Title
Metabolism of 1-13C-Propionate In Vivo in Patients with Disorders of Propionate Metabolism

Journal
Pediatric Research, 30(1)

ISSN
0031-3998

Authors
Barshop, Bruce A
Yoshida, Ichiro
Ajami, Alfred
et al.

Publication Date
1991-07-01

DOI
10.1203/00006450-199107000-00004

0031-3998/91/3001-0015$03.00/0
PEDIATRIC RESEARCH

Vol. 30, No. 1, 1991
Printed in U.S.A.

Metabolism of 1-^{13}C-Propionate *In Vivo* in Patients with Disorders of Propionate Metabolism

BRUCE A. BARSHOP, ICHIRO YOSHIDA,[1] ALFRED AJAMI, LAWRENCE SWEETMAN, JON A. WOLFF,[2] FRANCES RAY SWEETMAN, CHRISTINA PRODANOS, MOYRA SMITH, AND WILLIAM L. NYHAN

Department of Pediatrics and Institute of Molecular Genetics, University of California San Diego, La Jolla, California 92093 [B.A.B., I.Y., L.S., J.A.W., F.R.S., C.P., W.L.N.]; Tracer Technologies, Inc., Somerville, Massachusetts 02143 [A.A.]; and Department of Pediatrics, University of California Irvine, Irvine, California 92717 [M.S.]

ABSTRACT. Metabolism of propionate in human subjects was studied using bolus administration of 1-^{13}C-propionate i.v. or orally. The study population consisted of five patients with propionic acidemia (PA), eight with methylmalonic acidemia (MMA; four responsive to vitamin B_{12}), one each with multiple carboxylase deficiency and transcobalamin-II deficiency, and five healthy volunteers. Concentrations of 1-^{13}C-propionate were measured in blood in three patients with PA, two with MMA, and two controls. Breath samples were obtained at intervals during 3 h after the dose, isotopic enrichment of $^{13}CO_2$ was measured, and the cumulative percentage of recovery of ^{13}C was calculated from the individual's predicted resting energy expenditure. Recovery of $^{13}CO_2$ and half-time of 1-^{13}C-propionate in PA were significantly less than normal. The same parameters in MMA were below normal, but significantly greater than in PA. Recovery of $^{13}CO_2$ was well correlated with clinical severity in PA, but did not correlate in MMA. Differences between MMA and PA may indicate different distribution of propionate pools, differences in inducibility of residual enzyme activities, or an alternate pathway for decarboxylation of propionate available in MMA but not PA. Only one patient with PA demonstrated increased $^{13}CO_2$ production during biotin treatment. In a B_{12}-responsive MMA patient, no differences were noted within 2 d of initiating treatment with B_{12}, but there was an increase in $^{13}CO_2$ production after 4 mo. Recovery of $^{13}CO_2$ was normal in the patient with transcobalamin-II deficiency before and after treatment with vitamin B_{12}. In the patient with multiple carboxylase deficiency, $^{13}CO_2$ generation was nearly normal while he was receiving his maintenance dose of biotin, and was not significantly changed after 3 and 7 d without biotin treatment, despite a decrease of 30% in lymphocyte propionyl-CoA carboxylase activity. (*Pediatr Res* 30: 15–22, 1991)

Received January 16, 1990; accepted March 19, 1991.

Correspondence and reprint requests: Bruce A. Barshop, M.D., Ph.D., Department of Pediatrics, M-013 I, University of California San Diego, La Jolla, CA 92093.

Supported by U.S. Public Health Service Grants No. HD04068 from the National Institute of Child Health and Human Development, DK07318, NIH-HSSA AM07318, and AM-R43-33381 from the National Institute of Arthritis, Diabetes and Digestive and Kidney Disease, and RR00827 of the General Clinical Research Centers Program, National Institutes of Health, Bethesda, MD.

[1] Present address: Department of Pediatrics and Child Health, Kurume University, Kurume, Japan.

[2] Present address: Department of Pediatrics, University of Wisconsin, Madison, WI.

Abbreviations

PCC, propionyl-CoA carboxylase
PA, propionic acidemia
MCD, multiple carboxylase deficiency
MMA, methylmalonic acidemia
TC-II, transcobalamin-II
GCMS, gas chromatography-mass spectrometry
SIM, selected ion monitoring
APE, atom percent excess
V_{CO_2}, rate of CO_2 production
REE, resting energy expenditure

Disorders of propionate metabolism are potentially life-threatening conditions that present a considerable heterogeneity of clinical manifestations, particularly evident in the tolerance to protein and response to pharmacologic doses of biotin or vitamin B_{12}. Propionyl-CoA in human metabolism arises from the amino acids isoleucine, valine, threonine, and methionine, and to lesser extents from cholesterol and odd-chain length fatty acids. An additional source is propionate-producing bacteria in the gut. Propionyl-CoA is carboxylated by PCC, and the resulting S-methylmalonyl-CoA is converted by methylmalonyl-CoA racemase to R-methylmalonyl-CoA, which is isomerized by methylmalonyl-CoA mutase to succinyl-CoA, which is then oxidized in the tricarboxylic acid cycle to yield CO_2. In human disease, this pathway may be blocked at either the PCC or mutase steps. PA may arise from a defective PCC apoenzyme or from deficient holoenzyme formation, as in MCD due to holocarboxylase synthetase or biotinidase deficiency. MMA may arise from an altered mutase apoenzyme, from disordered cobalamin metabolism, which may give rise to MMA alone or in conjunction with homocystinuria, or from B_{12} deficiency, including that arising from TC-II deficiency.

Vitamin B_{12} can be lifesaving in certain complementation groups of MMA (1), and B_{12} is highly effective in TC-II deficiency (2). Treatment of MCD (3) and biotinidase deficiency (4) with biotin has been uniformly effective. There has been no evidence that patients with PA have improved clinically by treatment with biotin. However, reports that biotin treatment lowers the response of serum propionate concentrations to an isoleucine load (5) and increases cellular activity of PCC (6) provide an argument for testing the effects of biotin on each patient with PA.

Recent studies (7, 8) have established the utility of 1-^{13}C-propionate for estimating the rate of propionate oxidation *in vivo* through the rate of $^{13}CO_2$ evolution, and have indicated that the

estimated oxidative rate can be of prognostic value. Our study was undertaken to explore the use of bolus administration of 1-^{13}C-propionate as an adjunct in the evaluation of metabolic capacity *in vivo*, to compare the oxidation of propionate in patients with various defects of propionate metabolism, and to determine whether this methodology could be useful in evaluating vitamin responsiveness.

MATERIALS AND METHODS

Materials. Sodium 1-^{13}C-propionate (90 atom % ^{13}C) was purchased from MSD Isotopes, Pointe Claire-Dorval, Quebec, Canada or (99 atom % ^{13}C) was provided by Tracer Technologies, Somerville, MA. $^{2}H_6$-propionic acid (98 atom % ^{2}H) was purchased from KOR Isotopes, Cambridge, MA. Sodium propionate was obtained from Sigma Chemical Co., St. Louis, MO. Glass-distilled chloroform preserved with amylene was purchased from Burdick and Jackson Laboratories, Inc., Muskegon, MI; Bio-Sil A (200–400 mesh) silicic acid from Bio-Rad Laboratories, Richmond, CA; and Chromosorb 102 (60–80 mesh) for gas chromotography from Supelco, Bellefonte, PA. Twenty-mL evacuated tubes for collection of breath samples were non-silicon-coated Venoject tubes from Terumo Medical Corp., Elkton, MD.

Subjects. This research was approved by the University of California Human Subjects Committee, and informed consent was obtained from all patients or their parents. The patient population and sequence of tests are presented in Table 1. The patients studied included five with PA and six with MMA, of which three were responsive to vitamin B_{12}. Unless otherwise indicated, biotin was administered at 10 mg/d orally, vitamin B_{12} was given as 1 mg/d cyanocobalamin intramuscularly, and carnitine was given as 100 mg/kg orally, divided into two or three doses per day. All of the patients had not had an episode of acute acidosis for at least 1 mo before the studies, and all were well at the time of study, with two exceptions enumerated below. Patient L.S. developed a florid varicella eruption days after her first study and was convalescent at the time of the second study, while taking biotin. In one of the B_{12}-responsive patients with MMA (S.H.), the first ^{13}C-propionate breath tests were performed immediately before and 3 d after the initial trial of vitamin B_{12}, but his course during the initial days of therapy was complicated by a febrile illness, and his urinary methylmalonic acid excretion increased from 19 to 92 μmol/mg creatinine despite the initiation of intramuscular cyanocobalamin. Subsequently, his responsiveness to B_{12} was evident and his diagnosis was confirmed by enzyme assay of cultured fibroblasts. He was tested again after 4 mo of ongoing oral cyanocobalamin therapy (1 mg/d), at which time he was in good health. One patient (A.F.) (3, 10) with MCD was studied who had a defective activity of holocarboxylase synthetase. His Km for biotin was higher than that of any of the other patients studied (3), and his clinical response to biotin was less complete than that of any of the other patients. He was studied while receiving 75 mg of biotin p.o. per day and at three and seven days after cessation of treatment with biotin. One patient with TC-II deficiency (M.W.) (2) was studied immediately before and after 1 mo of B_{12} treatment, an interval over which her excretion of methylmalonate decreased from 13.3 to 0.14 μmol/mg creatinine.

Standard metabolic analyses. Urinary organic acids were quantified by the method of Hoffmann *et al.* (11). Lymphocyte carboxylase activities were assayed as previously described (12). Biotin in plasma and urine was determined immunochemically (Le TP, Sweetman L, Nyhan WL, unpublished data).

Studies of metabolism of sodium 1-^{13}C-propionate in vivo. Sodium 1-^{13}C-propionate was freshly dissolved in sterile water to make a 60 mmol/L solution, sterilized by filtration, and administered i.v. at 30 μmol/kg or orally at 100 μmol/kg body weight. The control individuals and the patients over the age of 3 were fasted overnight, and the other patients were fasted 3 h before the test; all were fasting during the tests. The subjects reclined in bed or engaged in quiet play throughout the period of collection of blood and breath samples. Blood samples of 3–5 mL were collected in acid citrate-dextrose solution for measurement of carboxylases in patients with PA or MCD. In six of the subjects, samples of heparinized blood (0.5 mL) were collected for determination of 1-^{13}C-propionic acid before giving the isotope and at 5, 10, 15, 20, 30, 45, 60, and 90 min after the isotope. The blood samples were placed on ice and then frozen at −20°C until analyzed. Two breath samples were obtained before the administration of the isotope and at 15-min intervals for 3 h after i.v. injection or 1, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 min after oral administration. Fifty mL of end-respiratory breath samples were collected in a syringe with a three-way stopcock from a Haldane tube in the older subjects and from a face mask in the younger subjects. The breath samples were transferred to duplicate evacuated tubes and kept at room temperature until analysis.

Measurement of ^{12}C- and ^{13}C-propionic acid in blood. The method of Bachmann *et al.* (13) for the quantitation of short-chain fatty acids in plasma by adsorption to silicic acid and elution with chloroform was modified by the use of an internal standard of $^{2}H_5$-propionic acid and GCMS. To 0.4-mL samples of whole blood or plasma was added 25 μL of 2 μmol/L $^{2}H_6$-propionic acid (98 atom % ^{2}H) in 0.35 mol/L LiOH. The carboxy deuterium of $^{2}H_6$-propionic acid exchanges with the large amount of water to give Li·$^{2}H_5$-propionate. The samples, containing 50 nmol of $^{2}H_5$-propionic acid, were acidified to pH less than 1 with 25 μL of 6 M sulfuric acid, 1 g of Bio-Sil A was stirred in to give a free-flowing powder that was poured onto a 0.6-cm inner diameter × 15-cm glass column, and the column was filled with chloroform. Chloroform was pumped through the column at a flow rate of 2.5 mL/min for 6 min and the effluent was collected in vials containing 100 μL of 0.1 M LiOH in nanograde methanol. The alkaline effluent was evaporated in a stream of nitrogen to a small volume and transferred to 1.5-mL snap-cap conical tubes by rinsing with methanol, and the samples were dried completely in nitrogen, capped, and stored at −20°C. For analysis by GCMS, the samples were dissolved in 50 μL of 0.6 M HCl. Blank samples of distilled water were carried through the entire procedure to obtain corrections for the small amount of propionic acid in Bio-Sil A and chloroform.

Standards for GCMS were prepared that contained 0 to 250 nmol of sodium propionate (90 atom % ^{13}C) and 50 nmol of Li·$^{2}H_5$-propionate in 50 μL of 0.6 M HCl. A Finnigan (Sunnyvale, CA) model no. 4021 gas chromatograph-mass spectrometer with an INCOS data system was used for (SIM) chemical ionization GCMS analysis of propionic acid, 1-^{13}C-propionic acid, and $^{2}H_5$-propionic acid. Analyses were done on a GC column 2 mm inner diameter × 180 cm packed with Chromosorb 102 (60–80 mesh) with phosphoric acid-treated glass wool at the outlet end. The injector temperature was 200°C, the column temperature 180°C, and the direct transfer line to the MS source 280°C. The GC carrier gas was methane, which also served as the chemical ionization reagent gas, and the flow rate was 25 mL/min, with a pressure of 0.07–0.08 torr in the source. The ionizing voltage was 70 eV. Propionic acid eluted at about 5 min. For the injection of solutions of propionic acid, 1 μL of 0.6 M HCl was drawn into the syringe and then 2 μL of propionic acid in 0.6 M HCl. Between each sample 3 μL of 0.6 M HCl was injected twice to clean the column. The solvent fronts were diverted away from the source.

For SIM quantitation, the m/z were 75 for propionic acid, 76 for 1-^{13}C-propionic acid, and 80 for $^{2}H_5$-propionic acid. The dwell time for each mass was 100 ms. Peak areas of the SIM chromatograms were calculated by the computer with the baseline points selected by the operator. For each day's analysis, standard curves for propionic acid and 1-^{13}C-propionic acid (90 atom % ^{13}C) were determined. Linearity was excellent and the standard curve of the ratio of areas at m/z 75 to m/z 80 for ^{12}C-propionic acid *versus* nmol of $^{2}H_5$-propionic acid was fitted by

Table 1. *Summary of 1-^{13}C-propionate bolus load studies**

Group	Subject	Clinical severity†	Age at study (y)	Route	Treatment	Breath $^{13}CO_2$ CPD at 180 min	Blood propionate		
							Prebolus (μM)	Half-life (min)	Pool size (μmol/kg)
PA	EA	9	2.5	i.v.		ND	553	34.8	250.0
			2.6	i.v.	+Biotin	ND	322	26.1	102.0
	EN	5	0.2	i.v.		20.3			
			2.3	p.o.	+Carnitine	23.6			
			2.6	p.o.		9.5			
	JT	9	0.4	p.o.	+Biotin, +carnitine	10.0			
			0.5	p.o.	+Carnitine	10.4			
	LS	2	6.3	i.v.		21.7	27	ND	
			6.3	i.v.	+Biotin	30.5	64	ND	
	PS	8	0.3	i.v.		8.4	14	15.1	12.3
			0.5	i.v.	+Biotin	6.4	17	14.9	21.2
			0.8	i.v.		10.8			
			2.8	p.o.	+Carnitine	9.0			
			3.1	p.o.		10.4			
MMA	(mut) CH	9	2.5	i.v.		35.8	44	10.9	29.4
			2.6	i.v.	+B_{12}	40.5	57	11.8	23.1
			4.8	p.o.		36.0			
			5.1	i.v.	+Carnitine	25.6			
			5.2	i.v.		36.6			
	(mut) CM	9	0.8	p.o.	+Carnitine	46.8			
			1.2	p.o.		50.7			
	(mut) JB	6	0.8	i.v.	+B_{12}	39.1	9	6.4	UD
			0.9	i.v.		34.1	20	6.5	3.8
			3.3	p.o.	+Carnitine	28.6			
	(mut) JE	5	2.1	p.o.		35.2			
	(CblA) SH	3	0.7	p.o.		33.1			
			0.7	p.o.	+B_{12} × 2 d	31.9			
			1.0	p.o.	+B_{12} × 4 mo	41.5			
	(CblA) TC	2	2.1	p.o.	+B_{12} +carnitine	40.8			
	(CblA) TJ	3	1.4	i.v.	+B_{12}	34.0			
	(CblA) CF	1	13.3	p.o.	+B_{12} +carnitine	45.7			
MCD	AF		3.3	i.v.	+Biotin	47.9	12	ND	
			3.3	i.v.	−Biotin × 5 d	54.0	11	4.2	UD
			3.3	i.v.	−Biotin × 8 d	45.0	11	5.3	UD
TC-II	MW		0.7	p.o.		53.0			
			0.8	p.o.	+B_{12}	45.8			
Control	1		34	i.v.		53.2	5	5.5	UD
			34	i.v.		ND	8	4.4	UD
	2		11.3	p.o.		54.3			
			11.3	p.o.		46.3			
			12.1	p.o.		52.8			
	3		8.3	p.o.		63.0			
	4		3.0	i.v.		59.9	3	3.4	UD
	5		2.4	p.o.		69.4			

* CPD, cumulative percent dose; ND, not determined; UD, undefined (see text).

† Clinical severity scored as per Walter *et al.* (9).

linear regression and used to calculate the concentration of ^{12}C-propionic acid in the samples of blood. Similarly, the standard curve of the ratio of areas at m/z 76 to m/z 80 was used to calculate the concentration of ^{13}C-propionic acid in the samples of blood. The experimentally determined natural abundance of ^{13}C-propionic acid was 2.70 atom %. The blood samples were analyzed and the atom % ^{13}C-propionic acid was calculated as (nmol ^{13}C-propionic acid)/(nmol ^{13}C-propionic acid + nmol ^{12}C-propionic acid). APE ^{13}C was obtained by subtracting the natural abundance. The 1-^{13}C-propionic acid injected in these studies was determined to be 89.8 atom % ^{13}C.

Measurement of $^{13}CO_2$ in expired air. The $^{13}CO_2/^{12}CO_2$ ratio in breath samples was measured by Tracer Technologies (Somerville, MA) using a VG-ISOGAS SIRA-10 ratio mass spectrometer. The APE of $^{13}CO_2$ was derived from a comparison of ion currents at m/z 44, 45, and 46 (including correction for oxygen isotopes) relative to a standard sample (calibrated to Pee Dee Belemnite limestone). Access to an indirect calorimeter was not available throughout these studies, so each subject's endogenous V_{CO_2} was estimated based upon predicted REE. The REE (kcal/h) was calculated using the Bateman formula and coefficients derived (14) to describe published (15) age- and sex-dependent basal metabolic rates (kcal/m^2·h) and an estimate of body surface area (BSA) based on weight and height according to the Dubois formula (16). Endogenous CO_2 production was calculated from the REE assuming a RQ of 0.83 (L CO_2/L O_2), a heat production of 4.83 kcal/L O_2 (15), and a density of 44.6 mmol CO_2/L at 37°C and 1 atm. Because the basal metabolic rate (BMR) applies only under specific conditions (awake, supine, calm, postabsorptive) that are not always feasible during testing of subjects of the pediatric age group, an activity factor correction is desirable under these experimental conditions (sub-

jects engaged in sedentary activity). Based upon indirect calorimetry, energy expenditure in adult subjects confined to bed and out of bed ranges from 1.2 to 1.3 times the basal rate (17), and similar measurements in another series (18) yielded an activity factor of 1.23 ± 0.08. Accordingly, we used an activity factor of 1.25 in the present study, and calculated V_{CO_2} (mmol/min) as

$$V_{CO_2} = BMR/60 \times BSA \times (0.83 \times 44.6)/4.83 \times 1.25$$

The rate of $^{13}CO_2$ production is normalized to % dose/h, calculated as

$$\%\ \text{dose/h} = APE \times V_{CO_2}/(60 \times \text{mmol } ^{13}C \text{ administered}) \times 100$$

To calculate the cumulative percent dose excreted, the area under the curve of APE *versus t* was numerically integrated with cubic splines, using algorithms of the International Mathematics and Statistics Library (Chicago, IL). The cumulative percent dose excreted at time t, CPD(t), is then

$$CPD(t) = V_{CO_2} \times \int_0^t APE(t)/(\text{mmol } ^{13}C \text{ administered}) \times 100$$

Calculated spline curves were also used to determine the maximum values for % dose/h and times of maxima, as presented below.

RESULTS

Concentrations of 1-^{13}C-propionate in blood and half-lives. Blood propionate concentrations were measured in three patients with PA, two with MMA, the patient with MCD, and two control subjects (Table 1). Among the patients with PA, the concentrations of ^{12}C-propionate in blood before administration of ^{13}C-propionate were 2.5 to 100 times the mean control levels. The 19- to 41-fold lower concentrations in patient P.S. than in E.A. were considered to reflect an excellent level of control in a rigorously compliant family. The concentrations of propionate were between 5-fold and 16-fold above the control mean in L.S., the patient with somewhat milder manifestations of PA; however, the second value was obtained during resolution of a varicella infection. The blood propionate concentrations in C.H., a patient with classic MMA, were as high as typical values from patients with PA, whereas in J.B., the patient with much milder B_{12}-unresponsive MMA, the concentrations were only approximately 2.5 times control.

The half-life of ^{13}C-propionate was between 4 and 5 min in each of the three control experiments carried out. Virtually identical results were obtained in A.F., the patient with MCD. In the patients with PA, the values ranged from about 3 to 7 times greater than control. The half-times in the two patients with MMA were shorter than in PA, and that was true even when comparing patient C.H. to patient P.S., although C.H. had zero-time propionate concentrations 2.5 to 4 times greater than P.S. Pool sizes were estimated for the patients with vitamin-unresponsive PA or MMA from the natural logarithm of APE ^{13}C-propionic acid extrapolated to zero time. Pool sizes were undefined in the control patients and the patient with MCD, because the extrapolated zero-time APE was greater than the APE of the infused ^{13}C-propionic acid (reflecting the great excess of the bolus relative to the pool and the noninstantaneousness of infusion). Turnover of propionate in the patients, estimated from the pool sizes and slopes of the first-order curves, ranged from 0.41–4.98 μmol/min/kg. This is comparable to the values of 0.92–3.10 μmol/min/kg found for five patients with MMA studied with continuous infusion of labeled propionic acid (19).

Conversion of 1-^{13}C-propionate to $^{13}CO_2$. The time course of isotopic enrichment of ^{13}C in expired CO_2 after i.v. and oral administration of 1-^{13}C-propionate is shown in Figure 1. In control individuals, the conversion to $^{13}CO_2$ was rapid, with the greatest enrichment observed at 15 min or less after i.v. administration. There was very little difference between the time courses after i.v. or oral administration, although the maximum enrichment was slightly later when the tracer was given orally. There was very little generation of $^{13}CO_2$ in patients with PA, and the curves were virtually flat. The $^{13}CO_2$ enrichment curves in patients with MMA were flatter than those for control subjects, but there was significantly more generation of $^{13}CO_2$ than there was in patients with PA. With only one exception (patient L.S. on biotin), the highest cumulative percent dose expired for any patient with PA was below the lowest value for any patient with MMA. The recovery of $^{13}CO_2$ correlated negatively with clinical severity (9) in subjects with PA, as shown in Figure 2. The regression coefficient of −0.849 was highly significant statistically. In contrast, the recovery of $^{13}CO_2$ did not correlate with clinical severity of MMA. The results were indistinguishable from normal in the patient with TC-II deficiency before B_{12} treatment, at a time when she was excreting modest but distinctly abnormal quantities of methylmalonate.

Effects of biotin, vitamin B_{12}, and carnitine. In the patients with PA whose blood propionate concentrations were measured, there appeared to be no real difference in the concentrations of propionate in the blood or in the half-life of ^{13}C-propionate before and after treatment with biotin (Table 1). In L.S., the patient with somewhat milder PA, the concentration of propionate appeared to correlate with the status of her varicella rather than to be an effect of biotin. Nevertheless, the conversion of 1-^{13}C-propionate to $^{13}CO_2$ was increased in L.S. during biotin treatment (from 21.7 to 30.5% at 3 h), whereas in all of the other patients with PA there was no appreciable change in the extent of conversion with biotin. Also, among the patients with PA, the lymphocyte carboxylase activities increased only in L.S. after treatment with biotin (Table 2).

In the two patients with B_{12}-unresponsive MMA whose blood propionate concentrations were studied, the half-life of propionate and the conversion of 1-^{13}C-propionate to $^{13}CO_2$ were virtually identical in the presence and absence of vitamin B_{12} (Table 1). In the patients with apparent mitochondrial cobalamin reductase deficiency, T.C., T.J., and C.F., who were first studied after 2 wk, 4 mo, and 10 y of oral cyanocobalamin treatment, respectively, the recoveries of $^{13}CO_2$ were among the highest for patients with MMA. S.H. was the only patient with MMA with whom there was an opportunity to test $^{13}CO_2$ production before and after initiation of vitamin B_{12} treatment. After 3 d of intramuscular cyanocobalamin, there was no improvement in his apparent 1-^{13}C-propionate oxidation, although that was a time when his course was complicated by a febrile illness. However, there appeared to be a modest increase in $^{13}CO_2$ recovery and a distinct increase in maximum enrichment after 4 mo of treatment with oral cyanocobalamin.

In A.F., the patient with MCD, the half-time of blood 1-^{13}C-propionate at 5 and 7 d after cessation of biotin therapy approximated the control value. Similarly, the cumulative percent of $^{13}CO_2$ formation at 180 min indicated that the overall metabolism of propionate *in vivo* did not change in 7 d without biotin. In contrast, the concentration of biotin in plasma and urine declined sharply during the period of study (Table 3). The half-life of the initial loss from the major biotin pool as measured in plasma or urine was 1.5 to 2 d. The plasma carboxylase activities were never normal. At the start, the values for PCC, 3-methylcrotonyl-CoA carboxylase, and pyruvate carboxylase were 16, 15, and 13%, respectively, compared to simultaneously measured controls. Furthermore, they did not change very much during the course of the study. By 7 d without biotin, the levels of PCC and 3-methylcrotonyl-CoA carboxylase were 13 and 16% of control, respectively. The activity of pyruvate carboxylase declined to a greater degree, to 7.4% of control on d 4 and to 5.0% on d 7. The levels of urinary organic acids were maintained at close to the initial levels, but there was a gradual increase in the urinary content of 3-methylcrotonylglycine, 3-hydroxyisovalerate, and methylcitrate.

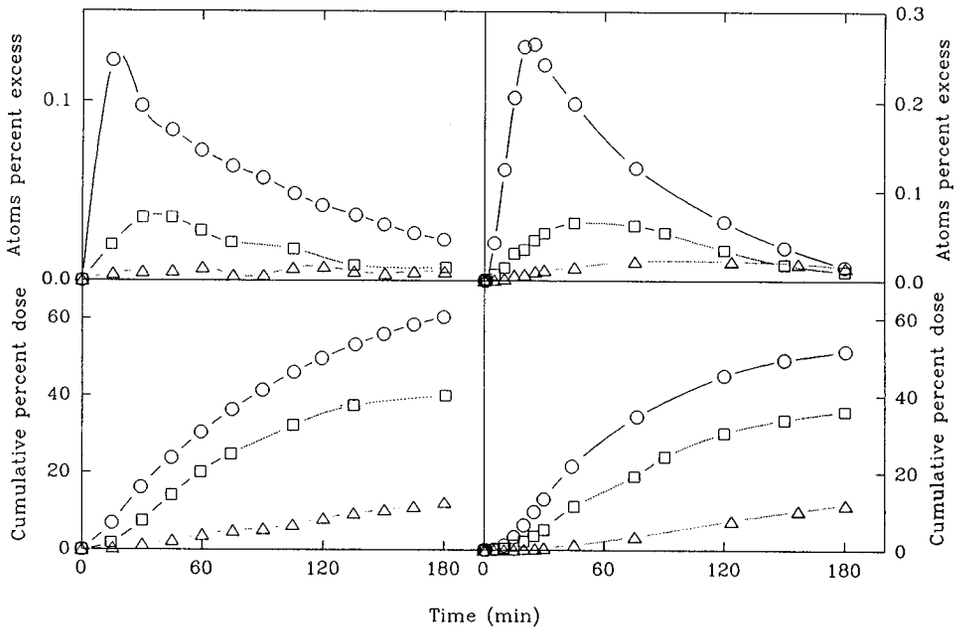

Fig. 1. Time course of $^{13}CO_2$ production after administration of sodium 1-^{13}C-propionate. *Left panels* show results obtained after administration of 30 μmol/kg 1-^{13}C-propionate i.v. and *right panels* show results after 100 μmol/kg isotope orally. *Upper panels* show the enrichment in APE ^{13}C measured in the breath samples. *Lower panels* show the calculated cumulative percentage of the isotope dose expired. *Circles* mark data from control subjects (subject 4 in i.v. study and subject 2 in oral study), *squares* from a subject with MMA (C.H.), and *triangles* from a subject with PA (P.S.).

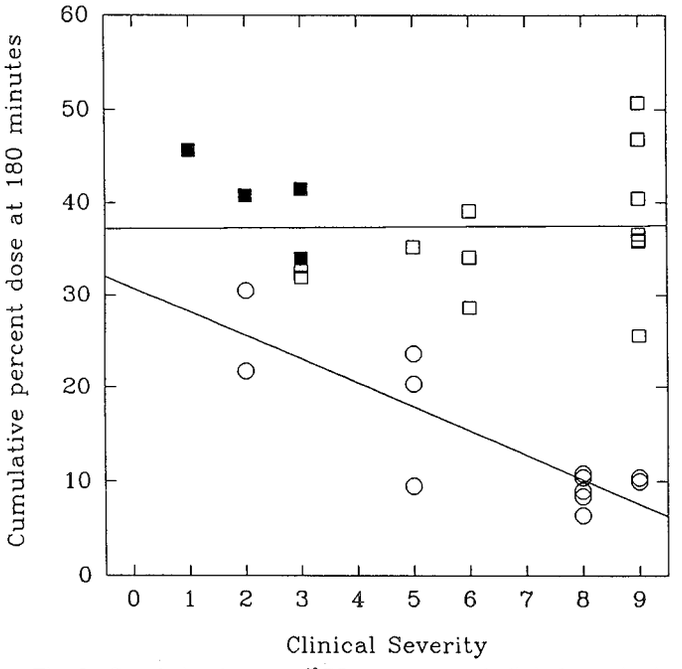

Fig. 2. Correlation between $^{13}CO_2$ recovery and clinical severity in PA and MMA. Clinical severity scores were tallied according to Walter *et al.* (9). *Circles*, patients with PA; *squares*, patients with MMA; and *filled squares*; patients with B_{12}-responsive MMA during B_{12} treatment. For PA, slope m = −2.57, regression coefficient $r = 0.849$, and significance $p = 0.00047$; for MMA, $m = 0.033$, $r = 0.015$, and $p = 0.89$.

Carnitine supplementation was without a consistent effect on the generation of $^{13}CO_2$ in any of the patients (Table 1).

DISCUSSION

This study design provides an estimate of relative propionate oxidation with a minimum of bedside procedures, but there are certain considerations that limit the interpretation of the data, in particular the use of VCO_2 predictions and the bolus administration of tracer. The unavailability of an indirect calorimeter dictated the requirement to predict VCO_2 from anthropometric data. This introduces a certain amount of uncertainty in the estimated isotope recovery, and it is of particular concern in comparing the patients with control, inasmuch as the validity of the Bateman formula (15) has not been confirmed in subjects with these disorders and with these therapies, including low protein diets. There is less concern using these predictions in comparing patient groups or therapeutic regimens in individual patients. The omission of indirect calorimetry did make it pos-

Table 2. *Lymphocyte carboxylase activities in subjects with PA**

Subject	Experiment	PPC (pmol/min·mg protein)	MCC (pmol/min·mg protein)	PCC/MCC	% Control
PS	−Biotin	0.67	250.00	0.0027	0.08
	Control	316.00	94.00	3.36	
PS	+Biotin	1.10	253.00	0.0043	0.20
	Control	187.00	89.00	2.10	
EA	−Biotin	1.24	217.00	0.0057	0.28
	Control	387.50	185.00	2.04	
EA	+Biotin	1.55	217.00	0.0074	0.36
	Control	292.00	144.00	2.04	
LS	−Biotin	0.68	31.00	0.0219	0.86
	Control	318.00	124.00	2.56	
LS	+Biotin	1.30	95.00	0.0137	0.58
	Control	295.00	125.00	2.36	
JT	−Biotin	7.20	16.40†	0.4390	6.65†
	Control	107.00	16.20†	6.62	
JT	+Biotin	4.90	127.00	0.0386	0.98
	Control	161.00	41.00	3.93	
EN	−Biotin	1.50	281.00	0.0053	0.22
	Control	295.00	125.00	2.36	

* All values were the means of duplicate assays. Each control was performed simultaneously with the experimental assay. The % control is the percentage of the ratio of PCC/MCC. MCC, 3-methylcrotonyl-CoA carboxylase.

† Sample and control frozen at −20°C for 6 wk before assay. All other assays performed within 48 h of collection.

Table 3. *Effects of cessation of biotin in a patient with holocarboxylase synthetase deficiency*

	Plasma biotin (mg/mL)	Urinary biotin (mg/mg creatinine)	Lymphocyte carboxylases* (pmol/min·mg protein)			Urinary organic acids (μmol/mg creatinine)			
			PCC	MCC	PC	3-Methyl crotonyl glycine	3-Hydroxy-isovalerate	3-Hydroxy-propionate	Methyl-citrate
Day 0	67.0	43.1				2.23	19.33	1.54	0.16
Day 1	37.6	10.4	52.0	18.4	3.1	5.48	13.13	1.71	
Day 2		13.8	49.1	11.1	2.2	7.52	19.33	0.81	0.61
Day 4	6.4	0.9	50.4	14.2	2.4	4.30	>11.0		
Day 7	4.9	0.5	37.1	13.8	1.3	7.22	33.81	1.80	1.08
Control									
Day 1			318.0	124.0	23.0				
Day 7			286.0	88.0	25.1				

* MCC, methylcrotonyl-CoA carboxylase; PC, pyruvate carboxylase.

sible to perform some of these studies at locations remote from a major medical center.

All previous studies of ^{13}C-propionate metabolism *in vivo* in patients with disorders of propionate metabolism have used the method of continuous tracer infusion. The bolus method as used here has distinct advantages in terms of noninvasiveness and acceptance by patients' families. In principle, it also permits the resolution of processes with different kinetic constants. However, there are certain assumptions that affect the kinetic interpretation of bolus tracer studies and that are difficult to validate. These limitations have been well discussed (*e.g.* reference 20). Of particular concern are *1*) the requirement that the tracer mix with the primary pool rapidly relative to its rate of metabolism, *2*) the requirement that the product move from the metabolic site to the sampling site rapidly with respect to the time of the sampling, *3*) the fact that the tracee pool size will affect the enrichment of tracer, and *4*) the possibility that a bolus of a relatively large amount of substrate will induce or stimulate residual enzyme or enzymes of alternate pathways. Because the first two assumptions cannot be validated with the available data, this study cannot be used to estimate the actual rate of propionate oxidation. The observed rate of $^{13}CO_2$ appearance includes undefined kinetic terms of substrate distribution and transport. Nevertheless, the recovery of ^{13}C should provide an index of the relative efficiency of propionate utilization, inasmuch as tracer mixing and $^{13}CO_2$ excretion are not slow in the 3-h time scale of sampling (manifest by the return of $^{13}CO_2$ enrichment to near baseline). It is assumed that the retention of $^{13}CO_3^-$ in slowly exchanging pools (21), which could account for up to 25% of the generated $^{13}CO_2$ (22, 23), is similar in all patient groups. The proportion of CO_2 release in expired air does not appear to be affected by protein intake in the neonate (24).

To address the effect of tracee pool size comprehensively will require more extensive blood propionate analyses than have been performed in this study to date. At least a part of the calculated decrease in ^{13}C recovery in the patients with increased propionate pools may arise from the dilution of the tracer. However, the observed differences in recovery between the patients with MMA and PA, taken as groups, are not likely to arise from differences in pool sizes (*e.g.* compare patients P.S. and C.H. in Table 1). Furthermore, there is a distinct delay of the appearance of $^{13}CO_2$ in PA patients with respect to MMA patients (Fig. 3). Although pool size would affect isotope recovery (decrease the cumulative percent dose), it should not affect the kinetic constants (alter the time of maximum $^{13}CO_2$ enrichment). Other possibilities to explain the differences between MMA and PA are that *1*) the rates of tracer mixing and/or substrate ingress or product egress at the mitochondrion are different (unlikely), *2*) residual methylmalonyl-CoA mutase but not PCC is inducible, or *3*) an alternate pathway of propionate decarboxylation is operant under these conditions in MMA but not in PA.

The shapes of the curves for $^{13}CO_2$ enrichment obtained in the patients with PA were so flat compared to those for controls that it appears likely that a different process or alternate pathway for the oxidation of propionate is involved in these patients. Abnormal oxidation of propionate to expiratory CO_2 was also observed in patients with pernicious anemia given 2-^{14}C-labeled propionate (25), and the kinetics observed in that study suggested the presence of two pathways, one prominent in normal individuals and another slower process assumed to arise from the β-oxidation

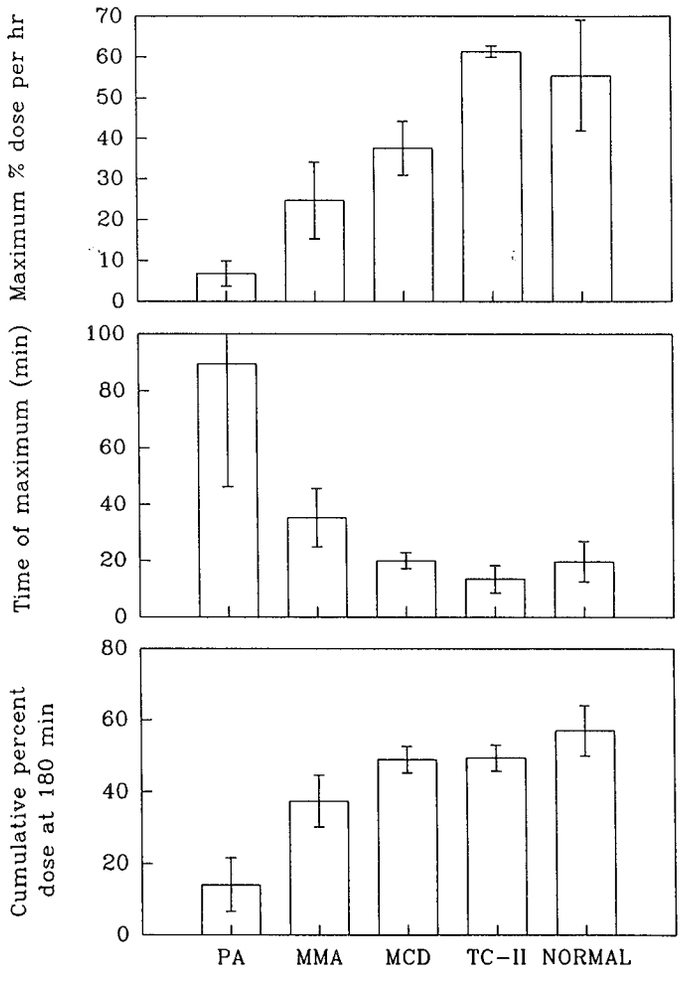

Fig. 3. Comparison of parameters of $^{13}CO_2$ generation between patient groups. *Upper panel*, maximum % dose ^{13}C expired per h. *Middle panel*, time of maximum ^{13}C enrichment. *Lower panel*, cumulative % ^{13}C dose expired at 3 h. The *bars* represent the means and the SD are shown by the *vertical brackets*. The data for MMA patients do not include those patients during treatment with vitamin B_{12}.

of propionyl-CoA to acrylyl-CoA (26), the pathway that appears to give rise to 3-hydroxypropionate in patients with PA (27). The slow rate of $^{13}CO_2$ production observed in the patients with PA in our study probably does arise from β-oxidation of propionyl-CoA. However, the rate of $^{13}CO_2$ production from 1-^{13}C-propionate that we observed in patients with MMA was very much greater than that observed in the patients with PA and must arise from yet another pathway or process. It is difficult to imagine that the net oxidation of propionate in patients with MMA is greatly enhanced relative to those with PA because the blood propionate concentrations and pool sizes in the former patients may be as high as those in the latter, as shown in Table 1 and in the data from the study of Thompson *et al.* (7). However, it is possible that nonoxidative loss of $^{13}CO_2$ could arise from isotope exchange through racemization of methylmalonate. The 1-^{13}C-S-methylmalonyl-CoA formed from 1-^{13}C-propionate is in equilibrium with 1-^{13}C-R-methylmalonyl-CoA through methylmalonyl-CoA through methylmalonyl-CoA racemase (26), and if the CoA moiety is transferred between the carboxylates of methylmalonate (concertedly or sequentially), 3-^{13}C-S-methylmalonyl-CoA would be formed, from which $^{13}CO_2$ would be released through PCC, which is known to be readily reversible (28). Indeed, isotope exchange between the 1 and 3 carbons of methylmalonyl-CoA has been demonstrated in the rat (29), where evidence was presented for sequential deesterification and reesterification of methylmalonate. However, data cited by Thompson *et al.* (8) showed only a small amount of $^{2}H_3$-propionate in only one of four children with MMA who were given continuous infusion of $^{2}H_5$-propionate. This would indicate that recycling by isotope exchange is not significant under the conditions of continuous infusion, but it is possible that this mechanism becomes kinetically significant only at the higher concentrations achieved in the present studies when the tracer was given as a bolus. It is possible that further studies of patients with MMA administered 2-^{13}C-propionate to eliminate $^{13}CO_2$ generation from isotope scrambling might distinguish that process from oxidation.

It is not clear from the available data whether the present methodology can discern an effect of cobalamin treatment in B_{12}-responsive MMA. There was no effect on $^{13}CO_2$ production after 2 d of B_{12} treatment in patient S.H. but there was an increase of about 25% after 4 mo of treatment. Unfortunately, we did not have the opportunity to test other B_{12}-responsive MMA patients before and after B_{12} treatment or to test patient S.H. at more dates early in his therapy. The data from vitamin B_{12}-deficient patients given 2-^{14}C-propionate (25) indicated that changes in propionate oxidation occurred later than changes in methylmalonic acid excretion or hematologic abnormalities.

In the patient with biotin-responsive MCD due to deficiency of holocarboxylase synthetase, the metabolism of 1-^{13}C-propionate was close to normal as measured by its half-life or its conversion to expiratory CO_2, and it remained so for 7 d after the cessation of biotin therapy. This was true even though the activity of PCC was only about 15% of the control level. The data provide information on how little carboxylase activity is required for normal metabolism of propionate. This sort of data could be of utility in the design of gene therapy, if that modality should become available for the management of human disease. These studies do not provide the answer as to how long a patient with MCD can go without biotin before propionate metabolism becomes abnormal, although they do indicate that 7 d is at least a minimum. This is probably a function of the half-life of the holocarboxylase itself, inasmuch as the levels of biotin decline promptly, with a half-life of 2 d or less. Among the carboxylases, the activity of pyruvate carboxylase appeared to decline more rapidly than that of PCC or 3-methylcrotonyl-CoA carboxylase.

There was no significant effect of biotin on the half-life of 1-^{13}C-propionate or its conversion to $^{13}CO_2$ in any but one of the patients with PA. This is in contrast with the observations of Wolf (6), who reported a significant increase in PCC activity in white cells of seven of eight patients with PA treated with 5–10 mg/d of biotin. In L.S., the single patient in whom an increased production of $^{13}CO_2$ was noted, the improved recovery was still as low as in the untreated or B_{12}-unresponsive patients with MMA, and her urinary excretion of hydroxypropionic and methylcitric acid did not change significantly despite the biotin treatment.

It is interesting to compare these results with those studies using continuous infusion of 1-^{13}C-propionate in similar patients (7, 8, 20). When studied by the continuous infusion method, there was little difference in $^{13}CO_2$ generation between patients with PA and those with MMA. In fact, there was considerable overlap with control populations. In contrast, after bolus administration there was no overlap of either disease population with controls and there was only one (posttreatment) overlap of the patients with PA with those with MMA. This may indicate differences in propionate pool distributions in PA and MMA or differences in inducibility of PCC and methylmalonyl-CoA mutase, or may reflect a role of free methylmalonate in the normal metabolism of propionate in man. In patients with PA, the recovery of ^{13}C in breath after a bolus dose correlates with clinical severity as does the plateau enrichment in the more demanding continuous infusion method (7). Bolus administration appears to better distinguish disposal of 1-^{13}C-propionate in PA from normal than does continuous infusion (7). Constant infusion studies have provided the important finding that a significant amount of residual activity is present in patients with MMA and PA, as well as phenylketonuria (20), with apparent oxidation rates that may be equal to control rates. However, even if ascribed to induction by high substrate concentrations, the apparently normal steady state enzyme activities do not explain the origin of the high concentrations. Because the differences in $^{13}CO_2$ generation may correspond more closely to differences in maximal velocities, there would appear to be a role for bolus tracer studies in determining metabolic capacities in biochemical diseases and their responses to therapy.

REFERENCES

1. Rosenberg LE 1983 Disorders of propionate and methylmalonate metabolism. In: Stabury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds.) The Metabolic Basis of Inherited Disease, 5th Ed., McGraw-Hill Co, New York, pp 474–497
2. Barshop BA, Wolff J, Nyhan WL, Yu A, Prodanos C, Jones G, Sweetman L, Leslie J, Holm J, Green R, Jacobsen DW, Cooper BA, Rosenblatt D 1990 Transcobalamin-II deficiency presenting with methylmalonic aciduria, homocystinuria and abnormal absorption of cobalamin. Am J Med Genet 35:222–228
3. Burri BJ, Sweetman L, Nyhan WL 1985 Heterogeneity of holocarboxylase synthetase in patients with biotin-responsive multiple carboxylase deficiency. Am J Hum Genet 37:326–337
4. Wolf B, Heard GS 1990 Screening for biotinidase deficiency in newborns: worldwide experience. Pediatrics 85:512–517
5. Barnes ND, Hull D, Balgobin L, Gompertz D 1970 Biotin-responsive propionicacidemia. Lancet 2:244–245
6. Wolf B 1980 Reassessment of biotin-responsiveness in "unresponsive" propionyl CoA carboxylase deficiency. J Pediatr 97:964–966
7. Thompson GN, Walter JH, Bresson JL, Bonnefont JP, Saudubray JM, Leonard JV, Halliday D 1990 *In vivo* propionate oxidation as a prognostic indicator in disorders of propionate metabolism. Eur J Pediatr 148:408–411
8. Thompson GN, Walter JH, Bresson JL, Ford GC, Bonnefont JP, Chalmers RA, Saudubray JM, Leonard JV, Halliday D 1989 Substrate disposal in metabolic disease: a comparison between rates of *in vivo* propionate oxidation and urinary metabolite excretion in children with methylmalonic acidemia. J Pediatr 115:635–639
9. Walter JH, Michalski A, Wilson WM, Leonard JV, Barratt TM, Dillon MJ 1989 Chronic renal failure in methylmalonic acidemia. Eur J Pediatr 148:344–348
10. Wolf B, Hsia YE, Sweetman L, Feldman G, Boychuk RB, Bart PD, Crowell DH, DiMauro RM, Nyhan WL 1981 Multiple carboxylase deficiency: clinical and biochemical improvement following neonatal biotin treatment. Pediatrics 68:113–118
11. Hoffmann G, Arakami S, Blum-Hoffmann E, Nyhan WL, Sweetman L 1989 Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatography-mass spectrometric analysis. Clin Chem 35:587–595
12. Weyler W, Sweetman L, Maggio DC, Nyhan WL 1977 Deficiency of propionyl-CoA carboxylase and methylcrotonyl-CoA carboxylase in a patient with methylcrotonylglycinuria. Clin Chim Acta 76:321–328

13. Bachmann C, Colombo JP, Bernter J 1979 Short chain fatty acids in plasma and brain: quantitative determination by gas chromatography. Clin Chim Acta 92:153–159
14. Jager-Roman E, Rating D, Platzek T, Helge H 1982 Development of N-demethylase activity measured with the ^{13}C-aminopyrine breath test. Eur J Pediatr 139:129–134
15. Brown AC 1973 Energy metabolism. In: Ruth TC, Patton HD (eds) Physiology and Biophysics, 20th Ed, Vol 3. Saunders, Philadelphia, pp 93–104
16. Dubois D, Dubois EF 1916 A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 17:863–871
17. Long CL, Schaffel N, Geiger JW, Schiller WR, Blakemore WS 1979 Metabolic response to injury and illness: estimation of energy and protein needs from indirect calorimetry and nitrogen balance. J Parenter Enteral Nutr 3:452–456
18. Webb P 1981 Energy expenditure and fat-free mass in men and women. Am J Clin Nutr 34:1816–1826
19. Walter JH, Thompson GN, Leonard JV, Bartlett K, Halliday D 1989 Contribution of aminoacid catabolism to propionate production in methylmalonic acidemia. Lancet 1:1298–1299
20. Thompson GN, Walter JH, Leonard JV, Halliday D 1990 *In vivo* enzyme activity in inborn errors of metabolism. Metabolism 39:799–807
21. Winchell HS, Wiley K 1970 Considerations in analysis of breath $^{14}CO_2$ data. J Nucl Med 11:708–710
22. Allsop JR, Wolfe RR, Burke JF 1978 Tracer priming the bicarbonate pool. J Appl Physiol 45:137–139
23. Hoerr RA, Yu Y-M, Wagner DA, Burke JF, Young VR 1989 Recovery of ^{13}C in breath from $NaH^{13}CO_3$ infused by gut or vein: effect of feeding. Am J Physiol 257:E426–E438
24. Van Aerde JEE, Sauer PJJ, Pencharz PB, Canagarayar U, Beesley J, Smith JM, Swyer PR 1985 The effect of energy intake and expenditure on the recovery $^{13}CO_2$ in the parenterally fed neonate during a 4-hour primed constant infusion of $NaH^{13}CO_3$. Pediatr Res 19:806–810
25. Fish MB, Pollycove M, Wallerstein RO 1968 *In vivo* oxidative metabolism of propionic acid in human vitamin B_{12} deficiency. J Lab Clin Med 72:767–777
26. Kaziro Y, Ochoa S 1964 Metabolism of propionic acid. Adv Enzymol 26:283–378
27. Ando T, Rasmussen K, Nyhan WL, Hull D 1972 3-Hydroxypropionate: significance of β-oxidation of propionate in patients with propionic acidemia and methylmalonic acidemia. Proc Natl Acad Sci 69:2807–2811
28. Land MD, Halenz DR 1960 Transcarboxylation and CO_2 "exchange" catalyzed by purified propionyl carboxylase. Biochemi Biophys Res Commun 2:436–439
29. Montgomery JA, Mamer QA, Scriver CR 1983 Metabolism of methylmalonic acid in rats: is methylmalonyl-coenzyme A racemase deficiency symptomatic in man? J Clin Invest 72:1937–1947