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Enzymatic and pharmacokinetic studies on the metabolism of branched chain α-keto acids in the rat

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Summary

Michaelis-constants and enzyme activities for dehydrogenation and transamination of the three branched chain α-keto acids in liver, kidney, skeletal muscle, and brain of rats are reported.

After oral load only 11–22 % of the keto acids pass the liver unchanged.

Blood levels in pharmacokinetic and absorption studies are related to the Michaelis-constants. At the low keto-acid concentrations after oral application, dehydrogenation in the non-hepatic tissues is supposed to prevail over transamination. Data on feed efficiency of branched chain α-keto acids reported in the literature support this view. The chance for transamination is better after intravenous administration.

The transferability of our data to humans, and various factors influencing the efficiency of branched chain α-keto acids are discussed in connection with data reported in the literature.

Zusammenfassung

Michaelis-Konstanten und Aktivitäten von Dehydrogenasen und Transaminasen der drei verzweigten α-Ketosäuren Keto-Valin, Keto-Leucin und Keto-Isoleucin in Leber, Niere, Skeletmuskel und Gehirn von Ratten werden mitgeteilt.

Nach oraler Zufuhr passieren nur 11–22 % der Ketosäuren unverändert die Leber.

Aus pharmakokinetischen und Resorptions-Untersuchungen erhaltene Blutspiegel an Ketosäuren werden zu den Michaelis-Konstanten in Beziehung gesetzt. Bei den geringen Konzentrationen an Ketosäuren nach oraler Zufuhr kann angenommen werden, daß die oxidativen Prozesse in den nichthepatischen Geweben über die Transaminierung überwiegen. Daten über die Wachstumseffizienz von verzweigtkettigen α-Ketosäuren im Vergleich zu den entsprechenden Aminosäuren stimmen mit dieser Vorstellung überein.

Bei intravenöser Verabreichung müßten die Voraussetzungen für Transaminierung besser sein als nach oraler Zufuhr. Auf der Basis von Daten aus der Literatur werden die Übertragbarkeit unserer Befunde auf den Menschen und die verschiedenen Faktoren, welche die Effizienz der verzweigten α-Ketosäuren durch Einwirkung auf ihren Stoffwechsel beeinflussen können, diskutiert.

Key words: branched chain α-keto acids; 4-methyl-2-oxopentanoate, 3-methyl-2-oxopentanoate; 3-methyl-2-oxobutyrate; dehydrogenation; transamination; pharmacokinetics; absorption

Introduction

Retention of products of nitrogen metabolism is responsible for the intoxication in renal insufficiency. Therefore dietetic restriction of protein

to a minimum is emphasized in the treatment of chronic uremia. The risk of development of protein deficiency during such a management can be diminished by substitution of essential amino acids (2, 8, 23, 34, 48). Further improvement was suggested by Walser (45) with the introduction of α-keto analogues of essential amino acids such as tyrosine, methionine, and the branched chain amino acids. The idea behind this proposal is that these keto acids could be converted in the organism to the corresponding amino acids by transamination and thus act like "amino acids without nitrogen". Several investigators reported positive effects with the use of keto analogues in the treatment of chronic renal failure (7, 26, 44). Others, however, could not observe advantages compared with the corresponding amino acids (11, 25).

Successful use of keto analogues will depend on whether transamination or other metabolic processes will prevail. The present study on the rat as a model was performed in order to find out whether for the keto analogues of the branched chain amino acids valine, leucine and isoleucine it can be predicted which one of the two possible metabolic pathways will exceed at a given mode of application: transamination or oxidative degradation. Enzymatic activities and Michaelis-constants of transaminase and dehydrogenase systems in liver, kidney, skeletal muscle and brain were measured and interpreted on the basis of results from pharmacokinetic and absorption studies. From data on blood and tissue concentrations of the branched chain α-keto acids and the Michaelis-constants of the competing enzyme systems it can be deduced whether transamination or oxidation will prevail.

Materials and methods

Male Sprague-Dawley rats weighing 250 ± 20 g were used, obtained from "Gesellschaft für Medizinische Versuchstierzuchten" (Dr. Ivanovas, D-7967 Kisslegg). They were fed on a standard pellet diet (Altromin 1324) ad libitum. All experiments began between 8 and 9 a.m.

Chemicals

The branched chain α-keto acids 4-methyl-2-oxopentanoate, 3-methyl-2-oxopentanoate and 3-methyl-2-oxobutyrate as sodium salts were a gift of J. Pfrimmer & Co., Erlangen. Nagarse for the preparation of brain mitochondria, and heparin were obtained from Serva, Heidelberg. Enzymes and coenzymes were purchased from Boehringer Mannheim GmbH; all other chemicals were products of E. Merck, Darmstadt.

Preparation of tissues

Liver mitochondria were prepared in the mannitol medium described by Chance and Hagihara (13). Liver was ground in a Potter-Elvehjem homogenizer with a teflon pestle in a proportion of 1 g wet weight to 10 ml medium. Nuclei and cell debris were sedimented by centrifugation at 6000 g·min. The supernatant was decanted and centrifuged at 75,000 g·min. The sediment (mitochondria) was twice resuspended and washed by centrifugation at 75,000 g·min. Finally the pellet was taken up and re-homogenized in mannitol medium.

Muscle mitochondria were prepared by the method of Ernster and Nordenbrand (22) with the modification that the muscles from the hind legs were ground with sea

sand in a mortar rather than minced with scissors before centrifugation. For grinding, washing and suspension a medium described by Chappell and Perry (14) was used, containing (final concentrations) 0.1 mol/l KCl, 0.05 mol/l TRIS buffer pH 7.4, 0.005 mol/l $MgSO_4$, 0.001 mol/l EDTA, and 0.001 mol/l ATP.

Brain mitochondria were isolated according to Basford (5).

Tissue extracts for measurement of transaminases

Liver, kidney, muscles and brain were homogenized in the same way as for preparation of mitochondria. The homogenates were exposed to sonic vibration at 0–2 °C for 2 min at 100 Watt (Disintegrator Labsonic, Braun Melsungen). Larger particles were removed by centrifugation for 10 min at 600 g. The supernatant was used for the experiments.

Assay of enzyme activity.

Branched chain α-keto acid dehydrogenase activity was assayed in a test system which was developed in studies on the influence of pH, ferricyanide concentration, need of cofactors and linearity of the reaction. As a result, the following test composition was used:

800 µl 200 mmol/l potassium phosphate buffer pH 7.4
50 µl 16 mmol/l $K_3Fe(CN)_6$
50 µl solution of keto acids, concentration ranging from 0.1 to 200 mmol/l
100 µl mitochondrial suspension with 2–4 mg protein.

The mixture was shaken for 5 min at 37 °C in a water bath and the reaction stopped by adding 1 ml TCA (10 g/100 ml). For determination of the ferricyanide concentration before the reaction, TCA was added before the mitochondria. Furthermore, a blank was used without keto acid.

The TCA-precipitate was removed by centrifugation and in the clear supernatant the amount of non-reduced ferricyanide was measured in a photometer at 436 nm. Ferricyanide exhibits a broad absorption maximum around 420 nm. From the difference of the absorption before and after incubation, and after subtraction of the blank, the amount of reduced ferricyanide was calculated using a ferricyanide standard or a standard curve. 2 moles of ferricyanide are reduced per mol keto acid dehydrogenated.

Transaminase activity was assayed in a two-step procedure. First the branched chain α-keto acid was incubated with glutamate and the enzyme preparation. After deproteinization the amount of α-ketoglutarate produced was measured in the supernatant. The composition of the reaction mixture was:

600 µl 250 mmol/l potassium phosphate buffer pH 8.4
100 µl 200 mmol/l sodium glutamate
50 µl keto acid solution, concentration ranging from 0.1 to 400 mmol/l
50 µl distilled water
200 µl enzyme preparation.

The blank contained distilled water instead of keto acids. The mixture was shaken for 5 min at 37 °C in a water bath and the reaction was stopped by the addition of 1 ml 1 mol/l perchloric acid. The precipitate was removed by centrifugation and the supernatant was neutralized with 0.8 ml 0.5 mol/l K_3PO_4. After cooling with ice the potassium perchlorate precipitate was removed by centrifugation. In the supernatant α-ketoglutarate formed during transamination was measured enzymatically according to Bergmeyer and Bernt (6).

Michaelis-constants and maximal reaction velocities of dehydrogenases and transaminases were obtained from a double reciprocal plot according to Lineweaver and Burk (35).

Pharmacokinetic experiments

Intravenous injections and infusions were applied through a small catheter, inserted in the inferior vena cava exposed at laparotomy in ether anaesthesia. The

animals were held in Bollman-cages. Blood samples were drawn from the tip of the tail, and urine was collected using a catheter. The branched chain α-keto acids in blood, urine and liver were measured with the method of Walser et al. (46). Elimination constants and half life were calculated from a semi-logarithmic plot of the decrease of the blood level after injection or after stop of a continuous infusion. The total clearance was determined under steady-state conditions as the quotient of rate of infusion to blood level (20). For the assay of keto-acid concentrations in the liver, samples were removed by the freeze-stop method and homogenized in perchloric acid. The blood content of the liver was determined according to Holzer et al. (28), and the keto-acid content of the blood-free liver was calculated with the formula of Hohorst et al. (27):

$$\text{Keto-acid content of blood-free liver} = \frac{\text{Tissue content} - (\text{weight} \cdot \text{keto-acid content})_{\text{blood}}}{1 - (\text{Weight})_{\text{blood}}}$$

Results

Tables 1–3 show the kinetic data (Michaelis-constants, maximal velocities, tissue capacities) for dehydrogenation and transamination of the branched chain α-keto acids 3-methyl-2-oxobutyrate (in the following named "ketovaline" for simplification), 4-methyl-2-oxopentanoate ("ketoleucine") and 3-methyl-2-oxopentanoate ("ketoisoleucine") in liver, skeletal muscle, kidney and brain of rats. The data confirm the well-known fact that transamination of branched chain keto acids or amino acids plays no important part in the liver (33); the method used in this study is not sensitive enough to obtain exact results. The Michaelis-constants for transamination are mostly higher than those for dehydrogenation. The maximal turnover capacity of tissues is not practically relevant, because it is measured or calculated at substrate saturation. It can be used, however, for comparison of the relative share of various organs in the total turnover.

Table 1. Kinetic parameters of dehydrogenation and transamination of 3-methyl-2-oxobutyrate (keto-valine) in various rat tissues. Michaelis constants (k_m) and maximal reaction velocities (V_{max}) of dehydrogenation have been measured in mitochondrial suspensions, of transamination in tissue homogenates. V_{max} have been calculated per g wet weight of tissues and for the total organ. It was assumed that muscle mass constitutes 40 % of body weight (41).

Enzyme	Liver	Muscle	Kidney	Brain
Dehydrogenase				
K_m (μmol/l)	38.3	112	52.6	179
V_{max}: μmol · g^{-1} · h^{-1}	82.6	23.3	79.3	14.7
activity of total tissue (μmol · h^{-1})	1225	2330	179	26.3
Transaminase				
K_m (μmol/l)	not detectable	151	120	78.5
V_{max}: μmol · g^{-1} · h^{-1}		30	51.3	71
activity of total tissue (μmol · h^{-1})		3000	118	150

Table 2. Kinetic parameters of dehydrogenation and transamination of 4-methyl-2-oxopentanoate (keto-leucine) in various rat tissues. Experimental conditions as shown in table 1.

Enzyme	Liver	Muscle	Kidney	Brain
Dehydrogenase				
K_m (μmol/l)	44.3	not detectable	44.6	81.4
V_{max}: μmol · g^{-1} · h^{-1}	23	1.7	18.6	8
activity of total tissue (μmol · h^{-1})	293	170	36.9	15,3
Transaminase				
K_m (μmol/l)	not detectable	116	255	95
V_{max}: μmol · g^{-1} · h^{-1}		34.3	102	88
activity of total tissue (μmol · h^{-1})		343	215	183

Table 3. Kinetic parameters of dehydrogenation and transamination of 3-methyl-2-oxopentanoate (keto-isoleucine) in various rat tissues. Experimental conditions as shown in table 1.

Enzyme	Liver	Muscle	Kidney	Brain
Dehydrogenase				
K_m (μmol/l)	61	94	215	103
V_{max}: μmol · g^{-1} · h^{-1}	41	9.7	16.7	11.9
activity of total tissue (μmol · h^{-1})	423	970	38.2	21.5
Transaminase				
K_m (μmol/l)	not detectable	385	153	39.5
V_{max}: μmol · g^{-1} · h^{-1}		17.5	63.7	29.6
activity of total tissue (μmol · h^{-1})		1750	141	60

Table 4. Elimination constants (k) and biological half life (t/2) of branched chain α-keto acids. Elimination constants have been calculated from the decrease of the blood level after intravenous loads with 1 mmol keto acid per rat, or after stop of intravenous infusion. $t/2 = \ln 2/k$

	k (min^{-1})	t/2 (min)
Keto-valine	0.066	10.5
Keto-isoleucine	0,051	13.6
Keto-leucine	0.066	10.5

For estimation of the actual turnover, the concentrations of the keto acids in blood or tissues should be known. For this purpose we performed pharmacokinetic studies. The elimination constants and data of half life in table 4 show that the branched chain α-keto acids are fast metabolized, although this gives no indication of the pathways of elimination.

Table 5. Blood level during steady state, total clearance (Cl_t) and metabolic clearance (Cl_{met}) of branched-chain α-keto acids at two different rates of infusion.
Cl_t has been calculated as $\frac{\text{rate of infusion } (\mu mol \cdot min^{-1} \cdot kg^{-1}}{\text{steady state blood level } (\mu mol \cdot ml^{-1})} = ml \cdot min^{-1} \cdot kg^{-1}$

	Rate of infusion = 37 μmol·min^{-1}·kg^{-1}			Rate of infusion = 77 μmol·min^{-1}·kg^{-1}		
	blood level μmol/l	Cl_t	Cl_{met}	blood level μmol/l	Cl_t	Cl_{met}
Keto-valine	184	201	195	658	117	114
Keto-isoleucine	487	76	74	1453	53	47
Keto-leucine	213	174	172	494	156	155

Data under steady-state conditions during continuous intravenous infusions are shown in table 5. The excretion of unchanged keto acids in the urine is minimal and therefore the metabolic clearance is close to the total clearance. The fact that the total clearance at the higher rate of infusion is lower than at the low rate proves that already at 37 μmoles $\cdot min^{-1} \cdot kg^{-1}$ the range of linear relationship between rate of infusion and blood level is exceeded (1). This becomes comprehensible if steady-state concentrations in table 5 are compared with the Michaelis-constants of the enzymes in tables 1–3: At a rate of infusion of 37 μmoles$\cdot min^{-1} \cdot kg^{-1}$ the keto-acid concentrations in blood are already in the range or above the Michaelis-constants of the dehydrogenases and transaminases.

Considering whether dehydrogenation or transamination predominates in total turnover, one can start from the principle that at low substrat concentrations the enzyme with the lowest Michaelis-constant gets the main share. At the intravenous dose rates used in this study, the difference of the Michaelis-constants of dehydrogenases and transaminases are not of great importance. Since the almost exclusive mode of application of branched chain α-keto acids till now is the oral route, the question arises what concentrations of keto acids can be reached in blood after oral administration. To answer this question, the extent of enteral absorption was determined by the method of Dost (21). If the concentration in blood is plotted against time, the areas under these time-concentration curves are in direct proportion to the amounts of substance that reached the blood, irrespective of mode and duration of application. In the case of intravenous application 100 % of the substance enter the circulation and therefore the time-concentration curve after an intravenous load can be used as reference for calculation of the percentage of substance absorbed via other routes.

We compared the areas under the time-concentration curves after intravenous injection of 1 mmol keto acid and after oral application of 2 mmol keto acid by gastric tube. An example is shown in figure 1. Half of the area under the curve after oral load (twice the intravenous dose!) is 22 % of the area after intravenous load. Apparently only 22 % of the oral dose reached the blood. Since there is no excretion in the feces, this low percentage evidently is due to removal of 78 % during liver passage.

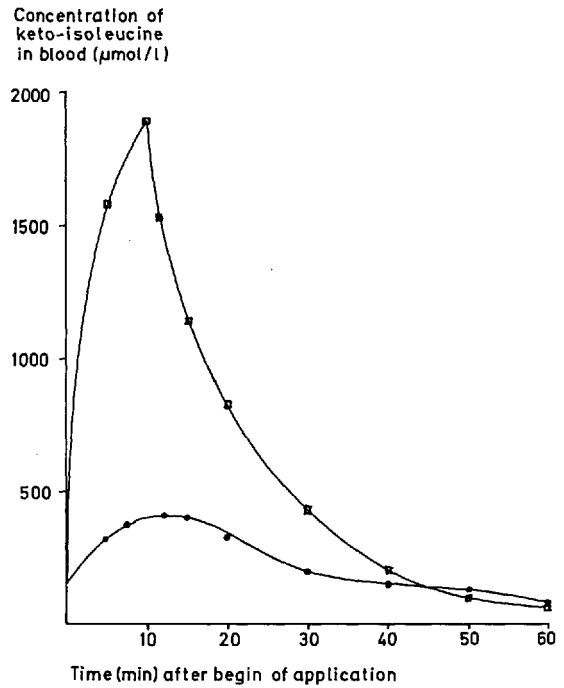

Fig. 1. Time-concentration curve of keto-isoleucine in blood after oral and intravenous load.
●——● oral (2000 µmoles), □——□ intravenous (1000 µmoles)

Transamination being negligible in liver, this portion must have been oxidized.

Data from this kind of experiments are summarized in table 6. The portion of branched chain α-keto acids passing the liver unchanged varies for the three keto acids from 11 to 22 %. The different situation after oral and intravenous application is also reflected in table 7, where a comparison is made of keto acid concentrations in blood and liver after both routes of administration. After oral load the concentrations in the liver are higher than in the blood; during intravenous infusion the concentrations in

Table 6. Percent of orally administered α-keto acids reaching the circulation after passage through the liver following an oral load with 2000 µmol by gastric tube. The figures have been obtained by comparison of the areas under the blood concentration curves after oral and intravenous loads.

	% of the dose having passed the liver unchanged
Keto-valine	11 ± 2.4
Keto-isoleucine	22 ± 5
Keto-leucine	12 ± 2.4

Table 7. Concentration of keto-isoleucine in liver and blood during intravenous infusion and after oral load by gastric tube. Values during infusion are steady-state concentrations. The values after oral load are taken 7–10 min after the application.

	α-keto acid concentration	
	liver µmol/kg	blood µmol/l
Intravenous infusion ($74\ \mu mol \cdot min^{-1} \cdot kg^{-1}$)	472	1600
Oral load (2000 µmoles)	384	273

Table 8. Peak concentrations of branched-chain α-keto acids in blood after oral load with 2000 µmoles in comparison with Michaelis-constants of dehydrogenases and transaminases in various tissues.

	Concentration in blood µmol/l		Michaelis-constants (µmol/l) Dehydrogenase			Transaminase		
	peak	30 %	M	K	B	M	K	B
Keto-valine	140	42	112	53	179	151	120	79
Keto-isoleucine	460	138	94	215	103	385	153	40
Keto-leucine	287	86	–	45	81	116	255	95

M = muscle; K = kidney; B = brain

blood-free liver tissue are only 30 % of the concentration in blood. It can be assumed with fairly good reason that similar to the liver other tissues reach also about 30 % of keto-acid concentrations of the blood. If one compares those peak concentrations in tissues after oral load (which are short-termed, see fig. 1) with the Michaelis-constants for dehydrogenation and transamination, predictions about the presumable fate of the keto acids can be made. Such a comparison is shown in table 8. With the exception of brain, the Michaelis-constants of transamination are higher than those of dehydrogenation. The concentrations of keto acids are lower or in the range of the Michaelis-constants. Therefore it can be expected that a greater portion is oxidized and not available for the desired transformation to amino acids.

Consequently there are two obstacles for a successful oral use of branched chain α-keto acids:

1. The high oxidative loss during liver passage.
2. The considerable portion of oxidative degradation in muscle and kidney.

Discussion

1. Dehydrogenases of branched chain α-keto acids

The dehydrogenase complex for branched chain α-keto acids is localized in the inner mitochondrial membrane. It is not known for certain till now whether there are three enzymes, one for each keto acid (42), or whether

one enzyme reacts with all three substrates with different affinity (19, 38, 40, 47). The existence of two enzymes, one for ketoleucine and ketoisoleucine, and one for ketovaline, is also discussed (9, 31).

Such a differentiation was not the aim of this investigation. The enzyme activity was measured in intact mitochondria, so as to imitate as close as possible in-vivo conditions. Ferricyanide has been used as electron acceptor to measure the dehydrogenation step of the complex. Measurement of the total complex with NAD as hydrogen acceptor proved to be unprecise in intact mitochondria due to mitochondrial swelling and turbidity changes under the influence of keto acids.

A survey over Michaelis-constants reported in the literature is given in table 9. In spite of the different methods used, many of the reported values

Table 9. Literature on Michaelis-constants for dehydrogenation of branched-chain α-keto acids.

Substrate	Organ	Method	K_m(μmol/l)	Author
Keto-valine	rat liver	Ferricyanide	38.3	this paper
		Ferricyanide	71	42
		$^{14}CO_2$	100	10
		$^{14}CO_2$	200	24
		NAD	13	39
	ox liver	NAD	15.6	38
	rat muscle	Ferricyanide	112	this paper
		$^{14}CO_2$	180	43
	rat kidney	Ferricyanide	52.6	this paper
	bovine kidney	NAD	40	40
Keto-isoleucine	rat liver	Ferricyanide	61	this paper
		Ferricyanide	60	42
		NAD	10.5	39
		2,6-DCIP	870	31
		$^{14}CO_2$	100	10
	ox liver purified	Ferricyanide	2500	18
		NAD	17.2	38
	rat muscle	Ferricyanide	94	this paper
		$^{14}CO_2$	30	37
		$^{14}O_2$	230	43
	rat kidney	Ferricyanide	215	this paper
	bovine kidney	NAD	37	40
Keto-leucine	rat liver	Ferricyanide	44.3	this paper
		NAD	14.6	39
		$^{14}CO_2$	200	47
		$^{14}CO_2$	60–110	24
		2,6-DCIP	2000	31
	ox liver	NAD	8,7	38
	ox liver purified	Ferricyanide	3500	18
	rat muscle	$^{14}CO_2$	17–27	36
		$^{14}CO_2$	25	37
	rat kidney	Ferricyanide	44.6	this paper
	bovine kidney	NAD	50	40

fall in the same order of magnitude. Large deviations are found mostly in higher purified preparations (18, 31). Those high Michaelis-constants suggest that the enzyme complex has been altered during the purification procedure.

A comparison of enzymatic activities reported in the literature is impossible not only because of lack of detailed data that would allow a recalculation of the activities per gram of wet tissue, but also because the nutritional state of the animals and the composition of the test systems are not comparable or not specified. Branched chain α-keto acid dehydrogenase is regulated by ATP as well as by hormonal and nutritional factors.

2. Transaminases

Studies on transaminases for branched chain amino acids (or keto acids) led to the discovery of three isoenzymes (29). The activity is distributed among mitochondria and cytosol (3, 4, 12, 30). In order to obtain the total activity, we used homogenates treated by sonic vibration. Comparison of our results with data from the literature is hardly possible since in almost all studies reported the branched chain amino acids were used as donor of the amino group and α-ketoglutarate as acceptor. For our problem, however, the opposite direction is relevant, with branched chain-keto acids as amino acceptors. Shinnick and Harper (41) report activities of the transamination from glutamate to ketoleucine in various tissues (Michaelis-constants have not been measured). These activities are slightly lower than ours: muscle 28,1, kidney 63,2, brain 46,7 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (for comparison see table 2).

3. Importance of the route of administration

According to table 6 only 11–22 % of orally administered α-keto acids cross the liver unchanged and are available for the remainder of the organism. In fair accordance with these findings are studies on growth efficiency of branched chain α-keto acids when substituted for branched chain amino acids. Hauschildt (24) reports a growth efficiency of 17 % if in a rat diet the branched chain amino acids are replaced by their keto analogoues. Chawla et al. (15) found 20 % to 27 % efficiency when substituting ketoleucine for leucine and 30 % with ketovaline for valine. The efficiency in the latter study, however, increased to 80 % if only ¼ of the daily valine requirement was provided as ketovaline. Chow and Walser (16) demonstrate a feed efficiency of 33 % and 50 % if leucine or valine is replaced by the appropriate keto analogue in an amino acid diet. Generally it shows up that the feed efficiency of branched chain α-keto acids is inversely related to total nitrogen intake and amount of keto acid given.

The somewhat better efficiency in these feeding studies in comparison to our results may be attributed to the fact that the keto acids are not given as a single load but rather distributed with the diet. Therefore it is likely that the keto acid concentrations reached in the liver during passage are lower and thus the oxidation slower. The high efficiency in Walser's experiments (16) may be explained by the fact that he used diets composed of free amino acids. Hauschildt (24) has shown that the activity of branched chain α-keto acid dehydrogenase of the liver is considerably

lower ($^1/_5$ to $^1/_6$) under an amino acid diet than under protein diet; moreover, protein restriction has a similar reducing effect on dehydrogenase activity. That means, the efficiency of keto acid supplementation can be varied by changing the nutritional conditions. There is no biochemical explanation, however, for the result of Walser (17) that valine can be completely replaced by ketovaline in a nitrogen-restricted diet. Even after a 100 % liver passage, transamination and oxidation processes in the other organs would compete.

From our results it can be deduced that the chances for a positive effect of branched chain α-keto acids are better on intravenous than on oral administration.

4. Transferability of the results to humans

Whether the results of the rat model can be transferred to the situation in man depends on the degree of comparability of the enzymatic outfit. To our knowledge systematic studies to this point are only reported by Khatra et al. (32). According to these, the activity of branched chain α-keto acid dehydrogenase in human liver is only about $^1/_{17}$ of rat liver. Of the total dehydrogenase activity of the organism in the rat are found in the liver 76 % (ketoleucine), 69.6 % (ketovaline), and 67.14 % (ketoisoleucine); in the muscles 7.91 % (ketoleucine), 14.19 % (ketovaline), and 7.91 % (ketoisoleucine). In man the liver contains 32.43 % (ketoleucine), 31.4 % (ketovaline), and 28.54 % (ketoisoleucine); the muscles contain 61.5 % (ketoleucine), 59.5 % (ketovaline) and 61.2 % (ketoisoleucine) of the total activity. These data show that the conditions for passing the liver are more favourable in man. Moreover, in liver cirrhosis branched chain α-keto acid dehydrogenase activity is considerably decreased (32), so that a better efficiency of orally administered keto acids can be expected. Portosystemic shunting too should increase the efficiency, since the dehydrogenation in the liver is by-passed. Taking further into account that protein deficiency decreases branched chain α-keto acid dehydrogenase in liver, and diet composition has influence on it (24), all these considerations show that there are many conditions that can change the efficiency of orally given keto acids and explain conflicting results as quoted in the introduction.

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