorella*, held that manganese brings about the reoxidation of iron after its reduction in the plant to the ferrous state; if the amount of manganese in the plant is deficient there results too high a proportion of ferrous iron, while if manganese is present in excess the reduction of ferric iron is prevented and the concentration of ferric iron is too high. In either condition the oxidation-reduction processes of the cell involving iron are disturbed.

On this view it is to be expected that the ratio of manganese to iron in the plant is of more importance than the absolute concentration of manganese. In this connexion it may be noted that Bertrand (1912 *b*, *c*) found the ratio of manganese to iron + zinc determined the development of conidia in *Aspergillus niger*, the capacity to produce these reproductive bodies being inhibited if the ratio Mn/Fe + Zn was too low. With higher plants the importance of the ratio of manganese to iron has been emphasized by a number of workers. Thus Pugliese (1913) and also Tottingham and Beck (1916) write of an antagonism between iron and manganese in the growth of wheat, and the former gives the optimum ratio of the two elements in the culture solution as 1/2·5. Scharrer and Schropp (1934) found that with maize in water culture the growth of the roots was at a maximum when the ratio of Mn/Fe in the culture solution was 1/7. That chlorosis might be induced by manganese when iron is deficient was reported by Gile in 1916 in the pineapple and again by Scholz in 1934 in the blue lupin.

The relation between manganese and iron in the plant has

more recently been dealt with by Shive (1941). This writer calls attention to two important points in this connexion, the first being that the active functional iron in the tissues is in the ferrous state, the second that the oxidizing potential of manganese is higher than that of iron. Shive holds that if iron is absorbed in the ferric state much of it is reduced in the plant by powerful reducing systems unless this is prevented by a counter-reactant. The manganese functions as such a counter-reactant, oxidizing ferrous to ferric iron which is precipitated, probably in organic complexes. Hence, if manganese is deficient in the plant, there will be an excess of active ferrous iron which induces chlorosis, a chlorosis due to iron toxicity. On the other hand, if the concentration of manganese is high the concentration of active ferrous iron is low, and if too low a chlorosis due to iron shortage will result. Thus it is necessary for healthy growth that the proportion of iron to manganese should lie within certain limits, and Shive concluded that, for the species investigated by him, the ratio of active iron to active (soluble) manganese in the plant should lie between 1·5 and 2·5. This conclusion was derived from the results of a series of culture experiments with soya bean described by Somers and Shive (1942) in which the quantities of both iron and manganese in the culture solutions were varied. They used in all eighteen different combinations of iron and manganese in their culture solutions. The iron content varied from 0·005 to 3·00 p.p.m. and the manganese content from 0 to 5·00 p.p.m.; the various combinations are shown in Table IX. Actually complete absence of manganese is never attained in the culture itself since there will be some present in the seed used and no doubt a little will also be introduced as impurity either from culture vessels or other nutrient salts used. The culture solutions also contained the usual major nutrients and boron. The concentrations of iron and manganese and a *p*H of 4·6–4·8 (to prevent precipitation of iron) were maintained approximately constant by the use of a technique in which a continuous flow of solution passed through the culture vessels, and the solutions were completely changed every other day.

Approximate determinations of both soluble and insoluble iron and manganese in the tissues were also made. To separate

the soluble and insoluble fractions the fresh material was frozen by means of an ice-salt mixture and from this, in thawing, the juice was expressed under a pressure of 1600 lb. per sq. in. applied for $2\frac{1}{2}$ min. The expressed juice together with washings of the press cake and muslin containing it were taken as containing the soluble iron and manganese, the press cake the insoluble fraction.

A study of Table IX shows at once how the dry weight and condition of the plants is related to the Fe/Mn ratio and not to the absolute concentrations of these nutrients. Thus plants growing in solutions containing 0·002, 0·250 and 2·00 p.p.m. manganese respectively were all normal and possessed about the same dry weight provided that in each case the ratio of soluble iron to soluble manganese in the leaves was within the range 1·5–2·5. Whenever the ratio was outside this range pathological symptoms tended to develop.

If the ratio were above 2·5 the symptoms were of one kind, if the ratio were below 1·5 the symptoms were of a different kind. The former were thus those of a too high Fe/Mn ratio, the latter of a too low Fe/Mn ratio. The first could be described either as manganese deficiency or iron excess, the second either as iron deficiency or manganese excess. The first sign of a too high Fe/Mn ratio was a fading of the green colour of the lower leaves which later developed into an intervenal yellowing on the basal part of the leaves. Next the upper leaves showed a fading of the green colour in the intervenal areas followed by the development of small brown necrotic spots. Finally, the new leaves opened with the necrotic areas already present, and these leaves might fail to develop and fall, also stem apices might die. Roots showed no visible symptoms apart from being smaller than those of normal plants.

The symptoms of a too low Fe/Mn ratio (iron deficiency, manganese excess) were quite distinct from those just described. The first sign was a slight brown discoloration of the roots followed by yellowing and slight curling of the upper leaves. As the condition developed the discoloration of the roots and chlorosis of the upper leaves continued until the newer leaves were almost white. The leaves curled downwards and sometimes the midribs darkened and their tissue broke down. Large

TABLE IX. Effects on soya beans grown in water culture of different proportions of iron and manganese. (From Somers and Shive)

Concentration in substrate p.p.m.		Ratio Fe/Mn in substrate	Ratio soluble Fe/soluble Mn		Dry weight per plant in g.	Condition of plants
Fe	Mn		In leaves	In roots		
0·005	0·000	—	2·65	5·04	3·47	Normal
0·005	0·002	2·50	2·37	3·36	3·78	Normal
0·005	0·010	0·50	2·05	2·73	3·62	Slight Fe deficiency (Mn excess)
0·005	0·250	0·02	1·00	1·44	2·86	Medium Fe deficiency (Mn excess)
0·005	2·00	0·0025	0·80	1·35	1·50	Severe Fe deficiency (Mn excess)
0·005	5·00	0·0010	0·83	1·29	1·57	Very severe Fe deficiency (Mn excess)
0·500	0·000	—	5·66	15·8	1·51	Very severe Mn deficiency (Fe excess)
0·50	0·002	250·0	5·44	14·5	2·22	Severe Mn deficiency (Fe excess)
0·50	0·010	50·0	4·47	12·4	3·01	Medium Mn deficiency (Fe excess)
0·50	0·250	2·0	1·95	2·24	3·78	Normal
0·50	2·00	0·25	0·72	1·26	3·16	Slight Fe deficiency (Mn excess)
0·50	5·00	0·10	0·58	1·17	3·02	Medium Fe deficiency (Mn excess)
3·00	0·000	—	9·82	17·4	1·37	Very severe Mn deficiency (Fe excess)
3·00	0·002	1500	9·85	15·1	1·39	Very severe Mn deficiency (Fe excess)
3·00	0·010	300	9·88	13·3	1·90	Medium Mn deficiency (Fe excess)
3·00	0·250	12·0	2·48	4·15	3·78	Normal
3·00	2·00	1·5	1·50	2·51	4·05	Normal
3·00	5·00	0·6	0·76	1·77	3·70	Slight Fe deficiency (Mn excess)

necrotic areas developed in the most chlorotic leaves, this being accompanied by death of the stem apices.

In both it was possible to correct the pathological symptoms by altering either the iron or manganese concentration in such a way as to bring the Fe/Mn ratio to a value between 1·5 and 2·5. A further indication of the importance of the Fe/Mn ratio was obtained by Somers, Gilbert and Shive (1942) in the respiration rate of soya beans in water cultures supplied with different proportions of the two nutrients. Respiration rates were always definitely lower when the Fe/Mn ratio was outside the range 1·5–2·5 than when it was within it.

In further support of the oxidation-reduction hypothesis outlined above, Somers and Shive mention a series of tests carried out with maize seedlings in which cobalt was substituted for manganese. The oxidation potential of cobalt is higher than that of manganese, so it should, on the hypothesis, have a greater tendency than manganese to lessen the metabolic efficiency of iron by effecting the oxidation of the latter to the insoluble ferric state, and this, Somers and Shive state, was so.

The work of Shive and his associates constitutes a very strong argument in favour of the hypothesis they present regarding the connexion between iron and manganese. An extension of such experiments to other species is clearly very desirable. If the connexion, which means that manganese deficiency is the same thing as iron excess and vice versa, should prove to be general, it must be admitted that it has not so far been realized by those workers who have had much experience with the effect of mineral deficiencies in the field. It would, however, afford an explanation of why a characteristic manganese deficiency has been observed in plants containing much manganese, while in others the symptoms of this deficiency have not appeared when the manganese content has been exceptionally low (cf. p. 59).

In a study of cation absorption by tobacco, Swanback (1939) made some observations on the effect of manganese on the absorption of potassium and calcium. The plants were grown in nutrient solutions containing calcium in three different concentrations. In the absence of manganese the symptoms of

deficiency of this element were most pronounced in the plants grown in the solutions containing the highest concentration of calcium (0·403 g. per litre), were less with a medium calcium concentration (0·143 g. per litre) and were not observed with low calcium content (0·042 g. per litre). The effects with potassium were in the reverse order, the symptoms of manganese deficiency being most pronounced with low potassium supply (0·026 g. per litre), less with medium potassium supply (0·082 g. per litre), and not noticeable in the plants supplied with the highest concentration of potassium (0·26 g. per litre). These results are interpreted as suggesting that there is an antagonism between calcium and manganese in their absorption, while there is none between potassium and manganese. Other observations by Swanback support this suggestion. Thus with a low supply of calcium the dry matter produced by the tobacco plant cultures was 6 times as much when manganese was not supplied as when 0·0054 millimol. per litre of this element was supplied to the culture solution. With a high supply of calcium the reverse resulted, the dry matter of the plants provided with manganese being 3·5 times that of the plants not supplied with it. These results are explained on the view that with low calcium supply the manganese retards the absorption and utilization of calcium, shortage of which results in the small amount of dry matter produced, while with high calcium supply the antagonistic effect of the manganese is insufficient to reduce the absorption and utilization of the calcium to a low level while the favourable effect of the manganese itself brings about an increase in growth and so of dry matter produced.

That calcium antagonizes the absorption and utilization of manganese while potassium is indifferent is shown by determinations of the manganese content of shoots and roots of tobacco plants grown in the solutions containing the various concentrations of calcium and potassium already mentioned and 0·0054 millimol. of manganese per litre. The results are shown in the following table. The values in the fourth and fifth columns of the table are of what Swanback calls the 'translocation quotient'. This is the ratio of the manganese content of the shoot to that of the root and is supposed to give a measure of the mobility or relative translocation of the manganese. They show

clearly the effect of calcium in reducing both the intake of manganese and its translocation to the leaves, while no such effect is suggested as regards potassium.

TABLE X. Effect of varying concentrations of calcium and potassium on the absorption of manganese by tobacco plants in water culture. (From Swanback)

		Manganese content in millimol. $\times 10^{-3}$ per g. after		Manganese translocation quotient after	
Treatment	Plant	45 days	60 days	45 days	60 days
Low calcium	Shoot	0·74	0·75	0·91	1·25
	Root	0·82	0·60		
Medium calcium	Shoot	0·25	0·36	0·70	0·07
	Root	0·36	5·00		
High calcium	Shoot	0·16	0·33	0·61	0·06
	Root	0·26	5·60		
Low potassium	Shoot	0·22	0·20	0·43	0·10
	Root	0·51	2·10		
Medium potassium	Shoot	0·25	0·36	0·70	0·07
	Root	0·36	5·00		
High potassium	Shoot	0·30	0·28	0·61	0·09
	Root	0·49	3·20		

Zinc. It has already been mentioned (p. 99) that Thatcher thought that zinc and copper were a pair of catalysts concerned together in oxidation-reduction reactions. That zinc, at any rate, is concerned in such reactions is the conclusion reached by Reed and Dufrénoy (1935) mainly as a result of their microscopical examination of mottled leaves of *Citrus*. They conclude that zinc is concerned with the functioning of sulphydryl compounds such as cysteine in their regulation of the oxidation-reduction potential within the cells. We have already noted that they found that the stromata of the chloroplasts in the palisade cells of such leaves are often rich in fat, while the vacuoles contain phenolic material and phytosterol or lecithin, which are absent from normal leaves. Reed and Dufrénoy interpret these substances as suboxidized products of carbohydrates and proteins, and their presence suggests a disturbance in the oxidation-reduction potential within the leaf cells.

For the view that the maintenance of the oxidation-reduction potential at its normal level depends on sulphydryl compounds

the following arguments are advanced. First, Hopkins and others have shown that such compounds appear to be present in all living cells and may control oxidation and reduction processes. Secondly, the oxidation of cysteine to cystine is catalysed by metals.[1] Thirdly, Giroud and Bulliard have shown that zinc has a specific effect in stabilizing the nitroprusside colour reaction of the sulphydryl (SH) group.[2] Reed and Dufrénoy also refer to Mazé's finding that roots of maize grown in a culture solution deficient in, though not completely free from, zinc, contain sulphides in the ash, indicating that the sulphur metabolism of the plant was adversely affected.

Chandler (1937) has pointed out that zinc deficiency has its most serious effects in plants where carbohydrates have accumulated, and he suggests that zinc deficiency brings about inhibition of some process of carbohydrate transformation. This, as Reed (1938) suggests, may mean that zinc catalyses oxidation processes which in its absence may run the other way.

Following on their earlier work, Reed and Dufrénoy (1942) have made a cytological study of catechol aggregates which arise in the vacuoles as a result of zinc deficiency. They consider that they form by a process which they call coacervation, in which disperse phase particles of the colloidal system constituting the vacuole become aggregated into spherical masses. This process is regarded as something more than a separation of phases, as the aggregates become surrounded by a precipitation membrane composed of orientated molecules of a phospholipoid. Tests for oxidase show (Dufrénoy and Reed, 1942) that these aggregates are not only centres of catechol derivatives, but also for catechol oxidase activity.

[1] It is stated, however (cf. Meldrum, 1934), that the most active metals are iron, copper and manganese.

[2] This test, due to Mörner, consists in adding a 5 or 10 per cent solution of sodium nitroprusside rendered alkaline with ammonia to the liquid to be tested, and shaking. A violet colour, which soon fades, is produced if a cysteine peptide such as glutathione is present. Some other substances, including creatinine and acetone, give somewhat similar colours. According to Giroud and Bulliard the addition of salts of zinc gives a red colour much more stable than the violet colour of the reaction as usually produced. It appears to be quite specific for the sulphydryl group and is not given by either creatinine or acetone.

Reed and Dufrénoy conclude that the vacuolar sap contains both oxidizable phenolic compounds and catechol oxidase capable of catalysing the oxidation of these compounds. Normally this oxidation is prevented owing to the presence of hydrogen donators which may include the ascorbic-dehydroascorbic acid system, dihydroxymaleic acid, cysteine and glutathione. During the earlier part of the growing season the relatively high concentration of hydrogen donators in the cell protects the catechol compounds from oxidation and they remain dispersed throughout the vacuole. With the approach of senescence, or with nutrient deficiency such as a shortage of zinc, the oxidation-reduction equilibrium is disturbed and coacervation results, the process, according to Reed and Dufrénoy, being a 'simple consequence of a gradient in the distribution of cations and correlative distribution of polyphenol oxidase'. They further suppose that a difference in electrical potentials will exist between the aggregations and the surrounding medium, since the former are foci for catechol oxidase, and that there will therefore be a tendency for cations to move into the coacervate, and this in turn will greatly influence the intake of dissolved material by the cell and consequent derangement of metabolism.

A somewhat more precise suggestion of the way in which zinc, through its effect on oxidation-reduction systems, may affect growth, has been put forward by Skoog (1940). Experiments made and described by this worker on tomatoes and sunflower grown in zinc-deficient culture solutions indicate a connexion between zinc and the growth-promoting substance auxin. Terminal buds and stems of such zinc-deficient plants appeared to contain no auxin or only a trace of it. Appreciable amounts were, however, found to be present in the leaves, although less than in leaves of control plants provided with an adequate supply of zinc. The visible symptoms of zinc deficiency only appear after the decrease in auxin content, and if plants in an extreme state of zinc deficiency are supplied with zinc, the auxin content of these plants increases considerably in one to a few days, while growth is resumed after the passage of several more days. These observations suggest that zinc is necessary for the maintenance of a normal auxin content.

Skoog also placed sections of stems from zinc-deficient and control plants, from which auxin had previously been removed, on agar blocks containing a known concentration of indole-3-acetic acid, and found that always more of this growth hormone was inactivated in the blocks in contact with tissue from zinc-deficient plants than in the blocks in contact with control tissue. This suggests that deficiency of zinc brings about excessive destruction of auxin, an effect attributed to an increased oxidative activity of the tissues, since these displayed increased capacity to oxidize benzidine in presence of hydrogen peroxide. That zinc functions as a catalyst in relation to oxidation-reduction processes in the cell is thus again indicated, while its relationship to auxin maintenance suggests why zinc deficiency may lead to retardation or cessation of growth.

As will be mentioned later, zinc enters into the composition of the molecule of the enzyme carbonic anhydrase, which catalyses the action $H_2CO_3 \rightleftharpoons CO_2 + H_2O$. As far as the writer is aware this enzyme has so far only been found in animals, but if it should occur in plants, it would suggest that zinc might be concerned in the excretion of carbon dioxide and possibly in other processes about which, in the absence of any information, it would be idle to speculate. It may, however, be pointed out that deficiency of zinc would then mean a shortage of the enzyme catalysing the release of carbon dioxide so that this end product of the oxidation of carbohydrate would accumulate as carbonic acid in the tissues. This would lead to a slowing down of the oxidation process and conceivably to just such an interference in the normal oxidation mechanism in the tissues as Reed and Dufrénoy hypothesize.

Copper. Precise information regarding the functions of copper in the plant is forthcoming on one point: it has been found that this metal enters into the composition of the oxidizing enzyme or enzymes known as catechol oxidase or polyphenol oxidase. This was shown by Kubowitz (1937) for the oxidase present in potato tuber and by Keilin and Mann (1938) for the similar oxidase present in the cultivated mushroom (*Agaricus campestris*). The enzyme, in fact, appears to be a copper-protein compound containing not less than 0·30 per cent of copper.

Catechol or polyphenol oxidase catalyses the oxidation of compounds with the orthodihydroxyphenol grouping such as catechol and pyrogallol. In the presence of the enzyme and a low concentration of catechol a number of other substances such as guaiacum, benzidine and ascorbic acid are also oxidized which are not oxidized by the pure enzyme alone. These further oxidations may require the presence of some substance such as orthoquinone, produced by the oxidation of catechol in the primary reaction, so the presence in plant tissues of a substance either of the catechol or orthoquinone type along with the oxidase would make possible the oxidation of a wide range of phenolic compounds.

Enzymes of this type are widely distributed in plants, but whether they are all copper compounds it is not yet possible to say. That the enzymes from plants so different from one another as the potato and mushroom both contain copper suggests that this is a possibility, and if this should prove to be so, the function of copper as a catalyst in vital oxidations can be regarded as established.

Reference has already been made to Thatcher's opinion that zinc and copper form a pair of mutually co-ordinating catalysts for oxidation-reduction reactions. It is therefore interesting to find that Reed and Dufrénoy connect the action of zinc in plants with the distribution of the copper-containing polyphenol oxidase.

Boron. Various functions have been ascribed to boron in higher plants. The effects attributed to the action of this element include an influence on the water relations of the protoplasm, a favourable influence on the absorption of cations and a retarding influence on the absorption of anions, a favourable influence on the absorption of calcium, a part in the formation of pectic substances in the cell wall, and an essential part in carbohydrate and nitrogen metabolism.

As regards the supposed effect of boron on the water relations of the protoplasm, it has already been pointed out that a very general symptom of boron deficiency, and often one of the earliest internal symptoms, is an enlargement of thin-walled cells. In this connexion some observations made by Schmucker (1933, 1935) on the germination of the pollen grains of tropical

water lilies and other species in presence and absence of boron are of interest. He found that when the pollen was placed in a drop of nectar from the flower, germination was normal, but when placed in a drop of sugar solution of the same concentration, the grains either failed to germinate or the pollen tubes produced quickly burst. But the pollen tube remained intact and its growth continued if the sugar solution contained 0·001 or 0·01 per cent of boric acid. From such observations Schmucker not only concluded that boron influenced the absorption of water by the protoplasm but that along with sugar it played some part in the formation of pectin in the cell wall. With regard to the latter supposition we have already noted that one of the most general features of boron deficiency is the breakdown of parenchymatous and other thin cell walls, especially those of the apical meristems in which pectic substances are, by some, supposed to predominate owing to the relative importance of the middle lamella in such cell walls. Other features of boron deficiency, such as discoloration of cell walls and the brittleness of petioles and leaf laminae, might also be held to support this view. On the other hand, Dennis (1937) has pointed out that discoloration of the cell wall is not confined to cases of boron deficiency, and he suggests that the effect of boron deficiency on the cell wall may be part of a more far-reaching effect of this deficiency on carbohydrate metabolism.

The view that boron as boric acid increases the intake of cations and decreases that of anions was put forward by Rehm (1937) as a result of experiments with *Impatiens balsamina* grown in water culture. The cultures were provided either with single salts or complete nutrient solutions with and without boron. The intake of the different ions was determined by analysis of the solution with the results shown in Tables XI and XII.

These data certainly indicate an increase of cation absorption and a relative or absolute decrease of anion absorption as a result of the presence of boron. At present it would seem to be extremely doubtful whether such an effect of boron can be accepted as a general one.

There is, however, much more evidence of a relation between boron and calcium. In a series of water-culture experiments

with *Vicia Faba*, Brenchley and Warington (1927) obtained results that suggested an association of boron with the absorption or utilization of calcium. Cultures without boron and with a very small supply of calcium as calcium sulphate (0·025 g. per litre) made poor growth and developed very small blackened

TABLE XI. Absorption by *Impatiens balsamina* of various ions from solutions of single salts in presence and absence of boron. (Data from Rehm)

Salt	Concentration	Concentration of boron, when present, in mg. per litre	Ratio of uptake of ions in presence and in absence of boron	
			Cation	Anion
K_2SO_4	0·001 *N*	0·5	1·378	1·05
KCl	0·001 *N*	0·5	1·718	1·07
KH_2PO_4	0·002 *N*	0·5	20·82	14·56
KH_2PO_4	0·002 *N*	10	95·00	27·10
$CaCl_2$	0·002 *N*	0·5	1·013	0·789
$MgSO_4$	0·002 *N*	0·5	3·022	1·244
$MgSO_4$	0·002 *N*	10	3·055	1·065
NH_4NO_3	0·002 *N*	1	1·038	0·872

TABLE XII. Absorption by *Impatiens balsamina* of various ions from solutions containing the major plant nutrients with and without boron. (Data from Rehm)

Ion	Ratio of absorption of ion with boron present (0·5 mg. litre) to absorption with boron absent	
	N supplied as $Ca(NO)_2$	*N* supplied as NH_4NO_3
NH_4	—	1·61
K	1·215	4·46
Ca	1·24	1·09
Mg	1·045	1·03
NO_3	1·834	1·05
Cl	0·788	1·127
SO_4	0·721	1·002
H_2PO_4	0·74	0·17

leaves, finally turning black at the stem apices and withering backwards from these. It will be noted that these are the symptoms of calcium shortage and not of boron deficiency (see p. 80). The blackening is attributed to the toxic effect of other nutrient salts, a toxicity which is normally counteracted by calcium, and the plants probably died before even the calcium in the seed was used up. In cultures containing the same supply

of calcium, but also containing boric acid, development was very much better, and, although there was some blackening of the leaves, the plants grew fairly tall, the stems did not blacken and normal flower buds developed. Brenchley and Warington interpreted these results as indicating that without boron the plants were unable to absorb or utilize sufficient calcium to prevent poisoning by the other nutrient salts, while when boron is present the latter enables the plant either to absorb calcium more rapidly or to utilize it more readily so that the toxic effect of the other nutrients is antagonized. It may be noted that in cultures without boron, as the supply of calcium is increased the symptoms of calcium shortage become less marked until with 0·1 g. of calcium sulphate per litre they disappear and the plants show typical symptoms of boron deficiency.

Later, Warington (1934), by actual determination of the amount of calcium absorbed by plants growing in culture solutions, showed that the presence of boron does indeed result in a very considerable increase in the amount of calcium absorbed by plants of *Vicia Faba*. The actual values she obtained are summarized in Table XIII.

Results comparable with those of Miss Warington have been obtained with soya bean by Minarik and Shive (1939). Plants were grown in sand cultures supplied with the usual major nutrients and manganese. Boron was supplied to the different cultures in concentrations varying from 0 to 10 p.p.m. As the results summarized in Table XIV show, the boron supply definitely influenced the amount of calcium which accumulated in the leaves, and that indeed the effect of boron on the growth of the plants was parallel with its effect on calcium uptake.

But this effect of boron in influencing calcium intake does not appear to be general. Indeed, Holley and Dulin (1937), working with cotton, could find no indication of a relationship between boron and calcium, and Morris (1938) found no difference in calcium content in normal and boron-deficient oranges, while Talibli (1935) actually found that addition of boron to the medium on which flax was growing brought about a reduction in the calcium content of the flax straw.

Work by Marsh and Shive (1941) on maize appeared to throw some light on the apparent divergence between the results

TABLE XIII. Absorption of calcium by *Vicia Faba* from nutrient solutions with and without boron. (Data from Warington)

Age of plant in weeks	Total calcium absorbed mg. per plant	
	With boron	Without boron
1930 series: Solutions not renewed		
2	2·2	2·6
3	8·3	5·1
4	15·1	7·1
5	25·1	6·6
1931 series: Solutions not renewed		
2	2·2	2·2
3	6·2	3·5
4	13·8	5·5
5	17·1	6·0
Solutions renewed fortnightly		
5	17·8	10·1
9	50·9	15·6
Solution renewed weekly		
9	54·83	26·65

TABLE XIV. Absorption of calcium by *Glycine hispida* from media containing various amounts of boron. (Data from Minarik and Shive)

Concentration of boron in medium p.p.m.	Average fresh weight of leaves per plant g.	Calcium in leaves mg. per g. of fresh weight
0·0	4·7	2·6
0·001	11·0	2·5
0·0025	15·8	2·6
0·005	16·2	2·9
0·010	24·0	3·0
0·025	32·8	3·5
0·05	38·7	4·5
0·1	32·6	4·0
0·25	28·4	3·9
0·5	28·1	3·4
1·0	31·5	3·5
2·5	13·6	4·2
5·0	12·8	3·6
10·0	0·8	2·0

obtained up to that time with these various species. Plants were grown in sand cultures and for the first week were supplied with a nutrient solution containing all necessary elements except boron, this being omitted so that any in the seed or in external sources should be exhausted. During the second week all the

cultures were supplied with a complete culture solution which included 0·25 p.p.m. of boron as boric acid. From the beginning of the third week, two series of cultures receiving no calcium and four supplied with a nutrient solution containing 170 p.p.m. of calcium, received different amounts of boron. These treatments were continued for 10 days, at the end of which time the dry weights of the shoots, the total calcium and boron content and the contents of soluble calcium and soluble boron of the shoots were determined. The various treatments and the results obtained from them are indicated in Table XV.

TABLE XV. Calcium and boron content of maize supplied with different amounts of boron. (Data from Marsh and Shive)

Concentration of boron supplied p.p.m.	Dry weight of shoot per plant g.	Total Ca per g. of dry matter mg.	Soluble Ca per g. of dry matter mg.	Total B per g. of dry matter mg.	Soluble B per g. of dry matter mg.
No calcium supplied in nutrient solution					
0·0	1·50	3·0	0·3	0·001	0·0005
0·25	2·60	3·0	1·0	0·008	0·0069
170 p.p.m. calcium supplied in nutrient solution					
0·0	2·85	7·6	2·1	0·002	0·0015
0·1	5·40	7·7	2·4	0·005	0·0042
0·25	5·30	8·0	2·8	0·008	0·0070
5·0	4·37	7·7	4·2	0·025	0·0232

Inspection of these results shows that the *total* calcium content of the shoots is independent of the amount of boron supplied; on the other hand, the *soluble* calcium content runs parallel with both the soluble boron content and the total boron content of the plant and also with the boron content of the medium. It is therefore concluded that the soluble calcium content is determined by the boron content, a large proportion of which is in a soluble form, and which is itself determined by the boron content of the medium.

There is thus, according to Shive (1941), a difference between dicotyledons as exemplified by *Vicia Faba* (and presumably *Glycine hispida*) and monocotyledons as exemplified by *Zea mais*, in that in the former the calcium and boron contents are generally much higher than in the latter, but in the dicotyledons studied only a small fraction of the boron is soluble, whereas in

monocotyledons practically all the boron remains in solution. In both groups the soluble calcium is directly related to the soluble boron which is itself determined by the total boron, which in its turn is determined by the concentration of the boron in the medium. This much smaller proportion of soluble boron in dicotyledons explains why the boron requirement of these plants is so much higher (5–10 times) than that of monocotyledons.

That this explanation is not of general applicability, however, appears from recent work on tomato by Reeve and Shive (1944), who investigated the relations of boron to potassium and calcium in this species. In their experiments on the relation of boron to potassium twenty cultures, each containing three plants, were grown in sand cultures which received a nutrient solution supplied in a continuous flow. Five different potassium concentrations were used, namely, 10, 50, 89, 250 and 500 p.p.m., there being thus four cultures receiving potassium in each one of these concentrations. The four cultures at each potassium level respectively received boron in the concentrations 0·001, 0·1, 0·5 and 5·0 p.p.m. The nutrient solution contained iron, manganese and zinc in addition to the major nutrients.

It was found that symptoms of boron deficiency appeared first in the culture supplied with 0·001 p.p.m. of boron and 500 p.p.m. of potassium, and last in the culture supplied with 0·001 p.p.m. of boron and 10 p.p.m. of potassium and that, in general, the severity of the symptoms of boron deficiency increased with increase in the potassium concentration. No symptoms, either of boron deficiency or excess, were observed in any of the cultures receiving the intermediate concentrations of boron (0·1 and 0·5 p.p.m.), but all cultures receiving 5 p.p.m. of boron developed symptoms of boron toxicity. With these, the severity of the symptoms increased with increase in potassium concentration. Thus, increasing potassium concentration brings about a progressive increase in the severity of the symptoms of boron deficiency in low boron concentrations and of the symptoms of boron excess in high boron concentrations.

Analysis of the plants showed that at each level of supplied boron the amount of both soluble and total boron in the tissues increases with increase in the potassium concentration.

This explains the accentuation at the high boron level of the symptoms of boron toxicity with increasing potassium concentration, but does not explain why the boron deficiency symptoms at the low boron level should be accentuated with increase in the potassium supply.

In their experiments on the relation of calcium to boron thirty cultures were used in which six levels of calcium supply (5, 10, 50, 100, 250 and 500 p.p.m.) and five of boron (0·001, 0·01, 0·5, 5·0 and 10·0 p.p.m.) were employed. The results show that calcium acts similarly to potassium in that with the lowest boron concentration (0·001 p.p.m.) the severity of the symptoms of boron deficiency increases with increase of the supply of calcium, the effect of the latter in this respect being, indeed, greater than that of potassium. In its influence on boron toxicity produced by the highest boron concentration (10 p.p.m.), however, calcium acts in exactly the opposite way to potassium, for with progressively increasing calcium supply the severity of the symptoms of boron toxicity becomes less. Chemical analyses of the plants show that with the lower concentrations of boron in the nutrient solution (0·001, 0·01 and 0·5 p.p.m.) the boron content (both total and soluble) is independent of the calcium concentration of the nutrient solution, but with the higher concentrations of boron in the nutrient solution (5·0 and 10·0 p.p.m.) increase in the concentration of calcium brought about a decrease of both total and soluble boron in the plants. This accounts for the effect of increasing concentration of calcium in reducing the toxicity of boron when supplied in high concentration. It may be noted that, in contrast to the earlier findings of Miss Warington with broad bean and of Minarik and Shive with soya bean, boron appears to have no effect on the absorption of calcium by tomato, and there does not appear to be any relation even between soluble boron and soluble calcium such as Marsh and Shive found in maize. However, the ratio of calcium to boron in the plant is influenced by the supply of potassium, increase of this cation in the nutrient solution bringing about a lowering of the calcium/boron ratio. Calcium appears to have no significant influence on the potassium/boron ratio.

The results obtained by Reeve and Shive are in harmony with the well-known fact that heavy liming of certain soils will

induce boron deficiency in a number of crop plants such as beet and swede. It will also be observed that if a soil contains sufficient boron to induce toxicity symptoms, heavy liming should reduce the severity of these. This was found to be so with oats by Jones and Scarseth (1944). These workers grew lucerne, oats and tobacco in pots of limed and unlimed soils to which various quantities of borax were added. The calcium and boron in the plants were determined. As a result the conclusion was drawn that a plant will only make normal growth when there is a certain balance between the intake of calcium and that of boron. From a consideration of their own data and those obtained by Cook and Miller (1939) for sugar beet, Muhr (1940) for soya bean and Drake, Sieling and Scarseth (1941) for tobacco, they come to the conclusion that the ideal balance for these various plants is attained when the ratios (in equivalents) of calcium to boron in the respective plants are 100 for sugar beet, 500 for soya bean and 1200 for tobacco.

While, then, there is a quite considerable amount of evidence of a relationship between boron and calcium, and also between boron and potassium, in plant nutrition, the results obtained with different species are so different that no generalization as to the nature of this relationship appears possible at present.

Microchemical tests made by Marsh and Shive on the apical meristem of maize plants supplied with different quantities of boron suggested a relation between boron and the pectin and fat contents of these tissues. Thus with a supply of 170 p.p.m. of calcium without boron tests for pectin in the cytoplasm were positive and for fats negative, but with 5 p.p.m. of boron tests for pectin in the cytoplasm were negative and for fats positive. With an intermediate supply of boron (0·1 and 0·25 p.p.m.) which was found optimal for growth (cf. Table XV) tests for both pectin and fat were positive. It is suggested therefore that boron plays some part in carbohydrate and fat metabolism.

Swanback (1939), to whose work on absorption of cations by tobacco reference has already been made, concluded from analyses of tobacco plants supplied with calcium and potassium at different levels with and without boron, that the latter element aids the absorption and utilization of calcium.

That boron is connected with carbohydrate metabolism was

indicated in the work of Johnston and Dore (1929), who found that in tomato plants suffering from boron deficiency there was a marked accumulation of sugars in the leaves and a corresponding reduction of the sugar content of the stems, indicating some considerable reduction below normal in the translocation of carbohydrates.

The work of Wadleigh and Shive (1939) on cotton seedlings is also important in this connexion. They examined the effects of boron deficiency in the seedlings by means of microchemical tests. For this purpose seedlings were grown in water cultures with and without a supply of boron. The first internal symptom observed was the increased acidity of a few cells scattered through the pith and cortex, the *p*H of these cells being from 3·8 to 4·4 as compared with the normal value of 5·8 to 6·4. As boron deficiency increased so did the number of these abnormally acid cells, which then also appeared in the pericycle and the older xylem parenchyma. When the majority of the cells of the pith had become very acid their cell walls began to break down, at the same time developing a deep brown colour. Next, some of the cells of the phloem and younger xylem parenchyma developed high acidity and ultimately a breakdown of cells occurred in these regions also.

While these changes were proceeding accumulation of sugars was observed in the boron-deficient plants, while starch was abnormally abundant in the endodermis. In the cells of the stem apices the nitrate-nitrogen content was much lower in boron-deficient than in normal plants. This was attributed to failure of nitrate absorption owing to death of the root apices. There was a very marked accumulation of ammonia nitrogen in the cells which develop high acidity. Wadleigh and Shive conclude from the fact that both sugars and ammonia nitrogen accumulate in boron-deficient plants that boron deficiency brings about a decreased rate of oxidation of sugars, and of amination of carbohydrate derivatives so that protein substances necessary for maintenance of protoplasm are not formed. Microchemical tests for proteins supported this view, for Millon's reagent, the xanthoproteic test and the biuret test all gave immediate results with the abnormally acid cells of boron-deficient plants, whereas in normal plants pre-treatment with

ether and alcohol was necessary to denature the proteins before these tests gave a positive result, thus indicating the degeneration of the proteins of the cells with high acidity and ammonia nitrogen content. The disturbance in the carbohydrate and nitrogen metabolism of the boron-deficient plants may be attributed to a disturbance of the normal oxidation-reduction relations of the cells, and it has been pointed out by Johnston and Dore (1929) and Eaton (1935) that boron has considerable affinity for the hydroxy groups of polyhydric alcohols.

Heggeness (1942) has recently suggested that boron may play a considerable part in protecting flax from attack by the rust *Melampsora Lini*. Borax was applied to the soil at the rate of 60 lb. per acre, 2 weeks after germination of the flax, which was sown on 9 May 1941. Some 10 weeks later plants on control plots which did not receive boron showed 100 per cent infection, while plants on the boron-treated plots showed very little infection in the field. When leaves from boron-treated plants were cleared with 80 per cent alcohol many points of infection could indeed be seen, but they mostly failed to develop. With a sowing later in the year, made on 16 June, the boron-treated plants were not so free from rust, but were yet very much freer from rust than the controls.

CHAPTER V

TRACE ELEMENTS IN ANIMALS

THE study of trace elements in animals has not proceeded so far as with plants, but analysis has shown that animals, no less than plants, may contain small amounts of many elements not generally regarded as essential. The well-established indispensable elements are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, sodium, potassium, magnesium, calcium, iron and chlorine. Fox and Ramage (1931),[1] by spectrographic examination of forty-three whole animals, separated tissues of eighteen others and blood of three more, found that in addition to the above-named essential elements copper was universally present. Manganese was also found to be widely distributed. Cobalt and nickel were also found to be present in a number of species, nickel being met with more frequently than cobalt, but the incidence of both appeared to be irregular. Lithium and strontium were also found in many of the species examined, while lead, silver, and rubidium were found in several. Cadmium and calcium fluoride were each found in one instance only.

The flame method of spectrographic analysis used by Fox and Ramage (cf. p. 28) is not applicable for the detection, and still less for the estimation, of a number of elements, including aluminium, arsenic, boron, antimony, bismuth, gold, molybdenum, tin, titanium, uranium, vanadium and zinc, all of which had earlier been recorded as occurring in at least one animal. In a later spectrographic examination of marine invertebrates made by Webb (1937) the silver and carbon arcs were used, by means of which these elements are detectable in small amounts. Twenty-one marine animals, as well as three algae, were examined; the animals included representatives of Gastropoda, Lamellibranchiata, Echinodermata, Pisces, Urochordata, Crustacea, Nemertea and Polychaeta.

As far as those elements which are also detectable by the flame

[1] References to earlier literature on trace elements in animals are to be found in this paper, and in that by Webb referred to below.

method are concerned Webb's results are in general agreement with those of Fox and Ramage. Copper and strontium were found in every animal examined, while manganese was detected in all except two of the species examined. Lithium was found to be widely distributed, although Webb was of the opinion that the concentration of this in the tissue fluids is never higher than it is in sea water. Cobalt was found in only one species, the gastropod *Pleurobranchus plumula*, while nickel was definitely found only in this and one other species, also a gastropod. It should be pointed out that neither cobalt nor nickel has been detected in sea water so that the concentration of these elements in the environment must be exceedingly low. Silver, lead and cadmium were found to be fairly widely distributed and the same was found for barium, which Fox and Ramage had been unable to detect in any of the specimens they examined. This, and the greater incidence of cadmium as established by Webb, may be due to the greater sensitivity of the arc method employed by the latter as compared with the flame method of Ramage.

As regards elements not detectable in small quantities by the flame method a measurable amount of boron was found in every species except two, while aluminium was detected in nine out of twenty species examined. Silicon was found in a number of species, but Webb concluded that apart from those in which there is a siliceous skeleton there is little tendency for the accumulation of this element. Chromium was found in four species, but unfortunately the data obtained for molybdenum by Webb are regarded as of uncertain value on account of the possibility of its presence in the electrodes used. Zinc in comparatively large quantities was found in some species, though in others this element was not detectable, while tin was found in small amount in several of the species examined. Fluorine in quantity was found in two gastropods. Vanadium is of interest because it is known to occur as a pigment in the blood of the ascidians (Henze, 1911). It appears, however, to be generally absent in recognizable amount from animals of other groups, the only example of an animal other than an ascidian in which Webb found it being the mollusc *Pleurobranchus plumula*. Finally, there is a group of elements for which Webb searched but which

were never found; these were antimony, arsenic, beryllium, germanium, gold, mercury and titanium. It will be observed that the spectrographic methods will not allow the detection of chlorine, bromine and iodine.

It thus appears that in addition to the generally recognized indispensable elements a large number of others have been found in animals. Of these copper would appear to be a constant constituent of animals while almost all animals examined contain manganese in significant amount. There is no indication that any other of the spectrographically recognizable trace elements are universally present although some of them may occur in relatively considerable amounts in a limited number of species. Of the few elements not recognizable by the spectrograph iodine is known to be widely distributed. Keilin and Mann (1940) state that 'it is now well established that zinc is a true and general microconstituent of living organisms'. In view of Webb's work, this statement would appear to be somewhat premature, though it may well be that the view of the universal presence of zinc in both plants and animals will ultimately prove to be correct, and the essentiality of zinc for mammals appears to be well established.

But as with plants, so with animals, the presence of an element in the organism is no evidence of its necessity. Thus although Webb found that lithium, boron, strontium, aluminium and silver were frequently present, he emphasized that there never appeared to be any appreciable accumulation of these elements from the environment. Even accumulation is no evidence of essentiality, which can only be proved by observation of the deleterious effects produced by the exclusion of the element from the animal's diet and the recovery of the animal from the ill-effects of deficiency when a supply of the element is restored.

Although the investigation of trace-element effects in animals has not been carried nearly as far as with plants, there are nevertheless a few trace elements which are well-established as essential for certain groups of animals. The best known of these are perhaps copper and iodine, the former of which is known to be needed for the utilization of iron in haemoglobin formation, while iodine is essential for the functioning of the

thyroid gland in higher animals. Cobalt is now known to be essential for sheep and cattle, for without a sufficiency of this element a serious disease which may prove fatal develops. Its wide distribution in animals suggests that manganese may be an essential element for animals as well as for plants, and there is evidence that a deficiency of it is associated with a disease of fowls known as perosis characterized by deformity of the leg bones. The essentiality of vanadium for the blood of ascidians has already been mentioned, but of the other elements, although a number of them may be constantly present in the bodies of man and the higher animals, nothing really definite is known regarding their indispensability.

Practically any trace element may produce poisoning if administered or presented to the animal in too great excess. Two diseases of animals, the causes of which have for long been obscure, have in recent years been tracked down to the effects of excess of two unexpected sources. One of these is the so-called 'alkali disease' affecting livestock in a number of the northern United States, the other is the scouring of cattle, and to a less degree of sheep, which occurs in England on certain land known as 'teart' in Somerset and a small area of Warwickshire and Worcestershire. The former disease has now been shown to be due to an excess of selenium, the latter to an excess of molybdenum, in the pasture plants growing, and consumed by the animals grazing, on the lands in which the two diseases are respectively incident.

In the following pages accounts will first be given of these two diseases due to trace-element excess, after which the trace-element deficiency diseases will be considered.

1. Diseases due to Trace-Element Excess

Alkali Disease (Selenium Poisoning). For many years a disease affecting horses, cattle, pigs and poultry has been known to occur in certain areas of the great plains of the Middle Western United States. The symptoms of the disease include loss of hair from the mane and tail of horses, from the switch of cattle and from the body of pigs, and a marked change in the growth of the horn of the hoof in all these animals which, when

severe, results in the sloughing of the hoof. General loss of condition involving emaciation and listlessness may follow, and in the worst cases the animals may die. There may be lesions in various internal organs, including the heart, liver, spleen and kidneys. With affected poultry eggs do not hatch. Further, the symptoms can develop in animals in non-affected areas when fed with hay or grain produced in the affected areas. Because of the popular opinion that these effects were due to alkali in the water drunk by the animals, the disease was generally known as 'alkali disease', but more than 30 years ago careful work by Larsen, White and Bailey (1912, 1913) showed that this conclusion was false and the cause of the disease must be sought elsewhere. That this was to be found in poisoning by selenium appears to have been first suggested in 1931 (see Byers, 1934). First investigations showed that selenium was present in the soils of the affected areas and that wheat grain from such areas might contain from 10 to 12 p.p.m. of selenium (Robinson, 1933). Nelson, Hurd-Karrer and Robinson (1933) found that wheat appeared to grow normally on soil to which 1 p.p.m. of selenium had been added in the form of sodium selenate, but the grain from this wheat was toxic to white rats fed with it, retardation of growth, and finally death, resulting. Schoening (1936) developed the typical symptoms of alkali disease in hogs by feeding them with maize grown in the affected area. Two lots of maize were used, one containing 5 p.p.m. the other 10 p.p.m. of selenium.

Further experiments on dosing animals with selenium leave no doubt that this element is responsible for producing the symptoms of alkali disease, and that this is, in fact, selenium poisoning. The animals used include rats (Franke and Moxon, 1936), rabbits (Smith, Stohlman and Lillie, 1937), swine (Miller and Schoening, 1938; Miller and Williams, 1940*a*), cattle, horses and mules (Miller and Williams, 1940*a*). As an example of the type of experiment employed may be cited one by Miller and Schoening on swine. In this eight pigs about 4 months old were separated into four pairs. All received a sufficient ration of grain, but the four groups received different amounts of selenium, the proportions of this, as sodium selenite in the ration, being respectively 392, 196, 49 and 24·5 p.p.m. Four of the animals

developed typical symptoms of alkali disease, namely, loss of hair and disturbance of the growth of the hoof, and all died in from 10 to 99 days. Post-mortem examination revealed lesions of internal organs, particularly of the liver, while kidneys, heart and spleen were also affected in some cases. Two control animals fed with similar grain without added selenium developed normally.

The minimum lethal dose of selenium for rats, rabbits, horses and mules appears to be of the order of 1·5 or 2 mg. per lb. of body weight, for cattle 4·5–5 mg. per lb. of body weight, and for pigs between 6 and 8 mg. per lb. of body weight.

By the continued administration of small doses of selenium to horses Miller and Williams (1940*b*) produced symptoms similar to, though not so striking as, those observed in chronic alkali disease under natural conditions. These symptoms included listlessness, loosening of hair in the mane and tail, softening of the horny wall of the hoof, and lesions in the liver, heart, kidneys and spleen.

It being thus clear that 'alkali disease' results from poisoning by grain or forage containing selenium, it is important to know whether there are differences in the selenium-absorbing capacity of different plants. There is no doubt that this is very much the case. Byers (1935) found notable differences in the selenium content of plants occurring naturally on seleniferous soils, while Hurd-Karrer (1935) also found marked differences in the selenium content of crop plants grown on artificially selenized soils. Thus Miller and Byers (1937) record a selenium content of 1110 p.p.m. in *Astragalus bisulcatus* and one of only 45 p.p.m. in wheat grown in the same area. In another area they found 1250 p.p.m. of selenium in *A. bisulcatus*, but only 3 p.p.m. in *A. missouriensis*. Byers had earlier found as much as 4300 p.p.m. of selenium in *A. bisulcatus*, while relatively enormous quantities of selenium have also been found in other species of *Astragalus*, as, for example, 5560 p.p.m. in *A. ramosus* and 1750 p.p.m. in *A. pectinatus*. On the other hand, *A. missouriensis*, *A. mollissimus* and *A. drummondii* absorb only little selenium. Prairie grasses in general have a very low capacity for absorbing selenium; thus *Andropogon scoparius* (little blue-stem) was found to contain only 0·8 p.p.m. of selenium.

Miller and Byers (1937) distinguish three classes of plants in regard to their capacity for absorbing selenium: (1) highly seleniferous plants which absorb selenium readily and which are either absent from or rare in neighbouring non-seleniferous areas. Plants of this group include *Astragalus bisulcatus*, *A. racemosus*, *A. pectinatus*, *A. carolineanus*, *Stanleya pinnata*, *S. bipinnata*, *Applopappus fremonti*, *Xylorrhiza parryi*; (2) plants capable of absorbing selenium, even in considerable amount, without severe injury, but which are widely distributed on both seleniferous and non-seleniferous soils. This group includes *Aster ericoides* (white wreath aster), *A. fendleri* (blue aster), *Gutierrezia sarothrae* (turpentine weed), *Helianthus annuus* (sunflower), *Agropyron smithii* (western wheat-grass) and the common cereals, wheat, rye, barley and maize; (3) plants with a low tolerance for selenium, which make poor growth at best on seleniferous soils and which absorb only small amounts of selenium. Plants in this group include *Bouteloua gracilis* (buffalo grass) and *B. curtipendula* (grama grass).

For the control of selenium poisoning the relation of selenium absorption to sulphur absorption by plants may be of great significance. Hurd-Karrer (1934, 1935) showed that increasing the sulphur content of the soil brought about a reduction in the quantity of selenium absorbed by plants. Similarly, with plants grown in water culture selenium uptake was reduced by increasing the concentration of sulphate in the nutrient solution. Hurd-Karrer and Kennedy (1936) found that grain from wheat grown on soil containing 2 p.p.m. of selenium was toxic to white rats, whereas grain from wheat grown on similar selenized soil treated with flowers of sulphur or gypsum was not toxic, the selenium content of the grain being reduced by such treatment from about 12 p.p.m. to about 4 p.p.m.

In 1937 Hurd-Karrer reported the results of experiments on the uptake of sulphur and selenium by some fifteen different crop plants grown in the greenhouse on soil artificially selenized by the addition of 4 p.p.m. of selenium as sodium selenate. The plants were known to differ widely with regard to their capacity for absorbing sulphur. The shoots were cut when 1, 2 and 3 months old and the sulphur and selenium determined. The results obtained after the first month are shown in Table XVI.

Inspection of this shows that although the species used exhibit a wide range in sulphur and selenium uptake, the ratio of sulphur to selenium absorbed is roughly the same in all.

TABLE XVI. Content of sulphur and selenium of a number of crop plants one month old. (From Hurd-Karrer)

Species	Sulphur per cent of dry weight	Selenium per cent of dry weight
Brassica oleracea capitata (cabbage)	3·37	0·0793
B. oleracea botrytis (cauliflower)	2·88	0·0710
B. nigra (black mustard)	2·78	0·0744
B. napus (rape)	2·39	0·0780
B. oleracea acephala (kale)	2·24	0·0615
Linum usitatissimum (flax)	1·28	0·0474
Helianthus annuus (sunflower)	1·32	0·0310
Trifolium pratense (red clover)	1·21	0·0315
Vicia villosa (vetch)	0·92	0·0280
Lactuca sativa (lettuce)	0·99	0·0195
Triticum sativum (wheat)	0·87	0·0240
Secale cereale (rye)	0·80	0·0280
Glycine hispida (Soya bean)	0·59	0·0130
Zea mais (maize)	0·42	0·0140
Sorghum vulgare	0·48	0·0130

Hurd-Karrer also found that the sulphur/selenium ratio was practically the same for stems and leaves and also that the ratio depended on the relative amounts of sulphur and selenium in the soil. If the former remained the same while the selenium content was varied, the sulphur/selenium ratio in the plant rose with a fall in the selenium content of the soil. Thus, with a sulphur content of the soil of 0·06 per cent, the average ratio of sulphur to selenium in the plants was found to be 114 when the selenium in the soil was 3 p.p.m., but when this latter was increased to 5 p.p.m. the sulphur/selenium ratio in the plants was only 22.

These results are readily understandable in view of the chemical similarity of sulphur and selenium. They indicate very definitely a way of reducing the selenium content of plants growing on seleniferous soils.

For a good detailed account of the earlier work on selenium poisoning reference may be made to a review by Trelease and Martin (1936).

The Scouring of Cattle on the Teart Lands of Somerset. On certain soils derived from the Lower Lias, where this is

directly exposed and not covered by drift or boulder clay, there are pastures which bring about the complaint of cattle known as scouring. Cattle, on being turned into these pastures, develop the symptoms of scouring within a few days. The symptoms are described by Ferguson, Lewis and Watson (1943) as follows: 'The dung becomes extremely loose and watery, yellowish green in colour, bubbly, and has a foul smell. The animals become filthy, their coats stare and they lose condition rapidly. Red Devon cattle turn a dirty yellow and black beasts go rusty in colour. Milk yields drop considerably.' Sheep are not affected to the same extent, but the dung is very soft and the fleeces sometimes become stained.

The principal area in which teart soils occur is in central Somerset, but smaller areas occur in Warwickshire and Gloucestershire. It is to be noted that an area in Glamorgan in which the soil is also derived from the Lower Lias does not exhibit teartness.

The effect of teart land on cattle has been known, according to Ferguson, Lewis and Watson, for over a hundred years, and has been attributed to a number of factors, including bacteria, parasites, particular species in the herbage, water supply, poor drainage, soil texture, a high proportion of non-protein nitrogen in the herbage, and some particular chemical constituent present in the herbage (Muir, 1936). Bacteria cannot be the primary cause of scouring because the symptoms cease directly the cattle are removed from teart pastures, while as regards parasites there is no abnormally high number of these in affected animals. Nor do the water supply, drainage and soil texture of teart pastures show constant and characteristic differences from non-teart areas. Ferguson, Lewis and Watson determined the nitrogen fractions in herbage from teart and non-teart areas but found no difference in the non-protein nitrogen of the two.

These workers were thus led to investigate the last of the suggestions listed above and made a spectrographic examination of a number of samples of herbage from different sources. As a result they found one constant difference between the samples from teart and from non-teart pastures, namely, in the content of molybdenum, which was considerably higher in the case of the teart herbage. Thus the mean molybdenum content

of the herbage from twelve teart localities was 33 p.p.m. of dry matter in 1937 and 38 p.p.m. of dry matter in 1938; the corresponding mean values for eleven non-teart localities were 5 and 4 p.p.m. for the respective years. This finding strongly suggested that molybdenum might be the cause of scouring, and this was confirmed by administering doses of ammonium or sodium molybdate to cattle when the symptoms of scouring were produced in a number of cases, although there was considerable variation in the degree to which different individual animals reacted to molybdenum. Scouring was also produced in cattle and sheep turned into non-teart pasture dressed with sodium molybdate.

Hay and frosted herbage do not have the same effect as the fresh material in inducing scouring. This appears to be due to the fact that fresh herbage contains a much higher proportion of the molybdenum in soluble form than does hay or frosted material, and it would thus appear that it is the soluble fraction of the molybdenum that is responsible for the effect on cattle.

It has been mentioned that the teart soils occur on the Lower Lias where this is directly exposed, but that such an area in Glamorgan does not exhibit the properties of teart land. Lewis (1943*a*) has examined a number of Lower Lias soils in their relation to teartness and finds that the teart soils contain about 20–100 p.p.m. of molybdenum in the surface horizon and are neutral or alkaline in reaction. The soils of the Glamorgan area, however, were found to contain only about 2–4 p.p.m. of molybdenum, which explains why they are not teart in character.

Very interesting are the results of experiments made by Lewis (1943*b*) to examine the uptake of molybdenum from teart soil by plants of a number of different pasture species. Ten pasture grasses and two species of clover were used in these experiments, the seeds being sown in pots of teart soil, four pots of each species being used. The herbage was cut when it was about 3–4 in. high and the molybdenum content determined. After a further period of growth it was again cut and the molybdenum content in the new sample determined. This procedure was repeated so that four samples were obtained in all for each species. The results are summarized in Table XVII.

TABLE XVII. Uptake of molybdenum by pasture grasses and clovers. (Data from Lewis)

Species	Molybdenum content in p.p.m. dry matter			
	May–June 1939	July–Aug. 1939	Sept. 1939	May–June 1940
Holcus lanatus (Yorkshire fog)	36	83	61	42
Agrostis alba (fiorin)	14	10	10	9
Phleum pratense (timothy)	8	8	4	6
Festuca pratensis (meadow fescue)	15	10	6	9
Dactylis glomerata (cocksfoot)	13	17	15	9
Poa trivialis (rough-stalked meadow grass)	12	18	15	11
P. pratensis (smooth-stalked meadow grass)	8	6	6	8
Cynosurus cristatus (crested dogstail)	10	8	5	9
Lolium perenne (indigenous perennial ryegrass)	11	11	11	11
L. italicum (Italian ryegrass)	12	13	11	10
Trifolium repens (wild white clover)	93	109	69	57
T. pratense (wild red clover)	87	103	62	59

These results show very clearly that the clovers and *Holcus lanatus* contain a much higher proportion of molybdenum than the other pasture plants examined. Teart pastures generally contain very little *Holcus*, but often a good deal of clover. Lewis is of the opinion that it cannot be assumed that the clovers are the only cause of teartness because analyses made of a number of grasses other than *Holcus* growing on teart land show that these can contain a much higher proportion of molybdenum than was found in the plants grown in pots (cf. Table XVIII).

TABLE XVIII. Molybdenum content in p.p.m. of dry matter of pasture plants growing on teart land. (Data from Lewis)

Species	Soil type		
	Hambridge	Evesham	Haselor
Dactylis glomerata (cocksfoot)	70	42	13
Poa pratensis (smooth-stalked meadow grass)	54	39	18
Lolium perenne (perennial ryegrass)	54	36	11
Cynosurus cristatus (crested dogstail)	60	36	—
Bromus mollis (soft brome)	60	11	18
Agrostis spp. (bent)	54	41	—
Phleum pratense (timothy)	—	30	8
Festuca spp. (fescues)	—	—	20
Arrhenatherum avenaceum (tall oat grass)	—	—	12
Trifolium spp. (clovers)	90	156	28

Ferguson, Lewis and Watson found that the scouring of cattle and sheep resulting from the absorption of molybdenum can be prevented and cured by dosing the animals with copper sulphate. On very teart land a daily dose of 2 g. of this salt for cows and 1 g. for young stock was found to be adequate for the purpose, while on mildly teart land a smaller dose would probably be sufficient. The mode of action of the copper is not evident.

Experimenting with six dairy cows, Ferguson (1943) found that giving a daily dose of 2 g. of copper sulphate to each cow for from 10 to 18 weeks produced no ill effects, the cows remaining in perfect health and calving normally. Having regard to the facts that (1) on most farms in teart areas there is a proportion of non-teart land, so that cattle would graze on the teart land for only a portion of their time, and (2) the scouring tendency of the herbage varies with the season, being greatest in early spring and September and less at other times, Ferguson considers that continuous daily dosing with copper sulphate would probably not be necessary for more than about 6 weeks, so that the cows used in the experiment actually received considerably more copper sulphate than would usually be necessary to prevent scouring.

Ways to reduce the teartness of pastures were also examined by Lewis. He found that application of acidic nitrogenous fertilizers containing ammonium sulphate reduced the proportion of molybdenum in the herbage. This was largely due to a reduction in the proportion of clover in the herbage, but the actual proportion of molybdenum in the grasses was also reduced. This might have been due to the ammonium sulphate bringing about an increase in the yield of the grasses without increasing the weight of molybdenum taken up. Also it has been noted that teart soils are neutral or alkaline. Rendering the soil more acidic will reduce the availability of the molybdenum and so induce a lowering in molybdenum absorption by the plants.

Another point observed by Lewis was that the molybdenum content of newly sown grasses is low, but increases with age, although clovers have a high molybdenum content from the beginning. A system of ley farming with short leys and with only a small percentage of clover would thus appear to be very suitable for teart land.

2. Trace-Element Deficiency Diseases of Animals

The Effects of Copper Deficiency. In certain Crustacea, Arachnida and Mollusca copper enters into the composition of a pigment haemocyanine which is concerned in the respiration of these animals.

Mann and Keilin (1938) have isolated two copper protein compounds, one from the blood, the other from the liver of mammals. The former of these, haemocuprein, is a blue compound present in both the red corpuscles and serum, and appears to account for all the copper in the corpuscles. The other copper-protein compound discovered by Mann and Keilin they obtained from ox liver and named hepatocuprein. This has several properties in common with haemocuprein, but is almost colourless. Both these copper-protein compounds contain 0·34 per cent of copper. It may be that these two compounds are intimately connected, but their precise function is not yet known.

The work of Hart, Steenbock, Waddell and Elvehjem (1928) and McHargue, Healy and Hill (1928) first showed that in mammals copper is necessary for the utilization of iron in the formation of haemoglobin. Later work by Waddell and Elvehjem and their collaborators, Cunningham (1931), Keil and Nelson (1931), Josephs (1932) and others has confirmed without doubt the essentiality of copper for haemoglobin formation, although the copper itself does not form part of the haemoglobin molecule as it does that of haemocyanine. Consequently deficiency of copper in mammals may lead to anaemia, and the cure of anaemia traceable to this cause can be effected by dosing with copper sulphate. For a general review of this question a paper by Elvehjem (1935) may be consulted.

A disease of cattle known as 'licking sickness', apparently attributable to copper shortage, has been described by Sjollema (1933) as occurring on farms in Holland where reclamation disease of cereals occurs. Reclamation disease, as we have seen (p. 95), is traceable to copper deficiency, and it was the occurrence of licking sickness of cattle along with reclamation disease that led Sjollema to connect the two.

The external symptoms of licking sickness are anaemia and

loss of appetite with a general degeneration in the condition of the animal. Internally, the proportion of dry matter in the blood is low, being only 13–14 per cent instead of the normal 18–20 per cent, while both the iron content and haemoglobin content are generally very low. From what is now known of the part played by copper in haemoglobin formation these are the symptoms which might be expected to result from a shortage of copper.

That a particular substance is concerned in the disease is indicated by the fact that moving affected animals to land on which reclamation disease does not occur brings about rapid improvement in the animals. That the shortage is not due to iron or manganese, but probably to copper, is indicated by analyses of the hay from farms in which the disease occurs. Such analyses show that there is no constant shortage of either iron or manganese, but that there is consistently an abnormally low content of copper, namely 2–3 p.p.m. and sometimes even less than 1 p.p.m., whereas hay from normal farms contains from 6 to 12 p.p.m.

Support for the attribution of licking sickness to copper deficiency was obtained by Sjollema by dosing affected animals with copper sulphate.

Later Sjollema (1938) described another disease of animals which he also attributed to copper deficiency, both cattle and goats being affected. The chief symptoms of this disease, which occurs only on fine dry sandy soils, are diarrhoea, wasting, and in black cattle, loss of colour in the coat which becomes brown-grey. The disease is apparently not the same as licking sickness.

The evidence adduced by Sjollema in support of copper deficiency as the cause of this second disease is similar to that respecting licking sickness. Thus the copper content of the blood, liver and milk of affected cows and goats was abnormally low, and so was the hair of affected cows. The grass and hay from farms on which the disease occurred were low in their content of both copper and manganese, but the quantity of the latter appeared more than adequate for the needs of the animals. The hay was also poor in zinc, but the blood of affected animals contained a normal amount of this element and the liver a higher content than the normal. As regards iron the content of

this element in diseased animals was abnormally high. Finally, dosing with copper sulphate brought about recovery of the affected animals.

If this latter disease is indeed different from licking sickness, and Sjollema's accounts appear to indicate this, it would appear quite unlikely that deficiency of copper alone could produce two distinct diseases in the same species. The fact that administration of copper sulphate can cure the disease cannot be regarded as sufficient evidence in itself that the disease has its origin in copper deficiency, for, as we have already seen, molybdenum poisoning of stock can be cured by dosing with copper sulphate. Indeed, the symptoms of the second disease described by Sjollema are rather reminiscent of those characteristic of the scouring of cattle, but the soils on which the disease described by Sjollema occurs suggest a deficiency, rather than excess, of some element as the cause. The possibility that the curative effect of copper sulphate might be due to traces of some other essential element present as an impurity in the copper sulphate administered is not to be ruled out. Altogether, the attribution of both licking sickness and the diarrhoea and wasting disease to copper deficiency should perhaps be treated with some reserve until confirmatory evidence is forthcoming and other possibilities eliminated.

A disease of cattle known as 'salt sick' has been known for many years in Florida. This disease, according to Becker, Neal and Shealy (1931), is a nutritional anaemia resulting from an insufficiency of copper and iron in the diet. Bryan and Becker (1935) reported that the disease occurs on certain sandy soils, the surface layers of which possess roughly only one-tenth of the iron content and one-half of the copper content of healthy soils of Florida. They found that cattle become salt sick on soils containing 0·036 per cent of iron and 3·85 p.p.m. of copper, and remain healthy on soils containing 0·42 per cent of iron and 8 p.p.m. of copper. The similarity of the disease and its method of cure with the licking sickness dealt with by Sjollema strongly suggests that the two diseases are the same. Whether, as suggested by Aston (1931) and Greig, Dryerre, Godden, Crichton and Ogg (1933), it is also identical with the disease known as bush sickness in New Zealand (see e.g. Askew and Rigg, 1932),

that known as Nakuruitis in Kenya (Orr and Holmes, 1931) and that called pining in Scotland (Greig *et al.* 1933) is another matter. If this should indeed be so it would mean that salt sick results, not from deficiency of copper, but from deficiency of cobalt. The *United States Department of Agriculture Yearbook for* 1939 records that although surveys were not then complete, observations of cattle indicate a few copper-deficient areas and a considerable cobalt-deficient area in Florida. Thus it may be that both copper deficiency and cobalt deficiency may occur in cattle in that State. The work of Rusoff, Rogers and Gaddum (1937) rather suggests that all the cases of salt sickness may not be due to the same cause. Thus they state that workers at the Florida Agricultural Experiment Station have found that cobalt is a limiting factor in a type of salt sick known as 'hill sick'. It would therefore appear likely that this is identical with pining or bush sickness. Rusoff, Rogers and Gaddum, however, were unable to detect cobalt spectrographically in the grasses of either salt-sick or healthy areas, but it may be, as they point out, that this element, as well as others, may be present in quantities too small to be determined by the method used. But as regards copper, they found the same content of this element in the grasses from salt-sick and normal areas, so that their analyses lend no support to the view that salt sick results from shortage of copper. But, as they point out, it may be that the ratio of various elements is the important factor and that the disease might occur where the iron content of the grass is high and the copper content low, or vice versa, or the ratio of iron or copper to some other element might be the determining condition.

A disease of lambs which may result from shortage of copper occurs widely in Great Britain, but appears to be most troublesome in an area of north Derbyshire, in part of the Cheviot Hills, in certain districts of Yorkshire, and in an area of Gloucestershire and Somersetshire where the Mendip Hills appear to provide the worst cases. The disease also occurs in Australia, while the same or a similar disease has been described as occurring in Sweden, in India and in Peru and other parts of South America. In this country it is known as swayback, swingback or swingleback, and in Australia as enzootic ataxia. In this country it has probably been known to farmers for a very

long time, but the first scientific description of it appears to have been recorded only some 14 years ago (Stewart, 1932). It was recorded for Australia at about the same time (Bennetts, 1932). Dunlop, who has specially studied the disease, thus (1939) described the symptoms: 'In most cases the symptoms are noticed in the lambs at birth. Some lie recumbent swaying their heads and make spasmodic efforts to rise and obtain milk from their dams. Such attempts frequently result in the lamb rolling over on its side, kicking vigorously with the hind legs. Others may be able to stand, but often the hindquarters swing about and eventually fall over drawing the rest of the body to the ground. Attempts to walk are characterized by incoordination of movement, a swaying gait, staggering and finally collapse.' Blindness may sometimes occur. In some cases the symptoms at birth may be slight and develop later. Dunlop states that the mortality of affected lambs is almost 100 per cent, so that adult animals showing symptoms of swayback are rare. However, according to Innes and Shearer (1940) mildly affected animals may survive and when later used for breeding may give birth to healthy lambs. Post-mortem examination of affected animals shows that lesions of the brain occur, the white matter disintegrating leaving cavities filled with a clear fluid or jelly. The degeneration may extend down the spinal cord. These lesions arise while the lamb is still *in utero*.

Investigations show that the disease is not hereditary, nor could any evidence be obtained to suggest that it was due to infection by micro-organisms. For example, no transmission of the disease results from inoculating new-born healthy lambs with extracts from affected tissues. All the facts, including the localization of the disease to certain limited areas, point to the disease being nutritional in origin. The coincidence of the disease in Derbyshire with soils containing lead might suggest that the disease results from lead poisoning, but this opinion has no experimental evidence to support it and is by no means generally held.

Dunlop describes a large-scale experiment carried out in Derbyshire designed to test the theory that swayback is a nutritional disease. In this experiment 1800 ewes distributed over fifty farms were used. Four groups of 300 ewes each, the

groups as equal as possible, were fed with mineral mixtures, made up into licks, the four mixtures containing respectively copper, cobalt, manganese or boron in the same amount. These were made available to the ewes on the various farms early in December, and by lambing time about the beginning of April practically all the licks had been consumed. As a result it was found that of the lambs born from ewes which received minerals with copper 1·34 per cent were affected with swayback as compared with 13·1 per cent of the lambs from ewes which received minerals without copper and 15·2 per cent where no minerals were supplied. While this experiment indicates that the addition of a small amount of copper to the diet of the ewes is effective in preventing swayback, it does not follow that the vegetation on which the ewes normally feed in swayback areas is necessarily deficient in copper. As Dunlop points out, the presence of excess of lead or other minerals may render the copper unavailable and it may not be absorbed in adequate quantity from the gut.

The favourable effect of feeding copper to pregnant ewes had also been demonstrated by Bennetts and Chapman (1937) in Australia and was confirmed in further work by Dunlop, Innes, Shearer and Wells (1939), but although it seems clear that the addition of copper to the diet is an effective preventive of swayback, it would be premature to assume that the disease is actually due to copper deficiency. It has already been pointed out that copper similarly prevents scouring of cattle, which, as we have seen, is due to poisoning by excess of molybdenum. At present therefore we cannot rule out the possibility that swayback may be due to poisoning by lead or some other metal, and that copper has a similar action in preventing the disease as it has in preventing molybdenum poisoning of cattle on teart lands. It may be significant that at least two of the areas in which swayback occurs, north Derbyshire and the Mendips, are areas in which lead occurs in sufficient quantity to be mined. It should also be observed that the symptoms in goats supposed by Sjollema to be suffering from copper deficiency are quite different from those of swayback. One might expect the symptoms produced in two such nearly related species as sheep and goats by deficiency of the same element to be similar.

Again, determinations of the copper content of the blood do not reveal any correlation between copper content of blood and incidence of swayback. Certainly Bennetts and Chapman (1937) in Australia found particularly low values for the copper content of the blood of four ewes that gave birth to affected lambs (less than 0·01 mg. per cent). On the other hand, Innes and Shearer (1940) found that in Derbyshire the average copper content of the blood of ewes giving birth to swayback lambs was 0·058 mg. per cent, while the value with ewes on swayback farms bearing normal lambs was actually somewhat lower, namely 0·045 mg. per cent, but in both groups there was quite a wide range of values, 0·037–0·070 mg. per cent in the swayback group and 0·034–0·061 mg. per cent with the ewes bearing normal lambs. Higher mean values were found for the copper content of the blood of ewes in non-swayback areas, but the range was even wider than in affected areas. There was thus no indication of any close relationship between copper content of the blood of the ewes and the incidence of swayback. This conclusion is supported by the results of the examination by Eden (1939, 1941) of the blood of more than 300 sheep in an area of Northumberland where swayback is unknown. Here very wide variations in copper content were found, the mean value for ninety-four sheep examined in 1940 being 0·080 mg. per cent with a range of 0·013–0·210 mg. per cent. Eden also points out that the pastures in this Northumbrian area have an even lower copper content (6–10 p.p.m. of the dry weight) than those of the swayback area of Derbyshire. It may be concluded that much work requires to be done to put our knowledge of the effects of copper deficiency in animals on a firm basis.[1]

The Effect of Iodine Deficiency. Iodine deficiency is generally regarded as the cause of goitre in man and other mammals. Goitre results from enlargement of the thyroid gland which contains the characteristic protein thyroglobulin, one of the constituent amino-acids of which, namely thyroxine, contains

[1] In a paper which appeared since the above was written Shearer and McDougall (1944) express the opinion that the Australian disease is due to an uncomplicated deficiency of copper in soil and herbage but that in this country this is not so although affected animals suffer from copper deficiency.

iodine in its molecule. In various parts of the world where iodine is deficient in the soil and where, in consequence of this, very little of this element is absorbed by pasture plants, farm animals may be liable to goitre, as, for example, in parts of California and the Middle Western United States. Affected animals are born with necks swollen as the result of enlargement of the thyroid gland and are generally weak or dead. According to Maynard (1937) iodine deficiency in dairy cows can be cured by adding 0·02 per cent to the food given to the animals.

Actually man appears to be more liable to goitre than farm animals, and in this country, where animals are rarely affected, there are areas, particularly in Derbyshire, where the incidence of goitre among the human population is quite high and where special measures in the way of administering iodine have to be taken to combat it.

It should be noted that although there is no doubt of the beneficial effects of iodine in the treatment of goitre doubts have been expressed as to whether iodine deficiency is the sole factor responsible for this condition. It has, for example, been quite widely held that the presence of radioactive material in the soil is also a factor responsible in part for goitre.

Manganese Deficiency in Chicks. Perosis. The very wide distribution of manganese throughout the animal kingdom suggests that this element may be as generally essential to animals as to plants. It is thought that manganese in small quantity is necessary for man although its function in the human body is not known. As a result of experiments with rats it has been suggested that manganese may be an essential catalyst in the utilization of vitamin B_1, since deficiency of manganese in the diet brought about similar changes, for example, loss of maternal instinct and progressive loss of fertility, as excess of vitamin B_1 (Perla, 1939).

The effect of manganese deficiency in bringing about a disease of young chickens known as perosis, hock disease or slipped tendon was first shown by Wilgus, Norris and Heuser in 1936. This disease is characterized by a deformity of the leg bones, and to some extent of the wing bones, of the chick, particularly of the tibio-metatarsal joint. The bones of the leg and wing are shorter than the normal. The relationship of manganese deficiency

to perosis was soon confirmed by Gallup and Norris (1937) and several other groups of workers. Thus Heller and Penquite (1937) found that addition of an aqueous extract of rice bran or of traces of manganese to perosis-induoing diet practically prevents the occurrence of the disease. Insko, Lyons and Martin (1938*a*) also showed that manganese exercised a protective action against perosis, whereas no such action resulted with aluminium or zinc, but rather the reverse. The same workers found (1938*b*) that a minimum quantity of 30 p.p.m. of manganese as manganese sulphate had to be added to an all-mash basal ration containing 6 or 7 p.p.m. of manganese, if growth was to be normal and perosis prevented. Amounts of manganese up to 646 p.p.m. were not toxic. With low contents of manganese they found that up to about 30 p.p.m. of manganese the degree of bowing of the legs of their experimental chicks was inversely proportional to the manganese content of the diet.

Wiese, Elvehjem and Hart (1938) also found both rice bran (15 per cent of the ration) and 0·0025 or 0·005 per cent of manganese as manganese sulphate gave protection against perosis. They found, however, that autoclaving the rice bran removed its protective property, from which they concluded that some thermolabile substance must be involved, perhaps in association with manganese, in preventing perosis. This finding would suggest then that perosis may not be an outcome of simple manganese deficiency, but that some other substance is involved as well.

Gallup and Norris (1938) also showed that a large proportion of the chicks hatched from eggs with a low manganese content have short leg bones, and Lyons and Insko (1937) showed that the embryos which were produced in eggs of hens fed on a manganese-deficient diet were characterized by abnormally short and thick legs and short wings. They found the deformities could be prevented either by adding manganese to the diet of the hen or by injecting manganese into the egg before incubation.

Caskey and Norris (1939) found that excess of calcium and phosphorus in the diet greatly reduced the availability of manganese, and they found that 15·5 mg. of manganese supplied orally over a period of 6 weeks with diets containing 1 per cent of calcium and 0·5 per cent of phosphorus was as effective in reducing perosis as 142 mg. of manganese supplied with 3 per

cent of calcium and 1·5 per cent of phosphorus. With neither diet, however, was perosis completely prevented, but when the manganese was supplied by intraperitoneal injection it was found that 10 mg. completely prevented perosis even at the higher levels of calcium and phosphorus supply.

Gallup and Norris (1938) have recorded the results of X-ray examination of the leg and wing bones of chicks fed on a diet low in manganese (10 p.p.m.), the X-ray photographs being taken at intervals from the time of hatching until the chicks were 7 weeks old. These were compared with similar photographs of chicks fed on a diet containing an adequate supply of manganese (100 p.p.m.). This X-ray examination confirmed the effect of manganese deficiency in producing shortened leg bones. Caskey, Gallup and Norris (1939) found that the ash content of the bones of chicks fed on a diet low in manganese was significantly below the normal. This was not due to a rachitic condition, since the chicks were supplied with ample vitamin D, while X-ray examination and staining with silver nitrate showed that calcification was normal.

Gallup and Norris point out that other investigators have reported measurable amounts of manganese in the bones of rabbits and rats, and they suggest that a small amount of manganese may not only be an essential constituent of bone in the chick, but that it may be essential for the development of bone in general.

Pining, Enzootic Marasmus, Bush Sickness or Morton Mains Disease. In countries as far apart as Scotland and Australia and New Zealand there has occurred for many years a disease of sheep characterized by the rapid deterioration of the animal. In Scotland, where the disease occurs in many parts of the country, it is known as pine or pining, vinquish and daising, and young cattle are also affected. Here the disease was recorded by Hogg, the Ettrick Shepherd, as long ago as 1831. The symptoms as they appear in the island of Tiree, Inner Hebrides, have been described by Greig, Dryerre, Godden, Crichton and Ogg (1933) as 'those of a progressive debility, accompanied by anaemia and emaciation. The onset is frequently insidious. The affected animal is dull, and the coat, in the case of cattle, is rough and staring, the visible mucous membranes, especially

the conjunctiva, become pale: physical condition is gradually lost, the eyeball becomes sunken, and there is commonly a serous discharge from the inner canthus. In young animals growth is markedly retarded, and they soon present a stunted, unthrifty appearance. Thereafter the anaemia and emaciation progress to the condition of cachexia, and, finally, as the result of extreme weakness, the animal is unable to rise. In severe cases the gait is stilted and somewhat incoordinated.' The disease usually ends fatally.

The disease of sheep in North Island, New Zealand, known as bush sickness, that in Southland, New Zealand, known as Morton Mains disease, and that in Australia called enzootic marasmus, all appear to be the same as pine in Scotland. Morton Mains disease in a bad year and in a bad locality was described by Wunsh (1937) thus: 'About midsummer a majority of the season's crop of lambs would fall off in condition. Their wool would become "chalky" and harsh, their eyes would water, and they would lose their activity. A large number—30 per cent or more—would gradually lose weight, become more and more helpless and finally die.'

A consideration of the facts known about Morton Mains disease in New Zealand suggests that it is a deficiency disease. Thus the disease does not appear on newly broken land, but only after several years of sheep farming on the same land. Also deep ploughing lessens the disease for a few years. Both these facts suggest a deficiency, and certainly not an excess, of some substance as the cause of the disease. That the disease is not due to bacteria or other parasites is indicated by the fact that a sick lamb transferred to healthy country recovers rapidly. Since the climate, physical conditions and type of herbage of healthy and affected farms might show no appreciable differences, there is a strong suggestion that some substance in very small amount, such as a trace element, is involved.

In 1935 it was reported by Underwood and Filmer that enzootic marasmus of sheep in Western Australia could be cured by administering a dose of 0·1–2 mg. of cobalt nitrate each day, and 2 years later it was reported that affected cattle could be cured in the same way (Filmer and Underwood, 1937). In the meantime Askew and Dixon (1936) had reported a similar

favourable effect of cobalt on sheep suffering from Morton Mains disease in New Zealand. This clearly suggested that deficiency of cobalt might be the cause of the disease, and this view was supported by the results of analyses of some New Zealand soils made by Ramage by means of his spectrographic technique (see p. 29), and which showed that two healthy soils each contained 7 p.p.m. of cobalt while a sick Morton Mains soil contained none of this element. Subsequent treatment of affected lambs in New Zealand with weekly amounts of 7 mg. of cobalt brought about a remarkable improvement after a fortnight.

In 1938, Underwood and Harvey showed that both soil and herbage of affected areas in Australia have a lower cobalt content than those of neighbouring healthy areas, but that the cobalt content of the herbage is considerably increased by dressing the soil with a little cobalt acetate. Similar results have been obtained in New Zealand (cf. Kidson and Maunsell 1939), although it would appear that there is not always a direct relation between cobalt contents of soil and herbage.

Experiments in Scotland on pining have similarly shown the relationship of this disease to a deficiency of cobalt. In south-east Scotland Corner and Smith (1938) have shown that pining could be cured and its reappearance prevented for 6 months by a daily dose of 1 mg. of cobalt for 14 days.

An examination of a number of soils in the north of Scotland, an area where pining occurs, made by Stewart, Mitchell and Stewart (1941), showed that the cobalt content of the soils varied from 1 to 300 p.p.m., and that most of the soils on which pining occurs have a cobalt content of less than 5 p.p.m. while some contain as little as 1 p.p.m. The same workers also carried out an experiment on the treatment of affected lambs with cobalt. In this experiment, which was started towards the end of June 1939, two sets of lambs were used, one comprising forty individuals which were treated with cobalt, the other of twenty-five which served as controls, and which at the beginning of the experiment were the best in the flock. Both groups ran together on bad pining land in which the cobalt content of the soil was only 1–2 p.p.m. The lambs of the experimental group were each given 10 mg. of cobalt as cobalt chloride each week for 10 weeks; the control animals received no cobalt. Apart from one lamb

which died from an unknown cause 3 days after the beginning of the experiment and which was immediately replaced by one from the control group, all the treated animals improved in condition and growth and were stated by the farmer to be the best set of lambs he had seen on the farm where the experiment was carried out. The control animals, on the other hand, rapidly lost condition, four died from pining, and by 4 September many others were in the last stage of the disease.

Stewart, Mitchell and Stewart also examined on a set of plots the effect on the cobalt content of soil and herbage which resulted from the application of various quantities of cobalt chloride to the soil, the amounts ranging from $\frac{1}{2}$ to 80 lb. of cobalt chloride ($CoCl_2.6H_2O$) per acre, superphosphate to the extent of $2\frac{1}{4}$ cwt. per acre being used as filling material for the small quantity of cobalt used. Ten weeks after the application of the dressings the total cobalt content of the soil and the amount soluble in dilute acetic acid were determined on each plot as well as the cobalt content of the herbage. The results, given in Table XIX, show how in this comparatively short time the application of cobalt to the soil affects the cobalt content of the herbage.

TABLE XIX. Effect of cobalt manuring on the cobalt content of herbage. (Data from Stewart, Mitchell and Stewart)

Cobalt applied to soil p.p.m.	Cobalt in soil in p.p.m.		Cobalt in herbage p.p.m.
	Total	Readily soluble	
0	2·9	0·177	0·11
	2·9	0·196	0·12
	2·8	0·200	0·20
0·6	—	—	0·23
			0·35
0·125	2·9	0·215	0·34
	2·9	0·230	0·41
0·25	3·25	0·255	0·51
	3·2	0·236	0·83
1·25	4·0	0·479	1·63
	4·2		2·28
5·00	5·8	1·75	3·94
10·00	9·6	1·96	8·17

The favourable effect on lambs produced by such manuring was shown by another experiment carried out by Stewart,

Mitchell and Stewart in which a field with a low cobalt content of the soil (about 1·8 p.p.m.) was divided into two halves, one of which was manured with 2 lb. cobalt chloride + 3 cwt. superphosphate per acre, the other with 3 cwt. superphosphate only. Four to five weeks after this manuring fifteen lambs were allowed to run on each half of the field, the two groups being of equal value at the start. After 6 weeks the difference in the appearance of the lambs of the two groups was striking, all those on the half of the field with added cobalt being in fine condition, whereas eight of the fifteen lambs on the other half of the field showed severe pining while the remaining seven animals were in poor condition.

While the effectiveness of cobalt manuring in increasing the cobalt content of the herbage of 'pining' lands can be regarded as established, work by Mitchell, Scott, Stewart and Stewart (1941) indicates that such manuring requires care. They find by spectrographic analysis that the herbage growing on cobalt-treated soil may take up an abnormally large amount of molybdenum. Where the herbage without cobalt treatment already had a fairly high molybdenum content, this latter may be so increased by cobalt manuring that it approaches the molybdenum content of the herbage of teart land, so that danger of scouring may ensue. Analyses of the herbage from two soils, one with a low, the other with a high molybdenum content, cut 15 months after various degrees of cobalt manuring, are shown in Table XX. Since Lewis has shown that molybdenum uptake is reduced by the use of acidic nitrogenous fertilizers (see p. 135), and since teartness occurs on neutral and alkaline soils, it would seem inadvisable to apply cobalt in the form of a cobalt-rich lime or in conjunction with fertilizers having an alkaline reaction.

TABLE XX. Cobalt and molybdenum contents of herbage from soils treated with cobalt chloride. (Data from Mitchell, Scott, Stewart and Stewart)

$CoCl_2.6H_2O$ added lb. per acre	Herbage from soil A		Herbage from soil B	
	Co p.p.m.	Mo p.p.m.	Co p.p.m.	Mo p.p.m.
0	0·08	1·7	0·07	6·3
2	0·22	2·2	0·20	9·2
10	0·63	2·4	0·89	10·0
80	3·20	7·5	2·75	14·2

The possibility that cobalt deficiency may also be met with in cattle in Kenya and in Florida has already been mentioned.

It is now recognized that pining in sheep occurs in various parts of England, notably in parts of Northumberland and Cumberland and on Dartmoor and Exmoor in Devonshire.

3. The Functions of Trace Elements in Animals

The trace elements which have been shown to be essential for animals of one or more species include iodine, manganese, copper, zinc and cobalt. It has already been mentioned that iodine enters into the composition of thyroxine, one of the amino-acids of thyroglobulin, the protein of the thyroid gland. This appears to be the only function of iodine in mammals, as far as is known at present. Copper has long been known to form part of the molecule of haemocyanine, a pigment concerned in the respiration of certain Crustacea and some other lower animals, while Mann and Keilin have shown that copper protein compounds, haemocuprein and hepatocuprein, occur in the blood and liver respectively of mammals. Although the actual part played by these compounds in the physiology of the animal is not clearly understood, it would appear to be established that the functions of copper, wholly or in part, are to be found in the roles of these copper-protein compounds.

Although diseases are now definitely associated with deficiencies of manganese and cobalt nothing precise is known of the functions of these elements nor in what form they are present in animals. With zinc, on the other hand, the reverse is the case, for whereas no condition has been associated with deficiency of this element, recent work by Keilin and Mann (1940) has thrown considerable light on the function of zinc in animals.

In 1933, Meldrum and Roughton showed that in the erythrocytes (red blood corpuscles) of mammals there occurs an enzyme which catalyses the reaction $H_2CO_3 = CO_2 + H_2O$. To this enzyme they gave the name carbonic anhydrase. The enzyme was shown by Davenport in 1939 to occur in relatively high concentration in the gastric mucosa of mammals. It has now been found by Keilin and Mann (1940) that preparations of this

enzyme, which they regard as practically pure, obtained from the erythrocytes of ox and sheep, contain constantly about 0·33 per cent of zinc, but no iron, copper, manganese, magnesium or lead. In preparations of the enzyme of varying degrees of purity the enzyme activity is directly proportional to the zinc content, which indicates that the zinc forms part of the enzyme molecule. The enzyme, in fact, appears to be a zinc-protein compound in which the protein in each molecule is combined with two atoms of zinc. It may be noted that no other zinc compound is known which has catalytic properties. Keilin and Mann found the concentration of carbonic anhydrase to be about 0·21 g. per 100 ml. of erythrocytes.

The function, or at any rate one function, of zinc in mammals, is to be found therefore in the action of carbonic anhydrase. In the blood this action accelerates the dissociation of carbonic acid into water and carbon dioxide and so furthers the escape of the latter from the blood into the alveolar spaces. As well as this function in carbon dioxide excretion from the blood, the presence of the enzyme in the parietal cells of the gastric mucosa suggests another function in the stomach, probably in connexion with the formation of hydrochloric acid.

CHAPTER VI

CONCLUDING REMARKS

It will be clear from the preceding review of the present position of our knowledge of trace elements in plants and animals that these elements present the biologist with two sets of problems, the one pathological, the other physiological. The pathologist is concerned with the abnormal conditions resulting in plants and animals from deficiency, and in a few cases from excess, of the various trace elements, and of the means by which the deficiency or excess can be removed. The problems of the physiologist are more subtle, for it is his business to discover the functions in the life of the organism of these various elements which are present in only minute amounts. The two sets of problems are of course interdependent, for the work of the pathologist in discovering the effects of deficiency greatly aids the physiologist, while knowledge of the part played by the trace elements in the life of the organism must necessarily help the pathologist in the diagnosis and treatment of deficiency diseases. But on the whole, knowledge of the pathology of trace elements, particularly in plants, is much more advanced than our knowledge of their physiological functions. A considerable number of well-recognized and defined plant diseases are now correctly attributed to various trace-element deficiencies, and means of controlling these diseases have been determined with considerable precision. Future work on the pathological side will no doubt extend the number of such known deficiency diseases. In addition to such work it would appear that two lines of research in the field of pathology would well repay attention. The first of these is the development of rapid means of early diagnosis. The usual method of observing deficiency symptoms, employed with outstanding success by Wallace, is naturally only applicable after visible external symptoms have developed. The injection methods elaborated by Roach appear to be particularly useful for trees, but although certainly usable for annual crops, and affording a means of early diagnosis, require some degree of technical skill for their employment with small herbaceous plants.

The second field of research which should afford valuable results in the control of deficiency diseases is the investigation of the relationship of soil and climatic factors to the incidence of these diseases. It is, for example, well known that the manganese available in the soil for absorption by plants may be only a fraction of the total manganese present, and that one of the factors determining the degree of availability of this element is the acidity (*p*H) of the soil, the availability in general decreasing with decreasing acidity (increasing *p*H). Hence liming the soil, by bringing about an increase in the *p*H value of the soil, may seriously affect the availability of manganese. This appears to be particularly so when the soil contains much humus, though the relations between *p*H, humus content and concentration of available manganese are at present far from being completely understood. Liming may also adversely affect the absorption of boron by plants, perhaps again because of its effect in lowering the availability of this element, perhaps partly because of the relations between calcium and boron. Again, it is well established that the severity of deficiency diseases may vary in different years, a variation which must be attributed to the differences in climate experienced in different seasons; the symptoms of boron deficiency, for example, are generally much more severe in periods of drought than in wetter seasons. It is to be presumed that the different water contents of the soil in the different seasons must affect the availability of the trace elements concerned.

Nor must the micro-organisms of the soil be forgotten. These may themselves play a part in determining the availability of various elements by bringing about oxidations and reductions of compounds of the different trace elements and so affecting their solubility, or they may play a more direct part in the deficiency diseases themselves as suggested by the work of Gerretsen and Ark referred to in an earlier chapter. There is here a wide field of investigation which at present has scarcely been entered.

In the physiological field the outstanding fact is, of course, that the trace elements as their designation implies are needed in very minute amount. This does not make the solution of the problems of their functions any easier. Because of the small quantities of them involved in the organism it has been usual to

assume that they play the part of catalysts, although there is quite a definite suggestion on the part of some workers that the function of boron is of a different kind from those of manganese, zinc and copper.

Two lines of attack on the role of the trace elements which have developed during recent years appear to give promise of fruitful results. The first of these is that displayed in particular by the work of Keilin and Mann in which compounds involving the trace elements in their molecules have actually been isolated and the chemical properties of these compounds determined. It has been mentioned earlier that several copper compounds and one zinc compound have been isolated in this way. They all appear to be protein compounds of the metal concerned, and two of them, the catechol oxidase of plants and carbonic anhydrase of animals, are, indeed, enzymes which had been previously known, and the properties of which were well established. As far as these results go they thus confirm the view of the catalytic nature of the trace elements in organisms; and suggest the possibility that manganese and molybdenum also will turn out to be essential constituents of enzymes. The further view that the trace elements are concerned in vital oxidations and reductions is also supported by our knowledge of the nature of at least two of these compounds, for catechol oxidase is definitely an oxidizing enzyme, while carbonic anhydrase is concerned in the release of an end-product of oxidation, carbon dioxide. The condition of boron is more problematic.

The second promising line of attack on the physiological aspects of trace elements lies in growing plants under experimental conditions in which the supply of various mineral nutrients is controlled and determining the resultant effects on growth and, as far as possible, the fate in the plant of the mineral elements concerned. The recent work of Shive and his associates along these lines, which has been dealt with in an earlier chapter of this book, has already produced very promising results with regard to the functions of manganese and boron in plants, and it would seem probable that a development of work along these lines will serve greatly to increase our understanding of the role of the trace elements in plants.

The realization of the importance of trace elements in plants

and animals is actually very recent, for, apart from a few pioneer observations, our present not inconsiderable knowledge of the subject is the result of work done during the last 25 years, and, indeed, for the most part, during the last decade. Of late, more and more workers have been attracted to the investigation of the problems these elements present to the biologist, and with the excellent research on these problems now being carried out in various parts of the world, we may or may not find the list of essential trace elements has to be increased, but we shall certainly see a rapid expansion in our knowledge of the physiology and pathology of plants and animals in relation to the trace elements.

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