Catabolism of L-Arginine by *Pseudomonas aeruginosa*

By ANNICK MERCENIER,^{1*} JEAN-PAUL SIMON,¹ DIETER HAAS²
AND VICTOR STALON¹

¹ Laboratoire de Microbiologie, Faculté des Sciences, Université Libre de Bruxelles and Institut de Recherches du C.E.R.I.A., Avenue Emile Gryzon 1, B-1070 Brussels, Belgium

² Mikrobiologisches Institut, E.T.H. Zürich, Universitätstrasse 2, CH-8006 Zürich, Switzerland

(Received 12 July 1979)

Pseudomonas aeruginosa is known to break down arginine by the arginine deiminase pathway. An additional pathway has now been found whereby arginine is converted to putrescine with agmatine and *N*-carbamoylputrescine as intermediates. The following enzyme activities belonging to this pathway were detected in crude extracts: arginine decarboxylase (EC 4.1.1.19), which catalyses the release of CO₂ from arginine to give agmatine; agmatine deiminase (EC 3.5.3.12), which degrades agmatine to *N*-carbamoylputrescine; and *N*-carbamoylputrescine amidohydrolase (EC 3.5.3.-), which then removes the ureido group of carbamoylputrescine. In crude extracts, arginine decarboxylase activity was stimulated by pyridoxal phosphate, Mg²⁺ and by the products of the catabolic pathway, putrescine and spermidine.

Growth of *P. aeruginosa* on arginine as the sole carbon and nitrogen source markedly increased the activity of arginine decarboxylase. Agmatine and *N*-carbamoylputrescine induced the synthesis of agmatine deiminase and *N*-carbamoylputrescine hydrolase. Addition of succinate or citrate to medium containing arginine or agmatine led to repression of the enzymes involved in the arginine decarboxylase pathway. Moreover, the repression of agmatine deiminase and *N*-carbamoylputrescine hydrolase was further increased when *P. aeruginosa* was grown in media with agmatine plus glutamine or agmatine plus succinate and ammonia. This suggests that the expression of the agmatine pathway may be regulated by carbon catabolite repression as well as nitrogen catabolite repression.

INTRODUCTION

Fluorescent pseudomonads possess the arginine deiminase pathway (Slade *et al.*, 1954; Stalon *et al.*, 1967) for the catabolism of arginine. This pathway leads to the formation of ATP by the combined action of arginine deiminase, catabolic ornithine carbamoyltransferase and carbamate kinase (Stalon *et al.*, 1967, 1972). The deiminase pathway is also a source of energy for fermentative organisms like *Clostridium* (Mitruka & Costilow, 1967) and *Streptococcus faecalis* (Bauchop & Elsdén, 1960).

In *Bacillus licheniformis*, arginase converts arginine to ornithine in well-aerated cultures, whereas the arginine deiminase pathway is induced in anaerobiosis (Broman *et al.*, 1975, 1978). A mutant of *B. licheniformis* displaying a 10-fold decreased level of carbamate kinase activity was unable to grow anaerobically on pyruvate plus arginine, but grew well aerobically on the same medium (K. Broman, unpublished results). This observation indicates that the arginine deiminase pathway can be an energy source in *Bacillus* growing anaerobically.

In pseudomonads, the enzymes involved in the arginine deiminase pathway have their highest activities in organisms deprived of oxygen (Haas *et al.*, 1979; Halleux *et al.*, 1972). A recently isolated mutant of *P. aeruginosa* lacking catabolic ornithine carbamoyltransfer-

ase (Haas *et al.*, 1979) grew aerobically on arginine as the only carbon and nitrogen source at the same rate as the wild-type. The properties of the mutant demonstrate that there must be at least one other arginine catabolic pathway in this organism. Kay & Gronlund (1969) showed that putrescine is a product of arginine catabolism by intact *P. aeruginosa*. Two modes of putrescine formation were considered: (i) arginine could be degraded via the arginine deiminase pathway to give ornithine, which would then be decarboxylated (Morris & Pardee, 1966); (ii) arginine could be degraded to putrescine via agmatine, without ornithine as an intermediate. In *Escherichia coli*, arginine can be decarboxylated to give agmatine and the direct conversion of agmatine to putrescine is catalysed by agmatine ureohydrolase (Morris & Pardee, 1966). In *Streptococcus faecalis*, agmatine induces an agmatine deiminase which produces *N*-carbamoylputrescine, and a catabolic putrescine carbamoyltransferase then catalyses the formation of putrescine and carbamoylphosphate (Roon & Barker, 1972; B. Wagnies, N. Lauwers & V. Stalon, unpublished observations). In higher plants, carbamoylputrescine is degraded by an *N*-carbamoylputrescine amidinohydrolase (Smith & Garraway, 1964; Smith, 1965).

In this paper, we show that in *P. aeruginosa* arginine can be converted to putrescine via two intermediates, agmatine and *N*-carbamoylputrescine. We have also examined the regulation of the two arginine catabolic pathways in this organism. A preliminary report of these results has been published (Mercenier *et al.*, 1978).

METHODS

Bacterial strains and growth of organisms. *Pseudomonas aeruginosa* wild-type strain PAO1 (ATCC 15692) and mutant strain PAO630 (*argF2*, *arcB9*) (Haas *et al.*, 1979) were used. These strains were routinely grown at 37 °C on minimal medium P, a nitrogen-free salts medium (Leisinger *et al.*, 1972). Carbon and nitrogen sources were added (each at 20 mM) after sterilization by autoclaving, except for agmatine, glutamine, *N*-carbamoylputrescine and putrescine which were sterilized by filtration. Cultures were grown in flasks fitted with side-arms on a rotary shaker. The volume of the culture fluid was 10% of the flask volume. Cells from exponential phase cultures (about 4×10^8 cells ml⁻¹) were harvested by centrifuging, washed with 0.9% (w/v) NaCl at 0 °C and, if not used immediately, they were stored frozen as a pellet.

Preparation of cell extracts. Extracts were prepared by suspending the cells in 2 ml 50 mM-phosphate buffer pH 7.5 supplemented with 0.5 mM-dithiothreitol and 5% (v/v) ethyleneglycol, subjecting the suspensions to 5 min disruption in a Mullard Sonic Oscillator and removing cell debris by centrifuging at 20 000 g for 10 min at 4 °C.

Synthesis of *N*-carbamoylputrescine. *N*-Carbamoylputrescine was synthesized from putrescine and cyanate as described by Smith & Garraway (1964) with some modifications. After precipitation twice with ethanol acidified with conc. HCl (1%, v/v), the preparation of *N*-carbamoylputrescine was still contaminated with putrescine and *N,N'*-dicarbamoylputrescine. The white precipitate was dissolved in water and chromatographed on a Dowex 50W-X2 column (H⁺ form, in water). *N,N'*-Dicarbamoylputrescine was eluted immediately, *N*-carbamoylputrescine by a linear gradient of KCl from 0 to 1.5 M, and putrescine with 2 M-KCl. The pooled fractions of *N*-carbamoylputrescine were at least 99% pure and stored at -20 °C after acidification to pH 4 with HCl.

The enzymic synthesis of *N*-[carbamoyl-¹⁴C]carbamoylputrescine was accomplished under the following conditions. The reaction vessel contained (in 2 ml) 10 μmol putrescine, 6.5 μmol [¹⁴C]carbamoylphosphate (total radioactivity 50 μCi, 1.85 GBq) and 40 μg purified putrescine carbamoyltransferase of *Streptococcus faecalis*. After incubation at 37 °C for 30 min, the reaction was stopped by boiling for 10 min to destroy the excess carbamoylphosphate and ¹⁴CO₂ was removed by bubbling air through the solution.

Separation of intermediates of arginine catabolism. The procedure used was based on that described by Moore *et al.* (1958), but several modifications were made to allow the identification of products related to arginine catabolism. A typical elution pattern of a mixture of various amino acids, guanidino compounds and polyamines from the cationic exchange resin Aminex A4 is shown in Fig. 1. Separation was at 73 °C using stepwise elution on a 0.9 × 20 cm column. The first elution was with 0.1 M-sodium citrate buffer pH 5.28 for 75 min. After this time, 0.066 M-sodium citrate buffer pH 6.0 containing KCl at 120 g l⁻¹ (buffer B) was passed through the column as proposed by Gehrke *et al.* (1977). The flow rate through the column was 120 ml h⁻¹ and the ninhydrin reagent pump delivered 60 ml h⁻¹. The time of the ninhydrin reaction in the coil at 100 °C was 10 min. For all products tested, the recovery after analysis was ≥ 95%.

Fig. 1. Ion-exchange chromatography of basic amino acids, amines and diamines. A mixture of 50 μmol of each compound was applied to an Aminex A4 column and eluted as described in Methods. Guanidinobutyrate was determined by the method of Sakaguchi as described by Ramos *et al.* (1970).

Assays of enzyme activities. Enzyme-catalysed decarboxylation was assayed by measuring the liberated $^{14}\text{CO}_2$ as previously described (Legrain & Stalon, 1976).

Arginine decarboxylase (EC 4.1.1.19; L-arginine carboxy-lyase). The standard incubation mixture contained (in 1 ml) 50 μmol Tris/HCl buffer pH 8.25, 10 μmol putrescine, 5 μmol L-arginine and DL-[1- ^{14}C]arginine (40 000 c.p.m. μmol^{-1}), 0.1 μmol pyridoxal phosphate, 4 μmol MgCl_2 , 0.5 μmol dithiothreitol and cell extract to attain about 1 mg protein. The incubation time was 10 to 25 min. The enzyme was labile and rapidly destroyed on freezing whole cells or cell extracts. Activity was conserved for at least 8 h by the addition of 0.5 mM-dithiothreitol and 5% (v/v) ethyleneglycol to the extraction buffer.

N-Carbamoylputrescine amidohydrolase (EC 3.5.3.-). The incubation mixture contained (in 1 ml) 50 μmol EDTA/NaOH buffer pH 7.25, 2 μmol N-carbamoylputrescine plus N-[carbamoyl- ^{14}C]carbamoyl-putrescine (35 000 c.p.m. μmol^{-1}) and extract. Enzyme activity was proportional to protein concentration and time for at least 60 min, provided less than 30% of the substrate was consumed.

Ornithine decarboxylase (EC 4.1.1.17; L-ornithine carboxy-lyase). The incubation mixture contained (in 1 ml) 50 μmol EDTA/NaOH buffer pH 7.0, 0.025 μmol pyridoxal phosphate, 0.1 μmol L-ornithine plus DL-[1- ^{14}C]ornithine (2×10^6 c.p.m. μmol^{-1}) and extract. Enzyme activity was proportional to protein concentration and time up to 30% substrate consumption.

Agmatine deiminase (EC 3.5.3.12; agmatine iminohydrolase). This enzyme was assayed by colorimetric determination of N-carbamoylputrescine. The standard incubation mixture contained (in 2 ml) 100 μmol EDTA/NaOH buffer pH 7.8, 10 μmol agmatine and extract. The reaction was started by adding the agma-

tine solution and stopped after 15 min by adding 2 ml of 1 M-HCl. *N*-Carbamoylputrescine formed was measured by the method of Archibald (1944). The activity was proportional to enzyme concentration and time at least within the range of 0.05 to 3 μ mol *N*-carbamoylputrescine formed.

Anabolic ornithine carbamoyltransferase (EC2.1.3.3) was assayed as described previously (Stalon *et al.*, 1972).

The protein content of extracts was determined by the method of Lowry, with bovine serum albumin as a standard.

Specific activity is defined as the amount of enzyme catalysing the formation of 1 μ mol product h⁻¹ (mg protein)⁻¹.

Chemicals. Amino acids, amines and polyamines were from Sigma. Agmatine sulphate contained about 25% of putrescine and was used without further purification. DL-[1-¹⁴C]arginine.HCl was purchased from CEA-IRE. L-[U-¹⁴C]Arginine.HCl, DL-[1-¹⁴C]ornithine.HCl, L-[U-¹⁴C]putrescine.2HCl and L-[U-¹⁴C]-citrulline.HCl were obtained from The Radiochemical Centre, Amersham.

RESULTS

Intermediates of arginine degradation

An extract of the wild-type strain of *P. aeruginosa* grown on arginine as the only carbon and nitrogen source was incubated with L-[U-¹⁴C]arginine at pH 8.25, and the incubation products were separated and identified by automated ion-exchange analysis. Almost 40% of the initial radioactivity was recovered as putrescine (Table 1). Traces of labelled agmatine, *N*-carbamoylputrescine, ornithine and citrulline were also found, but no urea was recovered. Added citrulline was not converted to putrescine. With ornithine as the substrate of incubation, only traces of putrescine were found. Furthermore, an extract of the mutant PAO630 (which is devoid of catabolic ornithine carbamoyltransferase activity) converted arginine to putrescine to the same extent as the wild type, when grown under identical conditions. Since citrulline and ornithine appeared not to be intermediates in putrescine formation from arginine, further incubations were carried out in the presence of agmatine. The two main products were *N*-carbamoylputrescine and putrescine (Table 1). With *N*-carbamoylputrescine as substrate, 1 μ mol putrescine was formed per μ mol *N*-carbamoylputrescine (Table 1).

Table 1. *Products of the enzymic conversion of arginine in vitro*

Cell extract (2 mg protein) of strain PAO1 grown on L-arginine was incubated at 37 °C for 1 h in a reaction mixture containing (in 1 ml) either 5 μ mol [U-¹⁴C]arginine (sp.act. 200000 c.p.m. μ mol⁻¹) or 5 μ mol agmatine or 5 μ mol *N*-carbamoylputrescine, 10 μ mol Tris/HCl buffer pH 8.25, 0.1 μ mol pyridoxal phosphate and 4 μ mol MgCl₂. At the end of the incubation, 0.1 ml 40% (w/v) trichloroacetic acid was added. The protein was removed by filtration and the supernatant solution was subjected to ion-exchange chromatography as described in Methods. With arginine as the incubation substrate, fractions of 2 ml were collected from the column and arginine, putrescine, *N*-carbamoylputrescine, agmatine and citrulline were measured by determination of radioactivity. With agmatine or *N*-carbamoylputrescine as incubation substrate, agmatine, *N*-carbamoylputrescine and putrescine were determined by the ninhydrin test.

Incubation substrate (5 μ mol)	Product (μ mol)	
Arginine	Residual arginine	2.5
	Agmatine	0.025
	<i>N</i> -Carbamoylputrescine	0.1
	Citrulline	0.05
	Ornithine	<0.05
	Putrescine	2.0
Agmatine	Residual agmatine	1.55
	<i>N</i> -Carbamoylputrescine	0.65
	Putrescine	2.8
<i>N</i> -Carbamoylputrescine	Residual <i>N</i> -carbamoylputrescine	1.1
	Putrescine	3.9

Conversion of N-carbamoylputrescine to putrescine

Extracts of organisms grown on arginine, agmatine or putrescine rapidly decarboxylated *N*-carbamoylputrescine. Maximum activity was observed at pH 7.2. The apparent K_m for *N*-carbamoylputrescine was 0.3 mM at pH 7.25. Pyridoxal phosphate or the divalent cations Mg^{2+} or Mn^{2+} were not required for activity. Putrescine (10 mM) and ammonia (100 mM) had a slight inhibitory effect on enzyme activity, as expected if they are reaction products. Phosphate (5 to 100 mM) or arsenate (5 to 100 mM) did not stimulate the formation of CO_2 from *N*-carbamoylputrescine. Thus, the ureido group of *N*-carbamoylputrescine appeared to be cleaved by an amidinohydrolase, as found in plants (Smith & Garraway, 1964; Smith, 1965). The products were putrescine, CO_2 and NH_3 . No evidence for phosphorolysis (or arsenolysis) of carbamoylputrescine by a catabolic putrescine carbamoyltransferase (Roon & Barker, 1972) was found. Since the equilibrium constant of the reaction catalysed by putrescine carbamoyltransferase is in favour of carbamoylputrescine synthesis (Roon & Barker, 1972), extracts were tested for their ability to condense putrescine with carbamoyl-phosphate; no reaction occurred. Citrulline, a substrate for the catabolic ornithine carbamoyltransferase, did not inhibit the carbamoylputrescine hydrolase reaction.

Conversion of agmatine to N-carbamoylputrescine

Extracts of organisms grown on arginine, agmatine or *N*-carbamoylputrescine catalysed the conversion of agmatine to *N*-carbamoylputrescine. This reaction, involving the hydrolysis of a C-N bond in the guanidino group of agmatine, is catalysed by an agmatine deiminase. Maximum activity was observed at pH 7.8. In contrast, arginine deiminase, which is also present in *P. aeruginosa*, had a pH optimum of pH 5.8 and was stimulated by $MnCl_2$ in crude extracts (results not shown). The apparent K_m for agmatine was 0.1 mM.

Conversion of arginine to agmatine

Recently, a constitutive arginine decarboxylase was found in a *Pseudomonas* species isolated from a stagnant pond (Rosenfeld & Roberts, 1976). Extracts of *P. aeruginosa* PAO1 grown on arginine catalysed the release of $^{14}CO_2$ from DL-[1- ^{14}C]arginine. The reaction was stimulated by pyridoxal phosphate and Mg^{2+} . Maximum activity was observed at pH 8.0. The apparent K_m for arginine was 5 mM. Putrescine (10 to 20 mM) and spermidine (2.5 to 4 mM) stimulated the enzyme about fivefold in crude extracts under our assay conditions. The enzyme assay was not linear with time or amount of crude extract added. Since putrescine formed during the course of the reaction would be expected to enhance enzymic activity, and may partially explain the lack of linearity, 10 mM-putrescine was routinely included in the assay mixture. Although complete linearity as a function of time and enzyme concentration was not achieved, specific enzyme activities were reproducible for the standard protein concentration (1 to 1.5 mg ml⁻¹) used in the assay.

Conversion of ornithine to putrescine

Ornithine was decarboxylated by extracts of *P. aeruginosa* grown on mineral medium supplemented either with glutamate or with succinate and ammonia. The enzyme required pyridoxal phosphate but not Mg^{2+} . Maximum activity was observed at pH 7.0. The apparent K_m for ornithine was 0.1 mM.

Control of synthesis of enzymes involved in the arginine decarboxylase pathway

The specific activities of the enzymes involved in the arginine decarboxylase pathway were measured under a variety of growth conditions (Tables 2 and 3). For comparison, ornithine decarboxylase and anabolic ornithine carbamoyltransferase activities were also examined (Table 2).

Table 2. *Activities of L-arginine catabolic enzymes, ornithine decarboxylase and anabolic ornithine carbamoyltransferase in extracts of organisms grown on various carbon and nitrogen sources*

Specific activities were determined as described in Methods.

Growth medium	Arginine decarb-oxylase	Agmatine deiminase	N-Car-bamoyl-putrescine hydrolase	Ornithine decarb-oxylase	Anabolic ornithine carbamoyl-transferase
Succinate + NH_4^+	0.53	0.8	2.2	1.0	44.0
Glutamate	0.5	1.0	1.9	1.1	19.6
Ornithine	2.2	0.9	2.2	0.3	4.3
Arginine	6.0	1.5	2.4	0.2	1.0
Agmatine	0.2	35.0	22.0	0.5	24.0
N-Carbamoylputrescine	0.2	26.0	16.7	0.6	25
Putrescine	1.4	0.9	2.5	0.8	21
Arginine + Succinate	1.14	—	—	0.7	18.7
Arginine + Citrate	1.3	—	—	1.0	8.5
Arginine + Agmatine	1.8	26.6	14.0	—	3.1
Arginine + NH_4^+	5.0	—	—	—	0.6

Table 3. *Activities of agmatine degrading enzymes in extracts of organisms grown on various carbon and nitrogen sources*

Specific activities were determined as described in Methods.

Growth medium	Agmatine deiminase	N-Car-bamoyl-putrescine hydrolase
Agmatine	35	22
Agmatine + Pyruvate	31	—
Agmatine + NH_4^+	32.5	—
Agmatine + Succinate	17.5	12
Agmatine + Citrate	22.5	—
Agmatine + Glucose	23	11.5
Agmatine + Glutamine	12	8.7
Agmatine + Glutamate	24	14.0
Agmatine + Succinate + NH_4^+	11.0	8.0
Agmatine + Citrate + NH_4^+	17.0	—
Agmatine + Succinate + Glutamine	8.7	6.0

Induction. Arginine decarboxylase was strongly induced during growth on arginine as the only carbon and nitrogen source, whereas the activities of agmatine deiminase and N-carbamoylputrescine hydrolase were highest with agmatine and N-carbamoylputrescine as growth substrates. The activities of the enzymes involved in the arginine decarboxylase pathway were low in cultures grown on minimal medium (succinate + NH_4^+), whereas the activities of ornithine decarboxylase and the anabolic ornithine carbamoyltransferase were high under these conditions. These two enzymes were repressed in organisms grown on arginine giving a presumptive anabolic function for ornithine decarboxylase. The same regulatory pattern was observed with ornithine as the growth substrate. Arginine decarboxylase synthesis was stimulated but ornithine decarboxylase and the anabolic ornithine carbamoyltransferase were repressed, presumably because ornithine was transformed to arginine. In addition, it has been demonstrated that with citrate as the carbon source activities of the anabolic ornithine carbamoyltransferase are high (Voellmy & Leisinger, 1978). This seems equally true for ornithine decarboxylase synthesis (Table 3).

Catabolite repression. Various carbon sources repressed the synthesis of the enzymes involved in the arginine decarboxylase pathway (Tables 2 and 3). Succinate appeared to be

Fig. 2. Pathways of L-arginine catabolism in *Pseudomonas aeruginosa*: (1) arginine deiminase (EC 3.5.3.6), (2) catabolic ornithine carbamoyltransferase (EC 2.1.3.3), (3) carbamate kinase (EC 2.7.2.2), (4) arginine decarboxylase (EC 4.1.1.19), (5) agmatine deiminase (EC 3.5.3.12), (6) *N*-carbamoylputrescine amidinohydrolase (EC 3.5.3.-), (7) ornithine decarboxylase (EC 4.1.1.17).

the most effective catabolite repressor. Intermediate activities of the agmatine degrading enzymes were observed when cultures were grown on agmatine plus glucose or citrate. Pyruvate had little effect. In addition to carbon catabolite repression, agmatine deiminase and *N*-carbamoylputrescine hydrolase synthesis were also subject to nitrogen repression by glutamine or ammonia in the presence of succinate. In the absence of a good carbon source, ammonia had little influence, but repression by glutamine occurred even in the absence of succinate.

DISCUSSION

Pseudomonas aeruginosa has long been known to break down arginine by the arginine deiminase pathway (Thornley, 1960). This paper shows the existence of an additional arginine catabolic pathway which leads to putrescine via agmatine and *N*-carbamoylputrescine (Fig. 2). This pathway was established by two methods: (i) the identification of the intermediates obtained after incubating crude extracts in the presence of arginine, agmatine and *N*-carbamoylputrescine; (ii) the characterization of the enzyme activities implicated in this pathway.

The arginine decarboxylase catabolic pathway in *P. aeruginosa* is similar to that found in higher plants (Smith & Garraway, 1964; Smith, 1965) but differs from pathways reported for other bacteria which catabolize arginine by different routes; in particular, *E. coli* has an agmatine ureohydrolase rather than an agmatine deiminase. In *P. putida*, another arginine catabolic pathway was found by Rodwell and coworkers (Miller & Rodwell, 1971; Vanderbilt *et al.*, 1975). Here the intermediate products of arginine degradation are 2-oxoarginine, guanidinobutyraldehyde, guanidinobutyrate and 4-aminobutyrate (Miller & Rodwell, 1971; Vanderbilt *et al.*, 1975). Our attempts to demonstrate such a pathway in *P. aeruginosa* have been unsuccessful. 2-Oxoglutarate, which could be an acceptor of an arginine amino-transferase reaction producing 2-oxoarginine, was without effect on the formation of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ arginine in extracts of strain PAO1 (unpublished observation). No guanidino compounds other than arginine and agmatine were detected under the conditions specified in Table 1.

In *E. coli*, polyamines act as feedback inhibitors of arginine decarboxylase (Wu & Morris, 1973); in *Pseudomonas*, on the other hand, they appeared to be positive effectors of the decarboxylase activity. It is interesting that polyamines are negative effectors of the catabolic ornithine carbamoyltransferase of *P. putida*, which is an allosteric protein (Stalon, 1972) involved in the arginine deiminase pathway.

Maximum induction of arginine decarboxylase occurred with arginine as the sole nitrogen and carbon source. Activity was decreased in the presence of succinate. Ammonia had no effect on the induced synthesis of the decarboxylase, whether or not succinate was present. Agmatine appeared to repress arginine decarboxylase synthesis. Maximum induction of agmatine deiminase and *N*-carbamoylputrescine hydrolase occurred with agmatine as the sole carbon and nitrogen source. In addition to carbon catabolite repression, nitrogen repression also occurred when glutamine or ammonia were added to a medium containing agmatine and succinate. These results suggest that there are at least two regulatory units in the decarboxylase pathway. The first unit is concerned with arginine decarboxylase and the second involves agmatine deiminase and *N*-carbamoylputrescine hydrolase. Our nutritional studies suggest that several systems of catabolite repression operate in the arginine decarboxylase pathway. Whether glutamine synthetase is involved in nitrogen repression (Nyberg & Clarke, 1978; Potts & Clarke, 1976) remains to be determined.

The fate of putrescine in *P. aeruginosa* was not studied here. We assume that most of this compound is catabolized to succinate (Voellmy & Leisinger, 1976). It is probable that some putrescine derived from arginine is also used for spermidine synthesis (Fig. 2). Putrescine can also be formed by ornithine decarboxylase. If the specific enzyme activities measured *in vitro* (Table 2) reflect the situation *in vivo*, we may assume that during growth on glutamate or succinate the polyamines are synthesized mainly via the ornithine decarboxylase reaction; during growth on arginine the arginine decarboxylase is expected to prevail in polyamine synthesis.

Pseudomonads are known to possess a great metabolic potential; often there are multiple pathways for the catabolism of one substrate. Three lysine catabolic pathways are known in *P. aeruginosa* (Fothergill & Guest, 1977). The example of arginine catabolism shows that complex regulatory systems control the biosynthesis (Haas & Leisinger, 1974) and degradation of a single amino acid.

We thank J. P. ten Have for his skilful assistance in performing the guanidino compounds analysis and E. Rinderknecht for doing preliminary experiments. This work was supported by the Belgian Fonds de la Recherche Fondamentale Collective (grants no. 2.4542.75 and 2.4529.79). D.H. was supported by the Swiss National Foundation for Scientific Research (grant no. 3.204-0.77) and V.S. is chercheur qualifié at the Fonds National de la Recherche Scientifique. A.M. was 'boursière' of the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture.

REFERENCES

- ARCHIBALD, R. M. (1944). Determination of citrulline and allantoin and demonstration of citrulline in blood plasma. *Journal of Biological Chemistry* **156**, 121-141.
- BAUCHOP, T. & ELSDEN, S. R. (1960). The growth of microorganisms in relation to their energy status. *Journal of General Microbiology* **23**, 457-460.
- BROMAN, K., STALON, V. & WIAME, J. M. (1975). The duplication of arginine catabolism and the meaning of the two ornithine carbamoyltransferases in *Bacillus licheniformis*. *Biochemical and Biophysical Research Communications* **66**, 821-827.
- BROMAN, K., LAUWERS, N., STALON, V. & WIAME, J. M. (1978). Oxygen and nitrate utilization by *Bacillus licheniformis* of the arginase and arginine deiminase routes of arginine catabolism and other factors affecting their synthesis. *Journal of Bacteriology* **135**, 920-927.
- FOTHERGILL, J. C. & GUEST, J. R. (1977). Catabolism of L-lysine by *Pseudomonas aeruginosa*. *Journal of General Microbiology* **99**, 139-155.
- GEHRKE, C. W., KUO, K. C., ELLIS, R. L. & WAAL-

- KES, T. P. (1977). Polyamines – an improved automated ion-exchange method. *Journal of Chromatography* **143**, 345–361.
- HAAS, D. & LEISINGER, TH. (1974). Multiple control of *N*-acetylglutamate synthetase from *Pseudomonas aeruginosa*: synergistic inhibition by acetylglutamate and polyamines. *Biochemical and Biophysical Research Communications* **60**, 42–47.
- HAAS, D., EVANS, R., MERCENIER, A., SIMON, J. P. & STALON, V. (1979). Genetic and physiological characterization of *Pseudomonas aeruginosa* mutants affected in the catabolic ornithine carbamoyltransferase. *Journal of Bacteriology* **139**, 713–720.
- HALLEUX, P., LEGRAIN, C., STALON, V., PIÉRARD, A. & WIAME, J. M. (1972). Regulation of the catabolic ornithine carbamoyltransferase of *Pseudomonas fluorescens*. A study of the quaternary structure. *European Journal of Biochemistry* **31**, 386–393.
- KAY, W. W. & GRONLUND, A. F. (1969). Amino acid pool formation in *Pseudomonas aeruginosa*. *Journal of Bacteriology* **97**, 282–291.
- LEGRAIN, C. & STALON, V. (1976). Ornithine carbamoyltransferase from *E. coli* W: purification, structure and steady-state kinetic analysis. *European Journal of Biochemistry* **63**, 289–301.
- LEISINGER, TH., HAAS, D. & HEGARTY, M. P. (1972). Indospicine as an arginine antagonist in *Escherichia coli* and *Pseudomonas aeruginosa*. *Biochimica et biophysica acta* **262**, 214–219.
- MERCENIER, A., SIMON, J. P. & STALON, V. (1978). Le catabolisme de l'arginine chez *Pseudomonas aeruginosa*. *Archives internationales de physiologie et de biochimie* **86**, 919–920.
- MILLER, D. L. & RODWELL, V. (1971). Metabolism of basic amino acids in *Pseudomonas putida*. Intermediates in L-arginine catabolism. *Journal of Biological Chemistry* **246**, 5053–5058.
- MITRUKA, B. M. & COSTILOW, R. N. (1967). Arginine catabolism by *Clostridium botulinum*. *Journal of Bacteriology* **93**, 295–301.
- MOORE, S., SPACKMAN, D. H. & STEIN, W. H. (1958). Chromatography of amino acids on sulfonate polystyrene resins. *Analytical Chemistry* **30**, 1185–1206.
- MORRIS, D. R. & PARDEE, A. J. (1966). Multiple pathways of putrescine biosynthesis in *Escherichia coli*. *Journal of Biological Chemistry* **241**, 3129–3135.
- NYBERG, K. & CLARKE, P. H. (1978). Glutamine synthetase activities of cultures of *Pseudomonas aeruginosa* grown in minimal media with histidine, nitrate or ammonium sulphate as nitrogen source. *Journal of General Microbiology* **107**, 193–197.
- POTTS, J. R. & CLARKE, P. H. (1976). The effect of nitrogen limitation on catabolite repression of amidase, histidase and urocanase in *Pseudomonas aeruginosa*. *Journal of General Microbiology* **93**, 377–387.
- RAMOS, F., THURIAUX, P., WIAME, J. M. & BÉCHET, J. (1970). The participation of ornithine and citrulline in the regulation of arginine metabolism in *S. cerevisiae*. *European Journal of Biochemistry* **12**, 40–47.
- ROON, R. J. & BARKER, H. A. (1972). Fermentation of agmatine in *Streptococcus faecalis*: occurrence of putrescine transcarbamylase. *Journal of Bacteriology* **109**, 44–50.
- ROSENFELD, M. J. & ROBERTS, S. (1976). Arginine decarboxylase from *Pseudomonas* species. *Journal of Bacteriology* **125**, 601–607.
- SLADE, H. D., DOUGHTY, C. C. & SLAMP, W. C. (1954). The synthesis of high energy phosphate in the citrulline ureidase reaction by soluble enzyme of *Pseudomonas*. *Archives of Biochemistry and Biophysics* **48**, 333–336.
- SMITH, T. A. (1965). *N*-Carbamylputrescine amidohydrolase of higher plants and its relation to potassium nutrition. *Phytochemistry* **4**, 599–607.
- SMITH, T. A. & GARRAWAY, J. L. (1964). *N*-Carbamylputrescine – an intermediate in the formation of putrescine by barley. *Phytochemistry* **3**, 23–26.
- STALON, V. (1972). Regulation of the catabolic ornithine carbamoyltransferase of *Pseudomonas fluorescens*. A study of the allosteric interactions. *European Journal of Biochemistry* **29**, 36–46.
- STALON, V., RAMOS, F., PIÉRARD, A. & WIAME, J. M. (1967). The occurrence of a catabolic and an anabolic ornithine carbamoyltransferase in *Pseudomonas*. *Biochimica et biophysica acta* **139**, 91–97.
- STALON, V., RAMOS, F., PIÉRARD, A. & WIAME, J. M. (1972). Regulation of the catabolic ornithine carbamoyltransferase of *Pseudomonas fluorescens*. A comparison with the anabolic transferase and with a mutationally modified catabolic transferase. *European Journal of Biochemistry* **29**, 25–35.
- THORNLEY, M. J. (1960). The differentiation of *Pseudomonas* from other gram-negative bacteria on the basis of arginine metabolism. *Journal of Applied Bacteriology* **23**, 37–52.
- VANDERBILT, A. S., GABY, N. S. & RODWELL, V. (1975). Intermediates and enzyme between α -ketoarginine and guanidinobutyrate in the L-arginine catabolic pathway of *Pseudomonas putida*. *Journal of Biological Chemistry* **250**, 5322–5329.
- VOELLMY, R. & LEISINGER, TH. (1976). Role of 4-aminobutyrate aminotransferase in the arginine metabolism of *Pseudomonas aeruginosa*. *Journal of Bacteriology* **128**, 722–729.
- VOELLMY, R. & LEISINGER, TH. (1978). Regulation of enzyme synthesis in the arginine biosynthetic pathway of *Pseudomonas aeruginosa*. *Journal of General Microbiology* **109**, 25–35.
- WU, W. H. & MORRIS, D. R. (1973). Biosynthetic arginine decarboxylase from *E. coli*. Purification and properties. *Journal of Biological Chemistry* **248**, 1687–1695.