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EFFECT OF MINERAL DEFICIENCY ON AMINE FORMATION IN HIGHER PLANTS

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Key Word Index—*Hordeum vulgare*; barley; *Raphanus sativus*; radish; *Pisum sativum*; pea; *Vicia faba*; broad bean; *Spinacia oleracea*; spinach; potassium and magnesium deficiency; agmatine, putrescine, spermidine, spermine and hordatine content; biosynthesis.

Abstract—Potassium deficiency caused putrescine accumulation in the leaves of barley, radish, pea, bean and spinach plants. Magnesium deficiency caused putrescine accumulation in barley, pea and bean leaves, and also in the leaves of older radish plants. In young radish plants less putrescine was found in magnesium deficiency, and in spinach magnesium deficiency was without effect on putrescine levels. Putrescine content may be a useful guide to the mineral status of legumes, since accumulation of this amine may be detected before deficiency symptoms appear. Radioactivity from L-arginine-[U-^{14}C] fed to barley seedlings was detected in agmatine within 2 hr, and probably also in the hordatines after 24 hr feeding. After 2 hr the label in the agmatine was greatest in the potassium-deficient plants, but after 24 hr the level declined to that found in the agmatine of the leaves of the magnesium-deficient and control seedlings. The rate of putrescine formation was high in both potassium and magnesium deficiency. Incorporation of radioactivity in spermidine and spermine on feeding putrescine-[1,4-^{14}C] to barley seedlings was estimated in the dansylated amines after separation by TLC. Activity was higher in spermidine and lower in spermine in the potassium-deficient plants than in the controls. The spermidine/spermine ratio declined on excision of barley leaves.

INTRODUCTION

ALTHOUGH magnesium deficiency in the fungus *Neurospora* and the bacterium *Escherichia coli* has a greater effect on polyamine levels than on levels of the diamine putrescine,[1,2] in barley magnesium deficiency has little effect on the polyamines; however, there is a significant increase in putrescine in barley plants under these conditions.[3] Since the discovery[4] by Richards and Coleman in 1952 of the accumulation of putrescine in potassium-deficient barley, a wide range of higher plants has been shown to accumulate this amine when grown in conditions of potassium deficiency.[5] Since the generality of the accumulation of this amine in magnesium deficiency was not known, in the present work the effects of both potassium deficiency (–K) and magnesium deficiency (–Mg) on putrescine and polyamine levels in several dicotyledonous plants, as well as in barley, have been investigated.

It was also of interest to study the effect of mineral deficiency on the metabolism of L-arginine-[U-^{14}C] since there is evidence that the putrescine in both barley[6] and

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[1] VIOTTI, A., BAGNI, N., STURANI, E. and ALBERGHINA, F. A. M. (1971) *Biochim. Biophys. Acta* **244**, 329.
[2] HURWITZ, C. and ROSANO, C. L. (1967) *J. Biol. Chem.* **242**, 3719.
[3] SMITH, T. A. (1973) *Phytochemistry* **12**, 2093.
[4] RICHARDS, F. J. and COLEMAN, R. G. (1952) *Nature* **170**, 460.
[5] MURTY, K. S., SMITH, T. A. and BOULD, C. (1971) *Ann. Botany* **35**, 687.
[6] SMITH, T. A. (1971) *Biol. Rev. Cambridge Phil. Soc.* **46**, 201.

tobacco[7] originates primarily from arginine via agmatine. Moreover, the further metabolism of putrescine into the polyamines in these treatments was investigated by feeding putrescine-[^{14}C] to the shoots of barley seedlings.

RESULTS AND DISCUSSION

Effect of mineral deficiency on amine levels in higher plants

The effect on the putrescine and polyamine levels of K and Mg deficiencies alone, and in combination, and in one case together with Ca deficiency, were studied in the leaves of barley plants and the results are presented in Table 1.

TABLE 1. EFFECT OF MINERAL DEFICIENCIES ON PUTRESCINE LEVEL IN BARLEY LEAVES (sown February 1973)

Age (days)	Treatment	Deficient ion(s)	Compensating ion(s)	Amine levels (nmol/g fr. wt) Putrescine	Spermidine	Spermine	Dry wt (%)
28	1	K, Mg	Na, Ca	1660	not determined		13·6
	2	K, Mg	—	1210	not determined		13·0
	3	K, Mg, Ca	—	3070	not determined		10·0
	4	K	—	1710	not determined		16·0
	5	K	Na	1720	79	30	11·0
	6	Mg	Ca	2030	110	56	10·8
	7	(Control)	—	280	93	51	9·6
49	5	K	Na	3960	78	6	21·4
	6	Mg	Ca	4090	121	34	15·4
	7	(Control)	—	1480	96	49	22·4

Means of duplicate determinations. For details of treatments see Table 5. Standard error of putrescine means (28 days) for treatments 1 to 7 = 13%. Standard error for means of amine levels (treatments 5, 6 and 7) = 10%.

The concentration of putrescine in the –K barley leaves, in which Na was used[3,8] as the compensating ion in the present work (Table 1, treatment 5), was *ca* 0·25 of that found by Hackett *et al.*[9] who gave mean values of 16 μmol/g fr. wt. In the latter work, sodium sulphate was not added to compensate for the reduced potassium sulphate in the deficient media. The possibility that the discrepancy in putrescine content could be explained on the basis of the suppression of putrescine accumulation by the Na^+, as suggested by Coleman and Richards,[10] was unlikely since on omitting the Na ions the level of putrescine declined (Table 1, treatment 4). Perhaps a more likely interpretation for the lower levels of putrescine in the –K barley in the present study may be found in the behaviour of the barley varieties used in the various investigations.

In the leaves from treatments 1 to 6 (28 days old) putrescine was significantly higher than in the control leaves ($P = 1\%$), but there was no significant difference in the putrescine level between treatments 1, 2, 4, 5 and 6. The highest concentration of putrescine was found in the plants suffering from K, Mg and Ca deficiencies in combination ($P = 5\%$) (treatment 3). The increased putrescine level found in the older control plants tended to diminish the ratio of putrescine between these and the –K and –Mg plants. A similar effect, though not so marked, was found in previous work.[3] The low level of spermine ($P = 1\%$) previously noted in the –K plants[3] was confirmed.

[7] YOSHIDA, D. (1969) *Plant Cell Physiol.* **10**, 923.
[8] SMITH, T. A. (1970) *Phytochemistry* **9**, 1479.
[9] HACKETT, C., SINCLAIR, C. and RICHARDS, F. J. (1965) *Ann. Botany* **29**, 331.
[10] COLEMAN, R. G. and RICHARDS, F. J. (1956) *Ann. Botany* **20**, 393.

The effect of K and Mg deficiency on the amine contents of radish, pea, bean and spinach leaves are given in Table 2. In radish, although putrescine levels are high in the -K leaves after 27 days' growth, in Mg deficiency the putrescine level is lower than that of the control at this time, and even after 40 days the putrescine level is only twice as high as that of the control. By contrast, in the pea leaves Mg deficiency induces significant putrescine accumulation, though only half that found in the -K material, and a similar relation was found in the bean plants. No consistent trend could be found in the relation between polyamine level and mineral deficiency in these plants. In spinach, although K deficiency raised putrescine 3-fold, Mg deficiency had virtually no effect on the level of this amine.

TABLE 2. EFFECT OF POTASSIUM AND MAGNESIUM DEFICIENCIES ON THE DI- AND POLY-AMINES OF RADISH, PEA, BEAN AND SPINACH LEAVES

Plant	Age (days)	Deficient ion	Treatment	Amine levels (nmol/g fr. wt) Putrescine	Spermidine	Spermine	Dry wt (%)
Radish* sown	27	K	5	1470^{xx}	339^{xx}	31^{xx}	8·0
15 March 1973		Mg	6	130	211^{xx}	38^{xx}	5·6
		Control	7	380	142	17	8·2
	40	K	5	1660^{xx}	485^{xx}	44^{xx}	3·8
		Mg	6	510	445^{xx}	58^{xx}	7·6
		Control	7	210	178	14	8·4
Pea* sown	34	K	5	2030^{xx}	504	51	9·8
30 March 1973		Mg	6	1230^{xx}	517	65	9·8
		Control	7	270	340	45	10·0
Pea*† sown	44	K	5	2900	349	46	—
19 February 1973		Control	7	380	197	59	—
Bean* sown	34	K	5	1120^{xx}	111	25	9·2
19 February 1973		Mg	6	350^{xx}	87^{x}	16^{xx}	11·4
		Control	7	110	164	41	10·0
Spinach† sown	64	K	5	860	137	68	13·6
15 March 1973		Mg	6	240	196	54	16·2
		Control	7	270	217	57	12·0

* Means of duplicated determinations.
† Not included in the statistical analysis.
Difference from control, xx = significant at 1% level; x = significant at 5% level. The results from the 27- and 40-day-old radish leaves were combined. Standard error of treatment means for radish is 12% and for pea and bean, 17%.

Hoffman and Samish[11] found that K deficiency, which caused a reduction in the leaf K of 50% induced a 15-fold rise in putrescine in apple leaves and a 20-fold rise in grape vine leaves. They concluded that the level of putrescine is a more sensitive indicator of K status than the actual K content of the plant, and the results obtained with barley,[9] rye grass[12] and blackcurrant[5] indicate that their idea justifies further investigation.

However, in barley there is a direct correlation between amine content (agmatine + putrescine) and the proportion of dead shoots[9] suggesting that amine content is no better than visual symptoms as an indicator of K deficiency, and Nowakowski and Byers[12] came to a similar conclusion for rye grass (*Lolium multiflorum*). In blackcurrant leaves also, visual symptoms are correlated with the increase in putrescine content.[5]

[11] HOFFMAN, M. and SAMISH, R. M. (1971) in *Recent Advances in Plant Nutrition* (SAMISH, R. M., ed.), Vol. 1, p. 189. Gordon & Breach, New York.
[12] NOWAKOWSKI, T. Z. and BYERS, M. (1972) *J. Sci. Food Agric.* **23**, 1313.

In the present study, although the pea and bean plants grown in the –Mg and –K (+Na) media (treatments 5 and 6) showed no visual symptoms at the time of harvest, the putrescine content was raised significantly in both deficiencies. This suggests that putrescine may be a better guide to mineral deficiency than visual symptoms in legumes. Similarly, in –K *Sesamum indicum*, putrescine accumulates before symptoms appear (Basso, unpublished). Later experiments indicated that visual symptoms of K deficiency in legumes could be induced more easily by the use of treatment 4 than with treatment 5, though putrescine was not estimated in these plants.

Possible reasons for the accumulation of the diamine putrescine in K and Mg deficiency in terms of the known functions of the amines and the nutrient elements have been discussed earlier.[3] Previous studies on the metabolic pathway of putrescine formation suggest that this amine is derived from arginine with the intermediate formation of agmatine and *N*-carbamylputrescine. The enhanced activity of the enzymes in this pathway induced by K deficiency[6] and probably also in Mg deficiency[3] provide an adequate mechanism for the accumulation of putrescine in K and Mg deficiency. Although *a priori* it is not necessary to postulate an additional mechanism for putrescine accumulation, another may be found in the metal activation of the enzymes required for the synthesis of the polyamines spermidine and spermine from putrescine, in which decarboxylated *S*-adenosylmethionine donates one or two propylamine residues to putrescine to form spermidine and spermine respectively. Although little is known about the enzyme forming *S*-adenosylmethionine (*S*-adenosylmethionine synthetase) in higher plants, this enzyme has been isolated from liver and yeast[13] and has been shown to require a monovalent cation which is satisfied equally by NH_4^+, K^+ or Rb^+, but not by Li^+, Cs^+ or Na^+, and it also requires unusually high concentrations of Mg^{2+} for optimal activity. In addition, the enzyme decarboxylating *S*-adenosylmethionine found in mung bean sprouts is strongly stimulated by Mg^{2+} ions, although this enzyme from carrot and cabbage leaves is apparently not dependent on Mg^{2+}.[14] Any reduction in the activity of these two enzymes caused by deficiency of K, or more especially of Mg, could lead to the accumulation of putrescine. Although this mechanism may contribute to putrescine accumulation in the deficient plants, the reduction in spermidine and spermine in K and Mg deficiency predicted by this hypothesis is not consistently found (see Tables 1 and 2). However, spermidine and especially spermine are low in –K barley. Moreover, both polyamines were reduced in –Mg bean leaves and this also occurred in –K bean leaves, though to a lesser extent.

Investigation of the metabolic pathway of putrescine formation and degradation in barley

In barley, Coleman and Hegarty[15] demonstrated that label from D,L-ornithine-[2-^{14}C] is incorporated into putrescine, though the rapid labelling of citrulline and arginine in their work raises the possibility of an indirect pathway for the conversion of ornithine to putrescine.

In the present study, extracts of –K barley leaves were dialysed to pH 4·5, 6·5 and 8·5 and putrescine was estimated by GLC on incubation with L-ornithine. Even after 48 hr incubation in the presence of 100 μg of pyridoxal phosphate no evidence could be found for putrescine formation, which might have indicated the presence of ornithine decar-

[13] Mudd, S. H. and Cantoni, G. L. (1958) *J. Biol. Chem.* **231**, 481.
[14] Coppoc, G. L., Kallio, P. and Williams-Ashman, H. G. (1971) *Int. J. Biochem.* **2**, 673.
[15] Coleman, R. G. and Hegarty, M. P. (1957) *Nature* **179**, 376.

boxylase. In similar extracts at pH 6·5, arginine decarboxylase is known to be active[16] and it therefore seems likely that arginine is more important than ornithine as a precursor of putrescine in barley leaves, since agmatine, the product of arginine decarboxylation, is readily converted to putrescine on being fed to intact barley seedlings.[17]

L-Arginine-[U-^{14}C] was fed to the cut leaves of 16-day-old barley seedlings which had been grown in −K, −Mg or full nutrient media (treatments 5, 6 and 7). Each treatment was duplicated and samples taken after 2 and 24 hr for analysis (see Experimental). Samples were also taken from the original barley material prior to feeding for estimation of putrescine, spermidine, spermine, agmatine, arginine and dry weight (Table 3).

TABLE 3. ARGININE AND AMINE LEVELS IN THE SHOOTS OF 16-DAY-OLD POTASSIUM- AND MAGNESIUM-DEFICIENT AND CONTROL BARLEY PLANTS, AND THE NET FORMATION OF AGMATINE AND PUTRESCINE DETERMINED BY FEEDING L-ARGININE-[U-^{14}C] TO THESE SHOOTS

Treatment	Arginine	Agmatine	nmol/g fr. wt Putrescine	Spermidine	Spermine	Dry wt (%)
Potassium-deficient	370	630	2890	95	39	11·4
Magnesium-deficient	230	290	770	71	45	9·1
Control	130	260	700	82	34	10·8

Time after feeding	Treatment	cpm/g fr. wt Agmatine	cpm/g fr. wt Putrescine	Net amine formation (nmol/g fresh wt) Agmatine	Net amine formation (nmol/g fresh wt) Putrescine
2 hr	Potassium-deficient	13200	15900	24·3	29·4
	Magnesium-deficient	3400	15600	3·9	18·0
	Control	5100	14200	3·1	8·5
24 hr	Potassium-deficient	2100	16300	3·8	30·2
	Magnesium-deficient	3800	3700	4·3	4·2
	Control	2400	1800	1·4	1·1

Each value is the mean of two estimations. Standard error of treatment differences for amine levels = 14%.

Label was incorporated into agmatine and putrescine, though no label could be detected in *N*-carbamylputrescine (NCP), (less than 3% of that in putrescine). This was unexpected since there is strong evidence that NCP is an intermediate in the conversion of agmatine to putrescine. Agmatine feeding is known to induce the accumulation of NCP in the cut shoots of barley seedlings,[18] and an enzyme specifically hydrolysing this amine has been demonstrated in extracts of barley leaves.[19] It appears that NCP has only a transient existence in barley, even in conditions of K deficiency. This may also be true in many other higher plants, since no NCP could be detected in the amine fractions of the −K plants investigated in Tables 1 and 2 (limit 50 nmol/g fresh wt). However, NCP has been found in sugar cane tissue grown *in vitro*[20] and it occurs at 500 nmol/g fr. wt in K-deficient *Sesamum*.[21]

[16] SMITH, T. A. (1963) *Phytochemistry* **2**, 241.
[17] SMITH, T. A. and RICHARDS, F. J. (1962) *Biochem. J.* **84**, 292.
[18] SMITH, T. A. and GARRAWAY, J. L. (1964) *Phytochemistry* **3**, 23.
[19] SMITH, T. A. (1965) *Phytochemistry* **4**, 599.
[20] MARETZKI, A., THOM, M. and NICKELL, L. G. (1969) *Phytochemistry* **8**, 811.
[21] CROCOMO, O. J., BASSO, L. C. and BRASIL, O. G. (1970) *Phytochemistry* **9**, 1487.

In the first 2 hr feeding, correcting for reduced arginine uptake by the –K and –Mg shoots and for isotope dilution in the endogenous arginine, incorporation of radioactivity from arginine into agmatine was 8-fold greater in the –K shoots than in the –Mg or control plants, and since the radioactivity in agmatine declines rapidly with time from 2 to 24 hr in the –K tissue turnover of this amine is probably very fast in this material. On correcting for isotope dilution in the non-radioactive agmatine, putrescine was found to be formed more rapidly in the –K and –Mg seedlings than in the control plants by a factor of 3- and 2-fold respectively. Although the –Mg seedlings did not show an enhanced putrescine level (and indeed did not show visual symptoms of Mg deficiency), putrescine was strongly labelled within 2 hr. However, unlike the label in the putrescine in the –K plants, this label was lost within 24 hr. In the normal seedlings, incorporation into putrescine is low after 2 hr and this level declines after 24 hr, indicating that there is a relatively rapid turnover of putrescine. The high level of label in putrescine in the –K leaves after 24 hr feeding may result from a reduction in the further metabolism of putrescine in the –K tissue, an explanation proposed previously for this phenomenon in tobacco tissue by Yoshida.[22] However, label may reach even higher levels between 2 and 24 hr and the rate of putrescine degradation may be unchanged with K deficiency. On the basis of the present results it is not possible to distinguish between these possibilities.

In barley, agmatine, besides being utilized in putrescine formation, is likely to be the precursor of the hordatines, which are anti-fungal dimers of coumaroylagmatine.[23, 25] In view of this possibility, some of the properties of these conjugates were studied in relation to the techniques used in the investigation of agmatine in the present work. In the estimation of arginine and agmatine, using IRC-50 resin for the separation of these guanidines, no hordatines were found in the arginine fraction, which is homogeneous, but hordatines are found in the agmatine fraction and contribute to the Sakaguchi colour. They probably comprise 50% of the total guanidino compounds in the normal plant extracts and about 5% in the extracts of the deficient plants, as assessed by the size and intensity of the spots on TLC. Moreover, they are present in the amine fraction eluted from Dowex-50 by 12 M HCl, and here they may be partially hydrolysed to free agmatine in the evaporation step. K deficiency increases the content of one of these hordatines by a factor of two, probably hordatine M,[25] but the level of hordatines A + B is apparently unchanged.

In solvent 2, which separates the hordatines from agmatine, putrescine and arginine, label in the hordatine spots was found to be negligible after 2 hr feeding, but label became significant after 24 hr feeding and was then comparable with that found in agmatine. This radioactivity is included with that for free agmatine in the present results, since the agmatine and the hordatines have similar R_f values in solvent 1. The agmatine combined in the hordatines is a considerable proportion of the total agmatine in the normal plants, and estimates of the incorporation of radioactivity into putrescine based on the estimates for total agmatine are probably more than the actual rates by a factor of about 2 in the normal plants. Since the hordatines comprise a smaller proportion of the total guanidines in the –K tissue the rate of synthesis of putrescine in –K plants is probably greater than 3·5 times that of the normal plants.

[22] YOSHIDA, D. (1969) *Plant Cell Physiol.* **10**, 393.
[23] STOESSL, A. (1965) *Phytochemistry* **4**, 973.
[24] STOESSL, A. (1967) *Can. J. Chem.* **45**, 1745.
[25] STOESSL, A. and UNWIN, C. H. (1970) *Can. J. Botany* **48**, 465.

In the plants fed with putrescine-[1,4-^{14}C], (Table 4), the high level of radioactivity recovered in the putrescine in the –K plants may be explained by a low turnover of this amine in K deficiency. However, the high level of putrescine in the –K tissue may also account for this.

TABLE 4. PUTRESCINE, SPERMIDINE AND SPERMINE LEVELS IN THE SHOOTS OF 15-DAY-OLD POTASSIUM- AND MAGNESIUM-DEFICIENT AND CONTROL BARLEY PLANTS, AND THE NET FORMATION OF SPERMIDINE AND SPERMINE DETERMINED BY FEEDING PUTRESCINE-[1,4-^{14}C] TO THESE SHOOTS

Treatment	nmol/g fr. wt Putrescine	Spermidine	Spermine	Dry wt (%)
Potassium-deficient	2540	37	28	9·8
Magnesium-deficient	1130	30	46	12·1
Control	870	41	47	10·1

Treatment	cpm/g fr. wt Putrescine	Spermidine	Spermine	Net amine formation (nmol/g fresh wt) Spermidine	Spermine
Potassium-deficient	72700	3180	1350	110	13
Magnesium-deficient	39700	2150	1690	61	24
Control	43600	2720	2010	55	30

Each value is the mean of two estimations.

After correction for the reduced uptake by the mineral-deficient leaves and for the dilution in the endogenous putrescine, the results suggest that more spermidine and less spermine is formed in the –K plants by comparison with the other treatments. However, unequivocal interpretation of the results is difficult, since sampling was made only after 24 hr feeding, during which the specific activity of the putrescine remained constant in the –K plants and fell quickly in the other treatments (Table 3). It is therefore necessary to analyze at close intervals of time in order to determine the kinetics of the system.

TABLE 5. COMPOSITION OF NUTRIENT SOLUTIONS IN mM CONCENTRATIONS

Compound	Treatment 1	2	3	4	5	6	7
K_2SO_4	—	—	—	—	—	2·0	2·0
$MgSO_4$	—	—	—	1·5	1·5	—	1·5
$CaCl_2$	5·5	4·0	—	4·0	4·0	5·5	4·0
$NH_4H_2PO_4$	1·33	1·33	1·33	1·33	1·33	1·33	1·33
$(NH_4)_2HPO_4$	0·07	0·07	0·07	0·07	0·07	0·07	0·07
NH_4NO_3	6·0	4·0	4·0	4·0	6·0	6·0	6·0
$(NH_4)_2SO_4$	—	2·0	2·0	2·0	—	—	—
Na_2SO_4	2·0	—	—	—	2·0	—	—

Micronutrients were added in each case.[3]

An added complication in the interpretation of these results and the extrapolation of them to natural conditions is the apparent loss of spermidine which occurs on excision of the shoots. This is almost certainly related to the loss of protein and nucleic acid known to take place on excision of the leaves of barley[26] and the probable association

[26] YEMM, E. W. (1949) *New Phytologist* **48**, 315; ATKIN, R. K. and SRIVASTAVA, B. I. S. (1968) *Physiol. Plant.* **21**, 1234; ATKIN, R. K. and SRIVASTAVA, B. I. S. (1970) *Physiol. Plant.* **23**, 304.

between the rate of protein synthesis and spermidine levels.[27] Additional problems in the interpretation of the results may arise from the segregation of metabolic pools, but with present knowledge it is difficult to assess the significance of this factor.

EXPERIMENTAL

Effects of mineral deficiency on amine levels. Barley (*Hordeum vulgare* L., cv. Zephyr), radish (*Raphanus sativus* L., cv. Round Black Spanish), pea (*Pisum sativum* L., cv. Meteor), broad bean (*Vicia faba* L., cv. Aquadulce Claudia) and spinach (*Spinacia oleracea* L., cv. Noorman) were sown in sand in a greenhouse. The sand was watered daily with the nutrients shown in Table 5 which included the micronutrients.[3] Putrescine, spermidine and spermine were estimated in duplicate 10 g samples of analogous leaf material. The amines were concentrated and purified by an ion exchange method and converted to their dansyl derivatives prior to chromatography and estimation by their fluorescence.[3] A paper pad was attached to the top of the TLC plates to increase the separation of the fluorescent spots. Other 5 g samples were taken for estimation of dry wt. In order to expedite harvesting, the leaves were immersed in the trichloroacetic acid for up to 24 hr prior to extraction. On comparison with immediate extraction, no significant effect on the di- and poly-amine levels was detected using this treatment. Direct dansylation of perchloric acid extracts[28] was also attempted. Tissue (5 g) was extracted in 0·2 N perchloric acid (20 ml), and after centrifugation aliquots (0·2 ml) of the supernatant were dansylated, and the dansyl amines extracted into 2·5 ml toluene (×2). The residue obtained on evaporating the toluene layer at 60° under vacuum was redissolved in 0·25 ml toluene and samples (2–10 μl) were chromatographed. Many fluorescent spots were obtained, some of which overlapped the polyamines and this interference was particularly severe with extracts of the barley leaves. However, it appears possible to isolate the dansyl putrescine in the radish leaf extracts by this technique and this may provide a rapid method for the investigation of putrescine in this plant. Evaporation of the toluene at 60° under vacuum may have caused the variation experienced in the recovery of the amines, and milder methods of evaporation would probably be needed to make this method quantitative. Evaporation of the acetone after dansylation and prior to extraction in a small vol. of toluene[29] may eliminate the losses associated with toluene evaporation.

Feeding of radioactive metabolites. Chromatographically pure L-arginine-[U-^{14}C] (1HCl), 336 mCi/mmol, and putrescine-[1,4-^{14}C] (2 HCl), 18·3 mCi/mmol, were obtained from The Radiochemical Centre, Amersham. Barley seedlings were grown for 16 days in treatments 5–7 (−K, −Mg and control; Table 1) in a greenhouse, and 2 μCi of each substrate was fed separately to the cut shoots (5 g samples) in glass phials. After total absorption of the labelled substrate the shoots were washed and placed in 10 ml H_2O in the greenhouse. Samples were extracted after 2 and 24 hr for arginine feeding, but for the putrescine feeding experiment the shoots were sampled only after 24 hr. The tissue was extracted in 40 ml 5% trichloroacetic acid, the amines purified on Dowex-50 resin[8] and the amine fractions made up in 0·5 ml 0·1 M HCl. Using the Sakaguchi reagent[16] recovery of agmatine in duplicate estimations was 81 and 106%, and recovery of arginine was about 50% in the amine fraction. In duplicate estimations, recovery of *N*-carbamylputrescine was 77 and 75% using the colorimetric procedure of Hunninghake and Grisolia.[30] The amine fraction from the barley fed with the labelled arginine was subjected to TLC on cellulose CC41 containing 10% soluble starch, with *n*-BuOH–EtMeCO–·88 NH_3–H_2O (5 : 3 : 1 : 1) [solvent 1]; *n*-BuOH–HOAc–H_2O (4 : 1 : 1) [solvent 2]; and methylcellosolve–HOPr–H_2O (70 : 15 : 15) saturated with NaCl [solvent 3]. In solvent 1 the EtMeCO was redistilled and the solvent made up immediately prior to use. R_fs for solvents 1 and 3 are given in Ref. 8. R_fs in solvent 2 were arginine, 0·08; agmatine, 0·07; *N*-carbamylputrescine, 0·15; putrescine, 0·06; spermidine 0·02; and spermine, 0·02. Solvent 1 separated arginine, agmatine, *N*-carbamylputrescine and putrescine. The polyamines spermidine and spermine migrated with the putrescine. Samples (2 μl) were chromatographed on layers 200 μm thick or up to 10 μl on layers 1 mm thick. With greater loadings than these the components streaked and the resolution was impaired. Solvent 2 separated the polyamines, *N*-carbamylputrescine and putrescine, and was used prior to solvent 1 for the 2-D separation of *N*-carbamylputrescine. Solvent 3 was used for the isolation of the polyamines in a single dimension. Authentic unlabelled amines were chromatographed to confirm identifications after ninhydrin treatment and prior to counting. Samples of the amine fraction (10 μl) were chromatographed in solvent 1; the amine spots were visualized with the ninhydrin reagent. The cellulose adsorbent bearing the agmatine and putrescine spots were dispersed in 5 ml toluene containing 1% PPO and 0·015% of POPOP (scintillating liquid) before scintillation counting. The radioactive materials would not dissolve in this liquid. Arginine and agmatine were determined by the Sakaguchi method[3] in separate samples taken from the seedlings, and putrescine, spermidine and spermine were estimated in 0·1 ml samples of the radioactive amine fractions by the dansyl method.[3] Radioactivity

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in *N*-carbamylputrescine, spermidine and spermine was negligible ($< 3\%$) by comparison with that in putrescine at both 2 and 24 hr. Hordatine-containing fractions of normal and –K barley leaves were prepared according to the method of Stoessl by elution of H_2O extracts from IRC-50 resin with 2 M HOAc.[25] The hordatines were separated in solvent 2, and the TLC plates were heated at 100° for 1 hr to remove traces of HOAc before applying the Sakaguchi reagent.[31] Samples (100 μl) of the amine fractions from the shoots fed with putrescine-[^{14}C] were dansylated and 60 μl samples of the toluene layer were separated by TLC on silica gel G using solvent 2 of Ref. 3. The plate was sprayed lightly with triethanolamine–isoPrOH (1 : 4) and the fluorescent radioactive spots were removed from the plate, added to 5 ml scintillation liquid and counted at 5°. All the radioactivity found in the dansyl spots was soluble in the scintillation liquid. Activity in the spots was at least 2× that of the background (50 cpm). Radioactivity in the regions between the amine spots on the TLC plate was less than 5% above background. Absolute levels of the amines were estimated in 10 μl samples similarly chromatographed and scanned in a fluorimeter.[3]

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