0031-3998/87/2205-0591$02.00/0
PEDIATRIC RESEARCH

Vol. 22, No. 5, 1987
Printed in U.S.A.

Comparison of the Catabolism of Branched-Chain L-Amino Acids in Cultured Human Skin Fibroblasts

PETER SCHADEWALDT AND UDO WENDEL

Institut für Physiologische Chemie II [P.S.] and Kinderklinik C [U.W.], Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf, FRG

ABSTRACT. Using 1-^{14}C-labeled substrates, the metabolism of naturally occurring branched-chain L-amino acids was studied in incubations with cultured human skin fibroblasts derived from normal subjects and from a patient with maple syrup urine disease (variant form). Practically saturating conditions were reached at 1 mmol/liter of substrate and metabolic rates remained essentially constant up to 120 min. In control fibroblasts, the transamination of ^{14}C-labeled leucine, valine, isoleucine, and allo-isoleucine (1 mmol/liter) was about 26, 13, 12, and 5 nmol/90 min/mg of cell protein, respectively. The portion of transamination products undergoing oxidative decarboxylation within the cells was about 17, 43, 34, and 23%, respectively. With the maple syrup urine disease cell line, comparable transamination rates were found. As compared to the findings with normal cells, however, $^{14}CO_2$ production from the above mentioned substrates was reduced and amounted to 14, 11, 25, and 45%, respectively. Thus it appeared that residual branched-chain 2-oxo acid dehydrogenase activity was differently reduced towards the four 2-oxo acid substrates. (*Pediatr Res* 22: 591–594, 1987)

Abbreviations

MSUD, maple syrup urine disease
HEPES, (4-(2-hydroxyethyl)-1-piperazine-ethansulphonic acid
PBS, phosphate-buffered saline
HPLC, high performance liquid chromatography

In MSUD the activity of the mitochondrial branched-chain 2-oxo acid dehydrogenase complex (EC 1.2.4.4) is impaired and metabolites proximal to the decarboxylation step (branched-chain amino and 2-oxo acids) accumulate in tissues and body fluids (1, 2). *S*-2-oxo-3-methylpentanoate, the transamination product of L-isoleucine, is the only chiral branched-chain 2-oxo acid. At elevated serum levels, it has been shown to undergo racemization *in vivo*, apparently via nonenzymic enolization (3, 4). In MSUD, *R*-2-oxo-3-methylpentanoate thus formed may be oxidatively decarboxylated corresponding to the residual activity of the mutant enzyme and further degraded by the so called "*R*-pathway" of isoleucine catabolism (5, 6). A substantial portion, however, is retransaminated and L-allo-isoleucine accumulates (7–10). In healthy subjects, this amino acid is not found in the blood. Analysis of the patterns of L-isoleucine and L-allo-isoleucine levels in plasma of MSUD patients as well as loading experiments with *R,S*-2-oxo-3-methylpentanoate suggest a L-allo-iso-leucine pool with its own dynamics (8, 11, 12).

Little is known about the regulation of L-isoleucine and L-allo-isoleucine metabolism in human and animal tissues. In most studies on branched-chain amino and 2-oxo acid metabolism, solely L-leucine and/or L-valine or the corresponding 2-oxo acids were used as substrates. The aim of the present study was to demonstrate expected differences in the catabolism of the naturally occurring branched-chain amino acids including L-isoleucine and L-allo-isoleucine in human tissue. We therefore examined the transamination and oxidative decarboxylation rates in cultured skin fibroblasts of normal subjects and of a patient with variant MSUD using ^{14}C-labeled branched-chain amino acids.

MATERIALS AND METHODS

Chemicals. Unless otherwise noted, chemicals were purchased in the highest available purity from Merck, Darmstadt, or Sigma, Deisenhofen, FRG. Enzymes and coenzymes were from Boehringer, Mannheim, FRG, Acylase I (EC 3.5.1.14) was from Sigma, and Dowex 50 WX8, 50-100 mesh, was obtained from Serva, Heidelberg, FRG.

1-^{14}C-labeled (leucine, 2.1 GBq/mmol; valine, 2.0 GBq/mmol) and U-^{14}C-labeled (leucine, 12.9 GBq/mmol; valine, 10.7 GBq/mmol; isoleucine, 12.2 GBq/mmol) L-amino acids as well as $NaH^{14}CO_3$ (2.1 GBq/mmol), $K^{14}CN$ (370 MBq/mmol) and [methyl-^{14}C]toluene standard were from New England Nuclear, Dreieich, FRG, or Amersham-Buchler, Braunschweig, FRG. Prior to use, unlabeled and radioactive amino acids were checked on a LC 6000 amino acid analyzer (Biotronik, München, FRG) in runs with and without ninhydrin, respectively. The purity was as specified by the suppliers.

1-^{14}C-labeled L-isoleucine (8.6 MBq/mmol) and L-allo-isoleucine (13.3 MBq/mmol) were synthesized from $K^{14}CN$ and the respective unlabelled amino acid essentially as described in (13). The products were characterized by coupled gaschromatography-mass spectrometry of the trimethylsilyl-derivatives (*cf.* 14). Purity (98%) was checked by amino acid analysis as described above and by treatment with D-amino acid oxidase (EC 1.4.3.3) according to (15).

Cell culture. Human fibroblasts were obtained from skin biopsies of normal subjects (strain RE and WE, mixed at equal portions for assays) and of a patient with a variant form of maple syrup urine disease (intermittent type, strain JA). MSUD has been diagnosed in a 6-yr-old female, when she became ketoacidotic and lethargic triggered by a febrile illness. Cells were multiplied in monolayer culture in Eagle's minimum essential medium containing HEPES (20 mmol/liter), pH 7.2, 10% fetal calf serum, and penicillin and streptomycin. After 10–15 passages, fibroblasts at confluency were harvested by trypsinization,

Received February 20, 1987; accepted July 6, 1987.
Correspondence Dr. Peter Schadewaldt, Institut für Physiologische Chemie II, Universität Düsseldorf, Moorenstrasse 5, D-4000 Düsseldorf, West Germany.
Supported by Grant We 614/6-1 from the Deutsche Forschungsgemeinschaft.

suspended in culture medium, washed twice with NaCl solution (0.154 mol/liter) by centrifugation and resuspended in phosphate buffered saline, Dulbecco's modification (PBS), with bovine serum albumin (1.5 g/liter) and D-glucose (12.4 mmol/liter). In general, cell concentration was adjusted to 1–2 × 10^6 cells/ml and 0.4 ml of the suspension was used for one assay. Cell screening for mycoplasma contamination (16) was negative.

Incubations. Fibroblasts (0.2–1 mg of cell protein) were incubated in a final volume of 0.9 ml at 37° C in a shaking water bath. $^{14}CO_2$-tight incubation flasks were made from 22-ml glass scintillation vials essentially as described in (17). The medium comprised PBS, D-glucose (5.5 mmol/liter), bovine serum albumin (0.07%) and L-[^{14}C]amino acids as given in "Results." The reaction was started by addition of cell suspension and was terminated by injection of 0.3 ml $HClO_4$ (2 mmol/liter). Blanks were likely prepared, but cells were omitted. All samples were run in duplicate.

Assays. The $^{14}CO_2$ release during incubation was completely absorbed in 0.5 ml ethanolamine-ethylenglycol (1:2, v:v) and measured by liquid scintillation counting (17). Thereafter, the 2-oxo-[^{14}C] acids in the medium were oxidatively decarboxylated by addition of 0.3 ml H_2O_2 solution (30%) as in (14) and the liberation of $^{14}CO_2$ was determined with fresh trapping solution as described above. Stability of 2-oxo acids and the oxidation procedure were examined with 2-oxo-4-methyl[1-^{14}C]pentanoate (prepared according to Ref. 18). $^{14}CO_2$ recovery was checked with $NaH^{14}CO_3$ solutions and counting efficiency by internal standardization with [^{14}C]toluene.

Protein concentrations were estimated by the Lowry procedure (19). Bovine serum albumin was used as a standard. One mg of cell protein was equivalent to 2.1 × 10^6 fibroblasts.

Estimation of total 2-oxo acid content was a modification of the method described elsewhere (20). At the end of the incubation, 0.8 ml of the assay mixture was withdrawn from the incubation flask, mixed with 0.1 ml standard solution (2-oxopentanoate, 33 μmol/liter; 2-oxohexanoate, 66 μmol/liter) and deproteinized with 0.5 ml $HClO_4$ (2 mol/liter). After centrifugation, 1 ml solution was mixed with 0.2 ml *o*-phenylendiamine (5 g/liter) in HCl (37%) and incubated for 60 min at 90° C. Then, pH 6 was adjusted by addition of 0.5 ml sodiumphosphate (0.5 mol/liter) and an appropriate volume of NaOH (8 mol/liter) (21). After extraction with $CHCl_3$ (3 × 1 ml) and evaporation to dryness, the quinoxalinol derivatives were dissolved in 200 μl methanol and analyzed on a HPLC system (Merck, Darmstadt, FRG) using a Nucleosil $5C_{18}$ column (250 × 4 mm, Macherey-Nagel, Düren, FRG) equipped with a guard column (4 × 4 mm, Lichrosorb 100 RP-18, from Merck) and 27% CH_3CN at 1.3 ml/min. Fluorimetric detection was as described previously (20). Blanks and calibration standards (2-oxo-4-methylpentanoate and 2-oxo-methylbutanoate, 0–10 nmol/assay) with suspensions of unincubated fibroblasts were run in parallel.

Calculations. Metabolic rates were calculated from the appropriate $^{14}CO_2$ liberation on the basis of the specific radioactivity of the C_1-atom of the respective L-amino[^{14}C]acid in the medium. Transamination was calculated as the sum of $^{14}CO_2$ production and 2-oxo[^{14}C]acid release. Results are presented as means ± SD of n independent experiments performed on different days. Statistically significant differences were determined by Student's paired *t* test.

RESULTS

Time and concentration dependence. Validity of methods was tested with normal fibroblasts. At 1 mmol/liter, the rate of $^{14}CO_2$ and 2-oxo[1-^{14}C]acid release from all four 1-^{14}C-labeled branched-chain L-amino acids remained essentially constant up to 120 min of incubation and subsequent incubations were carried out for 90 min. The metabolic activity of incubated fibroblasts (0.2–1 mg of cell protein/ml) was linearly correlated to cell mass (data not shown).

In a number of incubations with L-[1-^{14}C]leucine and L-[1-^{14}C]valine (30–90 min, 0.01–1 mmol/liter), 2-oxo-[1-^{14}C]acid release was estimated in parallel samples by chemical decarboxylation (x) as well as by HPLC analysis of the quinoxalinol derivatives (y). The data showed a rather good linear relationship ($y = 1.3x - 1.1$, $r = 0.98$, $p < 0.001$). The oxidative method yielded somewhat lower values (−13 ± 14%), apparently irrespective of incubation conditions. Underestimation by the oxidative method due to spontaneous decarboxylation of ^{14}C-labeled 2-oxo acids could be excluded in experiments with authentic 2-oxo-4-methyl-[1-^{14}C]pentanoate.

Results of experiments on the concentration dependence of $^{14}CO_2$ production and 2-oxo-[1-^{14}C]acid release from branched-chain amino[1-^{14}C]acids (0.01–2 mmol/liter) are shown in Figure 1. The shape of curves appeared to be approximately hyperbolic. Although metabolic rates tended to be slightly enhanced further with increasing substrate concentrations, practically saturating concentrations were reached at 1 mmol/liter. Half maximal effects were obtained around 0.1 mmol/liter. $^{14}CO_2$ production was apparently determined by the transamination rate, since the relation of decarboxylation to transamination (sum of $^{14}CO_2$ and 2-oxo-[1-^{14}C]acid release) was essentially constant over the entire concentration range.

Under the conditions applied, the consumption of [^{14}C]leucine at 0.01 mmol/liter was 15 ± 2% (mean ± SD, $n = 4$). At higher concentrations and with the other substrates, consumption was substantially lower. Changes of substrate concentration during incubation were therefore considered negligible.

Comparison of catabolism. Catabolism of branched-chain L-amino-[1-^{14}C]acids in normal and MSUD fibroblasts was comparatively studied under standard incubation conditions (90-min incubation at 1 mmol/liter). The results are summarized in Table 1. In normal fibroblasts, $^{14}CO_2$ production from leucine, valine, and isoleucine was similar (about 5 nmol/90 min/mg of cell protein). $^{14}CO_2$ release from allo-isoleucine was distinctly lower and amounted to 28 ± 4% of the value obtained with isoleucine.

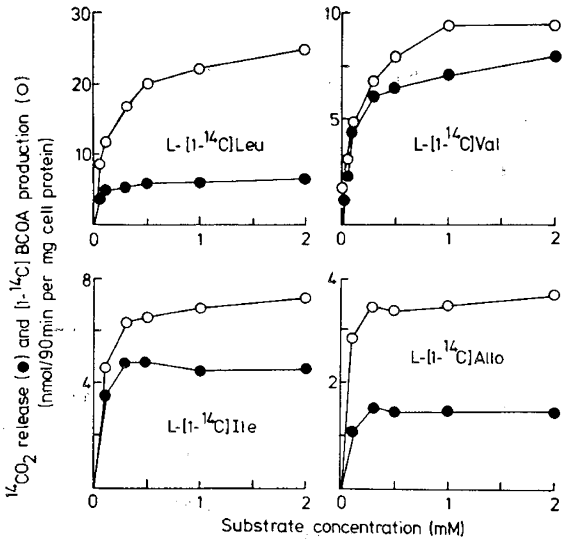

Fig. 1. Concentration dependence of $^{14}CO_2$ release and 2-oxo-[1-^{14}C] acid production from branched-chain L-amino[1-^{14}C]acids in cultured human skin fibroblasts derived from normal subjects. Means of two experiments are shown. Cells were incubated at 37° C for 90 min. In incubations with leucine and valine, 18.5 kBq of ^{14}C-labeled compound were added to each assay. Substrate concentrations ranged from 0.01 mmol/liter (2.1 kBq/nmol) to 2 mmol/liter (10.3 Bq/nmol). For the sake of clearness, some results have been omitted. In experiments with isoleucine (8.6 Bq/nmol) and allo-isoleucine (L-Allo, 13.3 Bq/nmol), the specific radioactivities remained constant over the entire concentration range (0.1–2 mmol/liter). Labeled 2-oxo acids (branched-chain 2-oxo acids) were determined by chemical oxidation. For details see "Materials and Methods."

Table 1. *Catabolism of 1-^{14}C-labeled branched-chain L-amino acids in cultured human skin fibroblasts derived from normal subjects (control) and from patient with variant MSUD**

[1-^{14}C]-labeled L-amino acid (1 mM)	(*n*)	$^{14}CO_2$ production	2-Oxo[1-^{14}C]acid release (nmol/90 min/mg cell protein)	[1-^{14}C]amino acid transamination	$^{14}CO_2$ production / transamination (%)
Control fibroblasts					
Leucine	(10)	4.3 ± 1.2	21.4 ± 5.1†	25.7 ± 5.6	17.0 ± 4.3
Valine	(7)	5.5 ± 1.7	7.2 ± 1.5‡	12.7 ± 3.0	42.6 ± 5.0
Isoleucine	(6)	4.0 ± 0.6	7.8 ± 1.2†	11.8 ± 1.7	33.8 ± 3.3
Allo-isoleucine	(6)	1.1 ± 0.3*	3.7 ± 0.6†§	4.8 ± 0.7§	23.5 ± 4.9§
MSUD fibroblasts					
Leucine	(3)	0.6 ± 0.2	27.0 ± 4.4	27.6 ± 4.5	2.2 ± 0.7
Valine	(3)	0.6 ± 0.2	11.2 ± 2.0	11.8 ± 1.9	5.1 ± 2.0
Isoleucine	(3)	1.0 ± 0.2	11.3 ± 0.8	12.4 ± 0.6	8.5 ± 2.3
Allo-isoleucine	(3)	0.5 ± 0.1‖	4.3 ± 0.3§	4.8 ± 0.3§	10.8 ± 1.6‖

* Cells were incubated with 1 mmol/liter of substrate (37° C, 90 min). Specific radioactivities (ile, 8.6 Bq/nmol; allo-ile, 13.3 Bq/nmol) of both leucine and valine in experiments with normal (strains RE and WE, mixed) and MSUD cells (strain JA) were 10–40 Bq/nmol and 40–45 Bq/nmol, respectively. For further experimental details see "Materials and methods." Transamination was calculated as the sum of $^{14}CO_2$ and 2-oxo[1-^{14}C] acid release. The ratio of $^{14}CO_2$ production to transamination represents the percentage of transamination products undergoing oxidative decarboxylation. Results are means ± SD, number of experiments in parentheses. Statistically significant differences (by Student's paired *t* test): 2-oxo[1-^{14}C]acid *versus* $^{14}CO_2$ release.

† $p < 0.001$.

‡ $p < 0.01$; allo-isoleucine *versus* isoleucine.

§ $p < 0.005$.

‖ $p < 0.05$.

With each substrate, the release of the corresponding 2-oxo[^{14}C]-acid was significantly higher than $^{14}CO_2$ production. The release of 2-oxo-4-methyl[^{14}C]pentanoate was three times that of 2-oxo-3-methyl-[^{14}C]butanoate and *S*-2-oxo-3-methyl-[^{14}C]pentanoate (from ile). *R*-2-oxo-3-methyl[^{14}C]pentanoate was released at 47 ± 8% of the rate of its enantiomer.

Thus, leucine was transaminated at about twice the rate of valine and isoleucine, and the transamination of allo-isoleucine was about half of isoleucine (40 ± 5%). The portion of transamination products undergoing further degradation within the cells varied considerably from about one-half in incubations with valine to one-sixth in experiments with leucine.

In MSUD fibroblasts, transamination of branched-chain amino[^{14}C]acids neatly corresponded to the appropriate data obtained with normal cells, suggesting that branched-chain amino acid aminotransferase activity (EC 2.6.1.42) remained unchanged by the mutation. As anticipated however, $^{14}CO_2$ production was lower. As compared to normal cells, the residual decarboxylation activity was differently reduced toward each substrate and amounted to 14, 11, 25, and 45% in incubations with leucine, valine, isoleucine, and allo-isoleucine, respectively. As related to the corresponding transamination rates, *R*-2-oxo-3-methylpentanoate was apparently the most suitable substrate for the mutant enzyme in this MSUD cell line.

In additional experiments with U-^{14}C-labeled L-leucine, L-valine, and L-isoleucine with both normal and deficient fibroblasts, the release of ^{14}C-labeled 2-oxo acids was comparable to that observed with 1-^{14}C-labeled substrates, whereas $^{14}CO_2$ production was approx. twice as high (data not shown).

DISCUSSION

To our knowledge, this the first study with intact human fibroblasts in which the transamination and the subsequent decarboxylation step in the catabolism of the four naturally occurring branched-chain amino acids, L-leucine, L-valine, L-isoleucine, as well as L-allo-isoleucine were investigated.

In our assay system, 2-oxo acids formed by transamination of the respective branched-chain amino acids were estimated by chemical oxidation. By this method we found a considerably higher and far less variable 2-oxo acid production from leucine and valine than Yoshida *et al.* (22). In the latter study, 2-oxo-4-methylpentanoate and 2-oxo-3-methylbutanoate production was measured in a few experiments and differed by factors of about 5 and 10, respectively. As in numerous other studies (*cf.* Ref. 14), the chemical procedure proved reliable for 2-oxo acid determination under the present assay conditions. 2-Oxo acid production as estimated by HPLC analysis of the quinoxalinol derivatives was somewhat higher. The reason for these differences, however, remained unclear. Likely, 2-oxo-3-methylbutanoate production from valine was somewhat underestimated, since in fibroblasts up to 10% of the 2-oxo-3-methylbutanoate might be converted to the respective 2-hydroxy acid which escapes our detection system. In contrast, hydroxy acid formation from 2-oxo-4-methylpentanoate and 2-oxo-3-methylpentanoate seems negligible. This has been shown with incubated fibroblasts (22) and further evidence is provided by a variety of clinical chemical findings (23–27).

Decarboxylation of L-[1-^{14}C]leucine and L-[1-^{14}C]valine in skin fibroblasts has been examined by several investigators (1, 22, 28), L-[1-^{14}]isoleucine as a substrate has been used in only one study (1). Data for L-allo-isoleucine are not available. Under similar experimental conditions, in all studies, including the present one, $^{14}CO_2$ measurements yielded surprisingly comparable decarboxylation rates (3–6 nmol/90 min/mg of cell protein). Furthermore, when the oxidative decarboxylation step was comparatively studied with branched-chain amino acids in intact cells, no preference of any substrate could be established (1, 22). This is confirmed by the present results obtained with leucine, valine, and isoleucine.

With all substrates, transamination activity exceeded decarboxylation activity in fibroblasts (*cf.* Ref. 22). This is in accordance with all nonhepatic tissues so far studied including human muscle (17), and it is generally held that oxidative decarboxylation is the common rate-limiting step in branched-chain amino acid catabolism, except in the liver. In addition, apparently irrespective of the branched-chain amino acid concentration used or incubation time applied, a constant portion of the 2-oxo acid formed within the cells did not get access to the mitochondrial branched-chain 2-oxo acid dehydrogenase complex. This was

indicated by the constancy of the $^{14}CO_2$ production to transamination ratio.

The failure to detect significant differences in the oxidative decarboxylation rates and the distinct differences in transamination rates of individual branched-chain amino acids obtained with intact cells clearly contrast known properties of the (purified) enzymes involved. With branched-chain 2-oxo acid dehydrogenase, which has been isolated from a variety of animal tissues, a differential activity against the substrates in the order of 2-oxo-3-methylbutanoate > *S*-2-oxo-3-methylpentanoate ≥ 2-oxo-4-methylpentanoate > *R,S*-2-oxo-3-methylpentanoate has been observed (combined data from Refs. 29–32), whereas branched-chain amino acid aminotransferase has exhibited rather similar activities with the different branched-chain amino acids (33, 34). Thus it appears that additional regulatory mechanisms, *e.g.* amino and/or 2-oxo acid transport across cellular and intracellular membranes (35, 36), may be engaged in the regulation of overall branched-chain amino acid metabolism in the intact cell.

As compared to isoleucine catabolism, the rate of transamination and of oxidative decarboxylation of L-[1-^{14}C]allo-isoleucine catabolism were distinctly lower and amounted to 40 and 27%, respectively. Thus, the ratio of allo-isoleucine to isoleucine transamination in human fibroblasts was markedly lower than in rat tissue homogenates, the only data available for comparison (11). According to our findings, the significantly lower $^{14}CO_2$ production from L-[1-^{14}C]allo-isoleucine was due to the very low transamination rate and to the fact that only a minor portion (23%) of *R*-2-oxo-3-methylpentanoate was further oxidatively decarboxylated. The portion of *S*-2-oxo-3-methylpentanoate undergoing decarboxylation was significantly higher (34%). Whether the branched-chain 2-oxo acid dehydrogenase complex degrades *R*- and *S*-2-oxo-3-methylpentanoate at different rates, however, is virtually unknown. Work is now in progress to elucidate this point in experiments with l-^{14}C-labeled 2-oxo acids as substrates.

The degradation of the branched-chain amino acids was also studied in a cell line from a patient with variant MSUD. This strain with an only moderately reduced branched-chain 2-oxo acid dehydrogenase complex activity was deliberately choosen in order to obtain reliable $^{14}CO_2$ production. Branched-chain amino acid aminotransferase activity in these cells was apparently unchanged. As compared to controls, $^{14}CO_2$ formation was significantly lower and more 2-oxo acid accumulated. Moreover, the activity of the mutant branched-chain 2-oxo acid dehydrogenase complex toward the four substrates was found to be apparently altered. Whereas $^{14}CO_2$ production from l-^{14}C-labeled leucine and valine was reduced by about 90%, residual decarboxylation activity in experiments with isoleucine was 25% and with allo-isoleucine even 45%. Decarboxylase activity of different MSUD cell lines with three branched-chain amino acids (leucine, valine, isoleucine) has been examined in one study. Regularly, the activity has been found to be concurrently reduced with all substrates (1). Thus, it remains to be investigated whether the present finding is a more general feature in variant MSUD or whether it is specific for the cell line under investigation.

Acknowledgments. The authors are indebted to Dr. W. Radeck for the preparation of l-^{14}C-labeled isoleucine and allo-isoleucine, H.-W. Hammen for the amino acid analysis, Y. Susanto for some advice with the HPLC analysis, and Dr. U. Matthiesen for the mass spectrometric analysis.

REFERENCES

1. Dancis J, Hutzler J, Snyderman SE, Cox RP 1972 Enzyme activity in classical and variant forms of maple syrup urine disease. J. Pediatr 81:312–320
2. Tanaka K, Rosenberg LE 1983 Disorders of branched-chain amino acid and organic acid metabolism. In: Stanbury JB, Wyngaarden JB, Frederickson DS, Goldstein JL, Brown MS (eds) The Metabolic Basis of Inherited Disease, 5th ed. McGraw-Hill, New York, pp 440–473
3. Meister A 1951 Studies on *d*- and *l*-α-keto-β-methylvaleric acid. J Biol Chem 190:269–276
4. Weinberg RB, Walser, M 1977 Racemization and amination of the ketoanalogue of isoleucine in dogs. Biochem Med 17:164–172
5. Mamer OA, Tjoa SS, Scriver CR, Klassen GA 1976 Demonstration of a new mammalian isoleucine catabolic pathway yielding an R series of metabolites. Biochem J 160:417–426
6. Baretz BH, Lollo CP, Tanaka K 1979 Metabolism in rats in vivo of RS-methylbutyrate and n-butyrate labelled with stable isotopes at various positions. J Biol Chem 254:3468–3478
7. Norton PM, Roitman E, Snyderman SE, Holt LE 1962 New findings in maple syrup urine disease. Lancet 1:26–27
8. Snyderman SE, Norton PM, Roitman E, Holt LE 1964 Maple syrup urine disease, with particular reference to dietotherapy. Pediatrics 34:454–472
9. Halpern B, Pollok GE 1970 The configuration of alloisoleucine present in maple syrup urine disease. Biochem Med 4:352–356
10. Matthews DE, Ben-Galim E, Haymond MW, Bier DM 1980 Allo-isoleucine formation in maple syrup urine disease: isotopic evidence for the mechanism. Pediatr Res 14:854–857
11. Walser M, Sapier DG, Mitch WE, Chan W 1981 Effects of branched-chain keto acids in normal subjects and patients. In: Walser M, Williamson JR (eds) Metabolism and Clinical Implications of Branched Chain Amino and Keto-acids. Elsevier/North Holland, New York, pp 291–299
12. Wendel U, Langenbeck U, Luthe H, Seakins WT 1986 L-allo-isoleucine—an inert marker of isoleucine metabolism in maple syrup urine disease. Eur J Pediatr 144:523
13. Loftfield RB, Eigner EA 1966 The preparation of pure 1-^{14}C- and 1-^{3}H-labelled L-amino acids. Biochim Biophys Acta 130:449–457
14. Schadewaldt P, Oelers R, Radeck W, Staib W 1984 Oxidative determination of ^{14}C-labeled 2-oxo acids. Anal Biochem 143:308–315
15. Greenstein JP, Levintow L, Baker CG, White J 1951 Preparation of the four stereoisomers of isoleucine. J Biol Chem 188:647–663
16. Chan TR 1977 In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp Cell Res 104:255–262
17. Wagenmakers AJM, Veerkamp JH 1982 Degradation of branched-chain amino acids and derived 2-oxo acids in human and rat heart and skeletal muscle. Biochem Med 28:16–31
18. Rüdiger HW, Langenbeck U, Goedde HW 1972 A simplified method for the preparation of ^{14}C-labelled branched-chain α-oxo acids. Biochem J 126:445–446
19. Lowry OH, Rosebrough NJ. Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
20. Koike K, Koike M 1984 Fluorescent analysis of 2-keto acids in serum and urine by high-performance liquid chromatography. Anal Biochem 141:481–487
21. Mowbray J, Ottaway JA 1970 The preparation of 2-oxoacid derivatives suitable for specific radioactivity determination. Biochem J 120:171–175
22. Yoshida I, Sweetman L, Nyhan WL 1986 Metabolism of branched-chain amino acids in fibroblasts from patients with maple syrup urine disease and other abnormalities of branched-chain ketoacid dehydrogenase activity. Pediatr Res 20:169–174
23. Greer M, Williams CM 1967 Diagnosis of branched-chain ketonuria (maple-syrup-urine disease) by gas chromatography. Biochem Med 1:87–91
24. Gompertz D, Draffan GH 1972 The gas-chromatographic diagnosis of intermittent maple-syrup-urine disease (branched-chain ketoaciduria) Clin Chim Acta 40:5–11
25. Lancaster D, Mamer OA, Scriver CR 1974 Branched-chain alpha-keto acids isolated as oxime derivatives: relationship to the corresponding hydroxy acids and amino acids in maple syrup urine disease. Metabolism 23:257–265
26. Jakobs C. Solem E, Ek J, Halvorsen K, Jellum E 1977 Investigation of the metabolic pattern in maple-syrup-urine disease by means of glass capillary gas chromatography and mass spectrometry. J Chromatogr 143:31–38
27. Shigematsu J, Kikuchi K, Momoi T, Sudo M, Kikawa Y, Nosaka K, Kuriyama M, Haruki S, Sanada K, Hamano N, Suzuki J 1983 Organic acids and branched-chain amino acids in body fluids before and after multiple exchange transfusions in maple-syrup-urine disease. J Inher Metab Dis 6:183–189
28. Elsas LJ, Pask BA, Wheeler FB, Perl DP, Trusler S 1972 Classical maple syrup urine disease: cofactor resistance. Metabolism 21:929–944
29. Pettit FH, Yeaman SJ, Reed LJ 1978 Purification and characterization of branched-chain α-keto acid dehydrogenase complex of bovine kidney. Proc Natl Acad Sci USA 75:4881–4885
30. Danner DJ, Lemmon SK, Besharse JC, Elsas LJ 1979 Purification and characterization of branched-chain α-keto acid dehydrogenase from bovine liver mitochondria. J Biol Chem 254:5522–5526
31. Parker PJ, Randle PJ 1978 Branched-chain 2-oxo-acid dehydrogenase complex of rat liver. FEBS Lett 90:183–186
31. Danner DJ, Lemmon SK, Elsas LJ 1978 Substrate specificity and stabilization by thiamine pyrophosphate of rat liver branched chain keto acid dehydrogenase. Biochem Med 19:27–38
33. Taylor RT, Jenkins WT 1966 Leucine aminotransferase II. Purification and characterization. J Biol Chem 241:4396–4405
34. Ichiara A, Noda C, Tanaka K 1981 Oxidation of branched chain amino acids with special reference to their transaminase. In: Walser M, Williamson JR (eds) Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids. Elsevier/North-Holland, New York, pp 227–231
35. Gazzola GC, Dall'Asta V, Guidotti GG 1980 The transport of neutral amino acids in cultured human fibroblasts. J Biol Chem 255:929–936
36. Tarpey MM, Willis RC, Seegmiller JE 1977 Uptake of α-ketoisocaproic acid in lymphoblast line WI-L2. Biochem Biophys Res Commun 76:1267–1275