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Effect of Carbon Source on Enzymes and Metabolites of Arginine Metabolism in *Neurospora*

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The levels of enzymes and metabolites of arginine metabolism were determined in exponential cultures of *Neurospora crassa* grown on various carbon sources. The carbon sources decreased in effectiveness (as determined by generation times) in the following order: sucrose, acetate, glycerol, and ethanol. The basal and induced levels of the catabolic enzymes, arginase (EC 3.5.3.1) and ornithine transaminase (EC 2.6.1.13), were lower in mycelia grown on poor carbon sources. Arginase was more sensitive to variations in carbon source than was ornithine transaminase. Induction of both enzymes was sensitive to nitrogen metabolite control, but this sensitivity was reduced in mycelia grown on glycerol or ethanol. The pools of arginine and ornithine were reduced in mycelia grown in unsupplemented medium containing poor carbon sources, but the biosynthetic enzyme ornithine transcarbamylase (EC 2.1.3.3) was not derepressed. The arginine pools were similar, regardless of carbon source, in mycelia grown in arginine-supplemented medium. The ornithine pool was reduced by growth on poor carbon sources. The rate of arginine degradation was proportional to the level of arginase in both sucrose- and glycerol-grown mycelia. The distribution of arginine between cytosol and vesicles was only slightly altered by growth on glycerol instead of sucrose. The slightly smaller cytosolic arginine concentration did not appear to be sufficient to account for the alterations in basal and induced enzyme levels. The results suggest a possible carbon metabolite effect on the expression or turnover of a variety of genes for enzymes of arginine metabolism in *Neurospora*.

The metabolism of amino acids in most organisms includes biosynthesis, degradation, and incorporation into protein. In *Neurospora crassa*, control of arginine metabolism includes mechanisms observed in many procaryotes but also some which appear to occur exclusively in eucaryotes (reviewed in reference 10). The better understood mechanisms include: (i) feedback inhibition by arginine of acetylglutamate kinase (EC 2.7.2.8), the second enzyme in the biosynthetic pathway (8); (ii) repression of carbamyl phosphate synthetase (EC 2.7.2.5) by arginine; (iii) induction of arginase and ornithine transaminase by arginine; and (iv) nitrogen control in which the induction of the catabolic enzymes is impaired by the end products of arginine catabolism (ammonia, glutamate, or glutamine).

Two additional but poorly understood features also appear to contribute to the control of arginine metabolism in *N. crassa*. The first is a control system which has been termed cross pathway regulation and appears to involve a common regulatory element affecting the level of enzymes of arginine, histidine, lysine, and tryptophan metabolism (4, 5). A similar control system has been observed in *Saccharomyces cerevisiae* (25). The second feature, compartmentation of enzymes and metabolites, has been shown to affect arginine metabolism in *N. crassa* (10). In procaryotic cells, where less subcellular organization exists, most metabolic steps are carried out in one compartment of the cell, the cytoplasm. The early enzymes of arginine biosynthesis are located in the mitochondria of *N. crassa* (reviewed in reference 10). The last two biosynthetic enzymes and the degradative enzymes are found in the cytosol, where protein synthesis occurs. Most of the arginine pool is sequestered in organelles called vesicles (10). A simplified diagram of this intracellular organization is shown in Fig. 1. The vesicular arginine is not metabolized and does not appear to exert any regulatory influence (21). Alterations in the distribution of arginine between vesicles and cytoplasm can result in metabolic and regulatory changes (22).

In this communication another control process is shown to influence arginine metabolism in *N. crassa*. It is called carbon control. Carbon-limited growth is shown to affect the level of

ornithine transcarbamylase, arginase, and ornithine transaminase. Induction of the latter two enzymes is also affected.

MATERIALS AND METHODS

Strains, media, and chemicals. The wild-type strain (74A) and the urease-deficient strain, *ure-1A* (allele *9*), were obtained from R. H. Davis. Vogel minimal medium N (19) was used for growth. For arginine-supplemented medium, arginine was added to a final concentration of 5 mM unless otherwise specified in the text. Carbon sources were used at a final concentration of 1.5%. L-Arginine, L-ornithine, carbamylphosphate, and cycloheximide were purchased from Sigma Chemical Co. The isotopically labeled compounds L-[guanido-^{14}C]arginine (23.01 mCi/mmol) and L-[ureido-^{14}C]citrulline (45.75 mCi/mmol) were purchased from Amersham/Searle and purified by column chromatography before use. Ion-exchange resin AG 50W-X8 was purchased from Bio-Rad Laboratories.

Growth and sampling. Mycelia were grown in liquid medium from a conidial inoculum (approximately 5×10^6 conidia per ml). The conidia were germinated overnight at 15°C in 100 ml of appropriate growth medium in 250-ml baffled flasks on a shaking water bath. After approximately 16 h, the temperature was increased to 30°C. Growth was continued either on the shaking water bath or following transfer to 250-ml flat-bottomed boiling flasks. Cultures in boiling flasks were mixed with forced aeration. All experiments were carried out during the exponential growth phase of the culture. Growth rates were determined from the increase in cellular protein as previously described (3).

Mycelia for analysis of amino acid pools were collected by filtration on Whatman no. 540 filter paper. In some cases, the amount of urea which had accumulated in the growth medium was determined. Arginine and ornithine were solubilized from the collected mycelia by boiling water extraction (3). The extracted mycelia were suspended in 0.5 M NaOH for protein determination.

Arginine in protein was determined as follows: a sample of the liquid culture was added to tubes containing cold trichloroacetic acid (final concentration, 5%); the precipitated cells were washed once with 5% trichloroacetic acid, three times with ethyl-ether/ethanol (1:1, vol/vol), and twice with ethyl-ether; the pellets were suspended in 4 ml of 6 N HCl and hydrolyzed at 110°C for 18 h; the hydrolysates were filtered by using Gelman GA-6 cellulose-acetate membrane filters (0.45-μm pore size) and evaporated, and arginine was purified and determined as described below.

Two samples were collected from cultures labeled with radioactive amino acids. In experiments using radioactive arginine, mycelia from the first sample were collected on Whatman GF/A (glass microfibre) filter paper, washed with cold water containing 10 mM arginine, dried, and analyzed for radioactivity (cellular radioactivity). In experiments using radioactive citrulline, mycelia from the first sample were collected on Whatman no. 540 filter paper, washed with cold water containing 10 mM citrulline, and extracted with boiling water, and arginine was purified by ion-exchange chromatography as described below. The fraction containing arginine was assayed for radioactivity and arginine content as described below. The second sample from each culture was pipetted directly into tubes containing cold trichloroacetic acid (final concentration, 5%). After a minimum of 1 h, the precipitated protein was collected on Whatman GF/A filter paper, washed five times with cold 5% trichloroacetic acid, three times with ethanol/ethyl-ether (1:1, vol/vol), and finally with ethyl-ether. The samples were dried overnight and analyzed for radioactivity. This insoluble radioactivity is a measure of radioactive arginine incorporated into protein. Dry radioactive samples were counted in toluene scintillation fluid (4 g of 2,5-diphenyloxazole per liter of toluene). Aqueous samples were counted in Triton X-100-toluene (1:2) scintillation fluid.

Enzyme assays. Enzyme assays were performed, using mycelia permeabilized with toluene-ethanol as previously described (3). Mycelia were collected by centrifugation, washed with permeabilization buffer (0.02 M potassium phosphate, pH 7.5, 0.01 M EDTA), and suspended in 3.0 ml of permeabilization buffer. A 0.2-ml amount of toluene-ethanol (1:4) was added, and the suspension was mixed vigorously for 2 min. The permeabilized mycelia were collected by centrifugation and suspended in permeabilization buffer lacking EDTA. Arginase was assayed by the method of Davis and Mora (11). The product, urea, was determined by the method of Archibald (1). Ornithine transaminase was assayed by the method of Davis and Mora (11). Ornithine transcarbamylase was assayed by the method of Davis (9). The product, citrulline, was assayed by the method of Koritz and Cohen (13), with chloride added to intensify and standardize color values (7). One unit of enzyme activity equals the production of 1 μmol of product per min at 37°C. The specific activities were calculated as units per milligram of protein. Protein was determined by the method of Lowry et al. (14).

Fractionation and chromatography. Ornithine, arginine, and citrulline were separated on AG 50W-X8 (Na^+ form) as previously described (3). Ornithine was determined colorimetrically by the method of Chinard (6). Arginine was determined by the method of van Pilsum et al. (23). Urea was isolated from the medium on columns (0.7 by 12 cm) of AG 50W-X8 (H^+ form). The columns were equilibrated with 8 ml of 0.1 N HCl. The sample of medium (8 ml) was acidified with 0.2 ml of 4 N HCl and loaded on the column. The column was washed with 8 ml of 0.1 N HCl followed by 8 ml of 1.5 N HCl. Urea was eluted with an additional 10 ml of 1.5 N HCl. The urea separated by this method was determined by the method of Koritz and Cohen (see above).

Calculations. The radioactivity in the arginine pool at each time interval was calculated as the difference between cellular radioactivity and radioactivity in protein (mycelia labeled with radioactive arginine) or was determined by direct measurement (mycelia labeled with radioactive citrulline). The arginine pool (cytosolic plus vesicular) was determined from the measured arginine pool corrected for growth (see below) or by direct measurement. The specific radioactivity of the arginine pool was calculated as counts per minute per nanomole of arginine.

The average specific radioactivity of cytosolic arginine during a time interval was assumed to be equal to the specific radioactivity of arginine incorporated into protein during that interval. These values were calculated from the increase in radioactivity incorporated into protein during a time interval divided by the amount of arginine incorporated into protein during that time interval. The latter was determined from the growth equation as previously described (16).

The specific radioactivities of the arginine pool and of cytosolic arginine were used to calculate the fraction of the arginine pool in the cytosol as previously described (21). The specific radioactivity of the arginine pool (counts per minute per nanomole of arginine) divided by the specific radioactivity of cytosolic arginine (counts per minute per nanomole of cytosolic arginine) will be equal to the fraction of arginine which is cytosolic when all the radioactive molecules are in the cytosol. This will be true at very early times, since radioactive molecules must first pass through the cytosol before entry into the vesicles (16). Values obtained at the earliest times or by extrapolation to zero time estimate such a situation (21).

RESULTS

Characteristics of mycelia grown on various carbon and nitrogen sources. The generation times, specific activities of arginase, ornithine transaminase, and ornithine transcarbamylase, and the arginine and ornithine pools in wild-type (74A) mycelia grown on various carbon and nitrogen sources are shown in Table 1. Mycelia grew exponentially on the carbon sources tested: sucrose, acetate, glycerol, and ethanol. Their ability to serve as sole carbon sources varied largely, as measured by the generation times of mycelia during exponential growth. By this criterion, acetate, glycerol, and ethanol were poorer carbon sources than sucrose. Glucose gave results indistinguishable from sucrose.

In supplemented medium, the basal levels of both biosynthetic and degradative enzymes were lower in mycelia grown on acetate, glycerol, or ethanol as the sole source of carbon. In arginine-supplemented medium, the levels of both arginase and ornithine transaminase were elevated (Table 1). This induction was partially sensitive to the availability of alternative nitrogen sources, especially glutamine (Table 1). This phenomenon is termed nitrogen metabolite repression (17). The induced level of arginase was considerably lower in mycelia grown on acetate, glycerol, or ethanol as the carbon source. The same was true for ornithine transaminase in mycelia grown on glycerol or ethanol. The sensitivity to glutamine (or ammonia) was also reduced in mycelia grown on glycerol or ethanol. The level of ornithine transcarbamylase was reduced on all poor carbon sources and was not significantly affected by variations in nitrogen source(s) (Table 1).

The arginine pool was considerably reduced in mycelia grown on poor carbon sources in the

TABLE 1. *Enzyme activities and metabolite pool sizes in N. crassa grown on various carbon and nitrogen sources*[a]

Growth conditions			Enzyme activity (U/mg of protein)			Amino acid pools (nmol/mg of protein)[b]	
Carbon source	Nitrogen source	Generation time (min)	Arginase	Ornithine transaminase	Ornithine transcarbamylase	Arginine	Ornithine
Sucrose	NH_4^+	125 ± 7	0.92 ± 0.08	0.015 ± 0.003	0.50 ± 0.14	165 ± 26	95 ± 20
Sucrose	NH_4^+ + Arg	128 ± 4	2.54 ± 0.06	0.104 ± 0.012	0.52 ± 0.09	1110 ± 200	102 ± 7
Sucrose	Arg	240 ± 20	3.26 ± 0.35	0.112 ± 0.015	0.48 ± 0.06	1820 ± 100	156 ± 3
Sucrose	Arg + Gln	110 ± 5	1.38 ± 0.33	0.066 ± 0.022	ND[c]	ND	ND
Acetate	NH_4^+	200 ± 15	0.79 ± 0.13	0.010 ± 0.001	0.30 ± 0.03	90 ± 10	49 ± 6
Acetate	NH_4^+ + Arg	210 ± 15	1.26 ± 0.23	0.106 ± 0.025	0.40 ± 0.05	950 ± 40	45 ± 2
Acetate	Arg	430 ± 70	1.67 ± 0.08	0.116 ± 0.013	0.34 ± 0.05	1190 ± 140	68 ± 9
Acetate	Arg + Gln	165 ± 20	0.86 ± 0.44	0.057 ± 0.010	ND	ND	ND
Glycerol	NH_4^+	390 ± 30	0.75 ± 0.07	0.010 ± 0.001	0.12 ± 0.01	108 ± 9	18 ± 5
Glycerol	NH_4^+ + Arg	390 ± 30	1.10 ± 0.16	0.036 ± 0.001	0.15 ± 0.03	1110 ± 40	36 ± 2
Glycerol	Arg	540 ± 15	1.22 ± 0.25	0.036 ± 0.003	0.14 ± 0.02	1000 ± 30	32 ± 18
Glycerol	Arg + Gln	320 ± 40	1.20 ± 0.30	0.031 ± 0.002	ND	ND	ND
Ethanol	NH_4^+	470 ± 15	0.66 ± 0.25	0.009 ± 0.001	0.27 ± 0.08	55 ± 10	13 ± 3
Ethanol	NH_4^+ + Arg	460 ± 15	1.02 ± 0.06	0.029 ± 0.002	ND	ND	ND
Ethanol	Arg	485 ± 20	1.26 ± 0.42	0.031 ± 0.004	ND	ND	ND
Ethanol	Arg + Gln	385 ± 35	1.12 ± 0.32	0.023 ± 0.005	ND	ND	ND

[a] Wild-type (74A) mycelia were grown and enzyme activities and pool sizes were determined as described in the text. Values are the average and standard deviations of six separate experiments for mycelia grown on sucrose, acetate, or glycerol as the carbon source, and two experiments for ethanol-grown mycelia.

[b] Cytosolic plus vesicular.

[c] ND, Not determined.

absence of exogenous arginine. Variations in carbon source had little effect on the arginine pool of mycelia grown in arginine-supplemented medium containing ammonia. The pool was considerably larger in the absence of ammonia when sucrose was the carbon source. The ornithine pool was very small when cells were grown on poor carbon sources. It remained small, even in the presence of exogenous arginine.

Rates of arginine degradation. Arginine degradation can be measured by the accumulation of urea in the medium using a strain unable to degrade urea (Fig. 1) (21). Figure 2 shows the accumulation of urea during incubation of the *ure-1* strain in arginine-supplemented medium with sucrose or glycerol as the carbon source. Urea was produced more rapidly by mycelia grown using sucrose as a carbon source. The latter also exhibited a higher level of arginase (0.72 versus 0.27 U per sample). The rate of urea production (Fig. 2) divided by the activity of arginase yields 0.70 μmol/h per U (sucrose) or 0.61 μmol/h per U (glycerol). Similar values were obtained under uninduced conditions upon supplementation with arginine.

Measurement of cytosolic arginine concentrations. The cytosolic arginine concentrations of mycelia grown on sucrose or glycerol as the carbon source were determined by the pulse-label methodology previously described (16, 21). The technique involved exposure of exponential cultures to small amounts of highly radioactive amino acids and determining the degree of dilution by cytosolic amino acids before incorporation into protein (Fig. 3 and 4).

Figures 3A and 3B show the results obtained with mycelia growing in unsupplemented medium (sucrose or glycerol as the carbon source). Uptake of [^{14}C]arginine was essentially complete within 2 min. Radioactivity appeared quickly in protein despite the large arginine pool (Table 1). During the early period of rapid arginine uptake

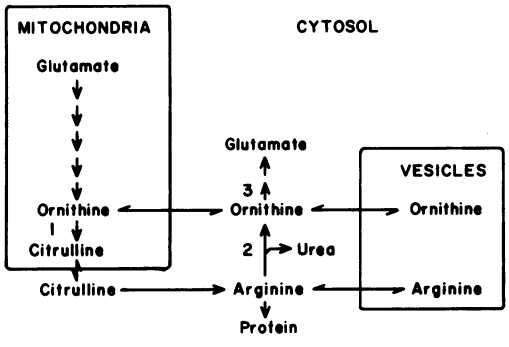

FIG. 1. *Organization of arginine metabolism in Neurospora. Enzymes: 1, ornithine transcarbamylase; 2, arginase; 3, ornithine transaminase.*

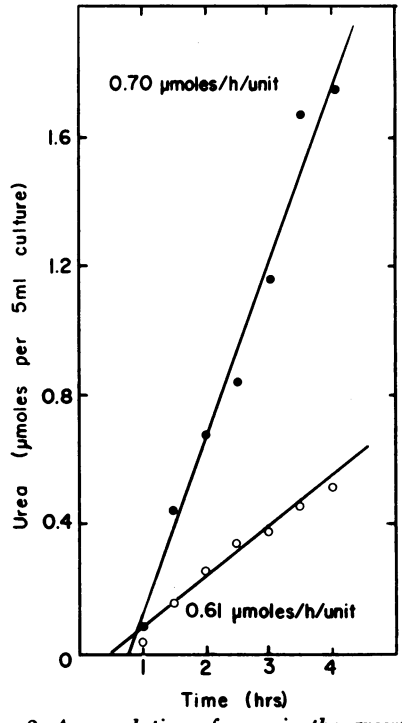

FIG. 2. *Accumulation of urea in the growth medium during incubation of a ure-1 strain in arginine-supplemented medium with sucrose (●) or glycerol (○) as the carbon source. Conidia were germinated overnight at 16°C in the appropriate medium supplemented with arginine. After an additional 3 h growth at 30°C, the mycelia were collected by filtration on Whatman no. 540 filter paper. A portion was used to determine arginase activity as described in the text, and the remaining mycelia were washed thoroughly to remove previously accumulated urea and suspended in fresh, prewarmed medium containing 10 μg of cycloheximide per ml. At the indicated times, samples were removed and assayed for urea in the medium as described in the text.*

(0 to 30 s), the specific radioactivity of arginine entering protein was greater than that of the arginine pool. These results are consistent with the vesicular compartmentation of a portion of the arginine pool (16).

Since arginine from the medium enters the mycelia via the cytosol, the radioactivity in cytosolic arginine should be approximately equal to the radioactivity in the arginine pool at very early times. Assuming this to be the case, the specific radioactivity of the arginine pool divided by the specific radioactivity of cytosolic arginine (arginine newly incorporated into protein) at the earliest time interval (10 s) should give a value very close to the fraction of arginine which is cytosolic (16). This value was found to be 0.024 for cells growing on sucrose as the sole carbon

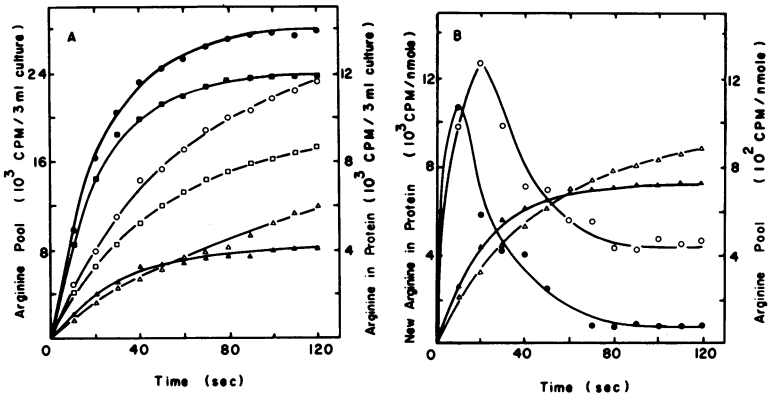

FIG. 3. *Uptake and utilization of radioactive arginine by mycelia growing in minimal medium with sucrose (closed symbols) or glycerol (open symbols) as the carbon source. Exponentially growing mycelia were exposed to 1.0 μCi of [guanido-^{14}C]arginine at time zero. At the indicated times, samples were removed and analyzed as described in the text. (A) Uptake and utilization of arginine: cellular radioactivity (●, ○); radioactivity in the arginine pool (■, □); and radioactivity in protein (▲, △). B, Specific radioactivities of cytosolic arginine (●, ○) and the arginine pool (▲, △) in mycelia from A.*

source and 0.022 for mycelia growing on glycerol as the sole carbon source. It was concluded that, in mycelia growing in minimal medium, the cytosolic arginine is approximately 2% of the arginine pool regardless of the carbon source. Similar values have been obtained previously (16, 21).

Results for similar experiments involving mycelia growing in arginine-supplemented medium are shown in Fig. 4. Increased time between samples and increased amounts of radioactivity were required because of the large dilution by exogenous arginine and the expanded arginine pool (Table 1). The uptake of radioactive arginine and its appearance in the arginine pool and in protein are shown in Fig. 4A. These curves, combined with the measured values for the size of the arginine pool and for arginine in protein (see Materials and Methods), were used to calculate the average specific radioactivity of the arginine pool and of cytosolic arginine (Fig. 4C). A similar experiment was performed, using radioactive citrulline (see Fig. 1) to avoid dilution by exogenous arginine (Fig. 4B and D). For either carbon source (sucrose or glycerol), the specific radioactivity of the cytosolic arginine increased more rapidly than that of the arginine pool (Fig. 4C and D).

Assuming all the radioactivity in the arginine pool is in the cytosol, one can calculate the apparent amount of arginine which is cytosolic at each time point, as described in Materials and Methods. Extrapolating to zero time, at which time this assumption should be correct, it was calculated that the fraction of arginine which is cytosolic on glycerol as the sole carbon source (9%) was lower than that of cells growing on sucrose as the sole carbon source (20%) (Table 2). The cytosolic arginine concentrations were calculated assuming that 80% of the cell water is cytosolic (15). The cytosolic arginine concentration of cells growing on glycerol appeared to be approximately 50% that of sucrose-grown cells, but still some 40-fold larger than the basal concentration.

DISCUSSION

The results described above indicate that the levels of arginine biosynthetic and degradative enzymes are significantly affected by variations in the carbon source. The basal levels of arginase, ornithine transaminase, and ornithine transcarbamylase were reduced when glycerol or ethanol replaced sucrose as the carbon source. The induced levels of the catabolic enzymes were also lower in mycelia grown on poor carbon sources. Reduced growth rates did not appear to be responsible for the lowered enzyme levels since mycelia grown on arginine as a sole nitrogen source had a reduced growth rate, but the arginine-degradative enzymes were maximally induced (Table 1). The results did not appear to be due to inducer exclusion, since no significant differences were observed in the arginine pool (arginine has been shown to be the inducer of arginase and ornithine transaminase [12]).

The levels of arginase and ornithine transaminase did not respond identically to variations in carbon source. This might be due to the existence of separate regulatory proteins with differing sensitivities to carbon metabolite levels. Alternatively, the unlinked genes for these two catabolic enzymes might have regulatory regions with different affinities for a common

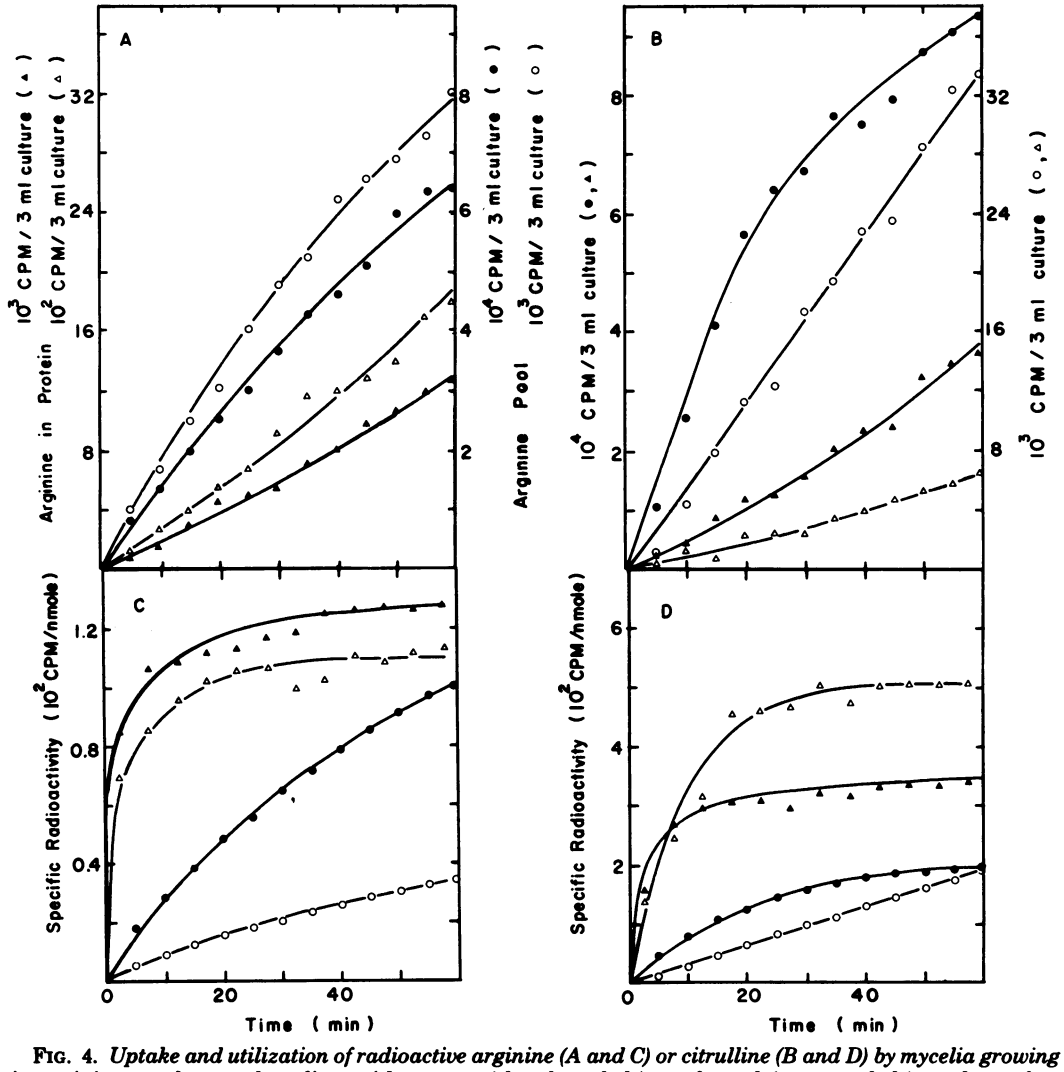

FIG. 4. *Uptake and utilization of radioactive arginine (A and C) or citrulline (B and D) by mycelia growing in arginine-supplemented medium with sucrose (closed symbols) or glycerol (open symbols) as the carbon source. Mycelia were exposed to 6.0 μCi of [guanido-^{14}C]arginine (A and C) or 6.0 μCi of [ureido-^{14}C]citrulline (B and D) at time zero. (A and B) Appearance of radioactivity in the arginine pool (●, ○) and in protein (▲, △). (C and D) Specific radioactivity of the arginine pool (●, ○) and cytosolic arginine (▲, △).*

regulatory protein. Another possibility is that the two enzymes vary in their sensitivity to proteolytic turnover. Whatever the mechanism of the carbon metabolite effects, both enzymes respond in a qualitatively similar but quantitatively different manner.

The arginine pool is not distributed randomly in *N. crassa*. The majority of the pool is sequestered in subcellular organelles, the vesicles, and is excluded from metabolic or regulatory processes (21). Thus, two hypotheses can be made concerning the effect of poor carbon sources: (i) a poor carbon source interferes with the synthesis or turnover of enzymes of arginine metabolism, or (ii) a poor carbon source alters the distribution of arginine between the cytosol and vesicles.

A number of observations suggest that the latter is not the case. First, a decrease in the effector pool of arginine is known to result in derepression of ornithine transcarbamylase (9). The existence of common regulatory elements for induction and repression of arginine metabolic enzymes is well documented in *S. cerevisiae* (24). Growth on a poor carbon source resulted in a decrease rather than an increase in

the level of this enzyme (Table 1). Second, arginine degradation, as measured by accumulation of urea, has been shown to reflect an increase in the cytosolic arginine concentration (21). The rate of arginine degradation (corrected for differences in enzyme levels) was not significantly different when glycerol replaced sucrose as the sole carbon source (Fig. 2). Finally, pulse-label experimental measurements of the cytosolic arginine concentrations (Table 2) indicated a 40-fold increase in this concentration upon arginine supplementation of glycerol-grown cells. Little induction of arginase was observed, and induction of ornithine transaminase was considerably reduced (Table 1). The induction of ornithine transaminase has previously been shown to be initiated in response to small changes in the arginine pool (20). The slightly lower cytosolic arginine concentration in glycerol-grown cells (versus sucrose-grown cells) would not appear to be sufficient to account for the lowered inducibility of the catabolic enzymes.

The results suggest that carbon limitation has significant effects on the rate of synthesis or turnover of specific protein molecules. Similar observations have been made in *S. cerevisiae* (23). The reduced basal levels of arginase and ornithine transaminase indicate that degradative enzymes are not derepressed in response to carbon limitation. This is consistent with the limited effectiveness of arginine and ornithine as sole carbon sources for *N. crassa*. The noninducibility of arginase and ornithine transaminase suggests that carbon limitation restricts the ability of *Neurospora* to respond to the availability of exogenous arginine. This is in contrast to the increased rate of induction of arginase and ornithine transaminase upon carbon starvation in *Aspergillus nidulans* (2). The specificity and mechanism of this reverse carbon metabolite effect is under investigation.

TABLE 2. *Cytosolic arginine concentrations in mycelia grown on various carbon and nitrogen sources*

Growth conditions		Cytosolic arginine	
Carbon source	Nitrogen source	% of total[a]	Concn (mM)[b]
Sucrose	NH_4^+	2	0.4
Glycerol	NH_4^+	2	0.3
Sucrose	NH_4^+ + Arg	20	26
Glycerol	NH_4^+ + Arg	9	13

[a] Total refers to arginine pool (cytosolic plus vesicular).

[b] Calculated assuming 80% of the cell water is cytosolic (15).

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LITERATURE CITED

1. **Archibald, R. M.** 1945. Colorimetric determination of urea. J. Biol. Chem. **157**:507–518.
2. **Bartnik, E., P. Weglenski, and M. Piotrowska.** 1973. Ammonium and glucose repression of the arginine catabolic enzymes in *Aspergillus nidulans*. Mol. Gen. Genet. **126**:75–84.
3. **Basabe, J. R., C. A. Lee, and R. L. Weiss.** 1978. Enzyme assays using permeabilized cells of *Neurospora*. Anal. Biochem. **92**:356–360.
4. **Carsiotis, M., and R. F. Jones.** 1974. Cross-pathway regulation: tryptophan-mediated control of histidine and arginine biosynthetic enzymes in *Neurospora crassa*. J. Bacteriol. **119**:889–892.
5. **Carsiotis, M., R. F. Jones, and A. C. Wesseling.** 1974. Cross-pathway regulation: histidine-mediated control of histidine, tryptophan, and arginine biosynthetic enzymes in *Neurospora crassa*. J. Bacteriol. **119**:893–898.
6. **Chinard, F. P.** 1952. Photometric estimation of proline and ornithine. J. Biol. Chem. **199**:91–95.
7. **Crokaert, R., and E. Schram.** 1958. Dosage des N-carbamoyl-dérivés d'acides aminés par la diacétylmonoxime. Bull. Soc. Chim. Biol. **40**:1093–1106.
8. **Cybis, J., and R. H. Davis.** 1974. Acetylglutamate kinase: a feedback-sensitive enzyme of arginine biosynthesis in *Neurospora*. Biochem. Biophys. Res. Commun. **60**:629–634.
9. **Davis, R. H.** 1965. Carbamyl phosphate synthesis in *Neurospora crassa*. II. Genetics, metabolic position, and regulation of arginine-specific carbamyl phosphokinase. Biochim. Biophys. Acta **107**:54–68.
10. **Davis, R. H.** 1975. Compartmentation and regulation of fungal metabolism: genetic approaches. Annu. Rev. Genet. **9**:39–65.
11. **Davis, R. H., and J. Mora.** 1968. Mutants of *Neurospora crassa* deficient in ornithine-δ-transaminase. J. Bacteriol. **96**:383–388.
12. **Davis, R. H., M. B. Lawless, and L. A. Port.** 1970. Arginaseless *Neurospora*: genetics, physiology, and polyamine synthesis. J. Bacteriol. **102**:299–305.
13. **Kortiz, S. B., and P. P. Cohen.** 1954. Colorimetric determination of carbamyl amino acids and related compounds. J. Biol. Chem. **209**:145–150.
14. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
15. **Slayman, C. W., and E. L. Tatum.** 1964. Potassium transport in *Neurospora*. I. Intracellular sodium and potassium concentrations and cation requirements for growth. Biochim. Biophys. Acta **88**:578–592.
16. **Subramanian, K. N., R. L. Weiss, and R. H. Davis.** 1973. Use of external biosynthetic, and organellar arginine by *Neurospora*. J. Bacteriol. **115**:284–290.
17. **Vaca, G., and J. Mora.** 1977. Nitrogen regulation of arginase in *Neurospora crassa*. J. Bacteriol. **131**:719–725.
18. **Van Pilsum, J. F., R. P. Martin, E. Kito, and J. Hess.** 1956. Determination of creatine, creatinine, arginine, guanidoacetic acid, guanidine and methylguanidine in biological fluids. J. Biol. Chem. **222**:225–236.
19. **Vogel, H. J.** 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. **98**:435–446.
20. **Weiss, R. L., and G. P. Anterasian.** 1977. Control of arginine metabolism in *Neurospora*. Induction of ornithine aminotransferase. J. Biol. Chem. **252**:6974–6980.

21. **Weiss, R. L.** 1976. Compartmentation and control of arginine metabolism in *Neurospora*. J. Biol. Chem. **126**: 1173–1179.
22. **Weiss, R. L., and R. H. Davis.** 1977. Control of arginine utilization in *Neurospora*. J. Bacteriol. **129**:866–873.
23. **Weiss, R. L., J. R. Kukora, and J. Adams.** 1975. The relationship between enzyme activity, cell geometry, and fitness in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **72**:794–798.
24. **Wiame, J. M.** 1971. The regulation of arginine metabolism in *Saccharomyces cerevisiae*: exclusion mechanisms. Curr. Top. Cell. Reg. **4**:1–38.
25. **Wolfner, M., D. Yep, F. Messenguy, and G. R. Fink.** 1975. Integration of amino acid biosynthesis into the cell cycle of *Saccharomyces cerevisiae*. J. Mol. Biol. **96**: 273–290.