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Peritoneal Dialysis in Maple-Syrup-Urine Disease: Studies on Branched-Chain Amino and Keto Acids*

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Abstract. We report biochemical data on a child with MSUD who underwent peritoneal dialysis for severe metabolic imbalance. In confirmation of earlier data, the BCKA/BCAA ratios in blood had been found to be fairly stable in this patient during long-term dietary therapy.

The child became comatose at comparatively low levels of leucine and KICA (ca. 2 mM each). At this time the blood/cerebrospinal fluid ratio for BCAA's and BCKA's was markedly diminished. During peritoneal dialysis, peritoneal clearance was highest for KIVA, but less for MEVA and BCAA's (40–50% or urea clearance), and least for the allegedly most toxic metabolite, KICA. The differences for BCKA's may be due to their differential protein binding. Given these individual differences, 1.8 to 8.7 initial plasma volumes were cleared in 14 h with 24.2 l of dialysis fluid. In the same time, urinary excretion of BCAA's and BCKA's was much less efficient.

The data are discussed with regard to the pathobiochemical significance of high tissue levels of branched chain acids. A quantitative comparison between peritoneal dialysis and exchange transfusion is not yet possible.

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Abbreviations: BCAA's = branched-chain α-amino acids, *BCKA's* = branched-chain α-keto acids, *KICA* = α-keto-isocaproic acid, *KIVA* = α-keto-isovaleric acid, *MEVA* = α-keto-β-methyl-n-valeric acid, *MSUD* = maple syrup urine disease

Key words: Maple syrup urine disease – Peritoneal dialysis – Peritoneal clearances – Branched-chain α-amino acids – Branched-chain α-keto acids.

Introduction

Even during long-term treatment of MSUD by a carefully balanced dietary intake of BCAA's, patients are always at risk of metabolic imbalance during the course of reduced caloric intake or acute illness. Due to a hypercatabolic state the patients break down their own tissue proteins. This leads to accumulation in the blood and tissues of metabolites proximal to the enzyme block which interfere acutely with brain function [9]. In MSUD the oxidative decarboxylation (BCKA-dehydrogenase) of the three BCKA's is deficient [5] and these compounds (KIVA, MEVA and KICA) as well as their amino acid analogues (valine, isoleucine, alloisoleucine and leucine) accumulate. Most of the potentially dangerous episodic imbalances can be reversed by a high calorie, high carbohydrate intake, and by temporarily omitting leucine from the diet. However, in some instances these measures are inadequate and a further increase of the metabolite levels—especially of KICA—occurs with the patient falling into coma. Moreover, in the newborn period at the time of initial diagnosis extremely elevated concentrations of BCAA's and BCKA's have been observed in the blood and cerebrospinal fluid. In these situations the low renal elimination of the "neuro-toxic" metabolites [16] can necessitate multiple exchange transfusions [10,23] or peritoneal dialysis [1,8,11,19–21].

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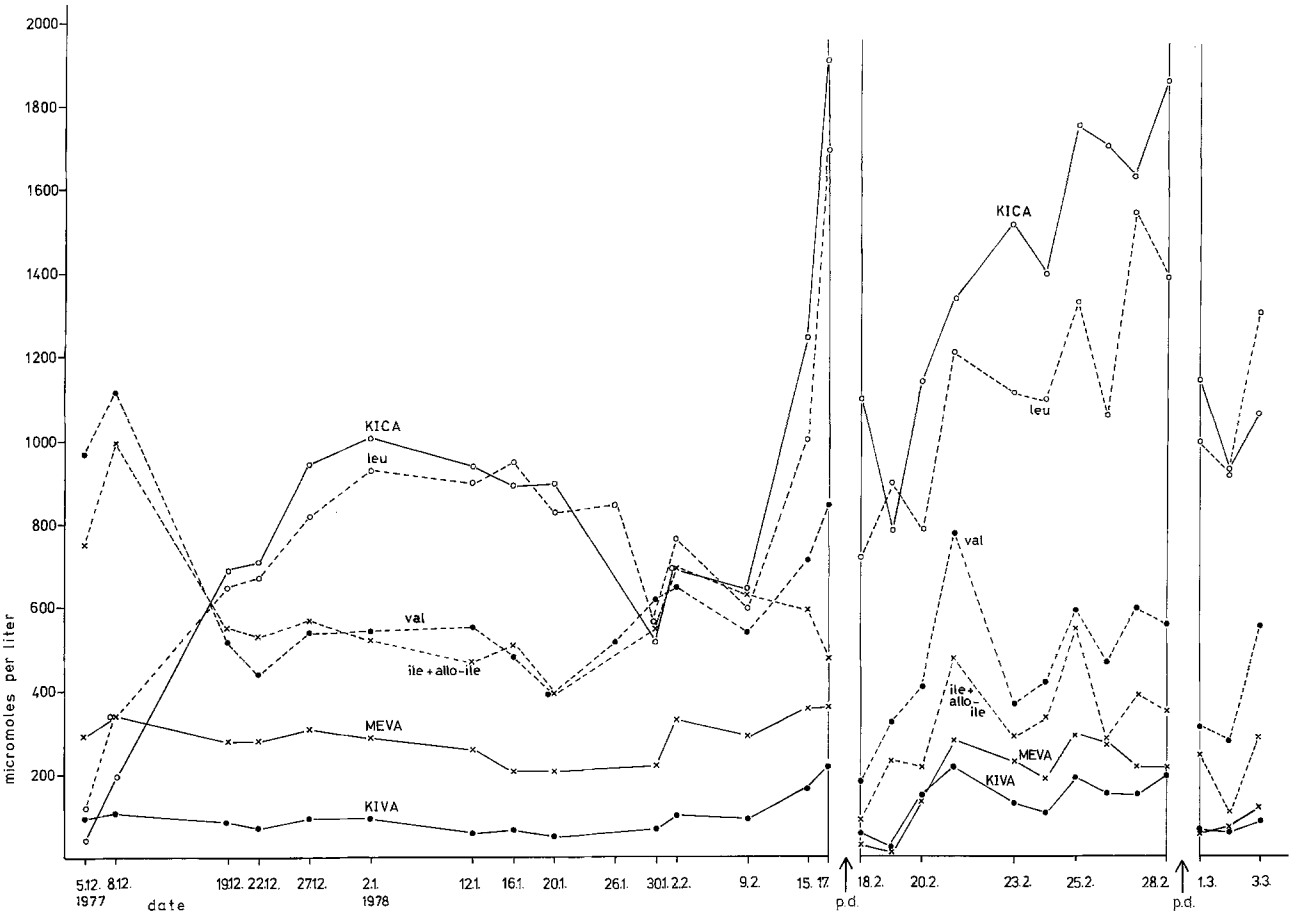

Fig. 1. Concentrations of the BCAA's and their keto-acid analogues in plasma during a 3-month-period. *p.d.* = peritoneal dialysis

We report the elimination of BCAA's and BCKA's during peritoneal dialysis, and their concentrations and ratios in the blood and cerebrospinal fluid of a patient with the classical type of MSUD during coma.

Case Report

The initial course of our patient C.P. has been reported by Hammersen et al. [10]. Dietary management in this child during the first 18 months of life was very difficult. Twelve episodes with markedly increased plasma leucine levels (up to 1.75 mM) occurred in connection with upper respiratory infections or small variations in the daily leucine intake. During one of these episodes at 6 months of age blood exchange transfusion was performed with a prompt clinical amelioration.

During a 3-month period we measured the plasma levels of BCAA's and their keto-acid analogues. The data are shown in Fig. 1. The different BCKA/BCAA pairs run parallel during the whole period with fairly constant ratios. The plasma concentrations of KICA and leucine were of the same magnitude, whereas the MEVA concentration was about half that for isoleucine + allo-isoleucine, and the concentration of KIVA was nearly one fifth that for valine [17].

At 18 months of age the girl was hospitalized because of a febrile upper respiratory tract infection. The plasma BCAA's were increased (leucine 1.3 mM, valine 0.6 mM, isoleucine + allo-isoleucine 0.56 mM). Initially the usual diet was administered in addition to a parenteral infusion of glucose, electrolytes, bicarbonate and antibiotics. Nevertheless, irritability and vomiting increased and a lethargic state developed within 24 h. A grand mal seizure occurred 48 h after hospitalization. Subsequently the patient was unresponsive and there was metabolic acidosis, hyperpnoea and a poor peripheral circulation. The plasma BCAA levels were greatly increased (leucine 1.7 mM, valine 0.85 mM and isoleucine + allo-isoleucine 0.48 mM). A lumbar puncture was performed to exclude meningitis. The cerebrospinal fluid was normal except for high concentrations of the three BCAA's and their ketoacids (see below). Peritoneal dialysis was initiated 5 h after the onset of coma.

Peritoneal Dialyses

The first dialysis was performed with the Peritosteril-IK solution (Fresenius, W.-Germany[1]) and lasted 14 h. The irrigation fluid was administered and removed through the same tube without interruption [4]. Each cycle of instillation and return of the fluid took from 8 to 10 min, amounting to 1.5 to 2 l/h. Five hours after the start of the dialysis the clinical state of the girl improved. She opened her eyes, the hypertonicity decreased, the tendon reflexes began to normalize, and peripheral circulation improved. After 9 h the child smiled, responded when spoken to, and was

1 Composition: per liter: 140.5 mVal Na, 4.0 mVal K, 3.5 mVal Ca, 1.5 mVal Mg, 105 mVal Cl, 44.5 mVal lactate, 1.5 g sorbite

obviously awake. The tendon reflexes and the muscule tone appeared normal. However, at the end of the dialysis the child became tachypnoeic. Respiratory difficulties started due to aggravation of the pulmonary infection and an effusion into the right pleural cavity. Drainage of the pleural effusion resulted in a pneumothorax which was effectively treated. Twelve hours later a cardio-respiratory arrest occurred and after resuscitation the child needed long-term artificial ventilation. During the following days, and while on a high calorie and balanced BCAA intake, the plasma leucine concentration remained constantly at 1.15 mM; valine and isoleucine + allo-isoleucine concentrations were low.

After 10 days the plasma leucine concentration again increased to 1.7 mM and a second peritoneal dialysis was performed. Unfortunately the dialysates after the 5th hour were lost and during the first 3 h the method of dialysis was changed: the fluid was left in the abdominal cavity for 20 min during each cycle. Within 10 h this second dialysis reduced the plasma leucine level to 1.1 mM.

During artificial ventilation a second pneumothorax occurred and 3 days later the pulmonary status deteriorated and the child died of cardiopulmonary insufficiency. At the time of death the plasma leucine level was 1.3 mM.

Methods

Venous blood samples were taken from scalp or antecubital veins between 8 and 9 a.m., at least 4 h after the last feed, for monitoring the blood BCAA's and BCKA's during dietary therapy. During peritoneal dialysis and intensive care treatment, blood was drawn from a catheter inserted into the jugular vein. Blood for amino acid analysis was heparinized and centrifuged immediately. The plasma was deproteinized with 4 volumes of 3% sulfosalicylic acid and after centrifugation the supernatant was analyzed on the same day. Blood for keto-acid analysis was centrifuged immediately and the serum stored at −70°C. Samples of cerebrospinal fluid and dialysis fluid were deproteinized with solid sulfosalicylic acid (50 mg/ml). During the peritoneal dialysis the irrigation fluid which returned from the abdominal cavity each hour was collected at 4°C. It was measured and aliquots were stored at −70°C. Further preparations of the samples were done immediately before analysis.

Amino acids were analyzed by column chromatography on an amino acid analyzer, using the short program for BCAA of Benson et al. [2].

Analysis of the BCKA's in the serum, cerebrospinal fluid and dialysis fluid was done by gas chromatography with nitrogen selective detection, according to Langenbeck et al. [14].

The concentration of urea in the plasma and irrigation fluid was analyzed with urease and the Berthelot reaction using the Boehringer set.

The peritoneal clearances of the individual BCAA's, their keto-acid analogues, and of urea as a reference compound for the effectiveness of dialysis were calculated from the hourly data with van Slyke's formula: $C = \frac{U \cdot V}{P}$, in which C = clearance in ml per min, U = concentration in irrigation fluid, P = serum or plasma concentration, and V = dialysis volume in ml per min.

Results

Comparison of the BCAA and BCKA Levels in Blood and Cerebrospinal Fluid. The concentrations of the BCAA's and their keto-acid analogues were measured simultaneously in blood and cerebrospinal fluid when the patient was comatose. The data are shown in Table 1. In both samples the concentrations of all the compounds were greatly increased. The blood/cerebrospinal fluid ratios for the individual BCAA concentrations, ranged from 1.4 to 2.4, and from 1.3 to 4.0 for the keto-acids. The ratios of the three BCKA/BCAA pairs in the cerebrospinal fluid differed from the ratios in blood. In the cerebrospinal fluid the KICA and MEVA concentrations were lower—and the KIVA level higher—than those in blood.

Peritoneal Dialysis. Figure 2 demonstrates the course of the serum level of each BCAA and BCKA during the first peritoneal dialysis. During dialysis the plasma concentrations of the three BCAA's decreased by 75% each, whereas a 55% decrease was found for KIVA and MEVA, and a 36% fall for KICA.

The elimination of each BCAA occurred at an almost constant rate (Fig. 3). For the BCKA's the

Table 1. Concentrations of the BCAA's and their keto-acid analogues in blood (plasma serum) and cerebrospinal fluid when the patient was in coma

	Valine	Isoleucine + allo-isoleucine	Leucine	KIVA	MEVA	KICA	Ratios		
							KIVA/ valine	MEVA/ isoleucine + allo-isoleucine	KICA/ leucine
Blood (μM)	844	477	1694	237	368	1918	0.28	0.77	1.13
Cerebrospinal fluid (μM)	359	338	892	189	101	483	0.53	0.30	0.54
Blood/cerebrospinal fluid ratio in C.P.	2.4	1.4	1.9	1.3	3.6	4.0			
Normal controls (mean ± s.d.)	9.8 ± 1.5[a]	10.1 ± 1.8[a]	7.7 ± 1.1[a]	3[b]	8[b]	6[b]			

[a] Humoller et al. [12]
[b] Langenbeck and Matthaei [15]

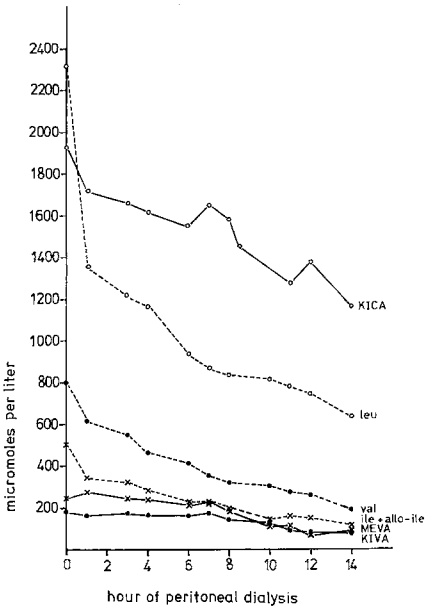

Fig. 2. Plasma (serum) levels of the BCAA's and BCKA's during the first peritoneal dialysis

elimination per hour decreased slightly with duration of the dialysis. During the first hour the removal was approximately twice as high as it was later. The hourly cumulative elimination of urea was nearly linear.

In Fig. 4 the respective peritoneal clearances calculated for each hour are given. The values for the three BCAA's increased with a reduced plasma concentration and varied from 0.9 to 3.8 ml/min for valine, from 0.8 to 4.0 ml/min for isoleucine + allo-isoleucine, and from 0.9 to 3.4 ml/min for leucine. The peritoneal clearances for the three BCKA's varied very little for each compound from the second hour onward (KIVA; 3.3 to 5.5 ml/min: MEVA; 2.2 to 3.5 ml/min: KICA; 1.1 to 1.9 ml/min). The clearance for urea increased during dialysis and varied from 4.2 to 8.0 ml/min. Thus, the peritoneal clearances of the BCAA's are very similar to each other. However, markedly different clearances were found for the BCKA's, the highest value being found for KIVA and the lowest for KICA.

In Table 2 the decrease of the blood levels of the BCKA's and BCAA's during dialysis is shown in

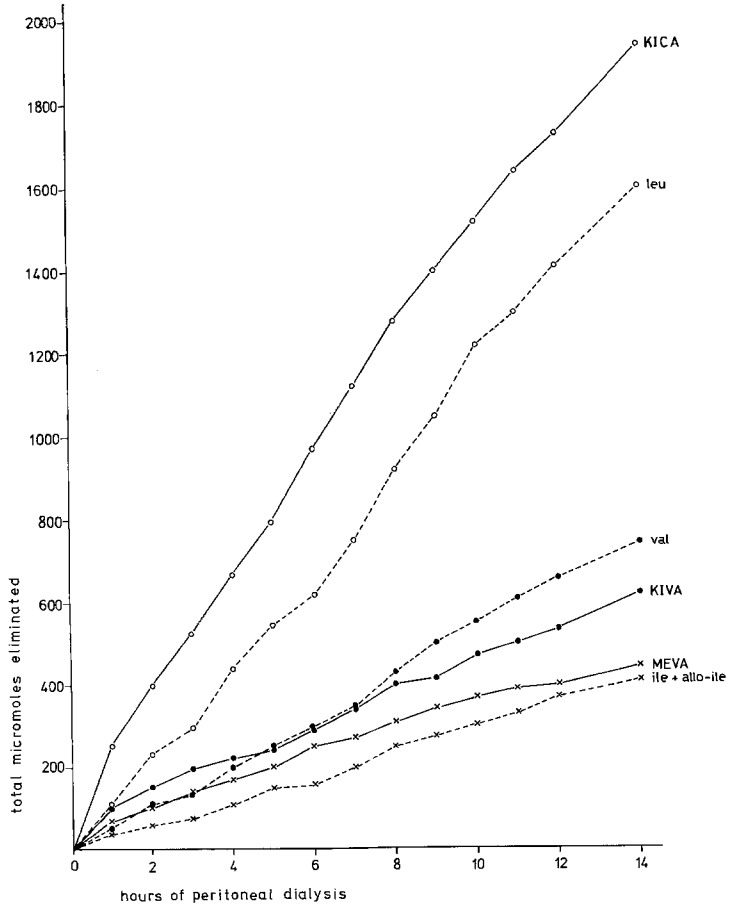

Fig. 3. Hourly cumulative elimination of the BCAA's and BCKA's during the first peritoneal dialysis

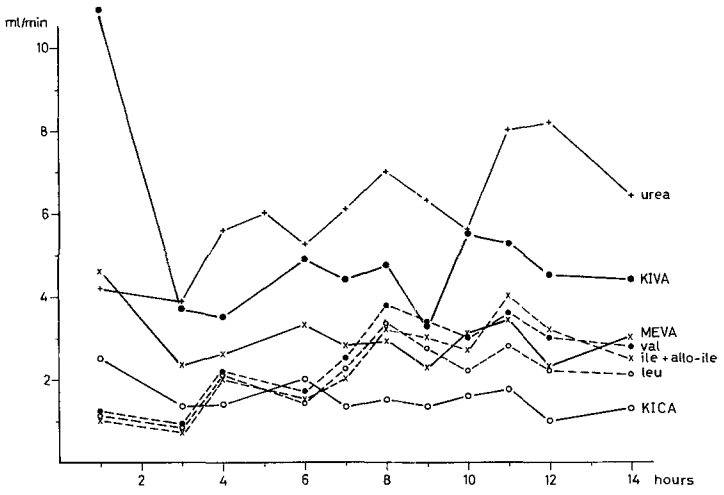

Fig. 4. Hourly clearance for BCAA's and BCKA's and urea during the first peritoneal dialysis

Table 2. Data for the first peritoneal dialysis. During 14 h 24.2 l of irrigation fluid were used. Body surface area was 0.36 m^2, plasma volume about 375 ml. For calculation of the cleared plasma volumes the initial plasma concentrations were assumed

	Valine	Isoleucine + allo-isoleucine	Leucine	KIVA	MEVA	KICA
Blood						
Initial concentration (μM)	802.9	506.9	2308.4	174.0	235.8	1923
End concentration (μM)	193.7	113.6	636.4	79.7	96.3	1167
Irrigation fluid						
Elimination total (μmoles)	739.7	408.4	1599.8	569	437	1943
		2748			2949	
Plasma volumes cleared	2.5	2.2	1.8	8.7	4.9	2.7
Urine						
Elimination total in 16 h (μmoles)	34.2	n.d.	100.8	76	775	1087
					1938	
Approximate clearances (ml/min) during 16 h	0.072	n.d.	0.071	0.45	3.42	0.59

relation to the total elimination (peritoneal and renal) of these compounds.

The data for the second peritoneal dialysis are incomplete. Figure 5 shows that there was only a small decrease in the blood concentrations of the BCAA's and BCKA's during the second dialysis although considerable amounts of BCAA's and BCKA's were removed from the body (Table 3).

Discussion

The steady state equilibria of BCAA's and BCKA's in blood reported here and in a previous paper [17] offer one explanation for the predominant effect of leucine on the clinical manifestations of MSUD. This amino acid is transaminated most efficiently to KICA, which in turn appears to be the most toxic of the BCKA's [9]. Accordingly, severe cerebral symptoms and coma may appear when both leucine and KICA reach blood levels of 2 mM.

The ratios of KIVA/valine and of MEVA/isoleucine + allo-isoleucine are much more in favour of the less toxic amino acids. Therefore, one of our patients (G.T.) was free of acute cerebral symptoms when her plasma concentrations were 4.69 mM for valine, 0.51 mM for KIVA, 3.08 mM for isoleucine + allo-isoleucine, 0.82 mM for MEVA but only 0.07 mM for leucine and 0.02 mM for KICA (unpublished observations). An earlier report [25] also points to the lesser clinical significance of valine and isoleucine.

There is a progressive increase of the BCKA/BCAA ratios in blood during peritoneal dialysis. At the same

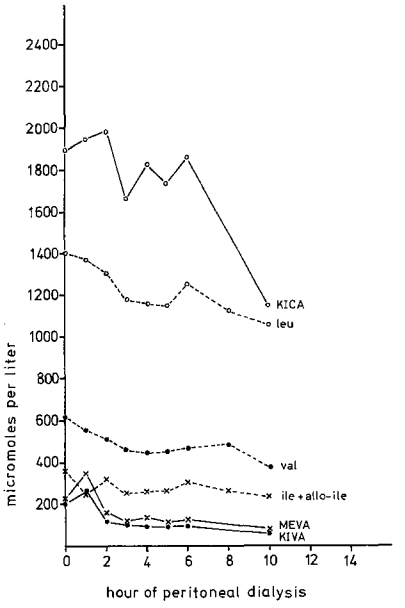

Fig. 5. Plasma (serum) levels of the BCAA's and BCKA's during the second peritoneal dialysis

time total elimination—renal and peritoneal—is higher for BCKA's than for BCAA's. This may be due to a preferential release of BCKA's from the tissues, either as a consequence of differing transport rates, the predominance of BCKA's in intracellular compartments, or both. Thus, the blood levels of BCKA's do not necessarily reflect the biochemical disturbances at the cellular level.

We do not yet know exactly the clinical significance of cerebrospinal fluid levels of either BCAA's or BCKA's. Normally, a high blood/cerebrospinal fluid ratio is found for BCAA's (lumbar cerebrospinal fluid [12]) and BCKA's (ventricular cerebrospinal fluid [15]). This is compatible with active transport from cerebrospinal fluid to blood at the blood-brain barrier. Such a transport system is known to exist for BCAA's (cf. ref. [18]). Very high tissue levels of branched-chain acids thus could interfere with active removal of these compounds from cerebrospinal fluid and lead to a diminished blood/cerebrospinal fluid ratio. Such a diminished ratio has been found in our patient C.P. for both the BCAA's and BCKA's. Plasma/cerebrospinal fluid ratios closer to normal have been reported for BCAA's in infants with MSUD at the time of initial diagnosis. They ranged from 2.8 to 5.8 and even to 40 [8] for leucine, from 2.6 to 5.5 for isoleucine, and from 3.6 to 7.6 for valine [24, 25]. Irrespective of the mechanisms involved, a very low blood/cerebrospinal fluid ratio of BCAA's may indicate a bad prognosis and may have contributed to the relatively early onset of coma in our patient when plasma leucine levels were 2 mM.

No detailed BCKA levels in cerebrospinal fluid in MSUD have been reported. Total BCKA's in plasma and cerebrospinal fluid had been shown to depend more on leucine than on valine [6] which is in agreement with our data (see above). Dreyfus and Prensky [7] found a ratio of 2.5 for total BCKA's in plasma and brain tissue. This is similar to our 3.3 plasma/cerebrospinal fluid ratio, calculated for total BCKA's.

In order to demonstrate the therapeutic efficiency of peritoneal dialysis we have presented a quantitative analysis for BCAA's as well as BCKA's. Like Gaull [8], we have taken urea as a reference for peritoneal clearance; it was about that reported by Gaull [8] and Boen [3]. In confirmation of Gaull's results [8] we found the BCAA clearances to be 40 to 50% of urea, without significant differences between the individual amino acids. There are large differences, however, in peritoneal clearance between the individual BCKA's, the highest clearance being found for KIVA, and lower rates for MEVA and KICA. The renal clearance rates are much lower for the BCAA's and for KIVA and KICA. Under the given biochemical condition of our patient, renal clearance of MEVA was higher than peritoneal clearance.

To fully appreciate the differences between peritoneal and renal transport the respective mechanisms must be taken into consideration. Peritoneal clearance reflects diffusion along a concentration gradient. The different rates for BCKA's may be a consequence of differential protein binding and are similarly during hemodialysis of uremic patients [22]. In contrast, the

Table 3. Incomplete data for the second peritoneal dialysis. Data for the first 5 h are shown during which 8.151 of irrigation fluid were used

	Valine	Isoleucine + allo-isoleucine	Leucine	KIVA	MEVA	KICA
Blood						
Initial concentration (μM)	570.8	359.0	1407.8	198.1	211.7	1872
Concentration after 5 h of dialysis (μM)	446.2	277.4	1148.6	92.4	117.6	1747
Removal within the first 5 h (μmoles)	358	211	894	349	425	1692
		1463			2466	

renal excretion rate reflects both glumerular filtration and tubular reabsorption and is rather inefficient [16]. There are mutual interactions of BCKA's in their renal clearance rates which depend on blood—and probably also on tissue—levels [16]. The data in Table 2 therefore cannot be generally applied. However, a higher renal clearance of BCKA's compared to BCAA's has already been reported [13].

High amounts of BCAA's, equivalent to the initial content of about two plasma volumes, and even higher equivalents of BCKA's were removed. The fact that only moderate diminution of the blood concentrations of branched-chain acids occurred during dialysis again points to the importance of cellular and intercellular pools in the pathogenesis of the acute clinical symptoms of MSUD [8].

According to our data and also to reports of other authors [1, 8, 11, 19–21], peritoneal dialysis is an effective treatment in life-threatening situations in MSUD with markedly increased metabolite levels. It is unfurtunate, however, that the most dangerous metabolites, i.e., leucine and KICA, have unfavourable kinetics not only in renal excretion but also in peritoneal dialysis.

Comparable data on BCAA's and BCKA's in blood exchange transfusion do not exist in the literature. Calculating the data for our own patient and for those given in another case in the literature [23] the amount of leucine removed by an exchange transfusion was equal in both instances (about 1250 µmoles), starting from different plasma leucine levels (1.6 to 3.5 mmoles/l, respectively) and using different exchange volumes of blood (400 and 200 ml/kg body weight, respectively). Data on BCKA's are as yet non-existent.

The therapeutic value of both procedures is unquestionable. The choice between these measures must be made by the clinician in the light of his own experience, the technical facilities, and the inherent risks.

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