Inhibition of oxidative metabolism by propionic acid and its reversal by carnitine in isolated rat hepatocytes

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The present study was designed to study the interaction of propionic acid and carnitine on oxidative metabolism by isolated rat hepatocytes. Propionic acid (10 mM) inhibited hepatocyte oxidation of [1-¹⁴C]-pyruvate (10 mM) by 60%. This inhibition was not the result of substrate competition, as butyric acid had minimal effects on pyruvate oxidation. Carnitine had a small inhibitory effect on pyruvate oxidation in the hepatocyte system (210 ± 19 and 184 ± 18 nmol of pyruvate/60 min per mg of protein in the absence and presence of 10 mM-carnitine respectively; means $\pm$ S.E.M., $n = 10$). However, in the presence of propionic acid (10 mM), carnitine (10 mM) increased the rate of pyruvate oxidation by 19%. Under conditions where carnitine partially reversed the inhibitory effect of propionic acid on pyruvate oxidation, formation of propionylcarnitine was documented by using fast-atom-bombardment mass spectroscopy. Propionic acid also inhibited oxidation of [1-¹⁴C]palmitic acid (0.8 mM) by hepatocytes isolated from fed rats. The degree of inhibition caused by propionic acid was decreased in the presence of 10 mM-carnitine (41% inhibition in the absence of carnitine, 22% inhibition in the presence of carnitine). Propionic acid did not inhibit [1-¹⁴C]palmitic acid oxidation by hepatocytes isolated from 48 h-starved rats. These results demonstrate that propionic acid interferes with oxidative metabolism in intact hepatocytes. Carnitine partially reverses the inhibition of pyruvate and palmitic acid oxidation by propionic acid, and this reversal is associated with increased propionylcarnitine formation. The present study provides a metabolic basis for the efficacy of carnitine in patients with abnormal organic acid accumulation, and the observation that such patients appear to have increased carnitine requirements ('carnitine insufficiency').

INTRODUCTION

Disorders of hepatic organic acid metabolism are associated with a number of disease states. In the hereditary organic acidaemias, specific enzyme deficiencies result in an accumulation of organic acids, which is associated with the clinical disease symptomatology. Several reports have demonstrated that patients with hereditary organic acidaemias can develop a secondary carnitine deficiency, and that administration of carnitine to these patients results in marked clinical improvement (Allen *et al.*, 1982; Chalmers *et al.*, 1983, 1984a,b; Roe *et al.*, 1983, 1984a,b; DiDonato *et al.*, 1984; Sugiyama *et al.*, 1984). Evidence suggests that carnitine's efficacy is a result of acylcarnitine formation from the acyl-CoAs that accumulate secondary to the metabolic defect (Secombe *et al.*, 1982; Roe *et al.*, 1983, 1984a,b; DiDonato *et al.*, 1984; Sugiyama *et al.*, 1984; Chalmers *et al.*, 1984a,b; Stumpf *et al.*, 1985). This may remove acyl-CoAs that are metabolic inhibitors, such as propionyl-CoA (Glasgow & Chase, 1976; Gregersen, 1979, 1981; Stumpf *et al.*, 1980), as well as liberating CoA for utilization in other biochemical pathways not affected by the primary defect.

The present study was designed to test directly the hypothesis that carnitine can improve hepatic oxidative metabolism under conditions of organic acid accumulation. Propionic acid has been shown to inhibit oxidative metabolism in several systems (Glasgow & Chase, 1976; Gregersen, 1979, 1981; Stumpf *et al.*, 1980), and was chosen as a model compound for organic acid accumulation. The effect of propionic acid on pyruvate and palmitic acid oxidation by isolated rat hepatocytes

was determined. The results demonstrate that propionic acid can inhibit pyruvate or palmitic acid oxidation and that this inhibition is diminished in the presence of added carnitine.

MATERIALS AND METHODS

Hepatocytes and incubations

Hepatocytes were isolated from male Sprague-Dawley rats by a modification of the collagenase perfusion technique of Berry & Friend (1969) as previously described (Brass *et al.*, 1984). Rats were allowed Wayne rat chow (Agway Co.) and water *ad libitum*. Hepatocyte isolations from fed rats were begun at 07:00 h. Starved animals had food removed from their cages 48 h before hepatocyte isolation. Fed rats weighed 275 ± 6 g (mean $\pm$ S.E.M., $n = 30$) at the time of isolation. Starved rats had initial and final body weights of 260 ± 12 g and 218 ± 13 g respectively (means $\pm$ S.E.M., $n = 5$). Cells isolated from fed rats had 2.67 ± 0.11 mg of protein/ 10^6 cells (means $\pm$ S.E.M., $n = 30$) and their wet weight was 17.2 ± 0.76 mg/ 10^6 cells (mean $\pm$ S.E.M., $n = 24$). Hepatocytes isolated from fasted rats contained 1.21 ± 0.13 mg of protein/ 10^6 cells (mean $\pm$ S.E.M., $n = 5$) and their wet weight was 6.97 ± 0.53 mg/ 10^6 cells (mean $\pm$ S.E.M., $n = 4$). Hepatocyte viability based on Trypan Blue exclusion averaged $93 \pm 1\%$ (mean $\pm$ S.E.M., $n = 35$), and in all cases was above 85%.

For experimental incubations hepatocytes were placed in 25 ml Erlenmeyer flasks with side arms and plastic centre wells. The flasks were sealed and preincubated at

37 °C under O₂/CO₂ (19:1) for 30 min. At the end of the preincubation, substrate was added through the side arm by syringe. The final composition of the incubation was 7.43 ± 0.58 mg of hepatocyte protein/ml (mean ± S.E.M., *n* = 35), 1% defatted bovine serum albumin, 134 mM-NaCl, 4.2 mM-KCl, 0.72 mM-MgSO₄, 0.9 mM-CaCl₂, 2.4 mM-Na₂HPO₄, 4.0 mM-glucose and 8.0 mM-tris(hydroxymethyl)aminoethane, pH 7.4, plus other compounds/substrates as indicated. When included in an experiment, carnitine, propionic acid or butyric acid was added at the start of the preincubation.

Reactions utilizing radiolabelled substrates were terminated by the addition of 30% (v/v) HClO₄ to a final concentration of 3% HClO₄ in the flask. Ethanolamine/methoxyethanol (1:2, v/v; 0.3 ml) was added to the centre well to collect ¹⁴CO₂, and the flasks were shaken an additional 60 min. The efficiency of the ¹⁴CO₂ trap was greater than 95%, as determined by recovery of ¹⁴CO₂ from NaH¹⁴CO₃. The centre well was then counted directly for radioactivity in Scintiverse II (Fisher Scientific). Acid-soluble product formation was determined by quantifying radioactivity in the HClO₄ supernatant from the flask contents. Values for radiolabelled product formation from control incubations terminated at zero time were subtracted from values obtained at later time points to yield net product formation. Zero-time blank values were always less than 10% of experimental values, and typically less than 5%.

Assays

β -Hydroxybutyrate was measured by the method of Olsen (1971). Samples were removed from the incubation and added to an equal volume of 5.1% HClO₄. The HClO₄ supernatant was then used for β -hydroxybutyrate analysis. Protein was quantified by the biuret method (Gornall *et al.*, 1949).

Fast-atom-bombardment mass spectroscopy

Incubations were qualitatively analysed for propionylcarnitine by a modification of the procedure described by Millington *et al.* (1984). A sample was removed from the incubation and added to an equal volume of 6.0% HClO₄. The HClO₄ supernatant was neutralized by adding 75 μ l of 1 M-K₂CO₃ to 350 μ l of the supernatant and leaving on ice for 30 min. Samples were further purified by the method of Hoppel & Genuth (1982) by loading a portion of the neutralized supernatant (375 μ l) on a Dowex 1 $\times$ 8 (Cl⁻ form) minicolumn, and the eluate plus two 500 μ l water washes was collected and pooled. Recovery of propionylcarnitine through this procedure was greater than 85%. Isobutyrylcarnitine (1.5 μ g) was added to the final sample as an external standard.

Samples were then analysed in a VG Analytical 7070E mass spectrophotometer equipped for fast atom bombardment. The atom beam was produced by an Ion Tech atom gun using xenon gas and operated at 7–8 kV. Samples (5 μ l) were loaded on to a stainless-steel fast-atom-bombardment target coated with 0.5 ml of glycerol. Scanning (10 s per decade) was controlled, and the data were acquired, by a VG DS 2000 Series data system. Data-system measurements of peak height were used. The molecular-ion signal was used to identify acylcarnitines as described by Millington *et al.* (1984). No interference was present from the matrix background at *m/z* values of interest. When incubation data were expressed relative to the isobutyrylcarnitine external

standard, the data were also normalized to 20 mg of hepatocyte protein/ml of incubation mixture.

Reagents

All chemicals used were of reagent grade. L-Carnitine, propionylcarnitine and isobutyrylcarnitine were gifts from Sigma-Tau (Rome, Italy). [1-¹⁴C]Palmitic acid and [1-¹⁴C]pyruvic acid were purchased from New England Nuclear Corp. β -Hydroxybutyrate dehydrogenase was from Sigma Chemical Co. Dowex 1 $\times$ 8 (Cl⁻ form) ion-exchange resin was obtained from Bio-Rad.

Statistics

'*n*' refers to observations made on separate hepatocyte preparations. Variables of interest were examined in simultaneous incubations from a single hepatocyte preparation. For example, simultaneous incubations including substrate, substrate plus propionic acid, substrate plus carnitine and substrate plus propionic acid plus carnitine were conducted. This permitted paired analysis of the data, and statistical significance was determined by Student's *t* test, with *P* < 0.05 considered significant. Values are presented as means ± S.E.M.

RESULTS

Hepatocyte pyruvate oxidation was measured as the formation of ¹⁴CO₂ from [1-¹⁴C]pyruvate. Pyruvate oxidation by hepatocytes isolated from fed rats was linear over a 60 min incubation period (Fig. 1). Addition of 10 mM-propionic acid to the incubation significantly decreased the rate of pyruvate oxidation (Fig. 1, Table 1). Lower concentrations of propionic acid (1 mM, 5 mM) also inhibited pyruvate oxidation (Table 1). In contrast, butyric acid (5 mM, 10 mM) caused a smaller inhibition of pyruvate oxidation (Table 1). These observations are consistent with reports by other investigators using isolated liver mitochondria (Gregersen, 1979; Stumpf *et al.*, 1980).

Carnitine (10 mM) had a small inhibitory effect on pyruvate oxidation in the hepatocyte system (210 ± 19 and 184 ± 18 nmol of pyruvate/60 min per mg of protein in the absence and presence of carnitine respectively; means ± S.E.M., *n* = 10; *P* < 0.05) (Table 1). However, in the presence of 5 mM- or 10 mM-propionic acid, addition of carnitine increased the rate of pyruvate oxidation by 19%. This increase in pyruvate oxidation was statistically significant when analysed on a paired basis. Thus carnitine appeared partially to reverse the inhibitory effect of propionic acid.

The interaction of carnitine, propionic acid and pyruvate in the hepatocyte system was further studied by examining propionylcarnitine production. Hepatocyte incubations containing pyruvate, propionic acid and carnitine were analysed for acylcarnitine content by fast-atom-bombardment mass spectroscopy. Incubations containing pyruvate and either propionic acid or carnitine generated very little propionylcarnitine, as evidenced by low *m/z* 218 (propionylcarnitine molecular ion) to 232 (isobutyrylcarnitine standard) intensity ratios, and low *m/z* 218 to 204 (acetylcarnitine molecular ion) intensity ratios (Table 2). In contrast, in incubations containing pyruvate, propionic acid and carnitine (conditions under which carnitine stimulated pyruvate oxidation), increases in propionylcarnitine production were observed (Table 2) by these same criteria. The

Fig. 1. Effect of propionic acid on [1-¹⁴C]pyruvate oxidation

Incubations were conducted as described in the text, with reactions terminated at the times indicated. Values for 10 mM-pyruvate (●) and for 10 mM-pyruvate plus 10 mM-propionic acid (○) are means $\pm$ S.E.M., with *n* shown in parentheses.

increase in propionylcarnitine relative to acetylarnitine (218/204 ratio) under these conditions demonstrates that the increased propionylcarnitine production is not a simple reflection of enhanced non-specific acylcarnitine generation in response to carnitine. The formation of propionylcarnitine in the hepatocyte system occurred in a time-dependent fashion during the preincubation and the incubation with substrate (Fig. 2).

Propionic acid also inhibited [1-¹⁴C]palmitic acid oxidation by hepatocytes isolated from fed rats (Table 3). Production of CO₂ from palmitic acid was not decreased by propionic acid, but generation of acid-soluble products (primarily ketone bodies and citrate; McGarry *et al.*, 1978) and total palmitic acid oxidation were significantly inhibited. This inhibition was not the result of alterations in the specific radioactivity of the label, as direct measurement of β -hydroxybutyrate generation confirmed the inhibition of palmitic acid oxidation by propionic acid (Table 3). As reported by others (McGarry *et al.*, 1975; Christiansen *et al.*, 1976; Christiansen, 1977), carnitine had a mild stimulatory effect on palmitic acid oxidation. However, in the

Table 2. Generation of propionylcarnitine by hepatocyte incubations

Incubations were conducted and fast-atom-bombardment mass spectroscopy was performed as described in the text. All incubations contained 10 mM-pyruvate added at the end of the preincubation. Additional compounds indicated were present at the start of the incubation. Peak height ratios were determined from signal intensities at *m/z* 204 (acetylarnitine molecular ion), 218 (propionylcarnitine molecular ion) and 232 (isobutyrylcarnitine external standard molecular ion). Intensity ratio at 218/232 is normalized to 20 mg of hepatocyte protein/ml of incubation. Data are means $\pm$ S.E.M., determined on samples obtained at the end of 60 min of incubation: **P* < 0.05 compared with pyruvate alone.

Additions	<i>n</i>	<i>m/z</i> ratio	
		218/204	218/232
None	4	0.693 $\pm$ 0.144	0.331 $\pm$ 0.157
Propionic acid (10 mM)	4	0.666 $\pm$ 0.085	0.317 $\pm$ 0.081
Carnitine (10 mM)	3	0.543 $\pm$ 0.070	0.296 $\pm$ 0.035
Propionic acid (10 mM) plus carnitine (10 mM)	4	2.19 $\pm$ 0.25*	0.952 $\pm$ 0.114*

presence of propionic acid, carnitine markedly increased palmitic acid oxidation (Tables 3 and 4). Thus propionic acid was much less effective in inhibiting palmitic acid oxidation in the presence of carnitine. Butyric acid (10 mM) did not inhibit palmitic acid oxidation by hepatocytes isolated from fed rats (results not shown). In contrast with the results in hepatocytes from fed rats, propionic acid did not inhibit palmitic acid oxidation by hepatocytes isolated from starved rats (Table 5).

DISCUSSION

Carnitine has been shown to be efficacious when administered to patients with a variety of disorders of organic acid metabolism (Allen *et al.*, 1982; Seccombe *et al.*, 1982; Chalmers *et al.*, 1983, 1984a,b; Roe *et al.*, 1983, 1984a,b; DiDonato *et al.*, 1984; Sugiyama *et al.*, 1984). The hypothesized mechanism for carnitine's action in these patients involves generation of acylcarnitines, with the resulting removal of acyl-CoAs permitting a

Table 1. Effect of propionic acid and carnitine on pyruvate oxidation by hepatocytes isolated from fed rats

Incubations were carried out as described in the text. Short-chain fatty acid (SCFA; either propionic acid or butyric acid) was added at the concentrations indicated. Pyruvate was always present at 10 mM. When included, carnitine was at a concentration of 10 mM. Values are means $\pm$ S.E.M.: **P* < 0.05 compared with pyruvate alone, †*P* < 0.05 compared with identical incubation without carnitine. All statistics were calculated by paired analysis.

Added SCFA	<i>n</i>	Pyruvate oxidation (nmol/60 min per mg of protein)		
		Pyruvate	Pyruvate + SCFA	Pyruvate + SCFA + carnitine
Propionic acid, 10 mM	7	210 $\pm$ 25	84 $\pm$ 8*	100 $\pm$ 12*†
Propionic acid, 5 mM	12	202 $\pm$ 14	80 $\pm$ 6*	89 $\pm$ 7*†
Propionic acid, 1 mM	9	214 $\pm$ 12	166 $\pm$ 16*	166 $\pm$ 10*
Butyric acid, 10 mM	6	232 $\pm$ 14	166 $\pm$ 19*	—
Butyric acid, 5 mM	6	215 $\pm$ 26	157 $\pm$ 25*	—

Fig. 2. Formation of propionylcarnitine in hepatocyte incubations

Data were obtained as in Table 2; -30 min represents beginning of preincubation, 0 min the time at which pyruvate (10 mM) was added and +60 min the conclusion of the incubations. Data (means $\pm$ S.E.M.; n is shown in parentheses) are m/z 218/232 peak intensity ratios: ●, incubations containing pyruvate (10 mM), propionic acid (10 mM) and carnitine (10 mM); ○, incubations containing pyruvate (10 mM) and propionic acid (10 mM); △, incubations containing pyruvate (10 mM) and carnitine (10 mM).

partial normalization of hepatic function (Seccombe *et al.*, 1982; Roe *et al.*, 1983, 1984a,b; Chalmers *et al.*, 1983, 1984a,b; DiDonato *et al.*, 1984; Sugiyama *et al.*, 1984; Stumpf *et al.*, 1985). The present study utilized the effects of propionic acid on metabolism by rat hepatocytes as a model to demonstrate the potential inhibitory effects of organic acids on diverse pathways of oxidative metabolism and the partial reversal of this inhibition by carnitine. The beneficial effect of carnitine was associated with generation of propionylcarnitine. These results provide support for the concept that carnitine may improve overall hepatic metabolism under conditions of unusual organic acid accumulation, and that this effect is associated with acylcarnitine formation.

Propionic acid accumulates in a number of human disease states, most notably hereditary propionic-acidaemia and methylmalonicaciduria (Chalmers & Lawson, 1982). Previous studies by other investigators have shown that propionic acid inhibits mitochondrial oxidation of pyruvate (Gregersen, 1979; Stumpf *et al.*, 1980). Propionyl-CoA was hypothesized to be responsible for the inhibition, and pyruvate dehydrogenase (Gregersen, 1981) and succinate-CoA ligase (GDP) (Stumpf *et al.*, 1980) were suggested as sites of inhibition. The present study demonstrates that propionic acid inhibition of pyruvate oxidation occurs in intact hepatocytes (Table 1, Fig. 1). The magnitude of the inhibition and the propionic acid concentration-dependence were similar to those seen in the mitochondrial system (Gregersen, 1979). Although caution must be exercised in interpreting $^{14}\text{CO}_2$ formation from $[1-^{14}\text{C}]$ -pyruvate as a measure of pyruvate dehydrogenase activity (Myles *et al.*, 1984), the large degree of inhibition of $[1-^{14}\text{C}]$ pyruvate oxidation caused by propionic acid in the present study supports a site of inhibition at the level of pyruvate dehydrogenase. The small effect of butyric acid on pyruvate oxidation (Table 1) suggests that simple substrate competition is not responsible for the large inhibition seen with propionic acid.

Carnitine caused a small decrease in pyruvate oxidation in the hepatocyte system. The mechanism for this effect is unclear, but may be related to the stimulation of fatty acid oxidation in hepatocytes from fed rats caused by carnitine (Christiansen *et al.*, 1976; Christiansen, 1977). In contrast, carnitine caused a statistically significant increase of 19% in pyruvate oxidation in the presence of propionic acid (Table 1). This stimulation can be explained by removal of inhibitory propionyl-CoA through propionylcarnitine production. Generation of propionylcarnitine has been demonstrated in other systems (Bohmer & Bremer, 1968; Solberg & Bremer, 1970; Valkner & Bieber, 1982), and its formation was confirmed in the present study by fast-atom-bombardment mass spectroscopy (Table 2).

Similar results were obtained when the interaction of propionic acid and carnitine on palmitic acid oxidation was examined in hepatocytes from fed rats. Propionic acid significantly decreased palmitic acid oxidation, but this inhibition was dramatically decreased in the presence of 10 mM-L-carnitine (Tables 3 and 4). Because carnitine

Table 3. Effect of propionic acid and carnitine on palmitic acid oxidation by hepatocytes isolated from fed rats

Incubations were carried out as described in the text. Rates of $[1-^{14}\text{C}]$ palmitic acid conversion into CO_2 and into acid-soluble products and total conversion (CO_2 plus acid-soluble products) are shown in nmol of palmitic acid/40 min per mg of protein. Values for β -hydroxybutyrate are nmol of β -hydroxybutyrate/40 min per mg of protein. Values are means $\pm$ S.E.M.: * $P < 0.05$ compared with identical incubation conditions without propionic acid; † $P < 0.05$ compared with identical incubation conditions without carnitine.

Additions	$[1-^{14}\text{C}]$ Palmitic acid metabolism ($n = 6$)			β -Hydroxybutyrate generated ($n = 7$)
	CO_2	Acid-soluble products	Total	
Palmitic acid (0.8 mM)	2.8 ± 0.4	31.5 ± 3.8	34.3 ± 4.0	65.3 ± 9.2
Palmitic acid (0.8 mM) plus propionic acid (10 mM)	2.6 ± 0.4	$18.5 \pm 3.9^*$	$21.0 \pm 4.2^*$	$32.9 \pm 4.7^*$
Palmitic acid (0.8 mM) plus carnitine (10 mM)	2.8 ± 0.4	$36.0 \pm 4.2^\dagger$	$38.8 \pm 4.4^\dagger$	67.0 ± 9.3
Palmitic acid (0.8 mM) plus propionic acid (10 mM) plus carnitine (10 mM)	$3.5 \pm 0.6^\dagger$	$28.0 \pm 5.2^{*\dagger}$	$31.5 \pm 5.7^{*\dagger}$	$40.7 \pm 7.1^{*\dagger}$

Table 4. Interaction of propionic acid and carnitine in palmitic acid oxidation by hepatocytes isolated from fed rats

Oxidation of [1-¹⁴C]palmitate to CO₂ and acid-soluble products was determined as described in the text. Values are means $\pm$ S.E.M., with $n = 6$ under each set of conditions: * $P < 0.05$ for absent versus present in each set. Baseline oxidation values are given in Table 3.

Inhibition of palmitic acid oxidation by 10 mM-propionic acid (%)	
Carnitine absent	41.4 $\pm$ 5.8
Carnitine (10 mM) present	21.9 $\pm$ 7.3*
Stimulation of palmitic acid oxidation by 10 mM-carnitine (%)	
Propionic acid absent	13.7 $\pm$ 3.1
Propionic acid (10 mM) present	51.4 $\pm$ 10.9*

Table 5. Effect of propionic acid on palmitic acid oxidation by hepatocytes isolated from 48 h-starved rats

Incubations were carried out as described in the text. Rates of [1-¹⁴C]palmitic acid conversion into CO₂ and acid-soluble products and total conversion (CO₂ plus acid-soluble products) are shown in nmol of palmitic acid/40 min per mg of protein. Values are means $\pm$ S.E.M. ($n = 5$).

Additions	[1- ¹⁴ C]Palmitic acid metabolism		
	CO ₂	Acid-soluble products	Total
Palmitic acid (0.8 mM)	2.5 $\pm$ 0.6	27.0 $\pm$ 4.7	29.5 $\pm$ 4.6
Palmitic acid (0.8 mM) plus propionic acid (10 mM)	2.7 $\pm$ 0.4	30.7 $\pm$ 5.7	33.3 $\pm$ 5.5

directly increases fatty acid oxidation (McGarry *et al.*, 1975; Christiansen *et al.*, 1976; Christiansen, 1977), these same data may be viewed from the perspective of enhancement of carnitine's stimulation of palmitic acid oxidation in the presence of propionic acid (Table 4). This point is important to the concept of 'carnitine insufficiency' (Chalmers *et al.*, 1983, 1984a,b), which suggests that increased concentrations of carnitine, beyond those seen in tissues under normal conditions, may be required for optimal metabolic function in the presence of organic acid accumulation.

Propionic acid did not inhibit palmitic acid oxidation by hepatocytes isolated from 48 h-starved animals (Table 5). This may be related to the higher endogenous carnitine concentration in starved liver (McGarry *et al.*, 1975; Brass & Hoppel, 1978). Previous work has shown no effect of propionic acid on fatty acid oxidation by isolated liver mitochondria (Gregersen, 1979; Stumpf *et al.*, 1980), which behave like starved liver with respect to metabolic regulation of fatty acid oxidation (DiMarco & Hoppel, 1975).

In patients with disorders of organic acid metabolism, carnitine requirements may be increased not only from increased urinary excretion of acylcarnitines generated from the organic acids, but also owing to increased cellular requirements for carnitine to normalize metabolic function ('carnitine insufficiency'; Chalmers *et al.*, 1983, 1984a,b). Administration of carnitine to animals (Brass & Hoppel, 1980b) and humans (Secombe *et al.*, 1982; Chalmers *et al.*, 1983, 1984a,b; Roe *et al.*, 1983, 1984a,b; DiDonato *et al.*, 1984; Sugiyama *et al.*, 1984) results in

generation of acylcarnitines consistent with these concepts. Studies *in vitro* have also confirmed that intramitochondrial acyl-CoAs can be converted into acylcarnitines by increased amounts of extramitochondrial carnitine (Solberg & Bremer, 1970; Bremer & Wojtezak, 1972; Lopes-Cordozo *et al.*, 1978; Brass & Hoppel, 1980a). The present study documents the requirement for carnitine availability independent of its key fatty acid-shuttle function in conditions of unusual organic acid accumulation. Further work will be required to extend these observations to the situation *in vivo* and disease models.

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