Although the final identification must await further progress, available evidence indicates that this intermediate compound is clearly distinguishable from either 7- or 8-monohydroxy-KA and is probably identical with or at the same oxidation level as 7,8-dihydrodiol of KA. The mechanism of the conversion of this compound to DHKA, therefore, appears to be analogous to the enzymic formation of catechol from 3,5-cyclohexadiene-1,2-diol catalyzed by a TPN-linked dehydrogenase from rabbit liver[6].

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Enzymic formation of acetyl-CoA and CO_2 from glutaryl-CoA

Glutaric acid has been shown to be an intermediate metabolite of lysine degradation[1], and recently to be a product of tryptophan metabolism in the rat[2]. Although several investigators have described the conversion *in vivo* of glutaric acid to acetic acid[2,3], the exact pathway of glutaric acid metabolism is yet to be elucidated. In this communication, we wish to report that 1 mole of glutaric acid is converted to 2 moles of acetate and 1 mole of CO_2 by a partially purified enzyme preparation from *Pseudomonas*, and glutaryl-CoA is proposed to be an intermediate in this process.

Pseudomonas fluorescens (ATCC 11299) was grown as described previously[4], except that 0.1 % glutarate and 0.5 % $(NH_4)_2SO_4$ were used as major carbon and nitrogen sources, respectively. Cell-free extracts were prepared by extracting 5 g of acetone-dried cells with 50 ml 0.02 *M* potassium phosphate buffer, pH 6.8, for 20 min at 0°, followed by centrifugation at 20,000 × *g* for 30 min. The supernatant fraction thus obtained was treated with protamine sulfate.

The reaction mixture (1.0 ml) containing 100 μmoles potassium glutarate, 10 μmoles ATP, 0.5 μmole CoA, 10 μmoles of reduced glutathione, 5 μmoles of $MgCl_2$, 1,000 μmoles of salt-free NH_2OH, 100 μmoles phosphate buffer, pH 6.3, and 30 mg of the enzyme preparation which had been treated with both Dowex 1 and charcoal, was incubated at 35° for 60 min. 6.5 μmoles of a hydroxamate derivative were produced[5], which was tentatively identified as glutaromonohydroxamate by paper chromatography using four different solvent systems* and high-voltage paper electrophoresis**. In the absence of ATP or CoA, practically no glutaromonohydroxamate was produced.

When GP was incubated with the enzyme preparation under aerobic conditions, approximately 2 moles acetate and 1 mole CO_2 were produced for each mole of GP utilized (Table I). When [1,5-^{14}C]GP was employed as a substrate, total radio-

TABLE I

STOICHIOMETRY OF THE OVERALL REACTION

The reaction mixture contained 15 μmoles [1,5-^{14}C]GP (5,800 counts/min/μmole) or [3-^{14}C]GP (5,790 counts/min/μmole), 50 μmoles of phosphate buffer, pH 6.8, and 10 mg of the enzyme preparation in a final volume of 1.0 ml. After incubation was carried out for 60 min at 35°, the reaction was stopped by the addition of 0.2 ml of 2 *N* KOH, and the mixture was heated for 1 min in a boiling-water bath in order to hydrolyze the thiol esters. Then the mixture was acidified by the addition of 0.5 ml 2 *N* H_2SO_4, and CO_2 evolved was trapped in alkali, An aliquot of the remaining solution was chromatographed on a silicic acid column (20 × 1 cm) and was eluted with a chloroform–*n*-butanol mixture. 2-ml fractions were collected, and titrated with 0.002 *N* KOH. Radioactivity was measured with an aliquot of each fraction.

Substrate	*Gas phase*	*Δ Glutarate*		*Δ CO_2*		*Δ Acetate*		
		Total (counts/min)	*Titration (μmoles)*	*Total (counts/min)*	*Δ* (μmoles)*	*Total (counts/min)*	*Titration (μmoles)*	*Specific activity (counts/min/μmole)*
[1,5-^{14}C]Glutaryl-pantetheine	air	−12,400	−2.0	+5,660	+1.9	+ 5,520	+3.5	1,620
	N_2	−400	−0.2	+20	—	+20	0.2	—
[3-^{14}C]Glutaryl-pantetheine	air	−11,100	−2.0	+70	—	+10,210	+3.6	2,740
	N_2	−500	−0.2	0	—	+30	0.2	—

* Calculated from radioactivity.

activity of CO_2 was almost equal to that of acetate and the specific activity of acetate was approximately one fourth of that of the original substrate. With [3-^{14}C]GP***, however, CO_2 evolved contained essentially no radioactivity but the specific activity of acetate was diluted approximately 2-fold. When neutral NH_2OH was added after the incubation at a final concentration of 0.5 *M*, a new hydroxamate was formed which was identified as acetohydroxamate by paper chromatography and

Abbreviations: CoA, coenzyme A; GP, glutarylpantetheine; ATP, adenosine triphosphate.

* Paper chromatography was carried out on Whatman No. 1 paper. Spots were visualized by spraying with an acidic $FeCl_3$ solution. R_F values of hydroxamates of glutarate and acetate with xylol–phenol–formic acid (5:5:2) were 0.46 and 0.62; isobutyric acid–*n*-butanol–water (2:2:1), 0.40 and 0.50; with *n*-propanol–ammonia (3:2), 0.12 and 0.33; with ethanol–ammonia–water (20:1:4), 0.40 and 0.54, respectively.

** High-voltage paper electrophoresis was carried out on Whatman No. 3 paper (15 × 55 cm; pyridine–acetic acid buffer, pH 6.5[6]; coolant, *n*-hexane; 2,000 V; 40 min). Mobilities of glutaromono- and acetohydroxamate were 8.0 cm to the anode and 1.2 cm to the cathode, respectively.

*** The authors are indebted to Dr. R. E. KOEPPE for a generous sample of [3-^{14}C]glutaric acid.

high-voltage paper electrophoresis. Acetohydroxamate thus obtained from either [1,5-^{14}C]GP or [3-^{14}C]GP contained radioactivity. However, when free [^{14}C]acetate was incubated together with cold GP under the same conditions, no radioactivity was incorporated into the acetohydroxamate isolated. The radioactive acetate formed from either [1,5-^{14}C]GP or [3-^{14}C]GP contained almost all radioactivity in the carboxyl carbon[7]. These results indicate that free carboxyl carbon (C-5) of GP was converted to CO_2, and both C-3,4 and C-1,2 of GP were converted to C-1,2 of acetylpantheteine respectively. Under anaerobic conditions, essentially no acetate or CO_2 was produced, but oxygen could be replaced by pyocyanine and 2,3,5-triphenyltetrazolium chloride. Radioactive acetate enzymically formed was identified by paper chromatography using three different solvent systems*, and by partition chromatography on a silicic acid column[9]. Further evidence for the identity was provided by the constant specific activity upon recrystallization of its *p*-bromophenacyl ester[10].

Similar results were obtained with glutaryl-CoA, and acetyl-CoA enzymically formed was identified with *p*-nitroaniline-acetylating enzyme from pigeon liver[11]. Further studies are currently in progress in order to elucidate the intermediates from glutaryl-CoA to acetyl-CoA**.

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* Paper chromatography was carried out on Whatman No. 1 paper. Spots were visualized by spraying with reduced ninhydrin[8]. R_F value of acetate with ethanol–ammonia–water (20:1:4) was 0.52; with *n*-propanol–ammonia (3:2), 0.66 and with tetrahydrofuran–ammonia–water (15:2:3), 0.62.

** Recently it was reported that glutaryl-CoA was isomerized to ethylmalonyl-CoA, followed by decarboxylation to butyryl-CoA in animal tissues[12,13], but this isomerization of glutaryl-CoA was not observed in our bacterial system. Neither butyryl-CoA nor succinyl-CoA was converted to acetyl-CoA under the above conditions.