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Effect of Orally Administered L-Carnitine on Blood Ammonia and L-Carnitine Concentrations in Portacaval-Shunted Rats

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L-Carnitine (16 mmoles per kg, injected intraperitoneally) is reported to protect mice against subsequent injection of ammonium acetate given at the unprotected LD_{100}. The present studies in rats show a variable protective effect of L-carnitine (16 mmoles per kg) administered 1 hr prior to an LD_{100} dose of ammonium acetate. Survival ranged from 100% to 35%. In two experiments, protection was highly significant; in a third experiment, L-carnitine did not protect against death but did significantly prolong time to death. Although the cause of this variability is not known, the data establish the protective effect in rats of L-carnitine given 1 hr before ammonium acetate. D-Carnitine and deoxycarnitine, chemically related analogs unable to substitute for L-carnitine metabolically, are without protective effect. The protective effect of L-carnitine is short-lived and is, for example, completely lost if ammonium acetate is given 24 hr after L-carnitine administration. In contrast, the free carnitine content of brain rises slowly but continuously for at least 24 hr following a single dose of L-carnitine. The observation that protection from ammonia toxicity is not correlated with brain carnitine levels strongly suggests a major peripheral component to the protective effect. Chronically hyperammonemic (portacaval-shunted) rats were found to have significantly depressed total and free carnitine levels in blood compared to normal and sham-operated controls. The hypocarnitinemia, but not the hyperammonemia, was completely reversed in portacaval-shunted rats given drinking water containing 10 m*M* L-carnitine. No abnormal behavioral effects were noted in portacaval-shunted or control rats given L-carnitine for 16 weeks, and carnitine did not affect growth or fluid intake. Since carnitine levels are decreased in the serum of some patients with a variety of hyperammonemic syndromes, the present findings may be of clinical relevance to the long-term treatment of these diseases.

At elevated concentrations, ammonia is toxic to the central nervous system, and hyperammonemia is thought to be a major factor contributing to the encephalopathy associated with several disorders of the liver including cirrhosis and inborn errors of the urea cycle [for reviews, see Refs. (1, 2)]. Treatments designed to reverse the hyperammonemia associated with liver disease include the use of oral antibiotics to control urea-hydrolyzing bacteria in the gut and administration of lactulose to increase nitrogen trapping in the gut (1, 2). Infants with inborn errors of the urea cycle have been successfully treated by administration of benzoate, phenylacetate and arginine, compounds which increase the formation and excretion of nitrogenous products other than urea (3). Early intervention and reduction of the hyperammonemia in such children is associated with a more favorable neurological outcome by the age of 1 year (4). Although successful in these children, administration of benzoate to adults with hyperammonemic syndromes has been attempted only rarely (5). High levels of benzoate interfere with urea production and potentiate ammonia toxicity in mice (6) and cause an increase in plasma and liver ammonia levels in rats (7). Benzoate therapy in hyperammonemic adults may thus involve some risk. An alternative therapy is suggested by the recent reports of Grisolía and colleagues indicating that L-carnitine (16 mmoles per kg intraperitoneally) fully protects mice against subsequently administered ammonium acetate at doses up to the LD_{100} for control mice (8–12). L-Carnitine has also been shown to overcome the potentiation of ammonia toxicity by benzoate (13) and to protect isolated liver mitochondria from functional damage by ammonium acetate (14).

Carnitine levels are affected by both disease and therapeutic interventions. Blood carnitine levels are low in Reye-like syndrome, and in propionic and methylmalonic acidemias [reviewed in Ref. (15)]. Carnitine levels are also reportedly low in the serum of fasting, cachectic patients with cirrhotic liver disease and in several postmortem tissues from liver disease patients [(16); see Ref. (17), however, for a contrasting view]. Although epilepsy is not associated with carnitine deficiency, some epileptic patients given valproate exhibit low plasma carnitine levels. Interestingly, these patients also exhibit high plasma ammonia levels. Ohtani et al. (18) established that the inverse correlation between plasma carnitine and ammonia levels in valproate-treated patients was significant and showed further that oral administration of DL-carnitine reversed both the carnitine deficiency and the hyperammonemia.

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The underlying cause of most disease-related or iatrogenic carnitine deficiency states and the metabolic basis for the association between low carnitine levels and hyperammonemia has not been fully elucidated. Carnitine serves as a buffer for the acetyl-CoA pool and is also a carrier for transport of long-chain fatty acids into the mitochondrial matrix. Carnitine is thus required for mitochondrial β-oxidation of long-chain fatty acids, a process that provides the cell with acetyl-CoA and NADH. Both products are metabolized to produce ATP, a substrate required for ammonia detoxification via either glutamine or urea synthesis. Acetyl-CoA also supports the synthesis of *N*-acetylglutamate, an activator of carbamoyl-phosphate synthetase, the rate-limiting enzyme of urea synthesis. The body carnitine pool is normally derived both from the diet and by endogenous synthesis from ϵ-*N*,*N*,*N*-trimethyllysine, a posttranslationally modified lysine derivative released during protein turnover. Synthetic capacity, limited by trimethyllysine availability (19), is not large relative to the metabolic requirement, and carnitine deficiency has been reported in individuals on strict vegetarian diets and in patients relying on parenteral nutrition (20). In children with organic acidemias, acylcarnitines are excreted in the urine, and endogenous synthesis is inadequate to compensate for the increased carnitine loss [*cf.* Ref. (15)]. Other metabolic stresses such as trauma, diabetes, malnutrition, sepsis and organ failure also increase the requirement for carnitine, although the mechanisms resulting in carnitine loss are generally less clear (20, 21). Studies by Costell et al. (12) suggest that hyperammonemia *per se* may cause carnitine depletion. Thus, mice administered ammonium acetate acutely or made chronically hyperammonemic by administration of urease exhibited decreased carnitine levels in liver and muscle and decreased acetylcarnitine levels in brain; long-chain acylcarnitine levels were also decreased in some tissues.

In addition to the studies reviewed above, several considerations have encouraged the view that carnitine administration may represent a useful therapy in liver disease. In clinical trials, L-carnitine was found to be nontoxic when given chronically to renal dialysis patients or to patients with cardiovascular disease or hyperlipidemia (21). In studies with rats, dietary carnitine supplementation was shown to ameliorate alcohol-induced fat accumulation in liver (22) and to counteract other metabolic effects of prolonged administration and withdrawal of ethanol. In patients with alcoholic liver cirrhosis, L-carnitine administration may thus both reverse some of the secondary chronic effects of alcohol on neuronal membranes and fat deposition in liver and also favorably affect the characteristic hyperammonemia (23). Grisolía and coworkers have, in fact, suggested that carnitine may be of therapeutic value in both hyperammonemic patients with some residual liver function and in children with inborn errors of the urea cycle (11–13). As a prelude to the possible use of L-carnitine in patients with liver disease, we have undertaken a study of long-term oral carnitine administration in the portacaval-shunted (PCS) rat, an established animal model of chronic liver disease (24).

MATERIALS AND METHODS

Reagents. L-Carnitine and D-carnitine hydrochloride were purchased from Chemical Dynamics Corp. (South Plainfield, NJ). Deoxycarnitine hydrochloride [(3-carboxypropyl)trimethyl ammonium chloride] was obtained from Aldrich Chemical Co. (Milwaukee, WI). L-[*N-methyl*-^{3}H]Carnitine hydrochloride (87 Ci per mmole, approximately 99% pure) was obtained from Amersham Corp. (Arlington Heights, IL). [*methoxy*-^{3}H]Methoxyinulin (270 mCi per gm, approximately 92% pure), 4-iodo[*N-methyl*-^{14}C]antipyrine (59.2 mCi per mmole, approximately 99% pure), Protosol and Omnifluor were obtained from New England Nuclear (Boston, MA). The purity of these radiolabeled compounds was determined by fractionating portions of each by ascending paper chromatography [solvent: *tert*-butanol:methylethylketone:formic acid:water (40:30:15:15) (25)], and then submitting sequential 1-cm segments of the resulting chromatograms to liquid scintillation counting in Aquasol-2 cocktail. [^{14}C]Acetyl-CoA was either obtained from New England Nuclear (40 mCi per mmole) or prepared as described (26). Radiochemical purity was $\geq$81% as judged by conversion to [^{14}C]acetyl-L-carnitine in the presence of carnitine acetyltransferase and excess L-carnitine.

Carnitine Uptake Studies. The brain uptake index (BUI; defined as the uptake of a radiolabeled test substance relative to the uptake of a freely diffusible radiolabeled marker) was determined by a modification of the method of Oldendorf (27); iodo[^{14}C]antipyrine was used as the freely diffusible marker (28). Adult male Wistar rats (Hilltop Laboratories, Cincinnati, OH) weighing approximately 250 gm were anesthetized with halothane, and a cannula was inserted into the right common carotid artery. The wound was closed and infiltrated with lidocaine, and the animals were allowed to recover. Three to 5 hr later, the animals were given injections of a bolus (0.2 ml) of heparinized saline containing 100 m*M* potassium phosphate buffer (pH 7.2), 0.1 μCi of L-[^{3}H]carnitine·HCl or [^{3}H]methoxyinulin and 0.025 μCi of 4-iodo[^{14}C]antipyrine. Five seconds later, the animals were decapitated. One-half of the right forebrain was removed and dissolved in 1 ml of Protosol, 10 ml of Omnifluor were added and the ^{14}C and ^{3}H content was determined by liquid scintillation counting.

Hyperammonemic Rats. Chronic hyperammonemia was induced in adult male Wistar rats (about 200 gm) by construction of an end-to-side portacaval anastomosis under halothane anesthesia according to the method of Lee and Fisher (24).

Metabolite Determinations. For measurements of total acid-soluble carnitine in brain, the animals were killed by "freeze-blowing," a procedure wherein the forebrain is extruded through a hollow needle and rapidly ($\approx$1 sec) frozen (29). The brain tissue was deproteinized with 3 *M* perchloric acid, and the homogenate was neutralized as described (29). In other studies, blood (0.5 ml) was collected from the neck following decapitation and added to 1.0 ml of 1.2 *M* perchloric acid; the precipitate was removed by centrifugation at 10,000 $\times$ *g* for 2 min in a Beckman microfuge. To the supernatant (800 μl) were added 50 μl of 1.5 *M* potassium phosphate buffer, pH 7.5, followed by 125 μl of 5 *M* KOH (final pH: about 6.8). The resulting precipitate of $KClO_4$ was removed by centrifugation, and the supernatant was stored at −80°C until analyzed. Ammonia was determined by the fluorometric/enzymatic method of Nazar and Schoolwerth (30) using reaction mixtures containing 2 μ*M* ADP to activate the glutamate dehydrogenase. Free carnitine was determined in neutralized extracts by the enzymatic ^{14}C-acetylation method of Cederblad and Lindstedt (31); the reaction of L-carnitine with [^{14}C]acetyl-CoA was drawn to completion by the inclusion of *N*-ethylmaleimide in

the reaction mixtures as recommended by Parvin and Pande (32). Total acid-soluble carnitine (*i.e.* free carnitine + acid-soluble acylcarnitines) was determined similarly using portions of the same extracts in which the acylcarnitines were first hydrolyzed by treatment with alkali [2 *M* KOH for 2 hr at 37°C (33)]. The concentration of "short-chain acylcarnitines" was estimated as [total acid-soluble carnitine] − [free carnitine]. Long-chain acylcarnitines, precipitated with the protein pellet by acid (34), were determined after resuspension in 2 *M* KOH, followed by incubation at 37°C for 2 hr and reneutralization with 3 *M* HCl (33).

These studies conformed to the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Animal Care Committee of Cornell University Medical College.

Statistics. Data are reported as the mean ± S.E.; except where noted, $n > 5$. Significance was established by the Mann-Whitney U test and by analysis of variance (ANOVA), followed by the Tukey (Honestly Significantly Different) test.

RESULTS

Protection of Rats against Acute Ammonia Intoxication by L-Carnitine Administration. Grisolía et al. reported that mice given 16 mmoles per kg of L-carnitine were fully protected against a lethal dose (12 mmoles per kg) of ammonium acetate injected intraperitoneally 30 min later (11–13). We find that intraperitoneal L-carnitine (16 mmoles per kg) is also protective in rats, but that the effect shows greater variation than reported by Grisolía et al. (Table 1). For rats given L-carnitine 1 hr prior to the administration of an LD_{100} dose of ammonium acetate (12 mmoles per kg), the percentage surviving varied from 35 to 100% (*i.e.* 5/5 in Experiment 1, 6/7 and 5/5 in Experiment 2 and 6/17 in Experiment 3). In groups of rats not given L-carnitine or saline before ammonium acetate, there were no survivors (0/9 in Experiment 2). In groups of rats given saline rather than L-carnitine before ammonium acetate, there were a few survivors (3/19 in Experiment 3). The results of Experiments 1 and 2 are thus in accord with the previous findings of Grisolía and coworkers, whereas the findings in Experiment 3 suggest protection but do not reach statistical significance. The protective effect does not appear to be due entirely to an osmotic effect, as suggested by Kloiber et al. (35), since three of four rats treated with D-carnitine and two of two rats treated with deoxycarnitine died when subsequently challenged with ammonium acetate (Table 1).

At present, we cannot explain the variability between Experiments 1 and 2, which indicate that L-carnitine can protect against death from ammonium acetate, and Experiment 3, which indicates that there is no significant protection. In Experiment 3, it is notable, however, that

TABLE 1. Effect of L-carnitine or L-carnitine analogs on the toxicity of a subsequent LD_{100} dose of ammonium acetate[a]

Compound given first (no. of animals)	Time between injections (hr)	Time until first clonic seizure (min)	Time until first tonic convulsion (min)	Time until death (min)	No. of survivors
Experiment 1					
L-Carnitine (5)	1				5
	24[b]				0
Experiment 2					
NH_4 acetate (9)[c]					0
L-Carnitine (7)	1				6
L-Carnitine (5)	24				0
L-Carnitine (5)	1				5
	24[b]				0
D-Carnitine (4)	1				1
Deoxycarnitine (2)	1				0
Experiment 3					
Saline (5)	0.5	10 ± 1 (5)	11 ± 1 (5)	16 ± 2 (5)	0
L-Carnitine (9)	0.5	17 ± 2† (8)	18 ± 2† (8)	24 ± 2† (8)	1
Saline (4)	1	9 ± 1 (3)	10 ± 1 (3)	13 ± 1 (3)	1
L-Carnitine (17)	1	26 ± 2* (11)	27 ± 2‡ (8)	31 ± 2* (11)	6
Saline (5)	1.5	9 ± 2 (4)	14 ± 6 (4)	18 ± 6 (4)	1
L-Carnitine (6)	1.5	24 ± 4† (5)	27 ± 4 (5)	32 ± 3 (5)	1
Saline (5)	2.5	8 ± 1 (4)	11 ± 1 (4)	14 ± 1 (4)	1
L-Carnitine (6)	2.5	12 ± 3 (6)	14 ± 4 (6)	18 ± 4 (6)	0
Na^+ acetate (3)[d]					3

[a] Rats were administered L-carnitine, L-carnitine analog or saline by intraperitoneal injection at a dose of 16 mmoles per kg. Except as noted below, ammonium acetate was subsequently given by intraperitoneal injection (12 mmoles per kg); this dose of ammonium acetate is the published LD_{100} (46) although, as the last column indicates, a few control animals survived if treated with saline. The time between first injection and the administration of ammonium acetate is shown as "time between injections." Times to onset of clonic seizures, tonic convulsions and death were recorded. Survivors either showed no signs of ammonia toxicity or made a full recovery (all lived until killed). Where indicated, times for carnitine-treated rats were significantly different from times for saline controls, with p values as follows: † $p \leq 0.05$; * $p \leq 0.005$; ‡ $p \leq 0.01$.

[b] Animals given ammonium acetate 1 hr after carnitine and which survived were given a second dose of ammonium acetate at 24 hr. L-Carnitine was not given again.

[c] Animals were given ammonium acetate (12 mmoles per kg) and no other treatment.

[d] Animals were given sodium acetate (12 mmoles per kg) and no other treatment.

when ammonium acetate was given within 2.5 hr of L-carnitine, the time to death was significantly extended (>2-fold) in the animals that were given L-carnitine but ultimately died. In addition, the times to first clonic seizure and first tonic convulsion, signs of central nervous system toxicity, were longer when ammonium acetate was given 0.5, 1.0, or 1.5 hr following L-carnitine than when saline was administered.

In summary, the protective effect of L-carnitine given 1 hr before an LD_{100} dose of ammonium acetate ranges from nearly total to quite small. Moreover, the protective effect appears to be short-lived and in no case extends to 24 hr following L-carnitine administration. There is a suggestion in our data that injection of saline may offer a small protective effect against subsequently administered ammonium acetate (compare Experiments 2 and 3), but osmotic effects do not account for the results with L-carnitine. Thus, L-carnitine cannot be replaced by its analogs, and the onset of central nervous system symptoms is more rapid in rats given saline than in those given L-carnitine.

Uptake of L-Carnitine by Rat Brain. Although L-carnitine is rapidly taken up by brain slices ($K_m \approx 3$ m*M*) (36), its accumulation by the brain from the blood *in vivo* is slow (37 and refs. cited therein). In mice given L-[*methyl*-^{14}C]carnitine, little radioactivity accumulates in brain at 1 hr (37). In the present studies with rats, we find that the BUI for 5.7 n*M* L-[*methyl*-^{3}H]carnitine (measured relative to iodoantipyrine, a freely diffusible marker) is 5.5 ± 0.5% (n = 11). Although small, this value is significantly greater ($p < 0.005$) than the apparent BUI value of methoxyinulin [BUI = 2.9 ± 0.9% (n = 3)]. Methoxyinulin does not cross the blood-brain barrier and is thus a marker for incomplete washout of the radiolabeled bolus from the brain. When the injected bolus of radiolabeled L-carnitine is supplemented with 1 or 10 m*M* unlabeled L-carnitine, the BUI values are 7.8 ± 1.2 and 7.9 ± 1.4%, respectively. These data are in reasonable accord with preliminary studies by Harris and Martin (38; Martin RJ, personal communication) indicating that the uptake of L-[^{14}C]carnitine (relative to [^{3}H]water) averages about 10% in a number of different brain regions (note that water is somewhat less diffusible into brain than is iodoantipyrine). In additional studies, we find that the uptake of 5.7 n*M* L-[^{3}H] carnitine is essentially unchanged (BUI = 5.8 ± 0.8%; n = 9) when 1 m*M* choline is added to the bolus.

In rats (≈250 gm) given a protective dose of L-carnitine (16 mmoles per kg intraperitoneally), the concentration of total L-carnitine in the brain increased slowly, as expected for a compound that penetrates the brain poorly. At 0, 1, 4 and 24 hr following injection, total acid-soluble carnitine in the rat forebrain was 81 ± 11 (n = 3), 168 ± 31 (n = 4; p = 0.05), 217 ± 62 (n = 4; p = 0.025) and 318 ± 74 (n = 4; p = 0.025) nmoles per gm wet weight, respectively (p values indicate the significance of change in comparison to the zero time data).

Blood Carnitine Levels in Control and Carnitine-Treated Portacaval-Shunted Rats. Control, sham-operated and portacaval-shunted rats were given either tap water or 10 m*M* L-carnitine as their only drinking water for 12 to 16 weeks. In each group, water consumption averaged 42 ± 1 ml per day per rat (range: 37 to 50 ml) indicating that animals in the treated groups received about 420 μmoles per day of L-carnitine (7.6 gm of L-carnitine in 16 weeks). Levels of free carnitine, short-chain acylcarnitine and long-chain acylcarnitine were determined in the blood of the various control and treatment groups; the results are reported in Table 2. Statistical analyses of the data (ANOVA) show a clear decrease in the content of both free and total L-carnitine in the plasma of PCS rats vs. both unoperated controls and sham-operated controls over the entire treatment period ($p \leq 0.001$). The Tukey test indicates that, at 8 and 12 weeks postsurgery, the plasma free and total carnitine levels in PCS rats are significantly ($p < 0.05$) decreased compared to unoperated and sham-operated controls (66 to 80% of controls); however, at 16 weeks, the differences are no longer significant. These studies thus establish that the PCS rats are significantly hypocarnitinemic for a period of at least 12 weeks following surgery. The apparent spontaneous correction of hypocarnitinemia by 16 weeks is consistent with the observation that adhesions form in the operative field of PCS rats and the suggestion by Kyu and Cavanagh (39) that these adhesions may allow significant reperfusion of the liver in some PCS rats after 10 to 16 weeks.

Blood levels of carnitine are normalized in portacaval-shunted rats given drinking water containing 10 m*M* L-carnitine. Unoperated control rats given carnitine-containing drinking water show significantly elevated blood carnitine levels in comparison to similar rats given normal drinking water ($p \leq 0.001$ by ANOVA). (A smaller but qualitatively similar increase in sham-operated control rats given carnitine-containing water is not statistically significant at the $p < 0.05$ level.)

Blood Ammonia Levels in Control and Carnitine-Treated Portacaval-Shunted Rats. Plasma ammonia levels were determined in groups of untreated and carnitine-treated PCS and in unoperated and sham-operated control rats at 8, 12 and 16 weeks (Table 3). It is known that the hyperammonemia associated with forming a portacaval shunt rises over the first 3 weeks following surgery and remains constant for 3 to 16 weeks (40). In the present study, the concentration of ammonia was significantly higher in the plasma of untreated PCS rats than in the plasma of untreated normal controls or in the plasma of untreated sham-operated control rats ($p \leq 0.001$ by ANOVA). Despite the normalization of plasma carnitine levels, PCS rats given drinking water containing carnitine had high plasma ammonia levels; these values do not differ significantly from those of PCS rats given ordinary drinking water ($p > 0.05$ by ANOVA). In three of the six sham-operated groups, plasma ammonia levels are appreciably lower than the average plasma ammonia levels in the unoperated controls. The reason for this finding is not clear at present.

Weight Gain in Control and Carnitine-Treated Portacaval-Shunted Rats. The presence of carnitine in the drinking water did not affect weight gain in unoperated or sham-operated control rats or in portacaval-shunted rats ($p > 0.05$); surgery delayed growth mod-

TABLE 2. Concentrations (μM) of free L-carnitine and short-chain and long-chain acylcarnitine esters in the blood plasma of normal, portacaval-shunted and sham-operated male Wistar rats provided with either water or 10 mM L-carnitine for 8, 12 or 16 weeks

Surgery	Oral L-carnitine	8 wk				12 wk				16 wk			
		FC	SCC	LCC	TotC	FC	SCC	LCC	TotC	FC	SCC	LCC	TotC
None	No	62 ± 3	25 ± 5	2 ± 0.5	89 ± 4	59 ± 4	24 ± 7	3 ± 0.7	92 ± 6	58 ± 3	26 ± 7	1	85 ± 6
		(70)	(28)	(2)		(69)	(28)	(3)		(68)	(31)	(1)	
	Yes	82 ± 5	36 ± 7	3 ± 1.6	121 ± 5	81 ± 5	30 ± 11	4 ± 0.3	114 ± 10	78 ± 3	29 ± 8	4 ± 1	112 ± 7
		(68)	(30)	(2)		(70)	(26)	(4)		(70)	(26)	(4)	
PCS	No	41 ± 1	26 ± 2	3 ± 0.4	70 ± 3	45 ± 3	25 ± 4	4 ± 0.6	74 ± 3	51 ± 4	27 ± 6	4 ± 0.9	81 ± 4
		(59)	(37)	(4)		(61)	(34)	(5)		(62)	(33)	(5)	
	Yes	57 ± 5	23 ± 13	5 ± 0.9	85 ± 12	54 ± 4	38 ± 9	5 ± 0.3	97 ± 8	70 ± 2	26 ± 4	4 ± 0.5	99 ± 4
		(67)	(27)	(6)		(56)	(39)	(5)		(70)	(26)	(4)	
Sham-op	No	60 ± 4	29 ± 6	3 ± 0.8	92 ± 4	66 ± 4	26 ± 5	3 ± 0.6	95 ± 3	63 ± 3	26 ± 5	2 ± 0.2	90 ± 4
		(65)	(32)	(3)		(69)	(27)	(3)		(69)	(29)	(2)	
	Yes	77 ± 3	35 ± 6	4 ± 0.9	116 ± 5	88 ± 6	35 ± 8	4 ± 0.5	126 ± 6	81 ± 4	29 ± 6	3 ± 0.2	113 ± 4
		(66)	(30)	(3)		(69)	(28)	(3)		(72)	(26)	(3)	

Percentages of total carnitine (TotC) represented by each fraction appear in parentheses below each respective fraction. Data are drawn from groups of five or six animals; all data are expressed as mean ± S.E. FC = free L-carnitine; SCC and LCC = short- and long-chain acylcarnitine esters; PCS = portacaval shunted; Sham-op = sham operated.

TABLE 3. Concentrations (μM) of total ammonia in the blood plasma of normal, portacaval-shunted and sham-operated male Wistar rats provided with either water or 10 mM L-carnitine for 8, 12 or 16 weeks

Surgery	Oral L-carnitine	8 wk	12 wk	16 wk
None	No	71 ± 24	94 ± 11	76 ± 9
	Yes	87 ± 22	84 ± 10	86 ± 2
PCS	No	147 ± 9	93 ± 8	120 ± 13
	Yes	133 ± 10	100 ± 9	126 ± 12
Sham-op	No	95 ± 21	33 ± 9	52 ± 5
	Yes	32 ± 2	22 ± 10	62 ± 6

Data are drawn from groups of five or six animals; all data are expressed as mean ± S.E. PCS = portacaval shunted; Sham-op = sham operated.

erately ($p < 0.05$). Thus, whereas unoperated control rats gained 50 ± 6 gm in the first week, sham-operated and portacaval-shunted rats gained 16 ± 3 and 1 ± 2 gm, respectively (the initial weight in all groups was 290 ± 14 gm; n = 105). By 4 weeks, rats in each group were gaining weight at about the same rate (30 ± 4 gm per week), and growth rates remained similar thereafter.

DISCUSSION

Grisolía et al. reported that mice given L-carnitine (16 mmoles per kg intraperitoneally) 30 min prior to an LD_{100} dose of ammonium acetate uniformly survived (8, 9). More recently, Deshmukh and Rusk (41) reported that L-carnitine is without protective effect in ammonia-intoxicated mice and ferrets (41). The present studies partially elucidate these contradictory findings. Our initial studies (Table 1, Experiments 1 and 2) showed that L-carnitine afforded nearly complete protection against an otherwise lethal dose of ammonium acetate. In a third study, carried out later, the number of survivors among carnitine-treated rats dropped to 35% even with an optimal delay of 1 hr between carnitine and ammonia administration (Table 1, Experiment 3). Even in this study, however, carnitine administration significantly prolonged survival and delayed convulsions in those animals that ultimately died. Our results clearly indicate that L-carnitine has a protective effect against subsequently administered ammonium acetate, but they also indicate that the effect is short-lived and not always easily demonstrated. Although the basis of the variability in our results [or those in the literature (8, 9, 41)] is not yet apparent, it is possible that subtle differences in genetic or nutritional factors can sufficiently affect the sensitivity of animals to ammonium toxicity or carnitine protection.

In other studies (Griffith OW, unpublished data), we find a variable but clearly protective effect of L-carnitine (16 mmoles per kg) in Swiss-Webster mice. However, in contrast to reports by Grisolía et al., we find that lower doses of L-carnitine are much less effective in both rats and mice. We also find that protection is lost as the dose of ammonium acetate is increased above the "minimal LD_{100}," a possible difficulty in the studies of Deshmukh and Rusk (41).

D-Carnitine and deoxycarnitine, metabolically inert compounds that are close structural and charge-type analogs of L-carnitine, do not appreciably protect against ammonia toxicity (Table 1). These results support the view that the protective effect of L-carnitine is mainly attributable to a metabolic effect and that protection via an osmotic or other physicochemical effect is less important. Our results thus contrast with those of Kloiber et al. (35), who concluded that the protective of L-carnitine is largely due to the "osmoprotectant" effect of quarternary amines. Those investigators confirmed the observation of Grisolía et al. regarding the protective effect of L-carnitine against ammonia toxicity in mice but found that choline, betaine and trimethylamine *N*-oxide also protected, albeit to a lesser degree.

In rats, the protective effect of L-carnitine with respect to acute and severe ammonia intoxication is short-lived; protection is maximal 1 hr after L-carnitine administration, is small and statistically insignificant by 2.5 hr and is undetectable at 24 hr (Table 1). Since the main symptoms of ammonia intoxication (including lethargy, seizures and coma) are clearly neurological in origin, it was

of interest also to determine the kinetics and extent of brain carnitine uptake following a single intraperitoneal injection of L-carnitine. The present findings indicate that L-carnitine crosses the blood-brain barrier poorly (BUI = 5.5%) but is nonetheless accumulated in moderately large amounts over several hours (*e.g.* a 3- to 4-fold increase in total carnitine over 24 hr). Importantly, the increase in brain total carnitine levels does not correlate temporally with the protective effect of L-carnitine against acute ammonia intoxication. Although increases in brain L-carnitine may contribute to its protective effect, the present studies suggest that protection from acute ammonium acetate toxicity depends mainly on peripheral rather than central L-carnitine-dependent mechanisms.

Cornford et al. (42) showed that DL-carnitine diminished the BUI of choline. The present studies show that choline does not affect the BUI of L-carnitine. These results suggest either that the D-isomer of carnitine is transported on the choline transporter or that our techniques are not sensitive enough to detect a partial inhibition of L-carnitine uptake. The finding that the BUI of L-[^{14}C]carnitine is not significantly diminished by the addition of up to 10 m*M* unlabeled L-carnitine indicates that, if L-carnitine transport is carrier mediated, the binding affinity is low. Failure of unlabeled L-carnitine to decrease the BUI of L-[^{14}C]carnitine suggests, in fact, that L-carnitine may enter the brain mainly by diffusion.

Most studies of the effect of L-carnitine on ammonia toxicity have focused on acute and severe ammonia intoxications (*i.e.* injection of ammonium salts). Grisolía and coworkers have, however, carried out studies of several hours duration in which mice administered L-carnitine were shown not to develop signs of ammonia intoxication when subsequently given intravenous urease despite the fact that blood ammonia reached levels usually associated with high morbidity (8). Our study examined the effects of long-term (16 weeks) L-carnitine administration on chronic, moderately hyperammonemic portacaval-shunted rats. These rats, which provide a useful model for chronic liver disease, were found to develop both hyperammonemia and hypocarnitinemia within 8 weeks of surgery. Oral administration of L-carnitine (about 0.42 mmole per day) to these rats completely reversed the hypocarnitinemia but had no effect on the hyperammonemia (Tables 2 and 3). This finding contrasts with the dramatic protection observed by Grisolía et al. with urease-treated mice, a shorter-term model of chronic ammonia intoxication. The results with PCS rats are, however, consistent with our acute ammonia intoxication studies which also showed that protection of L-carnitine against ammonia intoxication is short-lived and apparently dependent on large acute doses of L-carnitine.

As noted in the introduction, most (but not all) studies of patients with hyperammonemic syndromes (*e.g.* cirrhosis or urea cycle defects) or with hyperammonemia secondary to organic acidemia show blood concentrations of total carnitine (and especially free carnitine) to be low (15, 43, 44). A similar association between hypocarnitinemia and hyperammonemia was observed with PCS rats (Tables 2 and 3). In a more limited study, levels of plasma carnitine have ranged widely from low to above normal in a small population of patients with liver disease; the heterogeneity may reflect nutritional status or integrity of muscle mass (Hearn TJ, et al., unpublished data). Although the metabolic basis of the association between hyperammonemia and hypocarnitinemia is poorly defined, the present studies encourage the view that cirrhotic patients should be examined for carnitine status. Although L-carnitine administration did not improve the hyperammonemia of PCS rats, it did reverse the hypocarnitinemia. Carnitine deficiency may adversely affect the acyl-CoA/CoASH balance of tissues and thereby indirectly interfere with many metabolic processes including the citric acid cycle, gluconeogenesis, the urea cycle, fatty acid oxidation and the malate-aspartate shuttle (8, 45). Administration of L-carnitine may thus improve the metabolic status of hypocarnitinemic, cirrhotic patients for reasons not associated directly with their characteristic hyperammonemia.

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REFERENCES

1. Duffy TE, Plum F. Hepatic encephalopathy. In: Arias I, Popper H, Schachter D, et al., eds. The liver: biology and pathobiology. New York: Raven Press, 1982: 693–715.
2. Cooper AJL, Plum F. Biochemistry and physiology of brain ammonia. Physiol Rev 1987; 67:440–519.
3. Brusilow SW, Danney M, Waber LJ, et al. Treatment of episodic hyperammonemia in children with inborn errors of the urea synthesis. N Engl J Med 1984; 310:1630–1634.
4. Msall M, Batshaw ML, Suss R, et al. Neurologic outcome in children with inborn errors of urea synthesis. N Engl J Med 1984; 310:1500–1505.
5. Watson AJ, Karp JE, Walker WG, et al. Transient idiopathic hyperammonaemia in adults. Lancet 1985; 2:1271–1274.
6. O'Connor J-E, Ribelles M, Grisolía S. Potentiation of hyperammonemia by sodium benzoate in animals. A note of caution. Eur J Pediatr 1982; 138:186–187.
7. Palekar AG, Canas JA, Kalbag SS, et al. Failure of sodium benzoate to alleviate plasma and liver ammonia in rats. Biochem Med Metab Biol 1988; 40:133–145.
8. O'Connor J-E, Costell M, Grisolía S. Protective effect of L-carnitine in hyperammonemia. FEBS Lett 1984; 166:331–334.
9. O'Connor J-E, Costell M, Grisolía S. Prevention of ammonia toxicity by L-carnitine. Metabolic changes in brain. Neurochem Res 1984; 9:563–570.
10. Costell M, O'Connor J-E, Miguez M-P, et al. Effects of L-carnitine on urea synthesis following acute ammonia intoxication in mice. Biochem Biophys Res Commun 1984; 120:726–733.
11. O'Connor J-E, Costell M, Miguez M-P, et al. Influence of the route of administration on the protective effect of L-carnitine on acute hyperammonemia. Biochem Pharmacol 1986; 35:3173–3176.
12. Costell M, Miguez M-P, O'Connor J-E, et al. Effect of hyperammonemia on the levels of carnitine in mice. Neurology 1987; 37:804–808.
13. O'Connor J-E, Costell M, Grisolía S. The potentiation of ammonia toxicity by sodium benzoate is prevented by L-carnitine. Biochem Biophys Res Commun 1987; 145:817–824.
14. Bobyleva-Guarriero V, DiLisa F, Iannone A, et al. Ameliorating effect of carnitine on liver mitochondria functions in ammonium intoxicated rats. IRCS Med Sci 1985; 13:399–400.
15. Stumpf DA, Parker WD, Angelini C. Carnitine deficiency, organic acidemias, and Reye's syndrome. Neurology 1985; 35:1041–1045.
16. Rudman D, Sewell CW, Ansley JD. Deficiency of carnitine in cachectic cirrhotic patients. J Clin Invest 1977; 60:716–723.

17. Fuller RK, Hoppel CL. Elevated plasma carnitine in hepatic cirrhosis. Hepatology 1983; 3:554–558.
18. Ohtani Y, Endo F, Matsuda I. Carnitine deficiency and hyperammonemia associated with valproic acid therapy. J Pediatr 1982; 101:782–785.
19. Hoppel CL, Davis AT. Inter-tissue relationships in the synthesis and distribution of carnitine. Biochem Soc Trans 1986; 14:673–674.
20. Borum PR, Bennett SG. Carnitine as an essential nutrient. J Am Coll Nutr 1986; 5:177–182.
21. Kendler BS. Carnitine: an overview of its role in preventive medicine. Prev Med 1986; 15:373–390.
22. Sachan DS, Rhew TH, Ruark RA. Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. Am J Clin Nutr 1984; 39:738–744.
23. Corbett R, Leonard BE. Effects of carnitine on changes caused by chronic administration of alcohol. Neuropharmacology 1984; 23:269–271.
24. Lee SH, Fisher B. Portacaval shunt in the rat. Surgery 1961; 50:668–672.
25. Fink K, Cline RE, Fink RM. Paper chromatography of several classes of compounds: correlated R_f values in a variety of solvent systems. Anal Chem 1963; 35:389–398.
26. Griffith OW. Chemical mechanism of carnitine acetyltransferase. New York: Rockefeller University, 1975. Thesis.
27. Oldendorf WH. Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol 1971; 221:1629–1639.
28. Van Uitert RL, Sage JI, Levy DE, et al. Comparison of radiolabeled butanol and iodoantipyrine as cerebral blood flow markers. Brain Res 1981; 222:365–372.
29. Cooper AJL, McDonald JM, Gelbard AS, et al. The metabolic fate of ^{13}N-labeled ammonia in rat brain. J Biol Chem 1979; 254:4982–4992.
30. Nazar BL, Schoolwerth AC. An improved microfluorometric enzymatic assay for the determination of ammonia. Anal Biochem 1979; 95:507–511.
31. Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. Clin Chim Acta 1972; 37:235–243.
32. Parvin R, Pande SV. Microdetermination of (−)carnitine and carnitine acetyltransferase activity. Anal Biochem 1977: 79:190–201.
33. Rossle C, Kohse KP, Franz H-E, et al. An improved method for the determination of free and esterified carnitine. Clin Chim Acta 1985; 149:263–268.
34. Fishlock RC, Bieber LL, Snoswell AM. Sources of error in determinations of carnitine and acylcarnitine in plasma. Clin Chem 1984; 30:316–318.
35. Kloiber O, Banjac B, Drewes LR. Protection against acute hyperammonemia: the role of quaternary amines. Toxicology 1988; 49:83–90.
36. Huth PJ, Schmidt MJ, Hall PV, et al. The uptake of carnitine by slices of rat cerebral cortex. J Neurochem 1981; 36:715–723.
37. Brooks DE, McIntosh JEA. Turnover of carnitine by rat tissues. Biochem J 1975; 148:439–445.
38. Harris RBS, Martin RJ. Brain uptake index of L-carnitine and palmitic acid in brain sites associated with feeding in lean and obese Zucker rats. Soc Neurosci Abstracts 1986; 16:1452.
39. Kyu MH, Cavanagh JB. Some effects of porto-caval anastomosis in the male rat. Br J Exp Pathol 1970; 51:217–227.
40. Hindfelt B, Plum F, Duffy TE. Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. J Clin Invest 1977; 59:386–396.
41. Deshmukh DR, Rusk CD. Failure of L-carnitine to protect mice against ammonia toxicity. Biochem Med Metab Biol 1988; 39:126–130.
42. Cornford EM, Braun LD, Oldendorf WH. Carrier mediated blood-brain barrier transport of choline and certain choline analogs. J Neurochem 1978; 30:299–308.
43. Chapoy PR, Angelini C, Brown WJ, et al. Systemic carnitine deficiency—a treatable inherited lipid-storage disease presenting as Reye's syndrome. N Engl J Med 1980; 303:1389–1394.
44. Matsuda I, Ohtani Y, Ohyanagi K, et al. Hyperammonemia related to carnitine metabolism with particular emphasis on ornithine transcarbamylase deficiency. Enzyme 1987; 38:251–255.
45. Lai JCK, Rimpel-Lamhaouer K, Cooper AJL. Differential inhibition of mitochondrial dehydrogenases by fatty acyl CoAs. Proc NY Acad Sci 1989 (in press).
46. National Research Council, Subcommittee on Ammonia (Kamin H, Chair). Ammonia. Baltimore: University Park Press, 1979: 216.