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Biosynthesis of Carnitine and 4-*N*-Trimethylaminobutyrate from 6-*N*-Trimethyl-lysine

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The conversion of 6-*N*-[*Me*-^{14}C]trimethyl-lysine into carnitine and 4-*N*-trimethylaminobutyrate (butyrobetaine) was demonstrated in rats kept on a lysine-deficient diet. After the rats were given [^{14}C]trimethyl-lysine for 4 days, a total of 17% of the injected label was recovered as carnitine from carcass and urine extracts. Another 8% of the trimethyl-lysine label was converted into 4-*N*-trimethylaminobutyrate, most of which was recovered from the urine. The conversion of trimethyl-lysine into the above two metabolites supports the pathway of carnitine biosynthesis as lysine+methionine → 6-*N*-trimethyl-lysine → 4-*N*-trimethylaminobutyrate → carnitine. In addition, three other metabolites representing 2% of the injected dose were recovered. Only an insignificant portion of the label was recovered as free trimethyl-lysine from the carcass, whereas 22% of the injected label was recovered in the urine. A relatively low specific radioactivity in carnitine was found when 5-*N*-[*Me*-^{14}C]trimethylaminopentanoate and 6-*N*-[*Me*-^{14}C]trimethylaminohexanoate were administered to rats in amounts similar to the [^{14}C]trimethyl-lysine, suggesting that they were not free intermediates.

The biosynthesis of carnitine (3-hydroxy-4-*N*-trimethylaminobutyrate) has been shown to involve the methylation of a lysine-derived carbon chain by methionine. In the rat lysine was also incorporated into the butyrate carbon chain of 4-*N*-trimethylaminobutyrate (Cox & Hoppel, 1973), a compound rapidly hydroxylated to form carnitine in the mouse (Lindstedt & Lindstedt, 1965). Similarly, 6-*N*-trimethyl-lysine is formed by the addition of methyl groups from *S*-adenosylmethionine to peptide-bound lysine (Paik & Kim, 1971). Considering the common origins and structural similarities of 6-trimethyl-lysine, 4-trimethylaminobutyrate and carnitine, 6-trimethyl-lysine was proposed as an intermediate in the biosynthesis of 4-trimethylaminobutyrate and carnitine from lysine (Horne *et al.*, 1971; Cox & Hoppel, 1973).

The present study in the rat reports the conversion of 6-trimethyl-lysine into carnitine, 4-trimethylaminobutyrate and three other metabolites. 5-*N*-Trimethylaminopentanoate and 6-*N*-trimethylaminohexanoate were also tested as possible intermediates.

Materials and Methods

Animals and methods

Unless otherwise stated, these are as described by Cox & Hoppel (1973). Weanling rats were kept on a lysine-deficient diet for 3 weeks, and the average weight per animal decreased from 49.3±2.95g to 36.4±1.96g (±S.D., $n = 10$). Urine and carcass extracts were processed by method II (Cox & Hoppel, 1973) except that the AG-50 resin citrate buffer column used previously was replaced by a column (1cm×50cm) of AG-50 resin (X8; 200–400 mesh; H^+ form) eluted with a linear HCl gradient with a mixing flask containing 150ml of 1.5M-HCl and a reservoir containing 150ml of 4M-HCl. Elution proceeded at room temperature and 2ml fractions were collected. This system separated all the quaternary amines considered in the study (Fig. 1) and allowed t.l.c. without desalting the eluate.

The method of t.l.c., the methods of detection, and the determination of radioactivity were carried out as described by Cox & Hoppel (1973). The R_F values of the compounds examined in this study are given in Table 1.

To identify radioactivity specifically in biosynthesized 4-*N*-trimethylaminobutyrate, this compound was converted into carnitine by using the 4-trimethylaminobutyrate (butyrobetaine) hydroxylase assay developed by Lindstedt (1967). A 40–70%-satd.-$(NH_4)_2SO_4$ fraction of the 70000*g* supernatant fractions obtained from calf's liver served as the source of the hydroxylase (specific activity 0.27nmol/min per mg of protein). After the assay, carnitine and unchanged 4-trimethylaminobutyrate were separated on a column (1cm×50cm) of AG-50 (X8; H^+ form) and eluted with 2M-HCl. Synthetic 4-[*Me*-^{3}H]trimethylaminobutyrate was added to the incubation to monitor the conversion of biosynthesized 4-[^{14}C]trimethylaminobutyrate into carnitine. The amount of

standard 4-[^{3}H]trimethylaminobutyrate converted into carnitine was compared with that for 4-[^{14}C]-trimethylaminobutyrate.

Materials

[^{14}C]Methyl iodide was purchased from International Chemical and Nuclear Corp., Cleveland, Ohio, U.S.A. 4-*N*-[*carboxy*-^{14}C]Trimethylaminobutyric acid was a gift of D. B. Goodfellow (Case Western Reserve University, Cleveland, Ohio, U.S.A.). The primary amines 4-aminobutyrate, 5-aminopentanoate and 6-aminohexanoate as well as 6-*N*-monomethyl-lysine and 6-*N*-dimethyl-lysine were purchased from Cyclo Chemical Co., Los Angeles, Calif., U.S.A. The 2-*N*-acetyl-L-lysine was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. 2-Methylcholine propionate was purchased from Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A. The 2-methylcholine was converted into the Cl^- form by passing it through a small column of AG-50 resin (H^+ form) eluted with 2M-HCl.

The 6-*N*-trimethyl-lysine was synthesized by stirring 1.58g of 2-*N*-acetyl-L-lysine, 1.8g of $Ba(OH)_2$,-$8H_2O$, 6ml of boiled water, 24ml of methanol and 7.5ml of methyl iodide in a stoppered flask at room temperature for 20h. The solution was then filtered and the excess of methyl iodide removed by evaporation. The residue was taken up in 5–10ml of water and the Ba^{2+} ions were precipitated by adding 50mmol of H_2SO_4. After neutralization, excess of SO_4^{2-} was removed by applying the product to a column (2cm×30cm) of AG-1 resin (X8: 200–400 mesh; OH^- form) and eluting with water. This resin also adsorbed lysine, 6-*N*-monomethyl-lysine, 6-*N*-dimethyl-lysine and 2-*N*-acetyl-lysine. The quaternary amine was identified in the effluent by precipitation in 1% (w/v) acidified reinecke, or by spotting and spraying with iodoplatinate reagent. The AG-1 resin column effluent was evaporated, extracted with 5×20ml of ethanol, and the extract evaporated. This residue was taken up in 50ml of 6M-HCl and hydrolysed at 105°C for 24h to remove the 2-*N*-acetyl group. After hydrolysis the HCl was removed by evaporation, the residue taken up in 10ml of water, and chromatographed on a column (2cm×30cm) of resin AG-50 (H^+ form). Salt was eluted from the column with 200ml of 1M-HCl, and the 6-*N*-trimethyl-lysine was eluted with 4M-HCl, 10ml fractions being collected. Fractions containing 6-trimethyl-lysine were pooled, evaporated to a glass, triturated with diethyl ether, and dried *in vacuo* over KOH. The

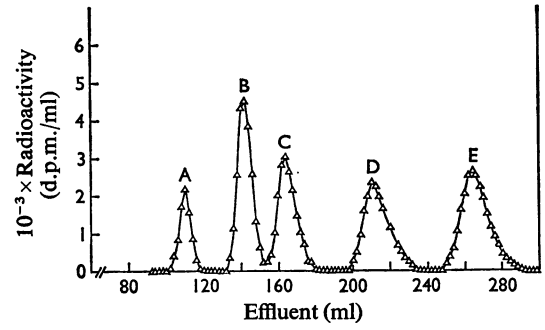


Fig. 1. *Chromatography of carnitine (peak A), 4-trimethylaminobutyrate (peak B), trimethylaminopentanoate (peak C), 6-trimethylaminohexanoate (peak D), and 6-trimethyl-lysine (peak E) on AG-50 ion-exchange resin*

The compounds were chromatographed on a column (1cm×50cm) of AG-50 (X8; H^+ form) and eluted with a gradient of increasing HCl concentration (150ml each of 1.5M-HCl and 4M-HCl as described in the Materials and Methods section). A solution containing the [^{14}C]methyl derivatives and 10mg of each compound was passed through the column at room temperature and 2ml fractions were collected. Radioactive peaks were identified by t.l.c.

Table 1. *T.l.c. of synthetic quaternary amines*

The t.l.c. plates and solvent systems are described by Cox & Hoppel (1973). The solvent front was allowed to run 15cm from the origin, and 25μg of each compound was chromatographed.

Compound	R_F values		
	System A	System B	System C
Carnitine	0.50	0.35	0.30
4-Trimethylaminobutyrate	0.50	0.35	0.20
5-Trimethylaminopentanoate	0.60	0.45	0.20
6-Trimethylaminohexanoate	0.65	0.50	0.25
6-*N*-Trimethyl-lysine	0.35	0.10	0.05
2-Methylcholine	0.75	0.40	0.20

6-trimethyl-lysine was chromatographically pure on t.l.c. (systems and R_F values in Table 1) as detected by iodine, ninhydrin and iodoplatinate sprays. A sample of 6-trimethyl-lysine kindly supplied by Dr. R. J. DeLange (DeLange *et al.*, 1969) chromatographed in the above systems in a manner identical with the compound synthesized by our method.

To detect incomplete acid hydrolysis, a sample of 6-*N*-trimethyl-2-*N*-acetyl-lysine was prepared. Before acid hydrolysis, a portion of the above reaction mixture was desalted on an AG-50 column. This sample, when analysed by t.l.c. had a major spot (6-*N*-trimethyl-2-*N*-acetyl-lysine, R_F 0.60 in system A) which reacted with I_2 and iodoplatinate sprays but gave no reaction with ninhydrin. A minor amount of 6-trimethyl-lysine ($R_F = 0.35$) was present but was well separated from the acetyl derivative. Thus incomplete hydrolysis of the 2-*N*-acetyl group was easily detected.

The 6-*N*-[*Me*-^{14}C]trimethyl-lysine was prepared on a smaller scale by the method used for non-radioactive 6-trimethyl-lysine. [^{14}C]Methyl iodide was made to react with 2-*N*-acetyl-lysine for 20h and then a 10-fold excess of nonradioactive methyl iodide was added and the reaction continued for another 20h. After hydrolysis the 6-[*Me*-^{14}C]trimethyl-lysine was purified by ion-exchange chromatography as described above. A 70–80% yield of radioactivity was obtained, and the labelled 6-trimethyl-lysine co-chromatographed with carrier in t.l.c. systems A, B and C. Specific radioactivity of the 6-[*Me*-^{14}C]trimethyl-lysine used in this study was determined to be 20.0μCi/mg (5.22μCi/μmol) by ninhydrin assay (Moore & Stein, 1954).

The 4-*N*-trimethylaminobutyrate, 5-*N*-trimethylaminopentanoate, and 6-*N*-trimethylaminohexanoate were synthesized from the parent primary amines by using an excess of methyl iodide in a solution of methanol, water and $Ba(OH)_2$ (Lindstedt & Lindstedt, 1965). The compounds were purified by the procedure used in the synthesis of 6-trimethyl-lysine. The quaternary amines in the HCl form were crystallized twice from ethanol and each compound was chromatographically pure in all three t.l.c. systems (Table 1). Melting points were determined and the values obtained agreed with the values reported by Lindstedt & Lindstedt (1965).

Methyl-labelled trimethylaminobutyrate, trimethylaminopentanoate and trimethylaminohexanoate were synthesized by the above techniques and the methods of Lindstedt & Lindstedt (1965). Specific radioactivity was determined by using the periodide assay (Wall *et al.*, 1960): 4-[*Me*-^{14}C]trimethylaminobutyrate, 5.62μCi/μmol; 4-[*Me*-^{3}H]trimethylaminobutyrate, 86.8μCi/μmol; 5-[*Me*-^{14}C]trimethylaminopentanoate, 0.78μCi/μmol; 6-[*Me*-^{14}C]trimethylaminohexanoate, 1.19μCi/μmol. These labelled compounds chromatographed with the appropriate carrier in all t.l.c. systems.

Results

Metabolism of 6-N-trimethyl-lysine in vivo

When lysine-deficient rats were given 6-*N*-[*Me*-^{14}C]trimethyl-lysine over 4 days, highly radioactive carnitine was isolated from the carcasses and urine of these animals. This labelled compound was identified by its elution from the AG-50 columns precisely with enzymically assayed carnitine (Figs. 2 and 3), and by crystallization with carrier without change in specific radioactivity (Table 2). Further, the labelled carnitine was chromatographically pure in t.l.c. systems A, B and C. The specific radioactivity of the carcass carnitine was 737000d.p.m./μmol, which was similar to the 730800d.p.m./μmol found in urine carnitine (Table 3). Therefore the injected 6-[^{14}C]trimethyl-lysine (specific radioactivity 11.6$\times$ 10^{6}d.p.m./μmol) was only diluted 16-fold as it was converted into carnitine. Approximately 14% of the administered ^{14}C label was recovered in the acid-soluble carcass carnitine, with an additional 3% in urine carnitine (Table 3). Thus carnitine was a major metabolite of exogenously administered 6-trimethyl-lysine in the rat.

By using four different methods, 4-*N*-trimethylaminobutyrate was established as a metabolite of 6-[*Me*-^{14}C]trimethyl-lysine in the rat. The resin AG-50 column chromatogram of the carcass extract (Fig. 2) and the urine (Fig. 3) showed a peak of radioactivity

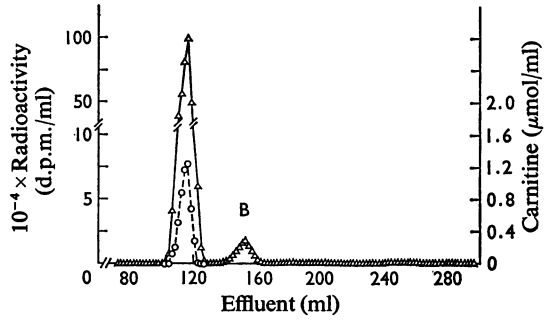


Fig. 2. *AG-50 chromatogram of the carcass extract from rats given* 6-[^{14}C]*trimethyl-lysine*

The extract was prepared from two lysine-deficient rats injected with 12.25μCi of 6-[*Me*-^{14}C]trimethyl-lysine/100g body wt. over 4 days. This carcass extract was prepared as described in the Materials and Methods section and then chromatographed through a column (1cm$\times$50cm) of AG-50 resin (X8; H^+ form) and eluted with the 1.5–4M-HCl gradient system. Fractions (2ml each) were collected. △, Radioactivity; ○, carnitine. Peak B was identified as 4-trimethylaminobutyrate.

where synthetic 4-trimethylaminobutyrate was eluted (Fig. 1). Samples from both of these peaks co-chromatographed with carrier trimethylaminobutyrate in all three t.l.c. systems. The carcass and urine trimethylaminobutyrate were also crystallized five times with carrier without change in the specific radioactivity (Table 2). In addition, a sample from the 4-trimethylaminobutyrate peak of Fig. 3 (urine) was converted into carnitine *in vitro* by 4-trimethylaminobutyrate (butyrobetaine) hydroxylase (Lindstedt, 1967). As described in the Materials and Methods section, 4-[*Me*-^{3}H]trimethylaminobutyrate was added to the ^{14}C-labelled metabolite and incubated with the enzyme preparation. After incubation 67% of the ^{14}C label and 69% of the standard 4-[^{3}H]trimethylaminobutyrate were converted into carnitine further demonstrating that this metabolite of 6-trimethyl-lysine was 4-trimethylaminobutyrate. Almost 30 times as much 4-[^{14}C]trimethylaminobutyrate was found in the urine compared with the carcass extract (Table 3).

Two unidentified peaks of radioactivity (Fig. 3, peaks I and II) were noted in the chromatogram of the AG-50 column of urine from the rats injected with 6-[*Me*-^{14}C]trimethyl-lysine. Peak I was eluted in a fashion similar to 5-*N*-trimethylaminopentanoate (Fig. 1). Radioactivity from this peak co-chromatographed in all three t.l.c. systems with carrier 5-trimethylaminopentanoate, and this label attained constant specific radioactivity with carrier after three crystallizations from ethanol–acetone. Therefore peak I in Fig. 3 was tentatively identified as containing 5-*N*-[^{14}C]trimethylaminopentanoate. When the carcass extract was chromatographed on an AG-50 column, a similar peak of radioactivity containing 7000d.p.m. total (peak too small to be illustrated in Fig. 2) was also detected.

A broad peak of radioactivity, identified as peak II in Fig. 3, was observed in the resin AG-50 chromatogram of urine, but a corresponding peak could not be detected in the carcass extract. Peak II of Fig. 3 appeared to be composed of two radioactive compounds which were eluted from the AG-50 resin in a

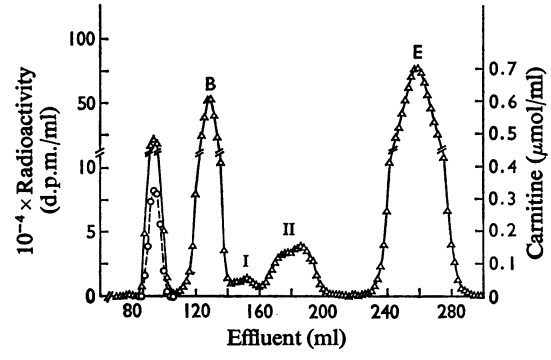


Fig. 3. *AG-50 chromatography of pooled 4-day urine collected from rats given 6-[^{14}C]trimethyl-lysine*

The urine from the rats described in Fig. 2 was evaporated to dryness and hydrolysed in 10ml of 0.1M-KOH for 1h at room temperature. After neutralization with 60% (w/v) $HClO_4$ and removal of the resulting precipitate, the supernatant was passed through a column (1.5cm×10cm) of AG-1 resin (X8; OH^- form) and eluted with water. The effluent was neutralized and chromatographed on AG-50 resin as described in the Materials and Methods section. △, Radioactivity; ○, carnitine. Peak B was found to be trimethylaminobutyrate and peak E was trimethyl-lysine.

Table 2. *Crystallization of [^{14}C]carnitine and 4-[^{14}C]trimethylaminobutyrate biosynthesized in vivo from 6-[^{14}C]-trimethyl-lysine*

Portions of the fractions from the following AG-50 columns were evaporated to dryness and crystallized with 150mg of the appropriate carrier: carcass (Fig. 2), carnitine 114ml elution vol. (fraction 57), 4-trimethylaminobutyrate 146–158ml (fractions 73–79); urine (Fig. 3), carnitine 90–94ml (fractions 45–47), 4-trimethylaminobutyrate 124–134ml (fractions 62–67). After each crystallization from ethanol–acetone the specific radioactivity was determined in 5–10mg of the crystals.

	Carnitine specific radioactivity (d.p.m./mg)		4-Trimethylaminobutyrate specific radioactivity (d.p.m./mg)	
Crystallizations	Carcass	Urine	Carcass	Urine
1	597	159	89	562
2	600	156	102	588
3	612	146	90	556
4	621	154	87	600
5	612	152	100	547

Table 3. *Labelled metabolites of* 6-[*Me*-^{14}C]*trimethyl-lysine*

The compounds were isolated from two lysine-deficient rats injected daily with 12.25μCi of 6-[*Me*-^{14}C]trimethyl-lysine/100g body wt. for 4 days. The results are calculated from Figs. 2 and 3.

	Carcass extract	Urine
Carnitine		
Specific radioactivity	737000d.p.m./μmol	730800d.p.m./μmol
Total radioactivity	4.68μCi	0.92μCi
Percent of injected dose	14%	2.8%
4-Trimethylaminobutyrate		
Total radioactivity	0.09μCi	2.55μCi
Percent of injected dose	0.26%	7.5%
6-Trimethyl-lysine		
Total radioactivity	0.0048μCi	7.31μCi
Percent of injected dose	—	21.5%

similar way to 6-*N*-trimethylaminohexanoate (Fig. 1). By t.l.c. two radioactive compounds were demonstrated in these fractions; however, these radioactivities did not co-chromatograph with carrier 6-trimethylaminohexanoate. T.l.c. of the fractions from peak II showed that most of the label in the early fractions chromatographed with $R_F = 0.60$, whereas the predominant label in the latter fractions of this peak had R_F about 0.15, which was lower than that of any of the synthetic compounds considered in this study. The identity of these metabolites of 6-[*Me*-^{14}C]-trimethyl-lysine in urine and their relationship with carnitine biosynthesis remains unknown.

Over 20% of the injected 6-[^{14}C]trimethyl-lysine was isolated unaltered from the 4-day urine sample (Table 3 and Fig. 3), but only a small amount of labelled 6-trimethyl-lysine (10000d.p.m. total) was recovered from the carcass. Thus free 6-[*Me*-^{14}C]-trimethyl-lysine was not retained by the rat carcass to any appreciable degree.

An attempt was made to measure the production of 2-methylcholine, a known metabolite of carnitine (Khairallah & Wolf, 1967), after the injection of [^{14}C]trimethyl-lysine. Synthetic 2-methylcholine is eluted from the AG-50 column system between trimethylaminobutyrate and trimethylaminopentanoate (Fig. 1), but it is not adequately separated from these compounds. However, 2-methylcholine is well separated from them by t.l.c. system A (Table 1). In the examination of the carcass and urine extracts no evidence was found for the accumulation of labelled 2-methylcholine after [^{14}C]trimethyl-lysine injection.

Conversion of 5-N-trimethylaminopentanoate and 6-N-trimethylaminohexanoate into carnitine in vivo

Two other compounds, 5-trimethylaminopentanoate and 6-trimethylaminohexanoate, were tested to determine if they might contribute to carnitine biosynthesis. Radioactive 5-trimethylaminopentanoate was tentatively identified in the urine of animals given 6-[^{14}C]trimethyl-lysine, and although Lindstedt & Lindstedt (1965) could not demonstrate conversion of these compounds into carnitine in mice, they were again tested in long-term experiments as possible intermediates in the conversion of 6-trimethyl-lysine into 4-trimethylaminobutyrate or carnitine.

When 5-*N*-[*Me*-^{14}C]trimethylaminopentanoate was injected into lysine-deficient rats (11.75μCi/100g daily over 4 days), the carcass carnitine was labelled to the extent of 2780d.p.m./μmol. This was 0.4% of the specific radioactivity attained in carcass carnitine after a similar dose of 6-[^{14}C]trimethyl-lysine (12.25μCi/100g daily over 4 days) was given. Radioactivity was recovered only in carnitine and 5-trimethylaminopentanoate when the carcass extract of these animals was chromatographed on the resin AG-50 column system (Fig. 4*a*).

Only a small amount of label was recovered in carnitine after lysine-deficient rats were injected with 6-[*Me*-^{14}C]trimethylaminohexanoate (11.5μCi/100g daily over 4 days). The specific radioactivity of carnitine isolated from the carcasses of these animals was 520d.p.m./μmol, which was only 0.07% of that in carcass carnitine after a similar dose of 6-[^{14}C]trimethyl-lysine. The AG-50 chromatogram of the carcass extract from animals given 6-[^{14}C]trimethyl-aminohexanoate showed another small peak of radioactivity where synthetic 5-trimethylaminopentanoate was eluted (Fig. 4*b*, peak I). This label co-chromatographed with 5-trimethylaminopentanoate in all three t.l.c. systems, but could not be crystallized to constant specific radioactivity with the appropriate carrier. Most of the radioactivity recovered from the carcass extract was unchanged 6-*N*-trimethylamino-hexanoic acid. Owing to the small incorporation of

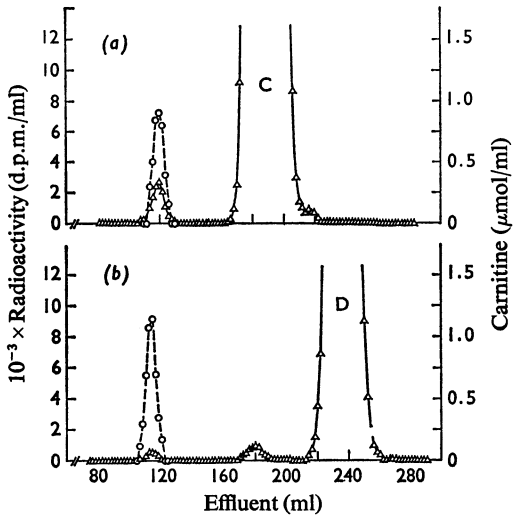


Fig. 4. *Chromatography of carcass extracts from rats injected with labelled 5-trimethylaminopentanoic acid or 6-trimethylaminohexanoic acid*

(*a*) Extract from two lysine-deficient rats injected daily with 11.75μCi of 5-[*Me*-^{14}C]trimethylaminopentanoate/100g over 4 days. The chromatography was performed on a column (1×50cm) of AG-50 resin (X8; H^+ form) eluted with an increasing concentration of HCl (1.5M to 4M as described in the Materials and Methods section. Peak C was trimethylaminopentanoate. (*b*) AG-50 chromatogram of the carcass extract from two lysine-deficient rats injected daily with 11.5μCi of 6-[*Me*-^{14}C]trimethylaminohexanoate/100g over 4 days. Chromatography conditions were the same as described in (*a*). △, Radioactivity ; ○, carnitine. Peak D was trimethylaminohexanoate.

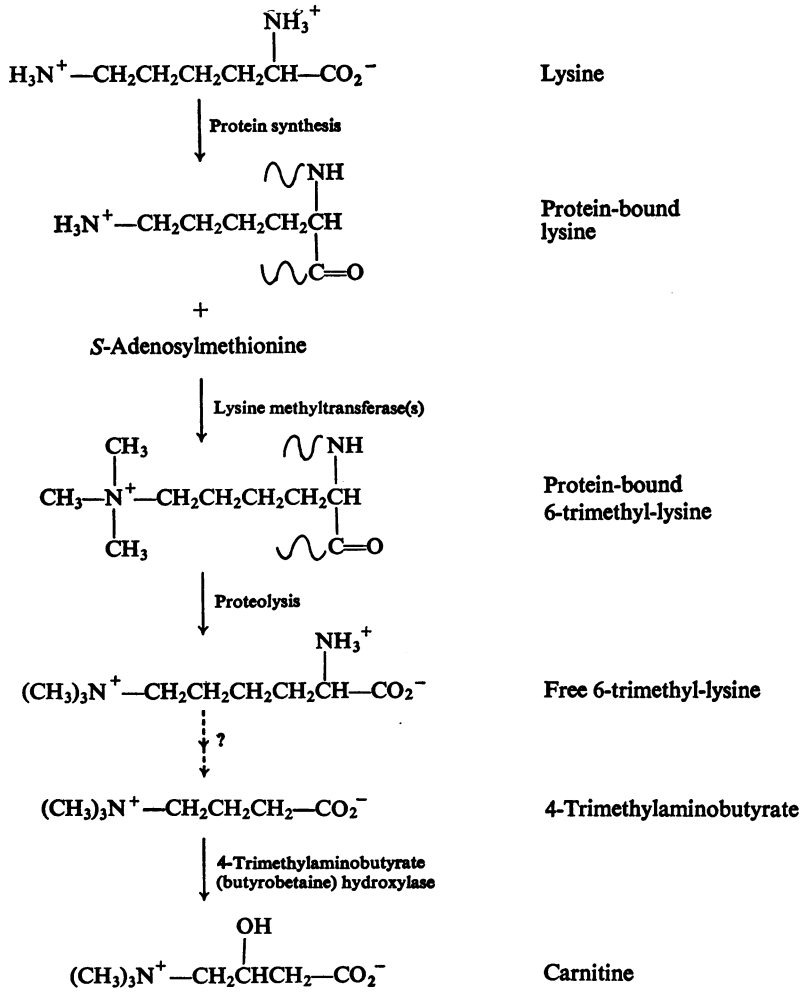
label into carcass carnitine, urine collected from the animals injected with 5-[*Me*-^{14}C]trimethylaminopentanoate and 6-[*Me*-^{14}C]trimethylaminohexanoate was not examined.

Discussion

The present study demonstrates that carnitine was a major catabolite of 6-*N*-trimethyl-lysine *in vivo*. In lysine-deficient rats 17% of the injected radioactive 6-trimethyl-lysine was recovered in the acid-soluble carnitine from carcass and urine. The next largest amount of 6-trimethyl-lysine-derived radioactivity was found in 4-*N*-trimethylaminobutyrate (8% of the injected dose). In the urine from the rats injected with 6-[*Me*-^{14}C]trimethyl-lysine three other metabolites were recovered which accounted for about 2% of the injected label. One compound could not be differentiated from 5-*N*-trimethylaminopentanoate by using ion-exchange chromatography or t.l.c. or by recrystallization with the synthetic compound. It is not known if the other two metabolites in urine are part of the pathway of carnitine or 4-trimethylaminobutyrate synthesis. Since 6-*N*-monomethyl-lysine and 6-*N*-dimethyl-lysine were adsorbed by the AG-1 (OH^- form) ion-exchange resin, the production of these compounds from 6-trimethyl-lysine was not studied. Still, a significant amount of the labelled 6-trimethyl-lysine was converted into other metabolites in the rat. This is in contrast with the findings of other workers, who have reported that 6-trimethyl-lysine was metabolized to only a small extent in the rat (Löwer *et al.*, 1972; Lange *et al.*, 1973).

Since 5-trimethylaminopentanoate is converted relatively slowly into carnitine in the rat, this compound does not appear to be an intermediate in the synthesis of 4-trimethylaminobutyrate or carnitine from 6-trimethyl-lysine. It is possible that 5-trimethylaminopentanoate is excluded from biosynthetically active compartments, or it is a bound intermediate. Thus it could be part of the 6-trimethyl-lysine → carnitine pathway even though it is not converted into carnitine as completely as would be expected for an intermediate. Conversely, this compound could be a metabolite of 6-trimethyl-lysine having nothing to do with carnitine biosynthesis. It has been postulated that when the 6-*N*-group of lysine is blocked, transamination with the 2-*N*-group is possible in mammals (Paik & Kim, 1964). A similar transamination with 6-trimethyl-lysine would produce 6-*N*-trimethylamino-2-oxohexanoate. This compound would probably be unstable under our methods of isolation and would decarboxylate to form 5-trimethylaminopentanoate. Thus the label that co-chromatographed with 5-trimethylaminopentanoate may not be a metabolite of 6-trimethyl-lysine but an artifact of the purification procedures.

In lysine-deficient rats we recovered most of the radioactive 4-trimethylaminobutyrate (97%) from the urine sample. Perhaps the 4-trimethylaminobutyrate that reaches the kidney is largely excreted, whereas that which is retained by body tissues is converted into carnitine. Similar results were obtained when radioactive 4-trimethylaminobutyrate was injected intraperitoneally into rats. After 30h 50% of the radioactivity was recovered from the carcass and 98% of this was identified as carnitine (Lindstedt & Lindstedt, 1961). Broquist & Tanphaichitr (1973) have recently proposed that tissues such as skeletal muscle, heart and testes can cleave 6-trimethyl-lysine to 4-trimethylaminobutyrate; however, only the liver can produce significant amounts of carnitine from 6-trimethyl-lysine. Further, 4-trimethylaminobutyrate hydroxylase activity is present in the liver but not in the supernatant fraction of kidney or heart homogenates (Lindstedt, 1967). Thus tissues peripheral to the liver may secrete 4-trimethylamino-



Scheme 1. *Biosynthesis of carnitine*

butyrate into the plasma, which would then be available for excretion by the kidney or hydroxylation in the liver. Starting with radioactive methionine (Strength *et al.*, 1965) or 6-trimethyl-lysine (Broquist & Tanphaichitr, 1973), the major site of carnitine biosynthesis appeared to be the liver. The biosynthesis was then followed by transport of the radioactive carnitine to other tissues. The relative contribution of peripheral tissues to carnitine biosynthesis through the production of 4-trimethylaminobutyrate is unknown.

Several pieces of information have accumulated to support the pathway of carnitine biosynthesis proposed in Scheme 1. Lindstedt (1967) has shown the rapid hydroxylation *in vitro* of 4-trimethylaminobutyrate to form carnitine. The 4-trimethylaminobutyrate hydroxylase purified from rat liver shows a high degree of specificity, and this compound is found in mammals (Broekhuysen & Deltour, 1961). In the present study and those by Tanphaichitr & Broquist (1973), it has been demonstrated that 6-trimethyllysine is readily converted into 4-trimethylaminobutyrate and carnitine in the rat; however, little is known about this conversion. Owing to the relatively slow conversion of labelled 5-trimethylaminopentanoate and 6-trimethylaminohexanoate into carnitine, it appears that these compounds are not free intermediates in the above reaction. A possible mechanism for the conversion of 6-trimethyl-lysine into 4-trimethylaminobutyrate was suggested (Horne &

Broquist, 1973) by reactions involved in the fermentation of lysine by species of *Clostridium* and *Pseudomonas* (Stadtman, 1963; Rimerman & Barker, 1968). These bacteria can metabolize lysine to acetate and butyrate through the formation of 3,6-diaminohexanoic acid (β-lysine). An analogous metabolism of 6-trimethyl-lysine would produce 3,6-diamino-6-*N*-trimethylhexanoate (6-*N*-trimethyl-β-lysine) which could undergo oxidative deamination or transamination and then cleavage to form a 2-carbon fragment and 4-trimethylaminobutyrate. It is not known whether peptide bound or free 6-trimethyl-lysine is cleaved to form 4-trimethylaminobutyrate. There is no evidence that 6-trimethyl-lysine is incorporated unchanged into protein (Löwer *et al.*, 1972; Paik & Kim, 1971), and a path for the conversion of free 6-trimethyl-lysine into 4-trimethylaminobutyrate and carnitine may be present in the rat.

Trimethyl-lysine has been identified in many proteins such as histones, cytochrome *c* and myosin (Paik & Kim, 1971) and has also been isolated in the free form from human plasma and urine (Kakimoto & Akazawa, 1970). In mammals, 6-trimethyl-lysine appears to originate only from the methylation of lysine residues after peptide-bond synthesis. The methylation of protein-bound lysine to form 6-trimethyl-lysine has been studied in partially purified preparations from Krebs ascites-tumour-cell chromatin (Burdon & Garven, 1971) and rat brain cytoplasmic fractions (Miyak & Kakimoto, 1973). In both enzyme preparations *S*-adenosylmethionine was the methylating agent. Several workers have shown that methionine is incorporated into the methyl groups of carnitine in the rat (Cox & Hoppel, 1973; Wolf & Berger, 1961). Therefore it was proposed that methionine enters into carnitine biosynthesis at the step of lysine methylation.

In the preceding paper (Cox & Hoppel, 1973), we demonstrated the conversion of lysine into the butyrate carbon chain of carnitine with the loss of C-1 and C-2 of lysine. Uniformly labelled lysine was also incorporated into the main carbon chain of 4-trimethylaminobutyrate, and in this experiment 4-trimethylaminobutyrate had a specific radioactivity 12 times that of carnitine isolated from the same animal (Cox & Hoppel, 1973). These data are consistent with a precursor–product relationship between 4-trimethylaminobutyrate and carnitine. In yeast 4-trimethylaminobutyrate has been shown to be an obligatory intermediate in the synthesis of carnitine from lysine or 6-trimethyl-lysine (Horne *et al.*, 1972).

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