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"SOME ASPECTS OF NITROGEN METABOLISM IN BARLEY, WITH PARTICULAR REFERENCE TO THE POTASSIUM-DEFICIENT PLANT."

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INTRODUCTION

In 1936, Richards and Templeman (35) at this Institute, made a general survey of certain nitrogenous compounds in barley leaves and of the modifications induced by potassium deficiency. This work, in which the organic nitrogenous fractions studied were protein-N, amino-N and amide-N, confirmed the fact that under potassium-deficient conditions, free amino-acids in plants accumulate. They pointed out that much of this accumulation arises from the rapid hydrolysis of protein in the older leaves rather than from any general disability of the deficient plant to synthesise protein, as had previously been assumed.

Following the comparatively recent introduction of the method of paper chromatography to amino-acid analyses, Richards and Berner (in the press) used this method in 1949-1950 to continue the nitrogen metabolism studies, making a qualitative survey of the free amino-acids present in leaves of barley, particularly in relation to potassium status. On chromatograms of potassium deficient plants they observed an unidentified ninhydrin-positive substance occurring at some stages in life-history in concentrations equal to and even exceeding those of the more abundant amino-acids; it was shown to be basic in character.

Part I of this paper describes the method

by which this substance has been isolated and identified as putrescine.

Earlier work had shown that in potassium-deficient barley, tillers fail to survive beyond a certain stage, but meristematic activity results in new tillers, which in their turn are similarly checked. This and other phenomena led to the view that under potassium-deficient conditions, certain substances accumulate until they reach toxic levels, when death of leaves and even of whole tillers might supervene. At first attention was focussed entirely on inorganic ions, and indeed it has been shown (Richards, unpublished) that inorganic phosphate at least does accumulate in this way to deleterious levels when potassium is deficient; under these conditions the simple restriction of phosphate supply (so that considerable accumulation becomes impossible) results in much improved growth and a far healthier appearance of the leaves. The identification of putrescine however introduces a new factor, and the possibility needs to be considered of the plant producing toxic organic substances in its own metabolism. Hence it seemed desirable to determine whether putrescine, at the concentrations in which it occurs under potassium deficiency, is actually harmful to the plant. To this end putrescine has been artificially introduced into high potassium plants, and the results of so doing are described in Part II of this paper.

Part II also sets out further experiments

designed to reveal the place of putrescine in the metabolism of potassium-deficient plants e.g. experiments concerned with the utilization of putrescine by high potassium plants, its distribution in low-potassium plants, and the effect of low-potassium nutrition on a few species other than barley.

Since Richards and Berner (in the press) found that rubidium and sodium largely prevent the accumulation of the unidentified substance now shown to be putrescine, more detailed results were now sought on the comparative effects of the alkali metals in this action.

Finally, experiments have been carried out in which certain amino-acids were fed to barley with the object of elucidating the metabolic changes giving rise to putrescine in potassium deficiency.

PART I

THE ISOLATION AND IDENTIFICATION OF PUTRESCINE FROM POTASSIUM-DEFICIENT BARLEY

ISOLATION AND IDENTIFICATION OF PUTRESCINE FROM POTASSIUM-DEFICIENT BARLEY

The isolation of putrescine from leaves of potassium-deficient barley has already been briefly reported by Richards and Coleman (33). A detailed account is given here.

The plant material used had been collected by Dr. F. J. Richards in 1951 from barley grown in sand culture by the method described in Part II (page 16) of this paper. Plants were of two nutrient types, a calcium type in which nitrates and phosphates were given in the form of calcium salts and an ammonium type in which they were given as ammonium salts. Potassium was supplied at the K_3 level, (or 1/9 of K_1, a level designed to give optimal growth). The plants had been harvested at about the eighth or ninth leaf stage, when symptoms of potassium deficiency were very pronounced. The tops were divided into green laminae, moribund leaves and stems, oven-dried at 80° and stored in air-tight jars.

Preliminary experiments

Two-dimensional chromatograms of extracts of this material established that the moribund leaves of plants grown in either calcium or ammonium type nutrient solutions contained the greatest amount of the unidentified substance. The oven-dried plant material was ground in a C & N Junior Mill (1 mm. sieve), re-dried, and again stored in air-tight jars.

Extraction. A sample (0.3 g.) of this material was ground in a mortar with 75% (v/v) aqueous ethanol. The grinding, which occupied 15-20 minutes, was continued until extraction was complete. The extract was filtered from the insoluble material, which was washed with additional solvent. The combined extract and washings (about 10 ml.) were evaporated to dryness under reduced pressure, and then taken into solution in 0.1 ml. water. Satisfactory two-dimensional chromatograms were obtained with 6 μl. of this solution.

Paper chromatography. The chromatograms were prepared by a modification of C. E. Dent's method (10). Whatman No. 1 filter paper (22½" x 18½") was used for two-dimensional chromatograms with the following solvents : (1) analytical grade phenol saturated with water at room temperature (2) 'collidine', a mixture of 2:4:6 'collidine'/2:4 lutidine :: 1/1 (v/v) shaken with an equal volume of water.

The chromatograms were developed by descending chromatography in cabinets manufactured by Shandon Scientific Company, London. These cabinets are designed to allow the development of up to ten chromatograms in one operation. A maximum of six were run in these experiments. For convenience of operation, a separate cabinet was used for runs in each solvent.

The plant extract was applied with a pipette to the paper near one corner, at distances, according to the number of sheets run in pairs, of 9, 10.5 or 12 cm. from either edge.

The application was controlled to give a spot of 15 mm. maximum diameter. The moist spot was dried by careful warming and the paper placed in the first cabinet for 2 hours, allowing it to approach equilibrium with an atmosphere saturated with water and phenol. This atmosphere was produced by placing a solution of water saturated with phenol in open glass dishes in the bottom of the cabinet. The phenol (30 ml. per sheet) was then run into the solvent trough, through a stoppered hole in the glass top of the cabinet, and the chromatogram allowed to develop for 24 hours. The cabinet was operated at room temperature, and the average effective distance of the run in the 24 hours was 13". The paper was then removed from the cabinet and partially dried in a current of warm air in a fume cupboard for about 3 hours. Remaining traces of the solvent were removed by heating in an oven at 90° for about 1 hour. The paper was then placed in the second cabinet, this time being brought to equilibrium with air saturated with water and 'collidine'. The 'collidine' was run into the solvent trough and the chromatogram allowed to develop for 40 hours. The average effective distance of the run in this time was 15". The paper was removed from the cabinet and dried in a current of warm air in a fume cupboard for about 5 hours. When dry, it was sprayed with a solution of 0.1 per cent. ninhydrin in ethanol and colour reactions were hastened by heating at 90° for 7 minutes. Fig. 3

shows diagrammatically the position on a two-dimensional chromatogram of putrescine in relation to the normally occurring amino-acids.

Some chemical properties of the unidentified substance

To determine if the unidentified substance was an α-amino-acid, use was made of the method of Crumpler and Dent (9). A two-dimensional chromatogram of the extract of moribund leaves was prepared in which basic copper carbonate had been dusted onto the paper in the path of the amino-acids in the phenol run. After this treatment, the unidentified substance gave a normal ninhydrin colour compound; no colour compounds were formed by the α-amino-acids, which were retained as copper salts. The unidentified substance was therefore not an α-amino-acid.

A comparison was made of the R_F values of this substance run in phenol, (with and without an atmosphere of ammonia), and in 'collidine', with the reported R_F values of naturally occurring amines (Bremner and Kenten), (5). Of these amines putrescine had R_F values which gave the closest agreement. To make a more accurate comparison of the R_F values of these substances in phenol and 'collidine', a two-dimensional chromatogram was prepared of a mixture of putrescine and an extract of green leaves containing only a moderate concentration of the unidentified substance. 30μg. of putrescine dihydrochloride (manufactured by Hoffman-La Roche & Co., Ltd., Switzerland) were used. As a control

a further chromatogram of the extract of green leaves was run without the addition of putrescine. From this experiment, the R_F values of the unidentified substance in the solvents phenol and 'collidine', were found to be indistinguishable from those of putrescine.

Isolation of unidentified substance by displacement chromatography on an ion-exchange resin

The isolation of the unidentified substance from an extract of leaves of potassium-deficient barley was first attempted by the use of a cation-exchange resin. A satisfactory separation was not effected with the elutant solutions tried and the use of this type of resin was discontinued. At this time, a strong anion-exchange resin ('Permutit' special sulphonated polystyrene resin) became available, and a satisfactory method of separation was developed using this resin. The method was an adaptation of that of Moore and Stein (24), who separated basic amino-acids on an anion-exchange resin column by displacement chromatography.

A resin column was prepared as follows : 50 g. moist sulphonated polystyrene resin (500 - 1500 mesh per inch) was purified by washing first with ten times its volume of 4N HCl and then with distilled water until all free acid was removed. A slurry (suspension of 1 part resin to 2 parts water) was poured into a glass tube, 0.9 cm. internal diameter and 30 cm. long, plugged at one end with glass wool. To this end, rubber tubing and a pinch-cock had been fitted to control the flow of effluent. Sufficient slurry was added to the tube to form a 15 cm. resin column. After filling with water to exclude air, and corking, the tube was inverted and the resin

allowed to settle. Return of the tube to the working position, with final settling of the resin, gave a uniformly packed column.

In an exploratory experiment, the free ninhydrin-reacting substances were extracted from 15 g. oven-dry moribund leaves of potassium-deficient barley by the method previously described. The ethanolic extract (750 ml.) after evaporation to dryness under reduced pressure, was taken into solution with 5 ml. water. This solution was passed through the resin column, with the addition of distilled water, at a rate of about 50 ml./hr. The effluent liquid was found by chromatography to be free from the unidentified substance. The ninhydrin-reacting substances were displaced from the column with solutions prepared from volatile constituents. These solutions were passed through the column at room temperature at a rate of about 50 ml./hr. in the following order : 0.1N hydrochloric acid (300 ml.), 0.2M ammonium acetate (250 ml.), 0.2M ammonium formate (200 ml.), dilute ammonium hydroxide (1% ammonia sp. gr. 0.880) (200 ml.), and 0.2M ammonium carbonate (1000 ml.). The column was washed with 250 ml. water before each change of elutant solution. The effluent was collected manually in about 50 ml. fractions.

The course of the separation of the unidentified substance from other ninhydrin-reacting substances in the barley extract was followed by means of one-dimensional paper chromatograms of the effluent fractions on Whatman

No. 1 paper, with the solvents phenol and 'collidine'. A large proportion of the ninhydrin-reacting substances was displaced by the hydrochloric acid. Small amounts were removed by the ammonium acetate and formate. The remaining impurities and a small amount of the unidentified substance was displaced by the ammonium hydroxide solution. The ammonium carbonate displaced the unidentified substance free from other ninhydrin-reacting compounds.

An aliquot (100 ml.) of the effluent ammonium carbonate was partially evaporated to remove the ammonia, acidified with hydrochloric acid and evaporated to dryness. A solution prepared from this residue gave a very intense colour with ninhydrin and the R_F values were found to be unchanged by the treatment.

To obtain a further amount of the unidentified substance for the purpose of identification, a sample (60 g.) of oven-dried moribund leaves, from plants grown in a calcium type nutrient solution, was ground and the free ninhydrin-reacting substances extracted with 75% ethanol. The extract (2000 ml.) was evapora ted to dryness under reduced pressure and taken into solution in 20 ml. water. This solution, with the addition of 0.1N hydrochloric acid was passed through a sulphonated polystyrene resin column (0.9 x 15 cm.) prepared by the method previously described, and regulated to flow at a rate of about 50 ml./hr. The effluent solution was found to be free from the unidentified substance.

The ninhydrin-reacting substances were displaced

from the column with 0.1N hydrochloric acid (500 ml.), dilute ammonium hydroxide (1% ammonia sp. gr. 0.880) (500 ml.), and 0.2M ammonium carbonate (2000 ml.) solutions. 500 ml. water was passed through the column between each pair of elutant solutions. The course of the separation was followed by means of one-dimensional chromatography as in the preliminary experiment. A good separation was obtained in this case without the inclusion of ammonium acetate and formate.

As before, the effluent ammonium carbonate solution was boiled until free from ammonia, then acidified with hydrochloric acid and evaporated to dryness. A solution of the unidentified substance was obtained by dissolving this residue in 20 ml. distilled water. An aliquot (5 ml.) of this solution was made strongly alkaline with sodium hydroxide and steam distilled. The distillate, which was strongly basic, was boiled to remove traces of ammonia, acidified with hydrochloric acid and evaporated to dryness. The crystalline solid obtained was dissolved in a minimum quantity of water, a little alcohol was added and the substance precipitated by the addition of acetone. The product was filtered, washed with acetone and dried.

The remaining 15 ml. of solution containing the unidentified substance was similarly treated, and the combined product was re-distilled and re-precipitated, to give approximately 100 mg. of a white crystalline solid.

Identification of the isolated substance

A solution of the free base of the substance was obtained by steam distilling 80 mg. of the crystalline solid and concentrating the distillate, at a low temperature, to 30 ml. This solution was used to prepare the picrate, chloroplatinate and the benzoyl derivative. The corresponding salts of putrescine were prepared for melting point comparisons. The melting points were determined on a microstage melting point apparatus.

Hydrochloride. The melting point of the hydrochloric acid salt of the unidentified substance was examined. The crystals turned brown at 285° and did not melt below 290°.

Picrate. Excess of a saturated solution of picric acid was added to 2 ml. of the solution of the unidentified base until precipitation was complete. The crystalline product was filtered, washed with cold water and re-precipitated from hot water. The crystals were dried in a desiccator and the melting point examined; they turned brown and decomposed at 250°.

Chloroplatinate. Excess of a saturated solution of chloroplatinic acid was added to 2 ml. of the solution of unidentified base and the mixture warmed until clear. On cooling in an ice-bath, crystals of chloroplatinate were formed. The product was filtered, washed with cold water, dried and purified by re-crystallization from

from alcohol. The crystals turned brown at 230° and did not melt below 275°.

From a comparison of the melting point data, the hydrochloride, picrate and chloroplatinate of putrescine were indistinguishable from the corresponding salts of the unidentified substance.

Benzoyl derivative. The dibenzoyl derivative of putrescine was prepared by adding 8 drops of benzoyl chloride to 100 mg. of putrescine dihydrochloride in 20 ml. of 5 per cent. sodium hydroxide solution. The mixture was shaken persistently, and the soft lumps of precipitate broken up with a glass rod, until the precipitate had become hard and granular and the odour of benzoyl chloride had entirely disappeared. The crystals were filtered and washed three times with 2 ml. cold water and dried in a vacuum desiccator. The product was re-crystallized by dissolving in 10 ml. hot anhydrous benzene, filtered while hot and allowed to cool. The crystals which formed on cooling were filtered, washed with cold benzene and dried at 100° for 10 minutes.

The benzoyl derivative of the unidentified substance was prepared by adding 4 drops of benzoyl chloride to 10 ml. of the solution of the free base to which had been added about 0.5 g. of sodium hydroxide. The mixture was treated as before and a crystalline product obtained. The melting points of the benzoyl derivatives of putrescine and of the unidentified

substance were identical. In both instances melting commenced at 177.5°, and at 178.5° the mass of fine crystals had melted. Equal quantities of the two products were finely ground in an agate mortar, and the melting points of the mixture determined. The melting point was unchanged by this treatment, giving a proof that the substances were identical.

X-ray powder photographs. Identical x-ray photographs from the hydrochlorides of the two substances confirmed the identification of the substance isolated from potassium-deficient barley as putrescine. Thanks are due to the Pedology Department of Rothamsted Experimental Station for taking these photographs, which are reproduced in Fig. 1.

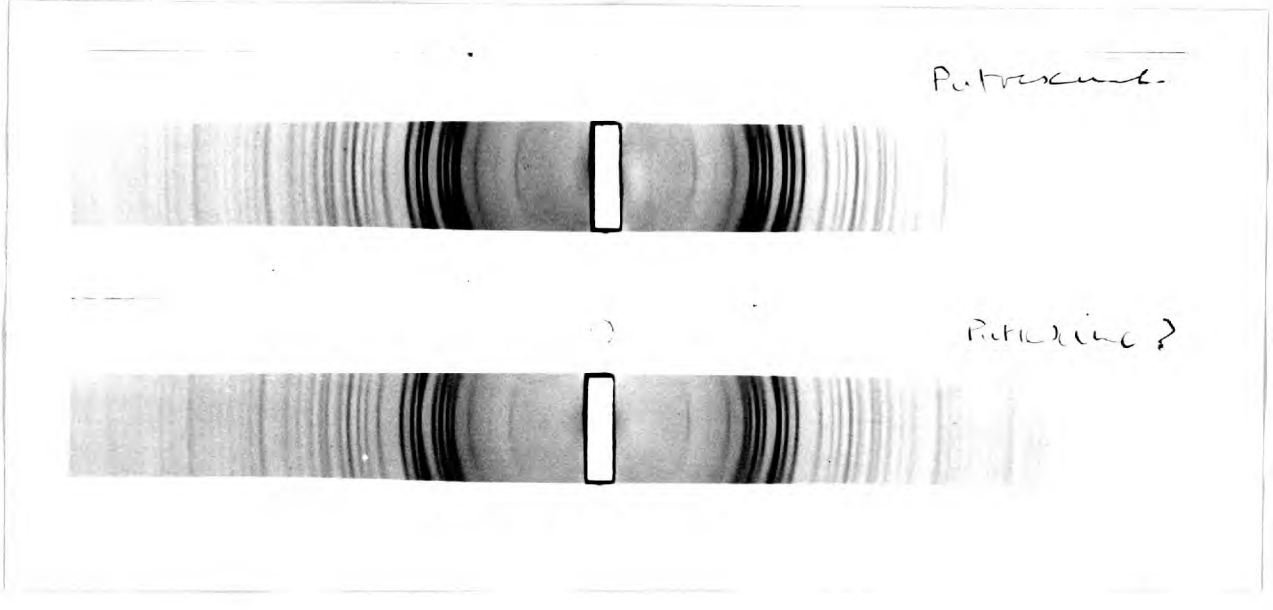


Fig. 1

PART II

THE PLACE OF PUTRESCINE IN THE NITROGEN METABOLISM OF POTASSIUM-DEFICIENT BARLEY

EXPERIMENTAL METHODS

Experiments were carried out with plants grown in sand culture in the summers of 1952 and 1953, and in water culture from February to July 1953, at Rothamsted Experimental Station, Harpenden.

Sand Culture - Summer 1952

Barley, var. 'Spratt Archer', flax, var. 'Royal', red clover (Trifolium pratense) and wheat, var. 'Atle Spring', were grown in sand culture in pots in the open. Ten inch glazed earthenware pots were used, containing about 30 lbs. of washed silver sand. To control the application of nutrient solutions, a 2½" porous pot was sunk almost to its rim in the sand in the centre of the larger pot. Each pot was provided at the bottom with a tubulure closed with a rubber bung, which carried an L-shaped glass tube leading to a glass bottle; this arrangement enabled any water percolating to the bottom to be drained, and subsequently to be returned to the pots.

Barley and wheat seeds of a uniform size were sown on May 2 at a depth of ¾", nine seeds per pot. Flax and red clover were sown at the same time at a depth of ¼", 36 seeds per pot. The seeds were planted in a circle between the rims of the 10" and 2½" pots. Nutrient solutions were added to all pots on May 9. Water when needed was given as tap water. About 2 week

weeks after germination, wheat and barley plants were thinned to the three best plants per pot. About 3 weeks after germination, flax and clover plants were thinned to 20 and 6 best plants per pot respectively. In all, 152 pots of barley and 12 pots each of wheat, flax and clover were sown, comprising 10 nutritional treatments.

A second set of barley seeds was sown on July 7; in all, 20 pots comprising 4 treatments.

Three main types of solution were employed : (1) a calcium type in which nitrate and phosphate were given in the form of calcium salts; (2) an ammonium type in which nitrate and phosphate were given as ammonium salts, with a small addition of calcium; (3) a sodium type in which nitrate and phosphate were given as sodium salts, with a small addition of calcium.

Two levels of potassium were used in each of the three types of solution: K_1, designed to give optimal growth, and K_3, (1/9 of K_1). To some of the pots at the K_3 level, sufficient sodium (or lithium, or rubidium) was added to bring the total alkali metal equivalent up to that of K_1.

Table I shows the various nutritional treatments and the number of pots of each used in the sand culture experiments in the summer of 1952. The symbols used to denote the treatments are composed as follows, after the method of Richards and Berner, (in the press). The solution type is indicated first and followed by a colon, after which are shown the potassium level and any addition

of lithium, sodium or rubidium. Thus the symbol Ca:K_3Na stands for the calcium type of solution, potassium at the K_3 level, and sodium equivalent to the difference in potassium between K_1 and K_3 added as a possible supplement to potassium.

Sand Culture - Summer 1953

Barley, (Cambridge mildew-resistant variety No.B2118), flax, var. 'Royal', red clover (Trifolium pratense) and wheat, var. 'Atle Spring', were grown in sand culture, following the same procedure as in the summer of 1952. The seeds were sown on May 4 and the nutrient solutions applied on May 11. Water when needed was given as tap water. The plants were thinned as in the previous year. In all, 65 pots were sown, comprising 4 nutritional treatments. The calcium type solution was used throughout. Barley, flax and red clover were grown with 2 levels of potassium (K_1 and K_3). Wheat was grown at 4 levels of potassium (K_1, K_3, K_4 and K_5). Table II shows the nutritional treatments and the number of pots of each used in this group of experiments.

TABLE I

Sand Culture Experiments - Summer of 1952

Nutritional Treatments and the Number of Pots of each

	Levels of alkali metals					Levels of phosphorus	
Solution type	K_1	K_3	K_3Li	K_3Na	K_3Rb	P_1	P_3
Ca	31	35	3	4	1	2	5
NH_4	33	35	3	4	-	-	-
Na	27	27	-	-	-	-	-

TABLE II

Sand Culture Experiments - Summer of 1953

Nutritional Treatments and the Number of Pots of each

	Levels of potassium			
Solution type	K_1	K_3	K_4	K_5
Ca	18	31	6	10

Table III gives the amount of each salt added per pot in the sand culture experiments. To all pots were also added $FeCl_3$ as required and 0.01g. $MnSO_4.4H_2O$.

TABLE III

Nutritional Scheme for Sand Culture Experiments - 1952, 1953

1. **Basal nutrients** (g. per pot)

Ca			NH_4	
$Ca(NO_3)_2 4H_2O$	..	12.64	NH_4NO_3	.. 4.00
$CaH_4(PO_4)_2 H_2O$	..	0.89	$(NH_4)_2HPO_4$	.. 0.05
$CaCl_2.6H_2O$	..	0.50	$NH_4H_2PO_4$	.. 0.77
$MgSO_4.7H_2O$	..	1.25	$CaCl_2 6H_2O$	.. 0.50
			$MgSO_4 7H_2O$	.. 1.25

Na	
$NaNO_3$	.. 9.10
$Na_2HPO_4 12H_2O$	.. 0.18
$NaH_2PO_4 2H_2O$	.. 1.04
$CaCl_2.6H_2O$	.. 0.50
$MgSO_4.7H_2O$	.. 1.25

2. **Potassium levels** (g. per pot)

	K_1	K_3	K_4	K_5
K_2SO_4	1.85	0.21	0.07	0.023

3. **Other alkali metals** (g. per pot)

Li_2SO_4	1.04
$Na_2SO_4 10H_2O$	3.04
Rb_2SO_4	2.52

4. **Phosphorus levels** (g. per pot)

	P_1	P_3
$CaH_4(PO_4)_2.H_2O$	0.89	0.099

Water Culture - Feb.-Jly. 1953

Barley, var. Cambridge B2118 was grown in water culture under glasshouse conditions from February until July, 1953. The plants were grown in tanks lent by Dr. F. C. Humphries of the Botany Department, Rothamsted Experimental Station, a type used by him in mineral nutrition studies. They are of 12.0 litres capacity, made from asphalt and asbestos. The plants were supported in the nutrient solution by waxed cotton netting suspended over a wooden frame ($17\frac{3}{4}$" x $7\frac{1}{2}$" x $\frac{3}{8}$"). The wooden frame, which contained four longitudinal slits ($16\frac{1}{2}$ x $\frac{1}{4}$") had been thoroughly impregnated with paraffin wax and then painted with bitumastic paint. 72 plants were grown in each tank. The seed was first germinated in germination dishes and uniform plants transferred to the tank on the 4th day. Plantings were done at 7 day intervals. Experiments were carried out on plants between the second and fourth leaf stage. Calcium type nutrient solution was used with two levels of potassium (K_1 and K_3).

Peas, commercial garden variety, were grown in water culture, under glasshouse conditions. 72 seeds were planted on waxed cotton netting in an asphalt tank. Calcium type nutrient solution was used with potassium at the K_1 level. The plants were given putrescine through the roots on the twentieth day.

Red clover, (Trifolium pratense) was grown in water culture under glasshouse conditions. About 200 seeds were germinated in germination dishes and 76 uniform plants transferred to a nutrient solution in an asphalt tank,

the plants being supported by waxed muslin (in place of the cotton netting used for the barley and peas), with the roots threaded through 1/10" holes. After three weeks, they were transferred to ½ litre wide-mouth bottles, one plant per bottle. Waxed corks with a large central hole, and halved vertically in the centre, supported the plants in the nutrient solution. Light was excluded from the roots by wrapping the bottles (54 in all) with heavy water-proofed paper. Calcium type nutrient solution was used with potassium at the K_1 level; the solution was renewed each week.

Table IV gives the amount of each salt added per 10 litres of solution.

TABLE IV

Nutritional Scheme for Water Culture Experiments

1. Nutrients other than potassium (g. per 10 litres of solution)[x]

Ca	
$Ca(NO_3)_2 \cdot 4H_2O$	8.43
$CaH_4(PO_4)_2 \cdot H_2O$	0.60
$CaCl_2 \cdot 6H_2O$	0.33
$MgSO \cdot 7H_2O$	0.83
$MnSO_4 \cdot 4H_2O$	0.007
$ZnSO_4 \cdot 7H_2O$	0.001
H_2BO_3	0.012
$FeSO_4$	0.021

2. (g. per Potassium levels 10 litres of solution)[x]

	K_1	K_3
K_2SO_4	1.23	0.13

[x] Distilled water was used in preparing these solutions and in watering the plants

BARLEY. SOME SYMPTOMS OCCURRING IN SAND CULTURE - 1952

Fig. 2 shows the differences in the growth of high (K_1) and low (K_3) potassium plants of the three solution types in the last week of June.



$Ca:K_1$ $Ca:K_3$ $NH_4:K_1$ $NH_4:K_3$ $Na:K_1$ $Na:K_3$

Fig. 2

The high-potassium plants of all three solution types made vigorous growth and generally had a healthy appearance. During July, those growing in the calcium and ammonium solutions were heavily infected with mildew. Slight differences between them were noticeable. Plants of the ammonium type had at first a more spreading habit than the other two types.

The $Ca:K_3$ plants made good growth in the first three weeks, but became yellowish in colour and developed brown speckles on the leaves by the fourth-fifth leaf stage. White areas had appeared on the leaf

blades by June 18, by which time the brown speckles which had been prevalent two weeks earlier, were no longer appearing on the newly developed leaves. Similar brown speckles again were seen on these plants during the first week of July.

The $NH_4:K_3$ plants made very poor growth during the first two weeks, but there was a marked improvement during the third week. Until the middle of June they were much greener than the $Ca:K_3$ plants, although many white areas had appeared on the leaves by June 18.

Fair growth was made in the $Na:K_3$ solution up to the middle of June, and many tillers were produced during late June and early July. The colour here was noticeably paler during early growth than in the $Ca:K_3$ or $NH_4:K_3$ plants. A few white areas were found on the leaf blades on June 18. Similar areas had only occasionally been seen on $Na:K_3$ plants in previous seasons (private communication from Dr. F. J. Richards.)

General procedure used in amino-acid analysis

Unless stated otherwise, analysis of the free amino-acids of the plants was made from extracts of the last fully expanded leaves.

In most cases fresh material was used for the extracts, which, after drying, were stored in a desiccator over phosphorus pentoxide until chromatographic analyses could be carried out. In a few instances, oven -dried material was extracted.

Two-dimensional chromatograms of these preparations were run, using the method described on page 5.

Quantitative determinations were not attempted, but some indication of the relative amounts of the various ninhydrin-reacting substances present was given by the sizes and colour intensities of the spots. The spots were graded into 9 intensity groups from 'extremely weak' to 'extremely strong'. Where a substance characteristically occurred on the chromatograms as a small spot, such as the unidentified substance "K" (See Fig. 3), only the intensity of the spot is taken into account. A good indication of the relative abundance of any one substance can be obtained by means of intensity groups, but quantitative comparisons between the several substances present are less reliable, since no account is taken of the different colour intensities formed with ninhydrin by similar amounts of these compounds.

Selection of solvent to replace 'collidine'. A lutidine/water solvent was used in place of 'collidine' for most of the analyses. This solvent was prepared by shaking 175 ml. of 2:4/2:5 lutidine (distilled once to remove impurities) with 100 ml. water. To test the suitability of this solvent for the separation of basic substances, one-dimensional chromatograms of ornithine, lysine and putrescine were prepared with its use and an improved separation was obtained. A two-dimensional chromatogram was then prepared of a mixture of 15 µg. each of aspartic acid, glutamic acid, glutamine, γ-aminobutyric acid, proline, putrescine, and arginine, using phenol as first solvent and lutidine as the second.

Lutidine gave a good general separation of the amino-acids. The dicarboxylic acids travelled much further in this solvent than in 'collidine', becoming quite clearly separated from the basic amino-acids. In addition it was later found that the separation of serine from glycine was also much improved. Considerable variability has since occurred in the effectiveness of this solvent, but it has not been ascertained under what conditions the best separation occurs. The temperature of the cabinet and the water content of the solvent may be important factors.

Fig. 3 shows the relative positions of the ninhydrin-reacting substances under discussion, when run on a two-dimensional chromatogram with phenol as first solvent and 2:4/2:5 lutidine as second solvent.

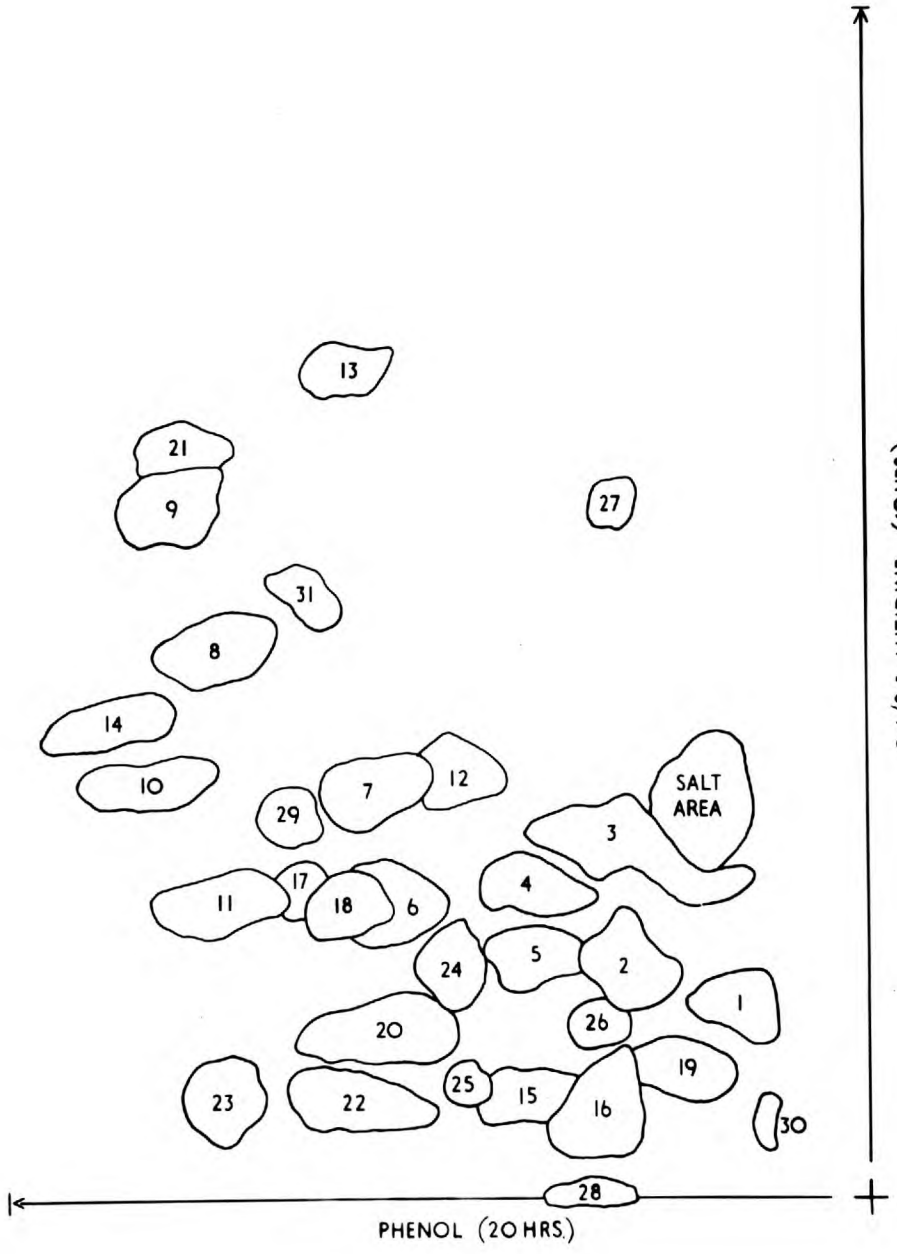


Fig. 3

1.Aspartic acid, 2.Glutamic acid, 3.Serine, 4.Glycine, 5.Asparagine, 6.Glutamine, 7.α-Alanine, 8.Valine, 9.Leucine Isoleucine, 10.Proline, 11.γ-Aminobutyric acid, 12.Threonine, 13.Tyrosine, 14.Pipecolinic acid, 15.Lysine, 16.Putrescine, 17.β-Alanine, 18.Citrulline, 19.Ornithine, 20.Arginine, 21.Tryptophane, 22.Unknown A, 23.Unknown B, 24. Unknown C, 25.Unknown D, 26.Unknown E, 27.Unknown F, 28.Unknown G, 29.Unknown I, 30.Unknown K, 31.Unknown L.

EXPERIMENTAL RESULTS

Toxic effects following the feeding of putrescine to barley

Previous work had suggested a correlation between the accumulation of putrescine in potassium-deficient barley, and the occurrence of the white areas often found on the leaves of these plants - a recognised symptom of deficiency. Experiments were commenced therefore to determine whether or not the amine is causally related to the development of this injury.

Putrescine was administered to the plants in a preliminary experiment through partially cut leaves and was readily absorbed in this way. The laminae were cut rather more than half-way across with a thin, sharp blade, at a distance of ¾" from the base, and then slit for a distance from the end of this cut towards the leaf tip; the midrib was included in the resulting flap, which was dipped into the feeding solution contained in a 1" x 1" glass specimen tube. The cut portion of the leaf was passed through a slit ⅛" x ⅝" in the cork and held in position by a plug of cotton wool. All corks were reduced in thickness to 1/3" for convenient operation, and impregnated with wax.

The plants used were grown in sand culture in calcium type nutrient solution with potassium at the K_1 level. Putrescine was given at the third leaf

stage, solutions of the dihydrochloride being presented at four concentrations (0.005%, 0.015%, 0.075% and 0.25%), using three plants at each concentration.

Effects of 0.25% and 0.075% solutions. Within a few hours white margins appeared at the cut edges of the leaves in the 0.25% solution and these were removed at intervals with a sharp, thin blade to allow absorption to continue. Similar white margins were formed on the leaves in the 0.075% solution after 24 hours. By the third day, sharply defined white areas, identical in appearance with those found on potassium-deficient plants, had developed on the distal part of the young leaves of those tillers directly fed with 0.075% solution, and on one occasion, also on leaves of a tiller only indirectly fed, i.e. the causal agent must have been translocated from one tiller to another. At the same time, the plants receiving the 0.25% solution were of a yellowish colour and growth was apparently checked. After a further two days the plants receiving the 0.075% solution were similarly affected. When putrescine was supplied in this way simultaneously to several leaves on one young high potassium barley plant, and time allowed for full development of the symptoms described above, the appearance of the plant suggested in quite a striking manner severe potassium deficiency.

No appreciable change could be seen in the

plants receiving either the 0.005% or 0.015% solution until the fifth day, when the fed leaves became moribund.

In the course of experiments conducted for other purposes, it was observed that in all cases where putrescine was administered in this manner at approximately 0.1% for several days, white areas developed on the young leaves. Plants similarly fed under glasshouse conditions developed these symptoms within 48 hours.

A two-dimensional chromatogram was prepared from a leaf extract of high potassium plants fed in this way, together with a control, to determine the free amino-acid composition. The chromatogram showed not only putrescine, but also an increase in glutamine, the formation of asparagine and of a substance not found in the control. The position of this substance (Unknown "B") on a two-dimensional chromatogram is shown in Fig. 3. An unidentified substance had already been found in the same position on chromatograms of potassium-deficient barley by Richards and Berner (in the press). Later work by the writer has confirmed the presence of this substance in low potassium plants.

Utilization of putrescine by barley

Graphical presentations of the free amino-acid composition of some of the plant materials analysed are shown on Figs. 4, 5, 6 and 8. The results are arranged in blocks headed by symbols showing the type of nutrient solution (Ca or NH_4) and the level of potassium supply, (K_1 or K_3). The columns within the blocks show the results of an analysis of a single plant extract. The relative amounts of the various amino-acids and related ninhydrin-positive substances per unit fresh weight, based upon intensity groups, are represented by shaded squares. A blank space indicates that no ninhydrin spot could be detected. Where a spot occurs on the chromatogram which cannot be identified with certainty, a query mark has been entered.

The changes in the free amino-acid composition resulting from feeding putrescine to high potassium plants

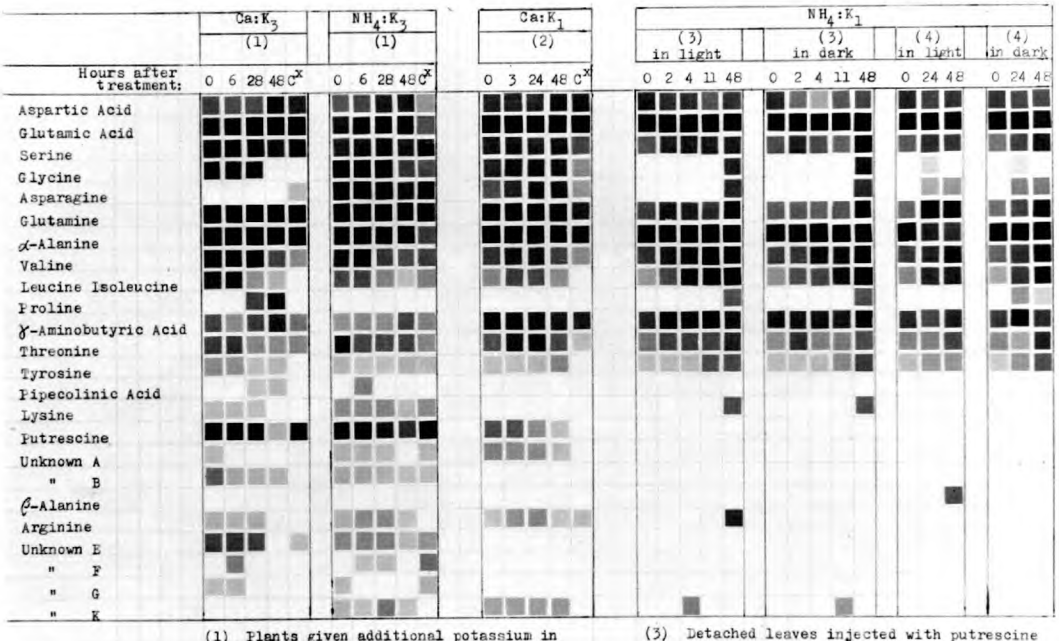


Fig. 4

suggest that putrescine can be utilized at least partially by these plants.

Fig. 4 shows the results of three types of experiment on putrescine utilization.

(i) Detached leaves injected with putrescine solution

The last fully expanded leaves of $\frac{NH_4:K_1}{(3)}$ plants were submerged in a solution of putrescine dihydrochloride (0.1%) immediately after cutting, and the air was removed from the intercellular spaces under reduced pressure. Injection occurred when the atmospheric pressure was restored. The leaves were then removed from the solution, washed, and supported in test tubes with their cut ends dipping into distilled water. In Fig. 4, the block headed $\frac{NH_4:K_1}{(3)}$ presents the amino-acid composition of the putrescine-injected leaves when maintained both in light and darkness for the periods shown. Block $\frac{NH_4:K_1}{(4)}$ shows a control experiment with water-injected leaves.

The experiment was unsatisfactory because insufficient putrescine was injected; it was not repeated. The increased concentrations of amino-acids within 48 hours suggest that marked proteolysis had occurred; and even if sufficient putrescine were introduced, this hydrolysis would be likely to prevent the detection of changes resulting from a slow rate of amine utilization.

(ii) Addition of potassium to K_3 plants. Sufficient potassium was added to the nutrient solution of potassium-deficient plants at a time when their putrescine content was high, to bring them up to three times the K_1 level. Fig. 4 $\frac{Ca:K_3}{(1)}$ and $\frac{NH_4:K_3}{(1)}$ shows the changes in the free amino-acid content of these plants during the 48 hours immediately following the addition of potassium.

In both sets of plants there was a marked reduction in putrescine content within 48 hours. Additional changes occurring in this experiment are discussed under the root feeding experiment below.

Because of the improvement in the growth of the plants, it is possible that sampling the last fully expanded leaves did not give accurate measurement of the reduction of putrescine, since putrescine distribution may not be uniform between young and old leaves. However, feeding experiments showed that putrescine is rapidly transported throughout the plants and therefore would soon pass into young tissue from the older leaves.

(iii) Feeding putrescine to high-potassium plants through the roots. $Ca:K_1$ barley grown in water culture was fed with putrescine at the third leaf stage. After removal from the nutrient solution, the roots were washed and placed in 0.1M putrescine dihydrochloride solution for two hours. They were then removed, again washed and placed in water for the periods

stated in Fig. 4, block $\frac{Ca:K_1}{(2)}$. Analyses were made from extracts of the whole plants, three plants being used on each occasion.

A moderate amount of putrescine was present at 0 hours; a noticeable reduction occurred during the first 24 hours, and a still further reduction during the second day.

Experiments (ii) and (iii) show that putrescine tends to disappear from barley supplied with adequate potassium, though it is accumulated there in potassium-deficiency.

Further, certain consistent changes were found in both these experiments. Where high potassium plants were given putrescine through the roots, valine, leucine, threonine and arginine increased in the first three hours and decreased in the period 24 to 48 hours. Asparagine and glutamine increased slightly within 48 hours. γ-Aminobutyric acid was probably reduced and an unidentified substance ("A" Fig. 3), presumably produced during the 2 hour feeding period, and present in the first sample, had decreased within 48 hours.

In experiment (ii) the potassium was applied on June 26 when the putrescine content in the plants was high, having slowly accumulated from early growth. In block $\frac{NH_4:K_3}{(1)}$ the amino-acid composition at 0 hours was typically that of a low potassium plant. The usual

modifications of the free amino-acid contents associated with potassium deficiency have been reported by Richards and Berner (in the press). They found that the changes which result from low potassium supply in barley include in general, decreases in the amount of dicarboxylic acids, a large increase of glutamine, variable increases in serine, glycine, asparagine, valine, leucine, tyrosine, arginine and lysine, and the occurrence of the substance now shown to be putrescine (Part I).

Taking into account the difference between the starting materials, the changes in amino-acid composition of the present experiment (ii), from 0 to 48 hours were consistent with those in experiment (iii). Asparagine, glutamine, valine, leucine and threonine were reduced and γ-aminobutyric acid increased. Further, in both the $Ca:K_3$ and $NH_4:K_3$ plants to which potassium had been added, the dicarboxylic acids increased during the 48 hours following the addition. The amino-acid composition at this time approached that of high potassium barley plants (Fig. 5). An unidentified substance - "A" (Fig. 3) was found in plants of both experiments (ii) and (iii); it was present in low potassium plants when their putrescine content was high, and was produced in high potassium plants when fed with putrescine. The unidentified substance "B" (see preliminary putrescine feeding experiment, page 30), also occurred in experiment (ii), in plants of both types. Other unidentified substances occurring in this experiment are referred to on page 62.

Pipecolinic acid. The identity of the substance reported as pipecolinic acid (Figs. 4, 5 and 6) has not been definitely established. Dr. F. J. Richards had observed it in 1949 (private communication) on chromatograms of 75% ethanol extracts of barley. Cook and Pollock (7) recorded an unknown amino-acid in barley steeping liquors, and this was later shown to be pipecolinic acid by Harris and Pollock (16). It was also found by Morrison in red clover(25) In the present experiments a substance which travelled to the same position on two-dimensional chromatograms, occurred occasionally in barley and frequently in wheat and red clover. That all these observations concern a single substance seems undoubted, for the ninhydrin product fades quickly in a very characteristic manner, just as/has been described for pipecolinic acid. Since the unknown compound and pipecolinic acid behave similarly in all these ways, there seems little doubt they are identical.

Recent observations of the rate of the reaction of this substance with ninhydrin throws doubt on the reliability of earlier records of its presence in barley extracts. It has been found to react with ninhydrin more slowly than other substances present, when developed at about 90°. When a chromatogram containing it was heated to a higher temperature, the intensity of the spot was markedly increased. Further tests showed that on many of the

early chromatograms, the colour reaction of the substance could not have been complete when recorded.

Some aspects of the occurrence of putrescine in potassium-deficient barley

The free amino-acid contents of high and low potassium barley sown on both May 2 and July 7, 1952, was investigated chromatographically, in order to determine the time of the first appearance of putrescine in $Ca:K_3$ and $NH_4:K_3$ plants, together with its distribution between the roots and tops. Chromatograms were also prepared of protein hydrolysates to determine whether the amine occurred as a constituent of the protein of these plants.

Putrescine was found in the first leaves of the $NH_4:K_3$ seedlings of both sowings, but could not be detected in either the first or second leaves of $Ca:K_3$ plants. The high proportion of basic substances generally in the first leaves of ammonium plants is also noteworthy. The calcium type plants nevertheless accumulated putrescine gradually during the first few weeks, and in fact the maximum concentration reached in both types at about the 7th leaf stage was approximately the same. While a moderate amount of putrescine was present in the 5th leaves of the $NH_4:K_3$ plants sown in July, the substance could only just be detected in the roots. No reason for this difference has been established, but a similar situation has been found in those K_1 plants fed with putrescine through the roots for 2 hours and subsequently transferred to water for 24 hours.

The tops of plants from each treatment ($Ca:K_1$ and K_3; $NH_4:K_1$ and K_3) sown on July 7 were sampled at the first, second and fifth leaf stages, extracts being made of the roots also at the final sampling. At this time, the K_3 plants were noticeably smaller than the K_1 but leaf symptoms had not developed. Samples were also taken on the fourth day from barley germinated in dishes without nutrients to compare their amino-acid contents with those of plants of the first leaf stage (i.e. 14 days from sowing). When sampling, the seedlings' seed reserves were removed.

The analyses showed that at the first leaf stage the $NH_4:K_1$ plants had a slightly higher total amino-acid content than the $NH_4:K_3$ plants, and that both of these had much greater contents than the $Ca:K_1$ and K_3 plants.

$NH_4:K_1$ plants. The very high total concentrations of amino-acids found in the early stages of these plants consisted mainly of an extremely large amount of glutamine, a large amount of glutamic acid, and moderate quantities of serine, glycine, asparagine, aspartic acid, α-alanine, valine and leucine. The most notable changes occurring between the first and the fifth leaf stages were a sharp fall in asparagine and glycine, a moderate fall in glutamine and a slight diminution of serine, valine, leucine, arginine and lysine. Asparagine indeed was not detectable in the fifth leaves, but was found at this time in the roots, which had less aspartic and glutamic acids and more glycine, asparagine and

glutamine than the leaves.

NH_4:K_3 plants. The composition of the NH_4:K_3 plants differed at the first sampling from that of the K_1, there being less glutamic acid and more glycine. General changes similar to those in the K_1 plants occurred between the first and fifth leaf stages. A small increase of valine and leucine between the second and fifth leaf stages is of particular interest. At the final sampling date the roots had greater amounts of asparagine, glutamine and γ-aminobutyric acid than the leaves. Putrescine, as mentioned above, was only just detectable in the roots though it occurred in moderate concentration in the leaves.

Ca:K_1 and K_3 plants. The amino-acid composition was similar at both potassium levels in these two plants. Unlike the NH_4:K_1 plants, lysine and leucine were absent, asparagine, valine and arginine greatly reduced, and glutamine, α-alanine and γ-aminobutyric acid somewhat diminished. Only small changes, consistent with the development of mild effects of potassium deficiency, occurred in the K_3 plants between the first and last sampling dates, (and none of note in K_1)

Seedlings. The amino-acid composition comprised a very large amount of glutamine, considerable amounts of glutamic and aspartic acids, valine and leucine, moderate amounts of asparagine, glycine, α-alanine and serine, and small amounts of threonine, proline, γ-aminobutyric acid, tyrosine, arginine and lysine.

The occurrence of much asparagine in the ammonium type plants at the first leaf stage, and its re-appearance later in plants affected by potassium deficiency, is consistent with the findings of Richards and Berner (in the press), who state that "in well-nourished barley, asparagine appears to be produced in more than trace quantities only when free ammonia is entering the plants more rapidly than it can pass into the usual channels of metabolism". Though the trends in glutamine were similar to those in asparagine, the changes were more gradual, and at the fifth leaf stage, the concentration was still high. Further, the distribution of these amides between the roots and leaves is noteworthy. In the NH_4:K_5 plants, the relative amounts were greater in the roots, and while the differences in their concentration were not so marked in ammonium plants sampled on later occasions, the substances at no time appeared in a higher concentration in the leaves than in the roots. There appeared to be a close parallel between the amounts of asparagine and glycine in the first and second leaves of the ammonium type plants, but this need not indicate any direct connection in metabolism, especially since considerable variability in their occurrence has been found on occasion. A relatively higher content of γ-aminobutyric acid was observed in the roots than in the leaves on a number of occasions.

Amino-acids in protein. To prepare protein hydrolysates, the plant residues left after extraction with 75%

ethanol were first freed from any possible remaining free amino-acids by additional grinding with 75% ethanol and filtering, and the insoluble fraction was hydrolysed by heating to 95° in a sealed tube for 48 hours with 6N hydrochloric acid. The free hydrochloric acid was then removed by evaporating to dryness under reduced pressure and a chromatogram prepared from a solution of the hydrolysate.

Chromatograms were run of hydrolysates from both high and low potassium plants of the May and July sowings. Putrescine was not detected on these papers. Moreover the chromatograms appeared identical, hence no evidence was obtained that potassium deficiency modifies in any way the basic protein structure. However, the relative amounts of the various amino-acids are interesting. Leucine, valine, tyrosine, lysine and arginine occurred in relatively larger amounts in the protein than in the free amino-acids; in fact, their higher content in the protein recalls strongly their similar comparative abundance in the free amino-acids of seedlings and of the first leaves of potassium deficient ammonium type plants, where excessive proteolysis would very likely be occurring.

The effect of low potassium supply on the free amino-acid composition of certain plant species

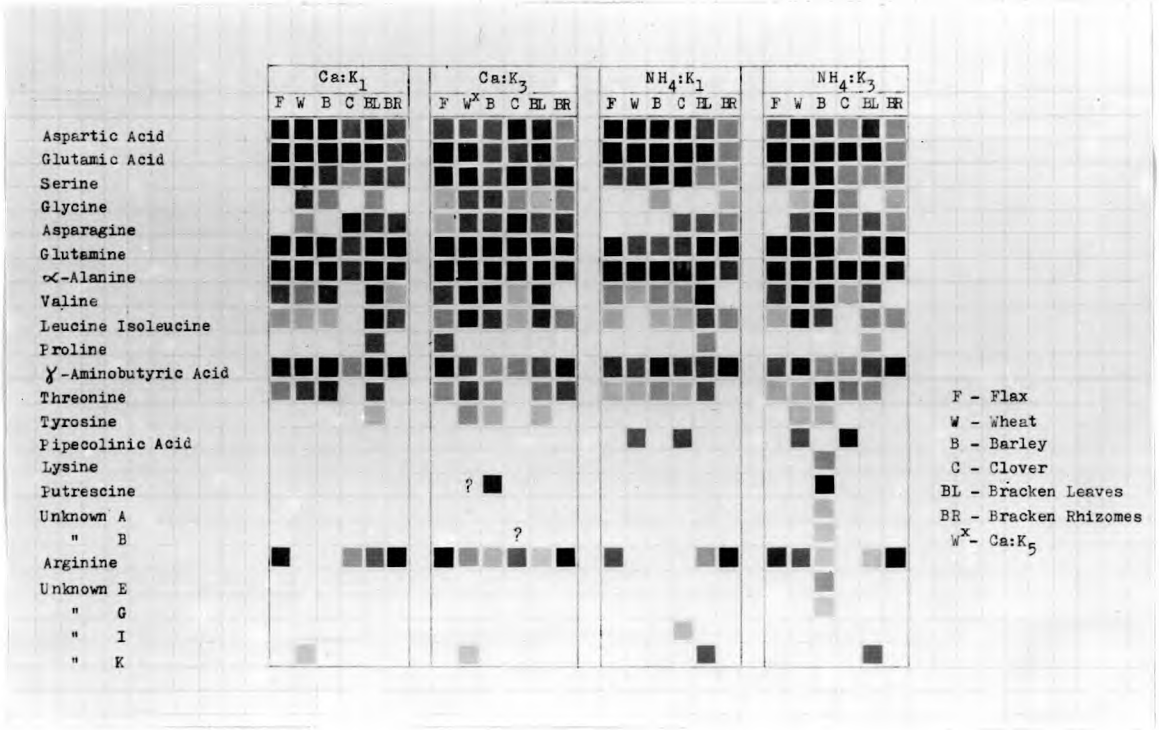


Fig. 5

Fig. 5 shows the free amino-acid composition of leaves of flax, wheat, barley, red clover and bracken grown in calcium and ammonium type nutrient solutions, at two levels of potassium (K_1 and K_3). The plants were sampled when those given the low supply showed symptoms of advanced potassium deficiency. Analyses were also made of the rhizomes of bracken. (Bracken material was supplied by Dr. W. W. Schwabe, of this Institute).

Wheat and Barley. Experiments with wheat ('Atle Spring') grown for two seasons at Rothamsted, indicate that its potassium requirement is much lower than that of barley. In 1952, wheat grown with potassium at the K_3 level, was reduced in size, but otherwise appeared healthy. When grown the following season with potassium at the much lower K_5 level, it showed comparatively slight leaf symptoms, while barley at the K_3 level was acutely affected. The chromatographic results for wheat and barley grown at these potassium levels in calcium type nutrient solutions are given in Fig. 5. The potassium-deficient wheat ($Ca:K_5$) shows an amino-acid content generally very similar to that of K_3 barley. The query mark indicates a very weak spot on the chromatogram in the position of putrescine : this was the first occasion on which evidence of putrescine production was detected in wheat. It has since been found in much higher concentration in a plant with characteristic white areas on the leaves; the amino-acid composition of this particular plant bore a striking similarity to that of typical low potassium barley. The unidentified substances "A" and "E" frequently found in K_3 barley were also present.

The $NH_4:K_3$ wheat was visually only slightly affected by potassium deficiency, but had higher contents of asparagine and glutamine and a lower content of glutamic acid than the corresponding K_1 plants. These and other differences caused by the low potassium supply are generally consistent with changes which occur in the amino-acid composition of barley under similar conditions.

Red clover. The chromatograms from red clover differed in a marked degree from those derived from the other species examined. Analyses of these plants at various stages of growth showed that where conditions favoured amide formation asparagine rather than glutamine accumulated; asparagine production is well-known to characterise the nitrogen metabolism of many legumes. The $NH_4:K_1$ and $NH_4:K_3$ plants (Fig. 5) which have lower asparagine contents than usual (cf. Fig. 5, $Ca:K_1$ and $Ca:K_3$), were sampled on August 11 when the nitrogen content of the nutrient solutions would be low, and therefore little nitrogen entering the roots. Very unsatisfactory growth was made by the $NH_4:K_3$ plants and symptoms of potassium deficiency were not well defined.

The amino-acid contents of the $Ca:K_1$ and $Ca:K_3$ plants sampled on July 16, 1953, were consistent with those found in 1952 in the corresponding calcium type plants and shown in the figure, though one or two extra substances were evident, / e.g. Unknown "I", Fig. 3. The amount of glutamic acid was reduced by potassium deficiency and aspartic acid, glutamine and serine were slightly increased, while lysine, valine and leucine, not visible on the K_1 chromatograms, were now detectable. A ninhydrin-reacting substance occurred on chromatograms of these plants in a position indistinguishable from an unidentified substance ("B"), found in K_3 barley. A chromatographically identical substance was produced in high

potassium red clover plants fed with putrescine, whether through the leaves or through the roots, (this is discussed in more detail on page 63). Further, the Ca:K_3 red clover plants grown in 1953 showed extreme symptoms of potassium deficiency and contained some putrescine, in addition to the unidentified substance "B".

Flax. Flax plants grown in either Ca or NH_4 type nutrient solutions had a much higher concentration of arginine than the species already mentioned, and the low potassium plants had even greater amounts. It occurs on some K_3 chromatograms as the most intense spot. In all the species examined, with the exception of bracken, potassium deficiency increased the content of arginine. It was observed that barley, wheat and clover (all of which under these conditions contain either putrescine or the substance "B" which is derivable from it cf. p 84) accumulate only small quantities of arginine, in contrast with flax. This suggests that putrescine may arise in these species from the breakdown of arginine.

Bracken. The free amino-acid composition of bracken does not appear to be affected greatly by the potassium supply. In the single comparison available the total concentration of amino-acids in the leaves of the high potassium plants was higher than in those from low potassium plants. The disproportion of the amino-acids in the rhizomes and leaves is striking.

Arginine, γ-aminobutyric acid and glutamine are apparently present in relatively higher concentrations in the rhizomes, but the remaining amino-acids are relatively more abundant in the leaves. It is possible that any or all of the three former substances are preferentially produced in the rhizomes, or they may be stored in this organ.

The free amino-acid composition of plants, other than barley, reported to contain putrescine

Analyses were carried out to determine whether or not the free amino-acid composition of plants which normally contain putrescine is similar in other details to that of barley plants containing putrescine. It had been anticipated that the unknown substances "A" and "B", Fig. 3 might be present. They were not found however; their apparent absence may possibly have been correlated with the low concentration of putrescine actually present; indeed this substance was itself often not detectable. The results of the survey made no direct contribution to the present study. Briefly, they are as follows :-

Ergot of Rye. (Claviceps purpurea).

The presence of putrescine in Ergot of rye was reported by Rielander (36).

In an extract of Ergot, the substances found were aspartic acid, serine, glycine, α-alanine, β-alanine, valine, leucine, proline, γ-aminobutyric acid and putrescine (?) γ-aminobutyric acid was present in an amount probably greater than the sum of all the remaining substances, and possibly was an end-product of catabolic processes involving host protein.

Atropa belladonna.

Putrescine was reported in Atropa belladonna by Goris and Larsonneau (15).

Chromatograms were prepared from extracts of leaves and roots of seedlings, and also from young leaves and roots of a mature plant. No putrescine could be detected from

the seedling, but a weak spot occurred in the position of putrescine on the chromatograms derived from both the roots and the leaves of the mature plant.

Citrus. (Orange juice)

Putrescine was isolated from orange juice by Herbst and Snell (18).

Chromatograms with well defined spots were difficult to prepare from the concentrated juice, but sufficient separation was obtained to show the presence of a large amount of proline, a moderate concentration of arginine, and of a substance occurring on the chromatogram in the position of the unidentified substance "D", Fig. 3, which gave a weak ninhydrin reaction and a strong Sakaguchi reaction. Putrescine was not present in sufficient concentration to be detectable.

Citrus. (Young leaves).

An extract was made from leaves of an actively growing shoot of an orange tree grown in a glasshouse at Rothamsted Experimental Station. The leaf sample was collected on January 30, 1953, at a time when light would be deficient. A particular feature of the amino-acid composition was an extraordinarily high content of proline and a moderate concentration of arginine. There were also two unidentified substances which occupied identical positions on the chromatogram to those of the Unknowns "C" and "D", (Fig. 3), found in barley plants artificially fed with arginine. Putrescine was again not found. It is of interest that asparagine and glutamine were present in about equal amounts in the actively growing leaves of this tree.

Addition of alkali metals to the nutrient solutions of barley as a supplement to low potassium supply

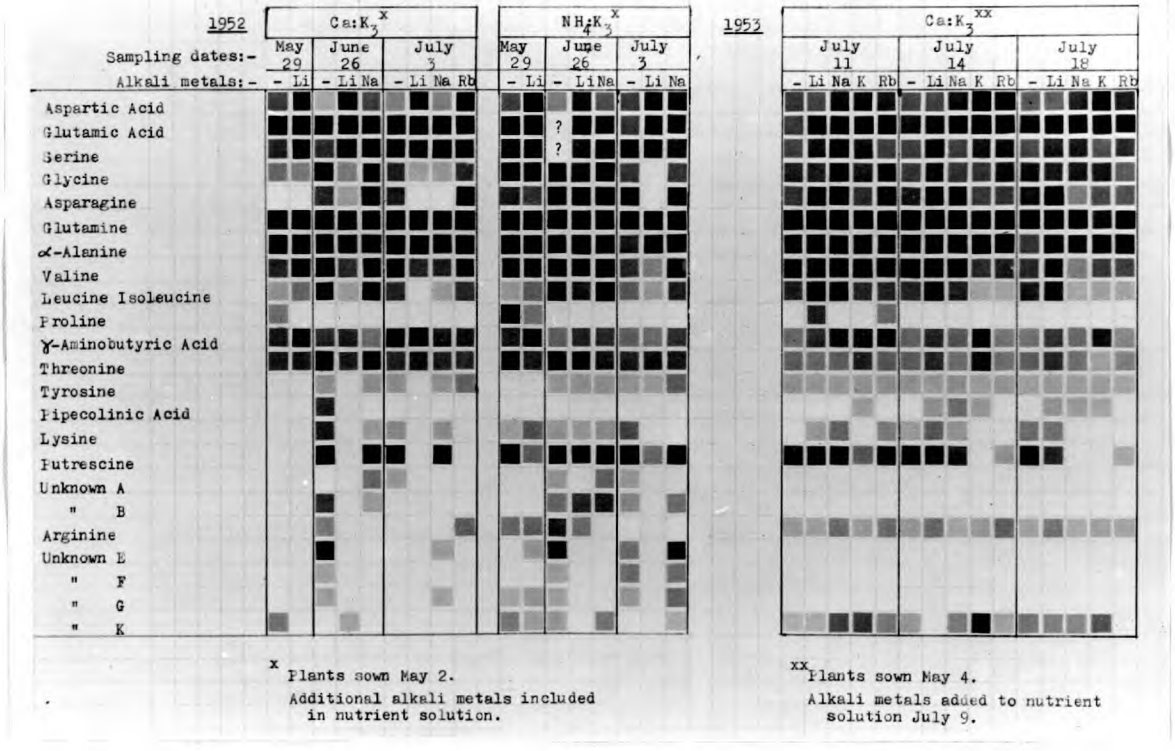


Fig. 6

Fig. 6 - (1952 experiment) shows the free amino-acid contents of treated plants at three dates of sampling.

Ca:K_3 and NH_4:K_3 plants. In the present experiments, the effect of the low potassium supply was not evident in the free amino-acid distribution of the Ca:K_3 plants on May 29, but the composition of the NH_4:K_3 plants was already somewhat affected. By June 26 the amino-acid

contents of these two sets of plants were however very similar, and characteristic of low potassium plants. From June 26 to July 3, the changes indicate a reduction in the severity of the disturbances. These plants served as controls in a number of small investigations designed to compare the efficiency of other alkali metals with that of potassium in preventing these aberrations in amino-acid metabolism, and especially in suppressing the accumulation of putrescine. The first series, whose results are described below, deals with barley grown throughout life at the K_3 level, but in the presence of sufficient lithium, sodium or rubidium to make the total alkali metal equivalent to that of K_1.

Effect of addition of lithium. The chromatogram from the Ca:K_3Li plants on June 26 suggests mild potassium deficiency, but no putrescine was detectable, nor indeed was it on any sampling occasion. The other basic substances associated with the Ca:K_3 papers (lysine and arginine) were similarly absent from those of Ca:K_3Li, as also were a series of spots due to unknown substances. Glycine, valine, leucine and asparagine seem usually to have been diminished by the lithium, but the dicarboxylic acids were increased. In all these respects the effect of the lithium is similar to that of extra potassium. The NH_4:K_3Li plants on the contrary had some putrescine at each sampling date, but the concentrations were low and the free aminoacid contents were not typical of low

potassium plants at either the May or July samplings. Even on June 26 when there was a moderate concentration of putrescine, the aspartic acid and glutamic acid contents were higher than in typical low potassium plants. Here again the changes consequent on lithium supply were generally in the same direction as those due to extra potassium, but were not so striking as where the basal solution contained excess calcium.

Effect of addition of sodium. The Ca:K_3Na and NH_4:K_3Na plants had free amino-acid contents similar to those of low potassium plants at each sampling date, (June 26 and July 3), but there was some reduction in the amounts of putrescine present as compared with the corresponding Ca:K_3 and NH_4:K_3 plants.

Effect of addition of rubidium. The Ca:K_3Rb plants made extremely poor growth (see page 56). Although leaf samples were taken on June 26 and July 3, difficulty was experienced in getting a good separation of their amino-acids and a satisfactory chromatogram was not prepared from the June sample. By July 3 growth had almost ceased, and the plants then sampled showed symptoms of extreme toxicity; hence these results were inconclusive. Nevertheless, putrescine was not detectable. The simultaneous accumulation to high levels of both the dicarboxylic acids and their amides is noteworthy, and according to Richards and Berner (in the press) is characteristic of rubidium toxicity.

When allowance is made for the difference in

composition of low potassium plants of the calcium and ammonium types, a close similarity is apparent in the effect of the addition of lithium to the Ca:K_3 and NH_4:K_3 plants. A similar relationship holds for those plants to which sodium had been added.

From the chromatograms of the Ca:K_3Li and NH_4:K_3Li plants, it is evident that their amino-acid distribution was little affected by potassium deficiency; at least they had sufficient alkali metal to perform whatever function in nitrogen metabolism is necessary to prevent putrescine accumulation, such as occurs in simple potassium deficiency. In the Ca:K_3Na and NH_4:K_3Na plants, there was also some reduction in the severity of these potassium deficiency effects. By reference to the size and condition of the plants during the last week of June (page 55) it will be seen, however, that in addition very marked adverse effects resulted from the specific toxicities of the alkali metals, and any beneficial effects they might have are consequently ill-defined. For example, those plants given rubidium were much reduced in size; therefore, although they were supplied with only the same amount of potassium as their K_3 controls, their internal content may well have been considerably higher, and the absence of putrescine be due to this cause instead of directly to the rubidium.

In a second small series of investigations designed for the same purpose, the technique was

consequently changed. Plants were grown at the K_3 level until severe symptoms of deficiency had appeared. An ample supply of one particular alkali metal was then made available to them, and the resulting changes in their free ninhydrin-reacting substances followed during the next few days. By these means varying dilution effects of the internal potassium due to differential growth were avoided.

External effect of addition of alkali metals to the original nutrient solutions of Ca:K_3 and NH_4:K_3 plants - 1952

Fig. 7 shows the growth differences induced in the calcium type plants by the last week of June.



Ca:K_1 Ca:K_3 Ca:K_3Na Ca:K_3Li Ca:K_3Rb

Fig. 7

In all cases treated plants were markedly different from their untreated controls. Sodium, (Ca:K_3Na and NH_4:K_3Na) caused the greatest increase in growth, especially in the early stages. On the other hand, lithium induced slower early growth but later a greater number of fully developed shoots. Both the Ca:K_3Na and the NH_4:K_3Na plants developed white areas on the leaf blades in the latter part of June, these being more prevalent in the ammonium type than the

calcium. In general appearance these plants closely resembled the Na:K_3 plants.

In the presence of lithium the margins of the leaves turned a dark brown between the second and third leaf stage, though the Ca:K_3Li plants developed the condition a few days sooner than the NH_4:K_3Li plants. This marginal necrosis appeared on all subsequent leaves.

Toxic symptoms developed even on the first leaves of the Ca:K_3Rb plants; growth was very slow and the plants turned a greyish-green colour. A large number of tillers were formed, but none survived to maturity.

Effect of addition of potassium to K_3 plants. In 1952 sufficient potassium was added on June 26 to the nutrient solution of K_3 plants, in an advanced stage of potassium starvation, to bring them up to 3 times the K_1 level. Fig. 4 ($\frac{Ca:K_3}{(1)}$ and $\frac{NH_4:K_3}{(1)}$) shows the resulting changes in the free amino-acid contents of these plants during the following 48 hours.

In both the Ca:K_3K and NH_4:K_3K plants a marked change was seen after 28 hours, an increase in glutamic acid and a decrease in the amounts of leucine and putrescine being evident. After 48 hours both dicarboxylic acids were increased, while their amides, together with putrescine, glycine, valine and leucine were diminished. Though a small amount of putrescine

still remained in both types of plants 48 hours after the addition of potassium, the general picture was already suggestive of that of high potassium plants and the appearance showed marked improvement. Existing leaves turned darker in colour and many more young leaves were developing. By the middle of July the shoots of these plants were almost as large as those of the original high potassium plants, though there was a purple colouring on the lower part of the stems. In this respect these plants closely resembled others grown under phosphorus deficiency.

Effect of addition of alkali metals to K_3 plants - 1953

Sufficient alkali metals were added to bring the total alkali equivalent up to that of K_1 level. Fig. 6 - (1953 experiment) shows the free amino-acid contents of treated plants at the sampling dates July 11, 14 and 18. Sulphates of alkali metals were added to the nutrient solutions of Ca:K_3 plants on July 9, when an advanced stage of potassium deficiency had been reached. In order to obtain representative data the various extracts were made from whole tillers.

Ca:K_3 plants. No important changes could be detected in the free amino-acid distribution of the Ca:K_3 plants (controls) during the week covering the sampling dates.

Ca:K_3Li plants. Two days after addition of lithium, the plants showed a very large increase in glutamine, a moderate increase in glutamic acid and γ-aminobutyric acid, and an increase in asparagine. By July 18, the only further important change was a slight fall in the putrescine concentration and a reduction of the excessive glutamine found a week earlier.

Ca:K_3Na plants. On July 11, the only noticeable change was an increase in asparagine, but since this amide later fell to below the control value, the significance of the rise is doubtful. By July 18 there was then a marked fall in asparagine, and also in valine, leucine, and putrescine (which in this particular sample was not detectable); some increase in glutamic acid, and possibly α-alanine, was also indicated.

Ca:K_3K plants. By July 11 (2 days after treatment), there were increases in the concentrations of glutamic acid, aspartic acid, γ-aminobutyric acid and threonine, reductions of glutamine and asparagine and a marked reduction of putrescine. By July 14, these changes were all accentuated, no putrescine at all being detectable; valine and leucine also had diminished in amount, and α-alanine increased. With the possible exception of threonine the amino-acid contents of these plants remained unchanged between July 14 and July 18.

Ca:K_3Rb plants. On July 11, the only considerable change which was maintained later was an increased glutamic acid content. By July 14 there had occurred a corresponding increase in aspartic acid, (also in α-alanine) and varying reductions in asparagine, valine, leucine and putrescine. The only notable further change which had occurred by July 18, was an increase in the concentration of glutamine. At this date, putrescine, though much reduced, was still present in detectable amounts.

Certain apparent changes due to these several additions have not been referred to in the general account given above. The most interesting of these refers to pipecolinic acid, which was evidently produced as a consequence of adding either potassium, sodium or lithium; rubidium appears curiously not to have had a similar effect. The presence of proline too on the two-day chromatograms from the rubidium and lithium treatments is suggestive, but it is impossible to state whether this is significant, or merely represents a sporadic appearance.

The Ca·K K plants therefore showed a marked

response to the addition of the alkali metal on the second day; the Ca:K_3Rb plants responded rather more slowly, not showing much change until the fifth day, and the Ca:K_3Na plants apparently slower still, though it is possible that their response occurred somewhat sooner than July 18 (the ninth day). In all these treatments putrescine either disappeared completely, or nearly so. A small reduction of putrescine in the Ca:K_3Li plants on July 18 may indicate some response to this element also, although no improvement could be seen in the appearance of these plants even three weeks after addition of the lithium.

The appearance of the treated plants described on page 61 indicates that while there was some response in growth to sodium and rubidium, correlated with the changes in their amino-acid contents, these elements can in no wise replace potassium fully.

External effect of addition of alkali metals to K_3 plants - 1953

Ca:K_3 barley sown on May 4 grew slowly during May and early June; the first and second leaves became moribund by the end of May. White areas developed on the leaf blades during the first week of July. Further alkali metal (either lithium, sodium, potassium or rubidium) was added to some of the plants on July 9. A marked improvement could be seen a few days later in the Ca:K_3 plants which had been given extra potassium, but not in those given any other supplement. After nine days all symptoms of potassium deficiency had disappeared from the Ca:K_3K plants and they were growing vigorously, though again a purple colouration had developed at the base of the stems just as in the previous season.

By now a noticeable improvement had occurred also in both the Ca:K_3Na and Ca:K_3Rb plants. The former were generally greener than their untreated controls, though the colour was not the dark green of high potassium plants; there was no noticeable increase in their size. The Ca:K_3Rb plants, which were improved in colour and appeared to have many active growing points on July 18, had developed symptoms of rubidium toxicity by July 23. At this time they were erect in habit and of a greyish-green colour. The developing leaves were a pale yellowish-green, and the internodes of the stems had become swollen.

The place of putrescine in the metabolism of potassium-deficient barley

Unidentified substances in K_3 barley. The use of lutidine as second solvent in place of 'collidine' in two-dimensional chromatography made possible the recognition of additional substances in low-potassium barley. These substances were previously mentioned when presenting the results of the putrescine utilization experiments (page 35). The substance designated "K" in this paper occurred in both high and low potassium plants, and has also been reported by Richards and Berner (in the press).

Unknown substances "E", "F" and "G" appeared as relatively weak spots on chromatograms from potassium deficient plants only, "F" being always very weak and "G" extremely so. All three substances when run in phenol with or without an atmosphere of ammonia, had R_F values very similar to those of putrescine, which suggests that they, like putrescine, have strongly basic properties. In extracts having high putrescine contents, a purple smear surmounted by an additional spot a little above serine at times joined putrescine and substance "F". Aronoff (2) and Miettinen and Virtanen (23) have shown that some basic amino-acids may take up two or more distinct positions on the same chromatogram, and it is possible that these additional spots are alternative positions of putrescine. However they were never seen on chromatograms of putrescine-free extracts

when putrescine was added to the papers. Suggestively, on several occasions a spot occurred in the position of "B" on chromatograms of barley fed with lysine.

Substances "A" and "B" (as referred to on page35-)occurred in low potassium plants in association with putrescine and what appeared to be the same compounds could be produced in high potassium plants by feeding them with putrescine. While it would be of particular interest to identify them, their presence in barley in only trace amounts made isolation impractical and any study of their properties unsatisfactory. Means were sought therefore to obtain material containing them in greater quantities.

Unknown "B". In 1952 a ninhydrin-positive substance, chromatographically indistinguishable from Unknown "B", was noted on papers of low potassium red clover. In the following summer clover was again grown in sand culture using calcium type solutions with potassium at the K_1 and K_3 levels. These plants all grew slowly during May and June and their leaves were pale green. Where potassium was abundant however, fair growth was made during July. In the low potassium clover small brown necrotic areas had appeared near the margins of the fully expanded leaves by July 13, and later in the inter-veinal spaces also. Both the high and low potassium plants were sampled on July 15, at which time the latter contained a moderate amount of Unknown "B" (red clover). A later sample, taken on July 27, contained some putrescine in addition to this substance.

High potassium red clover plants grown in a glasshouse were presented with a 0.1% solution of putrescine through partially cut leaves. Brown necrotic areas appeared within 48 hours in the interveinal spaces of the leaves fed. To determine whether these symptoms (which visually were indistinguishable from those of potassium deficiency in clover) could be produced by administering putrescine through the roots, Ca:K_1 plants grown in water culture were presented in this manner with putrescine dihydrochloride solution at the concentration 0.1% Small brown areas again developed near the leaf margins on the third day. Chromatograms run from leaf extracts of these plants contained a moderate amount of substance "B" (red clover) in addition to putrescine. The root feeding experiment was repeated, but this time the putrescine was given for 3 hours only, after which the plants were transferred to distilled water for a further 24 hours. Putrescine could not be detected in extracts made at the end of this time although they contained a moderate amount of substance "B". As had been anticipated, red clover utilizes putrescine at a much greater rate than does barley, making possible the production of a satisfactory quantity of substance "B" (red clover). This technique has therefore been adopted in order to produce a considerable amount of plant material containing the substance more or less free from putrescine. Unfortunately time has not

yet been available to attempt the isolation and identification of the compound from this material. Unknown "B" (red clover) travels slightly further on a chromatogram when run in phenol with an atmosphere of ammonia, suggesting weakly basic properties.

Kenten and Mann (20) have shown that aqueous extracts of pea seedlings oxidise putrescine rapidly, suggesting the presence of diamine oxidase. Red clover also contains this oxidase activity, though to a lesser degree. They did not find it in barley. On the assumption that this oxidase activity is responsible for the production of substance "B" (red clover) it was anticipated that peas might be a good plant to use for its production. Peas grown in water culture were therefore fed through the roots with putrescine on the 20th day after germination, and the feeding was continued for 6 days. These plants grew rapidly and at the end of the experiment were much larger than others which had been maintained for the same period with their roots in distilled water.

Though the putrescine-treated plants grew well, the chlorophyll had disappeared from the lower portions of their stems in some cases, this being accompanied by a collapse of the tissue structure.

The amino-acid content of the leaves was much greater in the treated than the untreated plants, the increased content consisting largely of asparagine, glutamine, valine and leucine. There was also present

yet another unidentified substance, "L", Fig. 3, which gave a pinkish colour with ninhydrin. When developed at 90° the colour reached its maximum intensity within 4-5 minutes, fading after a further 10-15 minutes. Also the γ-aminobutyric acid content was higher in the roots of these plants than in the leaves.

Tabor (47) demonstrated the presence of a diamine oxidase in extracts of hog's kidney; this quantitatively oxidises diamines to amine aldehyde, hydrogen peroxide and ammonia. He suggested that these aldehydes undergo rapid cyclization, giving in the case of putrescine, Δ pyrroline. O-Aminobenzaldehyde may be used as a test for these unsaturated ring compounds, with which it gives a yellow colour reaction.

Dr. P. J. G. Mann and Dr. W. R. Smithies (private communication), using a purified enzyme preparation from pea seedlings, have been able to obtain a small yield of pyrrolidine from putrescine by hydrogenation of the oxidation mixture, though Δ pyrroline has not been isolated from the original oxidation mixture.

In the present experiments, chromatograms from peas fed with putrescine were sprayed with a 0.01M aqueous solution of o-aminobenzaldehyde, but gave a negative test.

Substance "A" was found in greatest amounts in K_3 plants from the second to sixth leaf stages and in slightly higher concentrations in "ammonium" than in "calcium" plants. It occupies a position on the chromatograms almost directly beneath arginine.

With ninhydrin it gives a bluish-purple colour as compared with the reddish-purple of putrescine. Both in its position on the chromatogram and in the colour of its ninhydrin compound it is indistinguishable from agmatine.

Investigations as to source of putrescine in K_3 barley.

Feeding experiments designed to throw light on the fate of putrescine in the plant have already been described. A further series of similar experiments, aimed at elucidating the origin of the amine that occurs naturally in potassium deficient barley will now be dealt with.

Because of their chemical relationship to putrescine, the following substances were administered individually : ornithine, arginine, citrulline, proline, agmatine. Additional substances - glutamine, valine, leucine and lysine - were included because of their prominent position in the amino-acid changes associated with potassium deficiency.

The chemicals used in these experiments were manufactured by Hoffman-La Roche Co., Ltd., Switzerland, or B.D.H., England. Their purity with respect to other ninhydrin-positive substances was tested by one-dimensional chromatography. 50 μg. of the amino-acid or amine were run in each of the solvents phenol and 'collidine' separately, and the chromatograms developed by spraying with ninhydrin (1% in absolute alcohol.) Where only a single spot appeared on these chromatograms,

the chemical was taken to be pure.

By these means the agmatine procured for the work was shown to contain two ninhydrin reacting impurities. Since it was believed that this amine might well prove to be an immediate precursor of putrescine, it was eminently desirable to remove the impurities before presenting the substance to the plants. Purification was first attempted on a small scale by means of one-dimensional chromatography. A solution of the impure salt was prepared by dissolving 100 mg. of laboratory grade agmatine sulphate in a 5ml. distilled water. This solution was added from a pipette to sheets of Whatman No. 1 filter paper in a band of 1 cm. maximum width parallel to the long edge (22½"). A total of 18 sheets was used. The papers were dried, placed 6 sheets at a time in the chromatography cabinet and run for 24 hours in phenol, after which the solvent was removed from them. To determine the positions of the substances on the paper, three test strips were cut from each sheet (one from either end of the 22½" side and one from the centre.) These were sprayed with ninhydrin and the colour compound developed.

Additional test strips were sprayed with Sakaguchi-reagent as a specific test for guanido compounds. The paper was first sprayed with a mixture of 0.1% α-napthol in 50% ethanol and a 40% aqueous urea solution, 2:1 (v/v); when dry, it was sprayed with a sodium hypobromite solution (0.62 ml. bromine in 100 ml.

of 50% NaOH solution).

Three bands of colour were produced by the ninhydrin reaction. A very intense reddish-purple colour-reaction was given by a substance with R_F about 0.4 which was quite separate from the remaining two substances. This did not give the Sakaguchi reaction. A second substance, with an R_F value of about 0.7 gave a bluish-purple colour-reaction with ninhydrin and an intense Sakaguchi-reaction. A third substance, which was not clearly separated from the substance of R_F 0.7, gave weak reactions, both with ninhydrin and the Sakaguchi reagent.

The substance (R_F 0.7) giving the strong reaction with the Sakaguchi reagent was assumed to be agmatine. Its position was marked and the strips of paper containing it were cut from the sheets. To remove the agmatine from them, water was passed down the strips while they hung vertically, and the solution was collected as it drained from the lower edges. This solution was concentrated under reduced pressure and a two-dimensional chromatogram prepared of the concentrate, using phenol as first solvent and lutidine as the second. It was found that only an imperfect separation had been made. The substance with R_F 0.4 had been removed, but the remaining two substances in the mixture had not been separated.

The concentrated agmatine solution, which was brown in colour, was then acidified with hydrochloric acid to pH3 and evaporated to dryness. Further

purification was attempted by dissolving the salt in a minimum quantity of water, and after adding absolute ethanol, precipitating it with acetone. A brown amorphous solid was obtained which proved to contain the same amount of impurity.

An unsuccessful attempt was then made to separate these substances by displacement chromatography on an ion-exchange resin. A column (15 cm. x 0.9 cm. dia.) of 'Permutit' special sulphonated polystyrene resin was prepared in the manner already described in Part I (page 9). 1g. of impure agmatine sulphate was dissolved in 50 ml. of water and introduced on the column. The effluent solution was found to be free from ninhydrin positive substances. This and the elutant solutions used subsequently were passed at a rate of 25 ml. per hour. 1000 ml. of 0.2M ammonium formate was then passed through the column and the effluent fractions tested with ninhydrin and Sakaguchi reagent. No displacement was effected with this solution. The column was then washed with 250 ml. of water and 0.2M ammonium carbonate was passed. This solution displaced a substance giving a ninhydrin reaction, but no Sakaguchi reaction; it was found to have an R_F value in phenol of 0.4. 1500 ml. of effluent solution was collected before a fraction was obtained which was Sakaguchi positive.

25 ml. fractions were then collected which gave a good Sakaguchi reaction. 300 ml. of this solution was heated at 60° until the ammonia was removed, acidified with hydrochloric acid and evaporated to dryness. It was

found chromatographically that the second substance removed from the column had an R_F value in phenol corresponding to the second impurity previously shown to be present in the crude salt. An additional 1000 ml. of effluent was collected which was quite free from ninhydrin-positive substances, but further attempts to remove the agmatine from the column were not undertaken.

An additional amount of partially purified agmatine was obtained using the method of paper chromatography already described.

Feeding of organic substances to barley. As in the putrescine feeding experiments, the solutions of amino-acids were administered to the plants either through partially cut leaves, through the roots or to detached tops. The plants used in the root feeding experiments were grown in water culture, the roots being thoroughly washed in distilled water before placing them in the feeding solution. So far as metabolism in the tops is concerned, root feeding has the disadvantage that the substance under investigation may have been partly metabolised in the roots, and the products translocated to the tops. Hence there is no guarantee that any substance found in the tops was actually produced there. For this reason, direct feeding of the tops alone has also been undertaken. In treating the whole tops, the stems were severed a little above their base with a sharp blade and their cut ends immediately placed into the solution.

All feeding solutions were prepared immediately before use and, when necessary, were adjusted to pH 5. When an experiment was continued for several hours, the feeding solution was renewed frequently to prevent the accumulation of substances resulting from bacterial activity. After use in the feeding experiments, the purity of the solutions was tested by one-dimensional chromatography.

In certain preliminary experiments, the plants used were grown in sand culture in the field. They were fed in the first instance through partially cut leaves. It was first necessary to determine satisfactory amino-acid concentrations and presentation periods for the work. As already stated (p.28), the most suitable concentration of putrescine was determined in an initial experiment. Four concentrations were selected within assumed limits - 0.003%, 0.015%, 0.075% and 0.25% Three high potassium plants were treated in each case, two leaves being fed on each plant. It was found that those plants receiving the 0.075% solution of the dihydrochloride functioned apparently normally for at least twenty-four hours and a two-dimensional chromatogram established that they had assimilated sufficient putrescine to give a satisfactory ninhydrin reaction there.

Partly on the basis of these results with putrescine, arginine was presented to high potassium plants at two concentrations (0.25% and 0.1%), for two days. It was readily absorbed, and the arginine content of the plants receiving the 0.25% solution became several

times greater than that of the more abundant amino-acids. Those plants with leaves in the 0.1% solution developed a moderate arginine content and some fed for a further day were apparently unaffected by three days of continuous feeding.

In field experiments carried out during June and early July, 1952, arginine, ornithine and proline were each fed to both high and low potassium plants at a concentration of 0.1% for periods of 2 days and 5 days. This work was entirely preliminary and exploratory and the tentative results will not be presented. Nevertheless, two unidentified substances (Unknowns "C" and "D") were definitely recognised in plants fed with arginine, and on two occasions where plants were fed with this substance an increase occurred in the proline content.

The difficulties and limitations implicit in these field experiments were to some extent overcome when feeding techniques were resumed in 1953, by growing plants in water culture in the glasshouse in successive weekly sowings. By these means a continuous supply of suitable material was made available with little trouble; moreover the time-consuming manipulations involved in leaf-feeding could be discarded in favour of the quicker technique of presenting the various substances round the roots. Nevertheless in most of these experiments the tops were fed directly through the cut stems. High potassium plants were given amino-acids at the third leaf stage, when the plant has exhausted

the nitrogenous reserves in the seed.

To ascertain suitable concentrations for glasshouse conditions, experiments were carried out with amino-acid concentrations within the range 0.005M - 0.3M and for periods of from half-an-hour to three days. For a three day feeding period, a 0.005M concentration was found to be the most satisfactory, and 0.1M for a feeding period of half-an-hour. Short presentation periods were generally aimed at, with subsequent removal of the roots to water, since it appeared reasonable that the course of metabolism of the added substance might then be clearer than where the substance enters slowly but continuously. The latter situation indeed might easily lead to an early "dynamic equilibrium", in which no further changes could be detected; where a comparatively high concentration is introduced in a short time there is evidently a greater chance of finding rapidly-metabolised first products.

Fig. 8 shows a typical selection of the results of these feeding experiments

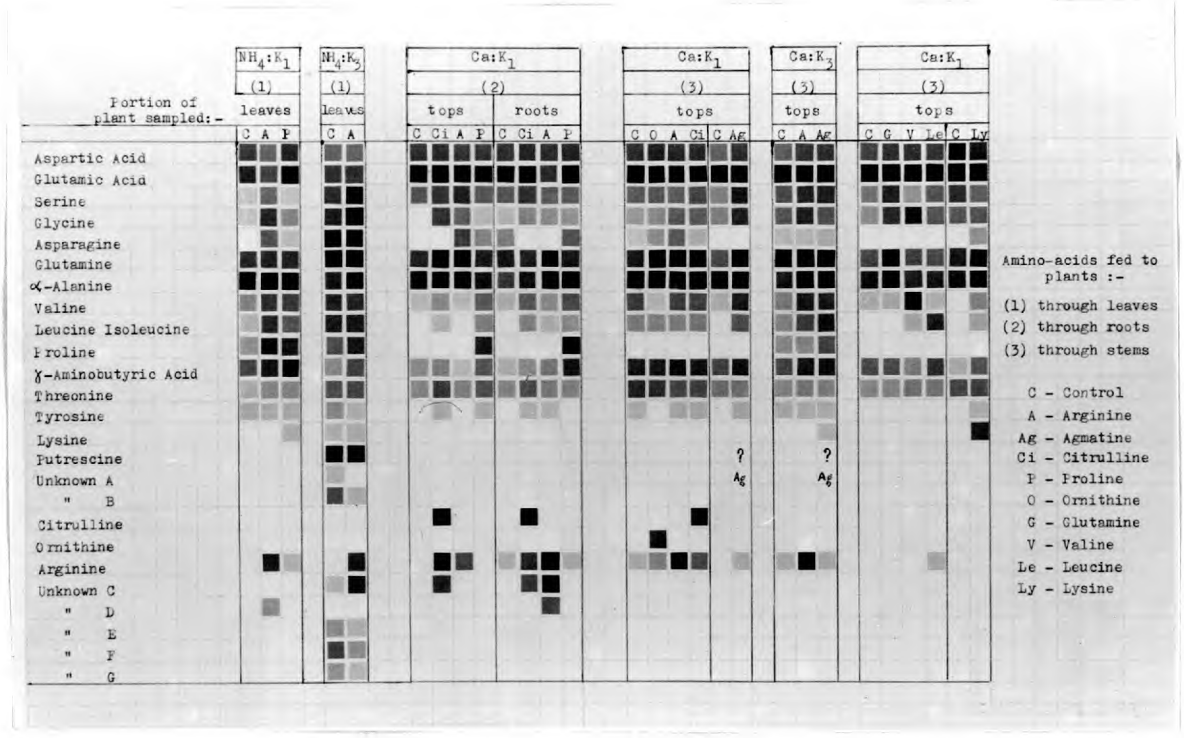


Fig. 8

The treatment of the plants given in the blocks from left to right are :

$\underline{NH_4:K_1}$ Plants fed continuously through the leaves with 0.1% solutions for two days (1952).

$\underline{NH_4:K_3}$ Plants fed through the leaves with 0.25% solutions for two hours (1952).

$\underline{Ca:K_1}$ Plants fed through the roots with 0.005M solutions for three days (with the exception of citrulline - two days) (1953)

$\underline{Ca:K_1 \text{ and } K_3}$ Plants fed through cut stems with 0.1M solutions for forty minutes, then transferred to distilled water for further six hours (1953)

$\underline{Ca:K_1}$ Plants fed through cut stems with 0.1M solutions for half-an-hour, then transferred to water for two hours (1953)

l-Glutamine. There was a large increase of α-alanine in plants fed with glutamine, as well as a small increase of glycine and serine. A similar increase of α-alanine appeared in root feeding, though in this case, there was also a high content of γ-aminobutyric acid. The small increases of glycine and serine, are probably of little significance, since many factors appear to influence their concentrations.

dl-Valine and l-Leucine. Since these substances occur in increased amounts in potassium-deficient plants, they were included in the experiment. However, apart from a small increase in glycine where valine was administered, no appreciable changes were detected.

l-Lysine. In experiments where other basic amino-acids or putrescine were fed to the plants, valine, leucine and tyrosine in many instances increased. This could be due to protein breakdown. A similar small accumulation was noted in the present experiments. The amides also increased (asparagine apparently at the expense of aspartic acid), probably as a result of de-amination of lysine.

l-Citrulline. There was an accumulation of arginine in all instances where citrulline was fed. While no exact comparison between the relative amino-acid concentrations of the roots and tops can be made, the much higher ratio of arginine to citrulline in the latter points to the conclusion that arginine is produced at least as rapidly in the leaves as in the roots. For

it is noteworthy that where arginine itself was fed through the roots, although its concentration in the roots became extremely high, the leaf content was invariably much lower. Some idea of these proportions can be obtained from the results of arginine feeding as shown in Fig. 8. That citrulline is readily converted to arginine in barley tops is also indicated by the results shown in block $\underset{(3)}{Ca:K_1}$. A very apparent increase of arginine had occurred in this experiment, after only 6 hours.

The appearance of substance "C" in conjunction with arginine accumulation in plants fed with citrulline is of interest in view of its presence on chromatograms of plants fed with arginine. It was also seen on papers of an extract of orange tree leaves where the arginine was naturally high

l-Arginine. The increase of proline found in some instances when plants were fed with arginine is difficult to explain. On several occasions, where this substance had increased following arginine uptake, the control plants contained at least a detectable amount of it as well, i.e. they contained more than usual. A parallel case was seen where glycine accumulated in plants fed with glutamine or arginine at a time when their initial content was moderately high.

The most striking change effected by arginine was the appearance of an additional ninhydrin-positive

spot, referred to in this paper as Unknown "C". While further evidence is needed to establish the identity of the substance, it has been observed to travel slightly further in phenol when ammonia is present; moreover, it could not we detected after acid hydrolysis.

Substance "D" sometimes appeared after feeding with arginine; it occurs on the chromatograms as a curiously circular spot adjacent to arginine. The presence of an apparently identical spot on papers from young leaves of the orange tree (which also have a relatively high arginine content) is noteworthy.

Ornithine. A small increase of arginine was found in plants fed with ornithine, and on one occasion a spot appeared on the chromatogram in the position of citrulline; this result did not recur. There was no obvious increase of putrescine when potassium-deficient plants were fed with ornithine. However, experiments with carbon marked ornithine would be worth while, since the amino-acid may be decarboxylated at a very slow rate. It is well-known that putrefying bacteria readily produce putrescine in this manner, but with the methods available no evidence has been obtained of a comparable enzyme activity in barley, even where a high internal concentration of ornithine had been artificially built up. This negative evidence is strengthened by the fact that barley is incapable of metabolizing at all rapidly any putrescine that might be produced. Hence it seems very improbable that the high putrescine concentrations

found in potassium deficiency can be due appreciably to ornithine decarboxylation. On the other hand it is probable that deamination of ornithine proceeds at a not inconsiderable rate, since the amide content increases after feeding with the amino-acid.

Agmatine. High and low potassium plants were fed with agmatine and in each case, a weak spot appeared in the position of putrescine. Unfortunately the results were not altogether conclusive, owing to the fact that only a few milligrams of sufficiently pure agmatine were available for the feeding solutions. Further investigations on this important point are desirable. Agmatine certainly increased the contents of glutamine and of several amino-acids, but it is quite unknown to what extent these changes are the direct result of its own metabolism, or of its toxicity leading to protein breakdown.

Proline. The introduction of proline led consistently to increases in asparagine, glycine, valine, leucine, γ-aminobutyric acid, and probably other amino-acids (e.g. α-alanine). The basic substances did not appear to be affected generally, though a little lysine and arginine appeared on one occasion. The significance of these effects is not apparent, but they probably have no immediate bearing on those aspects of metabolism under investigation.

DISCUSSION

The identification of putrescine as a substance accumulating in potassium-deficient barley, and the demonstration that it is directly responsible for a severe necrosis characteristic of advanced potassium deficiency, demands a re-consideration of our knowledge of this disorder.

Beginning with the studies of Shih, 1934 (41), work at this Institute on the physiology of potassium nutrition has been largely concentrated on the differential effects of deficiency of the element (1) at high levels of sodium and low levels of calcium, (2) at high levels of calcium in the absence of sodium, and (3) at high levels of both factors. Interactions with phosphorus supply have also been investigated.

In order to study the effects of potassium supply in low calcium conditions in the absence of sodium, it was necessary to resort to culture solutions having ammonium nitrate and ammonium phosphate to supply the nitrogen. Richards (37) reports the resulting "disastrous effects" in low potassium plants at an early stage, with the loss of many. Other workers (Turtschin, (48), 1934, Schropp and Arenz, (39), 1939, and Wall (50), 1940) using solutions based on ammonium salts found similar effects when potassium was deficient, and claimed that the disturbance was due to toxicity consequent on excess ammonium accumulation within the plant. Putrescine has been found to be present at the first leaf stage of

these plants, but only in amounts considered insufficient to produce the often fatal effects observed at that stage. It still seems likely therefore that the disastrous effect of this nutritional treatment on the plants in the first few days is largely attributable to excess ammonium. Richards (37) supplied other alkali metals to barley growing at low potassium levels with the object of determining whether they could perform to any extent the function of the missing potassium. It was found that rubidium when given in small amounts was very nearly as effective as potassium in removing immediate toxic symptoms, and in allowing early growth to proceed. Sodium on the contrary was valueless for this. Later experiments by Richards and Berner (in the press) revealed that rubidium and sodium both prevent the accumulation of the substance now shown to be putrescine, and hence its toxic effects. Putting these facts together it becomes evident that putrescine cannot be of prime importance in the toxicity associated with potassium-deficient seedlings growing in solutions containing ammonium salts. The present experiments corroborate the replaceability of potassium by rubidium or sodium in the prevention of putrescine accumulation, rubidium being the more effective.

The toxic effects so far referred to are largely confined to the seedling stage, before potassium deficiency can properly be said to have set in. True deficiency is only reached later, when the potassium available to the

plant has been internally "diluted" by growth. The plants growing in all solution types (not only those containing ammonium salts) now develop necrotic symptoms, and the mature leaves die off very rapidly, again suggesting toxic effects. Considerable evidence has been amassed that in these plants inorganic ions of various kinds accumulate to much higher levels than in high potassium plants, and that these accumulations may be deleterious. Thus in potassium-deficient barley, sodium, if available, accumulates so greatly as to cause a remarkable degree of succulence. Inorganic phosphate also piles up in very supra-normal amounts, and it has been demonstrated (Richards, unpublished) that this circumstance is detrimental to growth; if phosphate supply is reduced when potassium is deficient, the death-rate of leaves is diminished and considerably greater size is attained. One reason for the improvement appears to be that the accumulated phosphate leads to excessive respiratory losses, abnormally high respiration rates being very characteristic of potassium deficiency, at least in barley. If phosphate supply is still further reduced the respiration rate falls below that of the normal plant, and the type of growth and external symptoms change completely: the plant becomes typically phosphorus-starved in appearance, and growth is again reduced. At the optimum phosphate level for growth, then, the low potassium plant has the respiration rate of a healthy normal plant (neither excessive nor depressed)

and moreover the necrotic symptoms of typical potassium deficiency have all but disappeared; its growth rate is still not maximal for it will respond to potassium phosphate, but the plant has lost its unhealthy appearance.

Clearly then excessive phosphate content is an important primary factor in the adverse effects of potassium deficiency, and appears to be responsible not only for inordinate respiratory losses but also for the observed "toxic effects". Since it is now shown that some at any rate of these toxic effects (which disappear as phosphate supply is reduced towards its optimum) are in reality due to putrescine poisoning, the question arises whether the disturbances in nitrogen metabolism leading to putrescine production, or at least its accumulation, are themselves due rather to excess phosphate in the tissues than to, say, the need for a comparatively large amount of potassium by some particular enzyme system. The question cannot be answered at this stage, but it is proposed to investigate the probable effect of phosphate on the production of the amine in potassium deficiency.

In the meantime it may be noted that other alkali metals besides potassium repress putrescine formation, and of these rubidium is considerably more effective than sodium; in fact it appears to be quite as effective as potassium itself. It has been shown (Richards, 32) that one important effect of rubidium, when supplied to potassium-deficient barley, is to reduce very considerably the phosphorus uptake, and the phosphorus content of the tissues; the effect of

rubidium, like that of potassium, is therefore consistent with the idea that putrescine accumulation is consequent on the presence of excess phosphate. Sodium, on the other hand, when added to high calcium potassium-deficient cultures, appears from the rather meagre data available (Richards and Shih, Part II, (34) to exert little effect on the phosphorus content of the plants, and the question must be left unsettled. It may however be more than a coincidence that the greatly increased CO_2 production in respiration under potassium deficiency, due to phosphate, involves unduly high rates of de-carboxylation in the organic acid cycle, and that putrescine accumulation also must almost certainly arise by a similar process of de-carboxylation.

This paper establishes the presence of putrescine in plant species other than barley. It appears under conditions of low potassium in both wheat and/red clover, and may well prove to accompany potassium starvation in many plants. It has not, however, been found in flax, nor again in bracken, though the material used for the latter species had been stored for some years and may have changed during that time. Again, while some putrescine has been recovered from deficient red clover, it frequently could not be detected there; whether putrescine was obviously present or not, a further substance, Unknown "B", was always evident. Feeding experiments demonstrated that high-potassium clover converts putrescine rapidly into this same substance, hence there can be very little doubt that the Unknown "B", where it occurs naturally in low

potassium clover, has been derived from putrescine. Different species of plants therefore accumulate characteristically under deficiency different substances, either arginine, putrescine, or unknown "B". The unknown, where it occurs, is derived from putrescine, and the inference is strong that putrescine in its turn is derived from arginine. If this is so, the primary disturbance studied here due to potassium deficiency would seem to be one leading to arginine accumulation; what happens subsequently depends on the enzyme equipment of the particular species. No reasoned explanation of the cause of arginine accumulation under potassium deficiency can be offered at this stage of the research.

It is of very considerable interest that in barley under these conditions a toxic substance such as putrescine, produced by the plant's own metabolism, can accumulate to such high levels that it kills the leaf tissues almost before they have fully matured. In doing so, characteristic irregular areas from which all leaf pigments have gone are first developed, i.e. quite white areas. Similar areas have also occurred on wheat leaves containing putrescine. Clover on the other hand metabolises putrescine quite rapidly, yielding the basic Unknown "B". But this also would appear to be a toxic substance, for where it is present in considerable amount necrotic areas again appear on the leaf; these areas are however brown. Those appearing naturally on leaves of potassium-deficient

plants (from which putrescine may be virtually absent) resemble very closely indeed those induced on high potassium plants by treatment with putrescine. Rather similar brown leaf necroses also characterise potassium deficiency in flax and bracken, but in these plants neither putrescine nor Unknown "B" has been found in appreciable quantity. Whether or not arginine accumulation can be the responsible agent has not been determined, but clearly it is desirable to discover the immediate cause of these necroses.

It is of course true that all these accumulations associated with necrosis represent basic substances, and it would appear possible that the basicity is primarily responsible for the breakdown in all the plant types. No systematic investigation of this possibility has been attempted, but some preliminary observations of tissue pH in barley have been made, using Small's indicator methods (43), and comparing high potassium material with low potassium material collected at a time when severe deficiency symptoms were manifest. No difference in pH was detectable by the method, and it would appear certain that if any difference exists it must be slight. Claims that the pH of certain tissues is altered slightly by potassium deficiency have been made on one or two occasions (Reed and Haas (29), and Nightingale, Schermerhorn and Robbins (26), but the changes claimed are in the direction of increased acidity, not alkalinity. In the feeding experiments reported above, the basic substances were presented as hydro-

chlorides; it is conceivable that in these circumstances more HCl would be absorbed than of the substance intended, leading to the development of acidity in the tissues. Nevertheless plants treated with HCl alone in sufficient amounts to be toxic and eventually to kill several leaves did not develop necrotic areas in any way resembling those of potassium deficiency or those more artificially produced by feeding with putrescine. Nothing is definitely known therefore of the cause of the toxicity ascribable to putrescine accumulation, but it seems likely that something more than pH change is involved.

Although putrescine has not previously been associated with potassium deficiency, its presence has been recorded in a few plants. It was found in Atropa belladonna by Goris and Larsonneau (15). Later, Cromwell (8) working on the synthesis of alkaloids in this species found that putrescine and arginine when supplied experimentally enhanced alkaloid synthesis. The effect of putrescine, either alone, or in conjunction with glucose was especially marked, leading to the suggestion that the amine is the natural precursor of hyoscyamine. James (19) stated that the tropane alkaloids formed in young belladonna leaves may be increased by feeding the leaves with l-arginine or with l-ornithine, and claimed to have established the presence of ornithine in belladonna.

The suggestion was also made above that arginine is the precursor of the putrescine found

in potassium deficient barley. For direct production a choice of two routes is possible: (1) ornithine might be first formed (as by the action of arginase in Kreb's ornithine cycle) and subsequently decarboxylated, and (2) arginine itself might be decarboxylated prior to the splitting of the guanidine group; in this event the amine agmatine would be the intermediary. The results of feeding experiments designed to investigate the possibility of a route through ornithine have been largely negative, and no evidence has been found of putrescine production even when large amounts of ornithine were introduced into the tissues. Moreover ornithine has not been detected even in potassium-deficient barley, where putrescine is being accumulated. On the other hand evidence has been obtained that arginine may arise from introduced ornithine, but how directly is unknown; on one occasion citrulline was apparently formed, but this result was not repeated. If reliable, it presumably indicates the presence of some of the enzymes participating in the ornithine cycle of animals and micro-organisms. Further evidence of enzymes of this cycle was obtained from those plants fed with citrulline, which converted it quite readily into arginine. On the other hand no evidence has emerged that the cycle may be completed by arginase activity converting introduced arginine into ornithine.

The second direct route from arginine to putrescine involves agmatine as an intermediary, and it is unfortunate that the only available commercial

samples of this amine proved to be very impure. Efforts were made to purify it, but difficulties were encountered, and only a very small amount of the purified substance was available in time for the experiments this year. Though really insufficient for the purpose, this was administered to high potassium barley plants, and fairly distinct (but inconclusive) evidence was obtained that both putrescine and arginine were produced from it. Moreover, the chromatograms from potassium-deficient plants frequently showed a faint ninhydrin reaction in the region of agmatine, but the identity of the substance concerned has not been established as yet. During the present season a large quantity of potassium-deficient barley has been grown and extracted, and it is hoped that sufficient of the substance (and also of some of the other unknowns referred to in this thesis) can be isolated from it to render possible a definite identification.

Apart from some animal sources (e.g. sponges and cephalopods), agmatine has been identified by Engeland and Kutscher (11) as a natural product in the ergot of rye, in which also Goris and Larsonneau (15) found putrescine.

Gale (14) showed that in Bacterium coli, amines are produced by the action of specific amino-acid decarboxylases, listing six, including those which attack l-arginine and l-ornithine. The

possibility now arises that agmatine as well as putrescine may occur in potassium-deficient barley. Agmatine is not hydrolysed by arginase (Kossel and Dakin (22) and Kiesel (21), though it has been shown by Reinwein and Kochinki (30) to be converted by putrifactive bacteria to putrescine. Hellerman and Perkins (17) found that arginine may be hydrolysed in the absence of arginase by a catalytic process involving urease and one of the ions Co^{++} Mn^{++} Ni^{++} It may be that agmatine could be hydrolysed by a similar process.

At this point some of the numerous metabolic paths involving the basic amino-acids which have been described during recent years will be briefly outlined, though it must be confessed that at this stage they do not appear to help much in solving the immediate problems discussed above.

Little is known of the path of arginine formation in higher plants. Strassman and Weinhouse (46) conclude from work with isotopic carbon that arginine synthesis in Torulopsis utilis involves a direct conversion of α-ketoglutarate via glutamate, proline and citrulline.

Schmidt, Logan and Tytell (38) and Oginsky and Gehrig (27) working with Clostridium perfringens obtained evidence of the formation of citrulline from arginine by cell enzymes i.e. the inverse of a reaction occurring in the ornithine cycle. Also Slade (42) has shown that hydrolysis of arginine by cell free extracts of Streptococcus faecalis yields citrulline

and ornithine. Roche and Lacombe (37) again have obtained an enzyme from baker's yeast which quantitatively splits arginine into citrulline and ammonia; it has an optimum at pH 6.5. They were unable to detect arginase in these cells and infer that they metabolise arginine outside the ornithine cycle.

Turning to recent work on the ornithine cycle proper in animals, Ratner and Pappas (28) showed in 1949 by use of a purified enzyme preparation that imidation of citrulline to arginine is effected specifically by aspartic acid. Borsook and Dubnoff (4) had previously shown that the imidation of citrulline proceeds in animal tissues more rapidly with glutamic acid as the amino donor than with aspartic acid; Ratner and Pappas (28), in explanation of this fact, state that "glutamic acid is not only able to produce aspartic acid by transamination, but is more easily oxidized in the tissue than aspartic acid, thus also being able to produce the energy necessary for the reaction".

The formation of citrulline from ornithine in rat-liver residue was studied by Cohen and Grisolia (6). They found that if the liver is first incubated aerobically with glutamic acid, ATP, ammonium bicarbonate, magnesium, phosphate and potassium ions, then the addition of ornithine and further incubation under

anaerobic conditions leads to citrulline formation. The reaction was believed to be ornithine plus carbamyl glutamic acid giving glutamic acid and citrulline.

Srb and Horowitz (44), using arginine-less mutants of Neurospora crassa, have demonstrated the production of arginine from ornithine and citrulline.

As far as is known, citrulline has not been found in higher plants, except in the water melon (Citrullus vulgaris), from which it was originally isolated by Wada (49), and in the roots and nodules of the alders Alnus incana and A. glutinosa, where it was shown by Miettinen and Virtanen (25) to occur in abundance.

In the present experiments, the rapid appearance of arginine in barley fed with citrulline suggests the presence of a mechanism capable of directly converting the one into the other. Moreover, Arnow, Oleson and Williams ( ) have recorded that citrulline can replace inorganic N completely in the growth of Chlorella vulgaris. It was also shown by Fries (13) that when decotylized pea seedlings are maintained aseptically in darkness, root growth ceases unless arginine, glycine and adenine are supplied. The arginine is however replaceable by citrulline. It is probable that the

formation of arginine is the first step when, as in the above cases, citrulline is utilized.

To conclude this brief review it may be mentioned that the existence of an interesting cycle involving ornithine and proline, and therefore ring opening and closure, has been postulated. Weil-Malherbe and Krebs (51) and Bernheim and Bernheim (3) indicated the probability that proline, prior to deamination, is converted to glutamic acid, by a direct oxidative splitting of the ring. Stetten and Schoenheimer (45) confirmed the formation of glutamic acid from proline. Shemin and Rittenberg (40), from studies of the metabolism of ^{15}N fed to rats as isotopic glycine obtained evidence consistent with the existence of a cycle of transformation from proline via α-keto-δ-aminovaleric acid, ornithine, and α-amino-δ-iminovaleric acid to glutamic semialdehyde and from glutamic semialdehyde either to glutamic acid or via pyrrolinecarboxylic acid back to proline.

Fincham (12), using enzyme preparations of Neurospora crassa, obtained evidence for the synthesis of ornithine from glutamic δ-semialdehyde and glutamate.

As regards the formation and fate of putrescine, it would seem possible that diamines may arise in small amounts in normal nitrogen metabolism, at least in leguminous plants. From the very considerable diamine oxidase activity in the seedlings of peas and red clover, reported by Kenten and Mann (20), the natural occurrence of an appropriate substrate might well be expected. The present work shows that putrescine is metabolized

rapidly by living pea plants and to a lesser degree by red clover. Living barley metabolizes it much more slowly, and Kenten and Mann were unable to find diamine oxidase activity in preparations from this plant. The correlation found here between the presence of diamine oxidase activity and the ability to metabolize putrescine provides presumptive evidence that in these instances putrescine is in fact being oxidised in the plant by this particular enzyme system. Dr. P. J. G. Mann and Dr. W. R. Smithies (private communication) demonstrated that the mixture resulting from the action of diamine oxidase on putrescine gives a colour reaction specific for Δ^1 unsaturated cyclic compounds, though the product (presumably Δ^1 pyrroline) was not isolated. In the present work, Δ^1 pyrroline was not detected in extracts of peas fed with putrescine, nor was it found in barley similarly fed, in which case a ninhydrin-reacting substance occurred. What appears to be the same ninhydrin-reacting substance occurred likewise in red clover in fairly high concentrations. It is apparent that if such a spontaneous cyclization occurred of the amine aldehyde (γ-aminobutyraldehyde) presumably formed initially, then the resulting cyclic compound must immediately have undergone further transformation. Since the substance found in barley and clover gives a ninhydrin reaction, and must therefore have a free $-NH_2$ group, this would seem to be unlikely; the ring compound of Δ^1 pyrroline is relatively stable. Isolation and identification of the ninhydrin-positive substance derived from putrescine is clearly necessary before the

situation can be resolved, and plant material has been grown and preserved with this end in view.

Whatever the detailed biochemical routes taken in putrescine formation and disappearance, and whether or not the same routes are taken in high and low potassium plants, their importance can only be regarded as secondary to the main question posed by the known facts. This concerns the reason why it is only in potassium deficiency that amine accumulation is found, and that even there the presence of similar large amounts of other alkali metals can alleviate the condition. Reasons were given early in the discussion for suspecting that the prime cause of the disturbance might not be the insufficient internal concentration of potassium ions as such, but the resulting excess of phosphate ions. Nevertheless one piece of experimental evidence favours the view that phosphate is unlikely to be the important factor. This is given by those potassium-deficient barley plants which were provided with ample potassium (or rubidium, or sodium) at a time when they had accumulated a considerable putrescine content. The amine content of these plants immediately began to fall, and after a few days had reached a low level. While increased growth generally accompanied the fall, it is difficult to believe that the phosphate content could have been reduced sufficiently rapidly to account for the marked

change in metabolism. This fact therefore argues in favour of a more direct effect of the alkali metal ions themselves.

If this is so, a possible place to look for an explanation would seem to be some particular enzyme system which either requires these ions for its activity or else is inhibited by them. The need for potassium of some enzyme systems (concerned with sugar phosphorylations) has been discovered in recent years, but these systems would not seem to be related at all closely to putrescine formation. If however such a system should be responsible for the phenomena, it would appear to require quite large amounts of the metal, not comparable with the metal requirements of those enzymes associated with Fe, Mn, Cu, Zn etc. In view of this difficulty, the possibility might even be envisaged of the responsible system being one that is inhibited by high concentrations of potassium, so that when alkali metals are deficient the enzyme's activity becomes supra-optimal for balanced metabolism. Any such speculations are of course entirely lacking in factual support; and as a first step towards the elucidation of this field it is proposed to investigate how far internal phosphate concentration may be related to amine accumulation.

A further possible partial explanation of the phenomena may be kept in view. The various substances that have been found to accumulate in

different plants under potassium deficiency are all of a strongly basic nature, yet what evidence there is in the literature points to the fact that the tissues of these plants are actually more acid than those of high potassium controls. If it is true that alkali metal deficiency leads generally to increased acidity, basic metabolites might become important as compensatory factors, and their metabolic stability increased by the acid conditions. This would be the converse of the well-known balance between metabolically produced organic acids and ions of the alkali metals etc., when these latter are present in abundance. The potassium-deficient plant would on this view be compelled to accumulate toxic substances: this might still be teleologically advantageous however, for without the accumulations the greatly increased acidity might well be even more disastrous. The apparent relationship of free phosphate content to putrescine accumulation might also find its explanation here, for accumulated phosphate must be responsible for some of the excess acidity, and especially dangerous where metal cations are deficient.

Much work needs to be done before all these matters can be decided.

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S U M M A R Y

1. The isolation of an unidentified substance in potassium-deficient barley and its identification as putrescine are described.

2. Using a variety of nutrient solutions, with potassium as the main variable, barley and other plant species were grown in water and sand culture. Organic substances were fed to certain of these plants through the leaves, stems or roots. The amino-acids of plant extracts were analysed by means of paper chromatography.

3. A leaf symptom characteristic of potassium deficiency is produced by feeding putrescine to barley with a high potassium supply. After prolonged feeding, the appearance of the plants suggests severe potassium deficiency.

4. Putrescine is slowly utilized by barley which has a high potassium supply; at the same time, an unidentified substance is produced. A similar substance appears in quantity in red clover concurrently with a rapid utilization of putrescine.

5. In potassium-deficient barley, putrescine accumulates at an earlier stage in those plants having nitrates and phosphates added in the form of ammonium salts than in those having these salts added in the form of calcium. This substance occurs in smaller amounts in the roots than in the tops. It is not found in protein hydrolysates.

6. Under conditions of extreme potassium-deficiency, wheat and red clover accumulate putrescine. The amino-acid composition of potassium-deficient wheat

SUMMARY

bears a striking resemblance to that of deficient barley.

7. The alkali metals rubidium and sodium supplement potassium in some degree in barley, rubidium being the more effective.

8. An unidentified substance occurs in potassium-deficient barley which, when run on a two-dimensional chromatogram in the solvents phenol or lutidine, has R_F values identical with agmatine.

9. Two unidentified substances appear in barley fed with arginine.

10. Arginine accumulates when citrulline is fed to barley.

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