Rapid determination of whole-body bicarbonate kinetics by use of a digital infusion

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IRVING, C. S., W. W. WONG, W. M. WONG, T. W. BOUTTON, R. J. SHULMAN, C. L. LIFSCHITZ, E. W. MALPHUS, H. HELGE, AND P. D. KLEIN. *Rapid determination of whole-body bicarbonate kinetics by use of a digital infusion.* Am. J. Physiol. 247 (Regulatory Integrative Comp. Physiol. 16): R709–R716, 1984.—Accurate determination of substrate oxidation rates from breath $^{13}CO_2$ levels often requires information on the bicarbonate status of the subject. We have developed a rapid method to *1*) obtain a complete set of bicarbonate kinetic parameters, *2*) prime bicarbonate pools with ^{13}C, *3*) clamp breath $^{13}CO_2$ levels rapidly and accurately with predetermined ranges, and *4*) provide a steady base-line enrichment of ^{13}C for a subsequent substrate oxidation measurement. The method consists of administering $NaH^{13}CO_3$ intravenously as a combination of a bolus dose, an exponentially decreasing infusion, and a constant infusion. A Harvard model 2729 microprocessor-controlled syringe pump was modified for external control and coupled to a Hewlett-Packard HP-85 desk-top computer to deliver the complex infusion. An infusion algorithm that would rapidly attain and maintain an increase of 50‰ ^{13}C enrichment of breath CO_2 was derived by using the SAAM-27 program to interrogate a three-compartmental model of bicarbonate kinetics in normal, fasted, resting adult subjects. When the method was tested on five adult fasted subjects who had rested for 1.5 h, plateau enrichments were achieved within 10–20 min. The bicarbonate pool sizes and kinetic parameters obtained by compartmental analysis of their $^{13}CO_2$ data were used to obtain a refined infusion protocol, which resulted in more rapid attainment of plateau enrichments. If carried out immediately before a substrate oxidation test, the method can provide a complete description of bicarbonate kinetics for use in the compartmental and noncompartmental analysis of substrate catabolism.

carbon-13; compartmental analysis; pool sizes; primed constant infusion; primed-, exponential-, constant-infusion technique

DETERMINATION OF WHOLE-BODY RATES of oxidation of amino acids, carbohydrates, and fatty acids often is based on recoveries of labeled CO_2 in breath after administration of ^{13}C- or ^{14}C-labeled substrates (12). To convert the recovery of labeled carbon measured in breath into an estimate of the percent oxidation of labeled substrate, a correction must be made for losses that occur during the passage of labeled carbon through the bicarbonate pools. Estimates of these losses usually are obtained from the fraction of intravenously administered $NaH^{13}CO_3$, administered either as a bolus (7, 9, 10, 23, 24) or in a constant infusion (1, 2, 11) that is recovered as respiratory CO_2. In some studies, values used for the fractional recovery of labeled bicarbonate have been obtained from previously published studies, whereas in others these values have been derived from measurements carried out on a day other than the day of the substrate oxidation measurement (8–10). Our recent demonstration that large inter- and intraindividual variations occur in bicarbonate losses (9) suggests that the quality of substrate oxidation measurements might be improved by carrying out a determination of bicarbonate kinetics simultaneously with oxidation measurements. We report here a method that *1*) provides a rapid estimate of bicarbonate flux and pool sizes, *2*) rapidly clamps breath $^{13}CO_2$ levels close to a predetermined value, *3*) provides a steady base-line enrichment of breath $^{13}CO_2$ suitable for subsequent substrate oxidation measurements, and *4*) primes all relevant bicarbonate pools with ^{13}C. The method is based on a summed primed-, exponential-, and constant-infusion technique (14, 20) for the delivery of labeled bicarbonate from a computer-controlled infusion pump. This infusion system has been assembled from commercially available state-of-the-art components. Here we demonstrate how this computer-controlled infusion protocol can provide not only an estimate of bicarbonate flux and nonrespiratory losses, but also a complete description of bicarbonate kinetics, identical to that obtained by the intravenous bolus method and suitable for use in multicompartmental analysis of substrate oxidations.

METHODS

Materials. $NaH^{13}CO_3$ (Merck Sharp and Dohme, Montreal, Quebec) containing 90 atom % excess (APE) ^{13}C as assayed by gas-isotope ratio mass spectrometry was made up at a concentration of 50 mmol in saline and sterilized by filtration. The solution was tested for sterility and absence of pyrogens before use. The actual concentration and isotopic enrichment of the solutions were determined by using a previously described reverse isotope-dilution technique (9).

Computer-controlled syringe infusion pump. A Harvard microprocessor-based syringe infusion pump (model 2720) was modified (Devices for Science, Fairfax, VA) to include an auxiliary output-input connector on the rear panel of the pump, which provides external computer

control of the flow rate over 0.1–99 ml/h and computer monitoring of pump status (i.e., mode and safety limits such as "end-syringe," "near-end," "overpressure," and "low battery"). A shorting plug also was installed to allow normal operation of the pump. The modified infusion pump then was coupled through a Hewlett-Packard 82940A GP-I0 parallel interface to a Hewlett-Packard HP-85 desk-top computer. Software for the HP-85 computer was written that calculated the time-dependent volumetric infusion rate for delivery of a combination of a constant rate and up to three exponentially decreasing rates from input values of the concentration of the bicarbonate solution, the infusion algorithm, the length of the infusion period, and the weight of the subject. From these data, the program also calculated the total volume of labeled bicarbonate solution required. The program provided digital output for two modes of pump operation: *1*) a constant priming mode that operated at the initial maximal infusion rate and *2*) an exponentially decreasing rate mode. In the exponential mode the program printed out the current infusion rate at 1-min intervals and sounded an alarm 15 s before each breath collection time.

Derivation of infusion algorithm. The unified compartmental model of bicarbonate kinetics in humans (see 9) (Fig. 1), has served as the basis of all compartmental calculations of infusion algorithm. In the conventional mode of compartmental analysis, the input of tracer substance is an independent variable and the fractional rate constants are the adjustable parameters. The fractional rate constants are varied until the best fit is obtained between the tracer levels calculated from the set of fractional rate constants and the observed levels. To obtain an infusion algorithm, the appropriate set of fractional rate constants becomes the independent variable and the parameters that describe the dose flow become the adjustable parameters.

The dose flow for the primed + exponential + constant infusion is described by a priming bolus dose (μmol/kg) and an infusion (*Eq. 1*)

$$\text{infusion rate} = Ee^{(-kt)} + C \qquad (1)$$

where E ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) is the preexponential coefficient, and k (min^{-1}) is the decay constant for the exponential infusion, and C ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) is the rate of the constant infusion. The values for the dose flow of $NaH^{13}CO_3$ that would result in the rapid attainment and maintenance of a 56.2-milliatom % excess (mAPE) enrichment of breath CO_2 were determined by using the SAAM-27 program. The set of fractional rate constants used were those reported for normal adult subjects who were fasted overnight and remained in the resting state. (These data are shown as reference values in Table 4). The dose flow parameters were varied until the best fit was obtained between the calculated breath ^{13}C enrichment and the desired value of 56.2 mAPE above basal enrichment. The tolerance in these values is reflected in their standard deviations.

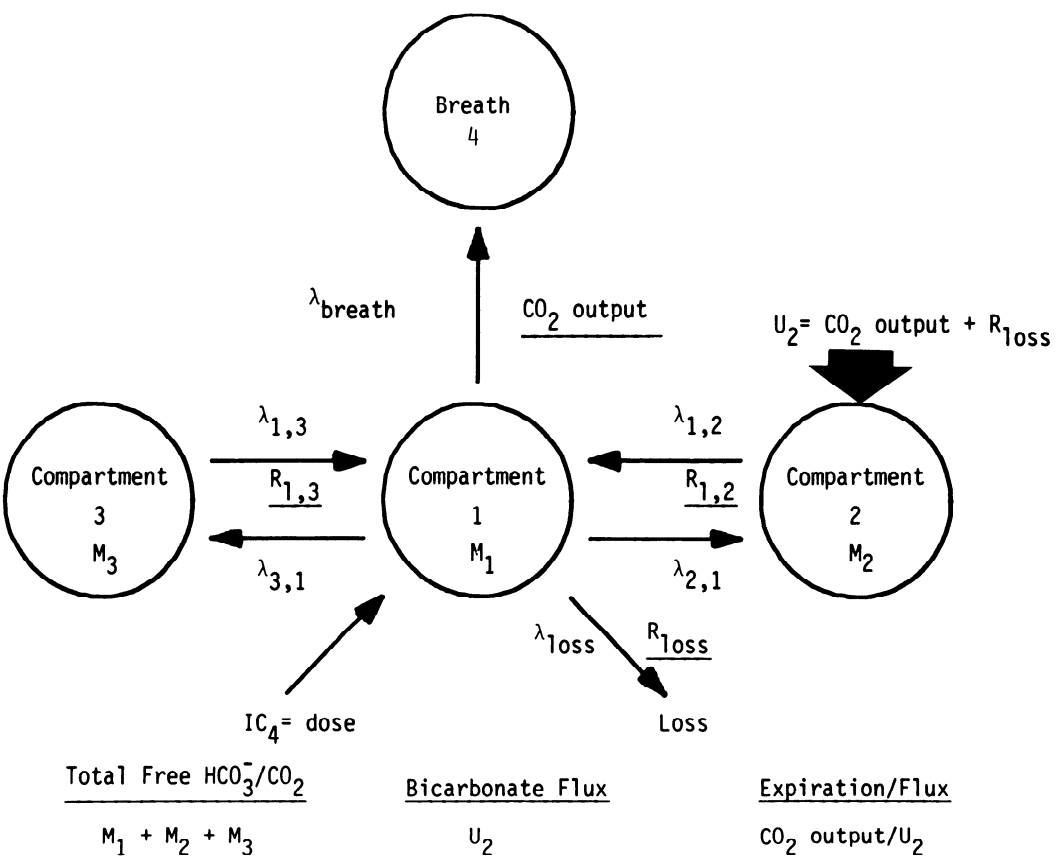

FIG. 1. Bicarbonate compartmental model and definitions of bicarbonate kinetic parameters taken from Ref. 9: fractional rate constants (λ, min^{-1}), pool sizes (M, $\mu mol \cdot kg^{-1}$), transfer rates (R, $\mu mol \cdot kg^{-1} \cdot min^{-1}$), endogenous input ($U_2$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and CO_2 output ($\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Verification of pump performance. To determine pump performance in the constant-infusion mode, a 50-ml disposable syringe (no. 5663, Becton-Dickinson) filled with water was placed in the pump and fitted with a 50-cm extension tube (no. K50, Pharmaseal) to which a 21-gauge Butterfly infusion set (no. 4492, Abbott Hospitals) was added, and syringe output was determined gravimetrically. At infusion rates of 50, 75, and 99 ml/h, approximately 5 min were required to achieve the expected infusion rate. This delay was the consequence of mechanical compliance in the syringe and the extension tube and of the spring-loaded plunger drive of the pump. It was necessary therefore to allow the pump to run several minutes at maximal rate before initiating the infusion. To verify performance in the exponential mode, the syringe was loaded with a dye that was delivered into a beaker containing 500 ml water, and the optical absorption was determined by continuous spectrophotometry. When the infusion algorithm for a 70-kg subject was simulated by using the dye solution and measurements were taken every minute for 30 min, there was agreement within 5% at all times between the estimated volume delivered and the amount predicted.

Subjects. The six volunteers described in Table 1 were recruited from laboratory personnel. All had normal medical histories and were free from any metabolic abnormalities. Informed consent was obtained from each in accord with the Helsinki Agreement. The protocol and use of the computer-controlled pump were approved by the Baylor Institutional Review Board for Human Research.

Protocol. After an overnight fast, each subject arrived at the laboratory at 8:00 A.M. and relaxed on a cot for the next 1.5 h, during which he or she was acquainted with the use of the face mask, which has been described previously (9). The subject donned the face mask 15 min before the start of the infusion and did not remove it for the next 45 min. Breath samples for the determination of the basal enrichment of respiratory CO_2 were collected 30 and 15 min before the start of the infusion.

To carry out the computer-controlled infusion protocol, a 50-ml disposable syringe was loaded with the amount of bicarbonate solution required for a 120-min infusion (usually 20–30 ml) plus an extra 10 ml to flush the infusion line and achieve maximal infusion rates. The syringe was fitted with a 50-cm extension tube that was connected to a Luer three-way nylon stopcock (no. 732-9009, Biorad). The two remaining ports of the stopcock were connected to an additional extension tube and to another three-way stopcock. Bicarbonate solution was purged through the system and out of the two ports of the second stopcock and then was diverted into the second extension and pumped through it at the starting infusion rate. A 5-ml syringe that contained the priming dose was connected to one port of the second stopcock. A 21-gauge Butterfly infusion set was placed into the subject's antecubital vein, flushed with saline, and then connected to the third port of the second stopcock. At the beginning of the infusion, the priming dose was administered rapidly and the bicarbonate solution from the pump was diverted from the second extension tube through the second stopcock into the subject. The exponentially decreasing infusion program was started immediately. This infusion protocol was executed rapidly and smoothly, with minimal discomfort to the subject, and allowed the computer-controlled infusion protocol to be started without delay to reach the maximal infusion rate. Breath samples for isotopic analyses were collected at 1, 3, 5, 7, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min, and CO_2 output was measured at hourly intervals as described previously (9).

TABLE 1. *Anthropometric and respiration data*

Subj No.	Sex	Age, yr	Height, cm	Weight, kg	$\dot{V}CO_2$, $\mu mol \cdot kg^{-1} \cdot min^{-1}$
1	F	34	160	57.3	114 ± 13
2	M	29	193	84.1	114 ± 23
3	M	36	183	88.6	132 ± 3
4	M	52	185	80.5	126 ± 10
5	F	24	165	57.7	125 ± 18
6	F	29	164	51.1	123 ± 14

$\dot{V}CO_2$, CO_2 output expressed as means ± SD.

Analytic procedures. The CO_2 concentration of breath samples taken during the respiratory volume measurements was analyzed by gas-solid chromatography on a Carle 111 instrument at 35°C and a helium gas flow of 90 ml/min by using a concentric CTR column that consisted of an external 183 × 0.63-cm molecular sieve column and an internal 183 × 0.32-cm Poropak mixture column (no. 8700, Alltech). The chromatographic separation of CO_2, O_2, and N_2 required 2 min. The composition of dried breath samples was calculated with a Hewlett-Packard 3390 integrating recorder. Previously described methods (9) were used to calculate CO_2 output from CO_2 concentration and respiratory volumes and to measure ^{13}C abundance of CO_2 by the automated gas-isotope ratio mass spectrometer. ^{13}C abundance was calculated as delta per mil vs. the limestone standard (PDB) and is expressed in units of atom percent excess × 1,000 (mAPE).

Data analysis and modeling were performed on the IBM 370 system operating in CMS previously described (9) with SPEAKEZ, a version of SAAM-27 modified to run interactively in CMS, and Tellagraf.

Compartmental analysis of computer-controlled infusion pump respiratory $^{13}CO_2$ data. The levels of $^{13}CO_2$ in breath obtained during the experiment for each subject were fitted by the SAAM-27 program (model code 10) to the three-compartmental model, shown in Fig. 1 and previously described for adult volunteers in the overnight-fasted and resting state. The compartmental analysis of computer-controlled infusion pump (CCIP) data differed from that of intravenous bolus measurements only in respect to the input of labeled bicarbonate, described by *Eq. 1* and Table 2. Estimates of pool sizes and transfer rates were obtained by steady-state solution by using each subject's measured CO_2 output rate. Convergence during the least-squares regression analysis was obtained in each case by using the criteria described previously for multicompartmental analysis of bicarbonate kinetic data (9). However, the standard deviations obtained for bicarbonate kinetic parameters were larger

TABLE 2. *Priming-, exponential-, and constant-infusion parameters*

Algorithm	P, μmol/kg	C,* μmol·kg^{-1}·min^{-1}	Exponentially Decreasing Rate	
			E, μmol·kg^{-1}·min^{-1}	k, min^{-1}
Initial	2.08	0.250	0.051	0.1156
	± 0.07	± 0.015	± 0.005	± 0.0016
Refined	2.00	0.264	0.074	0.0945
	± 0.05	± 0.013	± 0.005	± 0.0003

Values are means ± SD obtained from least-squares regression. P, priming dose; C, constant rate; E, initial rate; k, decay constant. * Values for the dose flow defined by $E\,e^{-kt} + C$.

than those obtained in intravenous bolus experiments. A "new" set of bicarbonate kinetic parameters, which represented the population of subjects studied, was determined by compartmental analysis of the averaged CCIP curve obtained from the data of the five subjects.

Refinement of the CCIP algorithm. The "new" set of averaged bicarbonate fractional rate constants together with the measured CO_2 production rates were used to derive a revised set of dose flow parameters. The procedure followed was the same as that used to obtain the initial set of dose flow parameters from the previously reported set of bicarbonate kinetic parameters (9) (see Table 4).

RESULTS

Simulated breath $^{13}CO_2$ levels from algorithm. The computer-simulated breath test curve that best approximated an instantaneous enrichment and a constant level of ^{13}C at 56.2 mAPE in breath CO_2 was obtained by using the values given in Table 2 for the priming dose, the preexponential coefficient, the exponential decay term, and the constant infusion. As seen in Fig. 2 the simulated breath $^{13}CO_2$ levels did not deviate from 56.2 mAPE by more than 2.2 mAPE. The curve rose slightly above 56.2 mAPE between 3 and 9 min and fell below 56.2 mAPE between 60 and 150 min. Plateau enrichments could have been attained earlier if additional exponential terms had been added to the infusion algorithm. However, the inclusion of such terms did not seem warranted at that stage of development.

To determine the amount of variation in the breath $^{13}CO_2$ levels that would result from departure of individual bicarbonate kinetics from the population values used to determine the infusion parameters, simulated breath test curves, which consisted of calculated values at the same sampling times used in the present study, were generated for the individual bicarbonate parameters reported (9) for normal, overnight-rested and fasted adult subjects. In all cases, plateau enrichments were obtained within 15 min and deviated less than 10 mAPE from the desired 56.2 mAPE.

Breath $^{13}CO_2$ curves obtained from infusion protocol. The levels of $^{13}CO_2$ in breath obtained during the infusion protocol are shown in Fig. 3 for the five subjects studied. In all subjects, constant levels of $^{13}CO_2$ were achieved between 10 and 20 min. The mean ^{13}C enrichment of

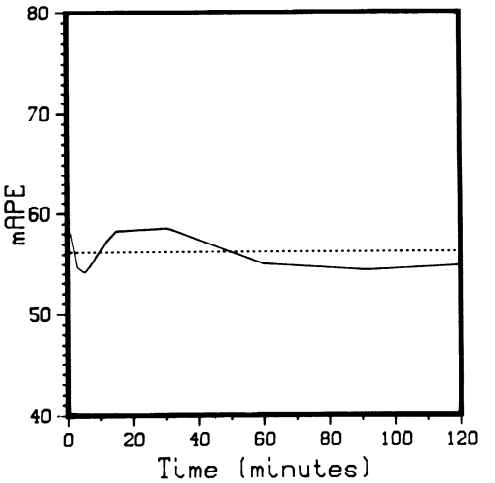

FIG. 2. Calculated best-fit curve (——) of ^{13}C enrichment of breath CO_2 expressed as milliatom % excess ^{13}C (mAPE = δ1.123‰ vs. limestone standard) obtained in determination of dose flow parameters that would result in immediate and constant labeling of breath CO_2 at 56.2 mAPE for published set of bicarbonate kinetic parameters.

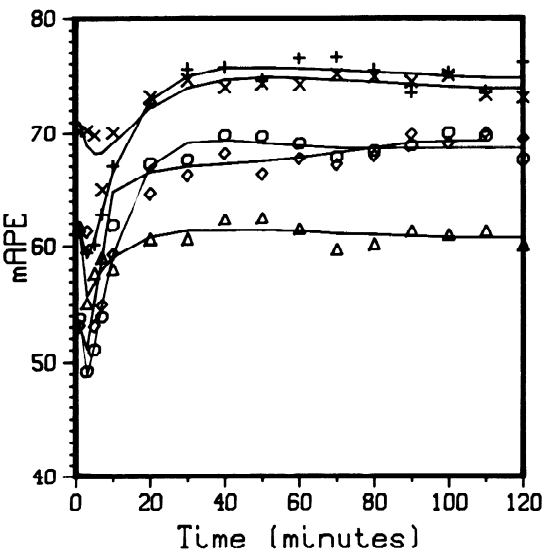

FIG. 3. Observed ^{13}C enrichments of breath CO_2 obtained in *subjs 1* (○), *2* (△), *3* (+), *4* (x), and *5* (◇) and calculated curves (——) obtained by fitting data to 3-compartmental bicarbonate model.

breath CO_2 between 20 and 120 min, shown in Table 3 for each subject, ranged from 59.3 to 72.6 mAPE. Within a particular subject, the variation in ^{13}C enrichment throughout the infusion was approximately 1.5% of the plateau enrichment. The values of bicarbonate flux (Table 3) estimated from the plateau enrichment ranged from 152.8 to 189.6 μmol·kg^{-1}·min^{-1}. The fractional recoveries of infused bicarbonate (Table 2), calculated from the ratio of $^{13}CO_2$ output at plateau to the rate of the constant infusion, ranged from 0.602 to 0.872.

The higher than expected plateau enrichment of breath CO_2 and longer than expected times required to reach these values suggested that *1*) the pump was malfunctioning, *2*) the five subjects studied represented a population that was not identical to that used to derive the infusion parameters, or *3*) the bicarbonate model was

TABLE 3. *Plateau ^{13}C enrichment, flux, and recovery estimated from breath $^{13}CO_2$ data for primed + exponential + constant infusion by using initial values*

Subj No.	mAPE	Flux, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	Fractional Recovery
1	67.26 ± 0.10	167 ± 2	0.682 ± 0.009
2	59.38 ± 0.61	190 ± 2	0.602 ± 0.007
3	76.67 ± 1.27	153 ± 3	0.872 ± 0.015
4	72.26 ± 0.82	155 ± 2	0.815 ± 0.009
5	67.26 ± 1.14	167 ± 3	0.749 ± 0.013
Mean ± SD	68.04 ± 5.69	166 ± 15	0.744 ± 0.107

Values are means ± SD. mAPE, milliatom % excess.

not sufficiently physiological to predict the result of a new dose input mode. Pump performance was verified as described in METHODS. The possibility that the five subjects had a different set of bicarbonate kinetic parameters from the group previously studied (9) was consistent with their higher rates of CO_2 output (122.4 ± 8.3 vs. 101.7 ± 4.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and higher fractional recoveries (0.744 ± 0.107 vs. 0.51 ± 0.09). It was not feasible to carry out additional intravenous bolus studies of bicarbonate kinetics in these subjects, and large day-to-day variations previously noted in bicarbonate kinetics suggested that such studies might be of limited value. Instead a method for direct derivation of bicarbonate kinetic parameters from $^{13}CO_2$ data collected during infusion was developed and tested.

Compartmental analysis of programmed influsion $^{13}CO_2$ data. Thus far we had used a set of bicarbonate kinetic parameters to derive a set of infusion parameters. We now approached the question of the fidelity of the reverse process in which infusion parameters and breath $^{13}CO_2$ levels were used to derive a set of bicarbonate kinetic parameters. The simulated $^{13}CO_2$ breath test curve, shown in Fig. 2, along with the infusion parameters given in Table 2, was fitted to a three-compartmental model of bicarbonate kinetics to obtain a new set of bicarbonate kinetic parameters. This regenerated set of bicarbonate kinetic parameters could be compared with the original parameters for accuracy and precision. Good convergence was obtained during the least-squares regression analysis carried out with the SAAM-27 program. The regenerated set of bicarbonate kinetic parameters agreed with the original parameters to within 3% and had a standard deviation of less than 0.1% of the values. The agreement demonstrated, both in principle and in fact, that bicarbonate kinetic parameters could be reconstructed from infusion data with a high level of accuracy and precision.

When the breath $^{13}CO_2$ data of the five subjects were fitted to a three-compartmental bicarbonate model, an excellent fit was obtained between the observed data and the calculated curve, as seen in Fig. 3. No systematic differences between the two were found as judged by coefficients of variation less than 0.015 and the absence of systematic differences between the observed and calculated curves.

The fact that introduction of small changes in the values of the bicarbonate kinetic parameters resulted in good fits of the observed $^{13}CO_2$ data indicated that the bicarbonate compartmental model was physiologically sound. It was concluded that this group of subjects had a set of parameters different from those previously described. Bicarbonate fractional rate constants, pool sizes, transfer rates, overall flux, and recovery obtained from compartmental analysis are given in Tables 3 and 4 along with their standard deviations. Compared with the group of normal adult subjects who had fasted overnight and remained in the resting state, the five subjects in this study had *1*) smaller central and peripheral bicarbonate pools (total free bicarbonate 11,306 ± 1,055 vs. 15,785 ± 2,350 $\mu mol/kg$), *2*) faster rates of transfer of bicarbonate between the central and peripheral pools, *3*) smaller nonrespiratory rate of loss of bicarbonate (44 ± 22 vs. 99 ± 25 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and *4*) smaller flux and larger fractional recoveries, as had been noted in the noncompartmental analysis of the $^{13}CO_2$ data at plateau.

TABLE 4. *Fractional rate constants for bicarbonate kinetics*

Subj No.	L_{21}	L_{12}	L_{31}	L_{13}	L_{loss}	L_{breath}
1	370 ± 74	400 ± 56	59 ± 9	36 ± 7	16 ± 2	36 ± 2
2	510 ± 200	890 ± 140	170 ± 24	55 ± 3	33 ± 4	47 ± 5
3	150 ± 30	320 ± 110	80 ± 19	53 ± 10	8 ± 1	42 ± 1
4	74 ± 32	98 ± 31	78 ± 32	53 ± 6	12 ± 9	44 ± 1
5	180 ± 34	333 ± 73	70 ± 10	38 ± 6	9 ± 1	34 ± 1
Mean ± SD	260 ± 180	410 ± 290	91 ± 44	47 ± 9	15 ± 11	41 ± 5
Averaged curve	140 ± 22	300 ± 64	87 ± 9	49 ± 4	14 ± 1	38 ± 1
Previously reported	138	187	63	26	29	30*

Values are means ± SD expressed as $min^{-1} \times 10^4$. * CO_2 output 101 $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

Derivation of readjusted infusion algorithm. The five sets of $^{13}CO_2$ data were averaged to obtain the averaged curve shown in Fig. 4. This curve, when fitted to a three-compartmental model of bicarbonate kinetics, yielded a set of kinetic parameters (Table 3) representative of all the subjects in this study. The new set of kinetic parameters, together with the averaged CO_2 output rates, was interrogated with the SAAM-27 program for the set of infusion parameters that would best result in the rise of breath $^{13}CO_2$ to an instantaneous and constant enrichment of 56.2 mAPE. As seen in Table 2, the new infusion algorithm consisted of a slightly smaller priming dose, an exponential rate that started at a slightly higher initial rate but decreased more rapidly, and a slower final rate of constant infusion.

Breath $^{13}CO_2$ levels obtained with readjusted infusion algorithm. Infusions using the new set of parameters were carried out on two occasions on *subject 1* and on a new volunteer, *subject 6*. As seen in Fig. 5, $^{13}CO_2$ rose more rapidly to a plateau value that was closer to the desired delta value of 56.2 mAPE. In the case of *subject 6*, a plateau enrichment of 52.9 mAPE was reached within 3 min.

DISCUSSION

An ideal method for the rapid determination of bicar-

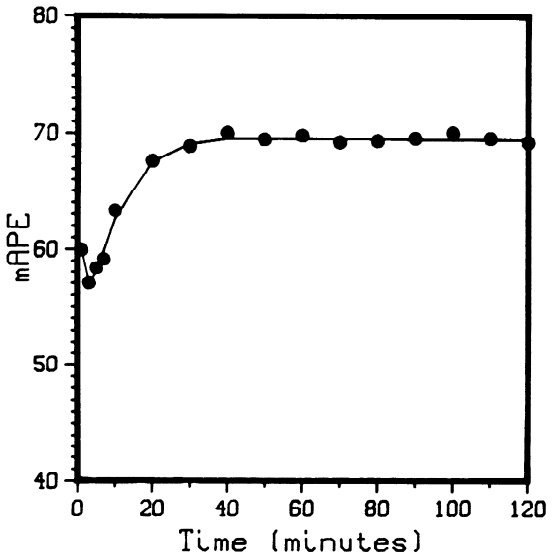

FIG. 4. Averaged ^{13}C enrichments of breath CO_2 for 5 *subjs* and calculated curve (——) obtained by derivation of new set of bicarbonate kinetic parameters from these data.

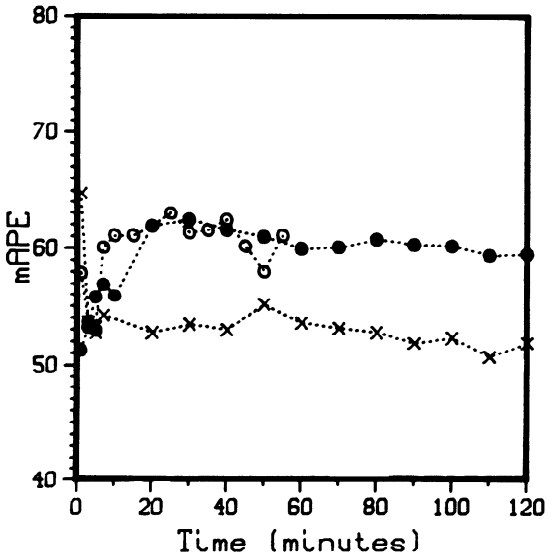

FIG. 5. Observed ^{13}C enrichments of breath CO_2 obtained for *1*) *subj 1* on 2 occasions (○ and ●) and *2*) for *subj 6* (x) with new dose flow parameters. Compared with curve for *subj 1* in Fig. 4, new dose flow parameters resulted in more rapid rise to plateau enrichment that was closer to 56.2 mAPE.

bonate kinetics in a subject immediately before substrate oxidation measurements would offer the three following advantages. *1*) It would provide an accurate and precise estimate of bicarbonate flux. *2*) Even more desirable, it would provide a complete set of fractional rate constants, pool sizes, and flux suitable for use in a more detailed kinetic analysis of substrate oxidation data. *3*) The method would not result in unpredictable changes in the ^{13}C enrichment of breath CO_2; preferably it would prime bicarbonate pools in a manner that reduces the time required for substrate oxidation measurements. Determinations of bicarbonate kinetics based on the output of $^{13}CO_2$ after intravenous administration of a single bolus of $NaH^{13}CO_3$ (7, 9, 10, 23, 24) require at least 120 min (24) and result in exponentially decreasing levels of breath $^{13}CO_2$. Superimposition of substrate oxidation measurements on nonconstant and unpredictable levels of $^{13}CO_2$ in breath would result in breath $^{13}CO_2$ data too difficult to analyze. The time required for $^{13}CO_2$ to return to pretest levels after an intravenous bolus is 6–8 h (9), which is too long to permit sequential measurements in the same subject to be carried out under the same metabolic conditions. On the other hand, constant infusions and primed constant infusions of $NaH^{13}CO_3$ also have been used to provide estimates of bicarbonate flux (1, 8, 10, 11). These infusions have the advantage that they result in constant levels of $^{13}CO_2$, once equilibrium has been achieved. Although 8 h are required for breath $^{13}CO_2$ levels to reach steady values during constant infusion of labeled bicarbonate (10), times as short as 40–60 min have been reported when the constant infusion is accompanied by an intravenous bolus injection of $NaH^{13}CO_3$ (1).

The use of a primed constant infusion is based on the assumption of a single homogeneous pool and first-order kinetics. Kruger-Thiemer (14) showed that for more than one body compartment, no combination of priming dose and constant infusion will result immediately in a constant level of drug in the central pool. This is appreciated readily in the series of curves they simulated for primed constant infusions in multicompartmental mammillary systems with irreversible loss from the central compartment (14). If the priming dose were chosen to set the zero-time plasma level of the drug to the level achieved at steady state, the plasma level then dropped to a minimum before a slow rise to steady state. The use of larger priming doses resulted in very high initial plasma levels that slowly approached the steady-state level. Mitenko and Ogilvie (16) subsequently showed that the quickest way to obtain steady-state concentrations was to let the ratio of priming dose to constant infusion rate be equal to the inverse of the terminal plasma decay rate observed after bolus administration of the drug. Allsop et al. (1) estimated, from literature values of bicarbonate pool size and rate of appearance, a value of 78–93 for the optimum prime-to-constant infusion ratio. This value has been used subsequently to prime bicarbonate pools (8, 15, 17, 18, 25–27). The mean values for total free bicarbonate and flux given in Table 3 for five subjects in this study yield a fractional turnover rate of 0.0146 min^{-1}, which is equivalent to the expected plasma terminal decay rate. The optimum prime-to-constant infusion ratio for these subjects would have been 68. The use of a priming dose to approach plateau concentrations more rapidly occasionally can lead to unpredictable results, with times required to reach equilibrium much longer than anticipated (14, 16, 20, 29). Since breath $^{13}CO_2$ levels are not monitored in real time, such protocols must be designed with long equilibration times if one does not wish to produce curves in which the achievement of plateau enrichments is problematic.

Kruger-Thiemer (14) and Vaughan and Tucker (22) derived infusion algorithms consisting of an initial intravenous bolus loading dose combined with an exponential-rate and a constant-rate intravenous infusion that would

achieve and maintain a constant plasma drug concentration. Many applications were proposed for this method; however, the lack of a pump capable of exponential infusions prevented such infusions from ever being carried out. Instead, approximate methods based on a series of stepped infusions were developed for rapid attainment of steady-state plasma levels of a number of drugs (19, 20, 29). Although the output of a number of pumps can be preprogrammed (3, 4, 13, 21), we are not aware of a syringe pump capable of delivering a smooth exponential infusion. The computer-controlled syringe infusion pump described in METHODS appears to be the first of its kind and now makes the execution of multiexponential infusions practical.

Clague et al. (6) proposed that errors in substrate oxidation measurements that result from large intra- and interindividual variations in bicarbonate kinetics might be minimized by the calibration of each subject's bicarbonate flux with a primed constant infusion of $NaH^{14}CO_3$ immediately before the infusion of the labeled substrate. The use of primed + exponential + constant infusion in place of primed constant infusion offers two advantages in carrying out such a protocol. *1*) Since the steady state is achieved much faster with the primed + exponential + constant infusion, the 60 min recommended by Clague et al. (6) to reach plateau could be reduced to less than 10 min and still meet their criteria for plateau enrichment, stable to within an 8% rise or fall in the sample enrichment. Reduction in equilibration time shortens the duration of the bicarbonate determination, which permits the study of more transient physiological and metabolic responses. *2*) The absence of large overshoots, such as those encountered in the initial phase of primed constant infusions, reduces the probability that a subject will not have achieved plateau enrichment at the time of sample collection.

To date, two experimental approaches have been taken to characterize bicarbonate kinetics. The first uses multicompartmental analysis of the decay of $^{13}CO_2$ in breath after a bolus dose of $NaH^{13}CO_3$ to obtain a set of pool sizes and transfer rates that provide a detailed mathematical description of bicarbonate kinetics. The second uses plateau enrichment of breath $^{13}CO_2$ achieved during the continuous infusion of labeled $NaHCO_3$ to obtain an estimate of overall flux of bicarbonate. This approach is not concerned with the internal structure of the bicarbonate system. Until now, the multicompartmental parameters could be obtained only from the bolus method, which cannot be applied immediately before substrate oxidation measurements. On the other hand, the primed continuous-infusion method, which *can* be carried out immediately before substrate oxidation measurements, was thought capable of yielding only a flux estimate and has been used mainly by investigators who were uninterested in the compartmental analysis of bicarbonate kinetics or substrate oxidation data. The extraction of pool size and transfer rate information from breath $^{13}CO_2$ levels measured during the primed + exponential + constant infusion of bicarbonate demonstrated here brings together the advantages of both methods. In multicompartmental studies of substrate oxidation, it now is possible to obtain a set of bicarbonate kinetic parameters that accounts for the losses and delays associated with passage of labeled carbon through the bicarbonate pools at the time of the substrate oxidation measurements. By reducing errors introduced into multicompartmental parameters from the use of a general or population value for bicarbonate kinetic parameters, it should be possible to resolve more subtle effects of metabolic state and dietary intake on substrate oxidation rates.

There are two possible explanations for the different set of bicarbonate pool sizes and kinetic parameters obtained for the five subjects in this study. The subjects were studied in the fasted state 1.5 h after arriving in the laboratory, whereas the previous values were reported for subjects who were studied in the fasted state after an overnight stay in the Clinical Research Center. One possible thesis is that labeled bicarbonate administered as a single bolus dose is distributed throughout the body and is eliminated in a manner different from that of labeled bicarbonate administered by continuous infusion. This is unlikely, since in both cases labeled bicarbonate follows the same circulatory route and the tracer doses of labeled bicarbonate were too small to perturb plasma bicarbonate homeostasis. A second and more likely explanation is that the physical activity associated with traveling to work in the morning affected bicarbonate pool sizes and transfer rates. The subjects who traveled to work tended to have smaller bicarbonate pool sizes and higher rates of CO_2 output, although they had been resting for more than 1 h before the start of respiratory measurements. One might speculate that if tissue bicarbonate levels rise during sleep, increased blood flow and respiration rates associated with traveling to work would tend to eliminate stored bicarbonate. These subjects also had smaller nonrespiratory losses of labeled bicarbonate. Part of the nonrespiratory loss is thought to be of metabolic origin (9, 20) and to result from the exchange of labeled CO_2 with labile carboxyl groups on metabolic intermediates such as oxaloacetate (5). The effect of increased activity on the metabolic state of the subjects is more difficult to predict. Further studies of the effects of moderate exercise and fasting on bicarbonate kinetics are necessary to make such predictions.

Even though multicompartmental models reduce complex patterns of tissue uptake and metabolism to a few compartments and transfer processes, they accomplish many useful objectives. One of these is the prediction of system performance under new conditions (28). The successful prediction of a set of primed + exponential + constant infusion parameters by the unified compartmental model of bicarbonate kinetics serves two purposes: *1*) it demonstrates the physiological relevance of the model, and *2*) it provides a new methodology that will aid in both the compartmental and noncompartmental analysis of bicarbonate and nutrient physiology.

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