THE DEGRADATION OF L-LYSINE IN GUINEA PIG LIVER HOMOGENATE: FORMATION OF α-AMINOADIPIC ACID*

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A summary of the little that is known of the metabolism of lysine in animals is as follows: it is indispensable in the diet, its α-amino group does not participate in reversible transamination reaction *in vivo* (2), neither the L nor D form is attacked by the appropriate amino acid oxidase, certain ε-nitrogen-substituted derivatives can replace lysine in the diet and their α-amino groups are oxidized by amino acid oxidases (3, 4), no α-nitrogen-substituted derivatives yet prepared can substitute for lysine in the diet (4–6).

Partly because so little was known, we have undertaken a study of the metabolism of lysine with the use of C^{14} as a tracer. The amino acid was synthesized with the isotope in the ε position and resolved into its L and D isomers. In order to observe in an initial exploration as many aspects of its metabolism as possible, one or the other isomer was made into a mixture with unlabeled amino acids, corresponding to the composition of casein, and incubated with guinea pig liver homogenate under different conditions.

The present communication deals with the finding of α-aminoadipic acid as a product of the degradation of lysine.

Preparations

Synthesis of C^{14}-Labeled Lysine—The steps in the synthesis are summarized in the following diagram; the position of the C^{14} is indicated by an asterisk.

$BaC^*O_3 \rightarrow C^*O_2 \rightarrow KC^*N \rightarrow NC^*\cdot CH_2\cdot CH_2\cdot CH_2Cl$ (γ-chlorobutyronitrile)
$\rightarrow NC^*\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH\cdot (COOC_2H_5)_2$ (Diethyl (3-cyano-*n*-propyl) malonate)
$\rightarrow NC^*\cdot CH_2\cdot CH_2\cdot CH_2\cdot C(=NOH)\cdot COOC_2H_5$ (ethyl 2-oximido-5-cyanovalerate)
$\rightarrow H_2N\cdot H_2C^*\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(-NH_2)\cdot COOH$ (DL-lysine)

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* This work is a part of that done under contract with the Office of Naval Research, United States Navy Department. A summary of this work has been reported (1).

The C^{14} used in this investigation was supplied by the Monsanto Chemical Company, Clinton Laboratories, Oak Ridge, Tennessee, and obtained on allocation from the United States Atomic Energy Commission.

*KC*N*—309.8 mg. of BaC^*O_3 was converted to KC*N by the method of Cramer and Kistiakowsky (7) and Loftfield (8, 9). The strongly alkaline solution obtained after decomposition of the excess potassium was concentrated to 4.7 gm. 90 per cent of the radioactivity was incorporated into the KC*N.

*γ-Chlorobutyronitrile**—KCN was converted to γ-chlorobutyronitrile by a modification of the procedure previously described (10). To the KC*N were added 1.253 gm. of inactive KCN dissolved in 4 ml. of H_2O and 28 ml. of absolute ethanol. The solution was titrated slowly at 0° to pH 10.8 with 6 N HCl; 3.8 ml. were consumed. The glass electrode was washed with 2 ml. of H_2O and the washings added to the solution, which was then refluxed with 19.2 gm. of trimethylene chlorobromide with vigorous stirring for $5\frac{1}{2}$ hours. After cooling, the solution was diluted with 45 ml. of water, and the two layers separated. To the lower layer was added a chloroform extract (12 ml.) of the top layer. The mixture was washed with 10 ml. of a 16 per cent $CaCl_2$ solution, then with 3 ml. of water, and dried over fused $CaCl_2$. The chloroform was removed by distillation at atmospheric pressure, and the residue distilled *in vacuo;* the product obtained at 89–93° and 24 mm. was used for the next step. Yield, 1.535 gm., or 75 per cent.

*Diethyl (3-Cyano-n-propyl) Malonate**—From the nitrile, DL-lysine was prepared by a modification of the method of Fischer and Weigert (11). 1.0 gm. of sodium in a Carius tube was dissolved in 10 ml. of absolute ethanol. After cooling there were added 13.3 gm. of diethyl malonate, then a mixture of 13.3 gm. of diethyl malonate and 2.74 gm. of chlorobutyronitrile*, followed by 13.3 gm. of diethyl malonate. The tube was sealed and heated for 15 hours at 95–98°. Ethanol, malonate, and remaining nitrile were removed by steam distillation, the residue extracted with ether, and the ether extract dried over potassium carbonate for 15 minutes, filtered, and distilled at 0.5 mm. Yield, 5.20 gm. (86 per cent); b.p. 128–131° at 0.5 mm.

*Ethyl 2-Oximido-5-cyanovalerate**—0.166 gm. of sodium was dissolved in 2.64 ml. of absolute ethanol and cooled to −18°. A mixture of 1.13 ml. of ethyl nitrite with 1.628 gm. of cold diethyl (3-cyano-*n*-propyl) malonate*, cooled to 0°, was then added dropwise into the ethylate solution and washed down with 0.66 ml. of cold absolute ethanol. After standing for 21 hours at −15° to −10°, the alcohol was removed at room temperature *in vacuo*. The residue, dissolved in 10 ml. of water, was extracted with 5 ml. of ether, and the ether washed with two 1 ml. portions of water. The combined aqueous solutions were cooled in ice, acidified with 10 per cent sulfuric acid, and extracted with ether. After removal of the solvent *in vacuo* and drying over sulfuric acid, the ester crystallized. Yield, 1.195 gm. (90.5 per cent).

DL-*Lysine**—In a flask with a reflux condenser 1.252 gm. of ethyl 2-oximido-5-cyanovalerate* were dissolved in 100 ml. of absolute ethanol, and 10 gm. of sodium added in small pieces as rapidly as possible. After 75 minutes the metal was almost completely dissolved. 10 ml. of water were added, and the solution refluxed for 45 minutes. It was then cooled in ice and acidified by adding 50 per cent H_2SO_4 slowly with vigorous stirring. The sodium sulfate was filtered off and washed several times by suspension in 95 per cent ethanol. Filtrate and washings were concentrated *in vacuo*, and the residual alcohol removed by steam distillation. The remaining aqueous solution was made alkaline to phenolphthalein with barium hydroxide, boiled, and the barium sulfate filtered off. The excess barium was removed with carbon dioxide and the filtrate concentrated to a syrup *in vacuo*. The syrup was taken up in ethanol, and a 5 per cent alcoholic solution of picric acid added dropwise with stirring until further addition caused no more turbidity. After standing at 0° overnight the crude picrate was filtered off and washed with cold absolute ethanol and ether. After two recrystallizations, first from 10 ml. and then from 5 ml. of hot water, 0.656 gm. of lysine* picrate was obtained. Yield, 26 per cent.

The picrate was converted to the hydrochloride by dissolving it in 13 ml. of hot water and 2.3 ml. of concentrated HCl. After the solution was cooled, the picric acid was extracted with ether. The aqueous phase, on evaporation *in vacuo* and drying over H_2SO_4 and NaOH, gave DL-lysine dihydrochloride* in quantitative yield.

Resolution of DL-*Lysine**—The DL-lysine was resolved by the carbobenzoxy-aniline-papain method of Bergmann *et al.* (12–14).

Carbobenzoxy-DL-*Lysine**—0.430 gm. of DL-lysine* dihydrochloride was dissolved in 2.9 ml. of 2 N NaOH and cooled in ice. 1.1 ml. of carbobenzoxychloride and 2.3 ml. of 4 N NaOH were added in four portions, and the mixture shaken vigorously for 25 minutes while cooling in ice. After extraction with ether the aqueous phase was acidified with HCl and the carbobenzoxylysine taken up with ether. It weighed 0.733 gm. after evaporation and drying *in vacuo;* m.p. 100–103°.

Carbobenzoxy-L-*Lysine Anilide**—0.730 gm. of carbobenzoxy-DL-lysine* was dissolved in 1.9 ml. of N NaOH and 1.8 ml. of water. To this solution were added 0.46 ml. of aniline, 7.1 ml. of a 0.3 per cent aqueous solution of cysteine hydrochloride, 8.2 ml. of citrate buffer solution (pH 5.0), 16.4 ml. of water, 2.5 ml. of a solution of 0.100 gm. of papain in 2 ml. of water, and 2 ml. of citrate buffer. After incubation at 40° for 19 hours, the L-anilide was filtered off and washed once with 1 per cent potassium bicarbonate solution and thrice with water. Yield, 0.465 gm. Recrystallized from 50 per cent ethanol, it melted at 121–122°.

Analysis—$C_{28}H_{31}O_5N_2$ (489.55). Calculated. C 68.70, H 6.38, N 8.59
Found. " 68.65, " 6.65, " 8.75

L-*Lysine Dihydrochloride**—The anilide was refluxed with 4.5 ml. of 6 N HCl for 2 hours. After cooling, the mixture was diluted with 30 ml. of water and made alkaline with freshly prepared silver oxide. After the silver chloride and excess silver oxide were filtered off and washed with water, the filtrate was extracted with ether. The clear aqueous phase was concentrated *in vacuo* to 20 ml., acidified with HCl, and allowed to stand for 30 minutes with occasional shaking. After filtration the liquid was evaporated to dryness *in vacuo* over NaOH and H_2SO_4 to give a quantitative yield of lysine dihydrochloride. $[\alpha]_D^{23} = +15.95°$, in water; $c = 5.46$. Berg (15) reports rotations from +15.63° to +16.55° for values of c from 3.00 to 16.00.

D-*Lysine Dihydrochloride**—The filtrate (without washings) of the L-anilide was incubated with 25 mg. of papain for 5 days at 40°. After removing the precipitated mixed L- and D-anilides, the filtrate was acidified with HCl and extracted with ether. 0.243 gm. of carbobenzoxy-D-lysine remained upon evaporation of the solvent. After refluxing with 3.0 ml. of 6 N HCl for 2 hours, extraction with ether, and evaporation of the aqueous phase, 0.130 gm. of D-lysine dihydrochloride was obtained. This was recrystallized from ethanol and yielded 99 mg. of purified material. $[\alpha]_D^{23} = -13.5°$, in water; $c = 4.47$. The rotation corresponds to approximately 92.5 per cent D and 7.5 per cent L form.

Analysis—$C_6H_{14}O_2N_2 \cdot 2HCl$. Calculated. N 12.79, Cl 32.36
Found. " 12.44, " 31.82

The total average yield of L- and D-lysine dihydrochloride obtained in several complete runs was 6 per cent, calculated on the KCN used. In an experiment in which $BaCO_3$ containing 4 millicuries of radioactivity was used, 230 mg. of L-lysine dihydrochloride with a specific radioactivity of 24,000 counts (corrected) per minute per mg. were obtained.

All the radioactivity measurements are expressed as counts (corrected) per minute. They were obtained with standard sample geometry, and corrected for background and resolving time. By means of empirically determined curves of self-absorption loss in different thicknesses of sample, they were corrected to maximum specific activity (16).

DL-α-*Aminoadipic Acid*—DL-α-Aminoadipic acid was prepared by a modification of the method of Sørensen (17). 1.228 gm. of chlorobutyronitrile and 2.060 gm. of diethyl sodium phthalimidomalonate were refluxed in a bath at 160–165°. After 4 hours the alkaline reaction had disappeared, and the excess nitrile was removed by steam distillation. The residue was cooled in ice and washed with water several times by triturating and decant-

ing. It weighed 2.050 gm. after drying. After solution in 12.5 ml. of absolute ethanol, it was heated under a reflux on a steam bath with 11 ml. of 4 N NaOH for 3 hours. 16 ml. of concentrated HCl were then added, the heating continued for 3 hours, and the solution evaporated on the steam bath. The residue was cooled in ice and extracted by repeated washing with ice-cold 33 per cent hydrochloric acid. The solution was filtered through a coarse fritted glass funnel, and evaporated. The residue, weighing 1.206 gm. after drying over H_2SO_4 and NaOH, was dissolved in 2.5 ml. of water, filtered into a 10 ml. beaker, and washed with 1.3 ml. of water. The aminoadipic acid was precipitated by titrating to pH 3.1 with 5 N NH_4OH. After standing for 4 hours at 22°, it was filtered off and washed with a few ml. of water, alcohol, and ether. Yield, 0.629 gm. (62 per cent based on diethyl sodium phthalimidomalonate).

The α-aminoadipic acid was resolved in the same manner as was lysine by the carbobenzoxy-anilide-papain method of Bergmann.

Carbobenzoxy-DL-α-aminoadipic acid, m.p. 124°.

Analysis—$C_{14}H_{17}O_6N$ (295.28). Calculated. C 57.28, H 5.82, N 4.75
Found. " 57.08, " 5.84, " 4.89

Carbobenzoxy-L-aminoadipic acid anilide, m.p. 170–171°.

Analysis—$C_{20}H_{22}O_5N_2$ (370.39). Calculated. C 64.85, H 5.99, N 7.56
Found. " 65.56, " 6.04, " 8.30

The L-α-aminoadipic acid melted at 205° with decomposition. Its specific rotation was $[\alpha]_D^{25} = +33.9°$, in 6 N HCl; $c = 5.49$.

Analysis—$C_6H_{11}O_4N$ (161.17). Calculated. C 44.72, H 6.88, N 8.70
Found. " 44.83, " 6.81, " 8.65

Procedure

Guinea pig liver was homogenized in the apparatus of Potter and Elvehjem (18) with a volume of saline solution equal to twice the weight of the liver. The composition of the saline solution was as follows: 0.123 M NaCl, 0.0128 M Na_2HPO_4, 0.005 M KCl, 0.0033 M $MgSO_4$. Unless stated otherwise the pH was 7.5.

The reaction mixture consisted of 2 ml. of homogenate, a sufficient quantity of a mixture of amino acids to provide a final concentration of 1.2 per cent, and 0.01 M α-ketoglutarate. The amino acid mixture corresponded approximately to the composition of casein. All of the lysine therein contained (10 mg. of the dihydrochloride) was labeled in the ε position with C^{14} (7400 counts (corrected) per mg. per minute). The final volume was 4 ml.; KOH was used for the neutralization.

The reaction mixture was incubated at 38° under oxygen for 6 hours,

after which the pH was adjusted to 5.0. The mixture was then placed in a boiling water bath for 10 minutes, filtered, the coagulated protein thoroughly washed with water, and the washings added to the main filtrate. The non-protein filtrate thus obtained was concentrated *in vacuo*.

Results

A small fraction of the non-protein filtrate from an experimental run conducted at pH 7.5 was chromatographed on filter paper with phenol and *s*-collidine, and the paper treated with ninhydrin (19). Two radioactive ninhydrin spots were found, one in the position of lysine, the other of glutamic acid. The radioactivity in the latter spot excluded its being glutamic acid, as the probable mechanism for the conversion of lysine to glutamic acid entails cleavage of the radioactive ε-carbon of lysine. It seemed likely that the substance in question was α-aminoadipic acid, $C^*OOH \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$, derived from lysine.

Accordingly, α-aminoadipic acid was synthesized and chromatographed on filter paper. It gave the same chromatogram as the unknown radioactive substance.

This lead, that the radioactive substance in question might be α-aminoadipic acid, was followed. The main portion of the non-protein filtrate, from a reaction mixture to which 20 mg. of radioactive L-lysine dihydrochloride had been added, was hydrolyzed by boiling overnight with 20 per cent HCl in order to hydrolyze any peptides present. The latter step was necessary for satisfactory chromatography on Lloyd's reagent (20); it also converted any of the piperidone of α-aminoadipic acid, which might have been formed, to the straight chain.[1] After removal of the HCl by distillation, the hydrolysate was chromatographed on Lloyd's reagent. The fraction containing all the amino acids except the bases had about 5 per cent of the radioactivity originally added as lysine. It was concentrated to dryness, extracted with ether, and the residue taken up in water and decolorized by boiling with charcoal. The combined filtrate and washings were evaporated to dryness, taken up in 3 ml. of water, and treated with solid $Ba(OH)_2$ until the pH was 6.0. A small amount of radioactivity was in the precipitate; by far the major portion remained in the solution. Absolute ethanol was added to the latter to a final concentration of 95 per cent. The barium precipitate contained all the radioactivity originally in the solution. The barium was removed with sulfuric acid, the precipitation and resolution repeated three times, and after final removal of the barium the filtrate was concentrated to near dryness. A drop was chromatographed on filter paper. Four ninhydrin spots were obtained; one

[1] α-Aminoadipic acid like glutamic acid cyclizes. Both forms of both amino acids chromatograph alike on filter paper with phenol and *s*-collidine.

in the glutamic acid region was radioactive, and the other three were in the alanine, aspartic acid, and threonine regions.

Concentrated HCl was added to the main portion of the filtrate and concentrated under a lamp until crystallization set in. About 100 mg. of crystals were collected. They gave a total of 630 counts (corrected) per minute. The mother liquor was found subsequently to have 7650 counts (corrected) per minute. The crystals gave three ninhydrin spots on the filter paper chromatogram; one in the glutamic acid region was radioactive, and the other two were in the aspartic acid and alanine regions.

The radioactive spot certainly contained α-aminoadipic acid mixed with glutamic acid. We have tried to separate α-aminoadipic and glutamic acids by chromatography on filter paper with a number of solvent mixtures; none effected a separation.

The hydrochloric acid mother liquor was evaporated to dryness and the dry residue dissolved in 0.1 N HCl. Solid $Ba(OH)_2$ was added until the solution was alkaline to phenolphthalein, and then ethanol to a concentration of 75 per cent. 351 mg. of barium salt were obtained, giving a total of 7650 counts (corrected) per minute. The barium was removed with H_2SO_4, the filtrate concentrated to dryness, and the residue dissolved in 0.1 N HCl. The presence of α-aminoadipic acid in it was determined by crystallization after adding to a portion of the solution, containing approximately 1650 counts (corrected) per minute, 100 mg. of non-radioactive α-aminoadipic acid as a carrier. The quantity of carrier was about 600 times that of the radioactive form.[2]

The solution was brought to pH 3.1 with ammonia and then concentrated slowly at room temperature under a low vacuum. The crystals which separated out were washed with a small amount of water and then ethanol, dried, and their radioactivity determined. Three recrystallizations were carried out, in the course of which 80 per cent of the carrier was left in the mother liquors. The specific activities of the crystals after each of the four crystallizations were consecutively 11.5, 13.7, 13.2, and 13.8 counts (corrected).

When 13.5 counts (corrected) are taken as the specific activity of the α-aminoadipic acid after addition of 100 mg. of carrier, 1350 counts (corrected) or 82 per cent of the radioactivity in the solution were in the α-aminoadipic acid formed from the radioactive lysine added to the reaction

[2] This estimate was made as follows. The lysine dihydrochloride used had a specific activity of 7400 counts (corrected). The equivalent specific activity as α-aminoadipic acid was, therefore $(217/161) \times 7400 = 9970$ counts (corrected). A total of 1650 counts in the solution would, then, be given by 0.165 mg. of α-aminoadipic acid derived from the radioactive lysine added. Its dilution by the carrier would be $100/0.165 = 606$.

mixture. Constant specific activity was obtained, evidently, on the first recrystallization.

The crystals remaining after the third recrystallization gave the following data, which correspond to those of α-aminoadipic acid. M.p., 204–205° with decomposition.

Analysis—$C_6H_{11}O_4N$ (161.17). Calculated. C 44.72, H 6.88, N 8.70
Found. " 44.84, " 7.03, " 8.69

A number of experiments were carried out with boiled liver homogenate and with D-lysine obtained from the resolution of the synthesized radioactive DL-lysine.

TABLE I

α-Aminoadipic Acid Formation from Lysine

The reaction mixture contained, in a total volume of 4 ml. of saline solution, 0.66 gm. (wet weight) of homogenized guinea pig liver, 10 mg. of radioactive lysine dihydrochloride (7400 counts (corrected) per mg. per minute), 1.2 per cent (based on the final volume) of an amino acid mixture having a composition corresponding to that of casein, and 0.01 M α-ketoglutarate. The mixture was incubated at 38° under oxygen for 6 hours.

The values are total counts (corrected) of the barium salt of the dicarboxylic acid fraction.

pH	Treatment of homogenate	Isomer of lysine used	Total counts per min.
7.5	Boiled	L	0
7.5	Unboiled	"	3690
8.5	Boiled	"	0
8.5	Unboiled	"	1250
7.5	Boiled	D	0
7.5	Unboiled	"	180
8.5	Boiled	"	0
8.5	Unboiled	"	115

The results are summarized in Table I. They are expressed as counts (corrected) per minute in the dicarboxylic acid fraction obtained after chromatography on Lloyd's reagent and precipitation from 75 per cent ethanol as the barium salt. Only the reaction mixtures with L-lysine and unboiled homogenate at pH 7.5 and 8.5 were carried through to the final stages of identification by dilution with carrier and recrystallization to constant specific activity. There was too little radioactivity in the others.

The data show that more α-aminoadipic acid was formed at pH 7.5 than at pH 8.5. In preliminary experiments at pH values 7.5, 8.2, 8.5, and 9.0, the non-protein filtrates were chromatographed on filter paper, the ninhydrin spots in the glutamic acid region dissolved off, and their radio-

activity measured. The highest counts were obtained at pH 7.5 and they diminished progressively with increasing pH.

The low counts (Table I) obtained when D-lysine was used may be ascribed to the 7.5 per cent of L-lysine in the D-lysine preparation.

No α-aminoadipic acid was formed when the homogenate was boiled before it was added to the reaction mixture and incubated.

A rough estimate of the rate of conversion of lysine to α-aminoadipic acid was obtained by comparing the total number of counts in the dicarboxylic acid fraction with that added as lysine. In a typical experiment at pH 7.5, 74,000 counts (corrected) were added as L-lysine dihydrochloride. 3700 counts (corrected) were found in the dicarboxylic acid fraction after 6 hours incubation with liver homogenate. In 6 hours, therefore, 5 per cent of the added lysine was converted to α-aminoadipic acid. Of the 10 mg. of lysine dihydrochloride (equivalent to 6.72 mg. of lysine) added, 0.336 mg. was converted to α-aminoadipic acid. Expressed as a Q value,[3] the rate was 0.065. This is about one-hundredth that of urea formation in liver slices or homogenates (21, 22) and about the same as that of the methylation of guanidoacetic acid by methionine (23).

This Q value is an underestimate. Some of the α-aminoadipic acid formed is converted to α-ketoadipic acid and to glutaric acid (24).

The solubilities of α-aminoadipic and of glutamic acids and their salts are so similar that the former could not be detected in analyses of the amino acid composition of proteins by any of the isolation methods in use. Nor have we been able to separate them by filter paper chromatography. The two amino acids are separated by chromatography on starch by the method of Moore and Stein (25), with a solvent consisting of 1 part of 0.1 N hydrochloric acid, 2 parts of *n*-propanol, and 1 part of *n*-butanol. Moore and Stein[4] and we have found that the α-aminoadipic acid emerges from the column considerably in advance of proline, whereas glutamic acid (with alanine) is in the effluent after proline. With this method we are now investigating whether or not α-aminoadipic acid is present in proteins.

DISCUSSION

The only previous report, of which we are aware, of α-aminoadipic acid in biological material is that of Blass and Macheboeuf (26). These authors isolated from cholera *Vibrio* two compounds whose elementary analyses, reactions on acetylation, and ultraviolet spectra corresponded to α-amino-

[3] Q is the change in amount of the substance in question expressed as if it were a gas in c.mm., at standard temperature and pressure, per mg. of dry weight of tissue used per hour. The dry weight of the liver used was 130 mg.; the time was 6 hours.

[4] Personal communication.

adipic acid and hydroxy-α-aminoadipic acid. Neither compound was identified with certainty, but there is little room for doubt that they were the amino acids named. The α-aminoadipic acid amounted to 1.6 per cent and the hydroxy-α-aminoadipic acid to 1.0 per cent of the dry weight of the organism.

Neuberger and Sanger (3, 4) have presented evidence that before the α-amino group of L-lysine can be attacked by animal tissue enzymes the ε-amino group must be masked, preferably by acylation. They discussed some possible pathways of the degradation of lysine *in vivo*. In one of them, formation of α-aminoadipic acid is the first step. The latter surmise is now substantiated by the evidence presented above. α-Aminoadipic acid, as the first (or one of the first) intermediate in the degradation of lysine, is in accord with the enzymatic findings of Neuberger and Sanger, in that conversion of the ε-amino group to a carboxyl group is analogous to acylation. It also accounts for the failure of the α-amino nitrogen of lysine to participate in reversible transamination reactions *in vivo*. It is converted to α-aminoadipic acid before it yields its α-amino nitrogen.

We have previously reported (27) evidence of the probable formation of α-aminoadipic acid from lysine in kidney.

Mitchell and Houlahan (28) have found that α-aminoadipic acid can replace L-lysine in one lysine-requiring *Neurospora* mutant. The accumulation of large quantities of α-aminoadipic acid and of hydroxy-α-aminoadipic acid in cholera *Vibrio* points to unusual features of lysine metabolism in that organism which is analogous to those found in mutants of microorganisms.

SUMMARY

1. The synthesis and resolution of lysine labeled with C^{14} in the ε position and the synthesis and resolution of α-aminoadipic acid are described.

2. α-Aminoadipic acid is formed from L-lysine in guinea pig liver homogenate. D-Lysine is inactive.

3. Over the pH range 7.5 to 9.0, the reaction is fastest at pH 7.5. Boiling destroys the catalytic activity of the homogenate.

4. α-Aminoadipic acid can be separated from glutamic acid by chromatography on starch.

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