Excretion and Metabolism of Propionyl-L-carnitine in the Isolated Perfused Rat Kidney

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ABSTRACT

Propionyl-L-carnitine (PLC) is an ester of L-carnitine (LC) under evaluation for the treatment of cardiovascular disorders. The renal disposition of PLC was studied in the isolated perfused rat kidney with deuterium-labeled derivative (PLC-CD3). Kidneys of male Sprague-Dawley rats were perfused at initial PLC-CD3 concentrations of 10 (n = 4) and 200 μM (n = 5). High-performance liquid chromatography/mass spectrometry was used to quantify PLC-CD3, deuterated L-carnitine (LC-CD3) and acetyl-L-carnitine (ALC-CD3) in perfusate and urine. Perfusion decreased in a monoexponential manner with a half-life of 90 ± 24 min (S.D.) (10 μM) and 94 ± 11 min (200 μM). The renal excretory clearance of PLC-CD3 was significantly lower (P < .05, unpaired t test) at an initial concentration of 10 μM (45 ± 23 μl/min) than at 200 μM (85 ± 28 μl/min), but in both cases it was substantially less than the glomerular filtration rate, which indicates extensive tubular reabsorption. The renal excretory clearance of PLC-CD3 represented less than 6% of the total clearance, which suggests that metabolism is the major renal elimination route for this compound. The appearance in perfusate and urine of LC-CD3 and ALC-CD3 provided additional evidence for a metabolic role of the kidney. The apparent renal excretory clearance values for these metabolites were always significantly higher than the values obtained for the corresponding endogenous compounds, which suggests that LC-CD3 and ALC-CD3, as formed metabolites, underwent passive or carrier-mediated movement directly into urine.

LC is an endogenous compound essential for the transport of long-chain fatty acids across the inner mitochondrial membrane (Bahl and Bressler, 1987; Bremer, 1983). In mammals, the body stores of LC are maintained by dietary intake and endogenous biosynthesis by numerous organs, including the kidney (Bremer, 1983; Rebouche and Engel, 1980; Rebouche and Paulson, 1986). Carnitine supplementation has also been used therapeutically for treating deficiency syndromes, and the compound may also be beneficially used for the treatment of cardiovascular conditions such as ischemic heart disease and angina pectoris (Bahl and Bressler, 1987; Goa and Brogden, 1987). PLC, a short-chain carnitine ester produced by esterification of the hydroxyl group of LC, displays an unique pattern of biochemical activity (Paulson et al., 1986; Sili prandi et al., 1987; 1991) and is currently being evaluated for the treatment of peripheral arterial diseases and other cardiovascular disorders (Bartels et al., 1992; Bre vetti et al., 1992; Greco and Mingrone, 1992).

LC and short-chain carnitine esters are highly polar compounds and at physiological pH possess both cationic and anionic functional groups. As a consequence, this class of compound is believed to move across biological membranes primarily by carrier-mediated transport (Bieber, 1988; Gross and Henderson, 1984; Vary and Neely, 1983). In the kidney, transport systems ensure that the body conserves LC, and at physiological plasma concentrations more than 95% of the filtered load is reabsorbed from the renal tubule (Engel et al., 1981; Harper et al., 1988; Mancinelli et al., 1995; Rebouche et al., 1993). ALC also undergoes extensive tubular reabsorption (Mancinelli et al., 1995), and studies with rat kidney brush-border membrane vesicles (Rebouche and Mack, 1984) and the IPK (Gross and Henderson, 1984; Hokland and Bremer, 1986; Mancinelli et al., 1995) indicate that the tubular reabsorption of LC and ALC exhibit typical characteristics of carrier-mediated processes, including saturation kinetics. Interconversion of LC and ALC in the IPK has also been observed (Hokland and Bremer, 1986; Mancinelli et al., 1995), and LC and ALC as locally formed, or ‘generated’ metabolites were found to undergo direct movement into tubular urine. These findings suggest that bidirectional transport systems are responsible for their renal metabolism.

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ABBREVIATIONS: PLC, propionyl-L-carnitine; LC, L-carnitine; ALC, acetyl-L-carnitine; PLC-CD3, propionyl-L-[N-methyl-D3]carnitine HCl; LC-CD3, acetyl-L-[N-methyl-D3]carnitine HCl; LC-CD3, L-[N-methyl-D3]carnitine; 14C-PLC, propionyl-L-[N-methyl-14C]carnitine HCl; CLT, renal total clearance; CLn, apparent renal (excretory) clearance; CLm, renal metabolic (non-excretory) clearance; fu, fraction unbound in perfusate; IPK, isolated perfused rat kidney; GFR, glomerular filtration rate; %TR, percent tubular reabsorption; HPLC, high performance liquid chromatography; MS, mass spectrometry; ANOVA, analysis of variance.
exist in the kidney and that the site of interconversion is, at least in part, distal to the major sites of tubular reabsorption (Mancinelli et al., 1995).

Currently, very little information exists on the disposition of PLC in the kidney. However, based on previous findings for LC and ALC, the kidney may play an important role in regulating the body stores of endogenous PLC and the pharmacokinetics of exogenous PLC administered for therapeutic aims. Similarly, the kidney may be involved in the metabolism of PLC. In 1986, Hokland and Bremer examined the distribution of LC and several short-chain carnitine esters in kidney extracts, urine and perfusate from IPK experiments in which PLC was added to the perfusion medium as the tritiated compound at a concentration of 30 μM. The findings suggested that PLC was converted to LC and ALC by the kidney. It was claimed that 'extensive' tubular reabsorption of PLC was noted in a single perfusion, but no data were provided.

With the recent use of PLC as a pharmacological agent, it is important that the processes involved in renal handling of this compound be clearly understood. Hence, in the present studies, the IPK was used to investigate the renal excretion and metabolism of PLC and to examine the fate of any renally formed metabolites. Studies were performed with deuterium-labeled PLC with analysis by HPLC/MS, which permitted discrimination between renally formed metabolites of the added substrate and the corresponding endogenous compounds released from the kidney during the perfusion experiments.

### Materials and Methods

**Chemicals.** l-Carnitine HCl, propionyl-l-carnitine HCl, acetyl-l-carnitine HCl and l-(−)-4-trimethylammonium-3-chlorobutyric acid (ST 1085, as internal standard) were obtained from Sigma Tau S.p.A (Pomezia, Rome, Italy). PLC-CD<sub>3</sub>, ALC-CD<sub>3</sub> and LC-CD<sub>3</sub> were synthesized by the Academy of Wissenschaften (Berlin, Germany) and their purity was >98% as judged by HPLC/MS. 14C-Carboxyinulin (3 mCi/g) and 14C-PLC were purchased from New England Nuclear Co. (Boston, MA) and Amersham (Buckinghamshire, UK), respectively. Fraction V bovine serum albumin (Miles Inc., Kaukaeeke, IL) was dissolved in Krebs-Henseleit buffer and purified by dialyzing against three exchanges of protein-free perfusate buffer over 3 days at 4°C. Before each perfusion, the concentration of bovine serum albumin in the perfusion medium was adjusted to 65 g/l. D-Glucose, l-cysteine, glycine, l-glutamic acid and D-mannitol were obtained from Sigma.

**Isolated perfused rat kidney.** Kidneys of male Sprague-Dawley rats (300—400 g; Charles River, Calco, Italy) were perfused as described previously (Mancinelli et al., 1995). Within a thermostatically controlled (37°C) cabinet, a rotary pump (Masterflex model 7518–10, Cole Palmer, Chicago, IL) was used to deliver recirculating perfusate through an 8-μm in-line filter (Millipore, Bedford, MA), a silastic tubing oxygenator, a glass bubble trap and finally a glass cannula inserted into the right renal artery of the excised kidney. Venous outflow was collected directly into the perfusate reservoir and urine was collected via a catheter inserted into the ureter. A constant renal arterial pressure of 110 to 120 mm Hg distal to the tip of the arterial cannula was maintained by adjusting the perfusion flow rate (30—40 ml/min). Urine was collected into preweighed 5-ml polypropylene tubes, and the volume was determined gravimetrically.

The perfusion medium (150 ml) consisted of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing bovine serum albumin (65 g/l), d-glucose (5 mM), l-cysteine (0.5 mM), glycine (2.3 mM) and l-glutamic acid (0.5 mM). Before use, the perfusate was filtered successively through a 1.2- and 45-μm filter (Millipore). Before cannulation of the renal artery, perfusate was recirculated through the perfusion system for at least 30 min to ensure adequate oxygenation and to adjust the pH to 7.4. 14C-Inulin (0.75 μCi) was added to the perfusion medium 15 to 20 min after the kidney was cannulated.

Urine flow rate was taken to be the volume of urine collected divided by the length of the urine collection interval. The functional viability of the kidney was assessed by measurement of the GFR (taken to be renal clearance of 14C-inulin) and the %TR of sodium and glucose, as described previously (Mancinelli et al., 1995).

Experiments were conducted to determine the disposition of PLC at initial (t = 0) nominal perfusate concentrations of 10 and 200 μM. After a 15- to 20-min conditioning period, and immediately after the addition of 14C-inulin, a dose of 1.5 μmol (n = 4) or 30 μmol (n = 5) of PLC-CD<sub>3</sub> was added to the (150 ml) perfusion medium, producing the desired initial concentrations. After a further 10-min equilibration period, urine was collected during six successive 15-min intervals. A sample of perfusate (0.5 ml) was collected from the reservoir 10 min after the addition of substrate and at the midpoint of each urine collection interval.

**Analytical methods.** 14C-Inulin in perfusate and urine was determined radiometrically with a 50-μl aliquot of sample diluted in 4.5 ml of liquid scintillation fluid (Optifluor, Packard, Milan, Italy), and radioactivity was measured by a Packard B1900-TR detector. An external standard method was used for quench correction. Sodium was measured in perfusate and urine by flame photometry (Perkin Elmer Atomic Spectrophotometer 1100B, Monza, Italy), and glucose was determined with use of a commercial kit (Sigma Diagnostic 510).

PLC-CD<sub>3</sub>, ALC-CD<sub>3</sub> and LC-CD<sub>3</sub> concentrations in urine and perfusate were determined by a validated atmospheric pressure ion-spray HPLC/MS/MS technique on a SCIEX API III triquadrupole mass spectrometer (Perkin Elmer, Monza, Italy). A 100-μl aliquot of freshly collected urine or perfusate, to which was added 300 μl of acetonate/methanol (3:1 v/v), was briefly vortex-mixed and centrifuged at 10,000 × g for 6 min. A 380-μl aliquot of the resultant supernatant was transferred to a clean tube and evaporated to dryness at 37°C under a gentle stream of purified air. The residue was dissolved in an aqueous solution (100 μl) of internal standard (275 μM) and sonicated for 20 sec. The sample (50 μl) was then injected onto a μBondapak-NH<sub>2</sub> (300 × 3.9 mm internal diameter) analytical column (Waters, Milford, MA) via an autosampler (model 9095, Varian, Milan, Italy). The mobile phase, consisting of 5 mM ammonium acetate/acetonitrile (30:70 v/v), was delivered at a flow rate of 1.5 ml/min, and the column effluent was interfaced to the ionspray probe by a 100 cm × 0.75 mm internal diameter fused silica tube. Daughter ion spectra of predetermined molecular ions were produced by collision-induced dissociation. Argon was used as the collision gas, with a ionization potential of 40 eV and collision gas cross-section of 250 × 10<sup>2</sup> molecules/cm<sup>2</sup>. Calibration standards of perfusate and urine containing known concentrations of PLC-CD<sub>3</sub> and ALC-CD<sub>3</sub> (0.5—20 μM) and LC-CD<sub>3</sub> (1.25—50 μM) were included in each analytical run, and where necessary unknown samples were diluted appropriately to fall within the calibration range.

The accuracy of quality control samples at low (0.5 μM for PLC-CD<sub>3</sub> and ALC-CD<sub>3</sub>) and high (20 μM for PLC-CD<sub>3</sub> and ALC-CD<sub>3</sub>, and 50 μM for LC-CD<sub>3</sub>) concentrations ranged from 97.1 to 100.2% with a coefficient of variation less than 10%.

**Protein binding of PLC.** The binding of PLC to perfusate protein was determined by ultrafiltration. 14C-PLC (0.02 μCi) and unlabeled PLC were added to blank perfusate to yield final concentrations of 1, 50 and 500 μM. After incubation at 37°C for 30 min, six replicate aliquots (1.2 ml) of each perfusate sample were transferred to Centrifree tubes (MPS-1, Amicon Co., Danvers, MA) and centrifuged at 1600 × g for 15 min at room temperature. Under these
conditions, approximately 300 µl of ultrafiltrate was obtained. Fraction unbound (fu) was taken to be the ratio of the concentration of radioactive \(^{14}\)C-PLC in ultrafiltrate to that in unfiltered perfusion medium.

**Calculations and statistical analysis.** The total clearance of PLC-CD\(_3\) by the perfused kidney (CLR) was estimated as the dose added to the perfusion medium divided by the total area under the perfusate concentration versus time curve from zero to infinite time (AUC\(_{0-\infty}\)), as determined by the trapezoidal method with extrapolation. The half-life (t\(_{1/2}\)) of PLC-CD\(_3\) was estimated from the slope of the terminal phase of the log plasma concentration versus time plot. Apparent renal excretory clearance (CLR) of PLC-CD\(_3\), LC-CD\(_3\), and LC-CD\(_3\) was estimated as the rate of excretion into urine divided by midpoint concentration in perfusate. CLR, the time-averaged renal clearance over the entire perfusion period, was calculated as the mean of individual time estimates. The nonexcretory (metabolic) clearance of PLC-CD\(_3\) by the perfused kidney (CLM) was taken to be the difference between the corresponding CLR and CLRave values. The renal excretory clearance of \(^{14}\)C-inulin was taken to be GFR and the %TR of PLC, sodium and glucose was calculated with equation 1.

\[
\%\text{TR} = 100 \left[ 1 - \frac{\text{CLR}}{\text{fu} \cdot \text{GFR}} \right]
\]

where fu-GFR represents the contribution of glomerular filtration to renal excretion. For sodium and glucose, fu was assigned a value of unity. For PLC, perfusate-binding experiments indicated that the extent of perfusate binding was negligible (see below) and so fu was again assigned a value of unity.

Data are presented as mean ± S.D. ANOVA, followed by a two-tailed Dunnett’s test was used in time-course experiments to compare the corresponding CLR estimates with the initial (0–15 min) value. Student’s t test for unpaired data was used to test for significant differences for pharmacokinetic parameters and urinary recovery between the low- and high-dose experiments. All differences at the .05 level were considered significant.

**Results**

In this study, a stable isotope marker was used to study the renal disposition of PLC. In this way, added substrate and its metabolites could be readily distinguished from the corresponding endogenous compounds released from the kidney during the perfusion experiments. Preliminary experiments, in which perfusate containing PLC-CD\(_3\) was recycled through the IPK system at 37°C for 2 hr, in the absence of a kidney, resulted in no detectable LC-CD\(_3\) and ALC-CD\(_3\). These experiments also confirmed that PLC was not taken up by the components of the perfusion system. The binding of PLC to perfusate protein was found to be negligible; the fu determined by ultrafiltration at 1, 50 and 500 µM was 1.05 ± 0.02, 1.07 ± 0.03 and 1.03 ± 0.03, respectively. Therefore, in the analysis of data on the renal handling of PLC, fu was taken to be unity. The functional parameters for the IPK experiments were similar to those reported previously (Mancinelli et al., 1995) with GFR values of 400 to 800 µl/min.

Semilogarithmic plots of the mean (± S.D.) concentration in perfusates of PLC-CD\(_3\), LC-CD\(_3\) and ALC-CD\(_3\) versus time, at initial nominal concentrations of 10 and 200 µM, are shown in fig. 1. Perfusion PLC-CD\(_3\) concentrations decreased in a monoexponential manner with mean half-lives of 90 ± 24 min and 94 ± 11 min for the 10 µM and 200 µM experiments, respectively (table 1). The gradual decrease in the perfusate concentration of PLC-CD\(_3\) (40–50% during the period of perfusion) coincided with a progressive rise in the perfusate concentration of LC-CD\(_3\) and ALC-CD\(_3\) (fig. 1). For example, in the 200 µM experiments, the perfusate concentration ratio of LC-CD\(_3\) to PLC-CD\(_3\) increased from 0.093 ± 0.001 at 10 min to 0.919 ± 0.172 at 92.5 min. The corresponding change for ALC-CD\(_3\) was from 0.014 ± 0.006 to 0.245 ± 0.047.

Urinary excretion of PLC-CD\(_3\) and both of the deuterated metabolites was observed during the perfusion experiments at both concentrations. Data on the urinary recovery of PLC-CD\(_3\), LC-CD\(_3\) and ALC-CD\(_3\) are summarized in table 2. The total recovery (sum of PLC-CD\(_3\), LC-CD\(_3\) and ALC-CD\(_3\)) represented 3.44% (low dose) and 12.71% (high dose) of the administered dose. The percentage recovery for all three analytes was significantly higher in all cases at an initial concentration of 200 µM.

The total, average renal excretory and metabolic clearance parameters for PLC-CD\(_3\) are summarized in table 1. The CLR values represented only 2.4% (10 µM) and 5.8% (200 µM) of the respective CLR values. Hence, the major clearance route of PLC-CD\(_3\) by the IPK was nonexcretory. The CLRave values for PLC-CD\(_3\) were substantially less than GFR and, at both concentrations, tubular reabsorption was extensive (table 1). Although CLR and CLM were not affected by changes in the initial concentration of PLC-CD\(_3\), a significantly higher CLR and a lower %TR were observed at the higher concentration (table 1).

The individual time-period estimates for the CLR of PLC-CD\(_3\), LC-CD\(_3\) and ALC-CD\(_3\) for the 200 µM experiments are
evidence for direct movement of renally formed LC-CD3 and metabolites appeared in perfusate and urine; and 4) there was ALC-CD3, leading to time- and concentration-dependent changes which can occur in the disposition of metabolites. Each of these pivotal finding are discussed in detail below.

**Tubular reabsorption of PLC.** The tubular reabsorption of PLC-CD3 was greater than 90% when the initial perfusate concentration was 10 μM (table 1). This extensive tubular reabsorption, which led to a low urinary recovery, was similar to that observed previously for LC and ALC in the IPK (Mancinelli et al., 1995). At an initial concentration of 200 μM, the %TR of PLC-CD3 was significantly lower than at the lower concentration (table 1). The magnitude of the change in the overall reabsorption process can be better appreciated by considering that the fraction of filtered PLC-CD3 escaping tubular reabsorption increased from 10% at 10 μM to nearly 20% at 200 μM. Hence, the tubular reabsorption of PLC is concentration dependent, with partial saturation between 10 and 200 μM. Although these concentrations are substantially higher than the base-line plasma concentrations of endoge- nous PLC in humans (0.6 μM, Millington et al., 1989; Minkler and Hoppel, 1993), they are similar to those observed in patients with metabolic disorders (Hoppel, 1991) and in individuals receiving oral or intravenous treatment with PLC (Longo and Pace, unpublished observations). Although we also conducted some experiments in which the initial perfus-

**Discussion.** The results of the present study provide a great deal of insight into the renal handling of PLC and of the time-dependent changes which can occur in the disposition of renally formed metabolites. The most notable findings can be summarized as follows: 1) PLC-CD3 was found to undergo extensive tubular reabsorption that was concentration dependent; 2) the excretory renal clearance of PLC-CD3 was small relative to the magnitude of nonexcretory (or metabolic) clearance; 3) PLC-CD3 was converted to LC-CD3 and ALC-CD3 by the perfused rat kidney and both of these metabolites appeared in perfusate and urine; and 4) there was evidence for direct movement of renally formed LC-CD3 and ALC-CD3, leading to time- and concentration-dependent changes in the apparent renal excretory clearance values for these metabolites. Each of these pivotal finding are discussed in detail below.

**Pharmacokinetic parameters of PLC-CD3 and GFR in IPK experiments in which the initial nominal concentration of PLC-CD3 was 10 and 200 μM (mean ± S.D.)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>10 μM n = 4</th>
<th>200 μM n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (μl/min)</td>
<td>652 ± 158</td>
<td>610 ± 130</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>90 ± 24</td>
<td>94 ± 11</td>
</tr>
<tr>
<td>CLT (μl/min)</td>
<td>1841 ± 318</td>
<td>1476 ± 299</td>
</tr>
<tr>
<td>CLtave (μl/min)</td>
<td>45 ± 23</td>
<td>85 ± 28*</td>
</tr>
<tr>
<td>CLR (μl/min)</td>
<td>1796 ± 304</td>
<td>1382 ± 319</td>
</tr>
<tr>
<td>%TR</td>
<td>92 ± 4</td>
<td>83 ± 7*</td>
</tr>
</tbody>
</table>

Significantly different from the corresponding value at 10 μM, as determined by the Student’s t test for unpaired data. * P < .05; ** P < .01.

**Urinary recovery (mean ± S.D.) of PLC-CD3, LC-CD3, ALC-CD3 and total deuterated analytes in IPK experiments in which the initial nominal concentration of PLC-CD3 was 10 and 200 μM**

<table>
<thead>
<tr>
<th>Compound</th>
<th>10 μM n = 4</th>
<th>200 μM n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLC-CD3</td>
<td>1.02 ± 0.46</td>
<td>3.10 ± 1.37*</td>
</tr>
<tr>
<td>LC-CD3</td>
<td>1.23 ± 0.42</td>
<td>7.65 ± 2.22**</td>
</tr>
<tr>
<td>ALC-CD3</td>
<td>0.53 ± 0.27</td>
<td>1.97 ± 0.77**</td>
</tr>
<tr>
<td>Total</td>
<td>3.44 ± 1.00</td>
<td>12.71 ± 4.25**</td>
</tr>
</tbody>
</table>

Data are expressed as a percentage of the administered dose. Total is the sum of PLC-CD3, LC-CD3, and ALC-CD3. Student’s t test for unpaired data. * P < .05; ** P < .01.

**Recovery of total deuterated analytes in IPK experiments in which the initial nominal concentration of PLC-CD3 was 10 and 200 μM (Mancinelli et al., 1995). At an initial concentration of 200 μM, the %TR of PLC-CD3 was significantly lower than at the lower concentration (table 1). The magnitude of the change in the overall reabsorption process can be better appreciated by considering that the fraction of filtered PLC-CD3 escaping tubular reabsorption increased from 10% at 10 μM to nearly 20% at 200 μM. Hence, the tubular reabsorption of PLC is concentration dependent, with partial saturation between 10 and 200 μM. Although these concentrations are substantially higher than the base-line plasma concentrations of endoge- nous PLC in humans (0.6 μM, Millington et al., 1989; Minkler and Hoppel, 1993), they are similar to those observed in patients with metabolic disorders (Hoppel, 1991) and in individuals receiving oral or intravenous treatment with PLC (Longo and Pace, unpublished observations). Although we also conducted some experiments in which the initial perfus-

**Fig. 2. Mean (± S.D.) apparent renal clearance estimates for PLC-CD3 (a), LC-CD3 (b) and ALC-CD3 (c) during successive urine collection intervals in experiments conducted at an initial nominal perfusate PLC-CD3 concentration of 200 μM (n = 5). Dashed line represents GFR values (μl/min). ANOVA (followed by two-tailed Dunnett’s tests) was used to test for changes related to the value for the initial (0—15 min) urine collection interval: * P < .05; ** P < .01.**
ate concentration of PLC-CD₃ was near the normal endogenous level of plasma PLC, extensive tubular reabsorption (>95%) resulted in urinary levels of PLC-CD₃ which were unquantifiable in many cases.

**Metabolic and excretory clearance of PLC.** During the IPK experiments, a substantial decrease with time in the perfusate concentration of PLC-CD₃ coincided with an increase in perfusate LC-CD₃ and ALC-CD₃ concentrations, as well as urinary excretion of these metabolites. Because no degradation of PLC-CD₃ was observed in the absence of a kidney, the enrichment of perfusate in LC-CD₃ and ALC-CD₃ must have been caused by the metabolic activity of the perfused kidney.

The relative contribution of metabolism and excretion to the renal elimination of PLC-CD₃ was assessed by comparing the respective CLRave and CLM values. Thus, it was found that the CLRave of PLC-CD₃ represented about 2.5 and 6% of CLM at the low and high concentrations, respectively (table 1). Based on urinary excretion and perfusate enrichment data, LC-CD₃ seemed to be the major metabolite. The appearance of ALC-CD₃ demonstrates that the IPK is also capable of converting PLC to ALC, either directly, or via LC. These findings support those of Hokland and Bremer (1986) who found that perfusion with PLC resulted in LC and ALC release into both the perfusate and the urine. A possible explanation for the formation of ALC could be the enzymatic hydrolysis of PLC to LC in the proximal tubular cells, followed by conversion of LC to ALC, as observed previously (Mancinelli et al., 1995).

A comparison of the relative magnitudes of GFR and CLM of PLC-CD₃ provides a unique insight into the mechanisms by which this compound gains access to the kidney cells for subsequent metabolic transformation. In theory, a highly polar compound such as PLC can access the renal tubular cells via two mechanisms: first, by uptake from peritubular capillaries, and second, by reabsorptive uptake from the tubular filtrate. If the renal metabolic clearance of PLC-CD₃ in the IPK had been less than GFR, then no firm conclusions could be drawn. However, at both concentrations the CLM values for PLC-CD₃ were 2- to 3-fold higher than GFR. The logical extension of this finding is that PLC-CD₃ must have been taken up into renal cells directly from the circulation as well as indirectly via reabsorptive uptake from the tubules. In view of the high polarity of PLC, it is likely that both components of renal cellular uptake would involve carrier-mediated transport events. Indeed, in terms of the reabsorptive uptake from the tubule, the concentration dependence of %TR (table 1) provides good evidence for the involvement of membrane transport systems.

**Disposition of the metabolites of PLC-CD₃.** When renal drug metabolism occurs, the traditional approach of estimating urinary clearance for the metabolite (e.g., the ratio of urinary excretion rate to the circulating metabolite concentration) may be misleading, because the observed excretion rate of the metabolite is the sum of the excretion rate of the circulating metabolite and that of the metabolite generated intrarenally and subsequently recovered in urine without entering the circulation. Because of the latter component, the measured renal clearance for the metabolite will often result in an overestimation of its true renal excretory clearance (Mancinelli et al., 1995). In addition, after administration of the parent drug, the renal clearance of a metabolite formed in the kidney and excreted into urine, will generally exceed that observed upon administration of the ‘pre-formed’ metabolite (Bekersky et al., 1984; Smith and Kugler, 1994; Von Lehmann et al., 1973; Wan and Riegelman, 1972a, b). The CLR results for LC-CD₃ and ALC-CD₃ (fig. 2) are in keeping with these concepts. Thus, considering that the concentration of the recirculating LC-CD₃ and ALC-CD₃ never exceeded 60 μM (fig. 1b), our results provide estimates of the ‘renal clearance’ of LC-CD₃ and ALC-CD₃ (fig. 2, b and c) which are 20 to 50 times greater than previously reported values applicable to the two compounds when added to IPKs in their preformed state (Mancinelli et al., 1995).

These findings have implications for the interpretation of pharmacokinetic data obtained after administration of carnitine esters for therapeutic uses. The renal clearance of LC increases substantially after an oral or intravenous dose of ALC (Marzo et al., 1989), and we now have preliminary data that suggest that the same phenomenon occurs in humans given PLC (Longo and Pace, unpublished observations). One interpretation of this finding is that the exogenous esters interfere with the tubular reabsorption of LC. An extension of this hypothesis is that by blocking the reabsorption of endogenous LC, the administration of short-chain carnitine esters may deplete the body stores of LC in a manner similar to that observed in some forms of carnitine-deficiency syndromes associated with impaired tubular reabsorption of LC (Bernardini et al., 1985; Engel et al., 1981; Gahl et al., 1988; Steinmann et al., 1987). However, on the basis of the current and previous findings (Mancinelli et al., 1995), it is likely that the observed increases in the ‘renal clearance’ of LC after dosing with ALC or PLC are largely caused by the conversion of the esters to LC in the kidney and excretion of a fraction of the so-formed LC into urine.

**Tubular leakage of renally formed LC and ALC.** An interesting finding from the present study is the decrease in the CLR of LC-CD₃ and ALC-CD₃ when the initial nominal concentration of PLC-CD₃ was 200 μM (fig. 2, b and c). It should be noted that a similar finding was observed in the 10 μM experiments, but because the perfusate levels of the metabolites were, in some cases, below the limit of quantification, the data set is not complete at this lower concentration. The decreased CLR of the metabolites with time can be explained by a change in time with the relative contributions of ‘generated’ and ‘preformed’ (circulating) metabolites to the renal clearance estimates. During the initial time periods, the concentration of the circulating metabolite in perfusate is low relative to that of the precursor. Hence, during this period, the renal clearance estimate essentially provides information on the leakage of the renally generated metabolite, because most of the metabolite appearing in urine is derived from a renally formed source rather than from the perfusion medium. With time, there is an increase in the concentration in perfusate of the metabolite and, as a consequence, there is an increase in the contribution of the ‘preformed’ species to observed renal clearance estimates. Because the reabsorption of the preformed metabolite is very high, the overall CLR estimate decreases (fig. 2, b and c). It should be noted that the observation for both metabolites could also be supported by a decrease with time in the perfusate concentration of the precursor (PLC-CD₃), and proportionally less generation of intrarenal metabolites which are secreted into urine. In reality, it is likely that both factors (i.e., the increase in the
perfusion concentration of the metabolite and a reduction in that of the precursor) contribute to the unique pattern observed during our experiments. Certainly, however, the observation has a sound pharmacokinetic basis. Again, this finding may have important implications for the interpretation of the pharmacokinetics of short-chain carnitine eaters and their metabolites in humans. Thus, a system could be operating under linear and time-independent conditions with respect to the renal handling of perfused and generated metabolites, but changes with time in the ratio of the precursor to the metabolite in plasma could lead to an apparent ‘time-dependent’ renal clearance.

The direct movement of renally formed LC and ALC from peritubular cells into urine raises several issues. First, there must be a mechanism for the movement of these metabolites into urine. Second, the site of metabolic conversion is likely to be distal to the site(s) of optimal tubular reabsorption, otherwise even secreted metabolite would be largely reabsorbed. It is unlikely that these issues can be resolved in perfused kidney systems. Rather, a combined approach, with use of perfused kidneys, cellular and subcellular systems will need to be used to progress further in understanding the spatial distribution along the renal tubule of the metabolic, secretory and reabsorptive processes for LC and its esters.

In summary, this study shows that in many ways the renal handling of PLC is similar to that observed previously for ALC. Thus, PLC is characterized by extensive and concentration-dependent tubular reabsorption and conversion to LC (and ALC); the metabolites of PLC, like those of LC and ALC, undergo direct movement into pre-urine. The interpretation of renal clearance estimates for the family of carnitines needs to take into account the differential handling of locally formed and circulating metabolites.

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