Carnitine and the Organic Acidurias

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The earliest observation of a deficiency of carnitine associated with an inherited metabolic disorder was with 5,10-methylene tetrahydrofolate reductase deficiency (1). A survey of carnitine status in patients with organic acidurias revealed an apparent secondary deficiency in all except maple syrup urine disease (2). There is considerable diagnostic value in the identification of acylcarnitine profiles for the recognition of organic acidurias. Furthermore, some of these disorders are effectively treated by supplemental carnitine therapy. The diagnostic and therapeutic applications of L-carnitine will be reviewed.

CARNITINE AS A DIAGNOSTIC TOOL

The development of fast atom bombardment–mass spectrometry (FAB-MS) facilitated the detection and analysis of specific acylcarnitines in urine (3). In particular, it enabled the recognition of new acylcarnitines, whose structures were subsequently investigated by auxiliary techniques (4). It was discovered that patients with inherited disorders of branched-chain amino acid and fatty acid catabolism excrete disease-specific acylcarnitines that reflect abnormal acylcoenzyme A (CoA) species accumulating at or near the site of the metabolic block (5). In situations where one would not expect unusual acyl-CoA thioester accumulation, as in maple syrup urine disease (MSUD) or phenylketonuria (PKU), for example, no unusual acylcarnitines are detected. In addition, the acylcarnitines were much more easily detected after loading patients with L-carnitine, which enhances the excretion of the abnormal, diagnostic species in patients with metabolic disorders.

In some cases, a single major, diagnostic acylcarnitine is predominant, facilitating the diagnosis, as exemplified by isovalerylcarcinine in isovaleric acidemia (6) and propionylcarcinine in propionic acidemia (7). In fat catabolism disorders, including medium chain acyl-CoA dehydrogenase deficiency and multiple acyl-CoA dehydrogenase deficiency (MADD), a specific pattern of acylcarnitines is excreted and the diagnosis cannot be reliably made on the basis of detection of any single species.
These acylcarnitine patterns are of considerable diagnostic utility because unlike branched-chain amino acid disorders, where the defects are expressed by a persistent and grossly abnormal organic aciduria, the levels of diagnostic organic acids vary considerably with clinical state (8). In our experience, the differential diagnosis of fatty acid catabolism disorders is greatly improved by including both GC-MS analysis of organic acids and FAB-MS analysis of acylcarnitines.

In an older child, or an infant not breast-fed, a significant carnitine deficiency can develop in MCAD deficiency, making the FAB-MS profile unclear or uninterpretable. The safe and simple expedient of collecting urine after an oral carnitine load of 100 mg/kg enhances the diagnostic acylcarnitine profile and removes ambiguity (9). This is illustrated in Fig. 1, which compares the FAB-MS results obtained on an affected presymptomatic boy aged 3½ years, whose first clinical symptoms did not occur until 7 years of age. The profile in Fig. 1A is uninterpretable, owing to chemical interference and low acylcarnitine concentration, but after the load (Fig. 1B) the characteristic pattern of MCAD deficiency (9) is clearly observed.

When a child with MCAD deficiency was given a bolus of [methyl-2H3]-carnitine, a stable-isotope-labeled form of carnitine, each of the diagnostic signals was accompanied by a "satellite" with a mass increment of +3 (Fig. 1C). Analogous results were obtained in a patient with propionic acidemia (11). This result is very significant for several reasons. It shows that exogenous carnitine equilibrates rapidly with the endogenous acyl-CoA pool and the percentage incorporation of labeled carnitine in each acylcarnitine is similar (Fig. 1C), indicating the general specificity of the carnitine acyltransferases to form short and medium chain length acylcarnitines in vivo. It also demonstrates a simple, definitive method of confirming the identities of acylcarnitine signals observed in the FAB-MS spectrum. This would be especially important in the identification of hitherto unknown acylcarnitines. The labeling experiments also confirm the existence of a general pathway for detoxification in several of the organic acidurias. Carnitine produces rapid transport of potentially toxic acyl groups out of the cell as acylcarnitines, which are then excreted as nontoxic metabolites. This is the biochemical rationale for l-carnitine therapy in these diseases.

Standard FAB-MS is limited to detection of acylcarnitines in urine when their individual concentrations exceed about 50 nmol/ml (4). The detection limit is a function of the chemical noise due in the biological matrix, which is variable. The absolute signal strength of acylcarnitines is also affected by the relative concentration of other surface-active components, including alkali metal cations. The successful detection of acylcarnitines at low concentration in urine, liver tissue (12), and blood plasma by FAB-MS has been possible in some cases, but only after extensive sample purification. Consequently, a more selective routine method was developed recently in our laboratory using a triple quadrupole—an example of a tandem mass spectrometer that incorporates two stages of mass analysis (MS/MS) in a single instrument. As with other combined techniques, such as GC/MS and LC/MS, MS/MS offers the potential for improving specificity and enabling the direct analysis of mixtures (13). This technique is particularly well suited to FAB-MS, since the appli-
FIG. 1. MCAD deficiency FAB-MS profiles (A) untreated patient (B) after carnitine load (C) after [methyl $^2$H$_3$]carnitine load showing incorporation of the label (*) into the acylcarnitines. From Roe CR, et al. (10).
cability of on-line chromatographic separation is very limited at present. The added specificity achieved by FAB-MS/MS has facilitated its application to the quantitative analysis of individual acylcarnitines in urine, plasma, and tissue using isotope dilution assay (14).

The FAB mass spectra of acylcarnitine methyl esters and the daughter ion spectra of the M⁺ ions generated by tandem MS show a prominent common fragment at m/z 99 (5,14). This ion is derived from the loss of both the acyl moiety, as the corresponding acid, and the quaternary ammonium function as trimethylamine. The structure of this highly characteristic fragment can be formally represented as \(^{1}\text{CH}_{2}-\text{CH}==\text{CH}-\text{CO}_{2}\text{CH}_{3}\) and represents the "backbone" of the acylcarnitine molecule. Isotopically labeled forms, having \(^{2}\text{H}\) or \(^{13}\text{C}\) in either the acyl or trimethylamino group, also exhibit the m/z 99 fragment. Because the precursors of m/z 99 are predominantly acylcarnitine molecular cations, the new scan function, when applied to a biological sample, generates a metabolic "profile" of acylcarnitines in the sample (14,15).

The most immediately obvious difference between acylcarnitine profiles obtained by FAB-MS/MS and those performed by the standard FAB-MS (5) procedure is a large reduction in the chemical "noise." This results from the increased selectivity (specificity) of the analytical procedure, which has improved the detection limit for individual acylcarnitine methyl esters in urine from 50 nmol/ml to better than 1 nmol/ml (14). It is possible to observe diagnostic acylcarnitine signals in urine samples even from neonatal MCAD-deficient patients (with carnitine deficiency) that were completely masked by chemical interference using the older method. When the availability of free carnitine is compromised, however, low levels of unusual acylcarnitine species, including dicarboxylic acylcarnitines, have been observed. Furthermore, the probability of interference from "background" ions or from compounds other than acylcarnitines is high in these cases. For these reasons, the interpretation of acylcarnitine profiles from urine is more straightforward and reliable when the patient is in a carnitine-sufficient state. This is assured if the sample is collected after the administration of a bolus of l-carnitine or if the patient is breast-fed or receiving carnitine supplement.

Most of the even-mass ions above m/z 200 in the m/z 99 precursor ion spectra of urine samples are derived from acylcarnitines. This is based on the profiles of patients with well-defined metabolic defects whose diagnostic acylcarnitines have been previously characterized and reported (3,8,16). Thus, as observed in these earlier studies (5), the normal profile (Fig. 2A) is dominated by acetylcarnitine (M⁺ = 218), with lesser amounts of C3, C4, C5, and C8:1 (M⁺ = 300) acylcarnitines. In isovaleric acidemia (IVA), isovalerylcarntiune (M⁺ = 260, Fig. 2B) is the dominant species (6), and in propionic acidemia (PA), propionylcarnitine (M⁺ = 232, Fig. 2C) is the major acylcarnitine (7). In methylmalonic aciduria (MMA), the profile of which is not shown here, propionylcarnitine is also very prominent and is accompanied by a prominent signal for acetylcarnitine (3). In many cases, a signal corresponding to methylmalonylcarnitine (m/z 290) is also observed. Similarly, the profiles of glutaric aciduria type I (glutaryl-CoA dehydrogenase deficiency GAI, Fig. 2D), 3-kettothio-
lase deficiency (KT, Fig. 2E), and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG, Fig. 2F) revealed the expected dominant molecular cations corresponding to glutaryl carnitine (m/z 304), tiglylcarnitine (m/z 258), and 3-methylglutaryl carnitine (m/z 318), respectively (16,17). The two remaining profiles are from patients with disorders of fatty acid catabolism. The profile in Fig. 2G is from a patient with medium chain acyl-CoA dehydrogenase (MCAD) deficiency and is characterized by the M⁺ ions of medium chain acylcarnitines (8,9): hexanoyl (m/z 274), octanoyl (m/z 302), octenoyl (m/z 300), and decenoyl (m/z 328). In Fig. 2H a patient with multiple acyl-CoA dehydrogenase deficiency (MADD) is presented, showing increased excretion of butyrylcarnitine (M⁺ = m/z 246) plus C5, C6, and C8 acylcarnitines.

These patients, with the exception of the one with KT, were receiving carnitine supplements when the urine collections were made. In patients with IVA, PA, MMA, and GAL, the profiles appear to be very clean even without carnitine supplement.

Using the "precursors of m/z 99" scan, individual acylcarnitines can be detected in blood plasma at concentrations of less than 0.5 nmol/ml (14). This compares with typical physiological concentrations for total acylcarnitines of 6–10 nmol/ml, which can increase two- or threefold in patients with metabolic disease (2).

The ability to detect acylcarnitines in blood is very important since it enables
recognition and diagnosis of inherited metabolic diseases after death. The overall composition of blood is much less variable than that of urine, and the range of acylcarnitine concentration is much narrower. Furthermore, the pattern of acylcarnitines in blood appears to be a better reflection of the status in tissue than that of urine. Initial results with blood have been very encouraging. For example, diagnostic profiles representative of the metabolic diseases summarized in Fig. 2 have been achieved, and in general, appear to be similar to the urine profiles after carnitine supplement. In Fig. 3 the profile of a metabolically normal child (Fig. 3A) is compared with that of a postmortem specimen from a carnitine-supplemented child with PA (Fig. 3B) and of another postmortem sample from a previously undiagnosed and
asymptomatic child that succumbed to "sudden infant death" (Fig. 3C). The classical profile of MCAD deficiency is evident from this profile. Clearly, such deaths could be avoided by neonatal screening of blood. An authentic "PKU card" which had been stored 6 months at room temperature was obtained from a patient later confirmed as MCAD deficiency. Although the recovery of acylcarnitines from the blood spots may deteriorate with age, the characteristic profile of MCAD deficiency was still detectable after 6 months. PKU cards from other organic acidurias stored in the freezer for 2 years still revealed the diagnostic profiles. It has already been demonstrated (14,15) that rapid sequential analysis of samples by FAB-MS/MS is feasible using a continuous liquid introduction system. A study has begun at Duke to determine the feasibility of large-scale neonatal screening.

Tissue samples have generally posed the biggest problem for the application of FAB-MS. Even after extensive cleanup, the acylcarnitine-enriched fractions are very complex matrices. The best success has been obtained with muscle, where the acylcarnitine concentrations are relatively high. Acetylcarnitine and butyrylcarnitine were detected and quantified by high-resolution FAB-MS (3) in muscle from normal mice and from a mutant strain with short chain acyl-CoA dehydrogenase deficiency (18). With the FAB-MS/MS technique individual acylcarnitines are detectable down to concentrations of 1 nmol/g wet weight in liver tissue (14), for example.

A number of liver samples from patients with known defects, including MCAD and PA, have subsequently been analyzed and revealed clear diagnostic profiles. Postmortem liver is therefore another useful material for the recognition of metabolic diseases by FAB-MS/MS. The liver must be deep-frozen soon after excision to preserve the integrity of the acylcarnitines. Tissue that has been preserved in formalin is unsuitable.

CARNITINE THERAPY

Early experience with carnitine loading in PA (7), MMA (19), and IVA (6) provided valuable biochemical clues to the potential therapeutic value of carnitine supplementation in these disorders. The dynamic biochemical effects of an oral carnitine challenge in a patient with isovaleric acidemia are illustrated in Fig. 4. Isotope-dilution assays showed a rapid increase in isovalerylcarnitine (IVC) excretion and a corresponding decrease in isovalerylglucose (IVG) excretion. This demonstrates effective competition for isovaleryl-CoA by carnitine. The increase in benzoyleglycine (hippurate) excretion concomitant with the changes in IVG and IVC concentration strongly indicates that the formation of IVC is largely intramitochondrial, since the liberated CoASH would be available for formation of benzoyl-CoA and hence hippurate, an exclusively intramitochondrial event.

Most of the organic acidurias are characterized by a "secondary" carnitine deficiency (2). In some instances the inability to conserve carnitine at the renal level offers a reasonable explanation (20). In several of these disorders, however, the total quantities of free carnitine and acylcarnitines excreted per kilogram of body weight
per 24 h are markedly below normal. Untreated patients with IVA (6) and MCAD deficiency (8,9), for example, have a marked reduction in plasma free carnitine associated with decreased excretion of total carnitine. Renal loss, therefore, is not an adequate explanation for their deficiency state. The integrity of carnitine biosynthesis or uptake by tissue may be compromised in these disorders.

In PA and MMA it is not unusual to observe relatively normal levels of total carnitine in the plasma and normal quantities excreted in the urine. However, the bulk of the carnitine is esterified, predominantly as propionylcarnitine. It is perhaps appropriate to describe this not as a true “deficiency” state, but rather as a relative insufficiency of carnitine to meet metabolic needs. Oral supplementation with L-carnitine results in increased excretion of propionylcarnitine and elevation of the free carnitine fraction, supporting the concept that additional carnitine is needed to handle the large quantities of propionyl-CoA being produced.

Despite widespread acceptance of the concept of carnitine deficiency or insufficiency and the compelling biochemical evidence for the detoxification pathway of carnitine conjugation, there is considerable controversy about the general use of L-carnitine as a therapeutic agent in the organic acidurias. This is surprising in view of the acceptance of glycine therapy in isovaleric acidemia (21), the purpose of which is solely to enhance the removal of a toxic metabolite. Carnitine supplementation in PA, MMA, and IVA is analogous, and in the latter case at least, has the bonus of correcting a known deficiency. In MCAD deficiency, carnitine supplementation would also seem to be justified on the basis of correcting a true deficiency state and for conjugation and excretion of medium chain acyl-CoA derivatives, especially octanoyl-CoA. Chronic oral carnitine supplementation of numerous patients with organic acidurias referred to Duke Medical Center has been taking place with close observation for up to 8 years. The range of disorders treated includes PA, IVA, MMA, HMG lyase, 3-ketothiolase deficiency (KT), MCAD deficiency, and MADD. Typical daily oral doses are 200 mg/kg for PA and MMA and 100 mg/kg for the others. It should be noted that only about 15% of the oral dose is absorbed, and the
daily dose is divided into four portions, owing to the speed of the biochemical response.

These experiences may be summarized as follows. In all cases, with the exception of MCAD deficiency, parents report increased interaction with and awareness of the environment and at least a subjective overall clinical improvement. The degree of compliance has been very high. The patients with IVA, KT, HMG, and MADD have experienced no further hospitalizations while on carnitine supplement. In IVA, carnitine supplementation was used instead of glycine. When carnitine supplementation was discontinued for lengthy periods in certain patients with IVA, KT, and MADD, serious deterioration requiring hospitalization did occur.

Carnitine supplementation in PA, MMA, and MCAD has not eliminated recurrent illness, but it has reduced the severity of the illnesses and reduced the number of hospitalizations, especially in PA. Only one of seven treated MCAD patients has had recurrent illnesses. Although four have had chickenpox, they did not require hospitalization. Because the clinical course is so variable in this disorder, it is difficult to determine the significance of these observations. However, there are compelling reasons to support chronic carnitine therapy for MCAD deficiency. A high proportion (~30%) of deaths attributable to the disease occur with the first episode of illness. We believe that "systemic" carnitine deficiency in an untreated child would seriously limit mobilization of sufficient carnitine to conjugate the rapidly accumulating and potentially fatal medium chain fatty acyl-CoA compounds during such an episode. Chronic and acute carnitine supplementation should protect against this. Observations in a family with four affected children, two of whom had died reportedly with a Reye-like episode and sudden infant death syndrome (9) before a diagnosis was made, tend to support these concepts. The data shown in Fig. 5 are from one

![Fig. 5. Carnitine supplementation and excretion in MCAD deficiency during acute illness and when clinically well. From Roe CR, et al. (10).](image-url)
of the survivors and one of the deceased children, in whom MCAD deficiency was diagnosed after death. MCAD was diagnosed at Duke in the surviving sibling at 2 months of age and prior to clinical symptoms by analysis of organic acids and acylcarnitines. The diagnosis was subsequently confirmed by enzyme assay in cultured fibroblasts. Before initiating carnitine therapy, this patient, who was breast-fed at the time, excreted 2 μmol/mg creatinine total carnitine, of which 43% was acylated. Quantitative analysis by isotope-dilution FAB-MS (3) showed that octanoylcarnitine exceeded the acetylcaritnine concentration in this urine by a factor of 3. When receiving oral L-carnitine supplement at 100 mg/kg-day but asymptomatic, the typical output of total carnitine was 7 μmol/mg creatinine, of which 0.5 μmol was acylated. During a severe clinical episode, she was given intravenous carnitine (30 mg/kg bolus followed by 30 mg/kg over the next 24 h) and recovered rapidly with regained sensorium within 5 h. The total carnitine excreted during the intravenous therapy was 61 μmol/mg creatinine, 48% of which was acylated. Octanoylcarnitine and acetylcarnitine concentrations were 13 and 16 μmol/mg, respectively. A repeat of the same intravenous carnitine regimen when the patient was clinically well revealed much lower acylcarnitine as a percentage of the total with octanoylcarnitine representing about 25% of the acylated fraction. Analysis of postmortem urine from the deceased, untreated sibling revealed a total carnitine excretion of only 3 μmol/mg, of which about 90% was acylated (Fig. 5).

We infer from these data that untreated MCAD-deficient patients are at increased risk owing to their inability to mobilize carnitine during clinical episodes. By maintaining a steady intake, some additional reserve of carnitine is available for detoxification during the onset of illness. The use of intravenous carnitine during acute illnesses has been conducted under our supervision with excellent clinical results in patients with MCAD, MADD, IVA, and PA. Patients typically recover within a few hours, compared with 1–3 days when intravenous glucose alone is employed acutely.

SUMMARY

The detection of acylcarnitines by FAB-MS in human physiological fluids has added another valuable diagnostic tool for the recognition of specific metabolic diseases. The newly developed technique of FAB-MS/MS, which embodies the principles of tandem mass spectrometry, affords a quantum leap in specificity, enabling the detection and quantification of acylcarnitines in concentrations well within the physiological ranges in urine, blood, and tissue. The therapeutic use of L-carnitine appears to offer protection against the harmful consequences of catabolism. A reduction in the number of clinical episodes requiring hospitalization has been observed in most cases. When such occurrences have necessitated intravenous fluids, the addition of L-carnitine to the intravenous infusate has led to dramatic reduction in recovery times. It has also been shown that there is no discernible toxicity associated with carnitine therapy, even under high-dose intravenous conditions.
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REFERENCES


DISCUSSION

Dr. Wendel: What carnitine doses do you recommend for the long-term treatment of an organic aciduria like β-ketothiolase deficiency?

Dr. Roe: We all have so little experience with large populations that it is hard to know exactly what would be the best level. Our β-ketothiolase patients have been treated with 100 mg/kg-day in four divided doses and that seemed to be sufficient. HMG and MCAD patients were given 50 mg/kg-day and propionics and methylmalonics 200 mg and higher. The dose is given every day since it is not possible to predict when crises will occur.

Dr. Van Hoof: With Professor E. de Hoffmann’s Finnigan-MAT TSQ 70 tandem mass spectrometer, we avoided the methylation step and obtained equally good results in the detection of carnitine esters. The advantage of this is that one can escape the alkaline step of methylation, during which methanalysis of carnitine esters is expected to occur. Another point: You did not mention the danger of using valproate in children suffering from these disorders.

Dr. Roe: Two cases (one an MCAD and the other a multiple acyl-CoA dehydrogenase deficiency) received valproate and both went immediately into coma. It is clearly contraindicated in these disorders.

Dr. Van Hoof: The use of valproate might be the cause of several episodes of Reye’s syndrome. Valproate is probably not the only culprit. The use of mosquito repellent has also been mentioned in the literature. These hazards, however, are expected to occur only in persons in whom mitochondrial β-oxidation is at the lower normal limit.

Dr. Roe: That is a very good comment. I would like to make two responses. In the biochemical literature it has been suggested that carnitine could enhance the branched-chain ketoacid decarboxylase, which would tend to suggest that it might produce a greater turnover of amino acids precursors. The same question has been raised in relation to the fatty acid pathway (i.e., that high-dose carnitine might enhance lipolysis). We have done this study on branched chain disorders and in the fat disorders, giving 100 mg carnitine per kilogram daily. It is clear that neither in propionic acidemia nor in the MCADs is there any enhancement of endogenous turnover as measured by the quantitative excretion of the acylcarnitine, nor any change in dicarboxylic acids. So I think that from a toxicity point of view it is good to know this. Referring to valproate, I think you are absolutely right. We are only beginning to understand what the contraindications would be in a number of these disorders. Tom Baillie, who is in Seattle, Washington with Dr. René Lévy, is very interested in the interactions of valproate in the inborn errors. This is one of the things we have worked on together recently and we have rationalized the origin of the intermediates that have been identified in valproate therapy.

Dr. Leroy: How sensible or how relevant is it to talk about normal carnitine levels? Can the hypothesis of carnitine deficiency be maintained in the case of myogenic hypotonia? Do we
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know really what normal serum levels of carnitine mean? Too few control data in children from infancy to late childhood are available. Do carnitine levels in plasma or muscle mean anything at all, and if so, how will they be useful? Can you please comment?

Dr. Roe: That is an excellent question. I get very concerned when I encounter situations where someone has measured the total carnitine and says it is normal. We don’t rely on “normal” values at all. We do plasma carnitine by radioenzymatic assay and also by the FAB technique with internal standards that are reliable for quantification. The species of acylcarnitine is more important than the total carnitine value. We frequently refer to ratios, (e.g., the acylcarnitine to free carnitine ratio), but those of us who do these tests know how rapidly they change in disease. But I think you have a valid point in doing carnitine measurements on the plasma of a child with hypotonia. It is a good starting point because there may be a secondary deficiency state. I would move just as quickly to the other studies, even if the carnitine levels were normal.

Dr. Bartlett: I agree with that statement. We have spent a lot of time and effort devising reliable assays of free carnitine, short chain acylcarnitine and long chain acylcarnitine. Our long chain acylcarnitine method relies on internal standardization with palmitoyl[methyl-3H]carnitine and is accurate and reproducible. However, it has become clear to us that in most instances the data generated are no more than confirmatory. The situation is particularly complicated during ketosis, when a high percentage of acylated carnitine is observed. This is largely due to short chain acylcarnitine, which can reach plasma concentrations in excess of 20 μmol/liter. This is nearly all acetylcarnitine and reflects a normal physiological response to fasting. However, in the absence of chromatographic analysis it would be very easy to confuse this normal physiological response with the pathological response, due, for example, to high octanoylcarnitine in medium chain acyl-CoA dehydrogenase deficiency. Under extreme conditions we could probably distinguish the two situations, but I would certainly not like to rely on analysis of carnitine and acylcarnitine alone.

Dr. Otten: Is there a defect of carnitine uptake into the cells? And if this is the case, does it make sense to supplement these patients with carnitine, and do you know the metabolic outcome? In patients with rickets with cardiomyopathy and low carnitine values, there is a high urinary carnitine output. When you supplement with carnitine there is a slight improvement in the cardiomyopathy.

Dr. Roe: Charles Stanley has definitely demonstrated the carnitine transport defect in terms of uptake, which I think is extremely interesting. In the cardiomyopathy area, we have had a similar experience. We have seen two types, one like the one you are talking about with excessive carnitine loss and others where there is really not an excessive carnitine loss, but there is a response to carnitine. I do not know the mechanism, but cardiomyopathy has improved with carnitine supplementation without any major dietary alteration. If one looks at the carnitine data, one can see that in MCAD and isovaleric acidemia, for example, the patients are actually excreting only a small fraction of the carnitine, maybe 10%, that normal children excrete in a 24-h period. These data suggest that the earlier work on the integrity of carnitine biosynthesis in “systemic carnitine deficiency” needs to be re-explored. There is probably some secondary problem in the biosynthetic pathway that may account for the so-called secondary carnitine deficiency producing a true deficiency state.

Dr. Saudubray: A very severe secondary carnitine deficiency is also observed in glutaric aciduria type I due to glutaryl-CoA dehydrogenase deficiency. We investigated three patients and found free and total carnitine plasma levels close to zero (around 1 μmol/liter). I guess these very low levels were not explained through enhanced urinary losses of glutaryl carnitine.
Of course, there was some glutaryl carnitine in the urine but only a small amount. So why are the carnitine levels so low in glutaric aciduria type I? What is your experience?

Dr. Roe: It is exactly the same as yours. There is an extraordinary deficiency that is not accounted for by enhanced excretion. We synthesized a CD3 carnitine for intravenous use, and we have shown that there is an inhibition of carnitine production in MCAD patients.

Dr. Mowat: This is a simple clinical question. When cases of MCAD present in the emergency room we do not usually know the diagnosis. Are there any conditions, for example Reye's syndrome, in which you think that carnitine might be dangerous if given as emergency treatment in this acute clinical presentation? I wonder also if the same would apply to the other clinical situations: for example, a child presenting with hypotonia, apnea, and bradycardia.

Dr. Roe: From our studies with high-dose intravenous carnitine and high-dose acetyl carnitine with stable isotope labels, there is absolutely no evidence of any toxicity at doses which would be far greater than one would employ in an acute emergency therapy situation. Intravenous carnitine has no obvious toxicity. I think its intravenous benefits need more documentation by other investigators. It could be an extraordinarily valuable emergency medication.