# Kinetic Compartmental Analysis of Carnitine Metabolism in the Human Carnitine Deficiency Syndromes

## Evidence for Alterations in Tissue Carnitine Transport

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**Abstract.** The human primary carnitine deficiency syndromes are potentially fatal disorders affecting children and adults. The molecular etiologies of these syndromes have not been determined. In this investigation, we considered the hypothesis that these syndromes result from defective transport of carnitine into tissues, particularly skeletal muscle. The problem was approached by mathematical modeling, by using the technique of kinetic compartmental analysis. A tracer dose of L-[*methyl*-$^3$H]carnitine was administered intravenously to six normal subjects, one patient with primary muscle carnitine deficiency (MCD), and four patients with primary systemic carnitine deficiency (SCD). Specific radioactivity was followed in plasma for 28 d. A three-compartment model (extracellular fluid, muscle, and "other tissues") was adopted. Rate constants, fluxes, pool sizes, and turnover times were calculated. Results of these calculations indicated reduced transport of carnitine into muscle in both forms of primary carnitine deficiency. However, in SCD, the reduced rate of carnitine transport was attributed to reduced plasma carnitine concentration. In MCD, the results are consistent with an intrinsic defect in the transport process.

Abnormal fluctuations of the plasma carnitine, but of a different form, occurred in MCD and SCD. The significance of these are unclear, but in SCD they suggest abnormal regulation of the muscle/plasma carnitine concentration gradient.

In 8 of 11 subjects, carnitine excretion was less than dietary carnitine intake. Carnitine excretion rates calculated by kinetic compartmental analysis were higher than corresponding rates measured directly, indicating degradation of carnitine. However, we found no radioactive metabolites of L-[*methyl*-$^3$H]carnitine in urine. These observations suggest that dietary carnitine was metabolized in the gastrointestinal tract.

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## Introduction

L-Carnitine (L-β-hydroxy-γ-*N*-trimethylaminobutyric acid) promotes mitochondrial β-oxidation of long-chain fatty acids by facilitating their transfer across the inner mitochondrial membrane (1). Two primary carnitine deficiency syndromes have been described: muscle carnitine deficiency (MCD)[1] (2, 3), and systemic carnitine deficiency (SCD) (4). Both syndromes are associated with reduced capacity for oxidation of long-chain fatty acids in skeletal muscle. MCD is characterized by mild to severe muscle weakness and lipid accumulation in muscle (5). Skeletal muscle carnitine concentration is depressed, but carnitine levels in liver and plasma are normal. SCD is associated with acute attacks of encephalopathy with hypoglycemia (often provoked by caloric deprivation), pronounced lipid accumulation in liver during attacks, and mild lipid excess in muscle and liver between attacks (5). In some SCD patients, cardiomyopathy is a dominant feature (6). Carnitine concentration is decreased in liver and skeletal muscle and is variable in plasma.

In the original case report of primary MCD, Engel and Angelini (2) suggested an abnormality of transport of carnitine into skeletal muscle. This hypothesis has never been tested in

1. *Abbreviations used in this paper:* $F$, flux of carnitine from one compartment to another; $F_{ao}$, rate of carnitine intake; $F_{oa}$, rate of carnitine excretion; $k$, fractional rate constant for each compartment or from one compartment to another; MCD, primary muscle carnitine deficiency; $Q$, quantity of L-carnitine in a designated compartment; SCD, primary systemic carnitine deficiency.

*Table I. Physical and Biochemical Data for Participants in the Study*

| Subject | Age | Sex | Weight | Plasma carnitine* | Muscle carnitine‡ | Urinary creatinine excretion |
|---|---|---|---|---|---|---|
| | *yr* | | *kg* | *μM* | *nmol/mg NCP* | *g/d* |
| Controls | | | | | | |
| 1 | 36 | M | 107 | 42.0±3.1 | 24.6 | 1.60 |
| 2 | 19 | M | 100 | 65.7±3.4 | 52.1 | 2.79 |
| 3 | 37 | M | 79.6 | 53.1±5.6 | 43.5 | 2.42 |
| 4 | 46 | F | 54.5 | 30.5±3.1 | 15.9 | 1.05 |
| 5 | 30 | F | 66.2 | 58.0±4.6 | 25.3 | 1.16 |
| 6 | 34 | M | 71.3 | 64.7±7.4 | — | 1.87 |
| MCD patient | | | | | | |
| 7 | 32 | F | 132 | 42.2±6.5 | 3.25 | 1.20 |
| SCD patients | | | | | | |
| 8 | 3.8 | F | 15.5 | 31.8±9.4 | 4.82 | 0.32 |
| 9 | 6.2 | F | 24.4 | 32.3±12.2 | 6.20 | 0.57 |
| 10 | 6.0 | M | 23.2 | 24.1±10.1 | 3.15 | 0.51 |
| 11 | 8.5 | F | 23.2 | 14.9±4.8 | 8.06 | 0.52 |

NCP, noncollagen protein. * Mean±SD of fasting, morning plasma total (free plus esterified) carnitine determinations for 27–29 consecutive days of the study. ‡ Total carnitine concentration measured in a muscle (vastus lateralis) biopsy specimen taken at the conclusion of the study.

patients with confirmed primary MCD.[2] Five possible causes of SCD have been proposed (6). These include: (*a*) a defect in carnitine biosynthesis, (*b*) abnormal renal handling of carnitine, (*c*) altered transport mechanisms affecting uptake and/or release of carnitine from tissues, (*d*) excessive catabolism of carnitine, or (*e*) malabsorption of carnitine.

Studies in our laboratory have shown that in two SCD patients carnitine biosynthesis from ε-*N*-trimethyllysine in vivo was normal (8). Moreover, in three SCD patients, activities of hepatic enzymes subserving carnitine biosynthesis from ε-*N*-trimethyllysine were not decreased (9).

We studied renal reabsorption of carnitine in four SCD patients. The apparent renal plasma threshold for excretion, tubular maximum for reabsorption, and fractional reabsorption of carnitine were all lower in these subjects than in 9 of 10 normal subjects (10, 11). These studies also indicated that if the plasma carnitine concentration in an SCD patient is raised (by intravenous or oral administration of carnitine) to the range of values observed in normal subjects (30–90 μM), the SCD patient would reabsorb a lower percentage of filtered carnitine (fractional reabsorption) than normal subjects. Thus, SCD patients cannot maintain normal plasma carnitine levels without constant intravenous infusion or oral intake of carnitine. The renal carnitine leak is due to an abnormally low apparent renal plasma threshold for carnitine excretion in SCD (10). One control subject (with no clinical symptoms of SCD and normal muscle carnitine concentration) also had a renal carnitine leak. We concluded that the renal carnitine leak alone did not account for the low tissue carnitine levels in SCD, but it was likely to act in combination with other defect(s), perhaps of carnitine transport into other tissues. A recent independent study of a single case of SCD confirmed the abnormal renal reabsorption in SCD (12).

In this investigation, we used the technique of kinetic compartmental analysis to test the hypothesis that MCD and/or SCD is caused by defective transport of carnitine into muscle and other tissues. We have previously shown in dogs that this technique is applicable for determination of carnitine fluxes into and out of muscle and other tissues, and that estimates of carnitine pool sizes and turnover times derived by this technique correspond to results obtained by other methods (13).

## Methods

*Patients and normal subjects.* We studied one MCD patient, four SCD patients, and six normal volunteers. The MCD patient was a 32-yr-old woman whose clinical history was described previously (2, 3, 14). SCD patients were a 6-yr-old male, an 8.5-yr-old female, and two sisters, 3.8 and 6.2 yr old. Clinical histories of these subjects have been published (10, 15). Data for control subjects are tabulated in Table I. All subjects

2. In a previous study by Willner et al. (7), carnitine transport was measured in vitro in quadriceps muscle strips from a patient identified by the authors as suffering from muscle carnitine deficiency. Carnitine transport was interpreted as normal. However, subsequent review of the patient's history indicated that this subject probably suffered from a secondary rather than primary muscle carnitine deficiency.

(or parent, in the case of minors) gave informed consent prior to the start of the study. Studies involving human subjects were reviewed and approved by the Mayo Clinic Human Studies and Radiation Control Committees.

*Materials.* [$^3$H]Methyl iodide, [$^3$H]toluene standard, and [*acetyl*-l-$^{14}$C]acetyl-Coenzyme A were purchased from New England Nuclear Corp., Boston, MA. L-[*methyl*-$^3$H]Carnitine was synthesized by the method of Stokke and Bremer (16). Radiochemical purity was judged to be >99% by thin-layer and paper chromatography by using three systems (17). Solutions of L-[*methyl*-$^3$H]carnitine for injection were prepared in 0.9% NaCl, and sterilized by filtration through a 0.22-$\mu$m sterile filter (Millipore Corp., Bedford, MA). Solutions for injection were tested and certified for sterility and apyrogenicity. L-Carnitine and carnitine acetyltransferase were obtained from Sigma Chemical Co. (Saint Louis, MO).

*Protocol.* Each study was begun between 8 a.m. and 10 a.m. At zero time, a dose of L-[*methyl*-$^3$H]carnitine (0.082–2.44 Ci/mmol, 5.1–10.5 $\mu$Ci/kg body weight) was administered intravenously. Blood was sampled (1.0–5.0 ml, contralateral to injection site) every 5 min for the first hour, every 10 min for the second hour, every 15 min for the third hour, at 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, 40, and 48 h, every 12 h for the next 5 d, and every 24 h for the remainder of the study (27–29 d total). Urine was collected over 12-h (subjects 7–9 and 11, Table I) or 24-h (subjects 1–6 and 10) intervals throughout the study. Urine specimens were preserved with a few crystals of thymol. Aliquots were stored frozen at −70°C for subsequent analysis. Stools were collected daily and stored frozen at −20°C. Weekly collections were pooled and homogenized in one volume (wt/vol) of water in a Waring blender, and homogenates were stored at −70°C for subsequent analysis.

At the conclusion of the study, extracellular fluid volume was estimated by determining the inulin space (18). In the 11 subjects studied, extracellular fluid volume range from 15 to 25% of body weight (mean±SD, 19.8±3.2).

*Diets.* Throughout the study, each subject was maintained on an isocaloric diet appropriate for growing children or adults. The diets were designed to provide a normal daily carnitine intake (4–8 $\mu$mol/kg body weight per d) and to minimize fluctuations in day-to-day carnitine intake. Carnitine content of individual food items (Table II) and whole-meal homogenates was determined, and daily carnitine intake for each subject was calculated (Table III).

*Analytical methods.* Radioactivity in plasma and urine was determined by liquid scintillation counting of 0.05–1.0 ml aliquots in 10 ml of Insta-Gel (Packard Instrument Co., Downers Grove, IL). Counting efficiency was determined by using a [$^3$H]toluene internal standard. Aliquots of plasma and urine and of food and stool homogenates were prepared and assayed for free and total carnitine by previously described methods (10). Urinary creatinine was measured colorimetrically as described in Technical Bullectin No. 555 (Sigma Chemical Co., 1977). Inulin was determined by an automated resorcinol technique (10). Protein was measured by the method of Lowry et al. (19) after alkaline digestion (20). All calculations were performed with an appropriately programmed Hewlett-Packard 9821 calculator (Hewlett-Packard Co., Palo Alto, CA). Student's *t* test for unpaired data (21) was used for statistical comparisons. Differences were considered significant at $P < 0.05$.

## Results

*Plasma carnitine concentration.* Free and total carnitine concentrations in normal subjects were relatively constant over the period of the study (Table I and Fig. 1). The mean coefficient of variation (SD/mean) for six normal subjects was 0.09 (range, 0.05–0.11). By contrast, in SCD patients, wide variations in plasma carnitine levels were observed (mean coefficient of variation, 0.35; range, 0.29–0.42). "Peaks" and "valleys" in curves of carnitine concentration vs. time occurred unpredictably and generally spanned 2–5 d. In the MCD patient, a distinct diurnal variation in plasma carnitine concentration was observed. Fasting, morning plasma carnitine levels were consistently (26 of 28 d) higher than corresponding evening plasma carnitine concentrations (mean difference, 7.9 $\mu$mol/l; Fig. 1). No diurnal variation was observed in two SCD patients (Nos. 9 and 11; Table I) studied over 28 d. In two other SCD patients (Nos. 8 and 10; Table I) and in control subjects, twice daily plasma carnitine measurements during the first 7 d of the study also did not show diurnal variation.

*Table II. Carnitine Content of Diet Items*

| Food item | Carnitine content* | Food item | Carnitine content |
|---|---|---|---|
| **Meat products‡** | | **Vegetables** | |
| Beef steak | 592±260 (4) | Broccoli (fresh) | 0.0228 |
| Ground beef | 582±32 (3) | (cooked) | 0.0111 |
| Pork | 172±32 (3) | Carrots (fresh) | 0.0408 |
| Canadian bacon | 146±52 (3) | (cooked) | 0.0393 |
| Bacon | 145±24 (3) | Green beans (cooked) | 0.0189 |
| Fish (cod) | 34.6±11.7 (3) | Green peas (cooked) | 0.0369 |
| Chicken breast | 24.3±8.0 (3) | Asparagus (cooked) | 1.21 |
| | | Beets (cooked) | 0.0195 |
| | | Potato (baked) | 0.080 |
| **Dairy products** | | Lettuce | 0.0066 |
| Whole milk | 20.4 | | |
| American cheese | 23.2 | **Bread and cereal** | |
| Ice cream | 23.0 | | |
| Butter | 3.07 | White bread | 0.912 |
| Cottage cheese | 6.96 | Whole-wheat bread | 2.26 |
| | | Rice (cooked) | 0.090 |
| **Fruits** | | Macaroni | 0.780 |
| | | Corn flakes | 0.078 |
| Bananas | 0.0056 | | |
| Apples | 0.0002 | **Nondairy beverages** | |
| Strawberries | ND§ | | |
| Peaches | 0.006 | Grapefruit juice | ND |
| Pineapple | 0.0063 | Orange juice | 0.012 |
| Pears | 0.0107 | Tomato juice | 0.030 |
| | | Coffee | 0.009 |
| **Miscellaneous** | | Cola | ND |
| | | Grape juice | 0.093 |
| Eggs | 0.075 | | |
| Peanut butter | 0.516 | | |

ND, not detectable.

* Units are micromoles/100 grams (solid foods) or micromoles/100 milliliters (liquids). Values reported are total (free and esterified) carnitine.

‡ Values for meat products are mean±SD (number of determinations in parentheses) of total carnitine (free plus esterified) in the cooked meat product divided by the precooked weight of the meat product.

*Table III. Carnitine Intake and Excretion in Human Subjects*

| Subject | $F_{ao}$* | $F_{oa}$‡ | |
|---|---|---|---|
| | | By direct measurement | By kinetic analysis |
| Controls | | | |
| 1 | 23.6 | 15.9 | 75.2 |
| 2 | 33.5 | 25.4 | 67.3 |
| 3 | 26.4 | 35.1 | 46.8 |
| 4 | 20.0 | 8.07 | 31.8 |
| 5 | 21.9 | 12.7 | 55.0 |
| 6 | 23.8 | 22.7 | 54.5 |
| MCD patient | | | |
| 7 | 25.5 | 13.6 | 23.0 |
| SCD patients | | | |
| 8 | 2.89 | 3.39 | 3.83 |
| 9 | 5.07 | 8.75 | 5.94 |
| 10 | 5.73 | 5.45 | 11.3 |
| 11 | 7.34 | 2.56 | 7.03 |

* $F_{ao}$, total carnitine intake (micromoles/hour).
‡ $F_{oa}$, total carnitine excretion. Total carnitine was measured in urine and stools ("by direct measurement") throughout the period of the study and expressed as mean excretion (micromoles/hour). Total carnitine excretion (micromoles/hour) was also estimated by kinetic compartmental analysis.

*Balance studies.* Dietary carnitine intake for each participant was monitored throughout the study, and average intake per hour was determined ($F_{ao}$, Table III). Items providing significant amounts of carnitine in the diet were meats and dairy products (Table II). Carnitine content of fruits, vegetables, and cereals was very low by comparison. Daily urinary and cumulative fecal excretion of carnitine were determined throughout the study, and are expressed as average carnitine excretion per hour ($F_{oa}$, by direct measurement; Table III). For each subject, fecal carnitine was <2% of total carnitine excretion.

In 8 of the 11 subjects, dietary carnitine intake exceeded carnitine excretion over the 28-d period. Carnitine excretion was 35–173% of intake in 11 subjects (mean for controls, 78%; for SCD patients, 105%; for one MCD patient, 53%). Differences between SCD patients and controls were not statistically significant.

*Kinetic compartmental analysis.* A tracer dose of L-[*methyl*-$^3$H]carnitine was administered intravenously to each participant in the study. Specific radioactivity of carnitine in plasma was followed for 27–29 d. The data obtained were analyzed by kinetic compartmental analysis (22).

A three-compartment open-system model was adopted (Fig. 2). In physiological terms, the compartments were roughly defined as (*a*) extracellular fluid, (*b*) muscle, and (*c*) other tissues. Attempts to fit the data to two different four-compartment models produced less satisfactory results when compared with the three-compartment model. Plots of log plasma carnitine specific radioactivity vs. time were reduced, or "peeled" to three straight lines (compare Fig. 2, Reference 13). Intercepts ($H_1$, $H_2$, $H_3$) and slopes ($g_2$, $g_2$, $g_3$) of these lines are related by the equation:

$$SA_a/SA_{a,o} = H_1e^{-g_1t} + H_2e^{-g_2t} + H_3e^{-g_3t}$$

where $SA_a$ is the specific radioactivity of carnitine in compartment $a$ at time t and $SA_{a,o}$ is the specific radioactivity at time zero. Using the general equations for a three-compartment model (Ref. 22, pp. 217–218, equations 13–16 and 18 *a*), the rate constants for exit of carnitine from each compartment ($k_{aa}$, $k_{bb}$, and $k_{cc}$) and the rate constants for carnitine transfer from com-

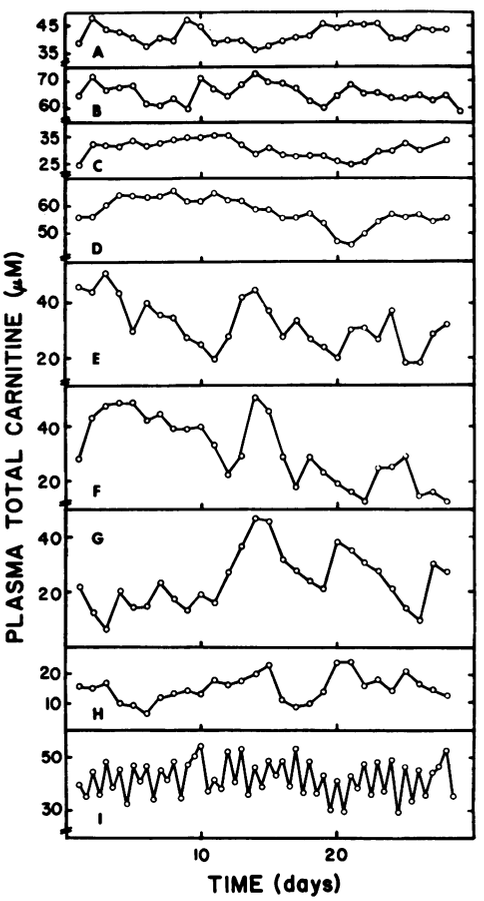


*Figure 1.* Plasma total carnitine concentrations in subjects participating in the study. Panels *A–D:* control subjects (Nos. 1, 2, 4, and 5, Table I); panels *E–H:* SCD patients (Nos. 8–11, Table I); panel *I:* MCD patient (No. 7, Table I).

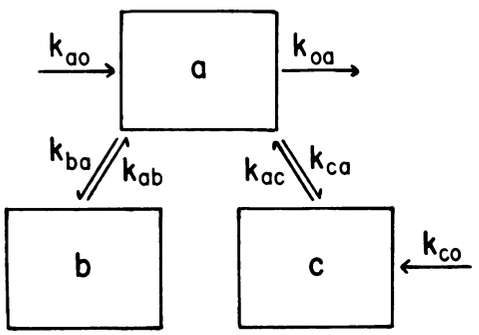


*Figure 2.* Schematic diagram of the three-compartment model. Carnitine pool *a* is extracellular fluid; pool *b* is muscle; and pool *c* represents other tissues, primarily liver and kidney. Rate constants for endogenous carnitine synthesis ($k_{co}$), dietary carnitine intake ($k_{ao}$), carnitine excretion ($k_{oa}$), and transfer of carnitine between compartments are shown as they relate to the various compartments.

partment *a* to compartment *b* ($k_{ba}$) and from compartment *a* to compartment *c* ($k_{ca}$) were calculated (Table IV).

The quantity of carnitine in extracellular fluid was determined from the mean carnitine concentration in plasma and the extracellular fluid volume (Table V). From the quantity of L-carnitine in compartment A ($Q_a$), $F_{ao}$ (dietary carnitine intake; Table III), and the individual rate constants, carnitine fluxes into and out of each compartment were calculated for each subject (Table VI). For compartment *b* (muscle), the rate of transport per unit mass of tissue was calculated from the flux ($F_{ba}$) and the muscle mass of each subject (Table VI). The latter was calculated from the following equation: muscle mass = 20 × creatinine excretion/day (22), where muscle mass is expressed in kilograms and creatinine in grams.

In the four SCD patients, carnitine transport rate into muscle was significantly lower in patients than in the controls. In the MCD patient, the rate of carnitine transport was less than half of the control mean. The difference between transport rates in SCD patients and control subjects was attributed to the reduced carnitine concentration in plasma of SCD patients (Table VI). However, the reduced rate of carnitine transport in one MCD patient was not related to a difference in plasma carnitine concentration compared with control subjects.

From the rate constants and $g_1$, $g_2$, and $g_3$, the amount of

*Table IV. Rate Constants and Turnover Times for Carnitine*

| | Rate constants | | | | | | Turnover times | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Compartment‡ | | | |
| Subject | $k_{aa}$* = $k_{ba} + k_{ca} + k_{oa}$ | $k_{bb} = k_{ab}$ | $k_{cc} = k_{ac}$ | $k_{ba}$ | $k_{ca}$ | $k_{oa}$ | *a* | *b* | *c* | Whole body§ |
| Controls | | | | | | | | | | |
| 1 | 0.919 | 0.00560 | 0.106 | 0.381 | 0.458 | 0.0807 | 1.09 | 179 | 9.43 | 38.1 |
| 2 | 0.996 | 0.00470 | 0.0894 | 0.660 | 0.285 | 0.0508 | 1.00 | 213 | 11.2 | 119 |
| 3 | 0.892 | 0.00455 | 0.0783 | 0.631 | 0.188 | 0.0734 | 1.12 | 220 | 12.8 | 80.8 |
| 4 | 0.834 | 0.00555 | 0.0774 | 0.514 | 0.243 | 0.0759 | 1.20 | 180 | 12.9 | 53.3 |
| 5 | 0.815 | 0.00629 | 0.0830 | 0.404 | 0.339 | 0.0720 | 1.23 | 159 | 12.0 | 40.4 |
| 6 | 0.860 | 0.00518 | 0.0869 | 0.531 | 0.258 | 0.0712 | 1.16 | 193 | 11.5 | 62.6 |
| Mean±SD | | | | | | | 1.13±0.08 | 191±20 | 11.6±1.2 | 65.7±27.8 |
| MCD patient | | | | | | | | | | |
| 7 | 0.841 | 0.0106 | 0.0741 | 0.145 | 0.668 | 0.0274 | 1.19 | 94.3 | 13.5 | 35.8 |
| SCD patients | | | | | | | | | | |
| 8 | 0.834 | 0.0156 | 0.0919 | 0.253 | 0.550 | 0.0312 | 1.20 | 64.1 | 10.9 | 31.2 |
| 9 | 1.24 | 0.00757 | 0.0669 | 0.530 | 0.672 | 0.0376 | 0.81 | 132 | 14.9 | 89.9 |
| 10 | 1.77 | 0.0125 | 0.116 | 0.724 | 0.947 | 0.101 | 0.56 | 80.0 | 8.62 | 27.8 |
| 11 | 1.13 | 0.00822 | 0.0783 | 0.487 | 0.539 | 0.102 | 0.88 | 122 | 12.8 | 27.4 |
| Mean±SD | | | | | | | 0.86±0.23‖ | 99.5±28.3‖ | 11.8±2.3 | 44.1±26.5 |

* The fractional rate constant for each compartment ($k_{aa}$, $k_{bb}$, $k_{cc}$) is the fraction of the carnitine pool in a compartment which exits from that compartment per hour. Rate constants for exit of carnitine from one compartment into another are designated, for example, $k_{ac}$, which is the fraction of carnitine in compartment *c* which leaves compartment *c* and enters compartment *a* per hour. Units are given per hour. ‡ Turnover time for a compartment is the reciprocal of the fractional rate constant for that compartment. Units are hours. § Whole-body turnover time is calculated from values for carnitine excretion, muscle, and "other tissues" carnitine pool sizes estimated by kinetic compartmental analysis and experimentally determined carnitine pool size for extracellular fluid. Units are days. ‖ Significantly different from controls ($P < 0.05$).

*Table V. Carnitine Pool Sizes*

| Subject | $Q_a$* | $Q_b$ | $Q_c$ | $Q_b$ |
|---|---|---|---|---|
| | *μmol* | *μmol* | *μmol* | *% of total* |
| Controls | | | | |
| 1 | 932 | 63,328 | 4,519 | 92 |
| 2 | 1,327 | 186,305 | 4,594 | 97 |
| 3 | 637 | 88,320 | 1,788 | 97 |
| 4 | 419 | 38,821 | 1,469 | 95 |
| 5 | 764 | 49,050 | 3,519 | 92 |
| 6 | 767 | 78,535 | 2,632 | 96 |
| MCD patient | | | | |
| 7 | 837 | 11,458 | 7,515 | 58 |
| SCD patients | | | | |
| 8 | 123 | 1,997 | 747 | 70 |
| 9 | 158 | 11,061 | 1,643 | 86 |
| 10 | 112 | 6,464 | 966 | 86 |
| 11 | 69 | 4,086 | 471 | 88 |

* $Q_a$, $Q_b$, and $Q_c$ are quantities of L-carnitine in compartments *a*, *b*, and *c*, respectively, estimated by kinetic compartmental analysis.

tracer in each compartment at any given time was calculated from equations 19 and 21–23, Reference 22, p. 218. These values are plotted for subjects 6 (control), 7 (MCD), and 10 (SCD) (Fig. 3). During the initial 48 h, there was rapid loss of tracer from compartment *a* with concurrent rapid rise in tracer present in compartments *b* and *c*. For control subjects and SCD patients, radiolabeled carnitine in compartment *c* fell rapidly to a low level, but reached a plateau and was lost only very slowly from compartment *b*. For the MCD patient, tracer accumulated in compartment *b* relatively slowly and the percent of total dose taken up was reduced. Concomitantly, proportionately more tracer was taken up by compartment *c* and the rate of tracer loss from this compartment between 5 and 100 h was slower than in controls or in SCD. The quantities of carnitine in compartments *b* and *c* ($Q_b$ and $Q_c$, respectively) were estimated from $F_{ab} \times k_{aa}$ and $F_{ac} \times k_{ac}$, respectively (Table V). $F_{oa}$, the rate of carnitine excretion by kinetic analysis, was the product of $k_{oa}$ and $Q_a$ (Table III). Turnover times for carnitine in compartments *a*, *b*, and *c* (the time required for the quantity of carnitine in a given compartment to exit from that compartment) were the reciprocals of $k_{aa}$, $k_{bb}$, and $k_{cc}$, respectively (Table IV). Whole-body carnitine turnover time was calculated from $(Q_a + Q_b + Q_c)/F_{oa}$ by using the value of $F_{oa}$ obtained by kinetic analysis. Turnover times for carnitine in compartments *a* and *b* of SCD patients were significantly lower than for controls. Mean whole-body carnitine turnover in SCD patients was 67% of the mean in control subjects, but this difference was not statistically significant. For the MCD patient, turnover times for carnitine in compartments *a* and *c* were 105 and 116% of control means, respectively. Turnover time for carnitine in compartment *b* and whole-body carnitine turnover time were 49 and 54% of control means, respectively.

*Metabolic interconversion of carnitine.* In control subjects, acylcarnitine esters accounted for 20±4 and 43±8% (mean±SD) of total carnitine in plasma and urine, respectively. In SCD patients, acylcarnitine esters were 24±4 and 45±11% of total carnitine in plasma and urine, respectively, and in the MCD patient, the acylcarnitines were 26 and 52% of total carnitine in plasma and urine, respectively.

Radioactivity in urine samples from days 1, 14, and 28 was analyzed (after mild alkaline hydrolysis of acylcarnitine esters) by ion-exchange chromatography (Fig. 4). One major peak of radioactivity, which corresponds to carnitine, was observed in

*Table VI. Carnitine Fluxes in Human Subjects*

| Subject | $F_{ca}$* | $F_{ac}$ | $F_{ab} = F_{ba}$ | $F_{ba}$ / Muscle mass | $F_{ba}$ / Muscle mass / Plasma carnitine |
|---|---|---|---|---|---|
| | *μmol/h* | *μmol/h* | *μmol/h* | *nmol/h/g* | *nmol/h/g/μM* |
| Controls | | | | | |
| 1 | 427 | 478 | 355 | 11.1 | 0.264 |
| 2 | 377 | 411 | 875 | 15.7 | 0.239 |
| 3 | 120 | 140 | 402 | 8.32 | 0.157 |
| 4 | 102 | 114 | 216 | 10.3 | 0.338 |
| 5 | 259 | 292 | 308 | 13.3 | 0.229 |
| 6 | 198 | 229 | 407 | 10.9 | 0.168 |
| Mean±SD | | | | 11.6±2.3 | 0.232±0.061 |
| MCD patient | | | | | |
| 7 | 559 | 556 | 121 | 5.02 | 0.119 |
| SCD patients | | | | | |
| 8 | 67.7 | 68.6 | 31.1 | 4.86 | 0.153 |
| 9 | 106 | 107 | 83.7 | 7.41 | 0.229 |
| 10 | 106 | 112 | 81.1 | 7.95 | 0.330 |
| 11 | 37.2 | 36.9 | 33.6 | 3.20 | 0.215 |
| Mean±SD | | | | 5.86±1.93‡ | 0.232±0.064 |

* Flux of carnitine from one compartment to another is designated, for example, by $F_{ca}$, which is the flux of carnitine from compartment *a* to compartment *c*.
‡ Significantly different from controls ($P < 0.005$).

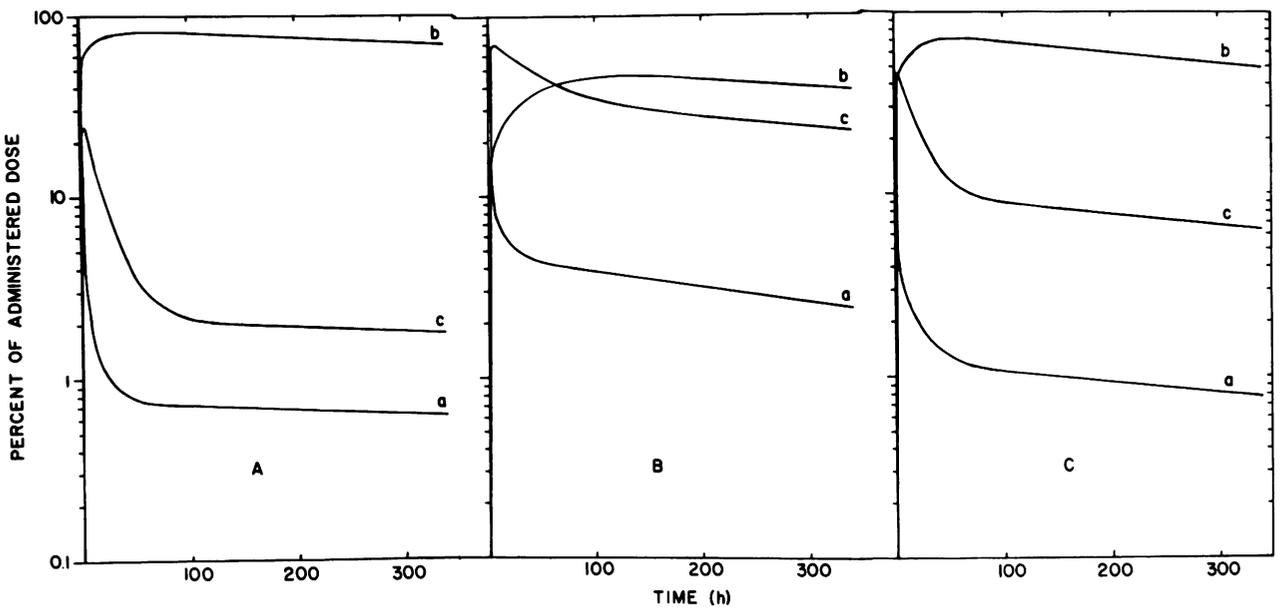


*Figure 3.* Curves for radiotracer content of compartments *a*, *b*, and *c*, as a function of time. Coefficients ($K_1$, $K_2$, $K_3$, $L_1$, $L_2$, $L_3$) were calculated (Reference 21, p. 218, equations 19, 21–23) and substituted into the following equations:

$$q_b/q_{ao} = K_1e^{-g_1t} + K_2e^{-g_2t} + K_3e^{-g_3t}$$

and

$$q_c/q_{ao} = L_1e^{-g_1t} + L_2e^{-g_2t} + L_3e^{-g_3t}.$$

Normalized (percentage of dose) values are plotted as a function of time ($t$). Curves shown are for control subject No. 6 (*A*), MCD patient No. 7 (*B*), and SCD patient No. 11 (*C*) (see Table I). Lower case letters indicate compartments to which individual curves correspond.

all chromatograms. Chromatographic profiles revealed no significant conversion of L-[*methyl*-$^3$H]carnitine to other metabolites.

## Discussion

This investigation was designed primarily to test the hypothesis that SCD and/or MCD result from abnormal transport of carnitine into and out of skeletal muscle. In the design of a protocol, we realized that techniques available for study of carnitine transport into muscle of experimental animals (perfusion of hindquarters, in vitro studies of isolated whole muscles or muscle strips) were not applicable to investigations in humans.[3] We previously studied carnitine transport into cultured muscle cells from SCD patients and normal subjects, and found no difference in kinetic parameters between the two populations (24). However, we reasoned that the putative defect in carnitine transport may not be expressed in immature myotubes in culture, or that contamination of muscle cell preparations with fibroblasts may mask any defect present in the myotubes.

3. Although suitable whole-cell preparations of human intercostal muscle strips may be obtained by biopsy, it is not possible to obtain a sufficient amount of tissue for complete and unequivocal analysis of carnitine transport parameters.

For these reasons, the technique of kinetic compartmental analysis was used to study carnitine transport in humans. By using this approach in dogs (13), we showed that values obtained for most parameters of interest were consistent with results obtained by direct chemical analyses. For this study, a three-compartment, open-system model was selected on the basis of several observations: (*a*) the obvious physical barrier between extracellular space and tissues; (*b*) the difference in carnitine pool sizes and intracellular carnitine concentrations between muscle and other tissues, such as liver and kidney in man and experimental animals (13, 25); and (*c*) the slower rate of transport of carnitine into muscle compared with liver and kidney in experimental animals (13).

A basic assumption for application of kinetic analysis to a given system is stationarity, or steady state, of the system (26). In biological systems, this condition may be approached, but is achieved only with great difficulty. In the present study, we identified two parameters which significantly deviated from stationarity. In the four SCD patients, plasma carnitine concentrations, and therefore, the quantity of carnitine in compartment *a*, varied significantly over the 28-d period. For the kinetic analysis, mean plasma carnitine concentrations were used. The variations in plasma carnitine were of relatively short duration with respect to the length of the study. Moreover, for each subject,

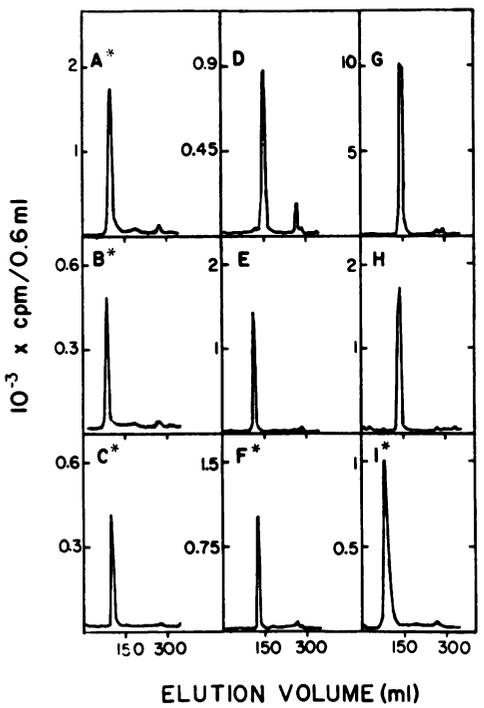


*Figure 4.* Chromatographic analyses of radioactivity in urine. Urine specimens from subjects 1 (*A–C*), 7 (*D–F*), and 10 (*G–I*) on days 1 (4.5–5.0 ml, *A, D,* and *G*), 14 (13.5 ml, *B, E,* and *H*), and 28 (13.5 ml, *C, F,* and *I*) were prepared and chromatographed as described in the legend to Fig. 5, Reference 12. Asterisk indicates that carnitine analyses were done on column fractions corresponding to the major peak. In each case, the peaks and shapes of the carnitine curves were identical to the radioactivity curves.

the mean plasma carnitine concentration was very close to the median value. For the MCD patient, diurnal variation in plasma carnitine levels was of lesser magnitude than fluctuation for SCD patients. Moreover, the fluctuations were regular and deviations above and below the mean were of approximately equal duration and amplitude. For these reasons, we believe that for the purpose of kinetic analysis in the SCD patients and the MCD patient, the mean plasma carnitine concentration is a satisfactory approximation of "steady state" carnitine in compartment *a*.

To assess the effects of variations in plasma carnitine concentration on results obtained by kinetic analysis, we performed sample calculations on data from different controls and patients, but systematically varied the plasma carnitine concentration (and hence, the value for $Q_a$). We found that calculated values for $Q_b$ and $Q_c$ and all the fluxes (except $F_{ao}$) varied roughly in proportion to the change in plasma carnitine concentration. However, the rate constants, turnover times, and the ratio [carnitine flux into muscle/plasma carnitine concentration] did not change. Thus, of the parameters used for comparisons between normal subjects and carnitine deficiency patients, only $F_{ba}$/muscle mass (Table VI) would be affected by variable plasma carnitine concentrations.

Stationarity of the experimental system was also altered by meal feeding. The diet introduced a bolus of carnitine with each meal. Dietary carnitine is rapidly taken up by intestinal epithelial cells. However, Gross and Henderson (27) found that carnitine taken up into the intestinal mucosa from the lumen was only very slowly released into the circulation. Indeed, in live rats, 90% of a dose of $^{14}$C-labeled carnitine introduced into the intestinal lumen was found in intestinal tissue 60 min later. After 7 h, 35% of the dose remained in the intestinal tissue. Thus, the slow release of carnitine from the intestine to the blood stream effectively dampens the bolus effect of a meal containing a significant amount of carnitine.

Another complication inherent in kinetic analysis of carnitine metabolism was the presence of esterified forms of carnitine in tissues and fluids. For the kinetic analysis, calculations were performed on the basis of total (free plus esterified) rather than free carnitine concentrations, for the following reasons: Studies of carnitine metabolism under a variety of conditions, including fasting (28) and exercise (29), indicate that the acylcarnitine esters turn over rapidly. Thus, administered free carnitine should equilibrate with the acylcarnitine ester "pool" relatively rapidly. The error introduced would only occur at the initial time points, and would diminish as the label completely equilibrated into the acylcarnitine ester "pool." Moreover, the error would only be present to the extent that acylcarnitine esters are handled differently from free carnitine. Studies of carnitine transport into several mammalian tissues and cell types indicate that short-chain acylcarnitines cross the plasma membrane via the same carrier and probably at nearly the same rate as free carnitine (30, 31, 32). Moreover, long-chain acylcarnitines usually account for <5% of total carnitine in human tissues and fluids (C. J. Rebouche, unpublished data).

Muscle carnitine concentration was significantly reduced in SCD and MCD patients compared with controls (Table I). These differences are reflected in values for carnitine pool sizes in muscle, as calculated by kinetic analysis (Table V) after correcting for differences in muscle mass. In normal subjects, muscle carnitine accounted for 92–97% of whole-body carnitine stores. This range is consistent with previously reported figures (25). In SCD patients, muscle carnitine was 70–88% of total body carnitine. This result indicates that in SCD muscle is relatively more affected than other tissues or extracellular fluid. For the MCD patient, muscle carnitine was only 58% of total body carnitine.

Carnitine transport into tissues has been studied in a variety of experimental systems. Virtually all have in common a saturable component which is optimally active at physiological plasma carnitine concentrations (10–100 $\mu$M). Saturability in such systems can be expressed in a manner analogous to Michaelis-Menten kinetics for enzyme catalysis. Thus, maximum velocity ($V_{max}$) and external carnitine concentration at which transport rate is half-maximal ($K_T$) may be determined. For carnitine transport into isolated rat muscle, $K_T$ was 60 $\mu$M (33).

In rat muscle, transport rates were almost linear with increasing external carnitine concentration in the range of 0–60 μM (33). Furthermore, rates of entry of carnitine into dog muscle in vivo varied linearly with increasing plasma carnitine concentration (13).

In the present study, rates of carnitine transport into muscle were calculated from $F_{ba}$ and the muscle mass for each individual. Assuming linearity over the range of 0–65 μM plasma carnitine concentration, the parameter "transport rate/plasma carnitine concentration" can be used to compare the efficiency of carnitine transport into muscle in the three groups of subjects.

For SCD patients and control subjects, mean transport rates/plasma carnitine concentration were not different. Thus, in SCD patients, even though transport rates for carnitine entry into compartment *b* from compartment *a* were significantly lower than in controls, the difference could be accounted for entirely by the reduced plasma carnitine concentration. By contrast, for the MCD patient, transport of carnitine as a function of plasma carnitine concentration was only 51% of the control mean and 24% lower than the lowest control value. The reduced rate of carnitine transport into muscle is evident by inspection of Fig. 3. Entry of radiolabeled carnitine into compartment *b* did not plateau until about 100 h into the study (compared with about 40 h for controls), and at the plateau, only ~45% of the administered dose was found in this compartment (compared with ~80% in controls). These results are consistent with an intrinsic defect in the mechanism of carnitine transport into muscle in MCD. In view of these results, it would be appropriate to study first degree relatives of the MCD patient. However, she was an adopted child, with no known brothers or sisters.

For SCD patients, kinetic analysis did not reveal an intrinsic abnormality of carnitine transport into muscle or other tissues. However, the marked fluctuations in plasma carnitine concentration (Fig. 1) in these subjects suggest an abnormality in regulation of the muscle/plasma carnitine concentration gradient. The following hypothesis is suggested: Plasma carnitine concentration is reduced, at least in part, due to the abnormally low renal plasma excretory threshold for carnitine in the SCD patients (10). To make up the deficit of carnitine in plasma, carnitine may be released from muscle (and perhaps other tissues) to achieve a "normal" plasma carnitine level. However, carnitine added to the extracellular fluid is lost during filtration of plasma through the kidney, creating a futile cycle which leaves muscle severely depleted of this compound. To date, factors which regulate the flow of carnitine into and out of muscle have not been clearly defined.

In the present study, carnitine excretion as a function of body mass was essentially equal for normal subjects and SCD patients (0.25±0.10 μmol/h per kg body weight for normal subjects and 0.23±0.09 μmol per kg body weight for SCD patients). These data in themselves do not indicate a renal carnitine leak in SCD patients. However, because the filtered load was reduced by low plasma carnitine concentrations in SCD patients, these subjects had higher than normal fractional excretion of carnitine. Abnormal renal handling of carnitine was demonstrated in each SCD patient in the present study. These results were reported previously (10).

In 8 of 11 subjects studied (5 control subjects, 1 MCD patient, and 2 SCD patients), carnitine intake exceeded carnitine excretion. Carroll et al. (34) recently reported a similar observation. In six patients with carnitine deficiency, the mean carnitine excretion (2.43 μmol/kg per d) was lower than the mean dietary carnitine intake (3.60 μmol/kg per d). These results suggest that significant amounts of carnitine were degraded. However, we observed no significant radioactive metabolites of L-[*methyl*-$^3$H]carnitine (other than acylcarnitine esters) in urine of any of our subjects. Thus, if degradation occurred, either the methyl groups were oxidized to volatile products ($CO_2$ and $^3H_2O$), or dietary carnitine (but not circulating or intracellular carnitine) was catabolized in the gastrointestinal tract.

Degradation of carnitine in mammals is a controversial issue. Reports from different laboratories over the last 20 years describe a number of metabolites in mammals presumably derived from orally or intravenously administered carnitine. These compounds include β-methylcholine (35), γ-butyrobetaine (36), trimethylamine (36), trimethylamine oxide (36), and trimethylacetonylammonium hydroxide (36). Much of this work has not been independently confirmed. Other studies in mammals failed to detect any metabolites of carnitine following intravenous administration of this compound (13, 38, 39, 40). A variety of microorganisms were shown to convert L-carnitine to one or more of the metabolites listed above (41, 42, 43, 44) as well as glycine betaine (45).

In 10 of 11 subjects in this study, kinetic analyses predicted higher carnitine excretion rates than were measured directly. These results are consistent with data obtained by kinetic compartmental analysis of carnitine metabolism in rats (39, 40) and dogs (13). Kinetic compartmental analysis did not discriminate between catabolic and excretory routes; these two parameters are lumped together in the mathematical model. Thus, overestimation of carnitine excretion by kinetic analysis in these species may reflect catabolic pathways for carnitine rather than problems inherent in the fit of the model to actual metabolic events. To investigate this question, preliminary experiments were done in rats to determine the fate of oral carnitine (C. J. Rebouche, unpublished data). In two rats, only 40 and 42% of an oral tracer dose of L-[*methyl*-$^3$H]carnitine was recovered as carnitine in tissues, urine, and stools. Another 12 and 21% of the radioactivity was recovered as nonvolatile metabolites of carnitine in urine, intestine, and stools. Data from the human studies do not indicate that excessive catabolism of carnitine is a factor in the pathogenesis of the primary carnitine deficiency syndromes. Indeed, normal subjects appear to have lost a slightly greater percentage of dietary carnitine by this route than the MCD and SCD patients.

*Note added in proof.* Medium-chain (general) acyl Coenzyme A dehydrogenase deficiency was found in cultured skin fibroblasts of patient No. 11 (Table I) (P. M. Coates and A. M. Glasgow, personal communication). This subject probably has secondary and not primary carnitine deficiency.

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## References

1. Fritz, I. B. 1968. The metabolic consequences of the effects of carnitine on long-chain fatty acid oxidation. *In* Cellular Compartmentalization and Control of Fatty Acid Oxidation. F. C. Gran, editor. Academic Press, New York. 39–63.

2. Engel, A. G., and C. Angelini. 1973. Carnitine deficiency of skeletal muscle associated with lipid storage myopathy: a new syndrome. *Science (Wash. DC).* 173:899–902.

3. Engel, A. G., C. Angelini, and R. A. Nelson. 1974. Identification of carnitine deficiency as a cause of human lipid storage myopathy. *In* Exploratory Concepts in Muscular Dystrophy II. A. T. Milhorat, editor. Excerpta Medica, Amsterdam. 601–617.

4. Karpati, G., S. Carpenter, A. G. Engel, G. Watters, J. Allen, S. Rothman, G. Klassen, and O. A. Mamer. 1975. The syndrome of systemic carnitine deficiency. Clinical, morphological, biochemical and pathophysiological features. *Neurology.* 25:16–24.

5. Engel, A. G., and C. J. Rebouche. 1982. Pathogenetic mechanisms in human carnitine deficiency syndromes. *In* Disorders of the Motor Unit. D. L. Schotland, editor. John Wiley & Sons, New York. 643–656.

6. Rebouche, C. J., and A. G. Engel. 1983. Carnitine metabolism and deficiency syndromes. *Mayo Clin. Proc.* 58:533–540.

7. Willner, J., S. DiMauro, A. Eastwood, A. Hays, F. Roohi, and R. Lovelace. 1979. Muscle carnitine deficiency. Genetic heterogeneity. *J. Neurol. Sci.* 41:235–246.

8. Rebouche, C. J., and A. G. Engel. 1981. Primary systemic carnitine deficiency. I. Carnitine biosynthesis. *Neurology.* 31:813–818.

9. Rebouche, C. J., and A. G. Engel. 1980. In vitro analysis of hepatic carnitine biosynthesis in human systemic carnitine deficiency. *Clin. Chim. Acta.* 106:295–300.

10. Engel, A. G., C. J. Rebouche, D. M. Wilson, A. M. Glasgow, C. A. Romshe, and R. P. Cruse. 1981. Primary systemic carnitine deficiency. II. Renal handling of carnitine. *Neurology.* 31:819–825.

11. Rebouche, C. J., and A. G. Engel. Carnitine biosynthesis and metabolism in the primary carnitine deficiency syndromes. *In* Neuromuscular Diseases. G. Serratrice, editor. Raven Press, New York. In press.

12. Waber, L. J., D. Valle, C. Neill, S. DiMauro, and A. L. Shug. 1982. Carnitine deficiency presenting as familial cardiomyopathy: a treatable defect in carnitine transport. *J. Pediatr.* 101:700–705.

13. Rebouche, C. J., and A. G. Engel. 1983. Kinetic compartmental analysis of carnitine metabolism in the dog. *Arch. Biochem. Biophys.* 220:60–70.

14. Engel, A. G., and R. G. Siekert. 1972. Lipid storage myopathy responsive to prednisone. *Arch. Neurol.* 27:174–181.

15. Glasgow, A. M., G. Eng, and A. G. Engel. 1980. Systemic carnitine deficiency simulating recurrent Reye syndrome. *J. Pediatr.* 96:899–891.

16. Stokke, O., and J. Bremer. 1970. A simple method for preparation of methyl-labelled (−) carnitine. *Biochim. Biophys. Acta.* 218:552–554.

17. Rebouche, C. J., and A. G. Engel. 1980. Significance of renal γ-butyrobetaine hydroxylase for carnitine biosynthesis in man. *J. Biol. Chem.* 255:8700–8705.

18. Schwartz, I. L., D. Schachter, and N. Freinkel. 1949. The measurement of extracellular fluid in man by means of a constant infusion technique. *J. Clin. Invest.* 28:1117–1125.

19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

20. Lilienthal, J. L., K. L. Zierler, B. P. Folk, R. Buka, and M. J. Riley. 1950. A reference base and system for analysis of muscle constituents. *J. Biol. Chem.* 193:265–275.

21. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. Iowa State University Press, Ames, Iowa. Seventh ed. 54–59.

22. Shipley, R. A., and R. E. Clark. 1972. Tracer Methods for In Vivo Kinetics: Methods and Applications. Academic Press, New York.

23. Graystone, J. E. 1968. Creatinine excretion during growth. *In* Human Growth. Body Composition, Cell Growth, Energy and Intelligence. D. B. Cheek, editor. Lea & Febiger, Philadelphia. 182–197.

24. Rebouche, C. J., and A. G. Engel. 1982. Carnitine transport in cultured muscle cells and skin fibroblasts from patients with primary systemic carnitine deficiency. *In Vitro (Rockville).* 18:495–500.

25. Rebouche, C. J. 1980. Comparative aspects of carnitine biosynthesis in microorganisms and mammals with attention to carnitine biosynthesis in man. *In* Carnitine Biosynthesis, Metabolism, and Functions. R. A. Frenkel and J. D. McGarry, editors. Academic Press, New York. 57–67.

26. Zierler, K. 1981. A critique of compartmental analysis. *Ann. Rev. Biophys. Bioeng.* 10:531–562.

27. Gross, C. J., and L. M. Henderson. 1983. Carnitine absorption by the rat small intestine. *Fed. Proc.* 42:1320.

28. Frolich, J., D. W. Seccombe, P. Hahn, P. Dodek, and I. Hynie. 1978. Effect of fasting on free and esterified carnitine levels in human serum and urine: correlation with serum levels of free fatty acids and β-hydroxybutyrate. *Metab. Clin. Exp.* 27:555–561.

29. Carter, A. L., D. L. F. Lennon, and F. W. Stratman. 1981. Increased acetyl carnitine in rat skeletal muscle as a result of high-intensity short-duration exercise. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 126:21–24.

30. Rebouche, C. J., and D. L. Mack. 1983. $Na^+$-gradient-stimulated transport of L-carnitine by rat renal brush border membranes. *Fed. Proc.* 42:1053.

31. Molstad, P., T. Bohmer, and K. Eiklid. 1977. Specificity and characteristics of the carnitine transport in human heart cells (CCL 27) in culture. *Biochim. Biophys. Acta.* 471:296–304.

32. Huth, P. J., M. J. Schmidt, P. J. Hall, R. G. Fariello, and A. L. Shug. 1981. The uptake of carnitine by slices of rat cerebral cortex. *J. Neurochem.* 36:715–723.

33. Rebouche, C. J. 1977. Carnitine movement across muscle cell membranes. Studies in isolated rat muscle. *Biochem. Biophys. Acta.* 471:145–155.

34. Carroll, J. E., M. H. Brooke, J. B. Shumate, and N. J. Janes. 1981. Carnitine intake and excretion in neuromuscular diseases. *Am. J. Clin. Nutr.* 34:2693–2698.

35. Khairallah, E. A., and G. Wolf. 1967. Carnitine decarboxylase. The conversion of carnitine to β-methylcholine. *J. Biol. Chem.* 242:32–39.

36. Strack, E., and H. Seim. 1979. Die Bildung von γ-butyrobetaine aus exogenem L(−)-carnitin in vivo bei maus und ratte. *Hoppe-Seyler's Z. Physiol. Chem.* 360:207–215.

37. Grüner, E., H. Aurich, and E. Strack. 1966. Über das Vorkomen von trimethyl-acetonyl-ammoniumhydroxide in menschlichen harn. *Hoppe-Seyler's Z. Physiol. Chem.* 343:240–245.

38. Yue, K. T. N., and I. B. Fritz. 1962. Fate of tritium-labeled carnitine administered to dogs and rats. *Am. J. Physiol.* 202:122–128.

39. Brooks, D. E., and J. E. McIntosh. 1975. Turnover of carnitine by rat tissues. *Biochem. J.* 148:439–445.

40. Cederblad, G., and S. Lindstedt. 1976. Metabolism of labeled carnitine in the rat. *Arch. Biochem. Biophys.* 175:173–180.

41. Seim, H., H. Löster, and H.-P. Kleber. 1982. Reduktiver stoffwechsel des L-carnitins und strukturverwandter trimethylammoniumverbindungen in *Escherichia coli*. *Acta Biol. Med. Ger.* 41:1009–1018.

42. Unemoto, T., M. Hayashi, K. Miyaki, and M. Hayashi. 1966. Formation of trimethylamine from DL-carnitine by *Serratia marcescans*. *Biochim. Biophys. Acta.* 121:220–222.

43. Seim, H., H. Löster, R. Claus, H.-P. Kleber, and E. Strack. 1982. Formation of γ-butyrobetaine and trimethylamine from quarternary ammonium compounds structure-related to L-carnitine and choline by *Proteus vulgaris*. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 13:201–205.

44. Seim, H., R. Ezold, H.-P. Kleber, and E. Strack. 1980. Stoffwechsel des L-carnitins bei enterobakterien. *Z. Allg. Mikrobiol.* 20:591–594.

45. Muira-Fraboni, J., H.-P. Kleber, and S. England. 1982. Assimilation of γ-butyrobetaine, and D- and L-carnitine by resting cell suspensions of *Acinetobacter calcoaceticus* and *Pseudomonas putida*. *Arch. Microbiol.* 133:217–221.