Annu. Rev. Nutr. 1991. 11:435–48

LYSINE–PIPECOLIC ACID METABOLIC RELATIONSHIPS IN MICROBES AND MAMMALS

Harry P. Broquist

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

KEY WORDS: lysine-pipecolate metabolism, hyperlysinemia, Zellweger syndrome, peroxisomal oxidation, pipecolatemia

CONTENTS

Introduction

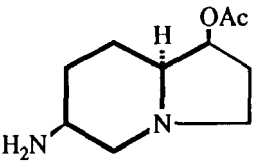
For about 40 years, L-pipecolic acid, 2-carboxypiperidine (Figure 1), has been known as a nonprotein amino acid that is widely distributed in higher and lower plants and is derived via lysine metabolism from both plants and animals. Indeed, the major route of mammalian lysine catabolism was first thought to be via pipecolate, although it is now apparent this is not the major route of lysine degradation in higher animals. However, there have been and continue to be reports in the clinical literature of pathological situations in which pipecolic acid accumulates in body fluids; these reports suggest intrigu-

0199-9885/91/0715-0435$02.00

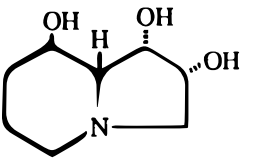
ing biochemical and clinical questions about the significance of such findings in man. Studies of lysine metabolism in diverse microorganisms, together with biochemical investigations of the genetic diseases of lysine metabolism and certain peroxisomal disorders, have contributed to an understanding of lysine-pipecolate relationships in both plant and animals. These studies, reviewed herein, illustrate important facets of lysine metabolism that encompass biochemical, nutritional, and clinical interests.

Pipecolic Acid in Higher Plants

In 1952, F. C. Steward's group identified L-pipecolic acid as a constituent of leguminous fruit and seeds (48). They isolated 800 mg of pipecolic acid from 10 kg of fresh green beans. R. I. Morrison also isolated pipecolate (500 mg) from 500 g of cut leaves of white clover (33) and showed that pipecolic acid is fairly widespread in angiosperms. Subsequent studies reviewed by Fowden (15) enumerate a number of compounds structurally related to pipecolic acid that are also present in various plants, e.g. 4- and 5-hydroxypipecolate, 4 keto- and 4-aminopipecolic acid, bakikiain (tetrahydropicolinic acid), and D-pipecolic acid. The function of these compounds and pipecolic acid in plant metabolism is not clear, although certain specialized roles have been discovered. For example, D-pipecolic acid has been found in the antibiotics aspartoxin and glumamycine [cf (15) for discussion]. D-pipecolate is a potent proline analog that inhibits growth of *Escherichia coli* and the development of seedlings and chick embryos. It may replace proline in critical plant proteins, thus giving rise to spurious proteins. L-pipecolic acid is incorporated entirely into the indolizidine ring system of slaframine and swainsonine in the fungal parasite ***Rhizoctonia leguminicola*** [cf (2) for discussion and references]; the bold-faced type in the drawing below indicates the contribution of pipecolate to these alkaloids.



Slaframine



Swainsonine

Pipecolic Acid as a Lysine Metabolite in Animals

Soon after the discovery of pipecolic acid as a plant constituent, its metabolic origin from lysine was established [cf Meister's text (28) for accounts of early work]. Experiments by Rothstein & Miller (37) were particularly noteworthy in showing that pipecolic acid played a role in lysine metabolism in the rat.

Rothstein & Miller administered [^{14}C]lysine together with unlabelled L-pipecolic acid (as a trap) to a rat and isolated radioactive pipecolic acid of relatively high specific activity from the urine. They also made the significant observation, in experiments employing [α-^{15}N] and [ϵ-^{15}N]lysine, that the α-amino group rather than the ϵ-amino group of lysine was lost during the conversion of lysine to pipecolate. On the other hand, administration of [α-^{14}C]aminoadipic acid did not lead to the formation of radioactive pipecolate; rather, [^{14}C]pipecolate was converted to α-aminoadipic acid (and glutarate) in the intact rat and also in a rat liver mitochondrial system (36). They postulated that lysine degradation in the rat proceeded as follows: Lysine $\rightarrow$ [α-keto-ϵ-amino-caproic acid $\rightleftharpoons$ Δ^1-piperideine-2-carboxylate] $\rightarrow$ pipecolic acid $\rightarrow$ [Δ^1-piperideine-6-carboxylate $\rightleftharpoons$ α-aminoadipic semialdehyde] $\rightarrow$ α-aminoadipic acid $\rightarrow$ CO_2, H_2O. These events are shown in Figure 1, reactions 1, 2, 5B, 6, 7, and 8. In this and in subsequent discussion, we note that Δ^1-piperideine-2-carboxylic (Δ^1-P2C) and Δ^1-piperideine-6-carboxylic acid (Δ^1-P6C) exist in chemical equilibrium with their open-chain hydrated forms α-keto-ϵ-aminocaproic acid and α-aminoadipic semialdehyde, respectively. Δ^1-P2C and Δ^1-P6C are also isomers, but they can be differentiated in biological systems via appropriate tracer methodology as their respective N-atoms derive from the ϵ-N or α-N atom of lysine.

Lysine Degradation via Saccharopine

Seemingly unrelated studies of lysine biosynthesis in yeast and fungi ultimately led to a clarification of the major pathway of lysine degradation in higher animals. In yeast and *Neurospora,* lysine was shown to be formed from α-ketoadipic acid via reverse reaction of 7, 6, 4, and 3 of Figure 1 [cf Trupin & Broquist (41) for discussion and references]. A unique feature of this pathway was the pyridine nucleotide–dependent transamination of aminoadipic semialdehyde with glutamate (GLU) to give lysine [Reaction (iii)] in which saccharopine (SAC) figured as a stable, transitory intermediate [Reactions (i) and (ii)].

Aminoadipic semialdehyde + glutamate + NAD(P)H,H+ $\rightarrow$ saccharopine + NAD(P)+ (i)

Saccharopine + NAD(P)+ $\rightarrow$ lysine + α-ketoglutarate + NAD(P)H,H+ (ii)

Aminoadipic semialdehyde + glutamate $\rightarrow$ lysine + α-ketoglutarate (iii)

Higashino et al (20) then made the important discovery that rat liver mitochondria readily catabolized lysine to CO_2 in a process requiring α-KG and in which saccharopine was formed. Hutzler & Dancis (21) then went on to purify lysine-α-KG reductase [reverse of reaction (ii)] from human liver

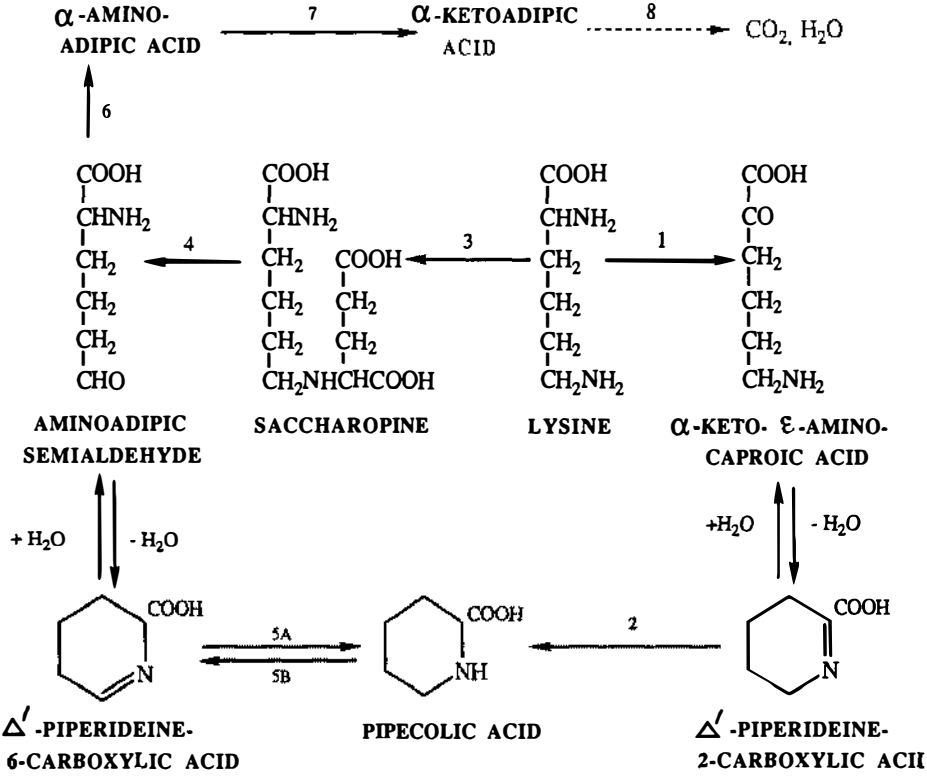


Figure 1 Lysine-pipecolic acid relationships in microbes and mammals.

and showed that the reaction required NADPH,H^+ and α-KG. These studies and subsequent investigation of the biochemical basis of familial hyperlysinemias (discussed below) led to the conclusion that the major route of lysine degradation in higher animals, including man, is via saccharopine and subsequent reactions 4, 6, 7, and 8 (Figure 1).

L. M. Henderson and his students evaluated lysine catabolism via the postulated saccharopine and/or pipecolate pathways in the rat (16) and the rabbit (18). Rat liver or kidney mitochondria readily formed saccharopine from L-lysine under anaerobic conditions; and saccharopine was metabolized aerobically to α-aminoadipic acid and glutamate. Consistent with these findings and pathway 3, 4, and 6 of Figure 1 was the demonstration that L-[α-^{15}N]lysine, *not* L-[ε-^{15}N]lysine, was incorporated into α-aminoadipic acid (17). Pipecolic acid was formed from DL-lysine in the rat (16), but calculations showed that 96% of the pipecolate formed was derived from D-lysine. Moreover, the L-pipecolate formed was shown to be relatively meta-

bolically inert. In the rabbit (18) the only endogenous source of L-pipecolate found was via the catabolism of D-lysine. But in contrast to the rat, L-pipecolic acid in the rabbit was readily catabolized to CO_2, thus indicating a route for the complete catabolism of D-lysine via pipecolic acid.

L-Pipecolic Acid Synthesis from L-,D-, and DL-Lysine

Over the years, investigators have sought to establish the precise route of pipecolic acid synthesis from lysine in both plants and animals. Only a few examples can be given. In a series of "metabolic overloading" experiments, Gupta & Spencer (19) administered DL-[6-^{14}C,6-^{3}H]lysine to a rat, to a lysine auxotroph of *Neurospora crassa,* to intact bean plants *(Phaseolus vulgaris),* and to excised shoots of *Sedum acre*. Pipecolic acid was subsequently isolated from the tissues, and radioactivity arising from the doubly labelled lysine was determined. In each case the $^{3}H:^{14}C$ ratio was very similar to that of the administered precursor. Figure 1 shows that if pipecolate had been formed from saccharopine and Δ^1-P6C, the $^{3}H:^{14}C$ ratio would be halved (loss of an H-atom at C-6 following saccharopine formation), whereas if pipecolate synthesis proceeds via Δ^1-P2C, the ratio would be unchanged. Such considerations led investigators to conclude that pipecolate synthesis in all cases proceeded via reactions 1 and 2 (Figure 1). Because racemic lysine was used in these studies, it is not known if the L- or D-isomer of DL-lysine was the exclusive pipecolate precursor or if lysine racemase (if present) influenced the result. To clarify this point, Chang & Adams (6) employed *Pseudomonas putida* mutants that were blocked at various loci from utilizing lysine as a sole source of nitrogen and energy for growth; they found that those mutants using D-lysine had induced a pathway via Δ^1-P2C and pipecolic acid. When L-lysine was the substrate, a pathway via α-aminovalerate was induced. Fangmeier & Leistner (13) working with *Neurospora crassa* showed that radioactivity from D-lysine was effectively incorporated into L-pipecolic acid; and in [^{15}N]lysine labelling experiments they showed that the α-amino group of D-lysine was lost in this conversion, i.e. reactions 1 and 2 in Figure 1 must have been operative.

Although it has been well established that L-lysine is catabolized in rat liver via the saccharopine pathway as discussed above, Chang showed (5) that both L- and D-lysine (^{14}C-labelled), when injected intraventricularly into rat brain, effectively labelled pipecolic acid. Small amounts of labelled aminoadipic acid were also detected in such brain samples. Lysine-ketoglutarate reductase [the reverse of Reaction (ii)] above) is of low activity in mammalian brain. Hence Chang concluded that rat brain contains enzyme systems for catabolizing both L- and D-lysine via reactions 1 and 2 of Figure 1 and that these reactions in mammalian brain may play a significant role in the nervous system.

The enzymatic conversion of lysine to α-keto-ε-aminocaproate (reaction 1, Figure 1) has been difficult to demonstrate in mammalian systems. It has long been known that the α-amino group of lysine does not readily enter into classical transamination reactions. Kusakabe et al (25) provided convincing evidence for an L-lysine α-oxidase catalyzing reaction:

$$\text{L-lysine} + O_2 \rightarrow \alpha\text{-keto-}\epsilon\text{-aminocaproate} + NH_3 + H_2O_2. \quad \text{(iv)}$$

The enzyme, a flavoprotein of 116,000 daltons (with two identical subunits of 56,000 daltons) was found in *Trichoderma viride* Y244-2. A heat labile antitumor substance was also present in wheat bran extracts of *T. viride,* and after purification of lysine oxidase to homogeneity, the antitumor activity copurified as well.

The origin of pipecolic acid from L-lysine metabolism in the fungal parasite *Rhizoctonia leguminicola* is of interest because such biosynthesis represents the initial steps in the biogenesis of the indolizidine alkaloids slaframine and swainsonine (4). Wickwire et al (45) could find no evidence for L-lysine oxidase activity and α-keto ε-aminocaproate formation in pipecolate synthesis in this fungus. Rather, cell-free extracts of *R. leguminicola* when incubated with L-lysine, α-ketoglutarate, and NADH,H+ formed saccharopine, Δ^1-P6C, and pipecolate. These products were identified unambiguously by appropriate chemical and biological means. Studies with cell-free enzyme systems established that pipecolic acid was formed from L-lysine via pathway 3, 4, and 5A of Figure 1. Additional studies using ^{15}N showed that the piperidine ring N-atom of pipecolic acid derived from the α-N atom of lysine. A previously unrecognized enzyme, termed saccharopine oxidase, that catalyzed Reaction (v) was found and purified to homogeneity (46).

$$\text{Saccharopine} + O_2 \rightarrow \Delta^1\text{-P6C} + \text{glutamate} + H_2O_2. \quad \text{(v)}$$

Saccharopine oxidase (45,000 daltons) contains bound flavin and apparently exists in *R. leguminicola* to shunt saccharopine, a major lysine metabolite, into a secondary pathway of lysine metabolism. It will be interesting to learn if pipecolic acid and/or its metabolites in plants have their penultimate origin in lysine and saccharopine via reactions 3, 4, and 5A of Figure 1. In this regard, Schütte & Seelig (39) reported that the α-^{15}N, not the ε-^{15}N, atom of lysine was incorporated into pipecolic acid in *Phaseolus vulgaris*. This incorporation pattern conflicts with the findings of Gupta & Spenser (19), discussed above, who claim that the ε-amino group of lysine supplies the nitrogen atom of pipecolic acid in *P. vulgaris*. This discrepancy needs to be resolved.

Familial Hyperlysinemia

Studies of hereditary disorders in lysine metabolism have contributed greatly to the elucidation of the degradative pathways for this amino acid. As discussed more fully elsewhere by Dancis & Cox (8), the initial clinical observation in familial hyperlysinemia is an elevated lysinuria (regularly exceeding 10 mg/dl) detected during biochemical studies of patients with presumed metabolic disease, often with neurological symptoms. Screening of other family members may reveal asymptomatic individuals with the same biochemical findings. Both sexes are affected, and consanguinity of parents has been reported, indicating autosomal recessive inheritance. Culture of skin fibroblasts in such instances and subsequent analysis of lysine catabolic enzymes has revealed multiple enzyme defects in familial hyperlysinuria. Dancis et al (12) obtained liver at autopsy from a patient with the disease and found unexpectedly that both lysine-α-ketoglutarate reductase [Reaction (vi)] and saccharopine dehydrogenase [Reaction (vii)] were absent.

$$\text{Lysine} + \alpha\text{-ketoglutarate} + \text{NAD(P)H,H+} \rightarrow \text{saccharopine} + \text{NAD(P)+} \quad \text{(vi)}$$

$$\text{Saccharopine} + \text{NAD(P)+} \rightarrow \text{aminoadipic semialdehyde} + \text{glutamate} + \text{NAD(P)H,H+} \quad \text{(vii)}$$

$$\text{Lysine} + \alpha\text{-ketoglutarate} \rightarrow \text{aminoadipic semialdehyde} + \text{glutamate} \quad \text{(viii)}$$

These enzyme activities and saccharopine oxidase [reverse of Reaction (vi)] were all less than 10% of normal in skin fibroblasts from two siblings with familial hyperlysinemia and from a third individual from an unrelated family. It is thought that saccharopine oxidase may be a distinctly different enzyme in ox liver (14), but saccharopine dehydrogenase in man likely catalyzes both forward [Reaction (vii)] and reverse [Reaction (vi)], but with the equilibrium toward saccharopine synthesis (12). Levels of both lysine catabolic enzymes [Reactions (vi) and (vii)] were surprisingly low in such a genetic disease, and these findings appeared to refute the dogma: one gene, one enzyme. To further add to the complexity of the problem, an unusual patient with familial hyperlysinemia, but presenting with cystinuria, was found to also have a saccharopinuria (8). Among six additional cystinurics who also had the double enzyme defect ascribed to familial hyperlysinemia, four had a detectable saccharopinuria.

The problem has been clarified by Markovitz et al (27), who found that lysine-α-ketoglutarate reductase and saccharopine dehydrogenase from mitochondrial extracts of baboon and bovine livers copurified throughout a 500-fold increase in activities. From these and other biochemical criteria it was concluded that a single protein, a multifunctional enzyme termed

aminoadipic semialdehyde synthetase, contains *both* enzymes and thus catalyzes Reaction (viii) via Reactions (vi) and (vii). The biochemical defect in familial hyperlysinemia I is now defined (11, 27) as associated with the loss of the entire synthase [i.e., Reactions (vi) and (vii)]; whereas familial hyperlysinemia II is viewed as a saccharopine dehydrogenase deficiency [Reaction (vii)].

Subsequently, Markovitz & Chuang (26) found that limited proteolysis of highly purified bovine aminoadipic semialdehyde synthetase resulted in the separation via acrylamide gel electrophoresis of the reductase and dehydrogenase with full retention of enzymatic activity of each polypeptide enzyme fraction. Such findings illustrate how saccharopine accumulates in familial hyperlysinemia II, since Reaction (vi) would be operating to form saccharopine, but Reaction (vii) would be inoperative, thus causing saccharopine to accumulate. A striking parallelism exists between hereditary orotic aciduria and familial hyperlysinemia (23). Thus, orotic aciduria type I involves a combined deficiency of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase, whereas in orotic aciduria type II, only the latter decarboxylase is deficient.

Pipecolatemia in Hyperlysinemia and in Zellweger Syndrome

Elevated pipecolic acid levels in urine (22) and plasma (8) are an intriguing facet of the hyperlysinemia syndrome. Improvements in the quantitation of pipecolic acid in physiological fluids (22) have led to more precise definition of clinical findings. For example (8), in seven patients with hyperlysinemia the mean plasma pipecolate concentration was 31.9 ± 10.7 μM versus 2.1 μM in normal individuals. And in these same patients the plasma lysine concentration was 1009 ± 240 μM. Such findings support the view that in hyperlysinemia the major lysine degradative pathway via saccharopine is blocked, but there is limited capacity for pipecolate synthesis, presumably by pathway 1 and 2 of Figure 1. Woody & Pupene suggest (47) that an alternate pathway for lysine catabolism via pipecolic acid, albeit limited, may help explain the fact that three of four children with hyperlysinemia in a family in their study had normal growth and were not mentally affected by their metabolic disease. Apparently, the high lysine concentrations present in hyperlysinemics are tolerated. In this regard, other lysine metabolites (47) in the urine of the hyperlysinemic children included α-N-acetyllysine, ϵ-N-acetyllysine, homocitrulline, and homoarginine. Such metabolism would serve to decrease circulating lysine levels in the diseased children. Woody & Pupene thought (47) that ϵ-*N*-acetyllysine may be involved in pipecolate synthesis as described in Reaction (ix). Subsequent transamination of ϵ-*N*-acetyllysine followed by respective deacetylation and reduction would yield

pipecolic acid [as in Reaction (x)], but such metabolism has not been rigorously demonstrated in man.

$$\text{Lysine} \rightarrow \epsilon\text{-}N\text{-acetyllysine} \rightarrow \rightarrow \rightarrow \text{pipecolic acid.} \quad \text{(ix)}$$

$$\epsilon\text{-}N\text{-acetyllysine} \rightarrow \alpha\text{-keto-}\epsilon\text{-}N\text{-acetylcaproate} \rightarrow \Delta^1\text{P2CA} \rightarrow \text{pipecolic acid.} \quad \text{(x)}$$

Interest in hyperpipecolatemia was heightened by the discovery that plasma and urinary pipecolate levels are elevated in Zellweger syndrome in the absence of hyperlysinemia (8, 40). In such instances, aminoadipic semialdehyde synthetase should be fully operative and "overflow" of lysine via the pipecolate pathway should be negligible. Instead, a block in catabolism of pipecolate is thought to account for its accumulation. Zellweger syndrome is a cerebro-hepato-renal syndrome with a distinctive constellation of multiple congenital anomalies, e.g. characteristic facies and hypotonia. A more detailed summary of this autosomal recessive disease is given in two excellent reviews dealing with peroxisomal disorders (38, 42). In a limited series of infants with Zellweger syndrome, Dancis & Hutzler (10) found that plasma pipecolate levels increased from normal levels at birth (4–10 days) to pathological levels with increasing age (2–4 months). However, major clinical manifestations of the disease were observed before the onset of severe pipecolatemia, making it difficult to asesss the significance of the latter in the development of the disease.

The most prominent morphological abnormality of Zellweger syndrome is an absence of peroxisomes in the liver and kidney (38). More than 150 patients have now been described in the literature (42). Indeed, this disease can be considered to be the prototype of genetic disorders in man in which peroxisomal functions are impaired. Schütgens et al (38) describe peroxisomes as subcellular organelles bounded by a single membrane, widely distributed in eukaryotic organisms, and ubiquitous in mammalian cells. At the biochemical level peroxisomes may be defined as membrane-bound organelles containing catalase and at least one hydrogen peroxide–producing oxidase. Recent clinical and biochemical research presumes that the enzyme responsible for pipecolate oxidation resides in the peroxisomes and seeks to clarify the relationship between hyperpipecolatemia and peroxisomal disorders, particularly in Zellweger syndrome.

L-Pipecolic Acid Oxidation: Microbes and Mammals

Early studies from Rodwell's laboratory (1, 4) of the oxidation of L-pipecolic acid to L-aminoadipic acid in *Pseudomonas putida* P2 showed the reactions to be 5B and 6 of Figure 1. Reaction 5B was catalyzed by a flavin adenine

dinucleotide (FAD)-requiring membrane-bound dehydrogenase associated with an electron transport particle (1). Subsequent oxidation via α-aminoadipic semialdehyde NAD+ oxidoreductase yielded α-aminoadipic acid (reaction 6, Figure 1) (4). In contrast, the yeast *Rhodotorula glutinis*, which utilizes pipecolate to meet its requirement for lysine for growth, has an L-pipecolate oxidase that directly accepts molecular oxygen as a substrate (24). These microbial systems served as a useful frame of reference for subsequent detailed studies of pipecolate oxidation in mammalian systems.

Mihalik & Rhead (31) developed a sensitive assay for the formation of [^{3}H]aminoadipic acid from [^{3}H]pipecolic acid by mitochondrial and peroxisomal fractions prepared from tissue samples from various species. They showed (32) that liver mitochondrial preparations from the rabbit, guinea pig, dog, pig, and sheep carried out the oxidation of pipecolic acid to yield aminoadipic acid (Table 1). Rat and mouse preparations were inactive. The results with the rabbit and rat preparations were consistent with whole animal observations (discussed above) that the rabbit (18), but not the rat (16), metabolizes L-pipecolate. Oxidation of pipecolate in a rabbit cortex mitochondrial preparation was enhanced by FAD (31), suggesting the involvement of a flavoprotein oxidase (reaction 5B, Figure 1) in a manner analogous to the *P. putida* system (1).

Of great interest was the finding by Mihalik & Rhead (31) that L-pipecolic acid oxidation by the monkey was carried out by a kidney cortex peroxisomal fraction. The system produced H_2O_2, suggesting that an oxidase was present, but no cofactors were identified in the crude system. Three groups have now demonstrated L-pipecolate oxidase activity in human liver (Table 1). Wanders et al (44) showed that the enzyme was localized in the peroxisomes but was deficient in liver from patients with Zellweger syndrome (43). Mihalik et al (30) measured L-pipecolic acid oxidation in peroxisome-enriched liver frac-

Table 1 Mitochondrial and peroxisomal oxidation of L-pipecolic acid in mammals

Species	Liver mitochondria	Liver peroxisomes	References
Rabbit	+		32
Guinea pig	+		32
Dog	+		32
Pig	+		32
Sheep	+		32
Monkey		+	31
Man		+	30, 44, 35
Mouse	–	–	32
Rat	–	–	32

tions in controls and in Zellweger patients. Results were expressed in terms of [^{3}H]aminoadipate formed from [^{3}H]pipecolate. Liver homogenates from adult and infant controls formed 47.1 ± 6.6 and 48.3 ± 10.0 pmol aminoadipate per milligram of protein per hour respectively, whereas Zellweger syndrome livers formed only 1.7 ± 0.3 pmol aminoadipate per milligram of protein per hour. The studies of these two groups (30, 43) thus establish on a biochemical basis that the high circulating levels of L-pipecolic acid in the Zellweger syndrome are caused by defective peroxisomal oxidation of pipecolic acid to aminoadipic acid.

Subsequently, Mihalik et al (29) purified peroxisomal L-pipecolic acid peroxidase to near homogenity from Rhesus monkey liver. The protein had a molecular weight of 46,000 (SDS gel-polyacrylamide gel electrophoresis) and contained covalently bound flavin. The authors followed the enzyme reaction (*a*) by measuring H_2O_2 produced by a spectrophotometric method and (*b*) by noting the appearance of an orthoaminobenzaldehyde adduct product presumed to be Δ^1P6C. Studies by Y. F. Chang and co-workers have contributed important insights toward understanding reactions 5B and 6 (Figure 1) in human liver (7, 35). Using a peroxisome-enriched human liver fraction, they rigorously established that the product of pipecolate oxidation was [Δ^1-P6C $\rightleftharpoons$ aminoadipic semialdehyde] as judged (*a*) by cochromatography (ion exchange and TLC) with authentic aminoadipic semialdehyde and (*b*) by borohydride reduction of product to pipecolic acid (35). Aminoadipic semialdehyde was oxidized to aminoadipic acid by a soluble enzyme, L-α-aminoadipic-δ-semialdehyde oxidoreductase, in human liver (7). The enzyme was localized in the cytosol, required NAD^+, free SH groups, and was stimulated by Mg^{2+}, Cu^{2+}, and Mn^{2+}. It can be seen (Figure 1) that the lysine-saccharopine pathway and the lysine-pipecolate pathway converge at aminoadipic semialdehyde. As noted herein, these pathways are operating in different species, in various organelles, and may reflect specific channeling devices to localize specific metabolic events.

Concluding Remarks

Nutritional metabolic research often follows the dictum: "And a little cell shall lead them" (3). Thus, the aminoadipate pathway of lysine biosynthesis was first elucidated in yeast and fungi; subsequently, reverse steps of this pathway (reactions 3, 4, 6, and 7 of Figure 1) were shown to constitute the initial major steps of lysine catabolism in higher animals including man. Likewise, the use of lysine auxotrophs in yeast and fungi to facilitate identification of blocked pathway intermediates has its counterpart in the metabolic consequences of the enzyme deletions in hyperlysinemias I and II. Then too, studies of pipecolic acid oxidation in *Pseudomonas putida* (1, 4)

foreshadowed similiar enzymatic findings in peroxisomal enzyme systems in the monkey (31) and man (30, 35, 44).

To the nutritionist the essential amino acid lysine has several crucial roles: Lysine is a building block in protein synthesis; it can form transitory complexes in various enzymes with cofactors including biotin, pyridoxal, and lipoate; and it is a precursor of carnitine. This review focuses on the ability of lysine to serve as a precursor of pipecolic acid, although the precise role of pipecolate in mammalian metabolism remains obscure. Dancis & Cox (8) point out that the consistency with which pipecolic acid is formed in the absence of lysine excess (as in the hyperpipecolatemias), and the preservation of the pathway in a variety of animal species (9), suggest that pipecolic acid may have a physiological function. No pathological significance seems to be associated with the accumulation of pipecolic acid in the inherited peroxisomal disorders that have been described (38). But the ability of the mammalian brain to synthesize pipecolic acid suggests a role as a neurotransmitter.

Literature Cited

1. Baginsky, M. L., Rodwell, V. W. 1967. Metabolism of pipecolic acid in a *Pseudomonas species*. V. Pipecolate oxidase and dehydrogenase. *J. Bacteriol.* 94:1034–39
2. Broquist, H. P. 1985. The indolizidine alkaloids, slaframine and swainsonine: Contaminants in animal forages. *Annu. Rev. Nutr.* 5:391–409
3. Broquist, H. P. 1989. And a little cell (child) shall lead them. *J. Nutr.* 119: 1071–74
4. Calvert, A. F., Rodwell, V. W. 1966. Metabolism of pipecolic acid in a *Pseudomonas* species. III. L-α-aminoadipate-δ-semialdchyde nictotineamide dinucleotide oxidoreductase. *J. Biol. Chem.* 241:409–14
5. Chang, Y.-F. 1976. Pipecolic acid pathway: The major lysine metabolic route in the rat brain. *Biochem. Biophys. Res. Commun.* 69:174–80
6. Chang, Y.-F., Adams, E. 1971. Induction of separate catabolic pathways for L- and D-lysine in *Pseudomonas putida*. *Biochem. Biophys. Res. Commun.* 45:570–76
7. Chang, Y.-F., Ghosh, P., Rao, V. V. 1990. L-Pipecolic acid metabolism in human liver: L-α-aminoadipate-δ-semialdehyde oxidoreductase. *Biochim. Biophys. Acta* 1038:300–5
8. Dancis, J., Cox, R. P. 1989. Errors of lysine metabolism. In *The Metabolic Basis of Inherited Diseases*, ed. E. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valde, pp. 665–70. New York: McGraw-Hill. 6th ed.
9. Dancis, J., Hutzler, J. 1982. Comparative rates of pipecolic acid metabolism in several animal species. *Comp. Biochem. Physiol. B*73:1011–12
10. Dancis, J., Hutzler, J. 1986. The significance of hyperpipecolatemia in Zellweger syndrome. *Am. J. Hum. Genet.* 38:707–11
11. Dancis, J., Hutzler, J., Cox, R. P. 1979. Familial hyperlysinemeia: Enzyme studies, diagnostic methods, comments on terminology. *Am. J. Hum. Genet.* 31: 290–99
12. Dancis, J., Hutzler, J., Woody, N. C., Cox, R. P. 1979. Multiple enzyme defects in familial hyperlysinemia. *Pediatr. Res.* 10:686–91
13. Fangmeier, N., Leistner, E. 1980. A ^{15}N NMR study on D-lysine metabolism in *Neurospora crassa*. *J. Biol. Chem.* 255:10205–9
14. Fellows, F. C. I. 1973. Biosynthesis and degradation of saccharopine, an intermediate of lysine metabolism. *Biochem. J.* 136:329–34
15. Fowden, L. 1964. The chemistry and metabolism of recently isolated amino acids. *Annu. Rev. Biochem.* 33:173–204
16. Grove, J., Henderson, L. M. 1968. The metabolism of D- and L-lysine in the intact rat, perfused liver and liver

mitochondria. *Biochim. Biophys. Acta* 165:113–20
17. Grove, J. A., Linn, T. G., Willett, C. J., Henderson, L. M. 1970. Retention of the α-nitrogen in the metabolic conversion of L-lysine to α-aminoadipate by the rat. *Biochim. Biophys. Acta* 215:191–94
18. Grove, J. A., Young, F., Roghair, H. G., Schipke, P. 1972. Lysine metabolism in rabbits. *Arch. Biochem. Biophys.* 151:464–67
19. Gupta, R. N., Spenser, I. D. 1969. Biosynthesis of the piperidine nucleus. The mode of incorporation of lysine into pipecolic acid and into piperidine alkaloids. *J. Biol. Chem.* 244:88–94
20. Higashino, K., Tsukada, K., Lieberman, I. 1965. Saccharopine, a product of lysine breakdown. *Biochem. Biophys. Res. Commun.* 20:285–90
21. Hutzler, J., Dancis, J. 1968. Conversion of lysine to saccharopine by human tissue. *Biochim. Biophys. Acta* 158:62–69
22. Hutzler, J., Dancis, J. 1983. The determination of pipecolic acid: Method and results of hospital survey. *Clin. Chim. Acta* 128:75–82
23. Kelly, W. N. 1983. Hereditary orotic aciduria. In *The Metabolic Basis of Inherited Disease*, ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, M. S. Brown, pp. 1202–26. New York: McGraw-Hill. 5th ed.
24. Kinzell, J. J., Bhattacharjee, J. K. 1982. Lysine biosynthesis in *Rhodotorula glutinis:* Properties of pipecolic acid oxidase. *J. Bacteriol.* 151:1073–77
25. Kusakabe, H., Kodama, K., Kuninaka, A., Yoshino, H., Misono, H., Soda, K. 1980. A new antitumor enzyme, L-lysine α-oxidase from *Trichoderma viride*. Purification and enzymological properties. *J. Biol. Chem.* 225:976–81
26. Markovitz, P. A. J., Chuang, D. T. 1987. The bifunctional aminoadipic semialdehyde synthase in lysine degradation. Separation of reductase and dehydrogenase domains by limited proteolysis and column chromatography. *J. Biol. Chem.* 262:9353–58
27. Markovitz, P. J., Chuang, D. T., Cox, R. P. 1984. Familial hyperlysinemias. Purification and characterization of the bifunctional aminoadipic semialdehyde synthase with lysine-ketoglutarate dehydrogenase activities. *J. Biol. Chem.* 259:11643–46
28. Meister, A., ed. 1965. *Biochemistry of the Amino Acids*, 2:928–51. New York/London: Academic. 2nd ed.
29. Mihalik, S. J., McGuinness, M., Watkins, P. A. 1991. Purification and characterization of peroxisomal L-pipecolic acid oxidase from monkey liver. *J. Biol. Chem.* In press
30. Mihalik, S. J., Moser, H. W., Watkins, P. A., Danks, D. M., Poulos, A., Rhead, W. J. 1989. Peroxisomal L-pipecolic acid oxidation is deficient in liver from Zellweger syndrome patients. *Pediatr. Res.* 25:548–52
31. Mihalik, S. J., Rhead, W. J. 1989. L-pipecolic acid oxidation in the rabbit and cynomolgus monkey. *J. Biol. Chem.* 264:2509–17
32. Mihalik, S. J., Rhead, W. J. 1990. Species variation in organellar location and activity of L-pipecolic acid oxidation in mammals. *J. Comp. Physiol. B.* In press
33. Morrison, R. I. 1953. Isolation of L-pipecolic acid. *Biochem. J.* 53:474–78
34. Nomura, Y., Schmidt-Glenewinkel, T., Giacobini, E. 1980. Uptake of piperidine and pipecolic acid by synaptosomes from mouse brain. *Neurochem. Res.* 5:1163–73
35. Rao, V. V., Chang, Y.-F. 1990. L-Pipecolic acid metabolism in human liver: Detection of L-pipecolate oxidase and identification of its reaction product. *Biochim. Biophys. Acta* 1038:295–99
36. Rothstein, M., Greenberg, D. M. 1960. The metabolism of DL-pipecolic acid-2-^{14}C. *J. Biol. Chem.* 235:714–18
37. Rothstein, M., Miller, L. L. 1954. Conversion of lysine to pipecolic acid in the rat. *J. Biol. Chem.* 211:851–58
38. Schütgens, R. B. H., Heymans, H. S. A., Wanders, R. J. A., van den Bosch, H., Tager, J. M. 1986. Peroxisomal disorders: A newly recognised group of genetic diseases. *Eur. J. Pediatr.* 144: 430–40
39. Schütte, H. R., Seelig, G. 1967. Zur biosynthese der pipecolinsaure in *Phaseolus vulgarus*. *Z. Naturforsch. Teil B* 22:824–26
40. Trijbels, M. M. F., Monnens, L. A. H., Bakkeren, J. A. J. M., Van Raay-selten, A. H. J. 1979. Biochemical studies in cerebro-hetato-renal syndrome of Zellweger: A disturbance in the metabolism of pipecolic acid. *J. Inherit. Metab. Dis.* 2:39–42
41. Trupin, J. S., Broquist, H. P. 1966. Amino acid metabolism. *Annu. Rev. Biochem.* 35:231–74
42. Wanders, R. J. A., Heymans, H. S. A., Schütgens, R. B. H., Barth, P. G., van den Bosch, H., Tager, J. M. 1989. Peroxisomal disorders in neurology. *J. Neurol. Sci.* 88:1–39
43. Wanders, R. J. A., Romeyn, G. J., Roermund, C. W. T., Schütgens, R. B. H., van den Bosch, R., Tager, J. M.

1988. Identification of L-pipecolate oxidase in human liver and its deficiency in the Zellweger syndrome. *Biochem. Biophys. Res. Commun.* 154:33–38

44. Wanders, R. J. A., Romeyn, G. J., Schütgens, R. B. H., Tager, J. M. 1989. L-Pipecolate oxidase: A distinct peroxisomal enzyme in man. *Biochem. Biophys. Res. Commun.* 164:550–55
45. Wickwire, B. M., Harris, C. M., Harris, T. M., Broquist, H. P. 1990. Pipecolic acid biosynthesis in *Rhizoctonia leguminicola*. I. The lysine, saccharopine, Δ^1-piperideine-6-carboxylic acid pathway. *J. Biol. Chem.* 265:14742–47
46. Wickwire, B. M., Wagner, C., Broquist, H. P. 1990. Pipecolic acid biosynthesis in *Rhizoctonia leguminicola*. II. Saccharopine oxidase: A unique flavin enzyme involved in pipecolic acid biosynthesis. *J. Biol. Chem.* 265: 14748–53
47. Woody, N. C., Pupene, M. B. 1971. Derivation of pipecolic acid from L-lysine by familial hyperlysinemics. *Pediatr. Res.* 5:511–13
48. Zacharius, R. M., Thompson, J. F., Steward, F. C. 1952. Detection, isolation and identification of (-)-pipecolic acid as a constituent of plants. *J. Am. Chem. Soc.* 74:2949