0031-3998/92/3104-0419$03.00/0
PEDIATRIC RESEARCH

Vol. 31, No. 4, 1992
Printed in U.S.A.

Amelioration of Adverse Effects of Valproic Acid on Ketogenesis and Liver Coenzyme A Metabolism by Cotreatment with Pantothenate and Carnitine in Developing Mice: Possible Clinical Significance

JEAN HOLOWACH THURSTON AND RICHARD E. HAUHART

Department of Pediatrics [J.H.T., R.E.H.] and Neurology and Neurological Surgery (Neurology) [J.H.T.], St. Louis, Missouri 63110

ABSTRACT. Very young children with organic brain damage, intractable seizures, and developmental retardation are at particular risk of developing fatal hepatic dysfunction coincident with valproate therapy, especially if the children are also receiving other anticonvulsant drugs. The mechanism of valproate-associated hepatic failure in these children is unclear. There are two major theories of etiology. The first concerns the manyfold consequences of depletion of CoA due to sequestration into poorly metabolized valproyl CoA and valproyl CoA metabolites. The other theory proposes that the unsaturated valproate derivative 2-*n*-propyl-4-pentenoic acid and/or metabolically activated intermediates are toxic and directly cause irreversible inhibition of enzymes of β-oxidation. The present study shows for the first time that in developing mice, when pantothenic acid and carnitine are administered with valproate, at least some of the effects of valproate are mitigated. Perhaps most importantly, the β-hydroxybutyrate concentration in plasma and the free CoA and acetyl CoA levels in liver do not fall so low. Cotreatment with carnitine alone was without effect. Findings support the CoA depletion mechanism of valproate inhibition of β-oxidation and other CoA- and acetyl CoA-requiring enzymic reactions and stress the role of carnitine in the regulation of CoA synthesis at the site of action of pantothenate kinase. (*Pediatr Res* 31: 419–423, 1992)

The new anticonvulsant drug, valproic acid (2-*n*-propylpentanoic acid, 2-*n*-propylvaleric acid), is particularly effective in the treatment of the types of seizures peculiar to infants and young children: infantile myoclonic spasms, febrile seizures, and petit mal (absence) (reviewed in Ref. 1). Yet this is the age group that is most susceptible to the hepatotoxic effects of the drug, particularly if the children have organic brain damage and are also receiving other anticonvulsant drugs (2). In a study of possible mechanisms of this complication, we found that valproate reduced the physiologic ketonemia of suckling mice (3, 4) and fasting epileptic children (5). In the livers of normal developing mice, valproate produced extraordinary decreases in the levels of free CoA, acetyl CoA, and free carnitine (4). Concomitantly, the concentration of medium-chain acyl CoA esters [including valproyl CoA and its metabolites (6)] increased some 6- to 7-fold (4).

As in our normal developing mice, valproate also reduced plasma β-hydroxybutyrate levels and liver CoA and acetyl CoA levels in fasted adult rats (7, 8). In isolated adult rat hepatocytes, valproate inhibited oxidation of fatty acids and pyruvate, fatty acid synthesis, gluconeogenesis, ketogenesis, and ureagenesis (6, 7, 9–11). In view of the 100 or so synthetic and catabolic enzyme reactions requiring CoA or acetyl CoA as substrates, cofactors, or activators, the above diverse ill effects of valproate on liver metabolism can largely be explained by sequestration of free CoA into nonmetabolized or poorly metabolized valproyl CoA and valproyl CoA ester metabolites. If this were the case, we reasoned that increasing liver CoA levels should lessen the valproate-induced metabolic aberrations. The hypothesis was tested by coadministration of pantothenate, carnitine, and cysteine with valproate in normal suckling mice. Pantothenic acid, cysteine, and ATP are precursors of CoA, and carnitine is known to overcome the strong inhibition of pantothenate kinase (first enzyme in this synthetic pathway) by CoA and all of its esters (see Ref. 12 for a review). No attempt was made to increase hepatic ATP levels, inasmuch as there is no reduction in liver ATP during valproate administration (3).

MATERIALS AND METHODS

Pantothenate (D-pantothenic acid, hemi-calcium salt), L-carnitine hydrochloride, and L-cysteine hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO; solutions of the latter two compounds were neutralized to pH 7.0 with NaOH just before use. Sodium valproate was a gift of Abbott Laboratories, Chicago, IL.

Preparation of Animals. All animal experiments were performed with the highest standards of humane care. Six litters of suckling 4- to 8-d-old Swiss-Webster mice were used; the total number of animals was 46. The mean weight (± SEM) of 28 of these mice was 3.95 ± 0.29 g. Weight-matched littermates were injected s.c. with 20 mL/kg of 0.9% NaCl or 15 mg/kg (0.09 mmol/kg) sodium valproate in an equivalent volume of 0.9% NaCl. Other littermates received valproate, pantothenate (2 mmol/kg), and L-cysteine (1 mmol/kg); valproate plus L-carnitine (2.5 mmol/kg); or valproate plus pantothenate, L-carnitine, and L-cysteine. Animals were killed by decapitation after 90 min; blood was collected from the severed neck vessels and the body was plunged into liquid nitrogen with rapid stirring to effect quick freezing.

Preparation of Plasma and Liver. Blood was promptly centri-

Received September 25, 1991; accepted December 10, 1991.
Correspondence: Jean Holowach Thurston, M.D., Department of Pediatrics, Washington University School of Medicine, 400 South Kingshighway Blvd., St. Louis, MO 63110.
Supported in part by the Abbott Laboratories, Chicago, and the Allen P. and Josephine B. Green Foundation, Mexico, Missouri.

fuged at 4°C. Plasma was deproteinized with 10 to 20 volumes of 0.5 M perchloric acid. It was not necessary to neutralize the acid extracts for assay of plasma metabolites. Aliquots of the acid extract required for fluorometric measurements of β-hydroxybutyrate did not change the pH of the reagent buffer or affect complete recovery of the standard. Liver was dissected free of membranes and visible blood vessels with sharp chisels in a cryostat at −35°C. Perchlorate extracts were prepared in a cold room at −20°C (13). Extracts were stored at −80°C until time of assay.

Analytical Methods. β-Hydroxybutyrate. The plasma β-hydroxybutyrate level was measured by a fluorometric adaptation of the method of Williamson *et al.* (14).

Free CoA and CoA esters. Free CoA and acetyl CoA were measured by the cycling procedures of Kato (15). Medium-chain acyl CoA esters were hydrolyzed by heating a portion of the perchloric acid extract for 60 min at 60°C in the presence of 6 mM DTT and 70 mM 2-amino-2-methyl-1-propanol buffer at pH 9.2 (16). The concentration of medium-chain acyl CoA esters was calculated by subtracting the previously determined concentrations of free CoA and acetyl CoA. The perchloric acid-insoluble precipitate was used for the measurement of long-chain acyl CoA esters.

Free carnitine and carnitine esters. Free carnitine was assayed fluorometrically by measuring the CoA formed (17) from the reaction of carnitine with acetyl CoA in the presence of carnitine acetyltransferase (EC 2.3.1.7). Short- and medium-chain acyl carnitine esters were hydrolyzed by heating a portion of the neutralized perchloric acid extract for 1 h at 37°C in the presence of 150 mM HEPES; the pH of the solution was adjusted to approximately 13 with 10 N NaOH (18, 19). After neutralization with 5 N HCl, the total acid-soluble carnitine content was measured as described above. The concentration of short- and medium-chain acyl carnitine esters was calculated by subtracting the concentration of free carnitine. The levels of long-chain acyl carnitine esters were similarly determined using the perchloric acid-insoluble precipitate.

Statistical Analysis. When only two treatment groups were compared, the statistical significance of the difference between the means of control and experimental values was determined by *t* test. When there were three or more experimental groups, statistical significance among the groups was determined by a one-way analysis of variance, followed by Tukey's honestly significant difference test (20) to identify differences between specific pairs of means.

RESULTS

Pantothenate, carnitine, and cysteine treatment of normal mice. Within 90 min of injection of a single dose of pantothenic acid, carnitine, and cysteine in normal suckling mice, liver free CoA and acetyl CoA levels increased 18 and 51%, respectively (Table 1). The 35% decrease in the content of long-chain acyl CoA esters was equal to the increase of medium-chain acyl CoA esters, although neither change was statistically significant. The total CoA content increased 20%. In these normal mice, the observed changes in liver did not affect the plasma β-hydroxybutyrate concentration.

In this protocol and those that follow, similar results were later seen by the administration of pantothenate and carnitine without the addition of cysteine. Apparently the endogenous level of cysteine (like ATP) in liver was sufficient for CoA synthesis in the presence of pantothenate and carnitine.

Valproate-treated mice. As we have originally observed in developing mice (3, 4), valproate treatment reduced plasma β-hydroxybutyrate levels 73% (Table 2). It should be recalled that ketonemia is physiologic in suckling rodents.

In the livers of these animals, free CoA levels fell 56% and acetyl CoA levels were reduced to one third of the control value (Table 2). Valproate administration increased the content of medium-chain acyl CoA esters almost 8-fold. Becker and Harris (6) have determined that the increase in this acyl CoA ester fraction was due to the accumulation of valproyl CoA and another valproyl CoA ester metabolite. The content of long-chain acyl CoA esters was reduced 50%.

Table 1. *Effect of pantothenate, carnitine, and cysteine on plasma β-hydroxybutyrate levels and liver CoA and carnitine metabolism in normal suckling mice**

Measurement	Control ($n = 4$)	CARN + B_5 + CYST ($n = 7$)	p
Plasma (mM)			
β-Hydroxybutyrate	1.24 ± 0.12	1.18 ± 0.09	NS†
Liver (μmol/kg)			
CoA	114 ± 4	135 ± 5	0.027
Acetyl CoA	22.5 ± 1.4	33.9 ± 1.2	<0.001
Medium-chain acyl CoA	25.7 ± 4.0	34.7 ± 3.1	NS†
Long-chain acyl CoA	15.8 ± 4.5	10.3 ± 0.9	NS†
Total CoA	178 ± 6	214 ± 7	0.008
CARN	263 ± 17	1598 ± 101	<0.001
Medium- and short-chain acyl CARN	313 ± 18	2616 ± 154	<0.001
Long-chain acyl CARN	71.4 ± 9.1	272 ± 18	<0.001
Total CARN	647 ± 12	4485 ± 193	<0.001

* Mice were treated as described in Materials and Methods. B_5, pantothenic acid; CYST, cysteine; CARN, carnitine. Values are means ± SEM.

† $p > 0.05$.

In valproate-injected mice, free carnitine levels fell 63% and there was a reciprocal 75% increase in long-chain acyl carnitine esters (Table 3).

Effect of pantothenate, carnitine, and cysteine on ketogenesis and liver CoA metabolism in valproate-treated mice. Injection of carnitine with valproate had no effect on the valproate-induced metabolic aberrations in plasma or liver (Table 2).

By contrast, in the presence of pantothenic acid, carnitine, and cysteine, the effect of valproate on the level of β-hydroxybutyrate in plasma was reduced almost 50%. Similarly, in liver, the reductions in free CoA and acetyl CoA produced by valproate were substantially smaller. Cotreatment of pantothenate/carnitine/cysteine with valproate elevated the concentration of medium-chain acyl CoA esters another 42%. One cannot exclude further activation of valproate to valproyl CoA.

Effect of pantothenate, carnitine, and cysteine on liver carnitine metabolism in valproate-treated mice. Although the doses of pantothenate and carnitine used were equal in control (Table 1) and valproate-treated mice (Table 3), the total hepatic carnitine concentration in control mice was 1 mmol/kg higher than in the valproate-treated animals. In both groups of mice, about 50% of the additional carnitine appeared in the hepatic medium-chain acyl carnitine fraction. In valproate-treated mice, the increase of medium-chain acyl carnitine esters does not appear to be due to conversion of the medium-chain acyl CoA esters [mostly valproyl CoA (6)] to valproyl carnitine. If this were the case, one would expect the content of the medium-chain acyl CoA esters to fall; in fact, this ester fraction increased significantly. In control mice (Table 1), the rest of the added carnitine appeared mostly as free carnitine, although long-chain acyl carnitine increased nearly 4-fold. In valproate-treated mice, the rest of the added carnitine appeared primarily in long-chain acyl carnitine esters (Table 3)—a 3-fold increase over the value obtained in the absence of valproate (Table 1). The finding suggests a pantothenate/carnitine/cysteine-induced increase in the flux of long-chain fatty acids to the liver in the latter animals. In neonatal humans, carnitine administration releases FFA and glycerol from adipose tissue (21–23). The effect of carnitine to stimulate lipolysis in adipose tissue of human newborns was not seen in adults (21). Whether carnitine plays a similar role in developing mice cannot be said. The near 3-fold elevation of the hepatic acyl carnitine/

Table 2. *Effect of carnitine or carnitine, pantothenate, and cysteine on plasma β-hydroxybutyrate levels and liver CoA metabolism in valproate-treated suckling mice**

Measurement	0.9% NaCl ($n = 6$)	Valproate ($n = 6$)	Valproate + CARN ($n = 6$)	Valproate + CYST + CARN + B_5 ($n = 6$)
Plasma (mM)				
β-Hydroxybutyrate	1.07 ± 0.20	0.29 ± 0.03†	0.30 ± 0.04†	0.66 ± 0.02†‡§
Liver (μmol/kg)				
CoA	88.6 ± 4.4	39.0 ± 1.5†	40.9 ± 1.6†	61.6 ± 2.1†‡§
Acetyl CoA	19.3 ± 1.9	6.3 ± 0.6†	7.8 ± 0.5†	10.7 ± 1.1†‖
Medium-chain acyl CoA	17.7 ± 3.8	137 ± 6†	152 ± 8†	195 ± 10†‡§
Long-chain acyl CoA	9.9 ± 0.8	4.9 ± 0.6†	4.8 ± 0.4†	7.6 ± 0.7‖¶
Total CoA	135 ± 10	189 ± 8†	205 ± 8†	275 ± 11†‡§

* Mice were treated as described in Materials and Methods. Abbreviations are the same as those used in Table 1. Values are means ± SEM.
† *p vs* control < 0.01.
‡ *p vs* valproate < 0.01.
§ *p vs* valproate + CARN < 0.01.
‖ *p vs* valproate < 0.05.
¶ *p vs* valproate + CARN < 0.05.

Table 3. *Effect of carnitine or carnitine, pantothenate, and cysteine on liver carnitine metabolism in valproate-treated suckling mice**

Measurement	0.9% NaCl ($n = 6$)	Valproate ($n = 6$)	Valproate + CARN ($n = 6$)	Valproate + CARN + B_5 + CYST ($n = 6$)
CARN	190 ± 14	69.7 ± 8.4†	540 ± 34‡§	641 ± 38‡§‖
Medium- and short-chain acyl CARN	294 ± 35	284 ± 25	2052 ± 51‡§	1915 ± 121‡§
Long-chain acyl CARN	94.5 ± 8.5	165 ± 15	1162 ± 80‡§	822 ± 57‡§¶
Total CARN	578 ± 49	539 ± 26	3754 ± 133‡§	3377 ± 146‡§‖
Acyl CARN/CARN	2.07 ± 0.16	5.50 ± 0.61‡	6.02 ± 0.43‡	4.40 ± 0.51†

* Mice are those used for Table 2. Abbreviations are the same as those used in Table 1. Values are μmol/kg (mean ± SEM).
† *p vs* control < 0.05.
‡ *p vs* control < 0.01.
§ *p vs* valproate < 0.01.
‖ *p vs* valproate + CARN < 0.05.
¶ *p vs* valproate + CARN < 0.01.

free carnitine concentration ratio in valproate-treated mice was not reduced by comedication with carnitine alone (Table 3). The acyl carnitine/free carnitine ratio in liver after cotreatment of animals with valproate, pantothenate, and carnitine was still significantly higher than in control animals (Table 3).

Effect of pantothenate alone or pantothenate and carnitine on ketogenesis and liver CoA metabolism in valproate-treated mice. Table 4 compares the effects of cotreatment of valproate-injected mice with pantothenate or pantothenate plus carnitine. By itself, pantothenate did not elevate the low level of β-hydroxybutyrate in the plasma; addition of carnitine with pantothenate increased the ketone body level 71%. In liver, pantothenate increased the free CoA content of valproate-treated mice 46%; addition of carnitine produced another 19% rise. The near doubling of liver acetyl CoA levels induced by pantothenate alone was not statistically significant. The additional 44% increase in the acetyl CoA content induced by the addition of carnitine to pantothenate was significantly higher than that in the valproate-treated mice. The hepatic medium-chain acyl CoA fraction was increased 31% by pantothenate; the addition of carnitine did not reduce this fraction.

DISCUSSION

Carnitine has been recommended as a supplement to all children under 2 y of age who are treated with valproic acid (24). It is postulated that potentially toxic valproyl CoA metabolites would be converted to valproyl carnitine esters via the activity of liver carnitine acyl transferases. The carnitine conjugate of valproic acid can be translocated across the mitochondrial mem-

Table 4. *Effect of pantothenate or pantothenate plus carnitine on plasma β-hydroxybutyrate levels and liver CoA metabolism in valproate-treated suckling mice**

Measurement	Valproate ($n = 6$)	Valproate + B_5 ($n = 7$)	Valproate + B_5 + CARN ($n = 7$)
Plasma (mM)			
β-Hydroxybutyrate	0.31 ± 0.03	0.35 ± 0.02	0.53 ± 0.04†‡
Liver (μmol/kg)			
CoA	38.9 ± 1.2	56.6 ± 2.1†	67.6 ± 2.8†‡
Acetyl CoA	6.6 ± 0.7	11.2 ± 1.4	16.1 ± 2.7‡
Medium-chain acyl CoA	151 ± 10	198 ± 6†	186 ± 7†

* Mice were treated as described in Materials and Methods. Abbreviations are the same as those used in Table 1. Values are means ± SEM.
† *p vs* valproate < 0.01.
‡ *p vs* valproate + B_5 < 0.01.

brane for excretion in urine. In consequence, free CoA would become available for β-oxidation and other essential CoA-requiring enzymic reactions. However, valproyl CoA did not readily react with acyl carnitine transferase in rat liver mitochondria (6). Carnitine esters of valproic acid may not be readily formed by human liver either. In one study of children receiving valproate and carnitine, valproyl carnitine accounted for less than 10% of the acyl carnitine in urine; the predominant acyl carnitine ester was acetyl carnitine (25). In another human study, valproyl carnitine excretion increased only from 0.4 to 1% after carnitine supplementation (26).

From the presently available data, a role for carnitine deficiency in valproate-associated hepatotoxicity and possible beneficial effects of carnitine supplementation is not clear. There are reports of decreased levels of free carnitine in the plasma of epileptic children receiving valproate (27–30). Ohtani *et al.* (27) found that cotreatment of these children with carnitine returned the valproate-induced decrease of the plasma carnitine level and the elevation of the ammonia concentration to normal. In valproate-treated mice, high levels of serum ammonia were seen in the face of normal free and esterified serum carnitine levels (31). Serum carnitine levels were normal in a child who developed hepatic dysfunction while receiving valproate, and carnitine treatment did not prevent liver failure (30).

Levels of ketone bodies in plasma are valid, albeit indirect, indicators of normal β-oxidation. Experimentally, valproate consistently inhibited β-oxidation and hepatic ketogenesis *in vivo* (7, 8) and *in vitro* (6, 11). In fasted adult rats, the valproate-induced hypoketonemia was not associated with a decreased liver carnitine content or an abnormal hepatic acyl carnitine/free carnitine concentration ratio (32). In other fasted adult rats receiving valproate, plasma β-hydroxybutyrate levels fell almost 90% despite 2- to 3-fold increases, not decreases, in the hepatic content of total and free carnitine; levels of acyl carnitine were unchanged (8). In epileptic children on chronic valproic acid treatment receiving carnitine, the 87% decrease of the β-hydroxybutyrate concentration in plasma remained unaffected by the carnitine supplementation (26).

Coadministration of a 28-fold greater dose of carnitine than valproate increased the level of free carnitine in the livers of young mice some 7-fold, yet the phenomenal increase had no effect on the reduction of the levels of CoA and acetyl CoA in liver or of β-hydroxybutyrate in plasma produced by valproate. Pantothenate alone did not block the effect of valproate on plasma β-hydroxybutyrate but did raise the liver CoA level. After coadministration of pantothenate and carnitine with valproate, there were significant elevations of free CoA and acetyl CoA levels in liver and β-hydroxybutyrate in plasma as compared with animals treated with valproate alone. [We have previously reported that at subclinical doses of valproate we were able to elevate the depressed plasma β-hydroxybutyrate level by coadministration of an 80-fold greater dose of carnitine (33).]

The beneficial effect of carnitine in valproate-treated mice in the presence of pantothenate emphasizes the lesser known role of carnitine in the control of CoA synthesis. Pantothenate kinase (ATP: pantothenate 4′-phosphotransferase; EC 2.7.1.33) is the rate-controlling first enzyme in the pathway of CoA synthesis. In kidney, liver, and heart, the enzyme is strongly inhibited by free CoA and all of its acyl esters (34–36). Carnitine de-inhibits the CoA-inhibited enzyme (36, 37). Unlike free carnitine, in heart acetyl carnitine does not de-inhibit the CoA-inhibited enzyme (36). It has been suggested that changes in the tissue concentration ratio of acetyl carnitine to free carnitine may control CoA synthesis in liver with high levels of acetyl carnitine reducing the activity of pantothenate kinase and high levels of free carnitine stimulating the enzyme (12, 36). Based on this premise, the extraordinary excretion of acetyl carnitine in the urine of valproate-treated patients (25) could promote CoA synthesis.

It is of interest that in rats (38) and guinea pigs (39) the greatest need of the liver for pantothenic acid and CoA is early in development, with a decreasing requirement with progressive age. If a similar developmental pattern of pantothenate and CoA requirement occurs in man, the data may relate to the peculiar susceptibility of very young children to valproate hepatotoxicity (2).

Findings in the present study support the CoA depletion theory of valproate hepatotoxicity. There is evidence that a similar mechanism may underlie the hepatotoxicity of the unsaturated metabolites of valproic acid. Based on the structural similarity of 2-*n*-propyl-4-pentenoic acid to the well-known hepatotoxins 4-pentenoic acid and hypoglycin A, it has been proposed that 2-*n*-propyl-4-pentenoic acid and/or metabolically activated intermediates alkylate and thereby specifically inhibit key enzymes of the fatty acid β-oxidation complex (40). Both 4-pentenoic acid and toxic metabolites of hypoglycin A must first be converted to their respective CoA derivatives to cause inhibition of β-oxidation (41, 42). Like valproate, 4-pentenoic acid administration reduced levels of free CoA and carnitine in liver (41–45), suggesting that the deleterious effect of 4-pentenoic acid on fatty acid β-oxidation is to a greater or lesser degree also due to sequestration of CoA and carnitine in relatively inert 4-pentenoyl CoA and carnitine esters. Reversal of the inhibitory effect of 4-pentenoic acid on fatty acid β-oxidation *in vitro* by the addition of free CoA and/or carnitine supports this hypothesis (43, 45).

It is well established that carnitine supplementation plays an important role in the management of children with disorders of organic acid metabolism (propionic acidemia, methylmalonic acidemia, glutaric acidemia type II). In rats injected with propionic acid or methylmalonic acid, there were profound decreases in liver free CoA and acetyl CoA levels and reciprocal increases in the content of medium-chain acyl CoA derivatives (46). In isolated rat hepatocytes, branched-chain α-ketoacids, α-ketoisocaproic, α-ketoisovaleric, and α-keto-β-methylvaleric acids, also caused an accumulation of medium-chain acyl CoA derivatives at the expense of free CoA (47). Because these biochemical changes are similar if not identical to those seen in our valproate-treated mice, the value of coadministration of pantothenate with carnitine in these clinical conditions merits consideration.

Acknowledgment. The authors thank Dr. David B. McDougal, Jr., for his continued interest and encouragement and his critical review of the manuscript.

REFERENCES

1. Simon D, Penry JK 1975 Sodium di-*n*-propylacetate (DPA) in the treatment of epilepsy: a review. Epilepsia 16:549–573
2. Dreifuss FE, Santilli N, Langer DH, Sweeney KP, Moline KA, Menander KB 1987 Valproic acid hepatic fatalities: a retrospective review. Neurology 37:379–385
3. Thurston JH, Hauhart RE, Schulz DW, Naccarato EF, Dodson WE, Carroll JE 1981 Chronic valproate administration produces hepatic dysfunction and may delay brain maturation in infant mice. Neurology 31:1063–1069
4. Thurston JH, Carroll JE, Hauhart RE, Schiro JA 1985 A single therapeutic dose of valproate affects liver carbohydrate, fat, adenylate, amino acid, coenzyme A, and carnitine metabolism in infant mice: possible clinical significance. Life Sci 36:1643–1651
5. Thurston JH, Carroll JE, Dodson WE, Hauhart RE, Tasch V 1983 Chronic valproate administration reduces fasting ketonemia in children. Neurology 33:1348–1350
6. Becker C-M, Harris RA 1983 Influence of valproic acid on hepatic carbohydrate and lipid metabolism. Arch Biochem Biophys 223:381–392
7. Turnbull DM, Bone AJ, Bartlett K, Koundakjian PP, Sherratt HSA 1983 The effects of valproate on intermediary metabolism in isolated rat hepatocytes and intact rats. Biochem Pharmacol 32:1887–1892
8. Kesterson JW, Granneman GR, Machinist JM 1984 The hepatotoxicity of valproic acid and its metabolites in rats. I. Toxicologic, biochemical and histopathologic studies. Hepatology 4:1143–1152
9. Coudé FX, Grimber G, Parvy P, Rabier D, Petit F 1983 Inhibition of ureagenesis by valproate in rat hepatocytes: role of N-acetylglutamate and acetyl CoA. Biochem J 216:233–236
10. Kingsley E, Gray P, Tolman KG, Tweedale R 1983 The toxicity of metabolites of sodium valproate in cultured hepatocytes. J Clin Pharmacol 23:178–185
11. Coudé FX, Grimber G, Pelet A, Benoit Y 1983 Action of the antiepileptic

drug, valproic acid, on fatty acid oxidation in isolated hepatocytes. Biochem Biophys Res Commun 115:730–736
12. Robishaw JD, Neely JR 1985 Coenzyme A metabolism. Am J Physiol 248: E1–E9
13. Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW 1964 Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J Biol Chem 239:18–30
14. Williamson DH, Mellanby J, Krebs HA 1962 Enzymatic determination of D(−)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J 82:90–98
15. Kato T 1975 CoA cycling: an enzymatic amplification method for determination of CoASH and acetyl CoA. Anal Biochem 66:372–392
16. McDougal Jr DB, Dargar RV 1979 A spectrophotometric cycling assay for reduced coenzyme A and its esters in small amounts of tissue. Anal Biochem 97:103–115
17. Tubbs PK, Garland PB 1969 Assay of coenzyme A and some acyl derivatives. In: Lowenstein JM (ed) Methods in Enzymology, Vol 13. Academic Press, New York, pp 535–551
18. McGarry JD, Foster DW 1976 An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J Lipid Res 17:277–281
19. Christiansen RZ, Bremer J 1976 Active transport of butyrobetaine and carnitine into isolated liver cells. Biochim Biophys Acta 448:562–577
20. Tukey JW 1953 The problem of multiple comparisons. [ditto] Princeton University, Princeton, NJ. Cited by Kirk RE 1968 Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole, Belmont, CA, pp 69–98
21. Novak M, Penn-Walker D, Hahn P, Monkus EF 1975 Effect of carnitine on lipolysis in subcutaneous adipose tissue of newborns. Biol Neonate 25:85–94
22. Orzali A, Donzelli F, Enzi G, Rubaltelli FF 1983 Effect of carnitine on lipid metabolism in the newborn. I. Carnitine supplementation during total parenteral nutrition in the first 48 hours of life. Biol Neonate 43:186–190
23. Borum PR 1985 Role of carnitine during development. Can J Pharmacol 63:571–576
24. Statement of the Pediatric Neurology Advisory Committee, DeVivo DC, Chairman. Symposium on Inborn Metabolic Errors and Seizures: The Role of Carnitine, Atlanta, GA, October 20, 1990, p 71
25. Millington DS, Bohan TP, Roe CR, Yergey AL, Liberato DJ 1985 Valproylcarnitine: a novel drug metabolite identified by fast atom bombardment and thermospray liquid chromatography-mass spectrometry. Clin Chim Acta 145:69–76
26. Melegh B, Kerner J, Jaszai V, Bieber LL 1990 Differential excretion of xenobiotic acyl-esters of carnitine due to administration of pivampicillin and valproate. Biochem Med Metab Biol 43:30–38
27. Ohtani Y, Endo F, Matsuda I 1982 Carnitine deficiency with hyperammonemia associated with valproic acid therapy. J Pediatr 101:782–785
28. Böhles H, Richter K, Wagner-Thiessen E, Schäfer H 1982 Decreased serum carnitine in valproate induced Reye Syndrome. Eur J Pediatr 139:185–186
29. Murphy JV, Marquardt KM, Shug AL 1985 Valproic acid associated abnormalities of carnitine metabolism. Lancet 1:820–821
30. Laub MC, Paetzke-Brunner I, Jaeger G 1986 Serum carnitine during valproic acid therapy. Epilepsia 27:559–562
31. Rozas I, Camina F, Paz JM, Alonso C, Castro-Gago M, Rodriguez-Segade S 1990 Effects of acute valproate administration on carnitine metabolism in mouse serum and tissues. Biochem Pharmacol 39:181–185
32. Sugimoto T, Woo M, Nishida N, Takeuchi T, Sakane Y, Kobayashi Y 1987 Hepatotoxicity in rat following administration of valproic acid. Epilepsia 28:142–146
33. Thurston JH, Hauhart RE, Carroll JE, Schiro JA 1984 Single doses of valproate reduced ketonemia in infant mice: reversal by L-carnitine. Pediatr Res 18:383A(abstr)
34. Karasawa T, Yoshida K, Furukawa K, Hosoki K 1972 Feedback inhibition of pantothenate kinase by coenzyme A and possible role of the enzyme for the regulation of cellular coenzyme A level. J Biochem 71:1065–1067
35. Halvorsen O, Skrede S 1982 Regulation of the biosynthesis of CoA at the level of pantothenate kinase. Eur J Biochem 124:211–215
36. Fisher MN, Robishaw JD, Neely JR 1985 The properties and regulation of pantothenate kinase from rat heart. J Biol Chem 260:15745–15751
37. Fisher MN, Neely JR 1985 Regulation of pantothenate kinase from various tissues of the rat. FEBS Lett 190:293–296
38. Unna K, Richards GV 1942 Relationship between pantothenic acid requirement and age in the rat. J Nutr 23:545–553
39. Hurley LK, Volkert NE 1965 Pantothenic acid and coenzyme A in the developing guinea-pig liver. Biochim Biophys Acta 104:372–376
40. Porubek DJ, Grillo MP, Baillie TA 1989 The covalent binding to protein of valproic acid and its hepatotoxic metabolite, 2-*n*-propyl-4-pentenoic acid, in rats and in isolated rat hepatocytes. Drug Metab Dispos 17:123–130
41. Holland PC, Sherratt HSA 1973 Biochemical effects of the hypoglycaemic compound pent-4-enoic acid and related non-hypoglycaemic fatty acids. Effects of the free acids and their carnitine esters on coenzyme A-dependent oxidations in rat liver mitochondria. Biochem J 136:157–171
42. Fukami MH, Williamson JR 1971 On the mechanism of inhibition of fatty acid oxidation by 4-pentenoic acid in rat liver mitochondria. J Biol Chem 246:1206–1212
43. Bressler R, Corredor C, Brendel K 1969 Hypoglycin and hypoglycin-like compounds. Pharmacol Rev 21:105–130
44. Corredor C, Brendel K, Bressler R 1967 Studies on the mechanism of the hypoglycemic action of 4-pentenoic acid. Proc Natl Acad Sci USA 58:2299–2306
45. Brendel K, Corredor CF, Bressler R 1969 The effect of 4-pentenoic acid on fatty acid oxidation. Biochem Biophys Res Commun 34:340–347
46. Stewart PM, Walser M 1980 Failure of the normal ureagenic response to amino acids in organic acid-loaded rats. Proposed mechanism for the hyperammonemia of propionic and methylmalonic acidemia. J Clin Invest 66:484–492
47. Walajtys-Rode E, Coll KE, Williamson JR 1979 Effects of branched chain α-ketoacids on the metabolism of isolated rat liver cells. II. Interactions with gluconeogenesis and urea synthesis. J Biol Chem 254:11521–11529