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The Biosynthesis of Putrescine in Higher Plants and its Relation to Potassium Nutrition

By T. A. SMITH and F. J. RICHARDS
Agricultural Research Council Unit of Plant Morphogenesis and Nutrition, Wye College, near Ashford, Kent

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Although potassium is an element required in relatively large amounts for the maintenance of normal growth in plants, little is known of its metabolic function. The discovery by Richards & Coleman (1952) that barley plants form the diamine putrescine when grown with a restricted potassium supply made it possible to study a metabolic pathway in which potassium may be directly involved.

Coleman & Hegarty (1957) showed that barley shoots produce radioactive putrescine when given labelled ornithine, but Coleman (1953) had indicated that agmatine might be a more usual precursor. For example, a ninhydrin-positive substance chromatographically similar to agmatine was sometimes found in extracts of potassium-deficient leaf material, whereas ornithine was not detected; moreoever, ornithine, when introduced into the plant by feeding techniques, was metabolized only slowly.

The occurrence of putrescine in other normal and potassium-deficient plant species has now been examined.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* L. var. Plumage Archer) and red clover (*Trifolium pratense* L.) were grown in sand culture in the open during the summer of 1961, with the ammonium nutrient solution of Richards & Berner (1954). Potassium was supplied at two concentrations, corresponding to optimum and deficient conditions respectively, the 'low-potassium' solution having only one-sixteenth of the concentration of the 'high-potassium' solution.

The barley was sown (nine seeds/10 in. glazed pot) during the third week of July, and the nutrients were applied in two equal portions, 1 and 2 weeks after sowing. Each pot received: NH_4NO_3, 4 g.; $(NH_4)_2HPO_4$, 0·05 g.; $NH_4H_2PO_4$, 0·77 g.; $CaCl_2,6H_2O$, 0·5 g.; $MgSO_4,7H_2O$, 1·25 g.; $MnSO_4,4H_2O$, 0·01 g. In addition, 1·76 g. of K_2SO_4 was supplied for optimum potassium supply and 0·11 g. for deficiency. After 2 weeks the plants were thinned to three per pot. The tops were harvested when the plants were 8 weeks old and were used fresh.

Red clover was sown (36 seeds/pot) in the second week of July and the same quantities of nutrients were again applied in two equal portions, 2 and 3 weeks after sowing. At the end of the third week the plants were thinned to six per pot, and after 10 weeks' growth the tops were removed and dried at 90° for 24 hr.

Barley seedlings used in the feeding experiments were grown in gravel for 12 days, nutrients being supplied with potassium at the optimum concentration.

Leaves of cabbage (*Brassica oleracea* L.) were obtained from the Horticultural Department at Wye College during October 1961. They were dried at 90° for 24 hr. and powdered before extraction.

The Sakaguchi test was conducted according to the method of Jepson & Smith (1953). The chromatographic solvent used to separate the agmatine from putrescine was butan-1-ol–ethyl methyl ketone–aq. NH_3 (sp.gr. 0·88)–water (5:3:1:1, by vol.) (Wolfe, 1957), and is referred to below as the 'butanol–ketone' solvent. The R_F values of agmatine and putrescine were 0·2 and 0·6 respectively. No amino acids in the plant extracts possessed R_F values above 0·25 in this solvent.

The weak cation-exchanger Amberlite IRC-50 (H^+ form) (analytical grade) was used, 3 g. being packed into columns 1 cm. × 20 cm. The resin was prepared for use by passing successively through the column: water, 5N-NaOH, water, N-HCl and finally water.

Infrared spectra were obtained on the Perkin–Elmer Model 21 spectrophotometer.

EXPERIMENTAL

Extraction of agmatine from potassium-deficient barley plants. Approx. 200 g. of fresh potassium-deficient barley leaves was macerated in 600 ml. of ethanol and filtered through nylon. After the volume had been reduced to 50 ml. by boiling, the solution was centrifuged and passed through a column of Amberlite IRC-50 (H^+ form). The column was washed with 4N-NH_3 until the effluent was colourless, and then saturated $(NH_4)_2CO_3$ soln., which elutes both agmatine and putrescine, was passed through the column until the effluent was no longer Sakaguchi-positive. The $(NH_4)_2CO_3$ was removed by heating to dryness at 100° *in vacuo*. Experiments showed that under these conditions little of the agmatine is lost. To the residue 3 ml. of 0·05N-H_2SO_4 was added, the resulting solution being centrifuged and the supernatant applied in a horizontal band near the base of five 20 cm. squares of Whatman no. 1 chromatography paper. The chromatograms were developed by the method of Datta, Dent & Harris (1950), with butanol–ketone as ascending solvent. After 3–4 hr., when the solvent had reached the top of the paper, the chromatograms were removed and dried, and the development process was twice repeated. Putrescine was now near the top of the paper and the agmatine had moved 5–10 cm. from the origin. The

agmatine was located by spraying with ninhydrin a vertical strip cut from the side of one of the sheets. The regions of the chromatograms containing the agmatine were cut out and eluted with 4N-NH_3 in three portions of 50 ml. each. These were combined and evaporated to dryness, the residue being dissolved in 0·1N-H_2SO_4 (1ml.). After addition of acetone (5 ml.), a white precipitate appeared which was redissolved in the minimum volume of water. After storage at 4° for 5 days, plate-like crystals of agmatine sulphate formed and were washed with acetone and dried. The yield was approx. 0·5 mg.

The same procedure was used for a second extraction with 300 g. of stem and leaf material from potassium-deficient barley, the yield being 2 mg.

The isolated material had m.p. 236–239° (uncorr.). The mixed m.p. with recrystallized commercial agmatine sulphate (m.p. 238–242°, L. Light and Co. Ltd.) showed no depression [Engeland & Kutscher (1910) reported m.p. 228–230° and Kiesel (1922) gave m.p. 226°]. The infrared spectra of the isolated material and of authentic agmatine sulphate determined by the KBr-disk method were identical.

Formation of putrescine by barley seedlings on feeding with arginine, agmatine and ornithine. Barley seedlings were grown for 12 days in a glass-house, when they were about 6 cm. high. Shoots (2 g. samples) were excised and the cut ends were placed in four phials containing 5 ml. of 25 mM-agmatine sulphate, of 25 mM-L-arginine monohydrochloride, of 25 mM-L-ornithine monohydrochloride or of water, the depth of liquid in each phial being about 1 cm. After 24 hr. in continuous light, the samples were washed with water and extracted separately in ethanol (10 ml.). Each extract was centrifuged and the supernatant concentrated to 2 ml. in a stream of warm air. Samples (100 μl.) of these solutions were chromatographed on Whatman no. 1 paper in the butanol–ketone solvent. The extract of the barley fed with agmatine contained a ninhydrin-positive compound with R_F corresponding to that of putrescine, the same substance being barely discernible on chromatograms of the control extract. This compound was also present in the extracts of the barley which had been given arginine or ornithine, but the amounts were much smaller than those in the shoots treated with agmatine.

A Sakaguchi-positive compound which co-chromatographed with authentic agmatine was present in the extracts of barley supplied with arginine, at a higher concentration than in the controls.

Chromatography of the solutions remaining in the phials at the end of the 24 hr. feeding period showed that bacterial breakdown of the substrates was negligible.

Variation in relative proportions of arginine, agmatine and putrescine with potassium supply in barley leaves. The concentrations of arginine in barley supplied with high and low concentrations of potassium differed much less than those of either agmatine or putrescine, confirming the observation of Coleman & Richards (1956) for putrescine and arginine in this plant.

Occurrence of agmatine and putrescine in red-clover and cabbage leaves. Dried red-clover leaves, grown in the high- and low-potassium media, were boiled with water (15 g. in 100 ml.) and filtered through nylon. On cooling, each solution was applied to a column of Amberlite IRC-50 (H^+ form) as above. The residues from the $(NH_4)_2CO_3$ soln. eluate were dissolved in 0·05N-H_2SO_4 (2 ml.). Spots corresponding to agmatine and putrescine were just detectable on chromatograms of extracts of clover grown at optimum potassium concentration, but considerably larger amounts of both substances were found in potassium-deficient plants.

Oven-dried leaves from cabbage plants grown in potash-fertilized ground were analysed similarly and weak spots corresponding probably to agmatine and putrescine were detected on the chromatograms.

From the intensities of the ninhydrin colours it seemed that the ratio of agmatine to putrescine was roughly the same in extracts of the barley, clover and cabbage leaves.

DISCUSSION

Agmatine is a metabolite in several invertebrates (Irvin & Wilson, 1939; Thoai, Roche & Robin, 1953; Lissitzky, Garcia & Roche, 1954), and has been detected in ergot (Engeland & Kutscher, 1910), in which putrescine also occurs (Rieländer, 1908). Agmatine has been isolated from ragweed pollen (Heyl, 1919) and detected chromatographically in the seeds and fruits of several other plants (Shibuya & Makisumi, 1953; Mourgue, Baret & Dokhan, 1953).

Certain bacteria produce agmatine from arginine (Sher & Mallette, 1954; Rodwell, 1953) and also convert it into putrescine (Nakamura, 1944; Møller, 1955). A further pathway for putrescine formation from arginine, with ornithine as the intermediate, has been demonstrated in bacteria (Gale, 1940). Coleman & Hegarty (1957) showed that [^{14}C]ornithine is converted into putrescine by barley shoots. This putrescine may be formed either by decarboxylation of the ornithine or by a less direct route, perhaps involving arginine and agmatine as intermediates.

The present results indicate that the preponderant immediate precursor is agmatine, which appears to be a product of arginine decarboxylation.

Evidence that the activity of at least one of the enzymes concerned in converting arginine into putrescine may be enhanced by lack of potassium was obtained by M. Bryant & F. J. Richards (unpublished work), who found in feeding experiments that uniformly labelled L-[^{14}C]arginine caused a more rapid production of radioactive putrescine in shoots of potassium-deficient barley than in shoots of barley grown with optimum potassium supply.

The increase in amines in deficiency conditions is apparently not a result of mass action due to increased arginine, since in barley the arginine concentration is less dependent on potassium supply than are those of agmatine and putrescine. Since both amines are increased in potassium deficiency it seems probable that potassium inhibits agmatine production.

SUMMARY

1. In potassium-deficient barley plants 1,4-diaminobutane (putrescine), which had been previously characterized, occurs together with a Sakaguchi- and ninhydrin-positive compound, whose infrared spectrum and chromatographic properties are identical with those of 1-amino-4-guanidinobutane (agmatine).

2. Agmatine and putrescine occur in potassium-deficient red-clover plants.

3. Under conditions of adequate potassium nutrition, agmatine and putrescine were found in barley and clover, and also in cabbage leaves, but the amounts were small relative to those in the potassium-deficient plants.

4. The putrescine concentration in barley seedlings was considerably increased by feeding with agmatine, a much smaller increase being induced by giving ornithine or arginine. Feeding with arginine increased the concentration of a Sakaguchi-positive compound, which was chromatographically indistinguishable from agmatine.

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Preparation and Properties of the Mucopeptides of Cell Walls of Gram-Negative Bacteria

BY J. MANDELSTAM
National Institute for Medical Research, Mill Hill, London, N.W. 7

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Salton (1953) and Cummins & Harris (1956) showed that the cell walls of Gram-positive bacteria consisted mainly of polymers which were later called mucopeptides by Mandelstam & Rogers (1959). These always contained glucosamine, muramic acid, glutamic acid, alanine, either lysine or $\alpha\epsilon$-diaminopimelic acid, and sometimes one or two additional amino acids. The mucopeptides of some bacteria such as *Micrococcus lysodeikticus* were completely digested by lysozyme, but others were attacked only slightly (Salton & Pavlik, 1960).

The walls of Gram-negative bacteria were more complex. They contained lipid (about 20%), polysaccharides and apparently all the amino acids normally present in protein (Salton, 1953). However, the existence of mucopeptide in these walls was suggested by the fact that both muramic acid and diaminopimelic acid were present. These compounds are not known to occur in any type of polymer except mucopeptide. In addition, Gram-negative cells could be lysed by lysozyme (Repaske, 1958; Zinder & Arndt, 1958), and the materials liberated by lysozyme action contained the hexosamines and amino acids characteristic of mucopeptide (Salton, 1958; Primosigh, Pelzer, Maass & Weidel, 1961).

Later the mucopeptide of *Escherichia coli* was sufficiently separated from protein and polysac-