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Regulation of Arginase Activity by Intermediates of the Arginine Biosynthetic Pathway in *Neurospora crassa*

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It has been found that, in *Neurospora crassa*, arginine synthesized from exogenous citrulline was not as effectively hydrolyzed as exogenous arginine. This was explained by the observed inhibition of arginase in vitro and in vivo by citrulline. The high arginine pool formed from exogenous citrulline feedback inhibits the arginine pathway. These two factors allow exogenous citrulline to be used adventitiously and efficiently as an arginine source. Finally, it was found that ornithine was a strong inhibitor of arginase. This suggests that the characteristically high ornithine pool of minimal cultures of *Neurospora* may act to control a potentially wasteful catabolism of endogenous arginine by arginase.

The auxotrophic *arg-8* strain of *Neurospora crassa* grows on proline (16). The mutant also grows on arginine and ornithine as a result of the action of a basic amino acid permease (13), an arginase (5, 11), and a ornithine-δ-transaminase (6). These functions lead to the formation of glutamic-γ-semialdehyde, a proline precursor which *arg-8* is unable to synthesize by the normal biosynthetic pathway (1). It has also been claimed that the mutant grows on citrulline via conversion to arginine, thence to ornithine and glutamic-γ-semialdehyde (Fig. 1).

The auxotrophic *arg-5* strain lacks the enzyme α-*N*-acetyl-γ-semialdehyde transaminase, required in the formation of ornithine (D. H. Morgan, Neurospora Newsl. 8:8, 1965). The *arg-5* mutant will grow on ornithine, citrulline, or arginine.

We found that the single mutant *arg-8* and the double mutant *arg-5;arg-8* do not grow on citrulline when the citrulline is sterilized by filtration. However, growth occured on autoclaved citrulline. We found that citrulline was partially decomposed to ornithine on autoclaving.

The fact that the double mutant grew on citrulline plus ornithine or on citrulline plus proline indicated that arginine synthesized from citrulline was not hydrolyzed by arginase efficiently enough to support proline biosynthesis in this mutant. Because the single and the double mutant grew on arginine alone we concluded that the arginine synthesized from citrulline was not as readily hydrolyzed by arginase as exogenous arginine.

These metabolic phenomena can be explained by the in vivo regulation of the activity of arginase by citrulline. In addition, we found that ornithine acts as a strong inhibitor of arginase in vitro and in vivo. This paper reports on these findings.

MATERIALS AND METHODS

Chemicals and growth conditions. *N. crassa* was grown in the minimal medium N of Vogel (15) supplemented with 1.5% sucrose. Crosses were made on 1.7% corn meal agar (Difco) in the dark at 25 C. Plating for ascospores was done on Vogel's medium supplemented with glucose and fructose (0.05% each) in place of sucrose, plus 1% sorbose. Conidia were harvested from solidified slants of Vogel's medium supplemented with 1.5% sucrose which were incubated for 3 days in the dark at 29 C and 2 days under visible light at 25 C. Growth in liquid medium was in 250-ml Florence flasks containing 100 ml of Vogel's minimal medium plus sucrose (1.5%). They were inoculated with filtered conidia and sparged with hydrated air at 25 C.

Stocks. All *N. crassa* stocks came from the fungal Genetics Stocks Center at Darmouth College, Hanover, N.H, or from the collection of the senior author. The basic stocks were the wild strain 74-A; *arg-5; arg-12*, which lacks ornithine transcarbamylase (OTC) and which grows on citrulline or arginine (7); *arg-12*^{s}, a strain that grows on minimal medium and has only 5% of normal OTC activity (3); *arg-1*, which lacks argininosuccinate synthetase and which requires arginine (12); *arg-8*; the prototrophic *ota* mutant, which lacks ornithine-δ-transaminase (6); the

prototrophic *aga* mutant, which lacks arginase (5); and the prototrophic *ure* mutant, which lacks urease (8). All multiple mutants were obtained from appropriate crosses of the aforementioned stocks.

Assay of in vivo arginase activity. The rationale for the assay was as follows when arginine-*guanido*-^{14}C is split by arginase, the ^{14}C-urea formed is hydrolyzed by the excess urease present in the cell. The $^{14}CO_2$ liberated in the medium can be trapped as $Na_2{}^{14}CO_3$. The same argument holds for citrulline-*ureido*-^{14}C since this amino acid is converted by the fungus to arginine-*guanido*-^{14}C. Ten milliliters of medium N (15) with 1.5% sucrose were incubated in side-arm tubes (18 by 150 mm) with perforated ground glass stoppers. They were sparged with hydrated air through a polyethylene catheter tube (1 by 130 mm). Each side-arm tube was connected in series to two side-arm tubes containing 16 ml of 2.5 N NaOH each. After the addition of the radioactive amino acid, the tubes were incubated with spores as previously described. Every 2 or 3 hr the traps were replaced by new ones. From each trap, the ^{14}C-carbonate of a 4-ml sample was precipitated with 10 ml of 2 N NH_4Cl and 6 ml of 1.5 N $BaCl_2$. The $Ba^{14}CO_3$ was washed twice with water and counted in a Nuclear-Chicago planchet counter (25% efficiency). Samples were counted at infinite thickness for a time sufficient to give at least 1,000 counts per min per sample. With this in vivo assay of arginase, no $^{14}CO_2$ evolution was detected in the strains *ure* and *aga*.

Assay of arginase activity in vitro. The wild strain 74-A or the *ure* mutant was grown in several 1-liter flasks with 800 ml of medium N (15) with sucrose (1.5%) and 0.5 mM arginine; they were incubated as described above. After 12 hr of incubation, the mycelium was filtered in Buchner funnels with Whatman paper no. 540 and washed profusely with distilled water at room temperature. Acetone powders were prepared from the mycelium and homogenized in the cold (4 C) with 0.025 M glycine-NaOH buffer-0.1 M KCl, *p*H 9.5, or in 0.025 M, tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer-0.1 M KCl, *p*H 7.5. The homogenates were centrifuged at 4 C at 12,500 × *g* for 20 min. The supernatant fluids (4 ml) were passed through a Sephadex G-25 column (35 by 35 mm) equilibrated with one of the above buffers. Fractions containing the protein peak were pooled and used as the enzyme source. The incubation mixture, in 10-ml Erlenmeyer flasks, contained in 1 ml: 10.5 μmoles of KCl, 0.25 μmoles of $MnCl_2$, and 5.0 μmoles of arginine-*guanido*-^{14}C (372 to 743 counts per min per nmole). The flasks had a well in the center holding a small tube of 0.25 ml of hyamine. The flasks were covered tightly with rubber stoppers (sleeve type). After 3 min in a water bath at 25 C, the reaction was started by injecting the enzyme solution (400 to 600 μg of protein). The flasks were incubated at 25 C for 1 to 2 min, and the reaction was stopped by injecting 2 ml of 0.2 M sodium citrate (*p*H 5.0) containing 28 units (10 mg) of urease. (One unit produces 1 mg of ammonia in 5 min at *p*H 7.0 and at 30 C from urea.) After the temperature was raised to 37 C, the flasks were incubated for 30 min in a rotary water bath shaker (100 rev/min), and the reaction was stopped by injecting 1 ml of 2.0 N $HClO_4$. After a further 45 min of incubation, the tubes with hyamine were removed and placed in a vial with 15 ml of scintillation fluid (0.4% 2,5-diphenyloxazole; 0.005% 1,4-bis-2-(phenyloxazolyl)-benzene in toluene). The vials were counted in a Nuclear-Chicago scintillation counter for a time sufficient to give at least 1,000 counts per min per sample (70% efficiency). With this method, the specific activity of arginase was similar in the wild-type and urease-less strains.

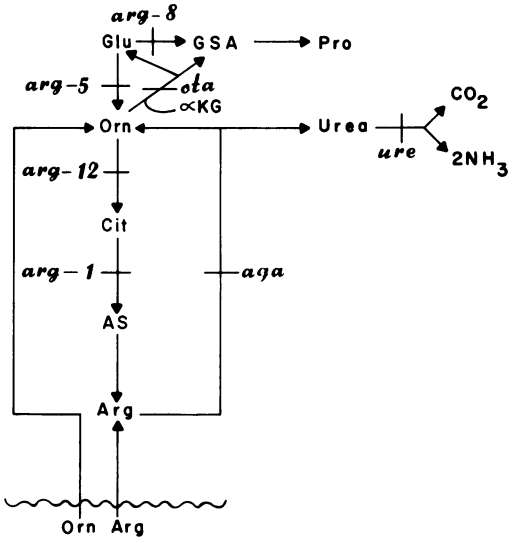

FIG. 1. *Schematic diagram of arginine and proline metabolism in Neurospora. Genetic blocks are shown in italics (see Materials and Methods). Abbreviations: AS, argininosuccinate; GSA, glutamic-γ-semialdehyde; αKG, α-ketoglutarate.*

Arginine pool, specific radioactivity of arginine and arginine incorporated into protein. The fungus was collected on membrane filters (Millipore type HA, 0.45 μm), washed with two volumes of distilled water and placed in 2 ml of 5% trichloroacetic acid. After centrifugation, arginine was measured in the supernatant fluid by the method of Van Pilsum et al. (14). The specific radioactivity of arginine was determined in the cell-free extract after removal of the trichloroacetic acid. This was accomplished by passing the cell-free extract (1.5 ml) through a column of AG1-X2, 200 to 400 mesh (15 by 15 mm) previously equilibrated with water, and eluting with 3.5 ml of 0.02 N HCl and 3 ml of water. The collected eluate was brought to 20 ml with 0.1 N sodium citrate buffer, *p*H 5.0, and was passed through a column of Dowex 50W-X2, 200 to 400 mesh (10 by 50 mm), previously equilibrated with 25 ml of the same buffer. After removal of citrulline, argininosuccinic acid, and urea with 20 ml of the same buffer, the arginine was eluted with 16 ml of 0.2 N NaOH and collected in 2-ml fractions. Arginine was measured colorimetrically in these fractions as described above and a sample was deposited on planchets, dried, and counted.

Citrulline and ornithine pools. Mycelial samples were collected on membrane filters as described above. Picric acid (1%) or trichloroacetic acid (5%) extracts were also prepared as above. After samples were centrifuged, the excess acids were removed from the supernatant fluids by passing them through AG1-X2 resin as described above. Citrulline was determined in a Technicon amino acid analyzer. Ornithine was purified by bringing the eluate from the AG1-X2 columns (8.5 ml) to 30 ml with 0.1 M ammonium formate and applying it to a column of Dowex 50W-X8, 200 to 400 mesh (12 by 70 mm) previously equilibrated with the same salt solution. After the column was washed with 30 ml of the same solution, ornithine was eluted with 0.3 M ammonium formate. Ornithine was measured colorimetrically by the method of Chinard (2).

Protein determination. Samples were collected on membrane filters, and the filters were immersed in 5% trichloroacetic acid. The precipitate was resuspended in 1.0 N NaOH, and the protein was determined by the method of Lowry et al. (10).

Chemicals. All amino acids were obtained from Calbiochem, Inc., with the exception of glycine, which was obtained from Sigma Chemical Co. The radioactive amino acids, arginine-*guanido*-^{14}C and citrulline-*ureido*-^{14}C, were obtained from New England Nuclear Corp. They were further purified by applying them to a Dowex 50W-X8 column, 200 to 400 mesh (1.0 by 5 cm). After the column was washed with 35 ml 2.0 N HCl, the amino acids were eluted with 35 ml of 4.0 N HCl. Only L-amino acids were used, and they were sterilized by filtration. Urease, bovine serum albumin and Tris were obtained from Sigma Chemical Co. POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene], PPO (2,5-diphenyloxazole), and hyamine hydroxide were obtained from Amersham/Searle Co.

RESULTS

Figure 2 shows the growth and arginine pool of the *arg-5;arg-8* double mutant in the presence of different combinations of amino acids. The double mutant grew poorly in citrulline and optimally in arginine, citrulline plus ornithine, and citrulline plus proline. These data suggested, as was mentioned above, that arginine was not as readily hydrolyzed in the presence of citrulline as in its absence (Fig. 2a). This occurred in spite of the fact that the arginine pool was higher in the presence of citrulline than in arginine (Fig. 2b). It was also found that citrulline partially inhibited the growth of the double mutant on arginine (Fig. 2a). The arginine pool again was higher in this condition than on arginine alone (Fig. 2b).

To test the hypothesis of the differential activity of arginase on arginine derived from citrulline versus exogenous arginine, we measured the activity of arginase in vivo and in vitro in the *arg-5* strain grown in the presence of ^{14}C-citrulline or ^{14}C-arginine. Since this strain synthesizes its own proline, it grows optimally on the above-mentioned amino acids. In Fig. 3, the height of the bars indicates the $^{14}CO_2$ evolved in 3-hr intervals per milligram of cell protein. The amount of $^{14}CO_2$ evolved by cells was approximately one-third lower in the presence of citrulline-*ureido*-^{14}C than in the presence of arginine-*guanido*-^{14}C, even though the arginine pool was much higher in citrulline. The arginase activity in extracts of 10-hr cultures was similar in the two cultures and corresponded to the value of a fully induced arginase (0.2 to 0.3 μmoles of urea per min per mg

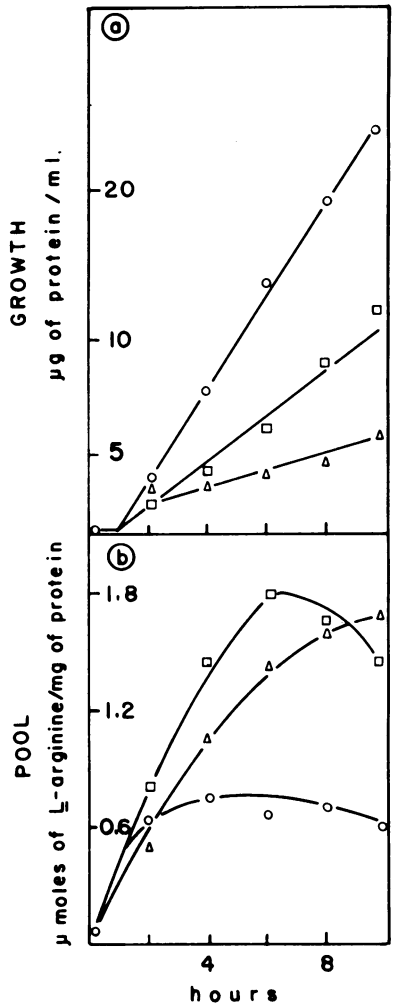

FIG. 2. *Growth (a) and arginine pool (b) of the arg-5;arg-8 strain. Additions to the medium (100 μg/ml): arginine, citrulline plus ornithine, or citrulline plus proline, ○; arginine plus citrulline, □; citrulline, △.*

of protein). The conditions which led to low rates of hydrolysis of citrulline-*ureido*-^{14}C in vivo were then explored.

To distinguish whether exogenous arginine is preferentially hydrolyzed by virtue of its origin (4), or whether arginine hydrolysis is merely inhibited by citrulline, the following experiment was performed. The *arg-5* strain was grown on equimolar concentrations of arginine-*guanido*-^{14}C plus citrulline-*ureido*-^{12}C or on arginine-*guanido*-^{12}C plus citrulline-*ureido*-^{14}C, and the arginine pool (column 1 of Table 1), the specific radioactivity of this pool (column 2), and the $^{14}CO_2$ evolved per milligram of cell protein (column 3) were determined at 3, 6, and 9 hr. As shown in Table 1, the amount of arginine hydrolyzed by arginase

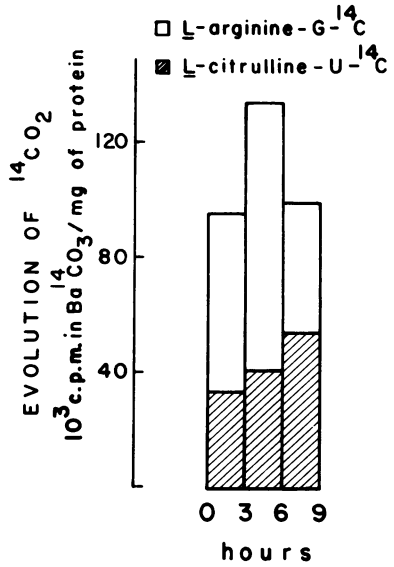

FIG. 3. *Evolution of $^{14}CO_2$ by the arg-5 strain when growing in the presence of 0.5 mM of arginine-guanido-^{14}C or citrulline-ureido-^{14}C (specific activity 260 counts per min per nmole).*

under these conditions was very similar regardless of whether it was endogenous or exogenously supplied (cf. last column, Table 1). These results unequivocally demonstrate that arginase does not distinguish between exogenous and endogenous arginine and that, under the conditions described, the arginine from the medium is not "channeled" for catabolism. If this were so, the label arising from hydrolysis of exogenously supplied ^{14}C-arginine would be much higher than that arising from ^{14}C-citrulline.

To determine whether citrulline itself or its derivative, argininosuccinic acid, was responsible for arginase inhibition in vivo, the growth of the *arg-1;arg-8* double mutant was measured on arginine in the presence and absence of citrulline. Since the *arg-1* mutation blocks the conversion of citrulline to argininosuccinic acid (12), it is possible, under the growth conditions described above, to distinguish between the alternatives mentioned. It was found that spores of the *arg-1;arg-8* double mutant collected from slants containing 500 μg of arginine-hydrochloride and 100 μg of proline per ml did not grow on arginine alone. This curious finding was more surprising in view of the adequate arginine pool. Growth was observed only when ornithine or proline were also added to the medium (Fig. 4a). [Since the *arg-1* mutation prevents any synthesis of arginine from its precursors, no growth was expected on citrulline (Fig. 4a, b).] The results were readily explained when it was found that spores of the *arg-1;arg-8* double mutant accumulated both citrulline and ornithine, both of which, as will be shown, inhibit arginase activity. Similar results were observed in the spores of the single mutants, *arg-1* and *arg-5*, in regard to the accumulation of citrulline and ornithine (Table 2).

To demonstrate directly that citrulline inhibits arginase, this enzyme reaction was

TABLE 1. *Hydrolysis of arginine by arginase in the strain arg-5 grown in citrulline plus arginine*

Growth condition of strain *arg-5*	Time (hr)	Micromoles Arg/mg P[b] (1)	Counts per min per nmole Arg (2)	$Ba^{14}CO_3$ counts per min per mg P (3)	Nanomoles Arg/mg P (3/2)
0.5 mM AG^{14}C[a], 0.5 mM CU^{12}C	3	0.609	74.5	20,300	273
	6	0.610	54.4	17,900	329
	9	0.520	55.8	24,200	434
0.5 mM AG^{12}C, 0.5 mM CU^{14}C[a]	3	0.518	144.3	34,600	242
	6	0.775	102.0	34,300	336
	9	0.565	139.0	60,000	432

[a] AG^{14}C = arginine-*guanido*-^{14}C; CU^{14}C = citrulline-*ureido*-^{14}C.
[b] Arg = arginine; P = protein.

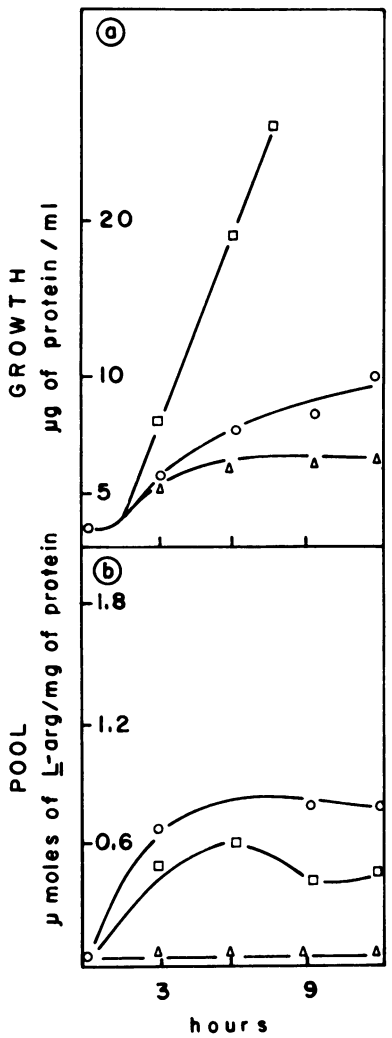

FIG. 4. *Growth (a) and arginine pool (b) of the arg-1;arg-8 strain. Additions to the medium (100 μg/ml): arginine, ○; arginine plus ornithine or arginine plus proline, □; citrulline, △.*

measured in vivo in the single mutant *arg-1* under different growth conditions.

Figure 5 shows the in vivo activity of arginase in strains *arg-1* and *arg-5*. Except for the first 3 hr, the activity in strain *arg-5* grown on citrulline-*ureido*-^{14}C was similar to that in strain *arg-1* grown on arginine-*guanido*-^{14}C. During the first 3 hr under the conditions described, the arginine pool and the activity of arginase were higher in strain *arg-5*. We propose that the citrulline concentrated from the medium by cells of strain *arg-5* and the citrulline synthesized endogenously by the *arg-1* mutant both inhibit the activity of arginase. In the *arg-5* strain grown on arginine, where citrulline is neither synthesized nor accumulated from the medium, the activity of arginase is optimal and several-fold higher than when citrulline is also present. When strain *arg-1* was grown in the presence of citrulline-*ureido*-^{12}C and arginine-*guanido*-^{14}C, the inhibition of arginase was maximal. This presumably occurred as a result of the greatly elevated citrulline pool derived from endogenous synthesis and uptake from the medium. Because the *arg-1* mutant cannot metabolize citrulline, the labeled arginine pool was not diluted with cold arginine. It is also apparent that the arginine pool of strain *arg-1*, under the conditions above described, was very similar to that of strain *arg-5* grown on arginine, where the arginase activity is optimal. In light of these results, it is clear why citrulline inhibits the growth of the *arg-1;arg-8* double mutant in arginine.

In addition to citrulline, ornithine was also accumulated by the spores of the *arg-1;arg-8* double mutant, and this amino acid is known to inhibit the activity of arginase in vitro (11). It was critical, therefore, to test the effect in vivo of the ornithine produced by arginase on the activity of this enzyme. To this end, strain *arg-12*, lacking OTC (7), and strain *arg-12^s;ota* were grown in the presence of arginine-*guanido*-^{14}C. Strain *arg-12^s;ota* has only 5% of the OTC activity and 5% of the arginine pool of wild type (7), and lacks ornithine transaminase activity entirely (6). The mutations therefore prevent disposal of ornithine produced by the arginase reaction. Therefore, when grown in the presence of arginine, the *arg-12^s;ota* strain accumulates ornithine mainly through the action of arginase. By contrast, while spores of the *arg-12* strain accumulated endogenous ornithine from glutamate (Table 2), little ornithine accumulates during growth on arginine (5).

In Fig. 6 we show that the activity of argi-

TABLE 2. *Citrulline and ornithine pools in spores of arginine auxotrophs*[a]

Strains	Pool (μmoles/mg of protein)	
	Citrulline	Ornithine
arg-1[b]	0.360	0.064
arg-5[b]	0	0.006
arg-1;arg-8[c]	0.130	0.015

[a] The spores were obtained from 5-day-old slants.
[b] Slants on 200 μg of arginine per ml.
[c] Slants on 500 μg of arginine plus 100 μg of proline per ml.

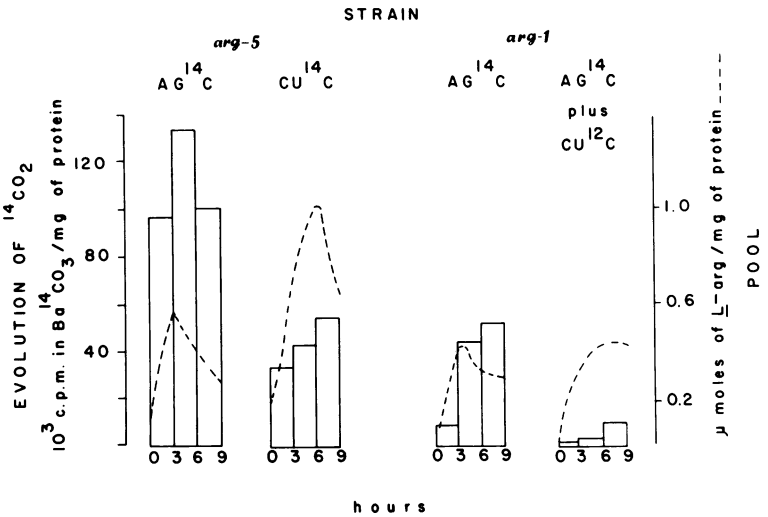

FIG. 5. *Evolution of $^{14}CO_2$ and arginine pool of the arg-5 and arg-1 strains when growing in the presence of the indicated radioactive amino acids at 0.5 mM. AG^{14}C = arginine-guanido-^{14}C (specific activity 260 counts per min per nmole); CU^{14}C = citrulline-ureido-^{14}C (specific activity 260 counts per min per nmole); CU^{12}C = citrulline-ureido-^{12}C.*

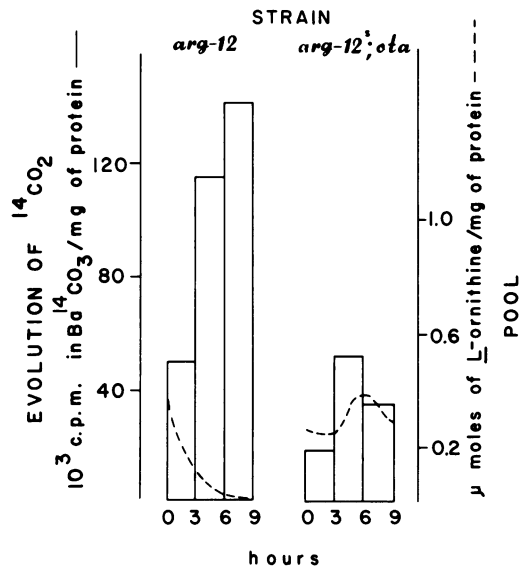

FIG. 6. *Evolution of $^{14}CO_2$ and ornithine pool of the arg-12 and arg-12^s; ota strains when growing in the presence of 0.5 mM arginine-guanido-^{14}C (specific activity 280 counts per min per nmole).*

nase in vivo was one-third lower in the *arg-12^s; ota* strain, which accumulates ornithine, than in the *arg-12* strain, which does not.

The activity in vivo of arginase in *arg-12* during the first 3 hr of growth was only one-half that found in strain *arg-5* under the same conditions (cf. Fig. 5). The reduced arginase activity in strain *arg-12* coincides with the grossly elevated pool of ornithine (formed from glutamate) found in the spores. The ornithine pool of spores of *arg-12* (Fig. 6) grown on arginine was 10-fold that of spores of the wild strain 74-A grown in minimal medium (0.04 μmoles per mg of protein). We conclude that this ornithine must have arisen from glutamate, since the *arg-5;arg-12* double mutant does not have a detectable ornithine pool (the *arg-5* mutation blocks ornithine biosynthesis from glutamate). The difference in the arginase activity in vivo of the *arg-12* and *arg-12^s; ota* strains occurred in the presence of similar arginine pools. Consequently, this effect cannot be attributed to differences in the levels of intracellular arginine.

All the evidence presented thus far is consistent with the hypothesis that ornithine and citrulline act as regulators of arginine catabolism. The possibility that this regulatory effect is accomplished by direct effects upon the arginase enzyme was tested directly in vitro.

We found that ornithine and citrulline competitively inhibit arginase at *p*H 9.5 (Fig. 7). Identical results were obtained at *p*H 7.5, where the V_m was 25-fold less that at *p*H 9.5. The K_m for arginine was 2.86×10^{-3} M at *p*H 7.5 and 1.92×10^{-3} M at *p*H 9.5. When the activity of arginase was measured in the presence of different concentrations of ornithine or citrulline we also found the competitive type of inhibition.

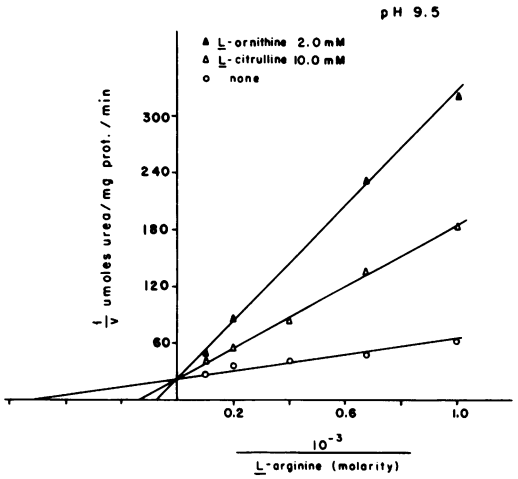

FIG. 7. *Competitive inhibition of arginine hydrolysis by ornithine and citrulline at pH 9.5. The reciprocal of the initial velocity is plotted versus the reciprocal arginine concentration.*

A possible mechanism to explain why citrulline inhibits the activity of arginase could be related to the fact that the adenosine triphosphate requirement for the formation of argininosuccinic acid is rate-limiting. As a consequence, exogenous citrulline would accumulate intracellularly. Thus the rate of flow of citrulline to arginine could only be sufficient to support the accumulation of a high arginine pool from citrulline if the activity of arginase is curtailed by citrulline.

To test this hypothesis, the distribution of exogenous arginine and arginine synthesized from citrulline was studied. Strain *arg-5* was grown on citrulline-*ureido*-^{14}C or on arginine-*guanido*-^{14}C, and the arginine pool, that incorporated into protein, and that catabolized by arginase were measured. In Table 3 we present the distribution of arginine from the medium and that synthesized from citrulline. It is apparent that the rate of flow of arginine from the medium into the cell was approximately double that seen from citrulline to arginine. If the activity of arginase was not susceptible to citrulline inhibition, then we would have expected that the arginine arising from citrulline-*ureido*-^{14}C would be catabolized to the same extent as that exogenously supplied. This was clearly not so. Moreover, an optimal arginase activity would have depleted the cell of any arginine from the pool, and possibly that found in protein. Again, this was clearly not the case.

DISCUSSION

The rise in the arginine pool which occurs when *N. crassa* is grown on citrulline leads to feedback inhibition of the arginine pathway and allows the cell to utilize citrulline as a sole arginine source. The advantages of this conversion are obvious since in this manner citrulline utilizes the final product of the pathway as a signal for preventing its own synthesis. A direct inhibition by citrulline of its own biosynthesis does not seem to prevail since under conditions in which arginine is not synthesized citrulline and ornithine accumulate (Table 2).

To understand how the arginine accumulates in the presence of citrulline, the following arguments must be taken into consideration. First, the conversion of citrulline to arginine has a rate-limiting step requiring energy in the formation of argininosuccinic acid. Second, although the elevated arginine pool induces the synthesis of arginase, we have shown that citrulline seriously inhibits the catalytic activity of this enzyme. The net effect is an accumulation of arginine.

There is no metabolic risk for the cell in catabolizing compounds it does not synthesize. However, the presence of catabolic pathways for compounds that the cell synthesizes and uses in macromolecular synthesis poses a metabolic problem. Microorganisms have solved this problem by repressing enzymes that catabolize such compounds except when they are in excess. However, it seems that such metabolic "safety switches" are not entirely sufficient, since in some microorganisms, catabolic enzymes are inducible not by their substrates but by the first intermediate of the catabolic pathway (9). This mechanism is apparently not operative in the catabolism of arginine by *N. crassa*; arginine induces the synthesis of the enzymes of this pathway (5). We would like to propose that the product inhibition of arginase by ornithine is a mechanism operative to regulate the catabolism of arginine: the high ornithine pool accumulated from glutamate of

TABLE 3. *Distribution of endogenously synthesized and endogenously supplied arginine*

Growth condition of *arg-5*	Time (hr)	Nanomoles of arginine			
		Pool (1)	Protein (2)	Catabolyzed (3)	Sum (1+2+3)
0.5 mM AG^{14}C[a]	3	55	36	365	456
	6	122	115	880	1117
	9	200	294	1265	1759
0.5 mM CU^{14}C[a]	3	92	23	130	243
	6	178	83	308	569
	9	317	223	522	1062

[a] AG^{14}C = arginine-*guanido*-^{14}C; CU^{14}C = citrulline-*ureido*-^{14}C.

minimal cultures serves in part to inhibit the hydrolysis of endogenous arginine by a noninduced arginase. In the presence of arginine in the medium, the production of ornithine from glutamate is inhibited (5), and, since ornithine is also the immediate product of arginine catabolism via arginase, we propose that the entire catabolic pathway is carefully regulated, even though arginine-originated ornithine never accumulates. No regulatory role can be assigned to citrulline under normal circumstances since no pool of this amino acid can be detected in *N. crassa* growing in minimal medium.

The question as to how an elevated arginine pool triggers the catabolism of this amino acid must be answered by further knowledge of arginine in its role as a substrate, an activator and an inducer of the synthesis of arginase. Because arginine is synthesized from glutamate, and because the catabolism of arginine ultimately yields glutamate and glutamine (Fig. 1), an "arginine cycle" must be controlled such that catabolism prevails only under conditions of excess arginine. This presumes a dual role for arginine in repressing its own biosynthesis and in inducing its own catabolism. At the same time the catabolism of arginine must be regulated not only by this substrate but also by the final products of its catabolic pathway.

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LITERATURE CITED

1. Castañeda, M., J. Martuscelli, and J. Mora. 1967. The catabolism of L-arginine by *Neurospora crassa*. Biochim. Biophys. Acta **141**:276–286.
2. Chinard, F. P. 1952. Photometric estimation of proline and ornithine. J. Biol. Chem. **199**:91–95.
3. Davis, R. H. 1962. A mutant form of ornithine transcarbamylase found in a strain of *Neurospora* carrying a pyrimidine-proline suppressor gene. Arch. Biochem. Biophys. **97**:185–191.
4. Davis, R. H. 1967. Channeling in *Neurospora* metabolism, p. 303–322. *In* H. J. Vogel, J. O. Lampen, and V. Bryson (ed.), Organizational biosynthesis. Academic Press Inc., New York.
5. Davis, R. H., M. B. Lawless, and L. A. Port. 1970. Arginaseless *Neurospora:* genetics physiology and polyamine synthesis. J. Bacteriol. **102**:299–305.
6. Davis, R. H., and J. Mora. 1968. Mutants of *Neurospora crassa* deficient in ornithine-δ-transaminase. J. Bacteriol. **96**:383–388.
7. Davis, R. H., and W. M. Thwaites. 1963. Structural gene for ornithine transcarbamylase in *Neurospora*. Genetics **48**:1551–1558.
8. Kølmark, H. G. 1969. Genetics studies of urease mutants in *Neurospora crassa*. Mutat. Res. **8**:51:63.
9. Lawrence, A. C., and B. Magasanik. 1968. Induction and repression of the histidine-degrading enzymes of *Bacillus subtilis*. J. Biol. Chem. **243**:5165–5178.
10. 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.
11. Mora, J., R. Tarrab, and L. F. Bojalil. 1966. On the structure and function of different arginases. Biochim. Biophys. Acta **118**:206–209.
12. Newmeyer, D. 1967. Arginine synthesis in *Neurospora crassa:* genetic studies. J. Gen. Microbiol. **16**:449–462.
13. Pall, M. L. 1970. Amino acid transport in *Neurospora crassa*. II. Properties of a basic amino acid transport system. Biochim. Biophys. Acta **203**:139–149.
14. Van Pilsum, J. F., R. P. Martin, E. Kito, and J. Hess. 1956. Determination of creatine, creatinine, arginine, guanidoacetic acid, guanidine and methyl-guanidine in biological fluids. J. Biol. Chem. **222**:225–236.
15. Vogel, H. J. 1964. Distribution of lysine pathways among fungi: evolutionary implications. Amer. Natur. **98**:435–446.
16. Vogel, H. J., and D. M. Bonner. 1954. On the glutamate-proline ornithine inter-relation in *Neurospora crassa*. Proc. Nat. Acad. Sci. U.S.A. **40**:688–694.