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ABSORPTION OF D- AND L-CARNITINE BY THE INTESTINE AND KIDNEY TUBULE IN THE RAT

CAROL J. GROSS and LaVELL M. HENDERSON

Department of Biochemistry, University of Minnesota, St. Paul, MN 55108 (U.S.A.)

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The process by which L- and D-carnitine are absorbed was investigated using the live rat and the isolated vascularly perfused intestine. A lumenal dose of 2–6 nmol in the perfused intestine resulted in less than 5% transport of either isomer to the perfusate in 30 min. The L-isomer was taken up by the intestinal tissue about twice as rapidly as the D-isomer by both the perfused intestine (52.8% and 21.6%, respectively) and the live animal (80% and 50%, respectively) in 30 min. After 1 h 90% of the L-carnitine had accumulated in the intestinal tissue and was released to the circulation over the next several hours. Accumulation of D-carnitine reached a maximum of 80% in 2 h and release to the circulations was similar to that of L-carnitine. Uptake of both L-[^{14}C]carnitine and acetyl-L-[^{14}C]carnitine was more rapid in the upper jejunal segment than in other portions of the small intestine. Acetylation occurred in all segments, resulting in nearly 50% conversion to this derivative in 5 min. Increasing the dose of L-carnitine reduced the percent acetylation. The uptake of both isomers was a saturable process and high concentrations of D-carnitine, acetyl-L-carnitine and trimethylaminobutyrate inhibited L-carnitine uptake. In the live animal after 5 h, the distribution of isotope from L-[^{14}C]carnitine and D-[^{3}H]carnitine differed primarily in the muscle where 29.5% of the L-carnitine and 5.3% of the D-carnitine was found and in the urine where 2.9% of the L-carnitine and 7.1% of the D-carnitine was found. The renal threshold for L-carnitine was 80 μM and for D-carnitine 30 μM, in the isolated perfused kidney. Approx. 40% of the L-carnitine but none of the D-carnitine excreted in the urine was acetylated. L-Carnitine and D-carnitine competed for tubular reabsorption.

Introduction

The transport of L-carnitine and its biologically inactive isomer, D-carnitine, have been studied in a number of tissues and isolated cell systems such as isolated rat liver cells [1], isolated rat muscle [2], isolated rat epididymal cells [3], rat kidney slices [4], and human heart cells [5,6]. All of the cell types studied developed a large tissue-to-plasma gradient for both L- and D-isomers. Frequently the D-isomer was not transported as efficiently as the L-isomer. It has also been suggested that both isomers and trimethylaminobutyrate compete for the same transport site [2,4,5].

Orally administered carnitine must first be transported to the circulation by the intestine before it can reach other tissues, making the intestine the first important organ in the use of exogenous carnitine. Little information is presently available concerning intestinal transport of carnitine. The kidney's role is also of concern, since it determines the rate of carnitine excretion. This study was concerned with the transport of both L-carnitine and D-carnitine from the lumen of the small intestine to the circulation, the reabsorption of these compounds by the kidney tubules and the effect of one isomer upon the other in both organs. The isolated vascularly perfused intestine and the live

rat were utilized for intestinal studies, and the isolated vascularly perfused rat kidney was used for excretion studies.

Methods and Materials

L-[*methyl*-^{14}C]Carnitine hydrochloride, specific activity 58 mCi/mmol, DL-[*methyl*-^{3}H]carnitine hydrochloride, specific activity 1.0 Ci/mmol, were radiochemically pure as revealed by paper electrophoresis at pH 3.5 and by HPLC described below. Inulin[^{14}C]carboxylic acid, specific activity 5 mCi/mmol, molecular weight 5175, and [^{3}H]inulin, specific activity 1.96 Ci/mmol, molecular weight 5200, were used for kidney clearance experiments. All isotopes were purchased from Amersham Corporation, Arlington Heights, IL. The following compounds were purchased commercially: L-carnitine from Sigma, St. Louis, MO; D-carnitine from Mann Research Laboratories, New York, NY, and acetyl-L-carnitine was a gift from Sigma Tau Chemical Co., Rome, Italy.

DL-[*Methyl*-^{3}H]Carnitine was resolved by the procedure described by Huth and Shug [4]. This procedure involved acetylation of the L-carnitine utilizing carnitine acetyltransferase purchased from Sigma, St. Louis, MO. The resulting mixture of D-[^{3}H]carnitine and acetyl-L-[^{3}H]carnitine was then resolved on a Dowex-50 column (1.2×70 cm) and the radiochemical purity of each isomer was determined by paper electrophoresis at 2000 V for 75 min in a pyridine acetate buffer (pH 3.5) (pyridine/acetic acid/water, 1 : 10 : 289). Acetyl-L-[^{14}C]carnitine was prepared from L-[*methyl*-^{14}C]carnitine by the procedures cited above for the resolution of DL-carnitine. Acetylation was done twice to maximize the yield. This produced acetyl-L-[^{14}C]carnitine that was 98% pure. Trimethylaminobutyrate was prepared according to the procedures of Mazzetti and Lemmon [7]. All other chemicals were reagent grade.

Intestinal perfusions and live-animal studies

Vascular intestinal perfusions were performed as previously described [8,9], using 18-h-fasted male rats of the Sprague-Dawley strain, weighing between 200 and 300 g (Biolabs, White Bear Lake, MN). Both the lumenal and vascular routes for administration of compounds were used. A series of live-animal experiments was performed as described previously [9], using both 18 h-fasted and unfasted male rats of the Sprague-Dawley strain. A midline incision was made and a cannula was placed in the intestinal lumen a few centimeters from the stomach, unless otherwise indicated. Segments of 25 to 30 cm were tied off, except for experiments lasting longer than 30 min when obstruction of the intestine seemed inadvisable. The incision was closed with surgical wound clips and the animals were allowed to recover from the anesthetic for those experiments lasting several hours. For most intestinal experiments the substrate was administered via the cannula in 1.2 ml of 0.9% NaCl. A second series of live-animal experiments was done to determine the rate of L-carnitine absorption and metabolism by three separate segments of the same animal. In these experiments, approx. 1-g segments of duodenal tissue only, upper jejunal tissue adjacent to the duodenum and distal ileal tissue were examined simultaneously. A cannula was placed at the proximal end of each of the three segments and a tie was placed 8–14 cm distal to each canula. D-[^{14}C]Carnitine in 0.2 ml 0.9% NaCl was administered into the lumenal contents of each segment. After 5, 10 or 30 min each segment was removed and the contents were separated by flushing with 13–15 ml of water. Each segment was boiled in a small volume of water, homogenized and centrifuged, and the supernatants of the contents and tissues were analyzed for labeled products by high-voltage paper electrophoresis and high-performance liquid chromatography without further treatment.

Hydrolysis of acetyl-L-carnitine to L-carnitine by intestinal contents alone was determined by incubating acetyl-L-[^{14}C]carnitine with contents obtained by tying off a 10-cm segment of the duodenum and removing 200–300 μl of contents with a syringe after 10 min. 1 nmol of acetyl-L-[^{14}C]carnitine was added to 200 μl of contents and aliquots were applied to Whatman 3MM paper for electrophoresis after 5, 10, 20 and 30 min. The transport of acetyl-L-[^{14}C]carnitine was examined in a 1 g upper-jejunal segment of the same rat. A dose of 2 nmol was injected in 0.2 ml of 0.9% NaCl according to the procedures described above.

HPLC analysis was done using a Beckman high-performance liquid chromatography system

with a 250 × 4.6 mm Rainin microsorb C-18 reverse phase column fitted with a guard column containing pellicular C-18 packing material from Alltech, Deerfield, IL. Compounds were eluted using an isocratic mobile phase consisting of 30 mM hexanesulfonic acid in 15 mM phosphate buffer (pH 2.5) (92%) and methanol (8%). Fractions were collected at 0.5 min intervals with a flow rate of 1 ml/min. Radioactivity peaks were determined by liquid scintillation counting. Aliquots of each fraction were added to 10 ml of aquasol purchased from New England Nuclear, Boston, MA, and counted using a Beckman LS-230 scintillation counter.

Kidney perfusions were done using a modification of the techniques described by Nishiitsutsuji-Uno et al. [10]. The right kidney of fasted male rats weighing between 300 and 400 g was perfused in situ and a heat lamp was used to maintain the temperature at about 37°C. The perfusion apparatus was similar to that described for intestinal perfusions. The arterial pressure was maintained between 100 and 110 mmHg by regulation of the perfusate flow which varied from 25 to 35 ml/min. The perfusate consisted of a modified Krebs-Ringer bicarbonate-phosphate buffer of the following composition: 0.581% (100 mM) NaCl, 0.035% (4.7 mM) KCl, 0.037% (2.5 mM) $CaCl_2 \cdot 2H_2O$, 0.016% (1.2 mM) KH_2PO_4, 0.029% (1.2 mM) $MgSO_4 \cdot 7H_2O$, 0.109% (7.7 mM) Na_2HPO_4, 0.038% (2.3 mM) $Na_3PO_4 \cdot 12\ H_2O$, 0.209% (24.9 mM) $NaHCO_3$, 6.5% bovine serum albumin (fraction V, Sigma St. Louis, MO), 90 mg% (5 mM) glucose, 11.7 mg% (0.8 mM) glutamine, 23000 units/100 ml penicillin, 23 mg% streptomycin sulfate purchased from GIBCO, Grand Island, NY and the following amino acids: 0.8 mM alanine, 0.8 mM proline, 0.4 mM isoleucine, 1.2 mM aspartate, 0.8 mM glycine, and 0.4 mM arginine. Recirculating perfusions were done with 130 ml of perfusate which had been equilibrated with 95% O_2/5% CO_2 prior to use. The pH was maintained at 7.4 by continuous oxygenation with this gas mixture during the perfusion. The kidney was allowed to recover for 30 min before addition of substrates. Urine collection was started 5 min after addition of substrates, to allow time for equilibration of the substrates with the perfusate, and urine was collected at 5-min intervals for the following 60 min. Inulin clearance was used to determine the glomerular filtration rate. Double-label counting techniques were employed for the determination of both inulin and carnitine excretion in the same perfusion and the following formulae were used for clearance and tubular reabsorption calculations:

$$\text{clearance} = \frac{\text{urine concn.} \times \text{urine vol.}}{\text{plasma concn.}}$$

$$\%\ \text{tubular reabsorption (carnitine)} = \frac{\text{clearance (inulin)} - \text{clearance (carnitine)}}{\text{clearance (inulin)}} \times 100$$

At the termination of the experiment, the kidney was flushed with 5 ml of 0.9% NaCl, boiled for 1–2 min in water and homogenized. The perfusate and kidney were analyzed for radioactivity and deproteinized by the addition of 2 vol. methanol. Most of the labeled inulin was also precipitated with methanol. Methanol was chosen rather than perchloric acid for precipitating the protein, since perchloric acid caused some hydrolysis of acetyl-L-[^{14}C]carnitine. This hydrolysis was directly related to the perchloric acid concentration. The protein was removed by filtration and the volume of the filtrate was reduced in vacuo. Radioactive compounds in these samples were analyzed by high-voltage paper electrophoresis and HPLC. Urine samples were analyzed for radioactivity and spotted for electrophoresis without further treatment.

Results

Results obtained with intestinal perfusions demonstrated that both L-carnitine and D-carnitine are taken up by the intestinal tissue, but are not transported into the perfusate to a significant extent for at least 30 min. L-Carnitine was taken up at more than twice the rate of D-carnitine; 53% of a 6-nmol dose of L-carnitine and 20% of the same dose for D-carnitine were removed from the lumen in 30 min (see Fig. 1). Recovery of radioactivity in these experiments was more than 95%.

Live-animal studies demonstrated a similar pattern with a 2.3 nmol dose, where 78% of the L-carnitine and 48% of the D-carnitine were found

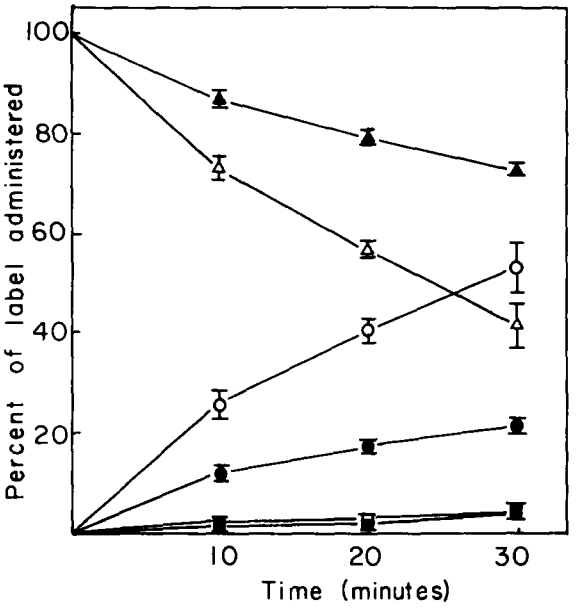

Fig. 1. Rate of uptake of 6 nmol L-[^{3}H]carnitine (open symbols) and D-[^{3}H]carnitine (solid symbols) from the proximal 25 cm of the intestinal lumen (△) to the mucosal tissue (○) and perfusate (□) of the vascularly perfused rat intestine. The curves represent three experiments for each time interval and vertical bars represent ± S.E.

in the intestinal tissue after 30 min. The live animal was used for the remainder of the intestinal experiments, unless otherwise indicated, for the following reasons: (a) carnitine uptake was more complete than in the perfused intestine; (b) the loss of carnitine to the general circulation was minimal (less than 5% in 30 min); (c) it is more nearly physiological; (d) this type of experiment can be done more easily than the intestinal perfusion. In several experiments mucosal tissue was separated from the muscle by scraping, and the distribution of radioactivity between these fractions was determined. More than 95% was associated with the mucosal cells.

Disappearance of radioactivity from the intestinal tissue was followed for up to 7 h. After 1 h, 88% of the L-isomer was found in the intestinal tissue and 7% in the contents, accounting for 95% of a 2.3 nmol dose. The label in the tissue was then gradually lost to the circulation over a number of hours (Fig. 2A). The D-isomer was removed from the lumen more slowly. A maximum of 81% was found in the tissue and 7% in the contents after 2 h, totaling 88% of the 2.3 nmol dose, before gradual loss to the circulation (Fig. 2B). A few experiments were done to determine the amount of label being removed through the lymph. For these experiments, unfasted male rats of the Wistar strain purchased from Harlen-Sprague Dawley, Indianapolis, IN were used since the lymph vessel was more easily cannulated in this strain. The amount of label found in the lymph was less than 0.25% of the dose after 2 h.

The uptake of L-carnitine was determined at various locations along the intestinal tract. The

TABLE I

RATE OF TRANSPORT AND ACETYLATION OF L-[^{14}C]CARNITINE BY ISOLATED INTESTINAL SEGMENTS OF THE LIVE RAT, WITH A DOSE OF 6.3 nmol

Values represent the mean ± S. E. of the number of experiments indicated in parentheses.

Segment	Time (min)	L-[^{14}C]Carnitine (nmol/g)	Acyl-L-[^{14}C]carnitine (nmol/g)	Recovery (%)
Duodenum	5	0.83 ± 0.07	0.92 ± 0.09	93.4 ± 4.5 (8)
(10.7 ± 0.4 cm/g)	10	0.97 ± 0.10	1.23 ± 0.23	89.7 ± 4.8 (8)
	30	1.44 ± 0.20	2.15 ± 0.22	93.0 ± 4.5 (6)
Jejunum	5	1.59 ± 0.29	1.47 ± 0.35	95.2 ± 3.1 (7)
(13.9 ± 0.4 cm/g)	10	2.02 ± 0.30	2.01 ± 0.17	100 ± 0 (7)
	30	1.75 ± 0.24	3.01 ± 0.47	95.5 ± 2.6 (5)
Ileum	5	0.99 ± 0.25	0.77 ± 0.29	91.0 ± 2.1 (7)
(14.5 ± 0.5 cm/g)	10	1.24 ± 0.32	1.29 ± 0.25	96.9 ± 2.2 (6)
	30	1.40 ± 0.32	1.55 ± 0.51	92.7 ± 1.1 (5)

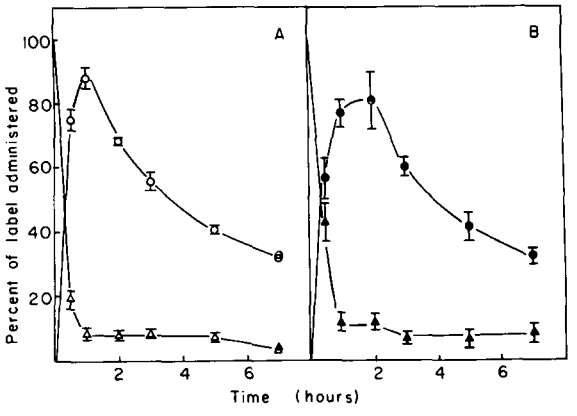

Fig. 2. Rate of transfer into and out of the intestinal tissue when a dose of 2.3 nmol of (A) L-[^{14}C]carnitine and (B) D-[^{3}H]carnitine were injected into the proximal intestinal lumen of the live rat. Each point represents the mean of three to ten experiments. △, lumenal contents; ○, intestinal tissue. The bars represent S.E.

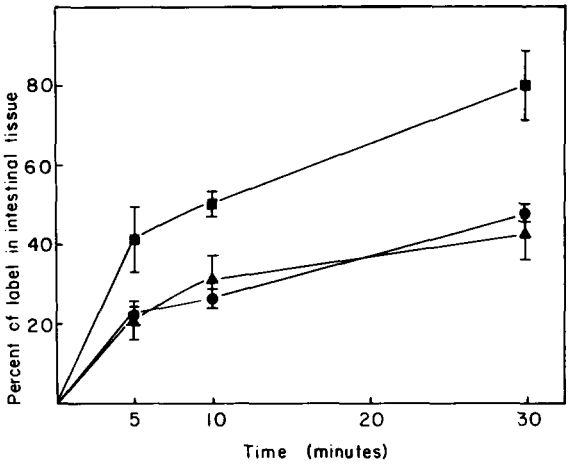

Fig. 3. Rate of uptake of a 6.3 nmol dose of L-[^{14}C]carnitine into approx. 1 g segments of the duodenum (●), upper jejunum (■) and lower ileum (▲). Points represent the mean and bars represent S.E. for five to eight experiments.

most rapid uptake occurred in the upper jejunum (Fig. 3). With a 6 nmol dose 80% was found in the tissue after 30 min. The duodenal and ileal segments took up significantly less. The duodenal tissue took up 3.59 ± 0.32 nmol/g in 30 min and the jejunal tissue took up 4.76 ± 0.83 nmol/g (Table I). When determined on a per centimeter basis, however, they were similar, namely 0.34 nmol/cm. The uptake of L-carnitine by the ileum was less than uptake by the jejunum when determined either per gram (3.05 ± 0.43 nmol/g) of per centimeter (0.21 nmol/cm) in 30 min.

Analysis of the intestinal tissue by electrophoresis and HPLC showed that more than 40% of the label was present as acetyl-L-carnitine after 5 min and more than 50% after 30 min for all segments. The jejunal segment displayed more acetylation (63%) than the other segments at 30 min (see Table I). The intestinal contents contained primarily L-carnitine, with small amounts of labeled acetyl-L-carnitine in some experiments, particularly at 30 min. The percent acetylation of L-carnitine decreased with increasing amounts of L-carnitine (Fig. 4).

Labeled authentic standards had the following electrophoretic mobilities (in cm per 75 min at 2000 V) under the conditions described above: L-carnitine, −21; D-carnitine, −21; and acetyl-L-carnitine, −16. The retention times for these standards using the conditions described for HPLC were as follows: L-carnitine, 10 min; D-carnitine, 10 min; and acetyl-L-carnitine, 21 min. Labeled inulin used in kidney perfusions did not interfere with the analysis of these compounds in either system.

Uptake of L-carnitine by the 25 cm duodenal-upper jejunal segment was found to be saturable. The percentage of labeled L-carnitine taken up in 30 min was reduced from 78% to 6% when the dose was increased from 2.3 nmol to 288 μmol by addition of unlabeled L-carnitine. D-Carnitine gave similar results where uptake decreased from 43% to 4% over the same concentration range (see Fig. 5). No significant difference between fasted and unfasted rats was found. A dose of 16 nmol gave the following percent distribution of label in three unfasted rats after 30 min: $75.4 \pm 7.0\%$ in the intestinal tissue and $20.4 \pm 3.1\%$ in the contents. Doses of 2.3 nmol and 30 nmol gave the following percent distribution of label in 18-hour fasted rats: $77.8 \pm 5.6\%$ and $75.4 \pm 4.9\%$, respectively, for intestinal tissue and $17.6 \pm 5.0\%$ and $24.1 \pm 4.5\%$, respectively, for lumenal contents. The total recovery of label was greater than 96% for each group of experiments.

The effect of unlabeled D-carnitine on the uptake of L-[^{14}C]carnitine was also examined. With increasing amounts of D-carnitine, the amount of

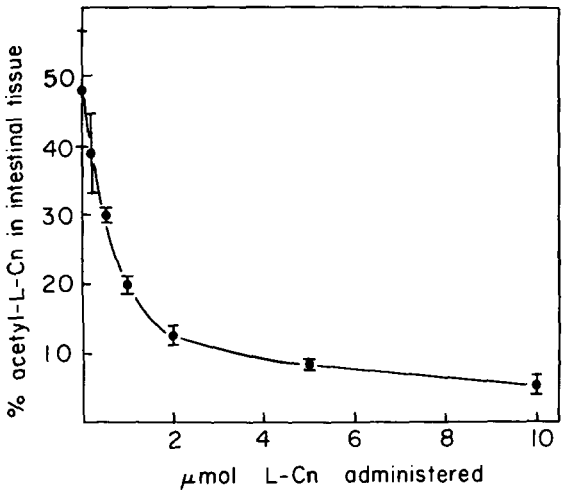

Fig. 4. Effect of increasing the dose of unlabeled L-carnitine (L-Cn) on the percent of isotope converted to acetyl-L-carnitine (acetyl-L-Cn) by the upper jejunal tissue 5 min after injection of L-[^{14}C]carnitine into the lumen. Each point represents the mean ± S.E. for three experiments.

L-[^{14}C]carnitine found in the intestinal tissue decreased from 75% with a 10-fold excess of D-carnitine to 10% with a 10000-fold excess. A 2500-fold excess was required to reduce the transport of a 2.3-nmol dose by 50%. Acetyl-L-carnitine similarly inhibited the transport of L-carnitine but required 10000-fold excess to produce 50% inhibition of uptake. Trimethylaminobutyrate was the most effective inhibitor of L-[^{14}C]carnitine uptake, requiring a 100-fold excess to reduce uptake of 2.3 nmol L-[^{14}C]carnitine into the intestinal tissue in 30 min by 50%.

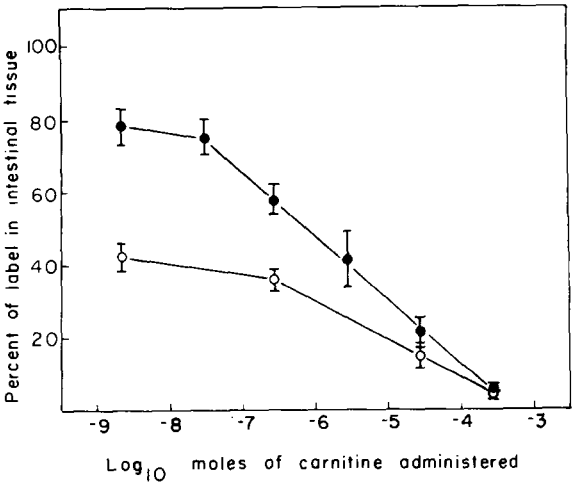

Fig. 5. Effect of concentration on the percent of isotope absorbed into the intestinal tissue in 30 min when L-[^{14}C]carnitine (●) or D-[^{3}H]carnitine (○) was injected into the lumen of the proximal intestine of the live rat. Each point represents the mean of three to ten experiments ± S.E.

Acetyl-L-[^{14}C]carnitine was used as substrate in both live-animal and intestinal-perfusion experiments, to determine whether the inhibitory effect was a result of the compound itself or a dilution effect resulting from the hydrolysis of acetyl-L-carnitine to L-carnitine by intestinal enzymes. The distribution of isotope in both live-animal and intestinal-perfusion experiments using acetyl-L-[^{14}C]carnitine was similar to results obtained when L-carnitine was the substrate with respect to the percent distribution of label. Experiments in which the lumenal contents was isolated and examined for the ability to hydrolyze acetyl-L-carnitine showed that almost no hydrolysis occurred. After 30 min, more than 96% of the label was still acetyl-L-carnitine. Three experiments were done in which 2 nmol acetyl-L-[^{14}C]carnitine was placed in the upper jejunal lumen. After 30 min, 87.8 ± 1.6% of the dose was to be found in the intestinal tissue. The distribution of label between L-carnitine and acetyl-L-carnitine was 0.53 ± 0.02 nmol/g and 1.77 ± 0.05 nmol/g, respectively. When D-[^{3}H]carnitine was used as the substrate, no significant conversion to any other products was detected.

A few experiments were done following the procedures described by Brass and Hoppel [11] to determine the extent of long-chain acylcarnitine

TABLE II

PERCENT DISTRIBUTION OF LABEL 60 min AFTER ADMINISTRATION OF 6 nmol OF L-[^{14}C]CARNITINE INTO THE PERFUSATE OF THE VASCULARLY PERFUSED INTESTINE

The numbers in parentheses represent individual values for two experiments.

Perfusate	87.8 (89.6, 86.0)
Intestinal tissue	5.8 (5.1, 6.5)
Colon	2.4 (2.3, 2.5)
Intestinal contents	1.0 (0.9, 1.1)
Intestinal bath	2.6 (1.5, 3.8)
Recovery	99.6 (99.4, 99.9)

formation by intestinal tissue. A dose of 16 nmol L-[^{14}C]carnitine was placed in the lumen of a 30 cm segment of the proximal small intestine. After 30 min, the intestine was flushed, boiled, homogenized and extracted with 3% perchloric acid. The precipitate was washed twice with 3% perchloric acid and then subjected to alkaline hydrolysis to release carnitine from the acid-insoluble acylcarnitine. Only 2–3% of the label was found in this fraction.

L-[^{14}C]Carnitine, when administered in the vascular perfusate, was taken up poorly by the intestinal tissue. After circulating a 6 nmol dose for 60 min, only 6% was found in the small intestine and 2.3% in the colon. Small amounts of ^{14}C-label were found in the intestinal contents and the bath and the remainder (87.8%) was in the perfusate (Table II).

Several 5-h experiments were conducted using L-[^{14}C]carnitine or D-[^{3}H]carnitine as substrates, where 3–4 nmol was injected into the intestinal lumen of the live animal and the distribution of label was examined in a number of tissues at the termination of the experiment. The percent distribution of isotope for both isomers (Table III) was similar for most samples. The exceptions were skeletal muscle and the urine. 6% of the D-carnitine was found in the skeletal muscle as compared to 25% of the L-carnitine and 14% of the D-carnitine was found in the urine as compared to 6% of the L-carnitine. This difference in the percentage of the two isomers which appeared in the urine was further investigated utilizing isolated kidney perfusions. For both D-carnitine and L-carnitine, greater than 95% of the radioactivity was reabsorbed at a perfusate concentration of 50 nM (Table IV). The renal threshold for L-carnitine was about 80 μM and for D-carnitine about 30 μM. When D-[^{3}H]carnitine was used as substrate this was the only labeled compound in the urine, but when L-[^{14}C]carnitine was the substrate, L-[^{14}C]carnitine and acetyl-L-[^{14}C]carnitine both appeared in the urine. Quantitation of the label in each compound was made after separation by

TABLE III

PERCENT DISTRIBUTION OF ISOTOPE IN TISSUES OF THE LIVE ANIMAL 5 h AFTER LUMENAL ADMINISTRATION OF 3 nmol OF D- OR L-CARNITINE TO THE LIVE ANIMAL

Data represent the mean ± S.E. of three experiments.

Tissue	L-[^{14}C]Carnitine	D-[^{3}H]Carnitine
Intestinal contents	5.1 ± 0.8	6.7 ± 2.7
Intestinal tissue	45.8 ± 5.9	41.4 ± 4.6
Liver	15.3 ± 2.8	14.3 ± 2.2
Kidney	2.2 ± 0.3	1.6 ± 0.9
Urine	2.9 ± 0.8	7.1 ± 0.9
Heart	0.5 ± 0.2	0.8 ± 0.4
Muscle	22.5 ± 5.2	5.3 ± 1.8
Testes	0.9 ± 0.1	0.7 ± 0.1
Recovery	95.2 ± 1.1	77.9 ± 4.6

TABLE IV

RAT KIDNEY PERFUSED FOR 60 MINUTES WITH L- OR D-CARNITINE

Values represent means ± S.E. for three determinations at each concentration. GFR, glomerular filtration rate.

Perfusate concentration (M)	Distribution of isotope			Physiological characteristics			
	Perfusate (%)	Kidney (%)	Urine (%)	Urine flow (μl/min)	Kidney wt. (g)	GFR (μl/min)	Carnitine clearance (μl/min)
L-[^{14}C]Carnitine						[^{3}H]inulin	
$5 \cdot 10^{-8}$	93.2 ± 0.1	5.9 ± 0.2	0.8 ± 0.3	57 ± 29	1.6 ± 0.1	404 ± 36	17 ± 7
$8 \cdot 10^{-5}$	94.4 ± 1.2	4.7 ± 1.3	0.8 ± 0.1	54 ± 8	1.7 ± 0.1	475 ± 76	20 ± 2
$8 \cdot 10^{-4}$	89.1 ± 1.0	2.3 ± 0.5	8.4 ± 0.6	77 ± 12	1.6 ± 0.3	314 ± 58	201 ± 33
D-[^{3}H]Carnitine						[^{14}C]inulin	
$5 \cdot 10^{-8}$	95.3 ± 0.6	4.0 ± 0.5	0.8 ± 0.1	35 ± 11	1.7 ± 0.0	385 ± 19	17 ± 3
$4 \cdot 10^{-5}$	95.4 ± 0.7	2.9 ± 0.5	1.7 ± 0.3	82 ± 2	1.5 ± 0.2	500 ± 46	41 ± 6
$8 \cdot 10^{-4}$	83.9 ± 2.0	2.3 ± 0.7	14.1 ± 2.0	79 ± 5	1.7 ± 0.0	405 ± 64	342 ± 67

TABLE V

SEQUENTIAL ADDITIONS OF UNLABELED SUBSTRATE TO THE PERFUSATE OF THE PERFUSED RAT KIDNEY AFTER ESTABLISHING THE PERCENT TUBULAR REABSORPTION OF LABELED SUBSTRATE

Values represent the mean ± S.E. for three to four experiments. Values in parentheses indicate the percent of acetyl-L-[^{14}C]carnitine in each fraction. n.d., not determined; GFR, glomerular filtration rate.

Labeled (nM)	Unlabeled (μM)	Time [a] (min)	% of tubular reabsorption of labeled substrate	% of acetylated urinary carnitine	Urine flow (μl/min)	GFR (μl/min)	% distribution of label after 60 min		
							Urine	Kidney	Perfusate
L-[^{14}C]Carnitine	D-Carnitine								
80	–	15	97.3 ± 0.6	n.d.	83 ± 32	694 ± 70	9.3 ± 0.9	6.5 ± 1.0	84.2 ± 1.3
	160	30	94.1 ± 3.0	n.d.				(36 ± 5)	(4 ± 2)
	400	45	72.4 ± 9.0	39 ± 2					
	800	60	43.1 ± 8.5	38 ± 2					
L-[^{14}C]Carnitine	L-Carnitine								
50	800	60	35.2 ± 2.2	38 ± 2	77 ± 12	314 ± 58	8.4 ± 0.6	2.3 ± 0.5	89.1 ± 1.0
								(n.d.)	(n.d.)
D-[^{3}H]Carnitine	L-Carnitine								
80	–	15	95.8 ± 0.3	n.d.	61 ± 24	518 ± 192	24.0 ± 10.6	3.0 ± 0.4	73.0 ± 10.9
	160	30	44.0 ± 11.5	0				(0)	(0)
	400	45	11.2 ± 2.8	0					
	800	60	9.2 ± 4.2	0					
50	800	60	16.3 ± 4.3	0	79 ± 5	405 ± 64	14.1 ± 2.0	2.3 ± 0.7	83.9 ± 2.0
								(0)	(0)
Acetyl-L-[^{3}H]carnitine	Acetyl-L-carnitine								
80	–	20	96.5 ± 0.4	n.d.	66 ± 9	349 ± 95	8.4 ± 2.2	3.8 ± 0.2	87.7 ± 2.1
	160	40	45.9 ± 6.4	68 ± 4				(47 ± 10)	(63 ± 1)
	400	60	7.8 ± 0.5	74 ± 3					

[a] Time after addition of labeled substrate.

high-voltage paper electrophoresis. Recovery of isotope from the paper was between 80 and 100% when the samples were allowed to stand overnight in 1 ml of water and 10 ml of aquasol before counting. Compounds in the radioactive zones were identified by cochromatography with radioactive standards. Further confirmation and quantitation of labeled compounds was done using HPLC. Urine samples were injected without prior treatment and eluted as previously described. Electrophoretic results and HPLC results were in very good agreement. At high perfusate concentrations of L-carnitine, about 40% of the label was excreted as acetyl-L-carnitine. Below approx. 80 μM there was not sufficient radioactivity in the urine to identify metabolites.

The effect of unlabeled D-carnitine on the reabsorption of L-[^{14}C]carnitine and the effect of L-carnitine on the reabsorption of D-[^{3}H]carnitine was examined by determining the reabsorption of the labeled compound during the initial 15 min of the experiment and the during three subsequent 15 min periods following administration of increasing amounts of the unlabeled isomer to give perfusate concentrations from 160 to 800 μM (see Table V). Unlabeled D-carnitine had slightly less effect in decreasing the reabsorption of L-[^{14}C]carnitine than did unlabeled L-carnitine (Table V) and unlabeled L-carnitine had a slightly greater effect in decreasing the reabsorption of D-[^{3}H]carnitine, than did unlabeled D-carnitine (Table V). However, these differences were not significant. When L-[^{14}C]carnitine was the labeled substrate and D-carnitine the unlabeled substrate, 38% of the label found in the urine was acetyl-L-carnitine and 62% was L-carnitine. This was similar to the distribution found when L-carnitine was both the labeled and the unlabeled substrate where 35–40% of the label was excreted in the urine as acetyl-L-carnitine and 60–65% as L-carnitine. When D-[^{3}H]carnitine was administered only D-[^{3}H]carnitine was detected in the urine when either L-carnitine or D-carnitine was the unlabeled substrate, showing conclusively that acetylation is specific for the L-isomer.

Acetyl-L-[^{3}H]carnitine was also administered to determine whether it was converted to carnitine before excretion. Additions of unlabeled acetyl-L-carnitine were made as described above, increasing the perfusate concentration to 160 μM at 20 min and to 400 μM at 40 min. Increasing the perfusate concentraton of unlabeled acetyl-L-carnitine resulted in a slight increase in the percentage of labeled acetyl-L-carnitine in the urine from 68 to 74%. More than 30% of the label in the perfusate after 60 min was L-carnitine, indicating that significant conversion to and reabsorption of L-carnitine was occurring.

Discussion

The rate of carnitine transport from the lumen of the perfused intestine was relatively slow compared to the transport of a number of other compounds. When a dose of 167 nmol of nicotinamide was placed in the lumen of the perfused intestine, only 6% of the dose remained unabsorbed after 15 min, while 18% was in the intestinal tissue and 76% in the perfusate. When the same dose of nicotinic acid was administered, 31% remained in the lumen, 17% in the intestinal tissue and 52% in the perfusate after 15 min. [9]. These compounds are thought to be transported by passive diffusion and facilitated diffusion, respectively. When a dose of 2.8 nmol of glucose was administered, only 9% remained in the contents and 6% in the tissue after 15 min. When 1 μmol of isoleucine was placed in the lumen, 26% was found in the contents, 10% in the tissue and 63% in the perfusate after 15 min (unpublished results). The latter two compounds are considered to be transported by an active process. L-Carnitine differed from these compounds primarily in the reduced rate of uptake from the lumen and the relatively large portion of label which was retained by the tissue. The distribution pattern of radioactivity was similar to that observed for trimethylaminobutyrate in the isolated perfused intestine [12].

The fact that D-carnitine was taken up by intestinal tissue about half as rapidly as was L-carnitine and interfered with L-carnitine transport suggested that the transport process is not affected much by the stereochemistry of the β-carbon atom. This view is supported by the effectiveness of trimethylaminobutyrate as an inhibitor of this transport process. It appears that if a transport protein is involved it does not distinguish between H and OH or between the L and D configuration

around the β-carbon atom of the substituted butyrate. On the other hand, the acetylation of the β-hydroxyl group is absolutely stereospecific for the L-isomer, since no acetyl-D-carnitine was detected in the intestinal tissue or in the urine, in contrast to the presence of substantial amounts of acetyl-L-carnitine.

Others have reported that more than one mechanism is involved in the transport of carnitine by the intestine. Studies using rabbit jejunal microvillus membrane vesicles [13] have provided evidence for a sodium-dependent, saturable process and a diffusion process. A significant amount of electrostatic binding was also found. Absorption of L-carnitine by a suspension of mucosal cells from human small intestine [14] resulted in an intracellular-to-extracellular ratio of 2.26 at a medium concentration of 20 μM. A K_m of 974 μM and a V_{max} of 27.4 nmol/ml per min was found, but there was a significant diffusional component at higher concentrations. Everted rings of rat intestine [15] developed an intracellular to extracellular gradient with L-carnitine of 2.25 for jejunal segments and 1.93 for duodenal segments.

Preliminary studies in this laboratory using isolated turkey enterocytes isolated from the proximal jejunum indicated that L-carnitine uptake is temperature-dependent and saturable, reaching equilibrium in 60 min and developing an intracellular to extracellular ratio of greater than 2 after incubation for 20 min in a medium containing 1.4 μM L-[^{14}C]carnitine.

In the present study both L-carnitine and acetyl-L-carnitine were taken up to the greatest extent by the upper jejunal segment. More than 80% of each compound was found in the tissue after 30 min when a dose of 6 nmol L-carnitine or a dose of 2 nmol acetyl-L-carnitine was given. The duodenal segment transported only 48% of a 6 nmol dose of L-carnitine under the same conditions. A significant amount of conversion of L-carnitine to acetyl-L-carnitine occurred at all locations in the small intestine at the lower concentrations. Approx. 50% of the label was acetyl-L-carnitine in the tissue 5 min after administration. Acetylation was probably taking place within the cells, since only traces of acetyl-L-carnitine were detected in the contents. As the concentration of L-carnitine was increased, however, the percent of acetyl-L-carnitine decreased, indicating that the carnitine acetyltransferase was becoming saturated with substrate.

Acetyl-L-carnitine did not undergo significant hydrolysis when exposed to intestinal contents alone; 96% of the label remained in acetyl-L-carnitine after 30 min. Approx. 20% hydrolysis of acetyl-L-carnitine to L-carnitine occurred in the intestinal contents when acetyl-L-carnitine was placed in the jejunal lumen of the live rat. The distribution of label in tissue metabolites was similar to that found in the contents removed from this segment, indicating that most of the acetyl-L-carnitine was probably being transported unchanged.

The threshold of appearance in the urine of the perfused rat kidney with increasing plasma concentrations of D-carnitine occurred at a lower concentration (30 μM) than that for L-carnitine. The threshold of appearance for L-carnitine was about 80 μM, which is slightly higher than the normal plasma L-carnitine concentration of 55 μM in normal adult male rats [16], which show a range of 30 to 80 μM [17]. This threshold value would be appropriate if the kidney were involved in the regulation of plasma carnitine. Frohlich et al. [18] showed that in humans, tubular reabsorption was more than 98% complete for free L-carnitine. The serum concentration of acylcarnitine and the ratio of acylcarnitine to carnitine increased with fasting, while the tubular reabsorption of acylcarnitine decreased, resulting in increased excretion of acylcarnitine.

Transport of L-carnitine by rat renal brush-border membrane vesicles was shown to be an active process, displaying Na^+-gradient stimulation with a K_m of 0.11 mM and a V_{max} of 11.6 pmol/s per mg protein. D-Carnitine, acetyl-DL-carnitine and trimethylbutyrate were inhibitory in this system [19].

A considerable amount (40%) of the labeled carnitine excreted was found to be acetylated by the perfused rat kidney. Huth and Shug [4] showed that kidney cortex slices converted 20% of the L-carnitine taken up to acetyl-L-carnitine in 60 min, with no significant conversion to long-chain acylcarnitine. No metabolic changes of D-carnitine were found.

The threshold of excretion for acetyl-L-carni-

tine, although not determined precisely in this study, occurred at a lower concentration than that for L-carnitine. Acetyl-L-carnitine was the major labeled product found in the urine (70%) when it was the compound added to the perfusate. The remaining 30% of the label in the urine was L-carnitine, indicating that the kidney can remove acetyl groups as well as add them. The presence of L-carnitine in the perfusate after 60 min indicated that some of the L-carnitine formed in the kidney was being reabsorbed. D-Carnitine was excreted and reabsorbed unchanged. Unlabeled D-carnitine increased the excretion of ^{14}C-label from L-carnitine to about the same degree as did unlabeled L-carnitine and gave the same distribution between L-carnitine and acetyl-L-carnitine. The plasma concentration of D-carnitine is therefore of significance when evaluating the use of DL-carnitine. Paulson and Shug [20] showed that tissue depletion of L-carnitine occurred in the rat heart and skeletal muscle when D-carnitine was injected intraperitoneally, which they suggest may result from diffusional exchange. Under normal conditions D-carnitine is excreted preferentially to L-carnitine, but when kidney function is impaired, D-carnitine can build up in the plasma and cause myasthenia-like symptoms [21]. The data reported here show that the D-isomer was absorbed by the intestine almost as efficiently as L-carnitine and gradually released to the circulation over several hours where it would be available for uptake by other tissues. The uptake by muscle was greater for L-carnitine than for D-carnitine and excretion of D-carnitine in the urine was greater than for L-carnitine in the live rat. The results from kidney perfusions also support the latter finding. This suggests that D-carnitine would be preferentially excluded from the muscle and excreted by the kidney under normal conditions. Therefore the use of DL-carnitine would probably not cause significant complications, except under circumstances where kidney function is impaired.

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