METABOLISM OF D,L-LYSINE-6-C^{14} AND D,L-LYSINE-2-C^{14} IN RATS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

L-lysine (1) is an indispensable constituent of the diet for all animals that have been studied. In rats the nutritional requirement for lysine is specific since only the L isomer and a few derivatives in which the epsilon amino group has been substituted (e.g., the ε-N-methyl and ε-N-acetyl compounds) support their growth (2, 3). The α-N-acetyl derivative of lysine is ineffective in this regard, but data from isotope experiments indicate that this derivative can be converted slowly to lysine in vivo (4, 5).

The intermediary metabolism of lysine has been the subject of considerable investigation. In 1954, Rothstein and Miller (6) found in rats that lysine is metabolically converted to pipecolic acid with the loss of the α-amino nitrogen, but not of the ε-amino nitrogen. Their data supported the view that pipecolic acid lies on the metabolic pathway between lysine and α-aminoadipic acid. This is shown in Figure 1.

Rothstein and Miller (7) also considered that lysine is metabolized in vivo as follows:

$$\text{Lysine} \longrightarrow \alpha\text{-Aminoadipate} \longrightarrow \text{Glutarate} \longrightarrow \begin{matrix}\alpha\text{-Ketoglutarate} \\ + \\ \text{Acetate}\end{matrix}$$

When L-lysine was incubated (8) with a guinea pig liver homogenate at 38°, α-aminoadipic acid was formed; but D-lysine was inactive. In Neurospora (9), the end product of L-lysine metabolism is L-pipecolic acid.

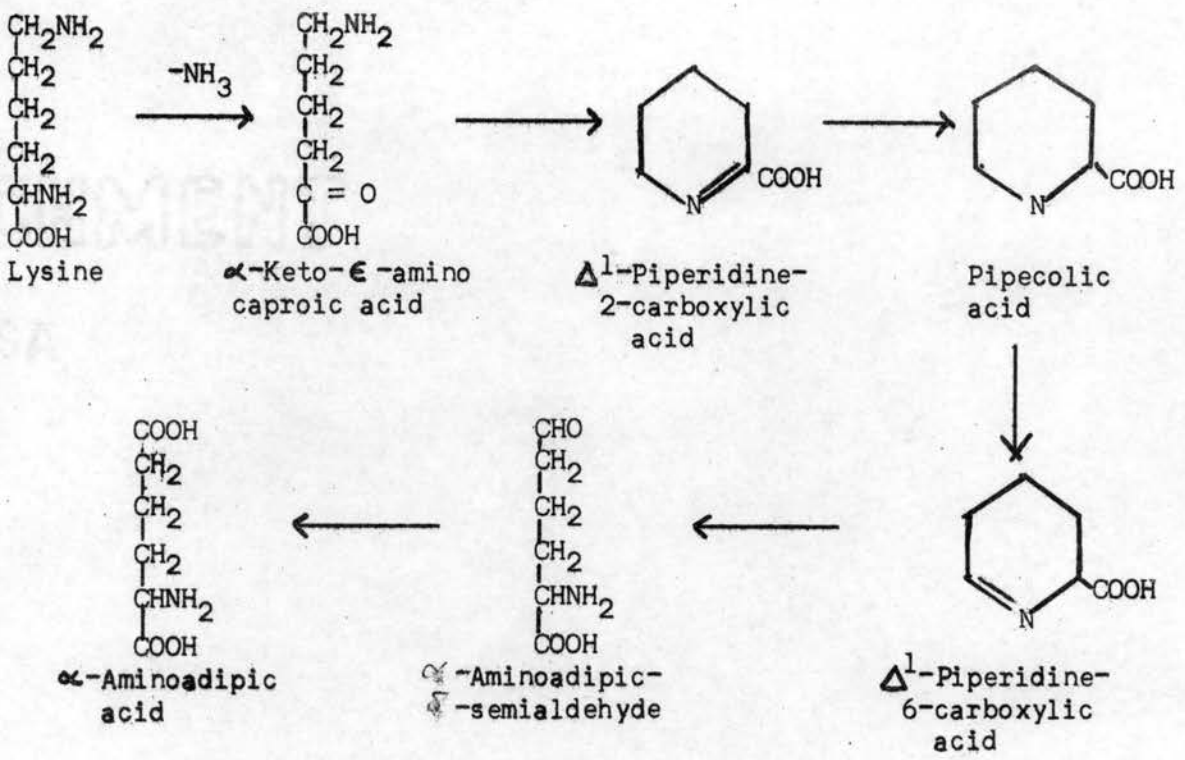
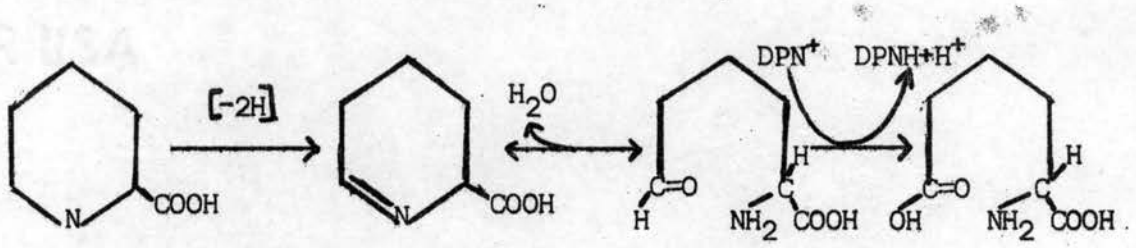


Figure 1. The Pathway of Metabolic Conversion of Lysine to α-Aminoadipic Acid

Pipecolic acid was reported by Rothstein and Greenberg (10, 11) to be an intermediate in the catabolic pathway of lysine, lying between L-lysine and α-aminoadipic acid. The suggestion that the interconversion of pipecolic acid and α-aminoadipic acid might involve the intermediate formation of Δ^1-piperidine-6-carboxylic acid and α-aminoadipic acid-δ-semialdehyde, has been studied by Rao and Rodwell (12). The Δ^1-piperidine-6-carboxylic acid, which is isolated in equilibrium with α-aminoadipic-δ-semialdehyde, is a product of the metabolism of pipecolic acid by resting cell suspensions of Pseudomonas P2 (12). The probable pathway (12) for pipecolic acid oxidation is shown in Figure 2.

That α-aminoadipic acid is converted to glutaric acid via α-ketoadipic acid was shown by Borsook (13). The proposed metabolic pathway of lysine in rats (10) is summarized in Figure 3. When incubated (10)

with rat liver mitochondria, glutaric acid, ∝-aminoadipic acid and pipecolic acid are oxidized to carbon dioxide in the presence of ATP. Hobbs and Koeppe (14) showed that glutarate-3-C^{14} is converted to acetate rather than directly to the carbon chain of ∝-ketoglutarate as postulated by Rothstein and Miller.(7).



Pipecolic acid — Δ^1-Piperidine-6-carboxylic acid — ∝-Aminoadipic acid-δ-semialdehyde — ∝-Aminoadipic acid

Figure 2. The Probable Pathway for Pipecolic Acid Oxidation

Rothstein and Miller (15) isolated urinary acetate-1-C^{14}, acetoacetate-1-C^{14}, and glucose-3, 4-C^{14} following the administration of glutaric acid-1, 5-C^{14} to normal and phlorhizinized rats. On the basis of the labeling pattern these authors postulated two possible pathways:

1. Glutaric acid ⟶ butyrate ⟶ acetoacetate ⟶ acetate
2. Glutaric acid ⟶ β-ketoglutarate ⟶ acetoacetate ⟶ acetate.

In 1960, Rothstein and Greenberg (10) were able to demonstrate that $C^{14}O_2$ was released from glutarate-1, 5-C^{14} by rat liver mitochondria.

Stern et al. (16, 17) suggested that acyl-CoA derivatives were the actual intermediate in glutarate metabolism based on the findings that various animal tissues, including liver, possessed a specific glutarate activating enzyme which catalyzed the conversion of glutarate to glutaryl-CoA in the presence of CoA and ATP.

While studing the enzymic carboxylation of crotonyl-CoA, Tustanoff

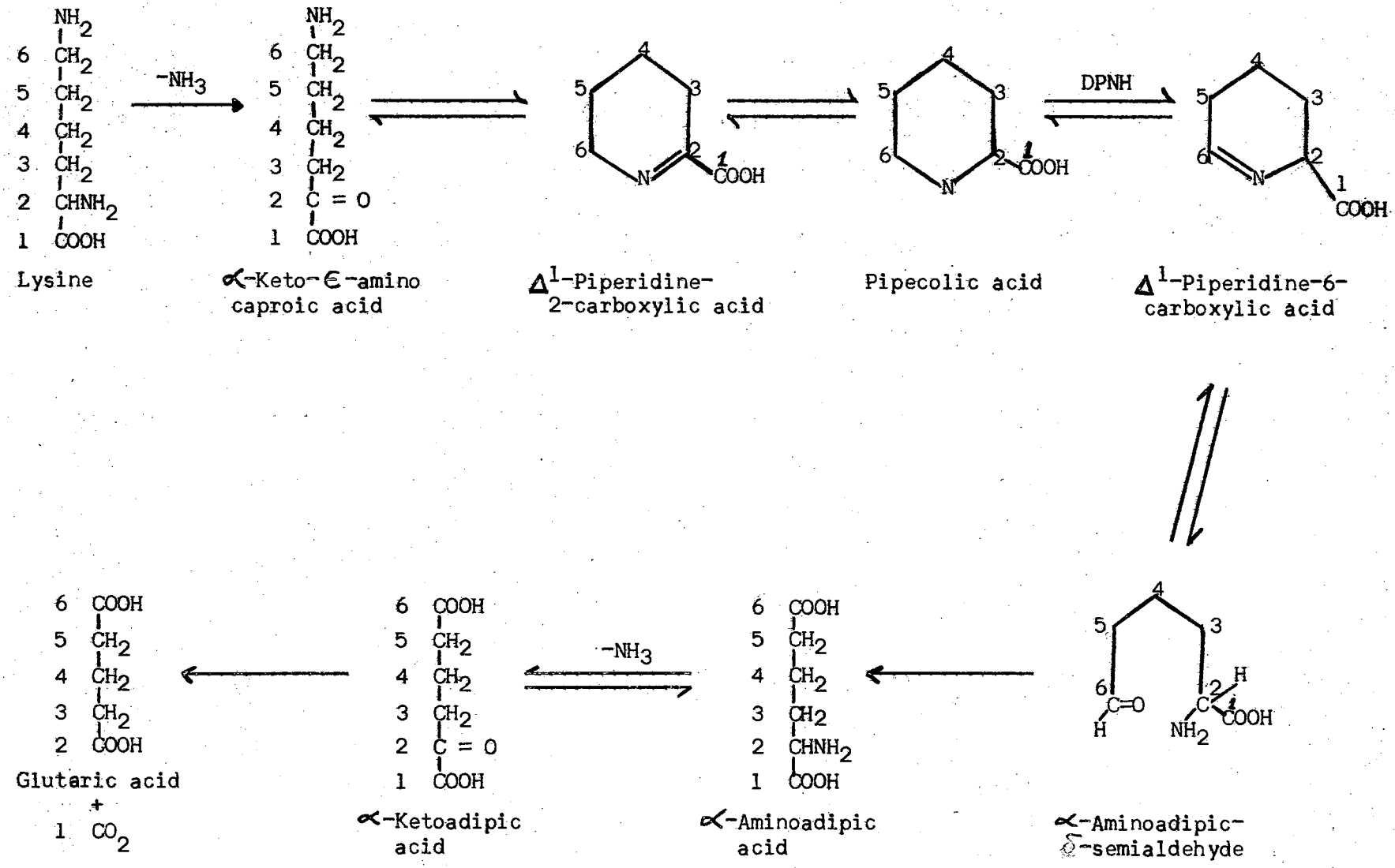
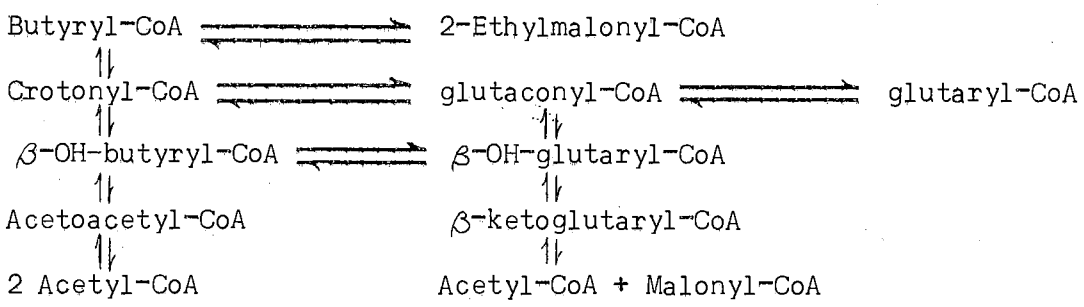


Figure 3. Proposed Metabolic Pathway of Lysine in Rats

and Stern (18) claimed they isolated radioactive glutaconyl-CoA, β-hydroxyglutaryl-CoA and malonyl-CoA. A scheme involving both carboxylation of crotonyl-CoA and glutarate metabolism was proposed.



The same authors presented some evidence suggesting that glutaconyl-CoA was an intermediate (19). However they were unable to prove that crotonyl-CoA was derived from the decarboxylation of glutaconyl-CoA. Wilson (20) has been unable to obtain any evidence to indicate that glutaconyl-CoA is an intermediate in glutarate catabolism.

Glutarate can be used by microorganisms (21, 22) as a hydrogen source. Of interest is the demonstration that extracts of Pseudomonas fluorescens convert glutaryl-CoA to carbon dioxide and two moles of acetyl-CoA (23).

Bagchi et al. (24) reported that when rat liver mitochondria were incubated with glutarate-3-C^{14}, glutarate was converted to acetate via acetoacetate.

Numa et al. (25) suggested that the glutarate catabolism proceeded via β-OH-butyryl-CoA, which might be formed through the decarboxylation of glutaconyl-CoA. Wilson (20) has worked on this question and found that β-OH-butyryl-CoA is an intermediate in animal and microbial enzymes systems. The whole scheme of carboxylation of crotonyl-CoA and glutarate metabolism still remains unresolved.

Studies on the catabolism of D,L-lysine in Clostridium sticklandii

by Stadtman (26) have provided evidence for another pathway in which both the D and L isomers of lysine are degraded anaerobically to butyric acid, acetic acid and ammonia.

$$CH_2(NH_2)CH_2CH_2CH_2CH(NH_2)COOH \longrightarrow CH_3CH_2CH_2COOH+CH_3COOH+2NH_3$$

This pathway differs from the catabolic pathway of lysine occurring in animals, Neurospora and certain bacteria.

In addition to lysine catabolism, tryptophan catabolism in mammals apparently also proceeds through α-ketoadipic acid (27). The proposal that α-ketoadipate goes to glutaryl-CoA (27) raises one question. If coenzyme A is attached to the glutarate molecule during its conversion to acetate (14), acetoacetate (24), and β-hydroxybutyrate (25), perhaps via glutaconyl (19), crotonyl (18) or β-hyroxyglutaryl-CoA (10), it would be expected that the carboxyl groups of glutaryl-CoA would be metabolized by different pathways. One might predict that the carboxyl not joined to coenzyme A would be released as bicarbonate and the other carboxyl would become the carboxyl of acetate. Bicarbonate-C^{14} and acetate-1-C^{14} injected into rats give very different labeling patterns in tissue glutamate (28). Bicarbonate-C^{14} gives the labeling pattern in glutamic acid of about 85 and 5 per cent radioactivity in carbons 1 and 5 respectively. Acetate-1-C^{14} gives the labeling pattern of about 30 and 70 per cent radioactivity in carbons 1 and 5 of glutamic acid respectively. Some years ago, Miller and Bale (29) studied the labeling patterns in glutamate isolated from the liver and plasma proteins of fasted spaniels fed D,L-lysine-6-C^{14} mixed with chopped lean beef. The pattern obtained was almost identical to that of acetate-1-C^{14} (28). These results of Miller and Bale are different than those expected if carbon 6 of lysine was metabolized via bicarbonate and raises the

question as to whether or not glutaryl-CoA is formed from α-keto-adipic acid.

It was of interest to know whether or not acetyl-CoA and carbon dioxide both come from carbon 2 and carbon 6 of lysine. Glutaric acid is a symmetrical molecule. Therefore the carbon atom of carbon dioxide and of the carboxyl group of acetate should both come from the symmetrical carbons 1 and 5 of glutaric acid. Carbons 1 and 5 of glutaric acid come from carbons 2 and 6 of lysine. The purpose of this investigation was to find out whether or not carbons 2 and 6 of lysine traversed a symmetrical structure (glutarate) during catabolism.

D,L-Lysine-6-C^{14} and D,L-lysine-2-C^{14} have been injected into rats; liver and carcass glutamate and aspartate have been isolated, degraded and assayed for radioactivity. The labeling pattern found in isolated glutamic acid indicated that carbon 2 of D,L-lysine-2-C^{14} is metabolized via the carboxyl of acetate, but that carbon 6 of D,L-lysine-6-C^{14} is probably converted primarily to bicarbonate.

CHAPTER II

EXPERIMENTAL

In each of the experiments performed, male rats from the Holtzman Company, Madison, Wisconsin, were used. The D,L-lysine-6-C^{14} and D,L-lysine-2-C^{14} were each dissolved in one ml of distilled water. Five tenths of a ml of one of the labeled lysines was administered to rats by intraperitoneal injection, except for Rat 233 which was injected subcutaneously. Rats 218B and 233 had been fasted 40 and 48 hours respectively before injection. After injection the rat was placed in a glass metabolism chamber which was swept with air in order to collect the exhaled carbon dioxide which was trapped in 1N NaOH. A summary of the animal data is presented in Table I.

A. Isolation Procedures

At a specific time interval (depending on the experiment, see Table II) after the injection of the labeled lysine, the rat was killed by decapitation. The liver and carcass protein powders were prepared by conventional means (28) involving extraction of the ground tissue with 10 per cent trichloroacetic acid, acetone and ether. "Carcass" refers to the entire animal, except liver, including the washed gastrointestinal tract.

1. Separation of Acidic Amino Acids from Protein Hydrolysates

The dried protein was heated under a reflux for 24 hours with

TABLE I

A SUMMARY OF THE ANIMAL DATA

Rat No.	Weight in gm	Compound Administered	Microcuries Injected	Exhaled Microcuries	Exhaled Per Cent	Times Fasted in Hours	Route of Administration
192	316	D,L-Lysine·2HCl-6-C^{14}	25	2	8	0	Intraperitoneal
195	195	D,L-Lysine·2HCl-6-C^{14}	23	1.7	7.4	0	Intraperitoneal
199	193	D,L-Lysine·HCl-2-C^{14}	56	1.7	3	0	Intraperitoneal
210	106	D,L-Lysine·HCl-2-C^{14}	34	0.88	2.7	0	Intraperitoneal
218B	90	D,L-Lysine·2HCl-6-C^{14}	39	9.2	23.8	40	Intraperitoneal
233	90	D,L-Lysine·HCl-6-C^{14}	104	17.1	16.4	48	Subcutaneously

100 ml of 6N hydrochloric acid for every 10 gm of protein powder. After filtration, the hydrolysate was evaporated *in vacuo* to a thick syrup three times to remove excess hydrochloric acid. The syrup was dissolved in 250 ml of water for every 10 gm of protein. The resulting solution, having a pH of approximately 2, was shaken for one hour with 20 gm of acetic acid-poisoned charcoal for each 10 gm of protein. This charcoal (Darco G-60) was prepared by shaking 20 gm with 250 ml of five per cent acetic acid for one hour, filtering and washing with water. The acetic acid-treated charcoal removes tyrosine, phenylalanine and coloring matter from the hydrolysate (30).

Glutamic acid and aspartic acid were isolated from the protein hydrolysate by ion-exchange chromatography (31). Because these two amino acids are acidic, an anion exchanger was used. The Dowex-1 resin (Cl^- form, 200-400 mesh) was washed with water to remove fine particles. It was added to columns of 3.6 by 21 cm and 4.8 by 55 cm respectively for liver and carcass protein hydrolysates.

The column was washed with 3N sodium acetate until the effluent gave a negative test for chloride ion. The resin, now in the acetate form, was washed with 0.5N acetic acid to remove sodium acetate which remained on the column and with water until a pH of approximately 4 was obtained.

The charcoal-treated hydrolysate was diluted to contain an equivalent of 20 gm of protein per liter of solution. It was then passed onto the column of Dowex-1 acetate at a rate of 1.5 ml per minute and 7 ml per minute for liver and carcass respectively. The column was washed with distilled water until the effluent was ninhydrin negative. The acidic amino acids were displaced with 0.5N acetic acid

at a rate of 1 ml per minute and 3.5 ml per minute for liver and carcass respectively. Fractions (5 ml for liver, 20 ml for carcass) were collected with the aid of an automatic fraction collector. The location of the acidic amino acids in the tubes was determined by the ninhydrin test. Only two peaks were observed.

The fractions were chromatographed in 76 per cent ethanol (32) by the ascending technique on Whatman No. 1 paper treated with 0.1 M sodium phosphate pH 7. The amino acids were detected with ninhydrin. By this procedure, liver and carcass glutamic and aspartic acids were identified (Figures 4 and 5).

2. Isolation of Glutamic Acid

The fractions from the Dowex-1 acetate column which contained only glutamic acid were combined, 5 ml of concentrated hydrochloric acid for every 10 gm protein were added, and the solution was evaporated to a volume of 1 to 3 ml. This solution was then transferred to a 50 ml beaker and evaporated to a volume of 1 to 2 ml. To this syrup were added 5 ml of concentrated hydrochloric acid, and glutamic acid hydrochloride was allowed to crystallize in the refrigerator overnight. The hydrochloride was filtered on a coarse porosity sintered glass filter funnel, washed with 2 ml of cold concentrated hydrochloric acid followed by acetone and ether, and then dried *in vacuo*.

The glutamic acid hydrochloride was dissolved in 10 ml of boiling water and a 25 per cent pyridine solution was added dropwise to adjust the pH to 3.5. The glutamic acid was allowed to crystallize for five minutes; then approximately two volumes of absolute ethanol were added, and the glutamic acid was allowed to crystallize overnight in the refrigerator. The glutamic acid was collected by filtration, washed

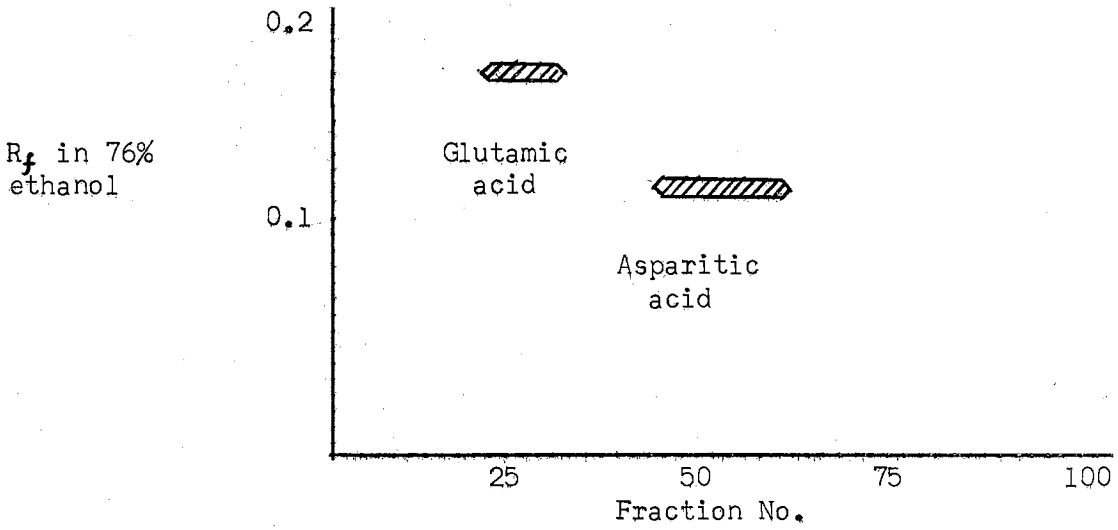


Figure 4. Distribution of Liver Acidic Amino Acids as Determined by Paper Chromatography after Displacement from the Dowex-1 Acetate Column

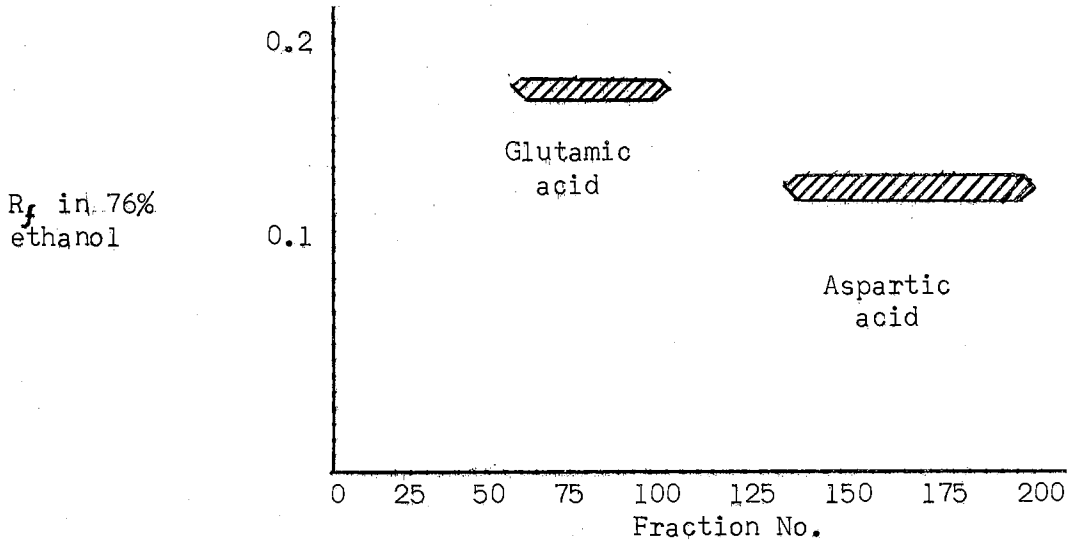


Figure 5. Distribution of Carcass Acidic Amino Acids as Determined by Paper Chromatography after Displacement from the Dowex-1 Acetate Column

two times with absolute ethanol and then with ether, and was dried in a drying apparatus *in vacuo* overnight. The yield of glutamic acid was approximately 10 per cent by weight of the initial protein.

3. Isolation of Aspartic Acid

All fractions from the Dowex-1 acetate column that contained aspartic acid were combined and the solution was concentrated *in vacuo* to a volume of 10 ml with the aid of rotatory evaporator. The solution was transferred to a 50 ml beaker and evaporated until crystals appeared. A minimum volume of boiling distilled water was added to dissolve the crystals. Two volumes of absolute ethanol were added, and the aspartic acid was allowed to crystallize overnight in the refrigerator. The aspartic acid was filtered, washed with absolute ethanol and ether and dried in a drying apparatus in vacuo overnight. The yield of aspartic acid was approximately eight per cent by weight of the initial protein.

B. Degradation Procedures

1. Decarboxlation of Glutamic Acid by the Schmidt Reaction

The degradation of glutamic acid to yield carbon 5 was carried out by the method described by Hahn (33). Carbon 5 of glutamic acid was liberated as CO_2 by treating this amino acid with hydrazoic acid (34). The yields of CO_2 obtained from carbon 5 by this method were in good agreement with those obtained from a Schmidt reaction on butyric acid during the complete degradation of glutamic acid (34, 35). The α,γ-diaminobutyric acid, the other product, was isolated as the dipicrate by a method similar to that described by Adamson (36).

Glutamic acid (100 mg, 0.68 mmoles) was placed in a 15 ml pear shape flask and 0.8 ml of 100 per cent sulfuric acid (36) was carefully added. The flask was then stoppered with a glass stopper and warmed

over a small flame to dissolve the amino acid. The solution was chilled in an ice-bath for 15 minutes. Three mmoles (about 200 mg) of sodium azide were added to the flask and the latter attached quickly to a distillation head (33). The air inlet to the reaction flask was kept closed and the flask was heated slowly, over a period of 30 minutes, to 50°. After the evolution of CO_2 ceased the temperature was raised to 100° C and kept there for an hour. The air inlet was then opened and carbon dioxide-free air was allowed to sweep through the system for one hour while heating at 100°. The sodium hydroxide solution in the carbon dioxide trap was then transferred to a Van Slyke apparatus for the manometric determination of CO_2 and subsequent C^{14} assay (37).

2. Preparation of α, γ-Diaminobutyric Acid Dipicrate

The solution remaining in the pear shape flask was dissolved in 3 to 4 ml of water and transferred to a 50 ml beaker. The solution was then neutrilized to pH 6 with hot saturated barium hydroxide and the barium sulfate was removed by centrifugation. The supernant was concentrated to 10 ml on the steam bath. After the addition of 300 mg (1.33 mmoles) of picric acid the solution was warmed until all the picric acid dissolved. The solution was allowed to cool at room temperature to crystallize the dipicrate of α, γ-diaminobutyric acid. The dipicrate was removed by filtration, washed with 2 to 3 ml of water and recrystallized from 5 to 8 ml of hot water. The dipicrates were recrystallized until the melting point was 180° to 181° (36).

3. Complete Degradation of Glutamic Acid

The degradation of glutamic acid was accomplished by the method of Mosbach, Phares and Carson (34, 35, 38) as modified by Koeppe and Hill (28). Usually two mmoles of glutamic acid were used. The series of

reactions is summarized in Figure 6.

4. Wet Combustion Of Amino Acids

In order to determine the total radioactivity of the various compounds, these substances were oxidized by the macrocombustion method of Van Slyke *et al.* (37, 39). The resulting radioactive carbon dioxide was measured manometrically, and assayed for radioactivity with a vibrating reed electrometer.

5. The Degradation of D,L-lysine-2 or 6-C^{14}.

The degradation of D,L-lysine was carried out by the method described by Strassman and Weinhouse (40). The lysine was oxidized to glutaric acid with hot acidic permanganate. The glutaric acid was extracted with ether before chromatographic purification (14) and then was decarboxylated in a Schmidt reaction. The resulting carbon dioxide represents carbons 2 and 6 of lysine. The reaction is shown in Figure 7.

a. Oxidation of Lysine to Glutaric Acid

Between 1.5 and 2.0 mmoles of D,L-lysine monohydrochloride were dissolved in 150 ml of water in a 300 ml 2-neck flask carring a dropping funnel and a condensor. Twenty ml of 9N H_2SO_4 were added, the solution was heated in a bath of boiling water, stirred vigorously with a magnetic stirrer, and a few drops of 1.5N $KMnO_4$ were added. When the reaction began (the color of $KMnO_4$ disappeared) the temperature of the bath was lowered to 85° and a total of 20 ml of 1.5N $KMnO_4$ was added dropwise over a period of an hour. The solution was kept at this temperature for one hour longer and after cooling, it was filtered to remove the MnO_2. The clear filtrate was continuously extracted with ether for 48 hours. The extract was transferred to a 20 ml beaker and evaporated to dryness on a hot plate.

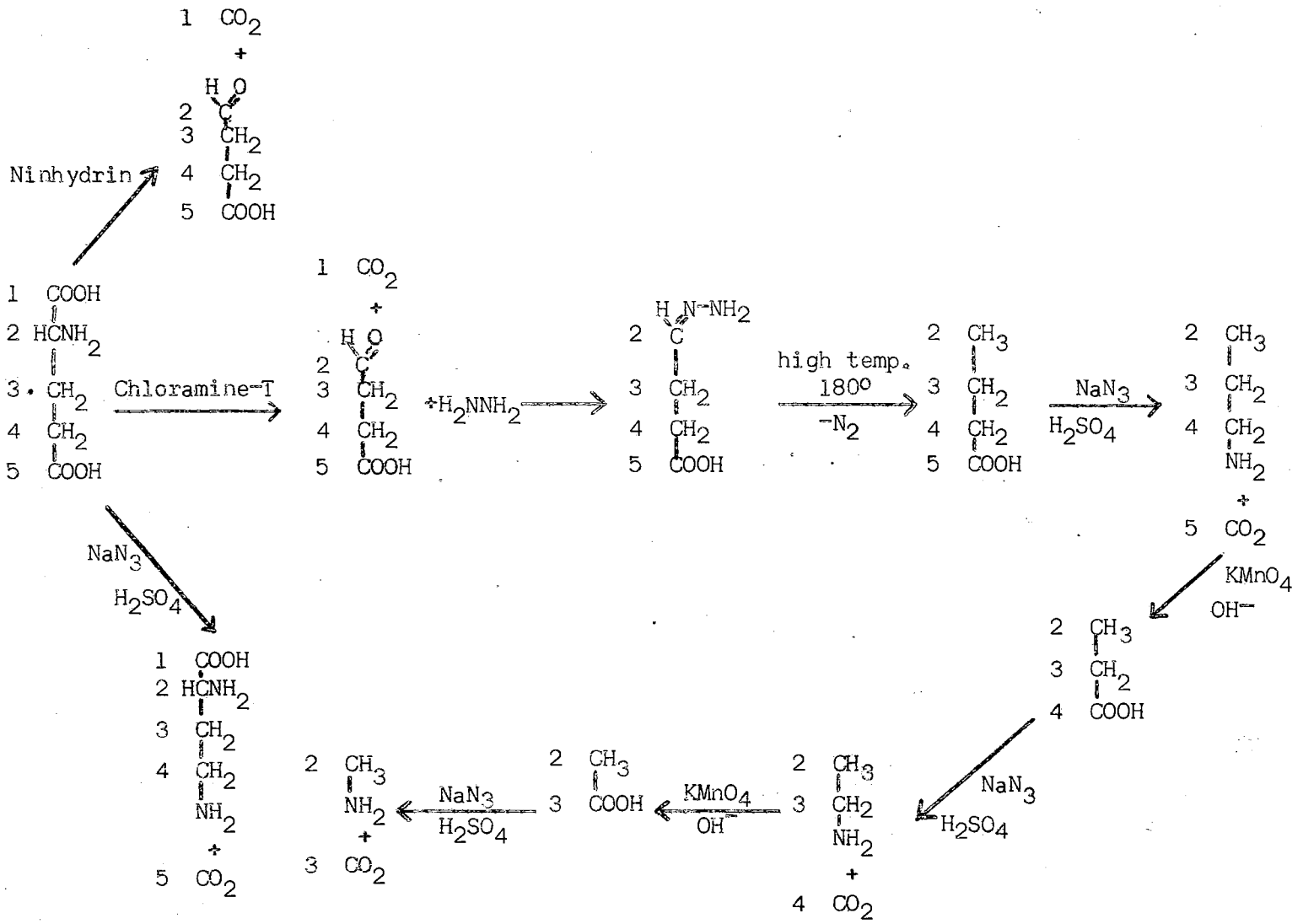


Figure 6. The Degradation of Glutamic Acid

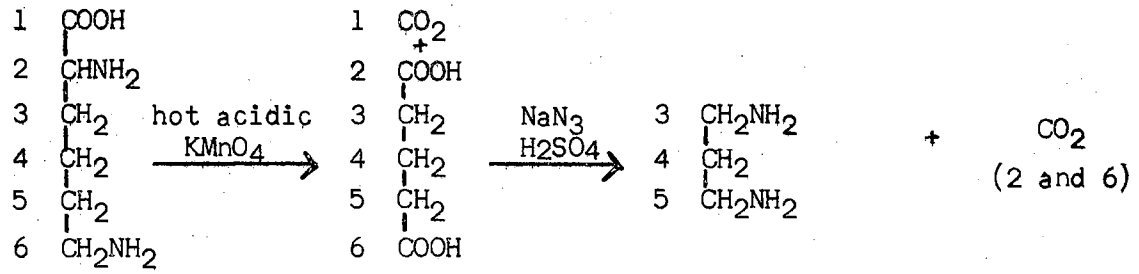


Figure 7. The Degradation of D,L-Lysine-2 or 6-C^{14}

b. Preparation of Celite Chromatographic Columns

A column, 0.8 by 30 cm in size was packed with Celite to a height of 25 cm by the addition of a slurry containing 5 gm of Celite (which had been mixed with 3 ml of 0.5N H_2SO_4) suspended in a 10 per cent solution of acetone in hexane. Approximately 40 ml of the eluting solvent (either chloroform or a one per cent solution of n-butanol in chloroform, both of which were saturated with 0.5N H_2SO_4) was run over the packed Celite column in order to remove the acetone-hexane solution.

c. Chromatographic Purification of Glutaric Acid

The dried glutaric acid obtained by ether extraction was slurried with 5 ml of chloroform and the solution decanted on to the Celite column. The chloroform layer was allowed to flow on to the Celite and the beaker was then rinsed three times with 5 ml of chloroform. Glutaric acid was eluted at a rate of 0.5 to 1 ml per minute with 100 ml each of 1,5 and 10 per cent (vol/vol) n-butanol in chloroform solution. Fractions were collected with the aid of an automatic fraction collector at five minute intervals and titrated with 0.1N carbon dioxide-free sodium hydroxide. Phenol red was used as an indicator in this titration. Figure 8 shows a diagram of the distribution of glutaric acid after chromatographic purification.

The solutions of these two peaks, shown in Figure 8, were

both concentrated to 5 ml for the identification of glutaric acid.

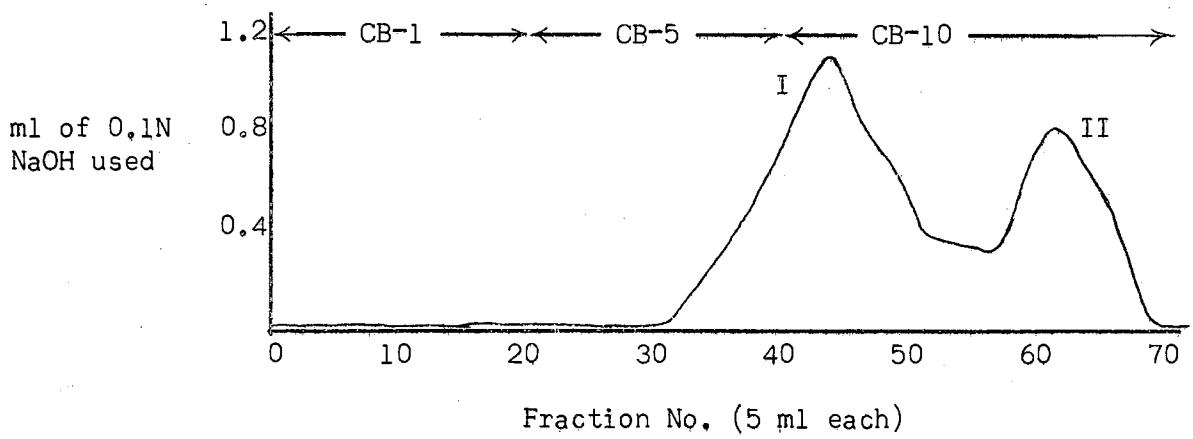


Figure 8. Glutaric Acid Chromatographic Purification

d. Paper Partition Chromatography (Identification of Glutaric Acid)

The ascending paper chromatographic technique was used to identify this organic acid (41).

Ten µl of each solution were spotted on Whatman No. 1 paper and developed in the solvent system of n-butanol : acetic acid : water (9 : 1 : 2.5 v/v). An indicator of 0.04 per cent bromocresol green (pH 6.0) was used to detect acidic compounds. In the case of paper chromatography in an acidic system, it is necessary to remove acetic acid before spraying the indicator on the paper. Therefore the chromatogram was steamed under an infrared lamp to vaporize the acetic acid. The dried chromatogram was sprayed with bromocresol green. The yellow spots showed the location of acidic compounds. The R_f of Peak I (0.75) was comparable to that of glutaric acid in this solvent system, therefore it was concluded that Peak I was glutaric acid. The compound in Peak II was not identified. Its R_f was 0.67. Radioactivity was found in both compounds.

The concentrated sodium glutarate was transferred to a 15 ml

of pear shape flask, evaporated to dryness, and then dried in the oven at 100° for two hours.

e. The Schmidt Reaction on Glutaric Acid

The apparatus for the Schmidt reaction on glutaric acid was the same as that mentioned above for glutamic acid. To 0.33 mmole of sodium glutarate in the pear shape flask was added 0.4 ml of 100 per cent sulfuric acid and one mmole (67 mg) of sodium azide. The flask was attached to a distillation head (33), heated at 55° for 30 minutes, and kept at this temperature for three hours. Then the temperature was raised to 70° or above and kept there for one hour. Carbon dioxide-free air was allowed to sweep slowly through the system for one hour in the boiling water bath. The sodium hydroxide in the second trap was transferred to a Van Slyke apparatus for the manometric determination of carbon dioxide and subsequent C^{14} assay.

C. Carbon Analysis and Radioactivity Assay

The total carbon content of the various compounds was determined by the macrocombustion method of Van Slyke and coworkers (34). Carbon dioxide liberated during degradation procedures was also measured manometrically.

The carbon dioxide from the wet combustion of samples and from the various degradations was swept into a Borokowsky (42) ionization chamber and assayed with a vibrating reed electrometer (43).

CHAPTER III

RESULTS AND DISCUSSION

A. Excretion of Radioactive Carbon Dioxide

A general summary of the experimental data is presented in Table I. Data relating to the rate of excretion of radioactive carbon dioxide may be found in Table II. From Tables I and II, it can be seen that the route of administration and the dietary condition of the animal did have an effect on the percentage of the injected isotope which was exhaled as $C^{14}O_2$.

In the case of D,L-lysine-6-C^{14}, fasting increased the rate of $C^{14}O_2$ release. Approximately 24 per cent of the injected dose was excreted as $C^{14}O_2$ from fasted Rats 218B and 233. This was three times as rapid as the rate of excretion in fed rats.

B. Results of Degradation Studies

In Table III are presented the results of degradation of D,L-lysine-2-C^{14} and D,L-lysine-6-C^{14}. It is apparent that almost 100 per cent of the radioactivity was located in carbons 2 and 6.

Table IV summarizes the results of the degradation of the carcass and liver glutamic acids. In the cases of fed Rats 199 and 210 which were administered D,L-lysine-2-C^{14}, carbon 5 of the carcass and liver glutamic acids contained significantly greater activity than did carbon 1. The ratio of radioactivity in carbon 5 to that in carbon 1 was approximately 2.5 to 1. This corresponds favorably with the value

TABLE II

FORMATION OF RADIOACTIVE CARBON DIOXIDE AFTER INJECTION OF D,L-LYSINE-6-C^{14} OR 2-C^{14} INTO RATS

Rat No.	Compound Administered	The Exhalation of $C^{14}O_2$, Expressed as Per Cent of the Injected Microcuries						
		15 min.	30 min.	1 hour	3 hours	4 hours	5 hours	24 hours
192	D,L-Lysine·2HCl-6-C^{14}	1.4	1.6	1.9	3.1			
195	D,L-Lysine·2HCl-6-C^{14}	0.6	1.2	1.3	1.0	0.4	0.3	2.6
199	D,L-Lysine·HCl-2-C^{14}	0.2	0.7	1.0	1.1			
210	D,L-Lysine·HCl-2-C^{14}	0.4	0.6	0.7	1			
218B	D,L-Lysine·2HCl-6-C^{14}	6.2	6.2	4.9	4.7		1.8	
233	D,L-Lysine·HCl-6-C^{14}	1.5	3.7	4.5	5.6	1.2		

TABLE III

DEGRADATION DATA OF D,L- LYSINE-2-C^{14} AND 6-C^{14}

Compound	D,L-Lysine		D,L-Lysine		D,L-Lysine		D,L-Lysine	
Position of C^{14}	6-C^{14}		2-C^{14}		6-C^{14}		6-C^{14}	
(Company) from	Calbiochem		Volk		Volk		Calbiochem	
Rat No.	192 and 195		199 and 210		218B		233	
	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total
Total	178.5		406		281.2		541	
Carbons 2 + 6	168	94.5	349	85	294	104	459	85

TABLE IV

C^{14} DISTRIBUTION IN RAT GLUTAMIC ACID AFTER INJECTION OF D,L-LYSINE-6-C^{14} OR D,L-LYSINE-2-C^{14}

Rat No.		192		195		199		210		218B		233	
Rat Weight (gm)		316		195		193		106		90		90	
Time Fasted (hours)		0		0		0		0		40		48	
Position of C^{14}		6-C^{14}		6-C^{14}		2-C^{14}		2-C^{14}		6-C^{14}		6-C^{14}	
Dose Injected (μc)		25		23		56		34		39		104	
Duration (hours)		3		24		3		3		5		4	
% Dose as $C^{14}O_2$		8		7.4		3		2.7		23.9		16.4	
		mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total
Carcass	Total	0.1		0.9		3.2		2.9		2.6		3.0	
	Carbon 1					0.8	25	0.6	21	1.7	65	1.8	60
	Carbon 5					2.1	66	2.4	83	0.7	27	0.8	27
	Sum $C_1 + C_5$					2.9	91	3.0	104	2.4	92	2.6	87
Liver	Total	2.7		2.5		17.9		22.6		12.4		16.6	
	Carbon 1	1.00	37	0.5	20	2.9	16	2.8	12	9.2	74	11.5	69
	Carbon 5	0.4	15	0.3	12	13.2	74	17.4	77	4.7	38		
	Sum $C_1 + C_5$	1.4	52	0.8	32	16.1	90	20.2	89	13.9	112		
	C 1-4					3.0	17	2.4	11	9.4	76		

obtained by Koeppe and Hill (28) after injecting acetate-1-C^{14} into rats. These results indicate that carbon 2 of lysine was metabolized in the Kreb's Cycle via the carboxyl of acetate and suggest that carbon 2 of lysine becomes the carboxyl of glutarate which is attached to coenzyme A prior to conversion to acetate. However, it should be noted that acetate-1-C^{14} labels glutamate much more readily than does bicarbonate-C^{14} (25). If equal amounts of these labeled compounds (acetate and bicarbonate) were given to rats, the labeling pattern in glutamate would probably be similar to that of acetate-1-C^{14} alone. Therefore the results with D,L-lysine-2-C^{14} do not rule out the possibility of free glutaric acid being an intermediate in lysine catabolism. If this were the case D,L-lysine-2-C^{14} could yield glutarate-1,5-C^{14} which could yield bicarbonate-C^{14} and acetate-1-C^{14}. The labeling pattern in glutamate would resemble that of acetate-1-C^{14}.

In the case of D,L-lysine-6-C^{14}, two different results were obtained, depending on whether or not the rat was fasted. When D,L-lysine-6-C^{14} was given to fed Rats 192 and 195, less than 60 per cent of the activity was in the carboxyls of liver glutamate, and about eight per cent of the total injected activity was exhaled as $C^{14}O_2$. Unfortunately, the specific activity of the carcass glutamate was too low to permit a complete degradation and an accurate C^{14} assay. In fact the low levels of C^{14} makes it necessary to repeat these experiments.

The use of fasted rats and larger amounts of D,L-lysine-6-C^{14} had increased threefold the percentage of the total activity exhaled as $C^{14}O_2$. In these rats the specific activity of the isolated tissue glutamate was increased to a level where complete degradation was possible. The results obtained with D,L-lysine-6-C^{14} were different in fed as compared with fasted rats (Tables IV, V). In the fasted

TABLE V

LABELING PATTERN OF CARCASS GLUTAMIC ACID

Rat No.	218B		233	
	muc/mmole	% total	muc/mmole	% total
Total	2.6		3.0	
Carbon 1	1.7	65	1.8	60
Carbon 2	0.1	4	0.16	5.4
Carbon 3	0.1	4		
Carbon 4	0.1	4	0.14	5
Carbon 5	0.7	27	0.8	27
Sum Carbons 1-5	2.7	104	2.9	97.4

Rats 218B and 233, the radioactivity was primarily in carbons 1 and 5 of glutamic acid. About 65 per cent of the radioactivity of the carcass and liver glutamic acids was in carbon 1 and 25 per cent was in carbon 5. Miller and Bale (29) working with D,L-lysine-6-C^{14} in fasted dogs, showed that about 30 per cent of the total activity was located in carbon 1 and 70 per cent in carbon 5 of glutamate.

Although the present results differ with those of Miller and Bale (29), they are similar to the values obtained by Koeppe and Hill (28). After injecting sodium bicarbonate-C^{14} into rats, they found 85 per cent of the total C^{14} in carbon 1 and about 5 per cent in carbon 5 of glutamic acid. Thus, with D,L-lysine-6-C^{14} in fasted rats, the labeling pattern found in tissue glutamate resembles that found with bicarbonate-C^{14} (28). Therefore the carbon 6 of D,L-lysine-6-C^{14} was probably metabolized into Kreb's Cycle via the intermediate formation of carbon dioxide in the fasted rat. The higher percentage of activity in carbon 5 of glutamate in the present results compared to those of Koeppe and Hill (28), when bicarbonate-C^{14} was injected to rats, suggests that a small portion of the D,L-lysine-6-C^{14} was catabolized via a symmetrical intermediate.

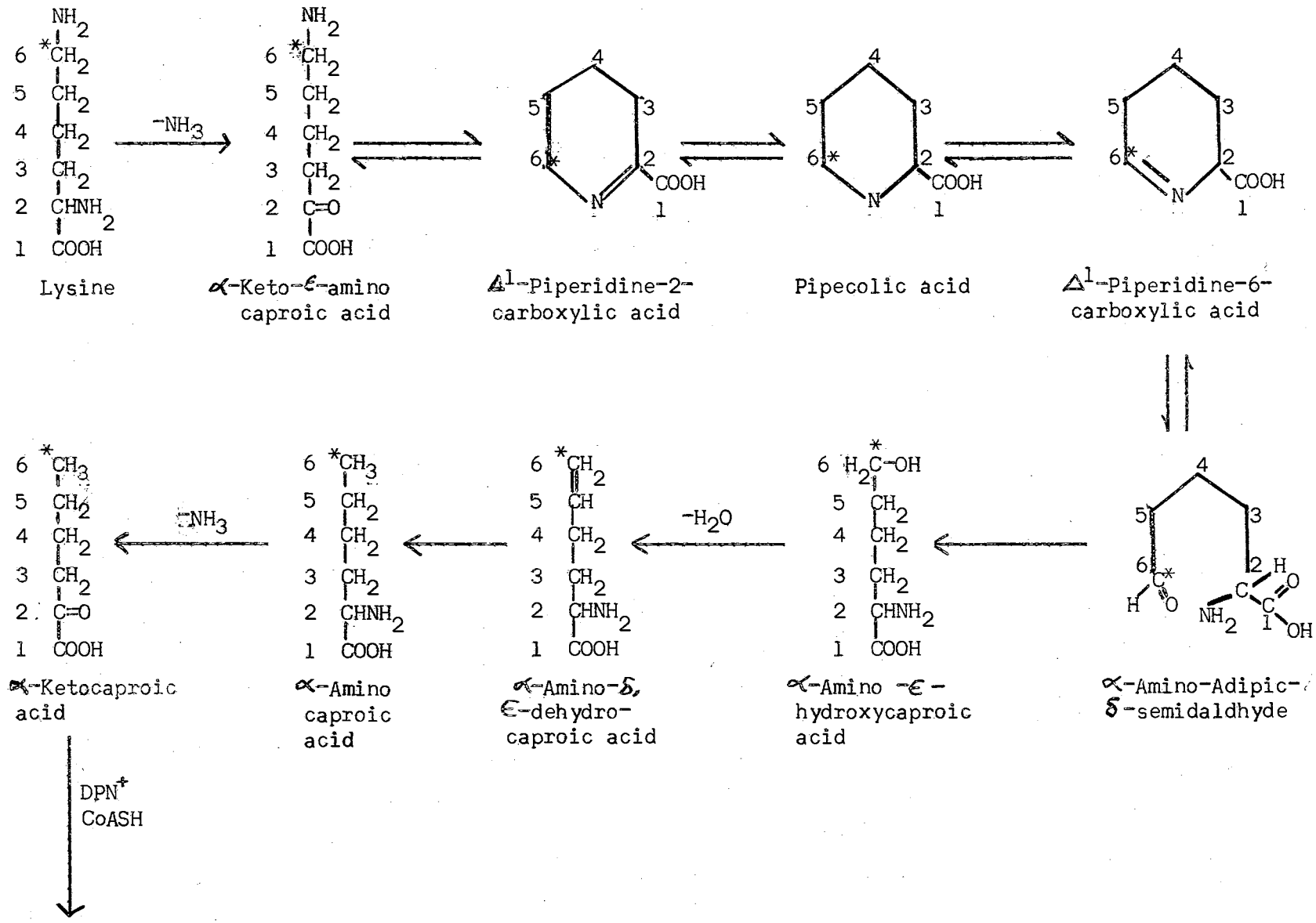
The results obtained from Rats 192 and 195 also do not agree with the values of Miller and Bale (29) who fed D,L-lysine-6-C^{14} to fasted dogs and obtained an acetate-1-C^{14} labeling pattern in glutamate. Moreover as mentioned above they are different from the results obtained in Rats 218B and 233. One reason for these findings could be an impure or incorrectly labeled lysine. However paper chromatography did not show the presence of substantial amounts of impurity and degradation (see above) indicated that the lysine samples were correctly labeled.

The labeling patterns observed (Rats 192 and 195) suggest that carbon 6 of D,L-lysine-6-C^{14} in fed rats was not metabolized in the Kreb's Cycle via either acetate or carbon dioxide and that there may be another pathway of lysine catabolism. One plausible explanation is that D,L-lysine-6-C^{14} was metabolized into the Kreb's Cycle via propionic acid. The fixation of CO_2 to this 3 carbon compound will yield succinic acid (44, 45, 46, 47) which is oxidized via the Kreb's Cycle. This possible alternate pathway(B) is shown in Figure 9. The results with Rats 192 and 195 were in agreement with the proposed pathway(B). However, our data provide little real evidence in support of such a catabolic route.

Degradation data of aspartic acid of liver and carcass are given in Table VI. In Rats 199, 210, 218B and 233, approximately 100 per cent of the total C^{14} was found in carbons 1 and 4. The glutamate from these animals was also labeled primarily in the carboxyl positions.

In Rats 192 and 195, the percentage of radioactivity in carbons 1 and 4 was less than 65. This finding correlates with the fact that the glutamate from these animals had considerable non-carboxyl labeling.

In Rats 199 and 210, the specific activity of glutamic acid was higher than that of aspartic acid, but in Rats 218B and 233 the reverse was found. Hill (48) observed that the specific activity of tissue glutamate was higher than tissue aspartate when acetate-1-C^{14} was injected to rats and that the reverse was true when bicarbonate-C^{14} was injected to rats. Our results suggest that acetate-1-C^{14} was formed from D,L-lysine-2-C^{14} in fed rats and bicarbonate-C^{14} from D,L-lysine-6-C^{14} in fasted rats. The results are in accord with the metabolic pathway (A) for D,L-lysine-2-C^{14} and 6-C^{14} as shown in Figure 10.


Lysine
-NH3
α-Keto-ε-amino caproic acid
Δ1-Piperidine-2-carboxylic acid
Pipecolic acid
Δ1-Piperidine-6-carboxylic acid
α-Amino-Adipic-δ-semidaldhyde
-H2O
α-Amino -ε- hydroxycaproic acid
α-Amino-δ, ε-dehydro-caproic acid
-NH3
α-Amino caproic acid
α-Ketocaproic acid
DPN+
CoASH

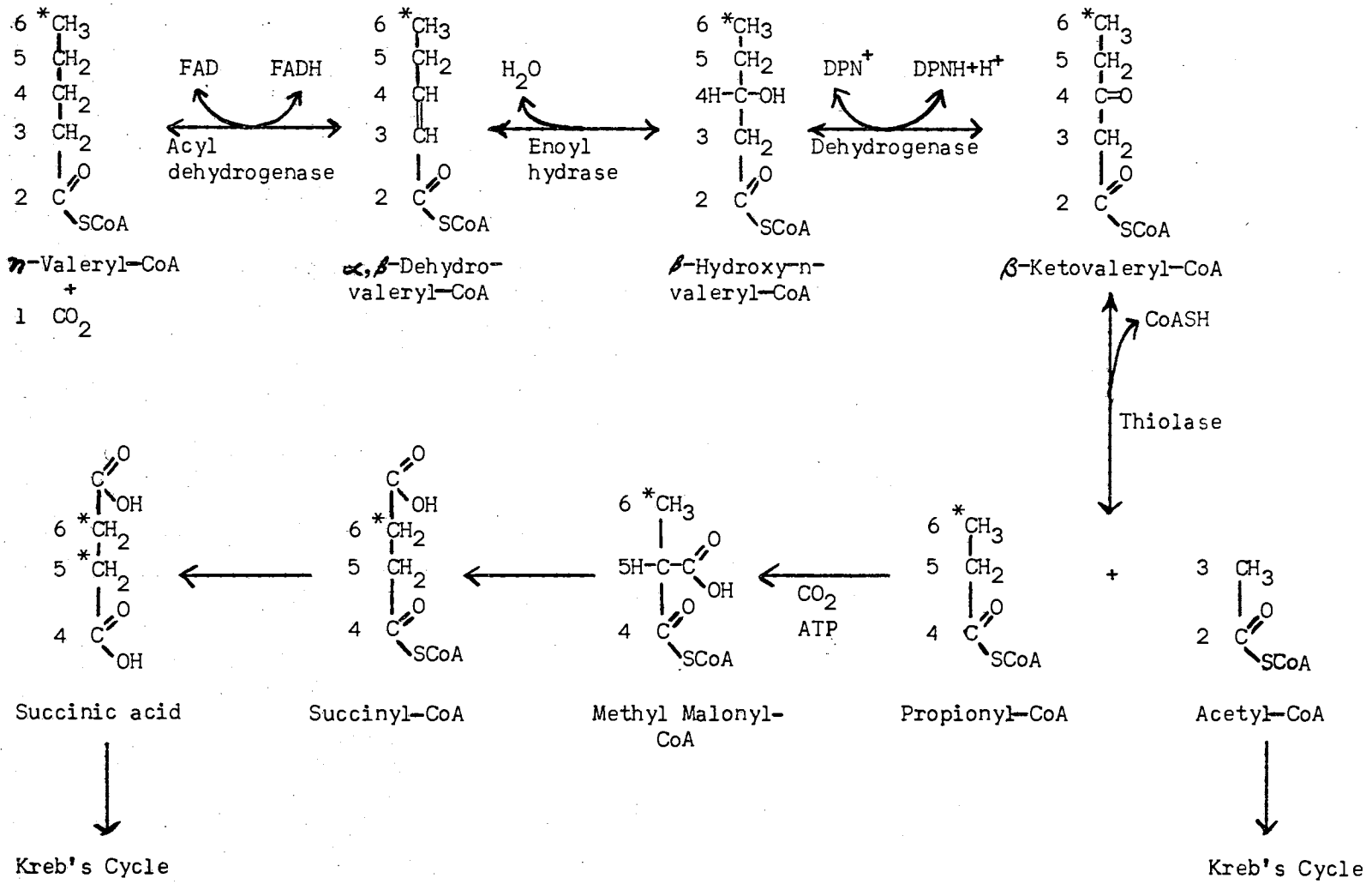


Figure 9. Possible Alternative Metabolic Pathway (B) of D,L-Lysine-6-C^{14} in Fed Rats

TABLE VI

C^{14} DISTRIBUTION IN RAT ASPARTIC ACID AFTER INJECTION OF D,L-LYSINE-6-C^{14} OR D,L-LYSINE-2-C^{14}

Rat No.		192		195		199		210		218B		233	
		mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total	mμc/mmole	% total
Liver	Total	2.2		1.1		4.3		5.7		13.1		20	
	Carbons 1 + 4	1.4	64	0.7	64	4.1	95	5.7	100	14.1	107	19	95
Carcass	Total					1.3		0.96		2.2		2.4	
	Carbons 1 + 4					1.3	100	0.93	97	2.1	95	2.2	92

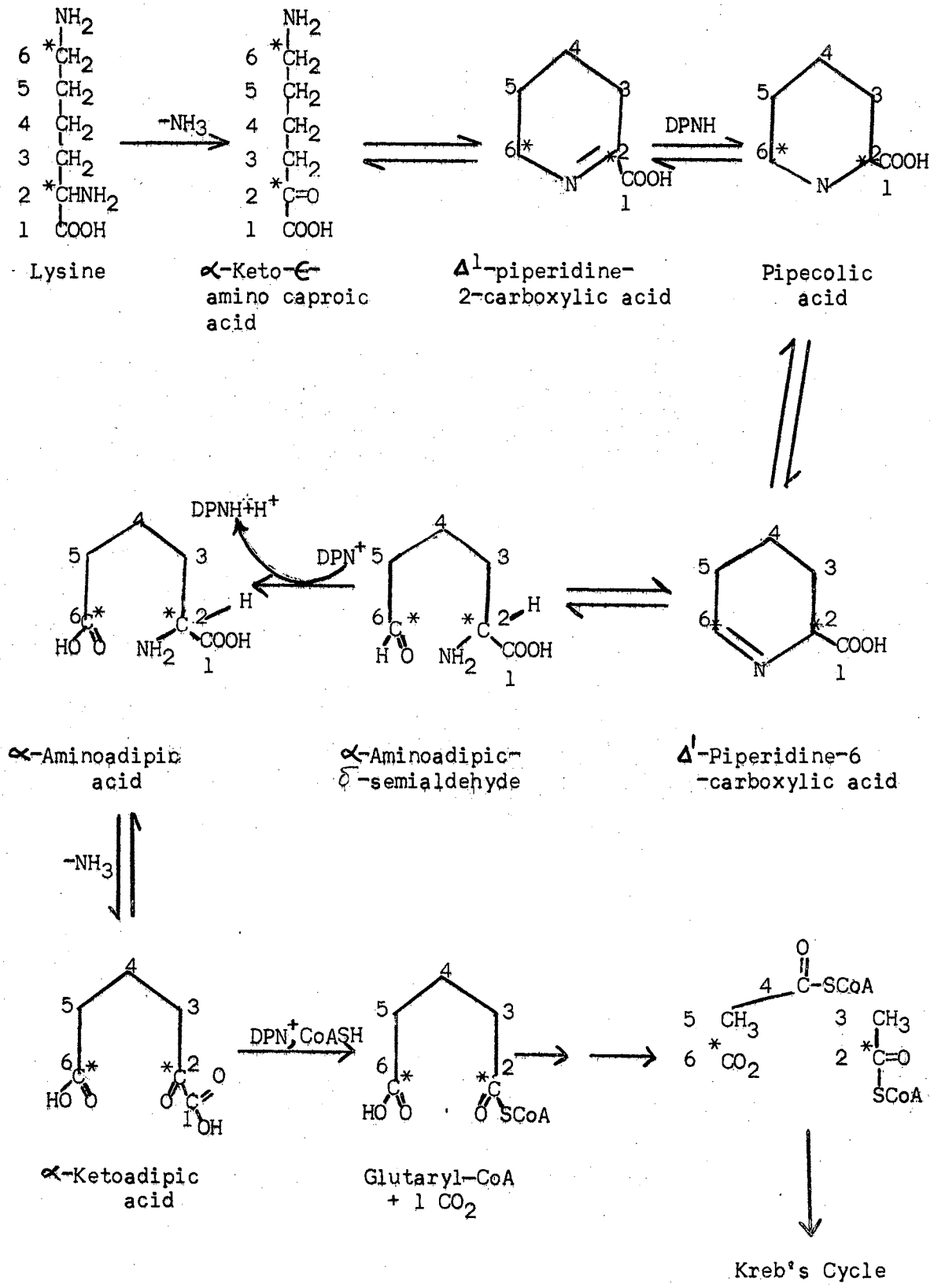


Figure 10. Proposed Metabolic Pathway (A) of D,L-Lysine-2-C^{14} and D,L-Lysine-6-C^{14} in Rats

CHAPTER IV

SUMMARY

When D,L-lysine-2-C^{14} was administered to fed rats, the labeling patterns found in isolated tissue glutamate and aspartate indicate that the major catabolic route of carbon 2 of lysine is via the carboxyl of acetate. Administration of D,L-lysine-6-C^{14} to fasted rats resulted in labeling patterns in tissue glutamate and aspartate which suggest that carbon 6 of lysine is catabolized via carbon dioxide. In the fed rats given D,L-lysine-6-C^{14}, the labeling patterns in isolated glutamate and aspartate suggested the possibility that an alternate metabolic pathway of lysine exists.

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