Occurrence of free basic amino acids in 5 varieties of lentil seed

Variety[a]	I	II	III	IV	V
Region	Balkans	USA	Algeria	India	France
Total nitrogen	4.10	4.49	4.00	4.26	3.80
Lysine	0.05	0.06	0.05	0.06	0.07
Histidine	0.02	0.02	0.01	0.03	0.01
Arginine	0.29	0.41	0.25	0.23	0.26
Ornithine[b]	n.d.	n.d.	n.d.	n.d.	n.d.
γ-Hydroxyornithine	0.06	0.08	0.04	0.06	0.06
γ-Hydroxyarginine	0.81	0.85	0.51	0.56	0.89
Homoarginine	0.02	0.07	0.02	0.02	0.01

All concentrations in % of dry substance. [a] For characterization of variety see reference[1]. [b] Present in small concentration; n.d. = not determined.

tioned that γ-hydroxyarginine and γ-hydroxyornithine were fond in several species of vetch (genus *Vicia*), and homoarginine, as a possible precursor of lathyrine, occurs in some species of the genus *Lathyrus*[6]. The presence in the lentil seed of both hydroxyarginine and homoarginine might be an incentive towards a review of the taxonomic position of the genus *Lens*.

Legumes constitute an important source of dietary protein for a considerable percentage of the world population. Certain peas of the genera *Lathyrus* and *Vicia*, which are of no commercial significance but which are eaten by sections of the population of India and the Mediterranean area, may cause neurological or muscular conditions, called lathyrism[7]. This disease is associated with the presence of toxic amino acids in these legume seeds, e.g. β-cyano-L-alanine, α,γ-diaminobutyric acid, β-N-oxalyl-α, β-diaminopropionic acid, and the related β-aminopropionitrile.

Lentil contains none of these toxic factors. Its characteristic and dominating amino acid within the legume family is γ-hydroxyarginine. Lentils have been eaten since ancient times, and have played an important role as a nutritional base in certain cultures of antiquity. This food has been proved 'historically' to be wholesome, yet its value has always been judged ambiguously. It is well known that some people suffer from vomiting, or allergic symptoms, after eating lentils. However, no scientific base, or experimental data, exists for considering a compound like hydroxyarginine as harmful, or as responsible for the phenomena mentioned. In preliminary inhibition studies with arginine antagonists, it was shown that γ-hydroxyarginine did not inhibit the growth of *Escherichia coli* or *Pseudomonas aeruginosa*[8]. Of lesser interest are similar experiments with homoarginine, which is a minor compound in the lentil seed[9,10].

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Assimilation of Ammonia and Growth of Biotin Deficient *Aspergillus nidulans*

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Summary. Biotin deficiency in *Aspergillus nidulans* has been found to increase the uptake of ammonium ions, associated with a marked increase in the activity of NADP-linked glutamate dehydrogenase, which is found to be the major route of ammonia assimilation in this culture. The results obtained are discussed with respect to the growth of *Aspergillus nidulans* during biotin deficiency.

Earlier studies in our laboratory indicated that biotin deficiency in *Aspergillus nidulans* gives rise to increased cellular synthesis, when grown on NH_4NO_3 as a sole nitrogen source[3,4]. Furthermore, it was observed that biotin deficiency in this culture caused marked increase in the growth rate, while the total period required for the completion of the growth cycle remained unaltered[5]. We have been able to demonstrate the remarkable increase in the uptake rate of ammonia attributable to biotin deficiency in *A. nidulans*[5,6]. Since biotin deficiency in this culture causes a marked increase (70%) in the protein content with a concomitant fall (65%) in the fatty acid content of the mold, it was of interest to study the assimilation of ammonia by this culture under these conditions. The present investigation suggests that the major route of ammonia assimilation is through NADP-linked glutamate dehydrogenase. The results obtained are discussed with respect to the growth of *A. nidulans* in a state of biotin deficiency.

The strain, media composition and the culture conditions used in the present investigation were the same as described earlier[5,7]. Culture grown in the presence of 5 units of avidin (General Biochemicals, Ohio) has a 65% lower fatty acid content than control. Biotin was not traceable in the mycelial extract, using the microbiological assay with *Lactobacillus arabinosus*, according to the

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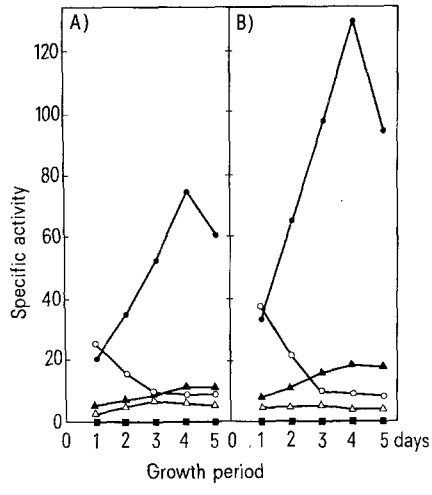
method of SKEGGS[8]. This culture is therefore referred to as 'biotin deficient' in this communication.

Cell free extracts for enzyme assays were prepared as described earlier[5]. Glutamate dehydrogenase (E.C. 1.4.1.3) and alanine dehydrogenase (E.C. 1.4.1.1) were assayed by the method described by THOMULKA and MOAT[9]. The method of ROON et al.[10] and FRY[11] was used for assay of glutamate synthase (E.C. 2.6.1.53) and glutamine synthetase (E.C. 6.3.1.2), respectively. Protein was estimated by the method of LOWRY et al.[12], using serum albumine as standard. A unit of enzyme was expressed as the amount of enzyme which causes a 0.001 change in the O.D. at 340 nm. Specific activity was expressed as units/mg of protein.

It has been demonstrated in a variety of microorganisms that inorganic nitrogen may be assimilated into amino nitrogen via glutamate, alanine, aspartate, carbamyl phosphate and glutamine, but no organism utilizes all these routes to the same degree[13,14]. Usually, a given organism will utilize one or two pathways predominantly, to the virtual exclusion of the others[15]. Since biotin deficiency in *A. nidulans* causes a marked increase in the protein content of the cells, it was of interest to study in detail the assimilation of inorganic nitrogen. Increased nitrate assimilation as a result of biotin deficiency and its regulation by ammonium ions has been demonstrated by us in this culture[5].

The results in the Figure show the specific activities of ammonia-assimilating enzymes. The data suggests that the major route of ammonia assimilation is through NADP-glutamate dehydrogenase. The NADP-linked alanine dehydrogenase and glutamine synthetase were present in both normal and biotin deficient cultures but their lower activity suggests their minor significance in ammonia assimilation. Alanine dehydrogenase was found to be NADP-linked and NAD-linked alanine dehydrogenase was not detected throughout the growth cycle. Similar results have been reported by Lamminnaki and PIERCE[16] and BURK and PATEMANN[17] in *S. cerevisiae* and *N. crassa*, respectively. Glutamate synthase was not detected in both the cultures throughout the growth cycle. This is in agreement with the results reported by BURN et al.[18], who showed absence of glutamate synthase in *N. crassa* and *A. nidulans*. Much of the information in the literature confirms the role of glutamate dehydrogenase in ammonia assimilation by microorganisms[19–23].

Thus, the results presented here suggest that the major route of ammonia assimilation in *A. nidulans* is via NADP-glutamate dehydrogenase. However, it is interesting to note the considerable increase in the activity of glutamate dehydrogenase in biotin-deficient culture as compared to that in normal culture of *A. nidulans*, which may be one of the factors for higher assimilation of ammonia[5,6]; and finally for the higher cellular synthesis as reported earlier[3,5]. However, at present it is not possible to elucidate the factor(s) responsible for the activation of this enzyme as a result of biotin deficiency in *A. nidulans*.



Specific activity of ammonia-assimilating enzymes in *Aspergillus nidulans*. Activities of NADP · glutamate dehydrogenase (●), NAD · glutamate dehydrogenase (○), NADP · alanine dehydrogenase (△), glutamine synthetase (▲), glutamate synthase (■) and NAD · alanine dehydrogenase (■) were determined on the different days of growth in normal (A) and biotin deficient (B) cultures.

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Effect of X-537A on the Phosphorylated Protein in Sarcoplasmic Reticulum Vesicles[1]

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Summary. The effect of the antibiotic X-537A on the phosphorylated ATPase (E∼P) was investigated. The results show that X-537A depresses the level of E∼P which is dependent on the Ca^{2+} gradient, while the Ca^{2+}-independent E∼P is not affected.

Sarcoplasmic reticulum (SR) regulates the concentration of free Ca^{2+} in myoplasm which controls muscle contraction and relaxation. This property of SR is governed by an ATPase which, in the presence of ATP, transports Ca^{2+} into the SR tubules with the formation of a phosphoprotein (E∼P) as an intermediate in the ATPase reaction[2–5].

In isolated preparations of SR vesicles, a Ca^{2+} gradient is generated after Ca^{2+} accumulation, and synthesis of E∼P and ATP can be coupled to the efflux of Ca^{2+} [6–13].