THE EFFECT OF MINERAL NUTRITION ON THE CONTENT OF FREE AMINO ACIDS AND AMIDES IN TOMATO PLANTS

I. A COMPARISON OF THE EFFECTS OF DEFICIENCIES OF COPPER, ZINC, MANGANESE, IRON, AND MOLYBDENUM

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Summary

The level and the quantitative composition of the free amino acid fraction of tomato plants grown in full nutrient and in cultures deficient in zinc, copper, manganese, iron, and molybdenum have been determined. The methods used in the investigation include the quantitative estimation of amino acids by a technique involving paper chromatography, and the culture of tomato plants in highly purified nutrient solutions.

All the deficiencies investigated except that of molybdenum brought about increases in the free amino acid fraction. In every case there was a change in the quantitative composition of this fraction. Iron and zinc deficiencies were associated with large increases in the free levels of the two amides asparagine and glutamine, while with copper and manganese deficiencies many individual amino acids increased in concentration but not the two amides. The level of total free amino acids and amides was low in molybdenum-deficient plants.

The qualitative differences between deficient- and full-culture plants were not large, but included the accumulation of β-alanine with deficiency of zinc, copper, or molybdenum, and of pipecolinic acid with deficiency of manganese or iron. Other differences were the absence of histidine with copper, manganese, iron, and molybdenum deficiency, of phenylalanine with copper deficiency, and of lysine with copper, manganese, and molybdenum deficiencies. Additionally many quantitative changes in amino acid concentration were found. In the deficient plants there were changes in the concentration of most amino acids relative to the levels in the controls. These changes were not uniform and the relative amounts of the different amino acids were altered with each deficiency.

The significance of these results is discussed in relation to the known interactions between the mineral nutrition and the amino acid composition of plants. Although a characteristic spectrum of free amino acids is associated with each individual deficiency, the differences are not considered sufficiently large to make analysis for these compounds an additional method for the diagnosis of mineral deficiencies in this plant.

I. INTRODUCTION

The importance of amino acids in the metabolism of plants has occasioned a number of investigations on the relation between these compounds and mineral nutrition. Richards and Templeman (1936) and Gregory and Sen (1937) were among the first to show an effect of the mineral nutrient supply on the concentrations of free amino acids and of amides. These workers found enhanced concentrations in phosphorus- and potassium-deficient, and depressed concentrations in nitrogen-deficient, barley plants. Subsequently accumulations of free amino nitrogen

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compounds were demonstrated by Eaton (1941) in sulphur-deficient sunflower plants, by Bean (1942) in zinc-deficient tomato plants, by Steinberg, Bowling, and McMurtry (1950) in calcium-, magnesium-, and boron-deficient tobacco plants, and by Hewitt, Jones, and Williams (1949) in manganese-deficient cauliflowers. Hewitt *et al.*, however, also showed that molybdenum deficiency is accompanied, not by a high, but by an abnormally low content of free amino nitrogen compounds.

Recently the effect of mineral nutrition on the composition as well as the concentration of the free amino acid fraction has been investigated by Richards and Coleman (1952) and Richards and Berner (1954), whose most important observation was that of an accumulation of the diamine putrescine in potassium-deficient barley leaves.

Hitherto, however, data have not been available on the relation between micronutrient deficiency and the level, as well as the composition, of the free amino acid fraction. It has been the purpose of this investigation to supply such quantitative data. Tomato was chosen as the test plant and the effects of deficiencies of copper, zinc, iron, manganese, and molybdenum were investigated.

The tomato has been used as the experimental material since it grows well in water culture (Hoagland and Arnon 1938), and the symptoms of deficiencies of all the known essential elements in this species have been described (Hambidge 1941; Wallace 1951). The analytical methods employed have included the technique of paper chromatography, which has enabled quantitative estimations to be made of all the individual free amino acids and amides present in these plants.

The primary significance of results from this, as from all other similar investigations, is that they show the extent to which particular elements are involved in the metabolism of amino acids in general. At the same time earlier results have shown that extensive changes are promoted by mineral deficiencies and the situation therefore holds the possibility that an analysis of the amino acid fraction may provide a basis for the identification of the deficiency. This was a primary consideration in the design of the investigation.

II. Methods

(*a*) *Culturing of Experimental Plants*

Seeds of tomato (*Lycopersicon esculentum* Mill. var. Pan America) were germinated on waxed mosquito netting over distilled water. After approximately 3 weeks, the seedlings were transplanted into nutrient solutions in 3-l. "Pyrex" beakers fitted with aeration tubes. Eight plants were grown per beaker and the basic composition of the culture solutions was essentially the same as that recommended by Stout and Arnon (1939).

Nutrient mixtures deficient in molybdenum were prepared by the method of Gentry and Sherrington (1950) in which this element is removed from macronutrient solutions by chelation with 8-hydroxyquinoline. It was found that this treatment also removes iron. Copper-, zinc-, and manganese-deficient nutrient mixtures were prepared by the method of Stout and Arnon (1939).

The control plants were grown in culture solutions prepared using the purified nutrient solutions, and adding a full complement of trace elements. The micro-

nutrient solutions were prepared from "Analar" grade salts without any prior purification.

(*b*) *Preparation and Purification of Plant Material Extracts*

Extraction of the free amino acids and amides from the plant material was carried out by grinding in cold 80 per cent. ethanol (Woodward and Rabideau 1953). The grinding was carried out for 10 min using a top drive homogenizer, operating at a speed of 1600 r.p.m. The alcohol suspension was allowed to stand overnight in the cold after which it was centrifuged. The insoluble material was resuspended in 80 per cent. ethanol and after centrifugation the two supernatant fractions were bulked. Approximately 5 g fresh weight of plant material were used to prepare one ethanol extract which had a final volume of 250 ml.

The ethanol extract, although it carried no protein, did contain various other substances which could disturb the chromatographic analysis for amino acids. These were separated by evaporating the extract to dryness at 40°C *in vacuo*, and redissolving the residue in water, which was then poured on to a column of "Zeokarb 225" which had previously been brought to the H^+ form. Sugars and salts were removed by washing the column with distilled water and finally the amino acids were released from the resin by washing with 1·5N ammonium hydroxide. This ammonia extract was evaporated to dryness *in vacuo* and the amino acids and amides were dissolved in a known volume, usually 1·0 ml of a 10 per cent. *iso*propanol–water mixture.

The sulphonated crosslinked polystyrene resin "Zeokarb 225" was used, as tests with standard amino acid solutions showed that all the amino acids and amides commonly occurring in plant material could be quantitatively eluted with 1·5N ammonium hydroxide. The only exception was cysteic acid which was completely lost in tests using standard solutions. This substance was either not held on the column or could not be eluted with ammonia. The closely related and naturally occurring amino acid cystine was not lost with resin column treatment.

(*c*) *Estimation of Amino Acids and Amides*

Qualitative analyses of unpurified plant material extracts were made by two-dimensional paper chromatography using the ascending method of solvent flow (Williams and Kirby 1948). A phenol–water mixture (80:20 w/v) was used as the first solvent, followed by the *n*-butanol–acetic acid–water mixture (4:1:1 v/v) of Reed (1950) in the transverse direction. To confirm the identity of various amino acids, *n*-propanol–water (70:30 v/v) and ethanol–water (15:25 v/v) were used as alternative second solvents. In some cases the phenol solvent was modified by the addition of acetic acid or ammonia vapours (Consden, Gordon, and Martin 1944; Dent 1948).

The general detecting procedure was by spraying with 0·1 per cent. ninhydrin in ethanol followed by heating. An isatin-in-ethanol spray followed by heating was used to detect proline. Modified Sakaguchi and Pauli tests were used to confirm the identity of arginine and histidine respectively (Sakaguchi 1950; Sanger and Tuppy 1951).

For the quantitative analyses two-directional paper chromatography was again employed, but under highly standardized conditions. All the chromatographic separations were carried out at a standard temperature which for ease of maintenance was $27 \pm 0{\cdot}5°C$.

Again a phenol–water mixture (80:20 w/v) was the first solvent, and using the ascending method of solvent flow, in an atmosphere of ammonia and cyanide vapours, a sufficient separation was obtained in 30 hr. In this case no prior equilibration of the paper with the atmosphere of the solvent tank was necessary. The second solvent of *n*-butanol–acetic acid–water mixture (4:1:1 v/v) was applied by the descending technique. With this more volatile solvent equilibration of the paper with the atmosphere of the solvent vapours for 4 hr prior to commencing the solvent run of 20 hr was essential for consistent results. Whatman No. 1 papers were used throughout as they gave reproducible results with this two-solvent system. Accurately-calibrated micropipettes were used to transfer the aliquots of between 5 and 50 μl of plant extract to the paper chromatograms. The papers, wet with solvent, were dried in a forced draught of air at 30°C and with this treatment decomposition of the amino acids was negligible (Novellie 1950).

The amino acids and amides separated by paper chromatography were estimated essentially as described by Wellington (1952). Papers were sprayed with a mixture of 2 per cent. ninhydrin and 0·1 per cent. pyridine in ethanol, and the colours were developed for 24 hr under the standard conditions of 40 per cent. relative humidity at a temperature of 20°C. The coloured areas which developed were eluted in 50 per cent. aqueous ethanol and colour intensity measurements were made using a Coleman spectrophotometer. All the measurements were made at 570 mμ, including that of the brown asparagine-ninhydrin complex.

Proline and hydroxyproline, which give yellow colours with ninhydrin, were not estimated with this reagent since they give a more sensitive reaction with isatin (Acher, Fromageot, and Jutisz 1950). When the papers are sprayed with a saturated solution of isatin in ethanol and heated at 65°C for 30 min no amino acids or amides other than proline and hydroxyproline give blue colours. Fortunately none of the plant material extracts, when analysed using a two-solvent system, showed the presence of hydroxyproline and the specificity of the isatin reaction with proline could be used to estimate this constituent directly. The intensity of the blue isatin-proline colour, which cannot be eluted from the paper, was measured directly on the paper by transmittance densitometry using a recording photoelectric densitometer.

The significance of the quantitative results obtained by the procedure outlined above was examined by preparing calibration curves with pure amino acids. A series of chromatograms was prepared using serial dilutions of standard amino acid solutions and the intensities of the blue amino acid-ninhydrin colours were measured spectrophotometrically. Individual calibration curves were obtained for each amino acid by plotting the mean values of six replicate estimations of percent. transmission at a series of six different concentrations.

With ninhydrin as the colour reagent the relationship of amino acid concentration to the logarithm of percent. transmission was a straight line but the slope of this line varied for each individual amino acid. Straight lines were fitted by the method of

least squares to the logarithms of percent. transmission against amount of amino acid in micrograms. The line passed in each case through the reading for the blank, which was based on a large number of observations, with high reproducibility. A test was made for excessive deviation from the line of the mean log transmissions at the concentrations used relative to the pooled replicate variance about the means. The deviations were significant only in the case of asparagine and leucine and there was no obvious curvilinear trend in any instance with the possible exception of leucine.

TABLE 1

RANGE OF RELATIVE PRECISION OF THE PAPER CHROMATOGRAPHIC METHOD FOR THE QUANTITATIVE DETERMINATION OF AMINO ACIDS

Amino Acid or Amide	Range of Concentration over which Relative Precision Estimated (μg)	Relative Precision of an Individual Estimation (μg)
Aspartic acid	0–19·94	0·920
Glutamic acid	0–22·08	1·118
Asparagine	0–19·80	1·198
Glutamine	0–21·90	0·679
Citrulline	0–26·28	0·771
Histidine	0–23·28	1·180
Lysine	0–21·94	1·303
Arginine	0–26·12	1·300
Serine	0–15·78	0·625
Glycine	0–11·28	0·424
Threonine	0–17·88	0·567
α-Alanine	0–13·38	0·238
β-Alanine	0–13·38	0·690
γ-Aminobutyric acid	0–15·48	0·487
Ethanolamine	0– 9·16	0·423
Pipecolinic acid	0–19·34	0·922
Phenylalanine	0–24·78	1·455
Valine	0–17·58	0·572
Leucine	0–19·68	0·847
Proline	At concn. of 4·0 μg	0·690

The mean square for deviates of individual logarithms of percent. transmission from the line, and their corresponding standard error were computed. This standard error divided by the slope of the line is an approximate estimate of the error of estimation of amino acid concentration corresponding to a particular logarithm of percent. transmission. A summary of this approximate precision with which each amino acid was estimated when using ninhydrin is given in Table 1.

Proline was considered separately as the relationship between the amount of proline and the logarithm of percent. transmission, as measured by the densitometer, was not a straight line. A smooth curve was fitted in this case and provided the calibration curve. An assessment of the accuracy of an individual proline estimation

at the set concentration of 4·0 μg was made, as many of the actual proline estimations were carried out in the range between 3 and 5 μg by carrying out serial dilutions of the extracts.

Although the individual calibration lines were all drawn through the reading for the blank, which corresponds to an amino acid concentration of zero, the smallest amount of each amino acid that could be detected was determined by the approximate precision with which each could be estimated. These values are presented in Table 1 and vary between 0·238 μg for α-alanine and 1·455 μg for phenylalanine. When analysing each plant material extract a series of chromatograms was prepared using different amounts of the extract. In this way most of the actual estimates of concentration were carried out using the central portions of the calibration lines and all the free amino acids present in the plant material at concentrations of greater than approximately 0·05 μg per mg dry weight were estimated. Accordingly amino acids referred to as absent may have been present at concentrations of less than this value.

(*d*) *Experimental Design*

The present investigation involved two different experimental series. In the first, the effects of molybdenum and iron deficiency were investigated and the plants were harvested 2 weeks after transplanting and 5 weeks after the seed was sown. In the second, the effects of zinc, copper, and manganese deficiency were examined, and the plants were harvested 4 weeks after transplanting and 7 weeks after sowing the seed. Each series involved a separate control culture provided with a complete nutrient mixture. In each series the harvest was delayed until the appropriate deficiency symptoms had been fully developed.

The analyses are based on the whole shoot. The stem was cut immediately above the cotyledons, and separate samples were taken for amino acid and dry weight determinations. The analytical results for molybdenum deficiency are based on five separate samples, those for iron and the corresponding control on single samples, and those for zinc, copper, and manganese deficiency and their corresponding control on two samples each. The samples taken for the determination of dry weight involved from four to six whole shoots taken at random from the plants that were harvested. The samples taken for the estimation of amino acids consisted of 15 shoots in each of the molybdenum-deficient samples, 24 shoots in the iron-deficient sample, and six shoots in the corresponding control sample. Six shoots were involved in each sample of the copper-, zinc-, and manganese-deficient material and six in the sample from their corresponding control.

III. Results

The primary data obtained in this investigation are given in Table 2. However, the significance of the quantitative differences can only be assessed from the logarithmic transformations of these figures which are given in Table 3 with the appropriate significance values. In the statistical analysis a log transformation to equalize variances within treatments for each constituent was made and the separate estimates of variance were pooled. These pooled variances were assumed to be applicable to all treatment comparisons. This assumption may not be strictly valid but the

significance levels given should serve as reasonable reference values. In Table 3 the values for the individual constituents of the iron- and molybdenum-deficient plants have been adjusted for the difference between the controls of series 1 and 2. This adjustment facilitates comparison of the iron and molybdenum deficiency values with the common control values and with the zinc, copper, and manganese deficiency values.

TABLE 2

COMPARISON OF THE EFFECTS OF DIFFERENT DEFICIENCIES ON THE CONCENTRATIONS OF FREE AMINO ACIDS AND AMIDES AND ON DRY WEIGHT

Results are expressed as μg of amino acid or amide per mg dry weight

Amino Acid	Control 2*	−Zn	−Cu	−Mn	Control 1**	−Fe	−Mo
Aspartic acid	1·526	4·408	5·553	4·996	0·985	2·303	0·152
Glutamic acid	5·107	7·624	7·116	6·683	5·848	6·911	2·500
Asparagine	0·343	16·58	0·738	0·545	0·415	6·307	0·161
Glutamine	3·850	26·27	2·277	1·400	2·180	10·27	0·306
Citrulline	0·447	Absent	0·399	0·407	0·199	0·582	0·254
Histidine	0·129	1·031	Absent	Absent	0·093	Absent	Absent
Lysine	0·068	0·749	Absent	Absent	0·063	0·340	Absent
Arginine	0·183	1·058	0·350	0·328	0·210	0·503	0·445
"Under arginine"	0·043	0·309	0·182	0·271	0·034	0·275	0·288
Serine	1·770	3·116	1·345	0·482	1·258	1·152	1·460
Glycine	1·810	2·474	0·755	0·202	0·797	0·548	0·191
Threonine	1·063	2·214	0·767	0·898	0·629	0·880	0·238
α-Alanine	1·730	1·823	1·359	1·166	1·299	0·895	0·885
β-Alanine	Absent	0·436	0·443	Absent	Absent	Absent	0·141
γ-Aminobutyric acid	0·439	0·527	0·328	0·532	0·184	0·495	0·924
Ethanolamine	0·288	Absent	0·241	0·330	0·220	1·125	0·226
Phenylalanine	0·194	1·355	Absent	0·192	0·239	0·329	0·178
Pipecolinic acid	Absent	Absent	Absent	0·385	Absent	0·178	Absent
Valine	0·234	1·026	0·295	0·288	0·189	0·453	0·203
Leucine	0·266	1·892	0·298	0·358	0·226	0·604	0·267
Proline	0·681	1·505	2·022	0·766	0·545	1·133	0·843
Total amino acids	15·98	31·57	21·45	18·28	13·01	17·71	9·195
Total amides	4·193	42·85	3·015	1·945	2·595	16·58	0·467
Dry weight (mg per plant)	213·6	65·95	75·77	69·41	79·52	10·18	17·98

* Control 2 comparable only with −Zn, −Cu, −Mn.

** Control 1 comparable only with −Fe and −Mo.

Before considering the changes promoted by the different deficiencies it may be noted that only one substance could not be identified. This substance reacted with ninhydrin but not with isatin, and had the same R_F as arginine with the phenol solvent but occupied a position below it with the butanol–acetic acid–water solvent. It was estimated with the arginine calibration curve and is referred to as "under arginine".

The effects of the deficiencies may be considered by comparing the data for each group with those for the control.

Molybdenum.—The most striking effect here is the low total amino acid content. Other effects include an absence of histidine and lysine which are present in the control and the presence of β-alanine which is absent from the control. Another feature is the very high level of γ-aminobutyric acid in these plants.

TABLE 3

COMPARISON OF EFFECTS OF DIFFERENT DEFICIENCIES ON THE CONCENTRATIONS OF FREE AMINO ACIDS AND AMIDES

Transformation log (×10,000) of values for μg of amino acid or amide per mg dry weight

Amino Acid	Control 2	−Zn	−Cu	−Mn	−Fe*	−Mo*	Least Diff. (5%) between Zn, Cu, Mn, and Control	Least Diff. (5%) between Fe, Mo, and Control	Least Diff. (5%) between Fe or Mo and Cu, Zn, Mn
Aspartic acid	3·184	3·644	3·745	3·697	3·553	2·372	0·098	0·139	0·240
Glutamic acid	3·701	3·881	3·851	3·815	3·773	3·332	0·259	0·366	0·634
Asparagine	2·534	4·220	2·868	2·715	3·716	2·123	0·278	0·393	0·681
Glutamine	3·565	4·418	3·357	3·137	4·239	2·713	0·259	0·366	0·634
Citrulline	2·645	Absent	2·602	2·608	3·111	2·751	0·170	0·240	0·416
Histidine	2·109	3·013	Absent	Absent	Absent	Absent	0·114	—	—
Lysine	1·829	2·869	Absent	Absent	2·561	Absent	0·258	0·365	0·632
Arginine	2·263	3·025	2·541	2·485	2·642	2·589	0·344	0·486	0·842
"Under arginine"	1·632	2·490	2·250	2·410	2·542	2·560	0·351	0·496	0·860
Serine	3·247	3·488	3·095	2·684	3·208	3·311	0·378	0·534	0·926
Glycine	3·242	3·393	2·868	2·250	3·080	2·622	0·536	0·758	1·313
Threonine	3·005	3·342	2·883	2·953	3·150	2·583	0·309	0·437	0·757
α-Alanine	3·238	3·261	3·134	3·064	3·076	3·071	0·105	0·148	0·257
β-Alanine	Absent	2·665	2·642	Absent	Absent	2·270	0·158	—	0·387
γ-Aminobutyric acid	2·642	2·722	2·516	2·725	3·072	3·343	0·089	0·126	0·218
Ethanolamine	2·460	Absent	2·382	2·492	2·215	2·472	0·342	0·483	0·838
Phenylalanine	2·274	3·132	Absent	2·285	2·413	2·146	0·243	0·344	0·595
Pipecolinic acid	Absent	Absent	Absent	2·050	2·371	Absent	—	—	—
Valine	2·367	3·010	2·463	2·455	2·747	2·399	0·236	0·334	0·578
Leucine	2·424	3·275	2·465	2·546	2·851	2·497	0·238	0·337	0·583
Proline	2·835	3·156	3·294	2·878	3·153	3·025	0·368	0·520	0·901

* The transformed values of each constituent of the −Fe and −Mo plants were adjusted for the difference between control 1 (see Table 2) and control 2. The three least difference values allow all combinations of comparisons between control, −Zn, −Cu, −Fe, −Mn, and −Mo to be made.

Zinc.—This deficiency, like all the others in the series except molybdenum, is accompanied by a high total amino acid content. It resembles iron deficiency in being characterized by a very high amide content. Increases in the majority of the individual amino acids contribute to the high total level, with the notable exceptions

of citrulline and ethanolamine both of which were absent. Additionally, β-alanine is clearly present in this material.

Iron.—With this deficiency there is a large increase in the total amide level with a smaller increase in the total amino acid level. Further differences are the accumulation of pipecolinic acid, a compound not present in the controls, and the absence of histidine.

Copper.—Here there is an increase in the total amino acid level but little change in the total amide level. Histidine, lysine, and phenylalanine are absent. Phenylalnine is present in the controls and in all the other deficiencies but is characteristically absent in copper-deficient plants.

Manganese.—With this deficiency, as with those of copper and molybdenum, the basic amino acids histidine and lysine are not present in the free pool of amino acids. There is a general increase in the total level of free amino acids, but again no change in the amide fraction. The compound pipecolinic acid, found in the iron-deficient plants, is also present with manganese deficiency.

IV. Discussion

The results obtained with the plants grown in full nutrient are of some interest. The range of compounds identified was similar to that found by Pearse and Novellie (1953) in tobacco leaves, by McKee and Urbach (1953) in apple leaves, and by Richards and Berner (1954) in barley leaves. The identification of citrulline and ethanolamine was unexpected, although the presence of citrulline as a major constituent of the amino nitrogen fraction in alder has been reported by Virtanen and Miettinen (1953). The presence of ethanolamine in plants has also been recorded by Woolley (1943). It is significant that cystine, methionine, tryptophan, and tyrosine were not identified, although tests with pure substances showed that they were not lost during extraction. The absence of these compounds from the free amino acid pool of plants has been recorded by Steward *et al.* (1954).

Certain amino acids have only been found in deficient plants, β-alanine in those lacking molybdenum, zinc, or copper, and pipecolinic acid in those deficient in manganese or iron. Certain other amino acids, although present in the controls, have not been found in the free pool of deficient plants. Phenylalanine was characteristically absent from the free amino acid fraction of copper-deficient plants although it was present in every other case. Histidine and lysine were not present in copper-, manganese-, and molybdenum-deficient plants, and lysine was not detectable in the iron-deficient material.

These qualitative changes were accompanied by extensive quantitative changes in the composition of the free amino acid fraction in the case of every deficiency investigated. However, the full significance of these differences is difficult to estimate from the present series of data, as it is recognized that the basis of comparison used here carries an important limitation.

Concentrations of different plant constituents change during ontogeny, and comparison of plants on a basis of temporal age may therefore be misleading if development proceeds at different rates within the same time interval. Physiological

age might be a more satisfactory basis of comparison. The identification of this, however, presents considerable difficulty and it may be questioned whether a plant deficient in one essential element can at any time be regarded as being the same physiological age as another which has not been grown with a corresponding restraint.

The differences due to development or physiological age are likely to be least extensive in the early phases of growth, and the basis of comparison that has been used here is therefore likely to be the least misleading, since the plants were young when harvested. The relatively close agreement, when the results were expressed on a concentration basis, i.e. μg of amino acid per mg dry weight, between the results for the two groups of control plants of differing age, weight, and development indicates that the concentration drifts with time over the range of the present experiments were not large.

Within their limitations the present series of data do show that micronutrients have a profound effect on amino acid metabolism. The most important of these is the general accumulation of free amino nitrogen compounds under all deficiencies tested except that of molybdenum. This result is in agreement with the recent work of Steinberg, Specht, and Roller (1955) with respect to all deficiencies except that of molybdenum. With regard to the molybdenum effect, the data assembled here agree with the earlier observations of Hewitt *et al.* (1949) and of Spencer and Wood (1954).

Molybdenum is the only element of those examined that is known to be directly involved in the nitrogen metabolism of plants (Nicholas, Nason, and McElroy 1954), and it is perhaps significant that it was only a deficiency of this element which led to a reduction in the level of free amino acids and amides.

There appear to be two groups within the other four deficiencies tested. Firstly, zinc and iron deficiencies which brought about large accumulations of asparagine and glutamine, and significant increases in the levels of many of the free amino acids. Secondly, deficiencies of copper and manganese which increased the free amino acid levels but had little effect on the total amide levels.

These differences may be due to variation in the stage of deficiency. This is improbable, however, since the symptoms in all cases were severe. In this connection it is of some significance that Prianischnikov (1922) showed that amides accumulate in plants in which proteins are being hydrolysed, and that Gregory (1937) has shown that amides are formed in the lower leaves of potassium-deficient barley at the expense of proteins which are degraded. Thus the amide accumulation noted here may correspond to an accentuated degradation of protein in the absence of iron and zinc.

The present series of results are of some significance in relation to the general character of the changes in the amino acid pool induced by different mineral deficiencies. Richards and Berner (1954) found that the general composition of the free amino acid fraction in leaves of barley does not change substantially with changes in nutrition. The results of this investigation are clearly consistent with this general conclusion. Except for relatively small anomalies the array of amino acids remains the same whatever the nutrient treatment. The suppressions of histidine with copper, manganese, iron, and molybdenum deficiency, of phenylalanine with copper, manganese, and molybdenum deficiency are of little significance, since the concentrations of these amino acids in the control plants are low. Again, the

emergence of β-alanine with zinc, copper, and molybdenum deficiency, and of pipecolinic acid with manganese and iron deficiency may be disregarded since the concentration of these is also low. Every major constituent in the pool of control plants is present in that of deficient plants. At the same time the quantitative data show that, while the general array of amino acids does not change with nutrient conditions, the quantitative relations of the amino acid pool do change very considerably.

Two aspects of the situation may be distinguished. Firstly, in terms of unit dry weight there may be either a general increase or a general decrease in the concentrations of all amino acids. Secondly, the extent of change may differ with each amino acid. For instance, with zinc deficiency in which there is a general increase in amino acid concentration the comparison with the control shows 10-fold increases with asparagine, glutamine, histidine, and phenylalanine, but only two- to five-fold increases with aspartic acid, serine, threonine, valine, and proline. The position is similar with every other deficiency.

It may be noted that the quantitative changes, while different with each deficiency, are in certain cases similar. Thus the effects of zinc deficiency are similar to those of iron deficiency with respect to the accumulation of amides and aspartic acid. This, and the circumstance that these deficiencies are not characterized by the suppression of any major amino acid or by the accumulation of any unusual substance, limits very considerably the possibility of using the composition of the amino acid pool as a diagnostic method.

Finally, the data of this amino acid survey of the one plant species grown under a series of nutritional conditions is important in relation to the many amino acid surveys made of other plant species (reviewed by Steward, Zacharius, and Pollard 1955). In general, no two plant species have identical free amino acid pools and usually the differences are quite large. Certain amino acids have been found in only a restricted few plant species, often very widely separated systematically, and additionally, compounds which are major components of the free amino acid fraction of one plant are often barely detectable in another plant species.

The present data suggest that, when a particular amino acid does not accumulate, this does not necessarily indicate that the catalytic systems required for synthesis are not available. In this investigation tryptophan and cystine have not been identified on the chromatograms. These acids, however, are certainly present in the proteins of tomato plants and the enzyme systems involved in their metabolism are therefore certainly available. Secondly, the work of Nason, Oldewurtel, and Propst (1952) has shown that deficiencies of micronutrient elements in plants, in general, bring about the retardation of certain enzyme-catalysed reactions and the acceleration of others. The nutrient deficiency usually does not involve the complete suppression of any system. Thus when phenylalanine is not developed in the absence of copper this may indicate a change in the rate of a reaction involved in the metabolism of this substance. With copper deficiency the appropriate catalytic system is still available, although the rate of some reaction which depends on it is altered. Again when a substance accumulates, as β-alanine does with zinc deficiency, this does not imply a corresponding change in the catalytic system. It is probably due to a change in the concentration of some

other reactant in a reaction chain, and it is probable that with respect to β-alanine the catalytic system is the same in the plants supplied with full and with deficient nutrients.

Clearly, the accumulation or depression of any particular substance does not necessarily correspond to changes in the catalytic system. Since this is the case, then it is clear that with any particular species the composition of the amino acid pool will only be characteristic of that species with a particular set of circumstances. It is certainly possible that an amino acid that is identified under certain temperature and nutritional conditions will not be found with different circumstances that do not affect the catalytic system but which influence the rates of the reactions which depend on this system.

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