Role of Lysine and e-N-Trimethyllysine in Carnitine Biosynthesis

II. STUDIES IN THE RAT*

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SUMMARY

We have previously reported that weaning rats fed a 20% wheat gluten diet, limiting in lysine and containing no detectable carnitine, have significantly lower levels of carnitine in skeletal and heart muscle than rats receiving adequate dietary lysine. Rats on 20% wheat gluten diets were administered appropriate levels of DL-[6-14C]lysine, L-[methyl-3H]-methionine, e-N-[methyl-3H]trimethyl-L-lysine, or [carboxy-14C]-butyrobetaine. Carnitine was subsequently isolated by ion exchange chromatography from a number of tissues and examined for radioactivity. In most trials about 0.1% of administered DL-[6-14C]lysine or L-[methyl-3H]methionine was incorporated into tissue carnitine. Dietary lysine significantly decreased the utilization of DL-[6-14C]lysine for carnitine synthesis. Radioactivity following administration of DL-[2,14C]lysine was not detected in biosynthesized tissue carnitine. e-N-[methyl-3H]trimethyl-L-lysine and [carboxy-14C]-butyrobetaine were converted in high yield to carnitine, e.g., 23 and 45% of administered dose per 100 g of skeletal muscle. Radioactive carnitine was also detected in the urine in these latter instances. It was shown that e-N-trimethyllysine is converted to γ-butyrobetaine in the rat. These data support the thesis that lysine is a precursor of carnitine in the rat, which together with similar labeling studies in Neurospora suggest the following transformations: lysine → e-N-trimethyllysine → γ-butyrobetaine → carnitine.

The preceding paper (1) presents evidence from studies in Neurospora that a moiety of lysine contributes to the biosynthesis of carnitine. Lysine carbon atoms 1 and 2 are not involved in this process, and it appears that carbon atoms 3, 4, 5, and 6 plus the e-N atom of lysine become the butyrate carbon chain and 4-N atom, respectively, of carnitine. Methionine, is the penultimate source of the N-methyl groups of carnitine in animals (2, 3) and in Neurospora (1). In the latter mold, e-N-trimethyllysine is a highly efficient precursor of carnitine (1). It is postulated that lysine is methylated via S-adenosylmethionine, giving e-N-trimethyllysine which is then cleaved in such a manner as to yield γ-butyrobetaine and thence carnitine by hydroxylation (cf. Fig. 4 in Reference 1).

We were interested to learn if evidence for these postulated relationships in Neurospora could also be found in the rat. Weaning males were fed a 20% wheat gluten diet, limiting in lysine and containing no detectable carnitine. Weanling male rats were fed a 20% wheat gluten diet, limiting in lysine and containing no detectable carnitine. Such nutritional conditions require de novo carnitine synthesis by the rat and minimize dilution by exogenous lysine of proffered radioactive test carnitine precursors. As reported briefly in our communication (4), following intraperitoneal administration in lysine-deficient rats, radioactivity from DL-[6-14C]lysine, but not DL-[2-14C]lysine, was significantly incorporated into carnitine in the liver and skeletal muscle. More recently we reported that e-N-[methyl-3H]trimethyllysine and 4-N-trimethylamino[1-14C]butyrate (γ-butyrobetaine) were incorporated approximately 100-fold more effectively than lysine into tissue carnitine by the rat (5). Full details of these findings are reported herein which establish unequivocally that lysine and e-N-trimethyllysine are involved in carnitine biosynthesis in the rat. A metabolic function for e-N-trimethyllysine in carnitine formation is thus defined in addition to the role it may play as a consequence of modifying the structure of certain proteins (6). Carnitine, an important biocatalyst involved in the transport of fatty acids across the mitochondrial membrane, is thus seen to derive in the rat from the essential amino acids, lysine and methionine, required for the synthesis of e-N-trimethyllysine.

EXPERIMENTAL PROCEDURE

Radioactive Materials and Methods—Sources of radioactive materials and a description of counting procedures are indicated in the accompanying paper (1).

Diet and Animal Care—Male weanling rats, Sprague-Dawley strain, were fed 20% wheat gluten diets containing minimal lysine (0.37%) and < 0.1 µg of carnitine per g for the time periods stated in the tables. With one exception noted in Table I the diet was in essence the diet of Gupta et al. (7) and is a 20% gluten, corn-starch, corn oil, salts, and vitamins diet further supplemented...
with certain limiting amino acids other than lysine. A complete
description of this diet together with other details of animal care
employed throughout the present investigation is given in Refer-
ence 8. Lysine deficiency induced from consumption of these
glutens diets is fully documented in Reference 8 and was recog-
nized by lack of growth, anemia, and hypoproteinemia.

Animal Experimentation and Tissue Analysis—At designated
time intervals, individual rats were placed in metabolism cages
with provision for collecting urine separate from the feces, and
the rats were injected intraperitoneally with respective radioac-
tive test carnitine precursors. Details of the injection schedule
and subsequent treatment of the animals are given in the tables.
On completion of the injection schedule, the animals were usually
maintained on their previous dietary regimen for a specified
number of days unless otherwise indicated and then killed. Cer-
tain tissues, and in some instances urine, were then examined for
from tissues (4, 8), the isolation of carnitine by ion exchange
chromatography (1), and the spectrophotometric assay of carni-
tine by the carnitine acetyltransferase assay (1, 4) appear in the
table of this diet together with other details of animal care
employed throughout the present investigation is given in Refer-
ence 8. Lysine deficiency induced from consumption of these
 glutens diets is fully documented in Reference 8 and was recog-
nized by lack of growth, anemia, and hypoproteinemia.

RESULTS

Labeling of Tissue Carnitine following Administration of Radio-
active Lysine—Rats A and B which had been maintained on a
20% gluten diet, limiting in lysine, for 99 days were administered
either DL-[6-14C]lysine or DL-[2-14C]lysine, respectively, as sched-
uled in Table I. Subsequently, the animals were killed, and
certain tissue extracts were fractionated by ion exchange chroma-
tography appropriate for the separation of carnitine from radio-
active lysine. The elution profiles of Fig. 1 clearly show coinci-
dence of carnitine activity (carnitine acetyltransferase assay)
with radioactivity derived from DL-[6-14C]lysine but not DL-[2-14C]-
lysine in extracts of skeletal muscle and liver. The specific ac-

### Table I

<table>
<thead>
<tr>
<th>Rat</th>
<th>Days on 20% gluten diet</th>
<th>Nutritional status</th>
<th>Isotope administered</th>
<th>Organ examined</th>
<th>Specific activity</th>
<th>Total dose</th>
<th>Type</th>
<th>Wet wt</th>
<th>Specific activity</th>
<th>Administered dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>99</td>
<td>Lysine-deficient</td>
<td>8-1-6[14C]lysine</td>
<td>Skeletal muscle</td>
<td>64.4</td>
<td>0.003</td>
<td>0.070</td>
<td>0.109</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>99</td>
<td>Lysine-deficient</td>
<td>DL-[2-14C]lysine</td>
<td>Liver</td>
<td>6.6</td>
<td>0.003</td>
<td>0.003</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>46</td>
<td>Lysine-deficient</td>
<td>DL-[6-14C]lysine</td>
<td>Epididymis</td>
<td>0.7</td>
<td>0.003</td>
<td>0.008</td>
<td>1.076</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>35</td>
<td>Normal control</td>
<td>DL-[6-14C]lysine</td>
<td>Skeletal muscle</td>
<td>36.5</td>
<td>0.01</td>
<td>0.050</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>55</td>
<td>Lysine-deficient</td>
<td>L-[6-14C]lysine</td>
<td>Liver</td>
<td>3.5</td>
<td>0.01</td>
<td>0.004</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rats A to D were fed the 20% wheat gluten diet, basal diet 1 in Reference 8, for the time periods indicated. The diet of Rat E
was basal diet 2 in Reference 8, which is similar to basal diet 1, but lacks an amino acid supplement present in basal diet 1.
* Rats A and B: 41 µCi every 12 hours for 3 days; animals killed 5 days later; Rat D: 41 µCi every 12 hours for 3 days; animals killed 3 days later; Rat E: 41 µCi every 12 hours for 3 days; animals killed 3 days later. Rat D: 125 µCi every 12 hours for 3 days; animals killed 12 hours later; during this 3½ day period the diet of Rat E was supplemented with 1% lysine.
* The assumption was made that skeletal muscle = 45% of body weight.

A second lysine-deficient rat (Rat C, Table I) was again ad-
dministered DL-[6-14C]lysine (cf. schedule, Table I), and extracts
of skeletal muscle, heart, liver, kidney, testes, and brain were
monitored for carnitine activity and radioactivity following ion
exchange chromatography. The results are plotted in Fig. 2
and provide confirmation for the utilization of DL-[6-14C]lysine in
carnitine biosynthesis in the rat and that such carnitine is widely distributed in rat tissue. Analytical data from the curves of Fig. 2 for Rat C are summarized in Table I. If the percentage of administered DL-[6-14C]lysine per whole organ for Rats A and C is summed, it is seen that about 0.08 and 0.06%, respectively, of the lysine administered was utilized for carnitine biosynthesis. The experiment with Rat C just discussed was repeated with Rat D by using DL-[6-14C]lysine of the same specific activity, but in this instance Rat D had been on a lysine-supplemented diet throughout the experimental period. The effect of such nutritional conditions was to reduce the specific activity of biosynthesized carnitine from radioactive lysine approximately 10-fold (compare Rat C with Rat D, Table I). In this experiment, Rat D was given the same amount of isotopic lysine as Rat A (cf. schedule, Table I). The level of biosynthesized carnitine, expressed as percentage of administered lysine per 100 g of skeletal muscle, liver, and epididymis was about 2.8- to 3.5-fold less for Rat D compared with Rat A. These data again reflect the diminution of radioactivity from isotopic lysine in newly formed carnitine by the unlabeled lysine supplement in the ration of Rat D. Such results would likely have been even more striking if Rat D had been on this diet for 99 days (the time schedule with Rat A) before the administration of isotopic lysine.

A second isotope dilution experiment was conducted with lysine-deficient Rat E (Table I), in which L-[2-3H]lysine of high specific activity was employed; however, during the administration of the isotope (3 days), the rat was allowed to consume a diet supplemented with 1.0% unlabeled lysine. Examination of biosynthesized carnitine under these conditions (Table I) revealed a tremendous dilution of specific activity of the initial isotopic lysine into tissue carnitine with a correspondingly minute amount of isotope incorporated into skeletal muscle and liver carnitine. The experiment illustrates the avidity of the tissues of the lysine-deficient rat for added lysine as evidenced by the pronounced dilution of radioactive lysine by dietary lysine into newly synthesized carnitine.

Labeling of Tissue Carnitine following Administration of L-NTrimethyllysine and γ-Butyrobetaine—Lysine-deficient Rat G was given a single injection of L-γ-[methyl-3H]trimethyl-L-lysine as detailed in Table II and killed after a 24-hour period, the carnitine content of certain tissue extracts was examined for radioactivity following purification by ion exchange chromatography. Fig. 3 illustrates coincidence of radioactivity derived from L-γ-trimethyllysine with carnitine fractions from liver and muscle of Rat G.

Under the conditions of ion exchange chromatography employed herein, γ-butyrobetaine is generally eluted about 10 fractions after carnitine. Radioactive peaks in this position were frequently noted in the experiments which have been discussed (e.g. cf. Figs. 2 and 3). Fractions 61 to 64 derived from the liver of Rat G (Fig. 3) were pooled, desalted, and evaporated to dryness, and the residue was taken up in methanol. Carrier γ-butyrobetaine (0.1 mg) was added, and the whole fraction was sub-
were applied in each instance to ion exchange columns. Tracts containing 3.5 g (wet weight) of skeletal muscle and liver extracts containing e-N-trimethyllysine were jetted to thin layer chromatography in two solvent systems and subsequently developed as described under "Experimental Procedure" and in Table II. The dose was such that each animal received an equal amount of radioactivity (microcurie per g of L isomer) on a body weight basis. Rat L was administered 6 &i every 12 hours for 1 day and was killed 3 days later.

The results shown in Fig. 4 illustrate that the only significant incorporation of radioactivity from profferred e-N-trimethyllysine into biosynthesized carnitine was into skeletal muscle. It is not surprising that dietary lysine had little effect on the incorporation of e-N-trimethyllysine into carnitine. It is noteworthy that more than 20% of the profferred e-N-trimethyllysine was only diluted out about 50 times (Rat G). Such findings are also borne out by the data of the last column of Table II, indicating that more than 20% of the profferred e-N-trimethyllysine was incorporated into carnitine per 100 g of tissue in contrast to about 0.01% of the administered lysine. Clearly e-N-trimethyllysine is a specialized lysine metabolite uniquely geared for carnitine synthesis. It is not surprising that dietary lysine had little effect on the incorporation of e-N-trimethyllysine into carnitine (compare Rat G with Rat H, Table II).

Other experiments which focus on the trimethyllysine-carnitine, precursor-product relationship were carried out with Rats F, H, J, and K (Table II) by using the same experimental procedures as described for Rat G, in which, following ion exchange chromatography of tissue extracts, it is possible to quantitate the incorporation of radioactivity from profferred carnitine precursors into biosynthesized carnitine. If the experiments with Rat F versus Rat G (Table II) are compared, it can be seen that the specific activity of DL-[6-14C]lysine was diluted out about 240,000 times in labeling skeletal muscle carnitine (Rat F), whereas the specific activity of e-N-[methyl-3H]trimethyllysine was only diluted out about 50 times (Rat G). Such findings are also borne out by the data of the last column of Table II, indicating that more than 20% of the profferred e-N-trimethyllysine was incorporated into carnitine per 100 g of tissue in contrast to about 0.01% of the administered lysine. Clearly e-N-trimethyllysine is a specialized lysine metabolite uniquely geared for carnitine synthesis. It is not surprising that dietary lysine had little effect on the incorporation of e-N-trimethyllysine into carnitine (compare Rat G with Rat H, Table II).

The incorporation of the N-methyl groups of e-N-trimethyllysine into carnitine must be direct, not only in view of the high efficiency of incorporation of the administered dose into tissue carnitine (Rats G and H, Table II), but when L-[methyl-3H]methionine was administered under comparable conditions to Rats J and K (Table II), only 0.05 and 0.07% of the administered dose was incorporated per 100 g of skeletal muscle (Table II). Rat K had been given a lysine supplement (cf. footnote a, Table II) prior to and during the administration of radioactive methionine to assure an adequate source in this instance of the carbon and nitrogen atoms of carnitine except for the N-CH3 groups.

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**Table II**

**Labeling of tissue carnitine following administration of test carnitine precursors**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Days on 20% gluten diet</th>
<th>Nutritional status</th>
<th>Isotope administered</th>
<th>Organ examined</th>
<th>Biosynthesized carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Specific activity</td>
<td>Type</td>
<td>Specific activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total dose</td>
<td></td>
<td>Per whole organ</td>
</tr>
<tr>
<td>F</td>
<td>83</td>
<td>Lysine-deficient</td>
<td>48.0 376</td>
<td>Skeletal muscle</td>
<td>0.0002 0.001 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>5.5 0.002 0.001 0.02</td>
</tr>
<tr>
<td>G</td>
<td>56</td>
<td>Lysine-deficient</td>
<td>43.0 172</td>
<td>Skeletal muscle</td>
<td>0.81 14.9 22.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>1.04 1.1 1.21 1.51</td>
</tr>
<tr>
<td>H</td>
<td>68</td>
<td>Normal control</td>
<td>43.0 364</td>
<td>Skeletal muscle</td>
<td>0.72 21.2 15.1</td>
</tr>
<tr>
<td>J</td>
<td>72</td>
<td>Lysine-deficient</td>
<td>49.7 182</td>
<td>Skeletal muscle</td>
<td>0.002 0.04 0.05</td>
</tr>
<tr>
<td>K</td>
<td>84</td>
<td>Lysine-deficient</td>
<td>40.7 295</td>
<td>Skeletal muscle</td>
<td>0.003 0.06 0.07</td>
</tr>
<tr>
<td>L</td>
<td>142</td>
<td>Lysine-deficient</td>
<td>1.6 12</td>
<td>Skeletal muscle</td>
<td>0.09 47.0 45.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heart</td>
<td>0.7 0.09 0.40 54.9</td>
</tr>
</tbody>
</table>

a All rats were fed the 20% wheat gluten diet, basal diet 1 in Ref. 8, for the time periods indicated. The diet of Rat H was supplemented with 0.8% L-lysine. Two days before Rat K was given isotopic methionine, 0.8% L-lysine was added to the diet for the duration of the experiment.

b All of the rats with the exception of Rat L were given a single injection of test radioactive material and killed after a 24-hour period. The dose was such that each animal received an equal amount of radioactivity (microcurie per g of L isomer) on a body weight basis. Rat L was administered 6 &i every 12 hours for 1 day and was killed 3 days later.

c The assumption was made that, skeletal muscle = 45% body weight.

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**Fig. 3.** Elution profile of radioactivity (-----) and carnitine (-----) following ion exchange chromatography of skeletal muscle and liver extracts of lysine-deficient Rat E, administered e-N-[methyl-3H]trimethyllysine. Conditions of the experiments are described under "Experimental Procedure" and in Table II. Extracts containing 3.5 g (wet weight) of skeletal muscle and liver were applied in each instance to ion exchange columns.
Fig. 4. Thin layer chromatography of radioactive \( \gamma \)-butyrobetaine biosynthesized from \( \epsilon-N-\)methyl-\( ^3\)Htrimethyllysine. Aliquots (90 \( \mu \)l) of a methanol solution (cf. text) containing carrier \( \gamma \)-butyrobetaine plus radioactive Fractions 61 to 64 (experiment of Fig. 3, Frame B) were applied to Eastman 6061 chromatography sheets and developed for 15 cm at room temperature in the solvent systems described in the figure. Following development, the sheets were air-dried and exposed to iodine vapor to visualize \( \gamma \)-butyrobetaine. The strips were then cut into 1-cm sections, immersed in a scintillation fluid, and counted in a Packard Tri-Carb liquid scintillation counter, and the counts in each fraction were plotted as shown.

It is apparent from these experiments that \( \epsilon-N \)-trimethyllysine is not serving merely as a source of labile methyl groups which might serve then in providing the methyl groups of carnitine via some other means, but rather all the evidence of Table II indicates that \( \epsilon-N \)trimethyllysine per se is a direct precursor of carnitine.

Fig. 5 describes an experiment with lysine-deficient Rat L (Table II) which, in agreement with studies of others (9, 10), shows that \( \gamma \)-butyrobetaine is a precursor of tissue carnitine in the rat. The dilution of the specific activity of \( \gamma \)-butyrobetaine into carnitine and the percentage of incorporation of the administered dose of \( \gamma \)-butyrobetaine into tissue carnitine per 100 g of tissue were of the same order of magnitude as encountered with \( \epsilon-N \)-trimethyllysine (Table II).

**Urinary Excretion of Carnitine and \( \gamma \)-Butyrobetaine following Administration of Test Carnitine Precursors**—In certain of the preceding experiments, specifically those concerned with Rats A and C (Table I) and Rats J, G, H, and L (Table II), during the administration of the test carnitine precursors, the rats were placed in metabolism cages with facilities for the collection of urine for the time periods indicated in Table III. In these instances, aliquots of urine were subjected to the standard ion exchange procedure employed throughout this study to effect the separation of carnitine and \( \gamma \)-butyrobetaine from test precursors. The radioactivity of such urine fractions, together with the carnitine content of appropriate fractions, was determined, and the results are summarized in Table III.

Although the intraperitoneal injection of isotopic lysine and methionine to lysine-deficient rats labeled tissue carnitine in these rats (Tables I and II), no radioactive carnitine or \( \gamma \)-butyrobetaine was excreted in these instances (Table III). Since the carnitine levels in skeletal muscle and heart in lysine-deficient rats have been shown to be low (8), it is likely that newly biosynthesized carnitine arising in response to the administration of a tracer quantity of lysine was being avidly taken up by the tissues and would not be expected to appear in the urine until the tissues were saturated. On the other hand, appreciable radioactive

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**Table III**

| Isotope administered* and nutritional status of rat | Urine collection period | Carnitine content | Radioactivity present in urine as
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotope administered and Specific activity</td>
<td>Administered dose</td>
<td>( ^{15}N ) Carnitine</td>
<td>( ^{14}C ) Butyrobetaine</td>
</tr>
<tr>
<td>DL-[( ^6)C]Lysine</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lysine-deficient Rat A</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>L-[methyl-( ^3)H]Methionine</td>
<td>5</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Lysine-deficient Rat J</td>
<td>1</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>e-N-[methyl-( ^3)H]Trimethyl-l-lysine</td>
<td>1</td>
<td>22</td>
<td>3.3</td>
</tr>
<tr>
<td>Lysine-deficient Rat G</td>
<td>1</td>
<td>100</td>
<td>3.8</td>
</tr>
<tr>
<td>[carboxy-( ^1)C]-( \gamma )-Butyrobetaine</td>
<td>1</td>
<td>246</td>
<td>0.2</td>
</tr>
<tr>
<td>Lysine-deficient Rat L</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The details concerning the administration of radioactive compounds for each rat are given in Tables I or II.

* The nutritional status of each rat is given in greater detail in Tables I or II.

* Determined by carnitine acetyltransferase assay.
carnitine was excreted following administration of labeled ε-N-trimethyllysine (Rats G and H) and γ-butyrobetaine (Rat L) (Table III). However, in these instances, the conversion of these carnitine precursors to carnitine is so efficient, as shown by the tissue findings in Table II, that it is not surprising that newly synthesized carnitine was detected in these cases in the urine (Table III). Indeed, in the case of Rats G and H receiving ε-N-trimethyllysine, more γ-butyrobetaine was found in the urine than carnitine (Table III), providing further evidence for a precursor-product relationship between ε-N-trimethyllysine and γ-butyrobetaine.

**Discussion**

The severity of the lysine deficiency that developed in the rats fed the unsupplemented wheat gluten diets herein has been fully documented elsewhere (8). An important finding, particularly relevant to this paper, is that these rats had significantly less carnitine in the skeletal muscle and heart than control rats supplemented with lysine. Such findings might be attributed to an impairment in the synthesis of carnitine biosynthetic enzymes, as a consequence of consuming a diet limiting in the essential amino acid lysine. However, the labeling of tissue carnitine in lysine-deficient rats administered DL-[6-14C]lysine and L-[G-3H]lysine shows that carnitine is being synthesized under these dietary conditions. The evidence for the postulated lysine-carnitine, precursor-product relationship in the rat is based then on (a) nutritional grounds (4, 8) and (b) the labeling studies presented herein.

Just as the carnitine content of the skeletal muscle and heart of the rat is related to the lysine content of the diet (4, 8), so the efficiency of conversion of DL-[6-14C]lysine to carnitine is related to the lysine content of the diet. This was illustrated in Table I, where (a) the reduction of specific activity of lysine into biosynthesized carnitine was greater in Rat D maintained on a lysine-supplemented diet than in the case of lysine-deficient Rat C, and (b) the extent of incorporation of the same amount of isotopic lysine into tissue carnitine was greater in lysine-deficient Rat A than in control Rat D.

In the experiment with Rat A (Table I), it was found that about 0.08% of the administered DL-[6-14C]lysine was incorporated into carnitine based on the tissues examined. This represents a minimal figure in quantifying the lysine → carnitine conversion as the skeletal muscle and liver together constitute no more than 50% of the body weight of the animal. Furthermore, based on food consumption records of Rat A and the lysine content of the 20% wheat gluten diet (0.37%), it was estimated that the diet met about one-third of the daily requirement of Rat A for lysine. With these figures in mind, it is interesting to note that in the studies with *Neurospora crassa* lysine auxotroph 33653 (1), about 0.22% of the lysine needed for the total metabolism of the mold was converted to carnitine. These data for the rat and the mold are of the same order of magnitude and illustrate that only a minute amount of the essential amino acid, lysine, required by these species is used for the biosynthesis of carnitine, which is consistent with the role of carnitine as a biocatalyst.

The biological role of the ε-N-methy1ated lysines is not completely understood, but the present view is that certain lysine residues after incorporation into proteins, e.g. histone, cytochrome c, and muscle protein, are methylated by S-adenosyl-l-methionine:protein-lysine methyltransferase and that such modification of protein structure may alter the biological activity of these proteins (6). Possibly, protein methylase III is operative in the biosynthesis of protein-bound trimethyllysine in rat liver and that certain of such trimethyllysine, consigned for carnitine synthesis, is released via protease activity as a substrate for carnitine biosynthesis. Alternatively, there may be an enzyme that can methylate the ε-amino group of free lysine. Carnitine is thought to be synthesized primarily in the liver (3), and in this respect it is interesting to note that Paik and Kim report (6) that the specific activity of protein methylase III is higher in the liver (1.07) than the muscle (0.05) and the heart (0.42).

In this regard, we found (4, 8) that the carnitine content in the liver of the lysine-deficient rat was significantly higher than in control rats receiving lysine. We interpreted these findings to mean that in the lysine-deficient rat an increased synthesis of carnitine by the liver is demanded to meet the diminishing supply of carnitine in tissues such as skeletal muscle and heart. This interpretation is consistent with the distribution of protein methylase III reported by Paik and Kim (6). Apart from the role that ε-N-trimethyllysine may play as a constituent of certain proteins, the data presented herein offer compelling evidence that ε-N-trimethyllysine functions as a substrate for γ-butyrobetaine synthesis (Figs. 3 and 4, Table III) and thence carnitine (Fig. 3, Tables II and III), presumably via the hydroxylation system demonstrated in rat liver by Lindstedt and Lindstedt (11). The inability of DL-[2-14C]lysine to label biosynthesized carnitine (Fig. 1, Table I) is interpreted to mean that ε-N-trimethyllysine is cleaved, losing carbon atoms 1 and 2, and that the remainder of the molecule then serves as the source of γ-butyrobetaine.

The cleavage of ε-N-trimethyllysine to γ-butyrobetaine must proceed with great facility in the rat, as evidenced by the ready conversion of trimethyllysine to carnitine in the tissues (Rat G, Table II) and the excretion of substantial γ-butyrobetaine in the urine (Rat G, Table III). The mechanism of the cleavage of ε-N-trimethyllysine to γ-butyrobetaine in rat tissues is presently under investigation.

**References**

Role of Lysine and ε-N-Trimethyllysine in Carnitine Biosynthesis: II. STUDIES IN THE RAT
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