Catabolism of L-Lysine by *Pseudomonas aeruginosa*

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SUMMARY

Pseudomonas aeruginosa PAC1 grows poorly on L-lysine as sole source of carbon but mutant derivatives which grow rapidly were readily isolated. Studies with one such mutant, *P. aeruginosa* PAC586, supported the existence of a route for L-lysine catabolism which differs from those reported previously in other species of *Pseudomonas*. The postulated route, the cadaverine or decarboxylase pathway, is initiated by the decarboxylation of L-lysine and involves the following steps: L-lysine → cadaverine → 1-piperidine → 5-aminovalerate → glutarate semialdehyde → glutarate. Evidence for this pathway is based on the characterization of the pathway reactions and the induction of the corresponding enzymes by growth on L-lysine. The first three enzymes were also induced by growth on cadaverine and to a lesser extent by 5-aminovalerate. No evidence was obtained for the presence of pathways involving L-lysine 2-monooxygenase or L-pipecolate dehydrogenase, but another potential route for L-lysine catabolism initiated by L-lysine 6-aminotransferase was detected. Studies with mutants unable to grow on L-lysine supported the existence of more than one catabolic pathway for L-lysine in this organism and indicated that all routes converge on a pathway for glutarate catabolism which generates acetyl-CoA. Pipecolate catabolism also appeared to converge on the glutarate pathway in *P. aeruginosa*. The results suggested that the growth rate of the parental strain is limited by the rate of transport and/or decarboxylation of L-lysine. The cadaverine pathway was present, but not so highly induced, in the parental strain *P. aeruginosa* PAC1. *Pseudomonas fluorescens* contained enzymes of both the cadaverine (decarboxylase) and oxygenase pathways, strains of *P. putida* (biotypes A and B) contained enzymes of the oxygenase pathway but not the decarboxylase pathway and *P. multivorans* appeared deficient in both. All these species possessed L-lysine aminotransferase activity.

INTRODUCTION

Three pathways for lysine catabolism by aerobic bacteria are outlined in Fig. 1. These are the 5-aminovalerate (or oxygenase) pathway, the pipecolate pathway and a transaminase pathway.

Evidence for the 5-aminovalerate pathway is based on the characterization of the individual enzyme-catalysed reactions in *Pseudomonas fluorescens* and *Pseudomonas putida* and also the induction of some of the enzymes by lysine (Reitz & Rodwell, 1970; Vandecasteele & Hermann, 1972). Likewise, the pipecolate pathway, containing cyclic intermediates, was defined initially by Rodwell and coworkers in *P. putida* P2, a strain isolated on pipecolate. The relevance of pipecolate to lysine catabolism was indicated by the finding that pipecolate-grown organisms were induced for lysine degradation and that L-lysine induced the synthesis of pipecolate dehydrogenase (Baginsky & Rodwell, 1966) and 1-piperidine-2-carboxylate reductase (Chang & Adams, 1974). Although the two pathways are connected by lysine racemase, it is now clear from isotopic labelling studies and by inhibition of the

Fig. 1. Scheme showing the initial steps in lysine catabolism by aerobic bacteria. The enzymes and corresponding references are: (1) lysine racemase (EC. 5.1.1.5), Ichihara, Furiya & Suda (1960); (2) L-lysine 2-monooxygenase (EC. 1.13.12.2), Takeda *et al.* (1969); (3) 5-aminovaleramide amidase (EC. 3.5.1.-), Reitz & Rodwell (1970); (4) 5-aminovalerate aminotransferase (EC. 2.6.1.-), Ichihara, Ichihara & Suda (1960); (5) glutarate-semialdehyde dehydrogenase (EC. 1.2.1.20), Ichihara & Ichihara (1961); (6) postulated reaction; (7) 1-piperideine-2-carboxylate reductase (EC. 1.5.1.-), Chang & Adams (1974); (8) L-pipecolate dehydrogenase (EC. 1.5.99.3), Baginsky & Rodwell (1966); (9) 1-piperideine-6-carboxylate dehydrogenase (EC. 1.5.1.-), Calvert & Rodwell (1966); (10) L-2-aminoadipate aminotransferase (EC. 2.6.1.39), Hartline & Rodwell (1971); (11) L-lysine 6-aminotransferase (EC. 2.6.1.36), Soda, Misono & Yamamoto (1968). Reactions 2 to 5 represent the 5-aminovalerate (or oxygenase) pathway, reactions 6 to 10 represent the pipecolate pathway and reaction 11 may be considered as a third route, the transaminase pathway. The cyclic compounds 1-piperideine-2-carboxylate and 1-piperideine-6-carboxylate are the dehydrated cyclic forms of 2-oxo-6-aminocaproic acid and 2-aminoadipate 5-semialdehyde, respectively.

racemase that degradation of L-lysine is primarily via 5-aminovalerate and degradation of D-lysine occurs via pipicolate, in *P. putida* P2 (Miller & Rodwell, 1971*a*). Furthermore, in another strain of *P. putida* biotype A (ATCC15070), studies with mutants blocked in the 5-aminovalerate pathway showed this route to be obligatory for growth on L-lysine (Chang & Adams, 1974). Growth of the mutants on D-lysine was unaffected, and it was concluded that although there is sufficient racemase activity to permit cross-induction of the pipicolate pathway by L-lysine (and the 5-aminovalerate pathway by D-lysine) it fails to support growth.

Transamination of L-lysine to form 1-piperidine-6-carboxylate has been reported in L-lysine-grown *Achromobacter liquidum* and *Flavobacterium* species (Soda & Misono, 1968; Soda, Misono & Yamamoto, 1968) and this provides a third route for lysine catabolism.

Glutarate, the product of the 5-aminovalerate pathway, is probably metabolized to acetyl-CoA via glutaryl-CoA, glutaconyl-CoA, 3-hydroxybutyryl-CoA and acetoacetyl-CoA, as described in *P. fluorescens* (Numa *et al.*, 1964). In contrast, the product of the other routes, 2-oxoadipate, appears to be metabolized via 2-hydroxyglutarate to 2-oxoglutarate and glutamate (Reitz & Rodwell, 1969; Hartline & Rodwell, 1971). It is not clear whether these compounds are of necessity the ultimate products because decarboxylation of any of the C₆ intermediates would yield products which would converge on glutarate.

Studies with *Pseudomonas aeruginosa*, originally intended to investigate the biochemical control and genetic organization of lysine catabolism and other converging pathways, have provided evidence for another pathway of L-lysine degradation. This pathway, the cadaverine or decarboxylase pathway, is initiated by decarboxylation and provides an alternative route to 5-aminovalerate and eventually glutarate.

METHODS

Bacterial strains. *Pseudomonas aeruginosa* PAC1 (NCIB10848) was a gift from Professor P. H. Clarke as was the isocitrate lyase mutant, PAC501, originally designated At1 (Skinner & Clarke, 1968). Mutant PAC586 was derived from PAC1 by selection for faster growth with L-lysine as sole source of carbon. Other cultures and their sources were: *P. aeruginosa* PAOI (ATCC17503), Dr M. B. Kemp; *P. putida* biotype B (ATCC17472), Professor R. Y. Stanier; *P. fluorescens* (ATCC11250), *P. putida* biotype A (ATCC12633) and *P. multivorans* (ATCC17759), the American Type Culture Collection (Stanier, Palleroni & Doudoroff, 1966). Stock cultures of *P. aeruginosa* PAC586 were maintained on plates of L-lysine minimal medium subcultured at monthly intervals, incubated for 30 h and stored at 2 °C. Other stocks were maintained on plates of nutrient agar and subcultured and stored in the same way during routine use. Stocks of each culture were also maintained on slopes of nutrient agar at 2 °C under paraffin for long-term storage. Strains of *P. aeruginosa* were cultured at 37 °C, but 30 °C was used for all other species.

Isolation of mutants. Spontaneous mutants (Lut⁺) of PAOI and PAC1 capable of faster growth on L-lysine as sole carbon source were isolated by direct selection of the larger colonies which appeared after plates of L-lysine minimal medium were spread with 10⁹ organisms. They were also selected by repeated subculture in lysine minimal medium. Five subcultures comprising a total of approximately 45 generations were used although no increase in growth rate was detected after 25 generations.

Mutants of *P. aeruginosa* PAC586 able to grow with succinate but not L-lysine were selected by treating exponential nutrient broth cultures with 1-methyl-3-nitro-1-nitrosoguanidine (100 µg ml⁻¹) for 40 min at 37 °C in 0.1 M-sodium citrate buffer pH 5.4. This was followed

by three cycles of mutant enrichment consisting of expression in succinate minimal medium and exposure of early-exponential phase cultures in lysine minimal medium to antibiotic for 18 h. The antibiotic concentrations were: 1st cycle, carbenicillin ($200\ \mu\text{g ml}^{-1}$); 2nd cycle, carbenicillin ($500\ \mu\text{g ml}^{-1}$); 3rd cycle, carbenicillin ($500\ \mu\text{g ml}^{-1}$) plus D-cycloserine ($100\ \mu\text{g ml}^{-1}$). After each cycle, survivors were collected by centrifuging, resuspended in phosphate buffer (0.05 M, pH 7.0) and plated on succinate minimal medium. Mutants were then detected by replica-plating to lysine minimal medium; approximately 17% and 90% of the survivors had the desired phenotype after the second and third cycles, respectively.

Media. The nutrient broth used routinely contained (g l^{-1}): Bacto nutrient broth (Difco), 8; Bacto yeast extract (Difco), 5; and NaCl, 8.5. The nutrient agar contained (g l^{-1}): Bacto yeast extract (Difco), 10; and Ionagar no. 2 (Oxoid), 10.

The basal minimal medium contained (l^{-1}): $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 6.0 g; K_2HPO_4 , 10.7 g; $(\text{NH}_4)_2\text{SO}_4$, 1.0 g; and 5 ml trace element solution. The trace element solution contained (l^{-1}): $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 82 mg; H_3BO_3 , 232 mg; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 96 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 8 mg; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 8 mg; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 30 mg; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 174 mg; and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g. Carbon sources were neutralized, sterilized and added to the sterilized basal medium to give final concentrations of 20 mM (or 40 mM for succinate and acetate). Solid media contained Ionagar no. 2 (Oxoid) at $12\ \text{g l}^{-1}$.

Growth of cultures. Cultures were grown aerobically at 30 or 37 °C as 500 ml batches in 2 l Erlenmeyer flasks on a rotary shaker. Larger batches of 1 l were grown in a Microferm Fermentor (New Brunswick Scientific Co.). Cultures were harvested in the exponential phase and washed at least twice using a total volume of 0.05 M-phosphate buffer pH 8.0 equal to that of the original culture.

Manometric experiments. Oxygen uptake was measured at 37 °C by conventional Warburg manometric techniques. Washed cultures were resuspended in 0.05 M-phosphate buffer pH 7.0 and samples containing 3 mg dry wt organisms and 95 μmol phosphate in 2.3 ml were added to the flasks. The centre wells contained 0.1 ml KOH ($400\ \text{g l}^{-1}$) and substrates (6 μmol in 0.6 ml) were tipped from the side-arms. Gas uptakes were calculated as $\mu\text{l O}_2$ absorbed ($\text{mg dry wt})^{-1}\ \text{h}^{-1}$ and corrected for the endogenous values obtained without added substrate.

Preparation of extracts and enzymology

Ultrasonic extracts were prepared by suspending 1.5 g wet wt of bacterial paste in 5 ml 0.05 M-phosphate buffer pH 8.0 and treating the suspensions for two 90 s periods in an ultrasonic cell disintegrator (M.S.E., 100 W). The suspensions were clarified by centrifuging at $15000\ g$ for 15 min at 2 °C. The supernatant fluids were used as crude extracts and their protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Soluble and particulate fractions were prepared by recentrifuging the crude extracts at $105000\ g$ for 2.5 h at 2 °C. The pellet obtained was resuspended in phosphate buffer to the original extract volume to give the particulate fraction and the supernatant fluid was used as the soluble fraction. The protein concentrations of the two fractions were the same, i.e. equal to half the concentration of the crude extract. Enzyme specific activities are based on the protein concentrations of the extracts or fractions used in specific assays and expressed as μmol of substrate transformed or product formed ($\text{mg protein})^{-1}\ \text{h}^{-1}$ at the temperature stated. Enzymes were assayed at their pH optima and in the region of proportionality between initial reaction velocity and protein concentration, and Michaelis constants were determined by double reciprocal plots (Lineweaver & Burk, 1934).

L-Lysine decarboxylase [EC. 4.1.1.18; L-lysine carboxy-lyase] was assayed in crude extracts by following the rate of release of labelled CO_2 from L-[U- ^{14}C]lysine. The reaction mixtures contained (μmol in 0.9 ml final volume): pyridoxal 5'-phosphate, 0.05; 2-mercaptoethanol, 10; sodium potassium phosphate buffer pH 6.8, 100; and crude extract equivalent to 1 to 2 mg protein. Mixtures were pre-incubated for 3 min at 37 °C. After cooling to 0 °C, 12 cm^2 glass filter paper wicks impregnated with 0.2 ml hyamine hydroxide (1 M in methanol; scintillation grade; Nuclear Enterprises, Beenham, Berkshire) were suspended above the mixtures. Substrate, 1.05 μCi L-[U- ^{14}C]lysine (20 μmol in 0.1 ml), was added, the tubes were sealed immediately and the reactions were started by warming to 37 °C. Reactions were stopped by injecting 0.5 ml 5 M-HCl through the gas-proof seal. After allowing 30 min for CO_2 absorption, the wicks were transferred to vials containing 7.5 ml NE213 scintillant (Nuclear Enterprises) and counted in Nuclear Chicago 6801 or Isocap 300 scintillation counters. Quench corrections were made by the channels ratio technique and the counts (d.p.m.) were related to the specific activity of L-lysine decarboxylase. Anaerobic assays were performed under N_2 (O_2 -free) in sealed tubes and lysine was added by syringe. Reactions stopped at zero time and controls containing heated extract or lacking lysine yielded no labelled CO_2 . A detailed kinetic study showed that the reaction quickly reached a constant rate which was maintained for over 30 min. The early lag was equivalent to 1 min and the routine incubation (16 min) corresponded to 15 min at the rate recorded.

L-Lysine 6-aminotransferase [EC. 2.6.1.36; L-lysine:2-oxoglutarate 6-aminotransferase] and *cadaverine aminotransferase* [EC. 2.6.1.29; diamine:2-oxoglutarate aminotransferase] were assayed by the same method based on those of Soda *et al.* (1968) and Kim (1964). The reaction mixtures contained (μmol in 2 ml final volume): 2-oxoglutarate, 25; *o*-aminobenzaldehyde, 5; sodium carbonate buffer pH 10.25, 250; pyridoxal 5'-phosphate, 0.25; and crude extract equivalent to 1.5 mg protein. The reaction was started with L-lysine (100 μmol) or cadaverine (300 μmol). After incubation at 37 °C for 8 min, the reaction was stopped by adding 0.5 ml trichloroacetic acid (200 g l^{-1}). Precipitated protein was removed by filtration and the extinctions of the dehydroquinazolinium derivatives were measured at 465 nm after 90 min (with lysine) or 60 min (with cadaverine); controls lacking the oxo-acid were included. Specific activities were calculated using $\epsilon_{465} = 2800 \text{ l mol}^{-1} \text{ cm}^{-1}$ for the chromogens obtained with the lysine and cadaverine products. This extinction coefficient was determined by relating the disappearance of 1-piperidine to NADH formation in the 1-piperidine dehydrogenase reaction. With other amino donors, the amounts added to the reaction mixtures were 50 μmol L-ornithine and 20 μmol putrescine, and the extinctions were measured after 90 min at 440 nm and 430 nm respectively, using $\epsilon = 1860 \text{ l mol}^{-1} \text{ cm}^{-1}$ (Holmstedt, Larsson & Tham, 1961).

1-Piperidine dehydrogenase [EC. 1.5.1.-; 1-piperidine:NAD $^{+}$ oxidoreductase] was assayed by a method analogous to that used for 1-pyrroline (4-aminobutyraldehyde) dehydrogenase (Jacoby & Fredericks, 1959). The reaction mixture contained (μmol in 3 ml final volume): NAD, 2; 1-piperidine, 2.5; 2-mercaptoethanol, 15; Tris/HCl buffer pH 9.0, 150; and 0.35 mg protein from the soluble fraction of crude extract. The reaction was started with 1-piperidine and the increase in extinction at 340 nm was recorded at 25 °C against a control without 1-piperidine.

L-2-Aminoadipate aminotransferase [EC. 2.6.1.39; L-2-aminoadipate:2-oxoglutarate aminotransferase] and *5-aminovalerate aminotransferase* [EC. 2.6.1.-; 5-aminovalerate:2-oxoglutarate aminotransferase] were assayed in crude extracts at 37 °C by a discontinuous method modified from Umbarger & Umbarger (1962). This is based on estimating the rate

of L-glutamate formation. The reaction mixtures contained (μ mol in 1 ml final volume): amino acid, 20; 2-oxoglutarate, 20; pyridoxal 5'-phosphate, 0.08; and sodium potassium phosphate buffer pH 7.5, 25. After pre-incubating the reagents at 37 °C for 1 min the reaction was started by adding the crude extract (1.5 mg protein). Incubation was for 5 min and the reaction was stopped by heating at 100 °C for 3 min. Precipitated protein was removed by centrifuging and the glutamate in the supernatant fluid was assayed with glutamate dehydrogenase and 3-acetylNAD according to Wyngaarden & Ashton (1959).

Glutarate-semialdehyde dehydrogenase [EC. 1.2.1.20; glutarate-semialdehyde:NADP⁺ oxidoreductase] was assayed in the soluble fraction of crude extracts at 25 °C using Tris/HCl buffer pH 8.5 and NADP by the method of Chang & Adams (1971), except that 1 mg bovine serum albumin was added to the reaction mixtures.

L-Pipecolate dehydrogenase [EC. 1.5.99.3; L-pipecolate:(acceptor) oxidoreductase] was assayed at 25 °C by the method of Rodwell (1971) using 2,6-dichlorophenolindophenol as electron acceptor. This activity was normally found in the particulate fraction but in *P. multivorans* it was present in the soluble fraction of the ultrasonic extracts.

L-Lysine 2-monooxygenase [EC. 1.13.12.2; L-lysine:oxygen 2-oxidoreductase (decarboxylating)] was assayed in crude extracts at 34 °C using a Beckman Oxygen Analyser (model 777) and the method of Takeda *et al.* (1969).

Isocitrate lyase [EC. 4.1.3.1; *threo*-D₅-isocitrate glyoxylate-lyase] was assayed in crude extracts at pH 6.8 according to the method of Dixon & Kornberg (1959), except that ethylenediaminetetraacetate was substituted for cysteine as suggested by Kennedy & Dilworth (1963).

Paper chromatography and high voltage electrophoresis. The reaction products of L-lysine, L-[U-¹⁴C]lysine and DL-[1-¹⁴C]lysine, and cadaverine metabolism were investigated by chromatography on Whatman no. 1 paper with solvents: A, t-butanol/formic acid (90 %)/H₂O (70:15:15, by vol.); and B, methanol/H₂O/HCl (35 %)/pyridine (160:36:4:3:20, by vol.). High voltage electrophoresis was carried out on Whatman 3MM paper using a Savant flat-plate instrument. The buffers used were: H₂O/pyridine/acetic acid, pH 6.4 (2792:200:8, by vol.) and 1 M-formic acid pH 1.9; a potential of 100 V cm⁻¹ was applied for 30 and 40 min respectively. Compounds were detected by spraying with ninhydrin [0.25 % (w/v) in acetone] or *o*-aminobenzaldehyde [0.1 % (w/v) in acetone]. Radioactive compounds were detected (before spraying) with an Actigraph III radiochromatogram scanner (Nuclear Chicago) or by autoradiography. Compounds were identified by comparison with the behaviour of authentic samples under several different conditions.

Materials. DL-Pipecolic acid was prepared by catalytic hydrogenation of α -picolinic acid hydrochloride (Rodwell, 1971) and purified as the hydrochloride by repeated recrystallization from methanol by the addition of acetone. The product was judged pure by melting point, infrared and n.m.r. spectra, and quantitative amino-acid analysis. 1-Piperidine and 1-pyrroline were prepared from L-lysine and L-ornithine by the method of Jacoby & Fredricks (1959). Glutarate semialdehyde was prepared from DL-2-aminoadipic acid by the method of Chang & Adams (1971). Glutamate dehydrogenase and 3-acetylNAD were from Boehringer, and all the other reagents and chemicals were of the highest purity obtainable commercially.

Fig. 2. Respiration of different substrates by washed suspensions of L-lysine-grown *Pseudomonas aeruginosa* PAC586. Exponential phase cultures were washed and bacteria equivalent to 3 mg dry wt were incubated at 37 °C in 0.05 M-phosphate buffer (95 μmol, pH 7.0) and substrate (6 μmol) was tipped from the side-arm to give a final volume of 2.9 ml. CO₂ was absorbed by 0.1 ml of KOH (400 g l⁻¹) in the centre well. Oxygen consumption was measured manometrically in an atmosphere of air and the results were corrected for endogenous respiration, typically 13 μl O₂ (mg dry wt)⁻¹ h⁻¹. The substrates were: ○, L-lysine; □, cadaverine; ■, 5-aminovalerate; △, glutarate; ▲, acetate; ●, D-lysine; ◇, DL-pipecolate; ◆, DL-2-amino adipate.

RESULTS

Studies with whole organisms

In preliminary experiments 17 out of 23 different strains of *Pseudomonas aeruginosa* were found capable of using L-lysine as sole carbon and energy source. These included *P. aeruginosa* PAOI and *P. aeruginosa* PACI. Their specific growth rates on L-lysine minimal medium were always low ($\mu \leq 0.035$ h⁻¹) compared with those observed for strains of *P. putida*, *P. fluorescens* and *P. multivorans* ($\mu = 0.35$ to 0.09 h⁻¹). However, stable faster-growing derivatives were readily obtained either by repeated subculture in L-lysine minimal medium or by direct selection of the larger colonies which appeared when plates of solid medium were spread with 10⁹ organisms. *Pseudomonas aeruginosa* PAC586 ($\mu = 0.14$ h⁻¹) was obtained from the parental strain PACI ($\mu = 0.03$ h⁻¹) by the latter method and was used for studying L-lysine catabolism in *P. aeruginosa*. Both strains (PACI and PAC586) grew at the same specific growth rates (h⁻¹) on cadaverine (0.14), 5-aminovalerate (0.3), glutarate (0.4), DL-pipecolate (0.9), acetate (0.4), succinate (0.4) and putrescine (0.4), but no growth could be detected on 2-amino adipate. Mutant PAC586 readily oxidized L-lysine, cadaverine, glutarate and acetate after growth on L-lysine (Fig. 2). 5-Aminovalerate was oxidized at an accelerating rate but it was still oxidized at a significant linear rate in the presence of chloramphenicol (50 μg ml⁻¹). In contrast, 2-amino adipate was not oxidized and D-lysine and DL-pipecolate were only oxidized after considerable lag periods (Fig. 2). The Q_{O_2} for L-lysine varied from 200 to 350 μl O₂ (mg dry wt organisms)⁻¹ h⁻¹ in different experiments and the net uptake of O₂ for each substrate followed to completion was between 60 and 70 % of the theoretical amount calculated for total combustion to CO₂. Very similar results

were obtained with the parental strain PAC1 and they suggest that L-lysine is degraded via 5-aminovalerate rather than via pipecolate and 2-aminoadipate.

Using the same substrates, comparable studies with L-lysine-grown cultures of *P. fluorescens*, *P. putida* (biotypes A and B) and *P. multivorans* gave patterns of oxidation which differed from each other and from that observed with *P. aeruginosa*. One of the most striking differences between *P. aeruginosa* and all the other species, except *P. fluorescens*, was its ability to oxidize cadaverine without lag. Furthermore, washed suspensions of cadaverine-grown PAC586 gave the same oxidation pattern as L-lysine-grown bacteria, suggesting either that L-lysine is metabolized via cadaverine or that metabolic routes for both substrates converge on the same intermediates. With 5-aminovalerate as the growth substrate, glutarate, 5-aminovalerate, L-lysine and cadaverine were all oxidized without appreciable lag and, although the oxidation rates for glutarate and 5-aminovalerate were increased up to twofold, the rates for L-lysine and cadaverine were only one-third of those observed with L-lysine or cadaverine as growth substrates. Growth on DL-pipecolate permitted immediate and rapid oxidation of D-lysine, DL-pipecolate and 5-aminovalerate; the oxidation of cadaverine and glutarate was normal after a delay of 20 min but L-lysine oxidation proceeded at a uniformly low rate from the time of addition. With glutarate, acetate or succinate as the growth substrate, fairly long periods (60 to 100 min) of adaptation preceded the oxidation of L-lysine, DL-pipecolate, cadaverine and 5-aminovalerate by mutant PAC586.

Further studies with washed suspensions of L-lysine-grown *P. aeruginosa* PAC586 confirmed the importance of cadaverine in L-lysine catabolism. Suspensions of organisms (1 mg dry wt ml⁻¹) were incubated aerobically at 37 °C with L-lysine (20 mM) in phosphate buffer (10 mM; pH 7.0) and samples were withdrawn at 20 min intervals over 3 h. Chromatographic (solvents A and B) and electrophoretic (pH 6.4) analyses of the supernatant fluids showed that as the lysine disappeared the major ninhydrin-positive material to accumulate was cadaverine, which was itself degraded on further incubation. Other minor components were detected and three of these were tentatively identified as 1-piperidine, 5-aminovalerate and glutamate. In similar experiments using L-[U-¹⁴C]lysine, [¹⁴C]cadaverine and five other labelled products were detected. With DL-[1-¹⁴C]lysine as substrate, no labelled cadaverine was formed (although the presence of cadaverine was confirmed with ninhydrin); three labelled products were detected but two of these were presumed to be specific metabolites of D-lysine because they differed from those formed with L-lysine.

Enzymology of L-lysine catabolism in P. aeruginosa

Extracts of L-lysine-grown *P. aeruginosa* PAC586 were examined for enzyme activities which could be responsible for the catabolism of L-lysine. No L-lysine oxygenase could be detected, nor could significant oxidase or dehydrogenase activities for L-lysine (or DL-pipecolate) be found, using a variety of conditions and electron acceptors. L-Lysine aminotransferase activity was detected but L-lysine decarboxylase was the most active enzyme using L-lysine as substrate (Table 1, column 1). Further metabolism of the decarboxylation product, cadaverine, did not appear to involve an amine or diamine oxidase, but an aminotransferase which converted cadaverine to 1-piperidine (5-aminoglutaraldehyde) was present. Further investigations revealed the presence of a NAD-dependent dehydrogenase which oxidized 1-piperidine to 5-aminovalerate. This product could then be converted to glutarate in two steps by enzymes of the 5-aminovalerate (or oxygenase) pathway, 5-aminovalerate aminotransferase and the NADP-dependent glutarate semialdehyde dehydrogenase, which were present at high specific activity (Table 1, column 1). Glutaryl-CoA synthase activity could not be detected but it was assumed from the relatively high specific

Table 1. *Enzyme activities detected in ultrasonic extracts of Pseudomonas aeruginosa* PAC586 after growth on L-lysine and other substrates

Cultures were grown with different substrates as sole carbon and energy sources and enzymes assayed as described in Methods. Specific activities are recorded as μmol substrate transformed $(\text{mg protein})^{-1} \text{ h}^{-1}$.

Enzyme	Specific activity after growth on:									
	L-Lysine	Cadaverine	Putrescine	5-Aminovalerate	Glutarate	Acetate	DL-Pipecolate	L-Glutamate	Succinate	Induction (Lysine/Succinate)
L-Lysine decarboxylase	7.5	9.1	0.1	3.3	0.5	0.3	7.5	0.2	0.1	75
Cadaverine aminotransferase	3.5	3.2	5.2	3.2	2.1	0.6	1.0	1.0	0.3	12
1-Piperidine dehydrogenase	10.0	9.1	14.6	6.9	5.1	1.1	3.6	1.2	1.3	8
5-Aminovalerate aminotransferase	7.5	7.7	6.8	7.9	6.1	1.1	2.0	0.5	0.9	8
Glutarate-semialdehyde dehydrogenase	27.2	32.7	25.4	23.2	15.1	1.2	9.2	2.8	1.5	18
Isocitrate lyase	11.8	8.2	0.9	24.2	19.0	18.6	3.0	1.7	1.7	7
L-Lysine 6-aminotransferase	2.3	2.1	3.6	2.5	1.4	0.3	0.7	0.4	0.3	8
L-2-Aminoadipate aminotransferase	1.0	1.2	1.3	1.3	0.8	0.3	0.6	0.4	0.4	2.5

activity of isocitrate lyase that L-lysine was metabolized largely via acetyl-CoA. L-Pipecolate dehydrogenase, a key enzyme of the pipecolate pathway, was just detectable and L-2-amino-adipate aminotransferase was present but not at a high specific activity.

These results indicated that L-lysine may be degraded by a new pathway, the cadaverine pathway, which is initiated by decarboxylation of the amino acid and converges with the oxygenase pathway at 5-aminovalerate after the diamine has been transaminated to 1-piperidine (Fig. 3). It can be calculated that the specific activities are sufficient to support the growth of mutant PAC586 on L-lysine at the observed rate. Another route initiated by transamination may also be present. New routes involving racemization to D-lysine were not excluded.

Some of the relevant enzyme activities were studied in more detail.

L-Lysine decarboxylase. Lysine decarboxylation by crude extracts was entirely dependent on the presence of pyridoxal 5'-phosphate, and the addition of 2-mercaptoethanol further improved the rate of decarboxylation by a factor of 1.7. The optimum pH was 6.8 and activity declined to very low values at pH 6.0 and pH 9.0. The K_m for L-lysine was 10 mM and V_{max} was $9.8 \mu\text{mol} (\text{mg protein})^{-1} \text{ h}^{-1}$. The product of the reaction was identified as cadaverine by chromatographic and electrophoretic techniques and by extraction and identification of its 2,4-dinitrophenyl derivative from reaction mixtures.

L-Lysine and cadaverine aminotransferases. These aminotransferases were assayed in extracts of L-lysine-grown organisms with 2-oxoglutarate as the amino acceptor and *o*-aminobenzaldehyde present. The optimum pH was 9.8 with both substrates. Both activities declined steadily at higher pH values, the rates being half-maximum at pH 10.5, but at lower pH values the rates with cadaverine fell more steeply (half-maximum at pH 9.3) than the rates with lysine (half-maximum at pH 8.8). Very little lysine decarboxylase activity was detected above pH 9.0 so the aminotransferase activity observed with lysine is specific for lysine and not due to the combined activities of lysine decarboxylase and cadaverine aminotransferase. No activity could be detected when 2-oxoglutarate was omitted, except in the

Fig. 3. Scheme showing the proposed cadaverine (decarboxylase) pathway of L-lysine catabolism. The enzymes involved are: (12) L-lysine decarboxylase; (13) cadaverine aminotransferase; (14) 1-piperidine dehydrogenase. 1-Piperidine is in equilibrium with the hydrated open structure, 5-aminoglutaraldehyde.

pH range 7 to 9 where very weak lysine and cadaverine oxidative deaminations seemed to occur. The activities of crude extracts were increased by approximately 10 % after passing through small columns of either Sephadex G-25 or Dowex-1 (X8, 100 to 200 mesh; H^+ form). No requirement for pyridoxal 5'-phosphate could be detected but it was routinely added to the reaction mixtures.

2-Oxoglutarate was the most active amino acceptor tested ($K_m = 1 \text{ mM}$). Glyoxylate, pyruvate and oxaloacetate gave between 10 and 40 % of the activities obtained with 2-oxoglutarate under comparable conditions. Studies with a range of amino donors showed that, in addition to L-lysine ($K_m = 11 \text{ mM}$) and cadaverine ($K_m = 67 \text{ mM}$), much higher affinities for transamination by the crude extracts were found with putrescine ($K_m = 0.8 \text{ mM}$) and ornithine ($K_m = 1.7 \text{ mM}$). However, the values for $V_{\max}$ were very similar with all four substrates, ranging from 2 to 6 $\mu\text{mol (mg protein)}^{-1} \text{ h}^{-1}$ with 6 mM-2-oxoglutarate.

Transamination of lysine could involve the 2- or 6-amino groups yielding 1-piperidine-2-carboxylate or 1-piperidine-6-carboxylate respectively. These differ in several respects (Soda *et al.*, 1968). The 2-carboxylate reacts rapidly with *o*-aminobenzaldehyde to produce a chromogen with an extinction maximum at 450 nm. The 6-carboxylate reacts slowly with *o*-aminobenzaldehyde and the corresponding chromogen has an extinction maximum at 465 nm; it also reacts rapidly with ninhydrin in glacial acetic acid to give a stable yellow complex. The two compounds also exhibit different electrophoretic mobilities in 1 M-formic acid. Based on these criteria, the reaction product was shown to be 1-piperidine-6-carboxylate, i.e. the enzyme activity corresponds to L-lysine 6-aminotransferase. Deproteinized samples of reaction mixtures containing L-[U- ^{14}C]lysine or DL-[1- ^{14}C]lysine plus 2-oxoglutarate incubated with crude extract at pH 9.8 were also examined and electrophoretic analysis confirmed that the major radioactive product corresponded to 1-piperidine-6-carboxylate in both cases. The product of cadaverine transamination was judged to be 1-piperidine by comparing the properties of its *o*-aminobenzaldehyde adduct with that of synthetic 1-piperidine. The formation of glutamate from 2-oxoglutarate with lysine and cadaverine was demonstrated by quantitative studies using glutamate dehydrogenase. Furthermore, this procedure provided an alternative but less convenient method for assaying the aminotransferases. It is not certain whether the lysine and cadaverine aminotransferase activities represent two discrete enzymes or one or more enzymes with wide substrate specificities. However, in this context it should be noted that the L-lysine 6-aminotransferase of *Achromobacter liquidum* was inactive with cadaverine (Soda & Misono, 1968) and the diamine aminotransferase of *Escherichia coli* was likewise inactive with lysine.

1-Piperidine dehydrogenase. This enzyme was best assayed by following the rate of NADH formation using the soluble fraction to limit NADH oxidase activity. The K_m for 1-piperi-

deine was 0.12 mM and $V_{\max}$ was 12 $\mu\text{mol (mg protein)}^{-1} \text{ h}^{-1}$. The enzyme could also be assayed discontinuously by measuring the disappearance of 1-piperidine using *o*-amino-benzaldehyde. A direct relationship between 1-piperidine disappearance and NADH formation was established and this was used to determine the molar extinction coefficient for the dehydroquinazolinium derivative. The coenzyme NADP could replace NAD but it gave activities which were only 10% of those obtained with NAD under comparable conditions. This contrasts with glutarate semialdehyde where NADP produced 10 times more activity than NAD. Attempts to demonstrate a 5-aminovalerate-dependent oxidation of NADH by the reverse reaction were unsuccessful. 1-Pyrroline was also oxidized by these extracts and, using the same conditions, the rate with this substrate (optimum at pH 7.5) was about one-third of the rate observed with 1-piperidine (optimum at pH 9.0). Extracts of putrescine-grown organisms possessed high 1-piperidine dehydrogenase (Table 1) and 1-pyrroline dehydrogenase activities in the same proportion as found in lysine-grown organisms. However, it is not known whether one or two dehydrogenases are involved.

*Status of L-lysine-degrading enzymes in P. aeruginosa PAC586
grown on different substrates*

In order to assess the importance of the proposed cadaverine pathway for L-lysine catabolism in *P. aeruginosa* PAC586, the specific activities of relevant enzymes were determined in organisms grown on a range of substrates (Table 1). The degree of induction for each enzyme is shown as the increase in activity in L-lysine-grown cultures relative to control cultures grown with succinate. All the relevant enzymes were induced by factors of 7 to 75 and this is consistent with the existence of an inducible pathway for L-lysine degradation via cadaverine, 5-aminovalerate, glutarate and acetyl-CoA. A very similar pattern of induction was observed with cultures grown on cadaverine and 5-aminovalerate, although in the latter case the induction of lysine decarboxylase was less pronounced. Growth on putrescine induced all the activities except the decarboxylase and isocitrate lyase, and with arginine (not shown) only isocitrate lyase was not induced. Cultures grown on glutarate contained even less lysine decarboxylase but still retained reasonably high levels of the other enzymic activities. In contrast, the acetate-grown organisms were only induced for isocitrate lyase. The results obtained are in reasonable agreement with the respiratory capacities observed with whole organisms grown on the same substrates. One exception appeared to be the DL-pipecolate-grown cultures which were highly induced for lysine decarboxylase but oxidized L-lysine very slowly. On the other hand, the cadaverine and 5-aminovalerate aminotransferases were low considering that these substrates were oxidized fairly readily. Attempts to compare and interpret such data are frustrated by the problems of cell permeability and also the unknown substrate specificities of enzymes assayed in crude extracts. Thus, it is possible that the observed 1-piperidine dehydrogenase activity of DL-pipecolate-grown cultures is really due to a soluble pipecolate dehydrogenase. The simplest interpretation of the high glutarate-semialdehyde dehydrogenase is that pipecolate is metabolized via glutarate semialdehyde or glutarate, but it could be due to a wide substrate specificity of a 2-amino adipate semialdehyde dehydrogenase. The weak induction of isocitrate lyase compared with glutarate-grown cultures may suggest that pipecolate is not primarily metabolized via glutarate and acetyl-CoA. L-2-Amino adipate aminotransferase was only weakly induced by growth on L-lysine and pipecolate. The induction of L-lysine 6-aminotransferase parallels the induction of other enzymes of the cadaverine pathway, particularly cadaverine aminotransferase (Table 1), but it is not possible to interpret this in terms of one or two discrete

aminotransferases. However, discrete enzymes may exist because lysine is not a substrate for the diamine aminotransferase of *E. coli* and cadaverine is not transaminated by the lysine aminotransferase of *Achromobacter liquidum*.

Studies with mutants of P. aeruginosa PAC586

Mutants of *P. aeruginosa* PAC586 which grew with succinate but not L-lysine as sole carbon and energy sources were isolated after mutagenesis and enrichment by penicillin selection (see Methods). Growth tests with 23 mutants isolated in one experiment indicated the presence of three basic groups. The largest group (Lut⁻), comprising 13 mutants, closely resembled the parental strain PAC1 in growing well on cadaverine, 5-aminovalerate, glutarate and acetate, but growing very slowly on L-lysine. Another group (Gut⁻), with eight representatives, grew on acetate but failed to grow on glutarate, cadaverine, pipecolate, 5-aminovalerate or lysine. Further tests showed that six of the Gut⁻ mutants failed to grow on glutaconate but grew on butyrate, whereas the remaining two mutants did not grow with either substrate. Consequently those growing on butyrate could have lesions affecting the conversion of glutaconyl-CoA to crotonyl-CoA whereas the other two may be blocked between crotonyl-CoA and acetyl-CoA. The third group (Aut⁻) consisted of two mutants which failed to grow on acetate and all the other test substrates, including pipecolate. The nutritional phenotype of the third group was also exhibited by a well-characterized isocitrate lyase mutant of *P. aeruginosa*, PAC501. However, the two Aut⁻ strains contained at least 50 % of the uninduced activity of isocitrate lyase of the parental strain so they were assumed to have some other lesion(s) affecting acetate metabolism.

The existence of alternative routes for L-lysine degradation to glutarate (or glutaconate) in *P. aeruginosa* PAC586 is strongly favoured by these studies. If there were just one route, mutants with the phenotypes expected for metabolic blocks between intermediate substrates cadaverine, 5-aminovalerate and glutarate should have been recovered. It may be possible to obtain such mutants by specific selections for failure to grow on cadaverine or 5-aminovalerate, and the existence of alternative routes of lysine catabolism would be confirmed if these mutants were capable of growing on L-lysine. The results clearly show that a functional glutarate catabolic pathway is essential for the catabolism of L-lysine, cadaverine and 5-aminovalerate and that glutarate in turn is degraded via acetyl-CoA. Also, despite the low activities of isocitrate lyase in DL-pipecolate-grown cultures, this enzyme and others involved in glutarate catabolism are essential for pipecolate metabolism, which appears to converge on glutarate (or glutaconate) and hence acetyl-CoA in *P. aeruginosa*.

L-Lysine catabolism in other species

The occurrence and significance of the cadaverine pathway in other species of *Pseudomonas* was investigated with L-lysine-grown bacteria and their extracts. Studies on the oxidation of relevant substrates (see Fig. 2) by washed suspensions gave some indication of the routes of lysine catabolism which might be induced in different species. For example, *P. fluorescens* oxidized all substrates including cadaverine, D- and L-lysine, DL-pipecolate and 5-aminovalerate without lag, indicating that several pathways may be operating. With *P. multivorans* only D- and L-lysine, DL-pipecolate and L-2-amino adipate were oxidized rapidly, suggesting that the major route for lysine catabolism is the pipecolate pathway; oxidation of cadaverine, glutarate, 5-aminovalerate and acetate was very slow and was always preceded by long periods of adaptation. It was difficult to interpret the results for the representatives of *P. putida* biotypes A and B. Neither oxidized cadaverine without lag. However, both were induced for D-lysine and acetate oxidation but DL-pipecolate was oxi-

Table 2. *Enzymes of L-lysine catabolism in different species of Pseudomonas*

Organisms were grown on L-lysine at 37 °C (*P. aeruginosa*) or 30 °C (all the other species), harvested in the exponential phase and extracts were assayed for enzyme activities as described in Methods. The results shown in parentheses were obtained under anaerobic conditions for L-lysine decarboxylase or with the soluble fraction for L-pipecolate dehydrogenase.

Enzyme	Specific activity of enzyme in extracts of:					
	<i>P. aeruginosa</i> PAC586	<i>P. aeruginosa</i> PAC1	<i>P. fluores-</i> <i>cens</i>	<i>P. putida</i> (biotype A)	<i>P. putida</i> (biotype B)	<i>P. multi-</i> <i>vorans</i>
L-Lysine decarboxylase	7.5	2.3	7.0 (5.5)	0.9 (0.03)	1.0 (0.06)	1.5
Cadaverine aminotransferase	3.5	2.6	2.6	1.1	2.6	0.5
1-Piperidine dehydrogenase	10.0	7.4	9.2	1.0	6.8	0.3
5-Aminovalerate aminotransferase	7.5	4.3	3.7	1.4	4.3	<0.2
Glutarate-semialdehyde dehydrogenase	27.2	19.9	15.9	5.5	27.9	2.6
Isocitrate lyase	11.8	14.4	15.2	3.9	12.7	2.3
L-Lysine 6-aminotransferase	2.3	1.5	1.5	0.5	2.1	0.5
L-Lysine 2-monooxygenase	<0.05	<0.05	12.6	4.4	4.2	<0.05
L-Pipecolate dehydrogenase	0.02	<0.01	0.02	0.9	0.03	0.02 (0.15)
L-2-Amino adipate aminotransferase	1.0	1.6	0.8	1.5	0.8	6.8

dized only by *P. putida* A although *P. putida* B was better adapted for oxidizing 5-aminovalerate.

Using extracts of L-lysine-grown organisms, enzymes of the cadaverine pathway and other relevant enzymes were assayed in several *Pseudomonas* species and in *P. aeruginosa* PAC1, the parent (Lut⁻) of strain PAC586 (Lut⁺) (Table 2). The parental strain had a similar distribution of enzyme activities to that of its derivative PAC586. Some of the enzymes, particularly L-lysine decarboxylase, were present at lower specific activities in strain PAC1.

In assessing the significance of the cadaverine pathway in organisms containing L-lysine 2-monooxygenase it was important to assay the decarboxylase under anaerobic conditions because the oxygenase also liberates CO₂. In *P. aeruginosa* the specific activities for L-lysine decarboxylase were the same under aerobic and anaerobic conditions. However, in *P. fluorescens* 20 % of the apparent decarboxylase activity could be attributed to the oxygenase and in *P. putida* of biotypes A and B the oxygenase could account for virtually all the decarboxylation (Table 2). The results indicate that *P. fluorescens* is potentially capable of metabolizing L-lysine by decarboxylation to cadaverine as well as by oxygenation. In contrast, the cadaverine pathway did not appear to be operating in either strain of *P. putida*. The oxygenase pathway was present and, in the representative of the A biotype, pipecolate dehydrogenase was also induced. In *P. multivorans* no oxygenase could be detected and, although a small amount of L-lysine decarboxylase activity was observed, other enzymes of the cadaverine and 5-aminovalerate routes were present at very low specific activities. A 'soluble' pipecolate dehydrogenase was detected and the high specific activity for L-2-amino adipate aminotransferase confirmed the indications from studies with whole cells, that the pipecolate route is probably important for L-lysine catabolism in *P. multivorans*. All the species tested possessed L-lysine aminotransferase activity.

DISCUSSION

The report of Stanier *et al.* (1966) that 90 % (26/29) of strains of *Pseudomonas aeruginosa* use L-lysine as sole carbon and energy source was confirmed by finding that 74 % (17/23) of the strains studied here possess this phenotype. These growth tests conceal the fact that the rate of growth on L-lysine is normally low. However, faster growing derivatives were readily isolated. Studies with extracts of one derivative, *P. aeruginosa* PAC586, indicated that it was deficient in key enzymes of the pathways used for D- and L-lysine catabolism in other *Pseudomonas* species. A search for alternative routes led to the discovery of reactions which constitute a new pathway, the cadaverine or decarboxylase pathway. This represents a variant on the 5-aminovalerate (or oxygenase) pathway because it converges on 5-aminovalerate but involves both acyclic (cadaverine) and cyclic (1-piperidine) intermediates which by-pass the route involving 5-aminovaleramide and the monooxygenase and amidase reactions (Figs 1 and 3). Enzymes for the conversion of 5-aminovalerate to glutarate were present, as in other species of *Pseudomonas* grown on L-lysine.

The cadaverine pathway (Fig. 3) is initiated by L-lysine decarboxylase. This enzyme was not detected in *P. aeruginosa* in early studies (Gale, 1946). One reason may have been the use of a low pH for the tests and this could explain the continued appearance of conflicting reports. For example, no lysine decarboxylase was detected in the *P. aeruginosa* strains tested by Steel & Midgley (1962) and Zolg & Ottow (1974) although all of 130 different cultures of *P. aeruginosa* were found to possess this activity in tests at pH 8.0 (Stewart, 1970). The ability of *P. aeruginosa* to oxidize cadaverine and other diamines by inducible enzymes is well established (Gale, 1942; Razin, Gery & Bachrach, 1959). The route proposed for the further metabolism of cadaverine to glutarate is directly analogous to that for the metabolism of putrescine to succinate via 1-pyrroline, 4-aminobutyrate and succinate semialdehyde in *P. fluorescens* and *E. coli* (Jacoby & Fredericks, 1959; Kim, 1964). The analogy may be extended because putrescine is the decarboxylation product of ornithine and consequently occurs as an intermediate in the catabolism of arginine and ornithine as well as spermine and spermidine in some pseudomonads. Growth of *P. aeruginosa* PAC586 on putrescine and arginine induced the enzymes for the conversion of cadaverine to glutarate, suggesting that either one group of enzymes, or two groups with overlapping specificities, may be responsible for the catabolism of the diamine. It is difficult to assess the most likely possibility because some of the relevant enzymes have fairly broad substrate specificities. Arginine, but not putrescine, also induced some lysine decarboxylase activity. The importance of cadaverine as an intermediate with regulatory significance is apparent from the fact that the respiratory activities (Fig. 2) and enzyme patterns (Table 1) of cadaverine- and lysine-grown cultures of mutant PAC586 are identical. Despite the difference in growth rates on L-lysine of the mutant PAC586 and its parent strain, PAC1, both grew at the same rate on cadaverine. The specific activity of lysine decarboxylase in lysine-grown strain PAC1 was less than one-third of that of mutant PAC586 and the slower growth of strain PAC1 may be a direct consequence of this. Alternatively, the growth of strain PAC1 may be retarded by a deficiency in the L-lysine transport system which limits the internal concentration of L-lysine and hence the degree of induction of the catabolic enzymes. However, it should be emphasized that synthesis of the decarboxylase is still inducible in the mutant PAC586. *Pseudomonas aeruginosa* may therefore be seen as having a very efficient inducible system(s) for diamine metabolism, and rapid growth on L-lysine can be accomplished by improving the flow of this substrate into the diamine pathway. In this respect L-lysine catabolism resembles L-histidine catabolism in *P. aeruginosa* strain PAC1, where histidine transport is

sufficient for its utilization as a source of nitrogen but not for supporting growth as sole carbon source (Potts & Clarke, 1974).

The transamination of L-lysine to 1-piperidine-6-carboxylate is also inducible and represents a second potential route for lysine catabolism in *P. aeruginosa*. Its physiological significance is difficult to assess. It could represent a mechanism for obtaining amino-nitrogen when *P. aeruginosa* is grown with other substrates plus L-lysine as sole source of nitrogen. The failure to isolate mutants unable to utilize lysine due to blocks between L-lysine and glutarate implies that there are at least two alternative and functional routes effecting the corresponding metabolic transformation. As an intermediate in the pipecolate pathway (Fig. 1), it might be assumed that 1-piperidine-6-carboxylate would be metabolized via 2-oxoglutarate. However, the dependence on a functional glutarate pathway for growth on L-lysine and DL-pipecolate and the induction of isocitrate lyase with both substrates imply that the product of the pipecolate pathway, 2-oxoadipate, can be converted to glutarate (or glutaryl-CoA) or that one of the C₆ compounds (1-piperidine-6-carboxylate or 2-aminoadipate) can be decarboxylated to converge on the 5-aminovalerate route (at piperidine or 5-aminovalerate). Studies with mutants specifically selected for blocks in cadaverine or 5-aminovalerate metabolism could be used for assessing the significance of the transamination route and the location of any metabolic bridge between the C₆ and C₅ routes. The affinities of some of the enzymes for their substrates appear low and their pH optima are rather high. However, high pH optima are often observed for enzymes concerned with the metabolism of basic substrates and high Michaelis constants for substrates of inducible pathways concerned with the catabolism of essential metabolites such as lysine are obviously advantageous (Miller & Rodwell, 1971*a, b*).

The results obtained with *P. fluorescens* indicate that it has the potential for using the cadaverine pathway; its existence may have been overlooked in previous studies due to the presence of the high lysine oxygenase activity. *Pseudomonas multivorans* had a weak decarboxylase activity and this has been noted previously in three strains including the one studied here (Samuels, Moss & Weaver, 1973). However, neither the cadaverine nor other routes involving 5-aminovalerate appear important, and this strain could rely entirely on the pipecolate route for lysine catabolism. Decarboxylation does not appear to be important in the catabolism of L-lysine by *P. putida*. The results are consistent with those of Chang & Adams (1974) and Miller & Rodwell (1971*a*); the oxygenase and pipecolate dehydrogenase routes both occur in strains of biotype A. The oxygenase route seems to be the main route present in the representative of biotype B. The results also show that the role of L-lysine 6-aminotransferase needs to be considered when assessing the physiological importance of different routes for lysine catabolism in all the strains. Kornberg (1960) used the specific activity of isocitrate lyase as an enzymic indicator of the metabolic pathways involved in L-lysine (and glutarate) catabolism by another *P. putida* of biotype A. He concluded that L-lysine was degraded via acetate, not 2-oxoglutarate. Using the same criterion this is true for all the *Pseudomonas* species examined here, except *P. multivorans*.

In view of the relatively small number of strains which have been examined it cannot be concluded that there is a rigid specificity for the routes of lysine catabolism possessed by different *Pseudomonas* species. However, it is clear that not only do the pseudomonads possess great metabolic potential but there is also a great diversity in the number of pathways for the catabolism of one substrate. Furthermore, in the case of lysine catabolism, multiple pathways can exist in one strain, posing interesting regulatory problems as well as those posed by the inter-relationships between the biosynthesis and degradation of the amino acid.

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