Nitrogen Accumulation in Mycelium of *Neurospora crassa*

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Mycelium of *Neurospora crassa* accumulates arginine and glutamine when deprived of an amino acid or pyrimidines, or in the presence of cycloheximide or at the end of exponential growth. A large proportion of the accumulated arginine is sequestered in an osmotically sensitive compartment. Electron microscopy of this fraction shows it consists of mitochondria and electron-dense bodies. Only a small part of the accumulated glutamine was sequestered in this fraction.

Mycelium of this fungus, when deprived of an amino acid, did not catabolize arginine due to a lack of arginase induction. A relationship is proposed between the sequestered arginine, its accumulation, and the lack of induction of arginase. The accumulated glutamine in the cytosol also prevents the catabolism of soluble arginine. Guanosine tetra- and penta-phosphate were not found in the mycelium of *N. crassa* during the deprivation of an amino acid or after cycloheximide treatment.

INTRODUCTION

It has been reported that starvation of histidine and tryptophan mutants of *Neurospora crassa* results in the derepression of histidine, tryptophan and arginine biosynthetic enzymes. This effect was not due to a lowered intracellular concentration of tryptophan or arginine (Carsiotis & Jones, 1974; Carsiotis, Jones & Wesseling, 1974). In *Saccharomyces cerevisiae*, it has been found that leaky mutants of amino acid biosynthetic pathways derepress their synthetic enzymes and the appropriate amino acids accumulate (Delforge, Messenguy & Wiame, 1975). In this paper we report the accumulation of arginine and glutamine in mycelium of *N. crassa* when growth is restricted.

It has been suggested previously that a substantial amount of the arginine found in *N. crassa* growing in minimal medium is localized within an osmotically sensitive compartment that sediments at 15000 g and which can be separated from mitochondria (Weiss, 1973). We have measured the degree to which accumulated amino acids are osmotically sequestered in such a compartment. We have also attempted to analyse the metabolic relationships between glutamine and arginine that result in the accumulation of these amino acids as nitrogen reservoirs in *N. crassa*.

METHODS

Organisms. Stocks came either from the Fungal Genetics Stock Center at the Humboldt State University Foundation, Arcata, California, U.S.A., or from the collection of J. Mora. The basic stocks were: wild type 74-A; *prol-3* which is unable to synthesize proline and grows in proline, ornithine or arginine (Vogel & Bonner, 1954); glutamine auxotroph *glm-1* which lacks glutamine synthetase; tryptophan auxotroph *tryp-2*; uridine auxotroph *pyr-3a*; and *arg-12^s* which has only 3 % of the ornithine transcarbamylase activity found

in the wild type (Davis, 1962). *Escherichia coli* mutants CP78 (rel⁺) and CP79 (rel⁻), and auxotrophs of leucine, threonine, histidine, arginine and thiamin were kindly provided by Dr F. Bastarrachea (Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, México).

Culture conditions. *Neurospora crassa* was grown in minimal medium N (MM) of Vogel (1964) supplemented with 1.5 % (w/v) sucrose or 2 % (v/v) glycerol. Conidia harvested from slants of supplemented MM were incubated in 250 ml Erlenmeyer flasks containing 50 ml of the same medium for 3 days in the dark at 29 °C followed by 2 days in the light at 25 °C. The final culture was inoculated with conidia harvested from the 250 ml flasks by filtration through glass wool, and was grown at 25 °C in 6 l Florence flasks containing 4 l medium, aerated with humidified air. The initial absorbance of conidia in the 6 l flasks was between 0.05 and 0.1 at 540 nm.

Growth determination. This was carried out as described for *N. crassa* conidia by Espín & Mora (1978).

Measurement of arginase activity. After growth for 12 h the mycelium was filtered (Whatman no. 41 filter paper) and thoroughly washed at room temperature with distilled water. Acetone powders were prepared from the mycelium, ground with dry ice and homogenized at 4 °C with 0.025 M-glycine/NaOH buffer (pH 9.5) containing 0.1 M-KCl, 0.01 M-dithiothreitol and 0.125 mM-MnCl₂. The homogenates were centrifuged at 4 °C at 12 500 g for 20 min and 4 ml of the supernatant was passed through a Sephadex G-25 column (35 × 35 mm) equilibrated with homogenization buffer. The void volume fractions which contained the protein peak were pooled and used as the enzyme source. The assay mixture (final volume 1.0 ml) contained 10.5 μmol glycine, 0.28 μmol MnCl₂ and 10 μmol L-[guanidino-¹⁴C]arginine (8 to 10 c.p.m. μmol⁻¹). The enzyme fraction (0.3 ml) was added to a 10 ml Erlenmeyer flask with a centre well holding a small tube containing 0.25 ml hyamine hydroxide. The flask was covered tightly with a rubber stopper (sleeve type). After 3 min in a water bath at 25 °C, the reaction was started by the injection of 0.7 ml of assay mixture. The reaction was stopped after 30 or 60 s by injecting 2 ml 0.2 M-sodium citrate (pH 5.0) containing 30 u. urease (1 u. at 30 °C produces 1 mg ammonia in 5 min at pH 7.0 from urea). The flasks were then incubated at 37 °C for 30 min in a rotary shaker water bath (100 rev. min⁻¹) and the reaction was stopped by injecting 1 ml 2 M-HClO₄. After a further 45 min incubation the tubes which contained the hyamine hydroxide were removed and placed in a vial with 15 ml scintillation fluid [0.4 % (w/v) 2,5-diphenyloxazole and 0.005 % (w/v) 1,4-di-2-(5-phenyloxazolyl)benzene in toluene]. The vials were counted in a Nuclear-Chicago scintillation counter (85 % efficiency) for a time sufficient to give at least 5000 c.p.m. per sample.

Preparation of soluble extracts for amino acid analysis. Mycelium samples were harvested by filtration (Whatman no. 41 filter paper) and washed with distilled water. Extracts were prepared and analysed for glutamic acid and glutamine as described by Espín & Mora (1978).

Mycelial arginine was extracted with 2 ml 5 % (w/v) trichloroacetic acid, and measured as described by Espín & Mora (1978).

Samples for amino acid analyses other than glutamic acid and glutamine were obtained by extracting mycelium with 1 % (w/v) picric acid. The extracts were analysed as described by Espín & Mora (1978).

Incorporation of [³²P]orthophosphoric acid into *E. coli* and *N. crassa*. *Escherichia coli* CP78 and CP79 were grown according to the method of Cashel (1969), except that ³²P at 500 μCi ml⁻¹ was added to the incubation medium. The cells from strain *prol-3* of *N. crassa* were grown in MM supplemented with 1.5 % (w/v) sucrose and proline (100 μg ml⁻¹). After 6 h incubation the mycelium was harvested by filtration through Millipore filters (type HA, pore size 0.45 μm), washed with distilled water and resuspended in MM containing lower concentrations of phosphate and supplemented with 1.5 % (w/v) sucrose. The specific radioactivity of ³²P was raised by lowering the concentration of KH₂PO₄ in MM from the normal 36.74 mM to 10 and 0.02 mM. Ten ml of medium with the two lower phosphate concentrations were incubated at 25 °C with agitation for 0.5, 1, 2 and 3 h with ³²P (250 μCi ml⁻¹) in the presence and absence of proline (100 μg ml⁻¹). Macromolecular synthesis was restricted by incubating mycelium with cycloheximide (10 μg ml⁻¹).

Autoradiography of labelled extracts. Acid extraction of ³²P-labelled bacterial and *Neurospora* cultures was performed as described by Cashel (1969), except that for *Neurospora* 10 ml of labelled culture was filtered through a Millipore filter, washed with cold distilled water and resuspended in 0.5 ml 2 M-formic acid, pH 3.4. The cell-free supernatants were spotted directly on to polyethyleneimine cellulose plates (Cashel, 1969) and subjected to two-dimensional thin-layer chromatography (Cashel, Lazzarini & Kalbacher, 1969). X-ray film was placed over the chromatograms and exposed for 18 h in the dark. Co-orientation of the films and chromatograms was accomplished with unlabelled standards of nucleotides (5 mM), including guanosine tetraphosphate.

Preparation and fractionation of sphaeroplasts. Procedures for the preparation of sphaeroplasts and the fractionation of *Neurospora* mycelium were as reported by Weiss (1973).

Electron microscopy. Mycelia from strain *prol-3* subjected to amino acid deprivation, and the 15000 g particulate fraction obtained from part of the same culture after the glucosylase treatment and homogenization procedure, were fixed at room temperature for 1 h with 2.5 % (v/v) glutaraldehyde in buffer containing 10 mM-KH₂PO₄, pH 7.5, and 1 mM-EDTA. Sorbitol (1 M) was added to the 15000 g fraction. The material

Fig. 1. Accumulation of arginine by mycelium of strains *prol-3* and 74-A. (a) Conidia from *prol-3* were grown in MM plus 1.5% (w/v) sucrose plus proline ($100 \mu\text{g ml}^{-1}$) for 12 h. The culture was then filtered, washed and resuspended in: ○, MM plus sucrose plus cycloheximide ($10 \mu\text{g ml}^{-1}$); ●, MM plus sucrose; □, MM plus sucrose plus proline; ■, MM plus 2% (v/v) glycerol; △, MM without NH_4NO_3 plus sucrose. (b) Conidia from 74-A were incubated in: ○, MM plus cycloheximide; ●, MM for 10 h and cycloheximide was then added.

was washed three times with the same buffer, post-fixed with 1% (w/v) OsO_4 in 0.15 M- NaH_2PO_4 , pH 7.0, for 1 h at 4 °C, dehydrated in a graded ethanol series and then embedded in Epon (Luft, 1961). Sections were cut with a Porter Blum II ultramicrotome, post-stained in 1% (w/v) uranyl acetate followed by lead citrate (Reynolds, 1966) and examined with a Jeol JEM-100B electron microscope.

Chemicals. All amino acids used were obtained from Calbiochem. Dithiothreitol, urease, glutaminase, bovine serum albumin and nucleotides were obtained from Sigma. L-[Guanidino- ^{14}C]arginine, L-[U- ^{14}C]glutamine, L-[U- ^{14}C]glutamic acid, and [^{32}P]orthophosphoric acid were purchased from New England Nuclear. L-[Guanidino- ^{14}C]arginine was further purified by passage over a 1.0×5.0 cm Dowex 50W-X8 column, 200 to 400 mesh. After washing the column with 35 ml 2 M-HCl, the amino acid was eluted with 35 ml 4 M-HCl. 1,4-Di-2-(5-phenyloxazolyl)benzene, 2,5-diphenyloxazole and hyamine hydroxide were obtained from Amersham/Searle Co., Arlington Heights, Illinois, U.S.A. Ninhydrin was purchased from Pierce Chemical Co., Rockford, Illinois, U.S.A. *Helix pomatia* was obtained from Endo Laboratories. Polyethyleneimine cellulose plates were purchased from Brinkman Instruments, Westbury, New York, U.S.A. X-ray film was from Kodak.

RESULTS

Mycelial accumulation of amino acids during restriction of protein synthesis

Mycelium of the *prol-3* auxotroph, after growth in proline for 12 h and transfer to medium without this amino acid, accumulated glutamine and arginine. This accumulation depended on the presence of good carbon and nitrogen sources (Fig. 1a, Table 1). Mycelium, but not conidia, of the wild-type strain 74-A accumulated arginine in the presence of cycloheximide (Fig. 1b). Glutamine was also accumulated under these conditions (Table 1). The wild type, in the presence of cycloheximide, accumulated other amino acids although to a lesser extent than glutamine and arginine (Table 2).

The accumulation of glutamine and arginine in mycelium was the result of a biosynthetic process, as indicated by the limited accumulation of arginine in the presence of cycloheximide in a mutant lacking glutamine synthetase (*glm-1*) and in one lacking an arginine biosynthetic enzyme (*arg-12^s*) (Fig. 2). The latter has only 3% of the wild-type ornithine transcarbamylase activity and is impaired in the conversion of ornithine to citrulline (Davis, 1962).

Table 1. *Mycelial accumulation of glutamine*

Strains were grown in MM or MM plus proline ($100 \mu\text{g ml}^{-1}$) for 12 h and transferred to MM with or without cycloheximide ($10 \mu\text{g ml}^{-1}$). Samples for glutamine analysis were taken before and 9 h after the transfer.

Strain	Condition	Glutamine [$\mu\text{mol (mg protein)}^{-1}$]
<i>prol-3</i>	MM + proline	0.075
	9 h after transfer into:	
	MM	0.550
74-A	MM + cycloheximide	0.735
	9 h after transfer into:	
	MM	0.071
	MM + cycloheximide	0.844

Table 2. *Amino acid analysis of wild-type strain 74-A*

Mycelium was grown for 12 h in MM and cycloheximide ($10 \mu\text{g ml}^{-1}$) was then added. Samples for amino acid analysis were taken before and 9 h after the addition of cycloheximide. Amino acids not shown were below detectable levels. Results are expressed as $\mu\text{mol (mg protein)}^{-1}$.

Concentration			Concentration		
Amino acid	Before cycloheximide addition	9 h after cycloheximide addition	Amino acid	Before cycloheximide addition	9 h after cycloheximide addition
Aspartic acid	0.015	0.057	Methionine	0.007	0.011
Threonine	0.008	0.037	Isoleucine	0.033	0.007
Serine	0.033	0.068	Leucine	0.005	0.010
Glutamine	0.061	0.840	Tyrosine	0.002	0.004
Glutamic acid	0.025	0.109	Phenylalanine	0.002	0.036
Glycine	0.025	0.017	Ornithine	0.004	0.066
Alanine	0.633	0.638	Lysine	0.013	0.020
Cysteine	0.005	ND	Histidine	0.189	0.034
Valine	0.015	0.095	Arginine	0.084	0.593

ND, Not detectable.

Macromolecular synthesis was also limited in mycelium by pyrimidine deprivation. Arginine accumulated in the mycelium of strain *pyr-3a* when deprived of pyrimidines in the presence or absence of cycloheximide (Fig. 3). Macromolecular synthesis was also restricted at the end of exponential growth. Figure 4 shows the accumulation of glutamine and arginine in strain *prol-3* when the growth rate was declining.

The accumulation of arginine by *prol-3* mycelium resembled that in conidia, except that in the latter the accumulation occurred only when protein synthesis was partially restricted due to the absence of an amino acid, and not when the cells were deprived of pyrimidines or in the presence of cycloheximide (Espín & Mora, 1978).

Catabolism of arginine in amino acid-deprived mycelium

Mycelium of strain *prol-3* grown in medium containing proline continued to grow after transfer to medium containing arginine, but not if glutamine was present or if the mycelium had been previously deprived of proline for 3 h in MM (Fig. 5), a condition under which glutamine accumulated (Table 1). The failure of *prol-3* to grow in the presence of arginine after incubation in MM alone resulted from the lack of arginase induction by arginine. When arginine was added to mycelium of the mutant strain *tryp-2* growing in MM plus indole, arginase induction started immediately and rose 2.5-fold above the basal level after 160 min. However, if the mycelium was first deprived of tryptophan for 3 h before the addition of arginine, only a limited increase in arginase activity was observed (Fig. 6).

Fig. 2

Fig. 3

Fig. 2. Accumulation of arginine by mycelium of strains *glm-1* and *arg-12^s*. Strain *glm-1* was grown in MM plus glutamine ($200 \mu\text{g ml}^{-1}$) as sole nitrogen source for 12 h. Cycloheximide ($10 \mu\text{g ml}^{-1}$) was added to part of the culture (○) while the rest was transferred to MM (●). Strain *arg-12^s* was grown in MM for 12 h (□) and cycloheximide was then added to part of the culture (■).

Fig. 3. Accumulation of arginine by mycelium of strain *pyr-3a*. Strain *pyr-3a* was grown in MM plus uridine ($100 \mu\text{g ml}^{-1}$). After 6 h (○), the culture was divided, filtered, washed and resuspended in: ●, MM; □, MM plus cycloheximide ($10 \mu\text{g ml}^{-1}$).

Fig. 4. Accumulation of arginine and glutamine by mycelium of strain *prol-3* in the pre-stationary phase of growth. (a) Growth of strain *prol-3* in MM plus proline ($100 \mu\text{g ml}^{-1}$). (b) Pools of glutamine (○) and arginine (●) measured at the times indicated in (a).

Fig. 5

Fig. 6

Fig. 5. Effect of proline deprivation on mycelial growth. Mycelium from strain *prol-3* was grown for 6 h in MM plus proline ($100 \mu\text{g ml}^{-1}$) (○). The culture was then filtered, washed and resuspended in: ●, MM; □, MM plus arginine ($100 \mu\text{g ml}^{-1}$); ■, MM plus arginine plus glutamine ($100 \mu\text{g ml}^{-1}$); △, MM for 3 h and then arginine was added.

Fig. 6. Arginase activity in mycelium deprived of an amino acid. Mycelium from strain *tryp-2* was grown in MM plus indole ($40 \mu\text{g ml}^{-1}$) for 10 h. Arginine ($1 \mu\text{mol ml}^{-1}$) was added to half the culture (○) and the other half was transferred to MM for 3 h after which indole plus arginine were added (●). Specific activity is expressed as $\mu\text{mol urea produced min}^{-1} (\text{mg protein})^{-1}$ at 25°C .

Intracellular distribution of glutamic acid, glutamine and arginine

Table 3 shows the distribution of glutamic acid, glutamine and arginine in mycelium of strain *prol-3* before and after proline deprivation. In non-deprived mycelium the recovery of arginine and protein from sphaeroplasts was similar (80.55 % and 84 % respectively) to that from whole cells, while there was a loss of glutamic acid (50.4 %) and of glutamine (76.5 %) which cannot be accounted for by experimental manipulation. According to the amount of protein and amino acids extracted into the 600 g supernatant, half of the non-deprived cells were broken by the homogenization procedure after glucuronide treatment. Some 7 % $[(1/13.22) \times 100]$ of the glutamic acid, 27 % of the glutamine and 59 % of the arginine present in the 600 g supernatant were found in the 15000 g particulate fraction. When L-[U- ^{14}C]glutamine was added to the 600 g supernatant and then centrifuged for 20 min at 15000 g only 1.4 % was bound to the 15000 g precipitate and the rest was soluble.

A similar distribution of these amino acids was found when mycelium was deprived of a nitrogen source for 1 h and then ammonium nitrate was added for 4 h, a condition which enhanced arginine and glutamine accumulation (data not shown). During the preparation of sphaeroplasts, approximately one-third of the protein, glutamic acid and glutamine were lost while the arginine was almost completely recovered. Some 27 % of the protein was solubilized after homogenization of the sphaeroplasts while 10 % of the glutamic acid, 17 % of the glutamine, and 60 % of the arginine present in the 600 g supernatant was found in the 15000 g particulate fraction. The amino acids present in the 15000 g supernatant were found in the soluble fraction after centrifuging for 60 min at 80000 g. When the 15000 g pellet was resuspended in 1 M-sorbitol and centrifuged again at 15000 g, no arginine was found in the supernatant, but when the pellet was resuspended in water, the arginine was completely solubilized. These results indicated that in the osmotically sensitive cells, part of the glutamine and most of the arginine were bound in the cytosol but almost all of the glutamic acid was soluble. The glutamine and the arginine levels in the 15000 g precipitate were 19- and 4-fold higher, respectively, than in non-deprived cells.

Table 3. *Intracellular distribution of glutamic acid, glutamine and arginine*

Mycelium of strain *prol-3* was grown for 12 h in MM plus proline ($100 \mu\text{g ml}^{-1}$). Part of this was transferred to MM without a nitrogen source for 1 h and then NH_4NO_3 ($25 \mu\text{mol ml}^{-1}$) was added for 4 h. Preparation of sphaeroplasts and fractionation were carried out as described by Weiss & Davis (1973).

Fraction	Protein (mg)	Glutamic acid (μmol)	Glutamine (μmol)	Arginine (μmol)
Before proline deprivation				
Whole cells	232.89	48.40	20.24	28.00
Sphaeroplasts	187.61	24.00	4.76	23.52
600 g pellet	100.51	12.30	1.94	12.30
600 g supernatant	95.74	14.60	2.70	10.00
15000 g pellet	22.00	1.00	0.51	5.00
15000 g supernatant	62.26	12.22	1.40	3.50
After proline deprivation				
Whole cells	465.50	75.32	268.52	175.41
Sphaeroplasts	328.30	48.01	168.07	169.45
600 g pellet	225.60	37.30	122.75	104.73
600 g supernatant	71.80	16.10	49.00	36.65
15000 g pellet	19.60	1.80	9.71	19.60
15000 g supernatant	48.85	16.00	46.20	13.44

Electron micrographs of the 15000 g particulate fraction (Fig. 7b) showed it to be composed of membrane-bound organelles: mitochondria, and some electron-dense bodies, both of which also appeared in whole mycelium (Fig. 7a).

Search for guanosine nucleotides during restriction of macromolecular biosynthesis

Mycelium was incubated in the presence or absence of proline for up to 3 h. Under these conditions six to nine spots containing ^{32}P were observed below the GTP marker after two-dimensional chromatography and autoradiography. None of them, however, co-chromatographed with standards of guanosine tetra- and pentaphosphate (Cashel, 1969) obtained from *E. coli* CP78 subjected to leucine deprivation. When macromolecular synthesis was completely restricted in *N. crassa* by the presence of cycloheximide, there was no effect upon the presence of the compounds that appeared during amino acid deprivation.

DISCUSSION

The accumulation of amino acids in mycelium in response to cycloheximide treatment and pyrimidine deprivation differs from that in conidia in that no arginine is accumulated in conidia (Espín & Mora, 1978). Furthermore, in the presence of cycloheximide amino acid-deprived mycelium accumulated more glutamine and arginine than when only deprived of an amino acid. Although accumulation of certain amino acids occurred in mycelium in the presence of cycloheximide or during uridine deprivation, it is possible that conidia require some nucleic acid and protein synthesis to synthesize the enzymes necessary for the production of glutamine and arginine. We propose that restriction of macromolecular synthesis in mycelium makes available carbon and nitrogen skeletons which, in the presence of the appropriate enzymes, allows the synthesis and accumulation of glutamine and arginine. The results presented in this paper differ from other reports of accumulation of amino acids in *Neurospora crassa* (Carsiotis & Jones, 1974; Carsiotis *et al.*, 1974) and *Saccharomyces cerevisiae* (Schurch, Miozzari & Hutter, 1974; Delforge *et al.*, 1975) in that the amino nitrogen was accumulated mainly in glutamine and arginine, and that this occurred when the mycelium of *N. crassa* was starved of amino acids or when cycloheximide was added.

It is known that when arginine is added to *Neurospora* in the exponential phase of

Fig. 7. Electron micrographs. (a) Mycelium of strain *prot-3* grown in MM plus proline ($100 \mu\text{g ml}^{-1}$) for 12 h, transferred to MM without a nitrogen source for 1 h and then NH_4NO_3 ($25 \mu\text{mol ml}^{-1}$) was added for 4 h. Note the electron-dense spherical bodies in the cytoplasm (arrowed). Bar marker represents $5 \mu\text{m}$. (b) Pellet from the $15000 g$ fraction obtained from the same mycelium. Mitochondria and spherical bodies can be seen, some of which are clearly surrounded by a membrane. Bar marker represents $1 \mu\text{m}$. M, mitochondrion; B, electron-dense spherical body.

growth, arginine synthesis ceases and its catabolism is induced (Castañeda, Martuscelli & Mora, 1967; Cybis & Davis, 1975; Davis, Lawless & Port, 1970). Recently it has been reported that most of the arginine and ornithine synthesized when *N. crassa* is growing exponentially is sequestered in vesicles (Weiss, 1973) and these amino acids are not associated with mitochondria in the 15000 g pellet. Our results show that, of the arginine recovered after fractionation (20 to 30 %), 60 % was found in the 15000 g particulate fraction, and also suggest that the dense bodies may contain sequestered arginine, although better techniques must be found to purify this osmotically sensitive fraction for a conclusive demonstration. If particle lysis during sphaeroplast preparation and leakage from intact particles during fractionation are taken into account, it is possible that the amount of sequestered glutamine and arginine is higher. Since the arginine biosynthetic enzyme whose activity is modulated by arginine is soluble (Cybis & Davis, 1974), our results may explain why endogenous arginine does not inhibit its own synthesis through a feedback mechanism and, hence, is accumulated.

A close metabolic relationship exists between glutamine and arginine, since both have a high nitrogen content and glutamine is the substrate of the arginine biosynthetic pathway as well as the product of the arginine catabolic pathway. The biosynthesis and accumulation of glutamine and arginine are coupled with the inability to catabolize arginine. We suggest that this results from the compartmentation of endogenously synthesized arginine which is thus unable to prevent its own synthesis and induce its own catabolism. We have found that glutamine prevents the catabolism of exogenous arginine by preventing the induction of arginase by arginine (Vaca & Mora, 1977). As demonstrated in this paper, endogenously synthesized arginine is not catabolized nor is arginase induced by exogenous arginine after the mycelia have been deprived of an amino acid, possibly as a result of the accumulation of glutamine. This metabolic mechanism also prevents the catabolism of arginine present in the cytosol and avoids a futile glutamine-arginine synthetic-catabolic cycle.

Alberghina and co-workers reported the absence of guanosine tetraphosphate during a shift-down transition of growth which restricts RNA synthesis in *N. crassa* (Alberghina *et al.*, 1973). These investigators used a phosphate label of specific activity 25 times below that used here. They found five spots which incorporated ³²P and appeared under the GTP marker and suggested that these may be polyphosphates (Harold, 1962). Buckel & Bock (1973) also reported the absence of guanosine tetra- and pentaphosphate during amino acid deprivation in *N. crassa*. There is only one report of the possible presence of guanosine tetraphosphate in *N. crassa* (DeCarlo & Somberg, 1974).

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