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

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ABSTRACT. Metabolism of propionate in human subjects was studied using bolus administration of 1-13C-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 B12), one each with multiple carboxylase deficiency and transcobalamin-II deficiency, and five healthy volunteers. Concentrations of 1-13C-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 13CO2 was measured, and the cumulative percentage of recovery of 13C was calculated from the individual's predicted resting energy expenditure. Recovery of 13CO2 and half-time of 13C-propionate in PA were significantly less than normal. The same parameters in MMA were below normal, but significantly greater than in PA. Recovery of 13CO2 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 13CO2 production during biotin treatment. In a B12-responsive MMA patient, no differences were noted within 2 d of initiating treatment with B12, but there was an increase in 13CO2 production after 4 mo. Recovery of 13CO2 was normal in the patient with transcobalamin-II deficiency before and after treatment with vitamin B12. In the patient with multiple carboxylase deficiency, 13CO2 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)

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Abbreviations

| Acronym | Definition |
|---------|------------|
| 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 |
| VCO2    | rate of CO2 production |
| REE     | resting energy expenditure |

Disorders of propionate metabolism are potentially life-threatening conditions that present a considerable heterogeneous of clinical manifestations, particularly evident in the tolerance to protein and response to pharmacologic doses of biotin or vitamin B12. 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 CO2. 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 disorders of cobalamin metabolism, which may give rise to MMA alone or in conjunction with homocystinuria, or from B12 deficiency, including that arising from TC-II deficiency.

Vitamin B12 may be lifesaving in certain complementation groups of MMA (1), and B12 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 1-13C-propionate for estimating the rate of propionate oxidation in vivo through the rate of 13CO2 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-

Materials and Methods

Materials. Sodium 1-13C-propionate (90 atom % 13C) was purchased from MSD Isotopes, Pointe Claire-Dorval, Quebec, Canada or (99 atom % 13C) was provided by Tracer Technologies, Somerville, MA. H3-propionic acid (98 atom % H) was pur-

used for 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 evaluat-

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**13C-PROPIONATE METABOLISM**

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

| Group | Subject | Clinical severity† | Age at study (y) | Route | Treatment | Prebolus (μM) | Half-life (min) | Pool size (μmol/kg) |
|-------|---------|--------------------|------------------|-------|-----------|---------------|-----------------|-------------------|
| PA    | EA      | 9                  | 2.5 i.v.         |       | +Biotin   | ND            | 553             | 34.8              | 250.0             |
|       | E       | 2.6 i.v.           | 20.3             |       |           | ND            | 322             | 26.1              | 102.0             |
|       | A       | 2.3 p.o.           | 23.6             |       | +Carnitine|               |                 |                   |                   |
|       |         | 2.6 p.o.           | 9.5              |       |           |               |                 |                   |                   |
| JT    | 9       | 0.4 p.o.           | 10.0             |       | +Biotin, +carnitine |               |                 |                   |                   |
|       | 0.5 p.o. |                   | 10.4             |       | +Carnitine|               |                 |                   |                   |
| LS    | 2       | 6.3 i.v.           | 21.7             |       |           | 27            | ND              | ND                |                   |
|       | 6.3 i.v. |                   | 30.5             |       | +Biotin   | 64            | ND              |                   |                   |
| PS    | 8       | 0.3 i.v.           | 8.4              |       | +Biotin   | 14            | 15.1            | 12.3              |                   |
|       | 0.5 i.v. |                   | 6.4              |       |           | 17            | 14.9            | 21.2              |                   |
|       | 0.8 i.v. |                   | 10.8             |       |           |               |                 |                   |                   |
|       | 2.8 p.o. |                   | 9.0              |       | +Carnitine|               |                 |                   |                   |
|       | 3.1 p.o. |                   | 10.4             |       |           |               |                 |                   |                   |
| MMA   | (mut) CH| 9                  | 2.5 i.v.         |       | +B12     | 40.5          | 57              | 11.8              | 23.1              |
|       |         | 2.6 i.v.           | 35.8             |       | +B12     | 44            | 10.9            | 29.4              |                   |
|       |         | 4.8 p.o.           | 36.0             |       |           |               |                 |                   |                   |
|       |         | 5.1 i.v.           | 25.6             |       | +Carnitine|               |                 |                   |                   |
|       |         | 5.2 i.v.           | 36.6             |       |           |               |                 |                   |                   |
|       | (mut) CM| 9                  | 0.8 p.o.         |       | +Carnitine| 46.8          |                 |                   |                   |
|       | (mut) JB| 6                  | 1.2 p.o.         |       | +Carnitine| 50.7          |                 |                   |                   |
|       |         | 0.8 i.v.           | 39.1             |       | +B12     | 9             | 6.4             | UD                |                   |
|       |         | 0.9 i.v.           | 34.1             |       |           |               | 20              | 6.5              | 3.8               |
|       |         | 3.3 p.o.           | 28.6             |       | +Carnitine|               |                 |                   |                   |
|       | (mut) JE| 5                  | 2.1 p.o.         |       |           | 35.2          |                 |                   |                   |
|       | (CblA) SH| 3                  | 0.7 p.o.         |       |           | 33.1          |                 |                   |                   |
|       |         | 0.7 p.o.           | 31.9             |       | +B12 x 2 d|               |                 |                   |                   |
|       | (CblA) TC| 2                  | 1.0 p.o.         |       | +B12 x 4 mo|              | 41.5            |                   |                   |
|       | (CblA) TJ| 3                  | 2.1 i.v.         |       | +B12     | 40.8          |                 |                   |                   |
|       | (CblA) CF| 1                  | 1.4 i.v.         |       | +B12     | 34.0          |                 |                   |                   |
|       |         | 13.3 p.o.          | 45.7             |       | +B12 +carnitine|              |                 |                   |                   |
| MCD   | AF      | 3                  | 3.3 i.v.         |       | +Biotin   | 47.9          | 12              | ND                |                   |
|       |         | 3.3 i.v.           | 54.0             |       | -Biotin x 5 d|              | 11              | 4.2              | UD                |
|       |         | 3.2 i.v.           | 45.0             |       | -Biotin x 8 d|              | 11              | 5.3              | UD                |
| TC-II | MW      | 0.7 p.o.           | 53.0             |       |           |               |                 |                   |                   |
|       | 0.8 p.o. |                   | 45.8             |       | +B12     |               |                 |                   |                   |
| Control| 1       | 34 i.v.            | 53.2             |       |           | 5             | 5.5             | UD                |                   |
|       | 2       | 34 i.v.            | ND               |       |           | 8             | 4.4             | UD                |                   |
|       | 11.3 p.o.|                   | 54.3             |       |           |               |                 |                   |                   |
|       | 11.3 i.v.|                   | 46.3             |       |           |               |                 |                   |                   |
|       | 12.1 p.o.|                   | 52.8             |       |           |               |                 |                   |                   |
|       | 8.3 p.o.|                   | 63.0             |       |           |               |                 |                   |                   |
|       | 3       | 8.3 i.v.           | 59.9             |       |           | 3             | 3.4             | UD                |                   |
|       | 4       | 3.0 i.v.           | 69.4             |       |           |               |                 |                   |                   |
|       | 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 13C-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 13C-propionic acid in the samples of blood. The experimentally determined natural abundance of 13C-propionic acid was 2.70 atom %.

The blood samples were analyzed and the atom % 13C-propionic acid was calculated as (nmol 13C-propionic acid)/(nmol 13C-propionic acid + nmol 12C-propionic acid). APE 13C was obtained by subtracting the natural abundance. The 1-13C-propionic acid injected in these studies was determined to be 89.8 atom % of 13C.

Measurement of 13CO2 in expired air. The 13CO2/12CO2 ratio in breath samples was measured by Tracer Technologies (Somerville, MA) using a VG-ISOGAS SIRA-10 ratio mass spectrometer. The APE of 13CO2 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 Vco2 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/m². h) and an estimate of body surface area (BSA) based on weight and height according to the Dubois formula (16). Endogenous CO2 production was calculated from the REE assuming a RQ of 0.83 (L CO2/L O2), a heat production of 4.83 kcal/L O2, and a density of 44.6 mmol CO2/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 VCO₂ (mmol/min) as

\[ VCO₂ = \frac{BMR}{60} \times BSA \times (0.83 \times 44.6)/4.83 \times 1.25 \]

The rate of \(^{13}CO₂\) production is normalized to % dose/h, calculated as

\[ \% \text{ dose/h} = \frac{APE}{VCO₂(60 \times \text{mmol \(^{13}C\) 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. The cumulative percent dose excreted at time \(t\), CPD(t), is then

\[ \text{CPD}(t) = VCO₂ \times \int_0^t \frac{APE(t)}{(60 \times \text{mmol \(^{13}C\) 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 \(^{1}\text{C}\)-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 \(^{1}\text{C}\)-propionate in blood before administration of \(^{1}\text{C}\)-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, these values were obtained during resolution of a urinary tract 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 vitamin B₁₂-reponsive MMA, the concentrations were only approximately 2.5 times control.

The half-life of \(^{1}\text{C}\)-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 B₁₂-unresponsive PA or MMA from the natural logarithm of APE \(^{1}\text{C}\)-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 \(^{1}\text{C}\)-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 \(\mu\text{mol/min/kg}\). This is comparable to the values of 0.92–3.10 \(\mu\text{mol/min/kg}\) found for five patients with MMA studied with continuous infusion of labeled propionic acid (19).

Conversion of \(^{1}\text{C}\)-propionate to \(^{13}CO₂\). The time course of isotopic enrichment of \(^{1}\text{C}\) in expired \(^{13}CO₂\) after i.v. and oral administration of \(^{1}\text{C}\)-propionate is shown in Figure 2. In control individuals, the conversion to \(^{13}CO₂\) 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 \(^{13}CO₂\) in patients with PA, and the curves were virtually flat. The \(^{1}\text{C}\)-enrichment curves in patients with MMA were flatter than those for control subjects, but there was significantly more generation of \(^{13}CO₂\) 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 \(^{13}CO₂\) 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 \(^{13}CO₂\) did not correlate with clinical severity of MMA. The results were indistinguishable from normal in the patient with TC-II deficiency before B₁₂ treatment, at a time when she was excreting modest but distinctly abnormal quantities of methylmalonate.

Effects of biotin, vitamin B₁₂, 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 \(^{1}\text{C}\)-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 \(^{1}\text{C}\)-propionate to \(^{13}CO₂\) 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 B₁₂-unresponsive MMA whose blood propionate concentrations were studied, the half-life of propionate and the conversion of \(^{1}\text{C}\)-propionate to \(^{13}CO₂\) were virtually identical in the presence and absence of vitamin B₁₂ (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 \(^{13}CO₂\) were among the highest for patients with MMA. S.H. was the only patient with MMA with whom there was an opportunity to test \(^{13}CO₂\) production before and after initiation of vitamin B₁₂ treatment. After 3 of intramuscular cyanocobalamin, there was no improvement in his apparent \(^{1}\text{C}\)-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 \(^{13}CO₂\) 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 \(^{1}\text{C}\)-propionate at 5 and 7 d after cessation of biotin therapy approximated the control value. Similarly, the cumulative percent of \(^{13}CO₂\) 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 decreased 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 $^{13}$C-propionate. Left panels show results obtained after administration of 30 µmol/kg $^{13}$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.).

Clinical Severity

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 | PCC/MCC | % Control |
|---------|------------|---------------------------|-----|---------|-----------|
| PS      | -Biotin    | 0.67 250.00               | 0.0027 | 0.08   |
| Control |            | 316.00 94.00              | 2.10 |
| PS      | +Biotin    | 1.10 253.00               | 0.0043 | 0.20   |
| Control |            | 187.00 89.00              | 2.04 |
| 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.
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 fact that the tracee pool size will affect the enrichment of tracer, and 3) 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$_2$ 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 these conditions in MMA but not in PA.

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-$^{13}$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 $\beta$-oxidation

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 (pmol/mg creatinine) |
|----------------------|----------------------------------|--------------------------------------------|------------------------------------------|
|                      |                                  | PCC | MCC | PC | 3-Methyl crotonyl glucose | 3-Hydroxyisovalerate | 3-Hydroxypropionate | Methylcitrate |
| 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  |    |    |
| Day 2                |                                  | 13.8 |     | 49.1 | 11.1 | 2.2  |    |    |
| Day 4                |                                  | 6.4  | 0.9  | 50.4 | 14.2 | 2.4  |    |    |
| Day 7                |                                  | 4.9  | 0.5  | 37.1 | 13.8 | 1.3  |    |    |
| Control              |                                  | 318.0 | 124.0 | 23.0 | 7.22 | 33.81 | 1.80 | 1.08 |
| Day 0                |                                  | 286.0 | 88.0  | 25.1 |    |    |    |    |

* MCC, methylcrotonyl-CoA carboxylase; PC, pyruvate carboxylase.
of propionyl-CoA to acetyl-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 \(\beta\)-oxidation of propionyl-CoA. However, the rate of \(^{13}\)CO\(_2\) production from 1-1\(^{13}\)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 1-1\(^{13}\)C-propionate is in equilib-rium with 1\(^{-13}\)C,S-methylmalonyl-CoA through methylmalonyl-CoA racemase (26), and if the CoA moiety is transferred between the carboxylates of methylmalonate (concertedly or sequentially), 1\(^{13}\)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, isotopic exchange between the methylmalonate and 1\(^{13}\)C,S-methylmalonyl-CoA has been demonstrated in the rat (29), where evidence was presented for sequential deesterification and res-terification of methylmalonate. However, data cited by Thomp-son et al. (8) showed only a small amount of \(^{13}\)H\(_2\)-propionate in only one of four children with MMA who were given continuous infusion of \(^{13}\)H\(_2\)-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 2-1\(^{13}\)C-propionate to eliminate 1\(^{13}\)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 cohabitation treatment in B,1\(^{13}\)-responsive MMA. There was no effect on 1\(^{13}\)CO\(_2\) production after 2 d of B 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\(_{1}\)-responsive MMA patients before and after B treatment or to test patient S.H. at more dates early in his therapy. The data from vitamin B\(_{12}\)-deficient patients given 2-1\(^{13}\)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 \(^{13}\)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 synthetase, 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 the PCC or 3-methylcrotonyl-CoA carboxylase (25).

There was no significant effect of biotin on the half-life of 1\(^{13}\)C-propionate or its conversion to 1\(^{13}\)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 1\(^{13}\)CO\(_2\) was noted, the improved recovery was still as low as in the untreated or B\(_{1}\)-unresponsive patients with MMA, and her urinary excretion of hydroxypropionic and methylycric 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 1\(^{13}\)CO\(_2\) generation between patients with PA and those with MMA. In fact, there was considerable overlap with control populations. In contrast, after bolus administra-tion there was no overlap of either disease population with controls and there was only one (posttreatment) overlap of the patients with PA and those with MMA. This may indicate differences in propionate pool distributions in PA and MMA or differences in inducibility of PCC and methylmalonyl-CoA mu-tase, or may reflect a role of free methylmalonate in the normal metabolism of propionate in man. In patients with PA, the recovery of 1\(^{13}\)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 1\(^{13}\)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.

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