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CARNITINE METABOLISM AND ITS REGULATION IN MICROORGANISMS AND MAMMALS

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ABSTRACT

In procaryotes, L-carnitine may be used as both a carbon and nitrogen source for aerobic growth, or the carbon chain may be used selectively following cleavage of trimethylamine. Under anaerobic conditions and in the absence of preferred substrates, some bacteria use carnitine, via crotonobetaine, as an electron acceptor. Formation of trimethylamine and γ-butyrobetaine (from reduction of crotonobetaine) from L-carnitine by enteric bacteria has been demonstrated in rats and humans. Carnitine is not degraded by enzymes of eukaryotic origin. In higher organisms, carnitine has specific functions in intermediary metabolism. Concentrations of carnitine and its esters in cells of eukaryotes are rigorously maintained to provide optimal function. Carnitine homeostasis in mammals is preserved by a modest rate of endogenous synthesis, absorption from dietary sources, efficient reabsorption, and mechanisms present in most tissues that establish and maintain substantial concentration gradients between intracellular and extracellular carnitine pools.

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HISTORICAL BACKGROUND AND FUNCTIONS

Discovery and Structure

Carnitine, a name derived from the Latin *caro* or *carnis* (flesh), was discovered in muscle extracts in 1905 (33). Soon after, the chemical formula $C_7H_{15}NO_3$ was accepted, and in 1927 its structure, a trimethylbetaine of γ-amino-β-hydroxybutyric acid, was correctly identified and published (102). In 1962 the configuration of the physiological enantiomer was determined (46), and in 1997 confirmed (4), as L(−)- or R-(−)-3-hydroxy-4-N,N,N-trimethylaminobutyrate (see Figure 1). Because of the vitamin-like properties of carnitine for the mealworm *Tenebrio molitor*, the name vitamin B_T was created, and the widespread distribution of carnitine was established in all organs of mammals and many lower animals, and in many microorganisms and plants (25).

Functions

L-Carnitine is essential to the importing of activated long-chain fatty acids from the cytosolic compartment into mitochondria. It is involved in the transport of activated medium- and short-chain organic acids from peroxisomes to mitochondria (the "carnitine shuttle"). Carnitine modulates the acetyl coenzyme A (CoA)/CoA ratio by acting as a reservoir for activated acetyl units. Carnitine can modulate the toxic effects of poorly metabolized acyl groups, either xenobiotic (e.g. pivalic acid) or arising from blockage of a normal metabolic pathway (e.g. propionic acid in propionic acidemia), by transesterification from CoA and subsequent removal by excretion of acylcarnitine esters. These functions are mediated by a group of carnitine acyltransferase enzymes that catalyze the following general reaction:

$$\text{acyl-CoA} + \text{L-carnitine} \underset{\longleftarrow}{\overset{\text{carnitine acyltransferases}}{\longrightarrow}} \text{acyl-L-carnitine} + \text{CoA}.$$

Three groups of carnitine acyltransferases have been identified: carnitine palmitoyltransferases (EC 2.3.1.21), carnitine octanoyltransferase (EC 2.3.1.137), and carnitine acetyltransferase (EC 2.3.1.7). In addition, carnitine-acylcarnitine translocase, located in the inner mitochondrial membrane, participates in the

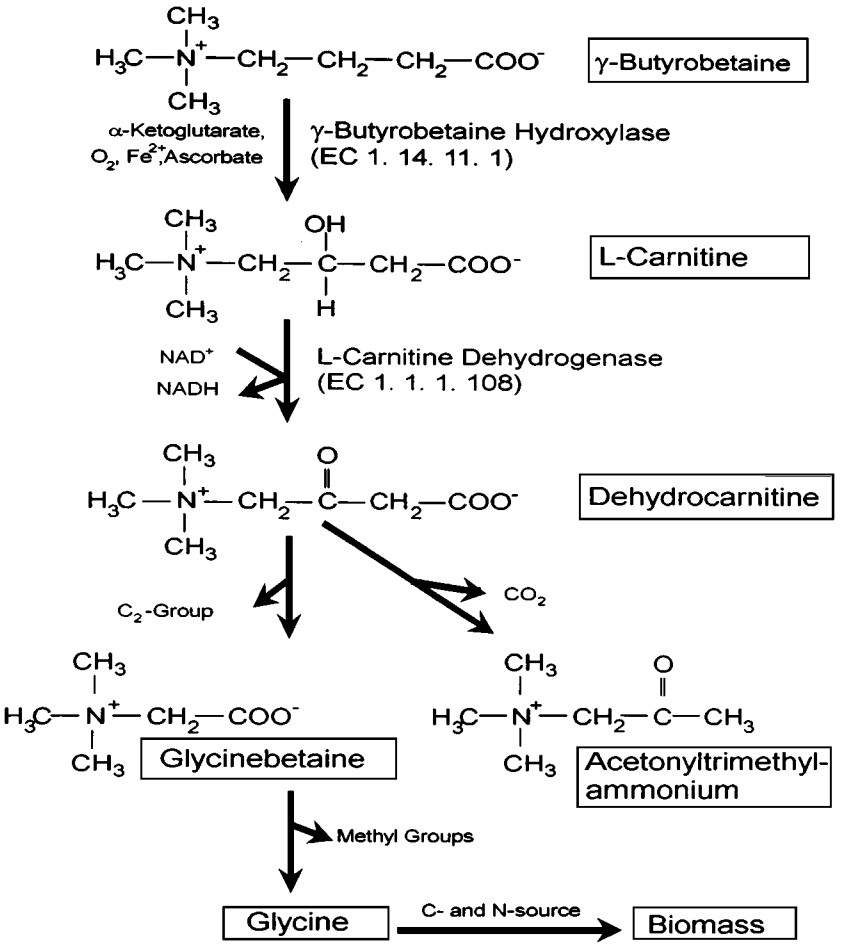

Figure 1 Pathway for aerobic degradation of γ-butryobetaine and L-carnitine by microorganisms grown with γ-butryobetaine or L-carnitine as the sole carbon and nitrogen source.

entry of long-chain fatty acylcarnitine esters into and the exit of carnitine and short-chain acylcarnitine esters out of mitochrondria. (For more extensive reviews on the functions of carnitine see References 6, 9, and 76).

PATHWAYS OF CARNITINE METABOLISM IN MICROORGANISMS

Carnitine's role in growth stimulation and metabolism of micororganisms varies depending on species and living conditions, e.g. salt concentration,

oxygenation, or anaerobic growth. In some yeasts, such as the carnitine-requiring *Torulopsis bovina* ATCC 26014, the limiting factor for growth is the stimulation of carnitine acetyltransferase–mediated transport of acetyl groups into mitochondria (20, 53).

In bacteria, betaines act as osmoprotectants. The osmoprotectant function of γ-butyrobetaine (the immediate biosynthetic precursor and a degradation product of carnitine) and carnitine has been demonstrated in *Escherichia coli* (45, 52), *Pseudomonas aeruginosa* (59), and *Listeria monocytogenes* (96, 105). *P. aeruginosa* grown in the presence of preferred carbon and nitrogen sources, high NaCl concentration, and L-carnitine accumulated betaine (59), indicating that the carbon chain of carnitine could be shortened to produce betaine to serve as osmoprotectant.

Betaines are accumulated specifically by bacteria through the action of membrane-associated permeases (49, 66). It has been suggested that in *E. coli*, CaiT, a 57-kDa protein product of the *cai* operon, is the transporter for carnitine (16). CaiT has significant sequence homology to a glycine betaine transporter (OpuD) from *Bacillus subtilis*, the choline transporter BetT from *E. coli*, a BetT-like protein from *Haemophilus influenzae*, and a glycine betaine carrier from *Corynebacterium glutamicum*. These membrane proteins form a family of transporters involved in the uptake of structurally related quaternary amines (47, 68).

Complete Degradation: Carnitine as the Sole Carbon, Nitrogen, and Energy Source

The complete utilization of L-carnitine by *Pseudomonas* was first described in 1959 (2). Under aerobic conditions, L-carnitine induces an enzyme reducing carnitine at carbon 3, producing dehydrocarnitine (Figure 1). Carnitine dehydrogenase (L-carnitine: NAD^+ oxidoreductase, EC 1.1.1.108) is highly specific for L-carnitine and NAD^+ (1). It has been purified from four different genera of bacteria: *Pseudomonas* (1, 28), *Xanthomonas* (64), *Alcaligenes* (100), and *Agrobacterium* (37). The enzyme has a K_m for L-carnitine between 1 and 10 mmol/l, a pH optimum between 9.0 and 9.5, and equilibrium constants for oxidation of carnitine of 1.3×10^{-11} (*Pseudomonas*) and 2.4×10^{-12} (*Agrobacterium*) (48). The gene from *Xanthomonas translucens* encoding carnitine dehydrogenase has been cloned and expressed in *E. coli* (65). Dehydrocarnitine subsequently is cleaved to form glycine betaine and presumably acetate. Formation of glycine betaine by enzyme extracts was stimulated by CoA and ATP (57) and occurred at the expense of the appearance of acetonyltrimethylammonium, which presumably is a nonenzymatic decarboxylation product of the unstable β-keto acid dehydrocarnitine.

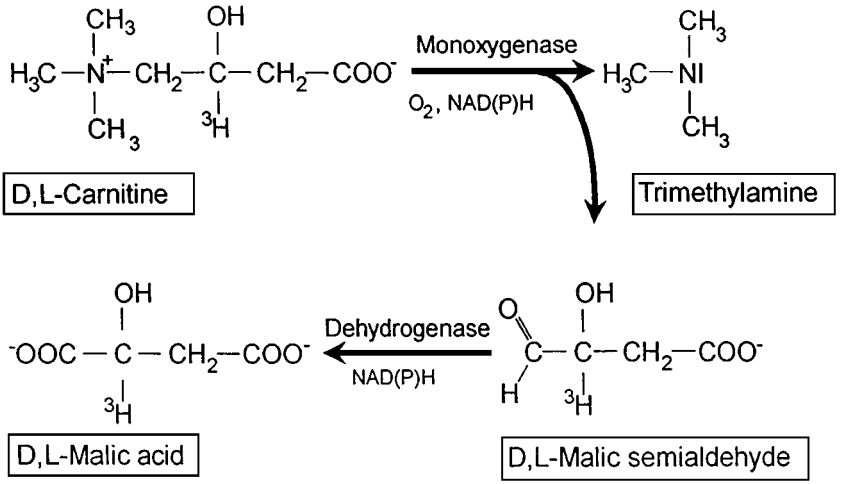

Figure 2 Trimethylamine formation in bacteria by aerobic cleavage of the C_4-N bond of carnitine. Retention of ^3H label at carbon 3 ruled out the possibility of a 3-oxo intermediate in this pathway (15).

Formation of Trimethylamine

In some bacterial species, carnitine is metabolized initially by cleavage of the C_4-N bond, forming trimethylamine and malate (Figure 2). This pathway was first demonstrated in *Serratia marcescens* by Unemoto and coworkers in 1966 (104). Subsequently it was shown that *Acinetobacter calcoaceticus* 69/V grown with DL-carnitine, acyl-L-carnitine esters, or γ-butyrobetaine plus an additional nitrogen source metabolized these betaines, producing trimethylamine in stoichiometric amounts (50, 63). D-Carnitine was metabolized if an additional carbon source such as L-carnitine was present in the incubation medium. Trimethylamine was not formed from choline or glycine betaine (50). This bacterium was able to grow on DL-malate and succinate, products of the splitting of the C_4-N bond of carnitine and γ-butyrobetaine. Resting cell suspensions of *A. calcoaceticus* grown on γ-butyrobetaine or DL-carnitine effectively degraded DL-3-fluoro-4-N,N,N-trimethylaminobutyric acid, 5-N,N,N-trimethylaminopentanoic acid, and crotonobetaine with stoichiometric formation of trimethylamine. The resting cells did not produce trimethylamine from glycine betaine or 3-N,N,N-trimethylaminopropionic acid (22). In cell-free extracts of *A. calcoaceticus* 69/V, enzymatic cleavage of the C_4-N bond of L-carnitine, D-carnitine, crotonobetaine, and γ-butyrobetaine (but not glycine betaine or choline) was demonstrated (90). Cleavage of the C_4-N bond of

crotonobetaine and the lack of stereochemical specificity with respect to the configuration at carbon 3 of carnitine are inconsistent with a mechanism involving a simple Hoffmann-type carbanion-mediated elimination. Therefore, a hydroxylation reaction was proposed, leading to the formation of trimethylamine and succinic semialdehyde from the splitting of γ-butyrobetaine (22) or of malic semialdehyde from carnitine (Figure 2).

Using cultures of *A. calcoaceticus* ATCC 39648, Ditullio et al (15) demonstrated monooxygenase-catalyzed cleavage of DL-carnitine resulting in formation of trimethylamine and malic acid. The initial step was a hydroxylation at carbon 4 requiring molecular oxygen. The resulting unstable intermediate spontaneously degraded to trimethylamine and malic semialdehyde, via a reverse aldol reaction. With DL-[3-^{3}H]carnitine as a substrate and malic acid as a trapping agent, [3-^{3}H]malic acid was identified as the product of the reaction sequence, definitively ruling out involvement of an oxo intermediate at carbon 3. The reaction sequence was dependent on both NAD^+ and NADPH in catalytic concentrations. Formation of trimethylamine from γ-butyrobetaine and crotonobetaine also was confirmed with this enzyme preparation.

Reduction of Carnitine to γ-Butyrobetaine via Crotonobetaine

Under anaerobic conditions enterobacteria do not assimilate the carbon and nitrogen of carnitine, but they do metabolize it via crotonobetaine to γ-butyrobetaine in the presence of other carbon and nitrogen sources (Figure 3). *E. coli* isolated from the intestinal lumen of a rat was first shown to reduce L-carnitine to γ-butyrobetaine (88). This conversion was linked with anaerobic growth stimulation of enterobacteria, including *Salmonella typhimurium* (91) and *Proteus vulgaris* (89), grown in complex or minimal media. Two enzymatic reactions, dehydration and reduction, are involved in the transformation. Reduction of the intermediate crotonobetaine is responsible for growth stimulation. Addition of nitrate or trimethylamine N-oxide to bacterial cultures completely repressed carnitine metabolism, and addition of fumarate significantly inhibited this pathway. Thus carnitine may serve as an electron acceptor during anaerobic growth of enterobacteria in the absence of preferred substrates (89, 91, 92). Indeed this may be the primary function of this pathway (91).

Carnitine dehydratase (L-carnitine hydrolyase, EC 4.2.1.89) from *E. coli* O44 K74 is an inducible enzyme detectable in cells grown anaerobically in the presence of L-carnitine or crotonobetaine (92). The purified enzyme is highly specific for L-carnitine: Acyl-L-carnitine esters of various chain lengths and carboxy esters of L- or D-carnitine were not substrates for the purified enzyme (44). An as yet unidentified cofactor necessary for the reaction was separated during purification. The purified enzyme is reversible, with an equilibrium constant

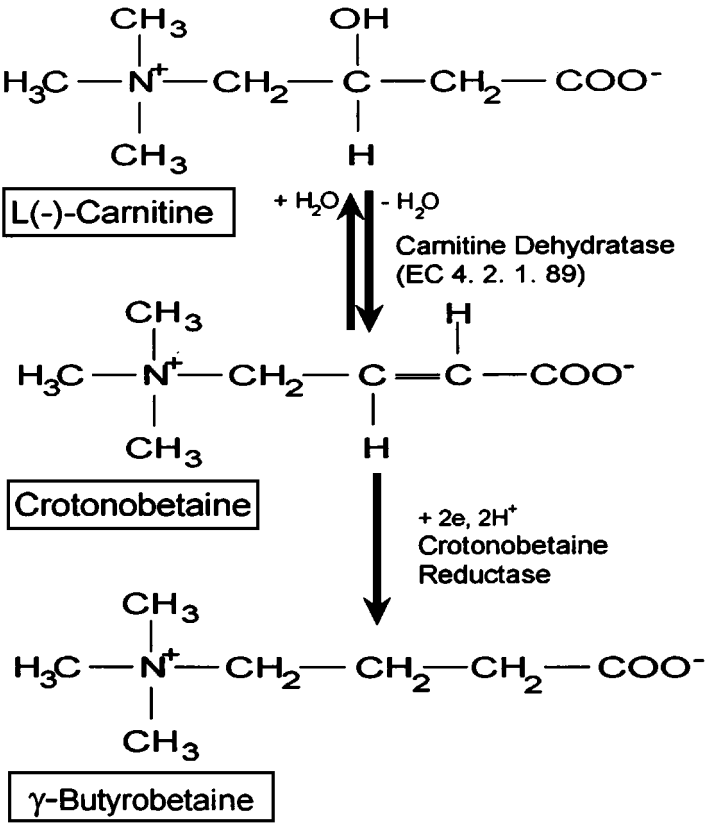

Figure 3 Pathway of anaerobic conversion of L-carnitine to γ-butyrobetaine in microorganisms.

for L-carnitine of 1.5. γ-Butyrobetaine, D-carnitine, and choline are competitive inhibitors of crotonobetaine hydration (44). Addition of water across the double bond of *trans*-crotonobetaine proceeds via a stereospecific *syn* pathway (4).

Crotonobetaine reductase activity was detected in extracts of *E. coli* O44 K74 grown anaerobically in media supplemented with L-carnitine or crotonobetaine (85). Enzyme activity was not found in extracts of cells grown in the presence of carnitine or crotonobetaine when glucose, nitrate, γ-butyrobetaine, or oxygen was present. The pH and temperature optima for this enzyme in vitro are 7.8 and 40°–45°C, respectively.

The gene encoding carnitine dehydratase of *E. coli* (*caiB*) has been identified (19). Expression of *caiB* confirmed the molecular mass of the enzyme (45 kDa)

and showed that no post-translational modification of the enzyme was necessary for catalytic activity. *CaiB* is part of the *cai* operon that contains five additional open reading frames (*caiTABCDE*) and is located at the first minute on the chromosome and transcribed during anaerobic growth in the presence of L-carnitine (16). In vivo expression of the *cai* operon led to synthesis of five polypeptides in addition to CaiB. Amino acid sequence similarity or enzymatic analysis supported the function assigned to each protein. CaiT was suggested as the transporter for carnitine and/or other betaines, CaiA is an oxidoreductase, and CaiC is a crotonobetaine/carnitine:CoA ligase. CaiD bears strong sequence homology with enoylhydratases/isomerases. Over-production of CaiE was shown to stimulate the carnitine racemase activity of CaiD and to markedly increase the basal level of carnitine dehydratase activity. It was inferred that CaiE is an enzyme involved in synthesis or activation of the unidentified cofactor required for carnitine dehydratase (CaiB) activity (16).

The 5′ end of the *cai* locus of *E. coli* contains four open reading frames organized as an operon and designated *fixABCX*, analogous to the *fixABCX* operons of *Azorhizobium caulinodans* and *Rhizobium meliloti* (17). The first two gene products of this operon share a high degree of sequence similarity with the β and α subunits, respectively, of mammalian electron transfer flavoproteins. Expression of a *fixA-lacZ* transcriptional fusion was induced by L-carnitine and crotonobetaine, but not by D-carnitine, γ-butyrobetaine, glycine betaine, or choline. The *fix* operon was repressed by glucose and nitrate. Eichler and colleagues speculated that the *fixABCX* gene products facilitate transfer of electrons to crotonobetaine reductase (CaiA) to reduce crotonobetaine to γ-butyrobetaine (17).

The 3′ region adjacent to the *caiTABCDE* operon contains a gene (*caiF*) that codes for a 16-kDa protein (CaiF) (18). Overexpression of CaiF resulted in stimulation of transcription of the *cai* and *fix* operons in the presence of carnitine. Expression of the caiF gene itself was not stimulated by carnitine but was subject to anaerobic induction by the fumarate-nitrate reduction transcriptional regulator and activation by the cyclic AMP receptor protein. The histone-like protein H-NS and the NarL (plus nitrate) regulator repressed expression. The *caiF* gene is transcribed in the direction opposite that of the *cai* operon. *CaiF* may be a key element in the regulation of carnitine metabolism in *E. coli*.

Reversible conversion of L-carnitine to γ-butyrobetaine is a common pathway in microorganisms. From a total of 706 strains (436 strains of bacteria, 37 strains of actinomycetes, and 233 strains of yeasts representing 68 genera) screened for carnitine dehydratase activity, 162 strains of bacteria, 20 strains of yeasts, and 2 strains of actinomyces were observed to form L-carnitine from *trans*-crotonobetaine (108). Strains belonging to genera *Escherichia* and *Proteus* produced carnitine in highest yields. A novel pathway now used for aerobic

production of carnitine in commercial quantities was identified by Kulla (51), in a microorganism isolated from a soil sample (genus not specified, but taxonomically similar to *Agrobacterium* and *Rhizobium*). In mutant strain HK13 of this organism, L-carnitine was efficiently produced from γ-butyrobetaine or *trans*-crotonobetaine via CoA esters of crotonobetaine and carnitine, in reactions analogous to the acyl-CoA dehydrogenase– and enoyl-CoA–catalyzed steps of mitochondrial fatty acid β-oxidation.

Integration of Bacterial Degradation in Carnitine Metabolism of Mammals

L-Carnitine is not degraded by enzymes of mammalian origin. Nevertheless, kinetic studies in rats, dogs, and humans consistently have shown that ingested carnitine is not quantitatively excreted (reviewed in 74). Using oral administration of pharmacological doses or radiolabeled forms of carnitine, it has been shown that carnitine is primarily degraded in nonruminant mammals to trimethylamine and γ-butyrobetaine (78, 84, 93). Studies comparing metabolism of orally administered carnitine in germ-free and conventional rats led to the conclusion that microorganisms in the gastrointestinal tract were entirely responsible for metabolite formation (84, 93). Although it is clear that the trimethylamine- and γ-butyrobetaine–forming pathways (Figures 2 and 3) are active in intestinal flora, it has not been demonstrated that complete degradation of carnitine (Figure 1) also occurs.

CARNITINE METABOLISM AND HOMEOSTASIS IN MAMMALS

Carnitine homeostasis in mammals is maintained by a combination of absorption of carnitine from dietary sources, a modest rate of biosynthesis, and highly efficient reabsorption of carnitine (Figure 4). Mechanisms are present in most tissues that establish and maintain substantial concentration gradients between extracellular and intracellular carnitine pools. Intracellular and extracellular carnitine may be present as either nonesterified (free) carnitine or as esters of short-, medium-, or long-chain organic and fatty acids. Normally the short-chain acylcarnitine ester pool consists primarily of acetyl-L-carnitine.

Metabolic Fate of Dietary Carnitine

In rats and humans, approximately 54–87% of dietary carnitine is absorbed. The remainder is excreted as metabolites in urine and feces following bacterial degradation in the large intestine (78, 84). The mechanisms by which carnitine is absorbed in the small intestine have not been definitively identified and characterized. Carnitine transport and carnitine absorption have been studied

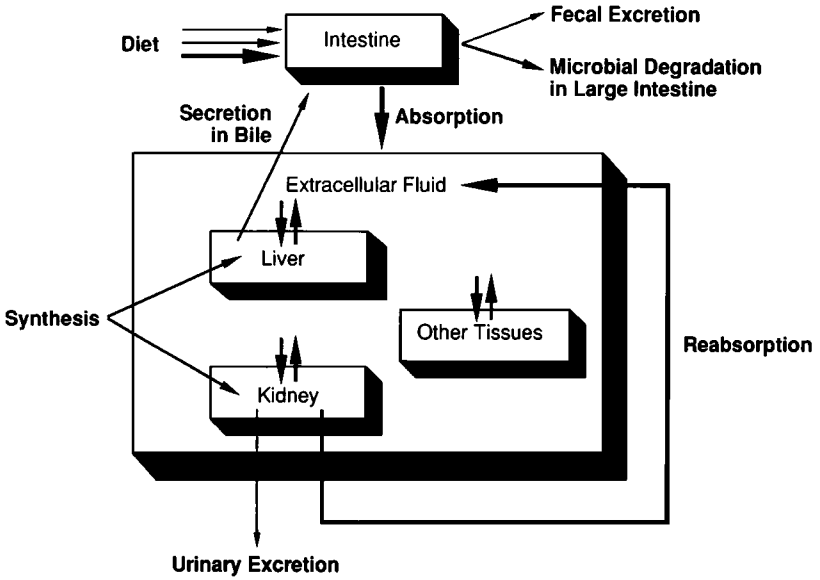

Figure 4 A schematic diagram of carnitine homeostasis and its regulation in mammals.

in a variety of intestinal preparations from rats, pigs, guinea pigs, and humans. L-Carnitine is taken up rapidly by the small intestinal mucosa of rats in vivo and released slowly into the circulation (29, 31, 32). Up to 50% of the carnitine taken up from the lumen into the intestinal mucosa is acetylated. A specific carrier for carnitine in the mucosal membrane was suggested by the observations that L-carnitine was taken up about twice as rapidly as the D isomer, and the process was saturable (29). Shaw and coworkers (94) observed active transport of L-carnitine into everted rat duodenal and jejunal sacs and rings. However, Li et al (54) found no evidence of active transport of carnitine into brush border membrane vesicles prepared from young adult male rats and concluded that no carnitine transporter, per se, exists in that membrane. Gross et al (30) concluded that carnitine is taken up by isolated guinea pig enterocytes by a facilitated diffusion process, whereas Hamilton and coworkers (36) reported active transport and passive diffusion of carnitine into human intestinal biopsy specimens. Li et al (55), using jejunal perfusions in vivo in human adults, concluded that carnitine in a normal meal is absorbed by active mechanism(s), whereas pharmacological doses of carnitine are absorbed primarily by passive diffusion. Li and colleagues (56) observed up-regulation of carnitine transport into jejunal segments from newborn pigs, unlike suckling and weanling

animals. Their work suggests that the greater rate of carnitine transport in newborn pig intestinal mucosa may be mediated by glucagon and insulin secretion (increased glucagon to insulin ratio).

The Caco-2 cell monolayer grown on a permeable surface has been used extensively as a model for investigation of small intestinal absorptive processes for a variety of nutrients, for other naturally occurring organic compounds, and for synthetic pharmaceuticals. L-Carnitine is actively transported into Caco-2 cell monolayers in vitro (61). But L-carnitine absorption across the Caco-2 cell monolayer appears to occur primarily, if not totally, by passive paracellular diffusion (CJ Rebouche, unpublished observations).

Clearly, the data and conclusions from these studies of carnitine absorption conflict in many respects, probably in large measure because of the vastly different techniques and preparations used, as well as possible inherent differences between species. Nevertheless, the majority, but not necessarily a consensus, of the results now available favor passive diffusion as the predominant mechanism for carnitine absorption in adult mammals. But the work of Li and colleagues (56) strongly suggests that active, carrier-mediated mechanisms have a significant role in carnitine absorption in newborn animals.

Carnitine that is not absorbed is almost totally degraded in the large intestine, by the action of bacterial flora. In rats (84) and humans (78), the degradation products identified following oral administration of [*methyl*-^{14}C]- or [*methyl*-^{3}H]L-carnitine were trimethylamine oxide (primarily in urine) and γ-butyrobetaine (primarily in feces). Trimethylamine oxide arises from cleavage of the quaternary ammonium moiety of carnitine, absorption as trimethylamine, and oxidation to trimethylamine oxide in the liver. In humans, trimethylamine oxide in urine accounted for 8–39%, and γ-butyrobetaine in feces accounted for 0.1–8% of total dietary carnitine (78). In rats, there was no evidence for excretion of volatile metabolites of carnitine ($^{14}CO_2$ or [^{14}C]trimethylamine) into expired air (84).

The postabsorptive fate of orally administered carnitine was studied in anesthesized rats (32). Absorbed carnitine appeared primarily in the portal circulation and was extracted by the liver. Enterohepatic circulation of carnitine was observed, with passive movement of carnitine, primarily as acylcarnitine esters, into bile. The rate of biliary carnitine secretion in rats was estimated to be 0.4–1.4 mmol per 100 g of body weight every 24 h, similar to the rate of urinary carnitine excretion (32, 35). Carnitine in rat and human bile collected in vivo was highly esterified (greater than 66% of total carnitine), and long-chain acylcarnitine esters accounted for 30–50% of total bile carnitine (34, 35). On the other hand, carnitine in bile collected postmortem was only 19% esterified in one study (70). In a separate study, 57% of postmortem bile carnitine was reportedly in the form of long-chain acylcarnitine esters (87). The concentration

of total carnitine in bile of both rats and humans was highly variable, even within the same study and within the same animal. Moreover, the rate of bile secretion varies with the physiological state of the animal. Consequently, the rate of secretion of carnitine in bile is likely to vary markedly, even within the same animal over both the short and the long term.

The function of carnitine and its esters in bile, if any, is not clear. It may simply provide the liver with a means to dispose of excess long-chain acylcarnitine esters. On the other hand, recent studies using in vitro cell culture models of intestinal mucosa suggest that long-chain acylcarnitine esters facilitate mucosal cell accumulation of calcium (99, 101) and enhancement of absorption of hydrophilic drugs (40). These findings suggest a role for hepatic esterification of carnitine and enterohepatic circulation of acylcarnitine esters in vivo.

IN VIVO KINETIC ANALYSIS Kinetics of systemic carnitine metabolism have been analyzed in rats, dogs, and humans, with radioisotope tracers of carnitine and mathematical modeling techniques. Cederblad & Lindstedt (13) estimated synthesis and turnover rates for carnitine using a two-compartment model following intravenous administration of a tracer amount of [*methyl*-^{14}C]L-carnitine. Daily whole-body turnover was 7% of the carnitine pool, and the calculated rate of carnitine synthesis was 20 μmol (kg of body wt)$^{-1}$ day^{-1}. Brooks & McIntosh (10) estimated compartment size, flux, and turnover time for carnitine in 14 rat tissues following intravenous administration of [*methyl*-^{3}H]L-carnitine. Turnover times ranged from 0.39 h in spleen to 903 h in the "slow" compartment of liver. The slow carnitine compartment in liver contained only 11% of total liver carnitine; the turnover time for the rapid compartment of liver was 1.31 h, consistent with observations in rats and other mammals that carnitine in liver as a whole is rapidly exchanged. By contrast, carnitine turnover was much slower in quadriceps muscle of rats: 13.4 h (18% of muscle pool) and 105 h (82% of muscle pool).

A three-compartment model was developed to describe carnitine metabolism in dogs and humans (80, 81). These compartments roughy corresponded to extracellular fluid (compartment *a*) and to two tissue compartments, one with a slow turnover and a relatively large pool of carnitine (compartment *b*) and the other with a high turnover and relatively small pool size (compartment *c*). Compartment *c* was predicted to be primarily liver and kidney [consistent with the flux and turnover estimates of Brooks & McIntosh (10) in rats, and rates of carnitine entry and exit measured in vitro], and it was suggested that compartment *b* corresponds to skeletal and heart muscle, which contain the vast majority of the total body carnitine pool. Whole-body turnover time in dogs and normal adult humans was estimated to be 63 and 65 days, respectively (range, 50–89 and 38–119 days, respectively). The carnitine pool in compartment *b*

comprised 95% and 97%, respectively, of the total body carnitine of humans and dogs.

No development of kinetic models for carnitine metabolism has appeared in the literature since 1984, a fact that may be due in part to the recognition that radiolabeled tracers are no longer acceptable for use in these investigations. However, availability of stable-isotope–labeled (^{2}H) forms of carnitine and recent development of methods to separate and quantify specific isotope-labeled forms of carnitine and its esters by coupled high-performance liquid chromatography and tandem mass spectrometry (23, 62) may generate a renewed interest in whole-body carnitine kinetics. Its usefulness was demonstrated in studies of patients with muscle carnitine deficiency and secondary systemic carnitine deficiency (81). Future uses of this technology may include investigation of the effects of diet composition (e.g. high fat versus low fat; vegetarian versus omnivorous) on whole-body carnitine metabolism in vivo, and effects of chronic exercise or endurance training on carnitine homeostasis. This method may be useful clinically to investigate the effects on carnitine metabolism of various genetic and acquired diseases that are associated with secondary carnitine deficiency or altered patterns of carnitine esterification.

METABOLIC FATE OF SYSTEMIC CARNITINE: TISSUE ACCRETION, ACYLCARNITINE FORMATION, AND METABOLISM Carnitine concentrations typically are higher in tissue than in extracellular fluid compartments. Indeed, the concentration of carnitine in skeletal and cardiac muscle of humans is normally over 50 times that in plasma. It has been shown that plasma carnitine concentrations correlate with tissue carnitine concentrations in rats of the same strain and maintained under identical dietary and environmental conditions (72). However, plasma carnitine concentrations within the normal range cannot be used to predict tissue carnitine concentrations in humans because of the variability in genetic factors affecting the mechanisms by which the tissue/extracellular fluid gradients are maintained, and because of environmental factors that may also affect the distribution of carnitine. On the other hand, plasma carnitine concentrations near or below the lower limit of the normal range often coincide with low carnitine concentrations in one or more tissues and, thus, provide a useful but nonspecific indicator of underlying pathology.

Most tissues accumulate carnitine from the extracellular milieu via specific transporters in the cell membrane. Carnitine transport has been studied in tissue, cell, and subcellular preparations of a wide range of organs from several mammalian species, including humans. In general, affinity for transporters in mammalian tissues is high, with measured K_T (concentration at half-maximal rates of transport) in the low- to mid-micromolar range (reviewed in 71). The exceptions include isolated rat liver cells and rat cerebral cortex slices, which

demonstrated lower affinity for carnitine (K_T in low-millimolar range). A generalized defect in carnitine transport into tissues is associated with sometimes fatal primary systemic carnitine deficiency in humans (103).

Acylcarnitine esters are formed intracellularly in the course of normal metabolic activity, in association with carnitine's well-known functions: Long-chain acylcarnitine esters (fatty acids greater than 12 carbon atoms in length) are generated in order to transport the fatty acyl moieties into mitochondria. Short- and medium-chain acylcarnitine esters are formed in mitochondria and peroxisomes, in part as a means of removing the organic acids from these organelles as high-energy compounds (7). These acylcarnitine esters may have other roles (either physiologic or pharmacologic) in cell and organ function. For example, long-chain acylcarnitine esters are involved in the deacylation/reacylation cycle in erythrocytes (69). Propionyl-L-carnitine improves the contractile function of the heart (86) and protects the ischemic heart from reperfusion injury (67). Acetyl-L-carnitine has putative antioxidant effects in mitochondria (95) and was shown to correct impaired mitochondrial DNA expression in brain and heart tissues of senescent rats (27).

Acetyl-L-carnitine is the predominant acylcarnitine ester, both intracellularly and in the circulation. Acetyl-L-carnitine participates in both anabolic and catabolic pathways in cellular metabolism. ^{14}C from [*acetyl*-1-^{14}C]acetyl-L-carnitine injected into mice appeared both as $^{14}CO_2$ and in the phospholipid and triacylglycerol fractions predominantly in liver (24). By contrast, ^{14}C from [1-^{14}C]acetate appeared almost exclusively as $^{14}CO_2$ under the same conditions.

In food-restricted mice, acylcarnitine ester concentration in extracellular fluid increases, with a concomittant increase in the concentration of acid-soluble (short- and medium-chain) acylcarnitine esters in liver (107). Refeeding results in a rapid decrease in serum acylcarnitine ester concentration and a marked increase in the concentration of liver acid-soluble acylcarnitine esters. In fasting humans, net uptake of short-chain acylcarnitine esters (predominantly acetyl-L-carnitine) by forearm muscle occurs with concomittant release of nonesterified carnitine (3), suggesting that these esters in blood contribute to the flux of metabolic fuels from the liver to muscle, at least during fasting. But the contribution of energy substrates to muscle from this source is relatively small (<5%) compared with that provided by β-hydroxybutyrate (3).

During high-intensity exercise, acylcarnitine esters accumulate in skeletal muscle. Hiatt et al (39) observed in humans that from rest to 10 min of high-intensity exercise (work load between lactate threshold and maximal work capacity), muscle short-chain acylcarnitine ester concentration increased 5.5-fold and nonesterified carnitine content decreased by 66%. By contrast, low-intensity exercise (work load equal to 50% of lactate threshold) produced no changes in carnitine metabolism. Friolet et al (26) reported a positive correlation

between the ratios of acetylcarnitine to nonesterified carnitine and acetyl-CoA to nonesterified CoA in skeletal muscle of humans at rest and during exercise. Furthermore, they reported an increase in plasma short-chain acylcarnitine ester concentration during exercise-to-exhaustion. Constantin-Teodosiu and colleagues (14) demonstrated that accumulation of acetyl-L-carnitine was greater (with a concomittant lowering of nonesterified carnitine) in type I than in type II muscle fibers of humans after exhaustive exercise, probably reflecting the greater mitochondrial content of type I than of type II muscle fibers. In general, carnitine metabolism in skeletal muscle during exercise provides a good model and strong support for the concept that carnitine and carnitine acetyltransferase offer a useful mechanism to modulate the acyl-CoA to nonesterified CoA ratio during metabolic stress. Recent reviews of the extensive literature on this topic are available (8, 38).

Carnitine Biosynthesis

Endogenous synthesis of carnitine contributes to carnitine homeostasis in mammals. Carnitine is synthesized in mammals from the essential amino acids lysine and methionine. The pathway has been reviewed extensively elsewhere (11, 73, 75). The rate of carnitine biosynthesis in humans is approximately 2 μmol (kg of body wt)$^{-1}$ day^{-1} and does not appear to be subject to significant fluctuation. The essentially constitutive nature of carnitine biosynthesis is thought to be due to a relatively constant low rate of ϵ-N-trimethyllysine availability from turnover of various proteins that contain this amino acid, and the accessibility of ϵ-N-trimethyllysine, both endogenously produced and that obtained from the diet, to the mitochondrial site of ϵ-N-trimethyllysine hydroxylase (75). In humans, 30–50% of total ϵ-N-trimethyllysine (from diet and endogenous production) is converted to carnitine, and the remainder is excreted in urine (77).

For strict vegetarians, who obtain less than 0.5 μmol (kg of body wt)$^{-1}$ day^{-1} of carnitine from their diet (58), endogenous synthesis contributes the vast majority of carnitine necessary to maintain normal homeostasis. On the other hand, for omnivorous individuals, particularly those who consume significant amounts of meat products (beef, lamb, pork), diet contributes the majority of carnitine available for maintenance of normal carnitine metabolism.

Contribution of the Kidneys to Carnitine Homeostasis

The kidneys have a pivotal role in establishment and maintenance of carnitine homeostasis in mammals. The functions of the kidney in carnitine metabolism are shown in a schematic diagram (Figure 5). The function of the kidney in highly efficient carnitine reabsorption has been well characterized in vivo in humans, and in rats at the subcellular level. The kidney also participates in the

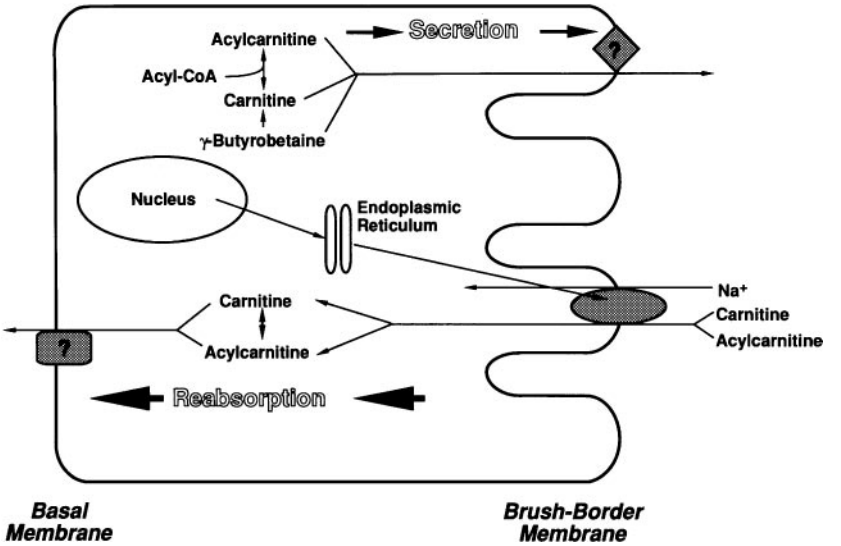

Figure 5 Carnitine metabolism in renal tubular epithelia. CoA, Coenzyme A.

synthesis (in some but not all mammals) and secretion (or alternatively, by leakage down a concentration gradient) of carnitine and acylcarnitine esters.

In humans, typically 90–98% of filtered carnitine is reabsorbed (21, 82). However, if the normal plasma carnitine concentration is increased—as, for example, by ingestion of a large amount of carnitine, or by infusion of carnitine into the blood stream—the rate of carnitine excretion increases disproportionately, and the efficiency of carnitine reabsorption decreases. These observations suggest that the efficiency of carnitine reabsorption contributes significantly to carnitine homeostasis by modulating the concentration of carnitine in plasma.

Physiological factors affect the rate of carnitine excretion. For example, a diet high in fat increases the rate of carnitine excretion, by increasing the plasma carnitine concentration, and thus the filtered load of carnitine (97). High-protein diets increase the rate of carnitine excretion by increasing the glomerular filtration rate (97). During pregnancy, circulating plasma carnitine concentration decreases significantly, in association with an increase in carnitine clearance (12).

Normal dietary carnitine intake also modulates the efficiency of carnitine reabsorption. Strict vegetarians were observed to excrete much less carnitine than did their omnivorous counterparts, at comparable plasma carnitine concentrations (58). Supplementation of strict vegetarian diets with carnitine resulted in a decrease in the efficiency of carnitine reabsorption, compared at the same

filtered load (e.g. the same plasma carnitine concentrations) (82). The kidneys adapt to dietary carnitine intake by reducing the efficiency of carnitine reabsorption. Because supplementation of strict vegetarian diets did not alter glomerular filtration rate, it was concluded that dietary carnitine must modulate specifically the process of carnitine reabsorption (82). The molecular mechanism for this effect has not been investigated.

Carrier-mediated transport of carnitine across the brush border membrane has been demonstrated with vesicle preparations from rat renal cortex (83, 98). Transport was in part dependent on an inwardly directed sodium ion gradient. K_T for carnitine transport was reported to be 55 (83) and 17 μmol liter^{-1} (98). Dietary L-carnitine (1% of diet for 10 days) specifically reduced by 52% the rate of carnitine transport across the brush border membrane in vitro (83). Qualitatively, this result correlates with modulation of carnitine reabsorption by dietary carnitine observed in vivo in humans.

Expression of the protein responsible for carnitine transport in rat renal brush border membranes was observed in *Xenopus laevis* oocytes following injection of mRNA isolated from rat renal cortex (5). Using size-selected mRNA (median length, 2 kb), a threefold higher rate of L-carnitine transport was observed compared with injection of total mRNA. To date, neither the transport protein nor the gene encoding the transport protein has been isolated or characterized at the molecular level.

The existence of a carnitine transporter at the basal and lateral membranes of renal cortical cells has not been clearly established. Huth and colleagues (43) demonstrated carnitine transport by rat kidney cortex slices, which presumably reflected transport into cells across the basolateral membranes. L-Carnitine was transported into cells of the kidney slice against a carnitine concentration gradient; dibutyryl cAMP stimulated this process. However, in the process of reabsorption, flux of carnitine must be in the outward direction across the basolateral membranes; the existence of a specific carrier for this process has not been demonstrated.

A mouse model of systemic carnitine deficiency has been identified. The juvenile visceral steatosis (*jvs*) mouse was shown to have a defect in reabsorption of carnitine both in vivo and in vitro using kidney slices (42). The molecular nature of the defect has not been identified; nevertheless, the existence of this mouse mutant underscores the importance of reabsorption for maintenance of carnitine homeostasis in mammals.

In normal humans, clearance of short-chain acylcarnitine esters often is greater than that for nonesterified carnitine. Acylcarnitine esters comprise about 22% of total circulating carnitine, but in urine they are, on average, 56% of total carnitine. This percentage varies greatly, from 3 to 91% in a study of 78 normal adults (58; see also 75). Two factors may contribute to this phenomenon. First, acylcarnitine esters may not be reabsorbed as efficiently as nonesterified

carnitine. Second, carnitine and its acyl esters are leaked (either by secretion or movement down a concentration gradient) from intracellular pools into the tubular lumen, where they at least in part escape reabsorption. In vivo studies by Rebouche & Engel (79) on the role of the kidney in carnitine biosynthesis in rats and humans provided indirect evidence that both carnitine and its immediate precursor, γ-butyrobetaine, were leaked directly from the kidney tubular cells into the lumen and excreted in urine. Using isolated rat kidney cortex tubules, Wagner and colleagues (106) demonstrated intracellular esterification of carnitine during perfusion with acetoacetate or β-hydroxybutyrate. More than 90% of the acetyl-L-carnitine formed was recovered in the extracellular fluid. The authors concluded that proximal renal tubule cells are the intrarenal site of carnitine acylation and may be involved in regulation of blood and/or urinary carnitine esterification.

Hokland & Bremer (41) observed that fractional reabsorption of carnitine, γ-butyrobetaine, and acylcarnitine esters was not different in the isolated, perfused rat kidney. When kidneys were perfused with carnitine plus α-ketoisocaproate or α-ketoisovalerate, increased amounts of acetyl-L-carnitine, isovaleryl-L-carnitine, and isobutyryl-L-carnitine were found. These investigators found a disproportionately high rate of excretion in urine of carnitine or acylcarnitine esters formed in the kidney compared with the same compounds when ultrafiltered. Mancinelli and coworkers (60), also employing the isolated, perfused rat kidney, found that the percentage of tubular reabsorption of carnitine and acetyl-L-carnitine, at concentrations normally found in rat plasma, were 94 and 97%, respectively. They also observed the capability of the rat kidney to acetylate carnitine and deacetylate acetyl-L-carnitine, and for intracellular carnitine and acetyl-L-carnitine to be leaked into the lumen and escape reabsorption.

It is not clear whether secreted or leaked acylcarnitine esters arise primarily from a pool synthesized in the kidney or from preformed acylcarnitine esters acquired by the kidney either directly from the circulation or from the glomerular filtrate. Results of in vivo studies (79) suggest that newly synthesized carnitine in the kidney of humans is in part leaked from intracellular pool(s), but it is not known what fraction of the total carnitine leaked arises from this pathway, or if separate pools of newly synthesized and preformed carnitine exist in kidneys. Likewise, the existence of a carrier mechanism for carnitine and acylcarnitine ester secretion across the brush border membrane is unknown.

CONCLUDING REMARKS

Although knowledge of carnitine metabolism in bacteria and higher organisms has advanced considerably over the last 30 years, gaps and unanswered questions remain. For example, in bacteria, although the pathway of carnitine

reduction to γ-butyrobetaine has been extensively characterized, many details of the routes to trimethylamine formation (Figure 2) and total dissimilation of carnitine (Figure 1), and their regulation, remain to be elucidated. In mammals, we still have limited knowledge of the structure and regulation of carnitine transporters in such organs as the kidney, liver, and heart and in skeletal muscle, although a defect in one or more of these transporters in humans and the corresponding mouse model has been known for 10 years. Little is known about the structure and regulation of enzymes in the pathway of carnitine biosynthesis. The role of carnitine and acylcarnitine ester secretion in bile remains speculative, and the roles of acylcarnitine esters in normal physiological processes (e.g. esterification and deesterification during exercise) and pathological conditions (e.g. diabetic nephropathy, cardiac ischemia) are incompletely understood. Thus research in this field should continue to be fruitful well into the twenty-first century.

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