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Permalink
https://escholarship.org/uc/item/4gv0k2rw

Journal
American Journal of Physiology, 259(1)

ISSN
0002-9513

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Publication Date
1990-07-01

DOI
10.1152/ajpregu.1990.259.1.r163

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Peer reviewed
Influence of increased metabolic rate on $[^{13}\text{C}]$bicarbonate washout kinetics

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Division of Respiratory and Critical Care Physiology and Medicine, Departments of Medicine and Pediatrics, Harbor-UCLA Medical Center, University of California, Los Angeles, School of Medicine, Torrance 90509; Department of Biomathematics, University of California, Los Angeles 90024-1766; and Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125

BARSTOW, THOMAS J., DAN M. COOPER, ERIC M. SOBEL, ELLIOT M. LANDAW, AND SAM EPSTEIN. Influence of increased metabolic rate on $[^{13}\text{C}]$bicarbonate washout kinetics. Am. J. Physiol. 259 (Regulatory Integrative Comp. Physiol. 28): R163–R171, 1990. The effect of changes in metabolic rate on the dynamics of CO$_2$ exchange among its various compartments in the human body is not well understood. We examined CO$_2$ dynamics in six healthy male subjects using an intravenous bolus of $[^{13}\text{C}]$bicarbonate. Subjects were studied while resting, during light exercise [50% of the lactate threshold (LT), 3–4 times resting O$_2$ uptake (VO$_2$)], and during moderate exercise (95% of the LT, 6 times resting VO$_2$). The sum of three exponential terms well described the washout of $^{13}\text{CO}_2$ in exhaled breath both at rest and during each exercise level despite substantial increases in metabolic rate accompanying the exercise studies. Average recovery of $^{13}\text{C}$ label rose from 67% during rest to 80% during light and moderate exercise ($P < 0.01$). The estimate of CO$_2$ elimination (VCO$_2$) calculated from the washout parameters and corrected for recovery was in very good agreement with the VCO$_2$ directly measured simultaneously by breath ($r = 0.999$, SE for VCO$_2$ = 0.079 l/min).

By use of a three-compartment mammillary model, the quantity of CO$_2$ in the central pool ($Q_1$) doubled from rest to light exercise (235 ± 60 to 479 ± 76 mmol, $P < 0.01$) but did not change further with moderate exercise (458 ± 74 mmol). Rate constants for exchange between pools and for irreversible loss in the system tended to increase with metabolic rate, but there was large variation in the responses. We conclude that the compartmental dynamics of CO$_2$ transport and storage are very sensitive to changes in metabolic rate induced by exercise.

THE STORES of O$_2$ in the body are relatively small; consequently, changes in O$_2$ uptake (VO$_2$) observed at the mouth parallel closely the simultaneous utilization of O$_2$ in metabolism by the various tissues of the body (2). In contrast, the body stores of CO$_2$ are large, so that changes in the metabolic production of CO$_2$ are not instantaneously translated to changes in CO$_2$ elimination (VCO$_2$) at the mouth (13). These stores of CO$_2$ and their effect on CO$_2$ transport in health and disease are not well understood.

CO$_2$ stores have been studied in the intact organism by the intravenous administration of radioactive $^{14}\text{C}$-, $^{15}\text{C}$-, or nonradioactive $^{13}\text{C}$-labeled bicarbonate (6, 19, 31). The subsequent washout of bicarbonate as labeled CO$_2$ in the breath during resting conditions typically has been described by the sum of three exponential terms (19, 20, 23, 32, 35), implying the presence of at least three major classes of kinetically distinct processes or pools, which affect the dynamics of VCO$_2$ at the mouth. These processes have been interpreted to represent washout of bicarbonate from a central pool in communication with two different tissue pools of bicarbonate with different perfusions (13, 19). Because exercise produces both an increased metabolic production of CO$_2$ and also significant changes in blood flow to several organs, we predicted that exercise would have a profound effect on the kinetics of bicarbonate washout.

We also wondered how exercise affects the following two aspects of CO$_2$ dynamics relevant to the evaluation of the oxidation of C-labeled substrates to CO$_2$: recovery of label and the average time a CO$_2$ molecule would reside in the exchanging CO$_2$ pools before being eliminated from the bicarbonate system (mean residence time; MRT). Recovery of injected bicarbonate as labeled CO$_2$ in the breath during resting conditions has been found to range between 50 and 90% (6, 11, 19-21, 34, 35) and is reported to decrease slightly with very mild exercise (31). No information is currently available regarding the effects of a wide range of metabolic rates on recovery of injected C-labeled bicarbonate or on MRT. To evaluate the influence of exercise on the dynamics of bicarbonate flux in the body and washout in the breath, we measured the washout of intravenously injected $[^{13}\text{C}]$bicarbonate as breath $^{13}\text{CO}_2$ during the following three metabolic states: rest, light exercise (three- to fourfold increase in metabolic rate), and moderate exercise (up to sevenfold increase in metabolic rate, but below an intensity that would result in sustained metabolic acidosis from lactic acid accumulation).

METHODS

Subjects. Six male volunteers, aged 21–34 yr, gave informed consent to participate in the study. Each was free from known cardiopulmonary or metabolic disease.
at the time of testing. All were physically active but none were undergoing extensive endurance training.

Protocol. Each subject performed a progressive cycle ergometer test to volitional fatigue, from which \( \dot{V}O_{2\text{max}} \) and the lactate (or anaerobic) threshold (LT, the maximum \( \dot{V}O_2 \) achieved before lactate begins accumulating significantly in the blood) were determined noninvasively from gas exchange and ventilatory patterns measured on a breath-to-breath basis as described below. Work rate on the cycle ergometer was increased in a ramp fashion by 30 W/min, following a 4-min period of unloaded cycling. Subject characteristics and \( \dot{V}O_{2\text{max}} \) are given in Table 1.

[\(^{13}\)C]bicarbonate washout kinetics were subsequently determined on separate mornings under one of the following three conditions: seated rest or during cycle ergometry at either 50 or 95% of the LT. The washout experiments in each subject were separated by \( \sim 1 \) wk. The experiments were performed in the morning, with the subject fasted for 10–12 h. Within 12 h before each turnover experiment, a 588 mM solution of NaH\(^{13}\)CO\(_3\) (Merck Sharp & Dohme lot no. 1931-I, 99.0%) in saline was made and sterilized by filtration. Chemical purity of the labeled bicarbonate was determined separately by back-titration with 0.05 N HCl, while the isotopic enrichment was confirmed by independent \(^{13}\)C nuclear magnetic resonance. For the studies at rest, the subject sat in a chair in the lab for 0.5 h before the start of the experiment and for the subsequent 4 h during the experiment. At time 0 a bolus injection over 5 s, containing 1,176 mmol [\(^{13}\)C]bicarbonate was made into an antecubital vein. Aliquots of exhaled gas (60 ml) for subsequent analysis for \(^{13}\)CO\(_2\) (described below) were drawn from the exhaled port of the breathing valve into plastic syringes and promptly sealed. Samples were drawn over 10 s, with the midpoint corresponding to the sample time, at the following times: \( \sim 5 \) min before the injection and 1, 3, 5, 7, 9, 12, 15, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min after injection. Metabolic rate (as \( \dot{V}O_2 \) and \( \dot{V}CO_2 \)) were determined by mass spectrometry from a sample drawn continuously from the mouthpiece at 1 ml/s. The inspired and expired volume and gas fraction signals underwent analog-to-digital conversion, from which \( \dot{V}O_2 \) (STPD), \( \dot{V}CO_2 \) (STPD), and minute expired ventilation (Ve; BTPS) were calculated on line with each breath, as previously described (4). The effect of the breathing valve on these calculations was evaluated by comparison with bag collection. A calibration factor was then used to obtain the final \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) reported here. Using this calibration factor, the SE values for \( \dot{V}CO_2 \) and \( \dot{V}O_2 \) were 27 and 45 ml, respectively. Heart rate during exercise was measured beat by beat using a modified V5 lead electrocardiogram.

Analysis of exhaled gas for \(^{13}\)CO\(_2\)/\(^{12}\)CO\(_2\). The \( \dot{V}CO_2 \) was isolated from the breath samples before analysis by ion ratio mass spectrometry by passage through a trap in dry ice (to remove water vapor) and then condensed in a trap in liquid nitrogen, allowing other gases to be evacuated (7). The \( \dot{V}CO_2 \) collected from the liquid nitrogen trap was further purified by passage over Cu turnings and MnO\(_2\) powder before isotopic analysis. This combined method of collection, isolation, and analysis led to greater precision (reduced variability) compared with commercial procedures. The ratio of \(^{13}\)CO\(_2)/^{12}\)CO\(_2\) in the exhaled gas samples was determined with a Nier 60\(^{\circ}\) double-collecting ion-ratio mass spectrometer, as modified by McKinney et al. (26). The ratio is reported in units of \( \delta^{13}\)C relative to the PDB (Belemnitella americana) standard (1.1235% \(^{13}\)C) and is defined as

\[
\delta^{13}\text{C}(\%n) = \left[ \frac{{^{13}\text{C}/^{12}\text{C}} \text{ sample}}{^{13}\text{C}/^{12}\text{C}} \text{ standard} \right] - 1.0 \times 10^{3} \quad (1)
\]

The value of the base line was subtracted from each value collected after injection of the [\(^{13}\)C]bicarbonate, yielding a net change in \( \delta \) (delta over base line; DOB). DOB can be converted to an equivalent excess specific activity (excess \(^{13}\)CO\(_2\)/total \( \dot{V}CO_2 \)) by multiplying DOB by 1.123 \( \times 10^{3} \).

General regression analysis of DOB data. For subsequent noncompartmental and compartmental analyses, it was necessary to find an empirical model that best fit the DOB washout data. The empirical models were se-
lected from among the following set:

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t}$$  \hspace{1cm} (2)

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} + L \cdot t$$  \hspace{1cm} (3)

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} + C$$  \hspace{1cm} (4)

$$\text{DOB} = \sum_{i=1}^{n} A_i e^{\lambda_i t} + L \cdot t + C$$  \hspace{1cm} (5)

where \(n = 1, 2, 3,\) or 4. Thus 16 candidate models were evaluated for each washout experiment. The \(A_i, \lambda_i, L,\) and \(C\) are referred to as the macroparameters of the model where \(A_i\) is the coefficient and \(\lambda_i\) the rate constant for the exponential process, \(L\) is the slope for a linear term, and \(C\) is a constant offset. Previous studies (19) included the linear trend \((L)\) and constant offset \((C)\) terms in Eqs. 3-5. For a specific candidate model with fixed value of \(n\), the best fit to the washout data was found using the weighted least squares (WLS) programs BMDP and BMDP3R (10). An optimal weighting scheme was used, namely weighting each datum inversely proportional to the measurement variance at that time (24). Preliminary analysis of residuals suggested that measurement error variance was approximately proportional to the square root of the observed DOB value. Alternate weighting schemes, including unweighted least squares, gave similar results to those reported below, suggesting robustness of our \(1/\text{DOB}\) weighting scheme. The BMDP programs provide point estimates and asymptotic standard errors for the model parameters and also for desired functions of the model parameters [e.g., area under curve (AUC) and MRT].

Because Eqs. 2-5 are from a series of nested models, the choice of the best fitting model was made by appropriate comparisons among the WLS fits of the 16 candidate models using an \(F\) test (5, 24) and assuming Gaussian errors. When comparing two nested models, we hypothesized that the simpler model is the true model and rejected this hypothesis in favor of the more complex model if the \(F\) statistic was sufficiently large (e.g., \(P < 0.05\)). For confirmation, we also used the Akaike Information Criterion and the Schwartz Criterion (24) to compare all 16 candidate models simultaneously. These results were very similar to the \(F\) test.

Noncompartmental analysis of washout kinetics. From the washout curve for \(^{13}\text{CO}_2\) in the breath, the following three important quantities were estimated: AUC, recovery of injected label, and the MRT. AUC was calculated by integrating to time = \(\infty\), the best fit regression equation for each washout experiment after subtracting out any linear trend or constant offset terms. For purely exponential models (from Eq. 2) this was equal to

$$\text{AUC} = \sum_{i=1}^{n} \left( A_i / \lambda_i \right)$$  \hspace{1cm} (6)

These regression-based AUC estimates compared well with results calculated directly from the DOB data using the trapezoidal rule with a single exponential extrapolation of the tail.

Recovery was calculated as

$$\text{Recovery (\%)} = \frac{\text{AUC} \times 1.123 \times 10^{-6} \times \dot{\text{VCO}}_2 \times 100}{\text{D}_0}$$  \hspace{1cm} (7)

where AUC is in units of \(\text{DOB} \cdot \text{min}\), \(1.123 \times 10^{-6}\) converts DOB to the fractional enrichment of total \(\text{CO}_2\) with added \(^{13}\text{CO}_2\), \(\dot{\text{VCO}}_2\) is the measured rate of \(\text{CO}_2\) elimination at the mouth in millimoles per minute, and \(\text{D}_0\) is the dose in millimoles of \([^{13}\text{C}]\)bicarbonate injected at time 0.

MRT for the whole bicarbonate system indicates the average time a labeled \(\text{CO}_2\) molecule, introduced into the central compartment as in this study, would remain in the exchanging bicarbonate system before being irreversibly lost either into the breath or via unaccounted loss.

MRT was estimated from the washout curves as the area under the moment curve (AUMC) divided by the AUC (9), assuming that the system is linear and stationary, that there are no \(\text{CO}_2\)-bicarbonate traps within the exchanging system, and that \(\text{CO}_2\) is eliminated only from the central pool. For the purely exponential model, AUMC = \(\Sigma (A_i / \lambda_i^2)\).

Compartmental analysis. Assuming that the washout data were adequately described by a sum of exponentials, we analyzed the data using a linear, mammillary compartmental system. For example, washout data with three exponentials would correspond to a three-pool model with one central pool (compartment 1; e.g., plasma) and two peripheral pools connecting only to compartment 1. Assuming tracer entry and \(\text{CO}_2\) loss is only via the central pool, this model is diagrammed in Fig. 1, where \(k_{ij}\) is the first order rate constant for transfer to pool \(i\) from pool \(j\), \(Q_i\) is the steady-state quantity of unlabeled \(\text{CO}_2\) stores in pool \(i\), and \(k_{0i}\) (not shown) is the fractional elimination rate for total irreversible loss of \(\text{CO}_2\) from the central pool. Because recovery of label as

![Diagram](https://via.placeholder.com/150)

**FIG. 1.** Representative 3-compartment mammillary model for the washout of \([^{13}\text{C}]\)bicarbonate using the sum of 3 exponentials. \(Q_i\) is the steady-state quantity of unlabeled \(\text{CO}_2\) in each pool, whereas \(k_{ij}\) represents 1st order rate constants for exchange of \(\text{CO}_2\) from pool \(j\) to pool \(i\). Irreversible loss (clearance) from system through central pool 1 occurs both by respiratory (measured; \(k_{01}\)) and nonrespiratory (unaccounted loss; \(k_{ai}\)) pathways. See text for further details.
The means were further evaluated using paired t-tests with repeated measures. Significant differences between model parameters was assessed by analysis of variance. The simple unweighted sample mean ± sample SD. The significance was declared for $P < 0.05$ after Bonferroni correction for multiple comparisons. Linear regression was used to examine any relationship between recovery of $^{13}$CO$_2$ in the exhaled breath and total VCO$_2$.

### RESULTS

**Metabolic responses.** The group mean for average $\dot{V}O_2$ over 4 h of rest was $281 \pm 20$ ml/min and for VCO$_2$ was $216 \pm 15$ ml/min (9.7 mmol/min; Table 2). Light exercise caused a three- to fourfold increase in both variables; mean average $\dot{V}O_2$ was $929 \pm 133$ ml/min, while VCO$_2$ increased to $840 \pm 113$ ml/min (37.7 mmol/min). Moderate exercise increased $\dot{V}O_2$ on average six times over rest (to $1,750 \pm 420$ ml/min) and VCO$_2$ over seven times above resting values ($1,639 \pm 454$ ml/min or 73.6 mmol/min). Light exercise represented 53% of the LT, or 30% of VCO$_{2\text{max}}$, while moderate exercise equated to 95% of the LT, or 57% of VCO$_{2\text{max}}$.

**Model identification.** The results from a typical washout experiment at each metabolic rate in one subject are shown in Fig. 2. Note that the washout dynamics at different metabolic rates were clearly distinguishable from each other and that even a mild increase in CO$_2$ production associated with light exercise caused a marked increase in the rate of loss of $^{13}$CO$_2$ into the breath. All 18 washout curves were well described by the sum of three exponential terms with no linear or constant terms (Eq. 2), as found previously by several investigators (19, 20, 23, 30-32, 35). In 12 of these experiments, Eq. 2 with $n = 3$ was also the statistically best description of the data.

$$\text{DOB} = A_1 \cdot e^{-\lambda t} + A_2 \cdot e^{-\lambda t} + A_3 \cdot e^{-\lambda t} \quad (12)$$

Whereas Eq. 12 resulted in excellent fits in the remaining six washout experiments, four of these curves were better fit statistically by the sum of four exponential terms (Eq. 2 with $n = 4$). These four more complex models were found in only two subjects, subject 3 (light and moderate exercise) and subject 6 (rest and moderate exercise). Two other experiments were best described by the sum of three exponentials plus a small constant offset term (Eq. 4 with $n = 3$). This occurred in subject 4 (moderate exercise) and subject 6 (light exercise).

When a three-compartment mammillary model was compared to a four-compartment model for the four data sets for which a sum of four exponentials was the better fit, it appeared that the fast peripheral pool of the three-compartment model had split into two intermediate pools, with little change associated either with the central pool or with the slowest peripheral pool. In addition, the dynamic characteristics and quantity of CO$_2$ in the entire exchanging system were similar between the three- and four-compartment models for the same data sets. For purposes of comparison, therefore, we chose to utilize the three-exponential description of the washout (Eq. 12) for all data sets in order to estimate noncompartmental parameters (e.g., AUC and MRT) and for deriving compartmental information. In all cases, the effect of increased metabolic rate with exercise on the washout and model parameters was much greater than any changes in the parameter estimates due to an extra constant or exponential term.

**Washout characteristics and noncompartmental analysis.** Table 2 gives the work rate performed by each subject, the resulting VCO$_2$, and the parameter estimates for Eq. 12 for each of the three metabolic conditions. Asymptotic standard errors of the parameter estimates were generally much smaller than the standard deviation across subjects. Increased VCO$_2$ associated with exercise resulted in significant reductions in $A_1$, $A_3$, and all three
TABLE 2. Work rate, metabolic rate, and washout parameters from three exponential functions (Eq. 12)

| Subject | Work Rate, W | VCO₂, mmol/min | A₁, DOB | λ₁, min⁻¹ | A₂, DOB | λ₂, min⁻¹ | A₃, DOB | λ₃, min⁻¹ |
|---------|-------------|----------------|--------|-----------|--------|-----------|--------|-----------|
| Rest    |             |                |        |           |        |           |        |           |
| 1       | 10.54       | 437.0±10       | -0.43±0.02 | 85.3±9.1 | -0.06±0.014 | 49.9±7.2 | -0.012±0.001 |
| 2       | 10.36       | 257.9±6.8     | -0.36±0.019 | 77.9±7.1 | -0.05±0.011 | 68.7±10 | -0.013±0.001 |
| 3       | 9.61        | 331.7±13      | -0.26±0.038 | 172.5±18 | -0.10±0.010 | 55.7±3.1 | -0.010±0.001 |
| 4       | 8.95        | 201.9±6.9     | -0.40±0.029 | 79.6±6.3 | -0.05±0.010 | 52.7±7.7 | -0.011±0.001 |
| 5       | 9.69        | 184.8±12      | -0.46±0.047 | 115.5±13 | -0.11±0.013 | 72.8±2.6 | -0.012±0.000 |
| 6       | 9.07        | 370.0±11      | -0.54±0.036 | 165.3±14 | -0.11±0.009 | 65.0±2.7 | -0.010±0.000 |
| Mean    | 9.70        | 297.2         | -0.45    | 116.7     | -0.084  | 59.1      | -0.012    |
| ±SD     | ±0.65       | ±99.3         | ±0.068   | ±44.2     | ±0.029  | ±28.5     | ±0.001    |
| Light exercise |       |                |        |           |        |           |        |           |
| 1       | 41.50       | 146.4±5.3     | -0.43±0.022 | 117.1±4.6 | -0.097±0.006 | 40.3±3.5 | -0.022±0.001 |
| 2       | 42.69       | 222.0±16      | -0.75±0.120 | 125.7±14 | -0.135±0.025 | 46.4±1.9 | -0.025±0.003 |
| 3       | 44.08       | 220.2±6.2     | -0.68±0.043 | 150.2±5.8 | -0.124±0.008 | 49.7±4.6 | -0.026±0.005 |
| 4       | 28.91       | 153.3±2.0     | -0.46±0.010 | 83.9±1.8 | -0.084±0.004 | 53.2±2.5 | -0.023±0.001 |
| 5       | 59.30       | 146.2±7.4     | -0.47±0.030 | 94.0±5.5 | -0.058±0.014 | 42.1±8.1 | -0.026±0.005 |
| 6       | 39.06       | 170.4±7.1     | -0.49±0.032 | 100.3±6.8 | -0.100±0.012 | 48.2±6.4 | -0.024±0.002 |
| Mean    | 37.76       | 177.4         | -0.54    | 111.9     | -0.106  | 46.8     | -0.024    |
| ±SD     | ±5.05       | ±35.6         | ±0.134   | ±24.2     | ±0.019  | ±4.8     | ±0.002    |
| Moderate exercise |       |                |        |           |        |           |        |           |
| 1       | 94.70       | 135.8±8.4     | -0.89±0.117 | 127.0±8.7 | -0.209±0.021 | 60.1±5.5 | -0.051±0.002 |
| 2       | 86.40       | 219.5±12      | -1.29±0.142 | 161.8±11 | -0.388±0.020 | 44.0±3.4 | -0.057±0.003 |
| 3       | 79.76       | 209.5±5.5     | -0.72±0.038 | 141.3±5.9 | -0.155±0.010 | 12.5±2.2 | -0.027±0.005 |
| 4       | 82.45       | 223.5±6.4     | -0.61±0.035 | 117.3±6.4 | -0.127±0.011 | 34.6±4.2 | -0.027±0.002 |
| 5       | 104.55      | 124.8±5.7     | -0.99±0.102 | 119.0±6.5 | -0.238±0.014 | 24.2±2.0 | -0.046±0.002 |
| 6       | 56.40       | 201.1±3.7     | -0.65±0.026 | 122.3±3.8 | -0.128±0.006 | 28.0±2.2 | -0.025±0.001 |
| Mean    | 73.63       | 189.2         | -0.85±0.058 | 127.0     | -0.19±0.019 | 33.9±6.4 | -0.039±0.009 |
| ±SD     | ±20.39      | ±46.8         | ±0.212   | ±17.6     | ±0.065  | ±16.6     | ±0.014    |

Values are means ± SE unless otherwise indicated. VCO₂, CO₂ elimination; A₁, and λᵢ, washout parameters DOB, δ over base line. Asymptotic SE values of estimate were generally much smaller than SD values across subjects. * Actual dose was 1.76 mmol, not 1.176; A₁ divided by 1.5. ¹P < 0.05 vs. rest; ²P < 0.05 vs. light exercise; ³P < 0.01 vs. rest; ⁴P < 0.01 vs. light exercise.

rate constants (λ₁, λ₂, λ₃) compared with rest.

The AUC, using Eq. 6 and normalized for a standard injection of 1.76 mmol [¹³C]bicarbonate, fell dramatically for light exercise compared with rest conditions (10.787 ± 1.195 DOB·t for rest vs. 3.308 ± 267 DOB·t at light exercise, P < 0.01). Moderate exercise caused a further significant decrease in AUC (1.761 ± 445 DOB·t, P < 0.01 compared with light exercise and rest).

MRT fell significantly from 65 ± 7 min (mean ± SD) at rest to 27 ± 2 min with light exercise (P < 0.01) and fell significantly lower still during moderate exercise (16 ± 5 min, P < 0.01 compared with rest and light exercise).

Recovery of [¹³C]CO₂ in the exhaled breath for all of the 18 individual experiments ranged from 59 to 90% (Fig. 3). Recovery rose significantly from a mean of 66.8 ± 5.3% at rest to 79.6 ± 10.9% for light exercise (P < 0.01), with no further change during moderate exercise (80.8 ± 3.3%). Coefficients of variation across subjects for recovery were 7.9% at rest, 13.7% for light exercise, and 4.1% during moderate exercise.

Compartment analysis. The interindividual means and standard deviations for the estimates of rate constants for exchange of CO₂ among the three compartments in the mammillary model and for loss of CO₂ into the breath and via unaccounted routes are presented in

FIG. 2. Semilog plot of washout of breath [¹³C]CO₂ after intravenous injection of [¹³C]bicarbonate at rest and during light and moderate exercise. Enrichment of breath CO₂ with [¹³C] expressed in units of δ over base line.

FIG. 3. Effect of metabolic rate (VCO₂) on recovery of [¹³C]bicarbonate as breath [¹³C]CO₂. Each symbol represents results for 3 metabolic rates in same subject.
Table 3. Interestingly, while there was a tendency for the rate constants to increase with light exercise over rest, these differences were not statistically significant; only moderate exercise resulted in significant speeding of the exchange dynamics. All of the rate constants except $k_{31}$ were significantly greater during moderate exercise than during rest, whereas all but $k_{31}$ and $k_{13}$ were also greater than the corresponding values for light exercise (Table 3).

In contrast to the changes in rate constants with exercise, the quantity of exchangeable CO$_2$ in compartment $i$ ($Q_i$) rose dramatically with light exercise (from $294 \pm 60$ to $479 \pm 76$ mmol, on average, $P < 0.01$) but did not change further with additional increases in metabolic rate ($458 \pm 74$ mmol)(Table 3). As discussed in METHODS, the quantities in pools 2 and 3 can only be bounded by lower and upper limits ($Q_{min}$ and $Q_{max}$ for each pool). Mean estimates for CO$_2$ in the "fast" pool 2 ranged from 176 to 247 mmol at rest, from 267 to 428 mmol during light exercise, and from 226 to 421 mmol for moderate exercise. Ranges for CO$_2$ in the "slow" pool 3 were 496-947 mmol, 557-1,616 mmol, and 674-2,261 mmol for rest, light exercise, and moderate exercise, respectively. Table 3 also lists estimates for the total exchangeable CO$_2$ in all three compartments under three possible conditions; all of the natural metabolic production of CO$_2$ occurs either in pool 1 ($Q_1$), pool 2 ($Q_2$), or pool 3 ($Q_3$). If endogenous CO$_2$ production is spread among the pools (e.g., one-third in each), then total exchangeable CO$_2$ at steady state equals the appropriate weighted average of the $Q_i$ (e.g., $XQ_i/3$). If the fractional distribution of endogenous CO$_2$ production among the three pools remains constant with increasing metabolic rate, then two important observations can be made from Table 3: 1) irrespective of the compartmental source of endogenous CO$_2$, total exchangeable CO$_2$ increases dramatically with exercise and 2) the preponderance of change is from rest to light exercise, with a slight, non-significant further increase in total CO$_2$ from light to moderate exercise. This is shown graphically in Fig. 4A, where the mean values for $Q_1$, $Q_2$, and $Q_3$ are plotted at the three average metabolic rates.

**TABLE 3. Mean parameter estimates and average estimation errors from three-compartment mammillary model**

| Parameter | Estimation Error, % | Rest | Light Exercise | Moderate Exercise |
|-----------|---------------------|------|----------------|-------------------|
| $k_{01}$  | 2.9                 | 0.066±0.016 | 0.102±0.019 | 0.201±0.056*$    |
| $k_{11}$  | 0.055±0.015         | 0.089±0.024 | 0.170±0.045 |
| $k_{12}$  | 0.008±0.005         | 0.013±0.010 | 0.032±0.015 |
| $k_{13}$  | 12.7                | 0.215±0.062 | 0.304±0.064 | 0.494±0.175$^+$  |
| $k_{31}$  | 10.3                | 0.163±0.017 | 0.173±0.052 | 0.232±0.047$^+$  |
| $k_{32}$  | 12.6                | 0.032±0.007 | 0.045±0.004 | 0.066±0.029$^+$  |
| $k_{33}$  | 13.7                | 0.077±0.034 | 0.055±0.019 | 0.091±0.029      |
| $Q_1$     | 2.2                 | 233±60    | 479±76        | 458±74           |
| $Q_{min}$ | 10.7                | 195±82    | 367±44        | 296±46           |
| $Q_{max}$ | 10.5                | 268±107   | 428±66        | 421±96           |
| $Q_{med}$ | 6.7                 | 521±120   | 557±77        | 674±104          |
| $Q_{med}$ | 9.4                 | 1004±155  | 1616±136      | 2261±863         |
| $Q_t$     | 3.6                 | 948±65    | 1303±93       | 1358±234$^+$     |
| $Q_2$     | 3.7                 | 1023±67   | 1464±113$^+$  | 1643±234$^+$     |
| $Q_3$     | 7.7                 | 1431±146  | 2361±174$^+$  | 2906±867$^+$     |

Values are means ± SD. $k_{01}$ rate constants for transfer to pool 1 from pool 0 in min$^{-1}$; $Q_i$ quantity of CO$_2$ in pool i in mmol; $Q_{min}$ and $Q_{max}$, upper and lower limits of quantities of CO$_2$ in pool i; $Q_t$, total quantity of CO$_2$ in pool i in mmol given endogenous CO$_2$ production only in pool i. Estimation error is average across all 3 metabolic rates of the asymptotic SE values expressed as % of the estimate (i.e., coefficient of variation). $^* P < 0.05$ vs. rest; $^+ P < 0.05$ vs. light exercise; $^+ P < 0.01$ vs. rest; $^+$ $P < 0.01$ vs. light exercise.

FIG. 4. A: range of possible values for total exchangeable CO$_2$ at each metabolic rate. $Q_t$, $Q_2$, and $Q_3$ represent estimates for total CO$_2$ assuming that metabolic production of CO$_2$ occurs only in central compartment ($Q_t$), in fast peripheral compartment ($Q_2$), or only in slow peripheral compartment ($Q_3$). B: extremes in possible changes in CO$_2$ stores with exercise. If endogenous source of CO$_2$ production changes from compartment 3 at rest to compartment 1 during moderate exercise (dashed line), there is virtually no change in total CO$_2$ stores (1%). Conversely, if the endogenous source of CO$_2$ changes from compartment 1 at rest to compartment 3 with moderate exercise, total CO$_2$ increases over 17 liters (337%; solid line). Note, however, that no negative changes (i.e., loss of CO$_2$) are predicted.

**Estimation of VCO$_2$.** The rate of total VCO$_2$ ($k_0Q_t$) is analogous to clearance and can be estimated noncomparatively from the washout data (dose divided by AUC)(9). However, clearance will be greater than the measured VCO$_2$ because recovery is less than 100% [i.e., $k_0Q_t$, estimates all loss of label, both in the breath and any nonrespiratory (unmeasured) loss]. Figure 5 shows the clearance data, corrected for the average recovery at rest (0.67) or exercise (0.80), as a function of the measured VCO$_2$ at the mouth, both in units of liters CO$_2$ excreted per minute. The dashed line is the line of identity. Correction by the average recovery led to a very good prediction of the measured VCO$_2$ from the washout characteristics ($r = 0.999$, SE for VCO$_2$ = 79 ml/min).
which the central compartment represents vascular and possibly some interstitial bicarbonate and the two peripheral compartments under resting conditions is assumed to be breath. Dotted line is line of identity. Regression analysis: intercept $= -0.009$, slope $= 1.017$, $r = 0.993$, SE for $\dot{V}CO_2 = 79$ ml/min.

**FIG. 5.** Estimation of $\dot{V}CO_2$ calculated from $[13C]$bicarbonate washout as breath $^{13}CO_2$ and corrected for average recovery at rest (0.67) or exercise (0.80), compared with $\dot{V}CO_2$ measured directly by breath. Dotted line is line of identity. Regression analysis: intercept $= -0.009$, slope $= 1.017$, $r = 0.993$, SE for $\dot{V}CO_2 = 79$ ml/min.

**DISCUSSION**

In the present study, washout of labeled bicarbonate was consistently and sufficiently well described by the sum of three exponential terms across a wide range of metabolic rates. In contrast, Irving et al. (19) found a small linear term suggesting a base-line drift, in addition to the three exponential terms, in their study of subjects at rest, which they attributed to changes in oxidative substrate mix, since carbohydrate and lipid differ slightly but measurably in $^{13}$C/$^{12}$C (29). However, this term was only detectable between 4 and 6 h after the bolus injection of labeled bicarbonate. In the present study, respiratory quotient (RQ)($\dot{V}CO_2$/$\dot{V}O_2$) fell slightly on average from 0.88 to 0.72 over the first 2 h of rest experiments and from 0.91 to 0.85 during the 2 h of moderate exercise, with no consistent change during the light exercise protocols. This small change in RQ would suggest a change in background $^{13}$CO$_2$/$^{12}$CO$_2$ of only 1-2% (3, 29). However, no significant linear drift components to the washout characteristics were found in any of the 18 individual experiments in the present study. In addition, the infrequent occurrence (2 of 18) and small size (1-2 DOB) of the constant term suggest that it is most likely not a fundamental component of the washout characteristics.

There remains uncertainty regarding the physiological correlates of the three-compartment mammillary model derived from the washout of labeled CO$_2$. The earliest observers (15, 23, 30, 32) speculated that the capillary and cellular membranes represented a significant diffusion barrier for CO$_2$ equilibration between extra- and intracellular spaces. Thus the central compartment represented vascular and extracellular bicarbonate, the fast peripheral compartment was intracellular bicarbonate of soft tissues, and the slower peripheral compartment, when observed, was bone carbonate.

In contrast, other investigators have suggested a perfusion-limited, organ-based model (13, 19, 31, 35), in which the central compartment represents vascular and possibly some interstitial bicarbonate and the two peripheral compartments are composed of tissues distinguishable by different perfusions. The tissue compartment with relatively fast exchange with the central compartment under resting conditions is assumed to represent metabolically active tissue with high perfusion (heart, brain, kidney, etc.), while the slower equilibrating pool is hypothesized to consist primarily of resting skeletal muscle, which has a relatively low perfusion at rest. Exchange of labeled bicarbonate with bone carbonate was envisioned to represent an essentially unidirectional loss of label over the course of a typical experiment (several hours). This interpretation is based on the electrical analog model of Farhi and Rahn (13) and is consistent with the observation that the initial ability of the body to store CO$_2$ with rebreathing is greater when perfusion to resting skeletal muscle is increased either by vascular denervation or increased metabolic rate (16).

It is interesting to note, however, that washout of solutes (28) or inert gases (27) from isolated, resting skeletal muscle demonstrates multiexponentiality, which would argue against the simple electric analog model of Farhi and Rahn (13). Thus, the precise physiological location of the three compartments remains unresolved.

The increase in Q$_1$ seen with mild exercise may reflect an increase in total CO$_2$ content within the bicarbonate system or a shift of bicarbonate from one of the two peripheral pools into the central pool. This large increase in Q$_1$ is not consistent with the hypothesis that membrane transport of CO$_2$-bicarbonate is the rate-limiting process in CO$_2$ exchange. Rather, these findings support the notion that perfusion is an important determinant of exchange, especially if the additional bicarbonate in the central pool is associated with the contracting skeletal muscles. However, if tissue perfusion, per se, was the sole limiting factor governing CO$_2$ exchange, one would predict a linear increase in Q$_1$ with increasing metabolic rate, which would parallel the known linear rise in blood flow which accompanies increases in metabolism (14, 22). This, in fact, did not occur. An alternative interpretation is that with even small increases in metabolic rate above rest, most or all of the muscle capillaries [known to be partially closed under resting conditions (18)] are “recruited” (i.e., opened), so that the diffusion surface approaches its maximum and the resistance to exchange for CO$_2$ between the intracellular pool and the vascular compartment is minimized (16, 18).

In contrast to the shift from rest to mild exercise, the bicarbonate model predicts that the greater rate of $\dot{V}CO_2$ elimination with moderate relative to light exercise is associated solely with an increased rate of fractional elimination from the central pool (as $k_0$) with no further increase in the amount of CO$_2$ in that pool (Q$_1$). This suggests that convective processes (e.g., blood flow and pulmonary ventilation) may play a greater role in facilitating removal of CO$_2$ from the blood to the environment at this level of exercise.

If it were known in which pool(s) the endogenous production of CO$_2$ occurred in vivo, then the total mass of exchangeable CO$_2$ in the three pools could be explicitly defined. However, this information can not be derived from the washout data alone (9); hence, only lower and upper bounds could be estimated for CO$_2$ in the two peripheral pools and, thus, for total exchangeable CO$_2$. If all of the metabolic production of CO$_2$ occurs in the central compartment, then the total quantity of CO$_2$ is
explicitly defined by $Q_t$ (Eq. 9), can be estimated non-compartmentally as the product of MRT and $V_{CO_2}$, and represents the minimum possible total $CO_2$ content. If endogenous production of $CO_2$ occurs in one of the peripheral compartments, with clearance remaining only from the central compartment, then the enrichment of the various $CO_2$ pools will not be uniform. In this case, the total $CO_2$ in the system is greater than $Q_{ts}$, with production of $CO_2$ entirely in the slow pool (compartment 3) yielding the largest estimate of exchangeable $CO_2$ ($Q_{ts}$). This is reflected by a 50% range in the estimates of total $CO_2$ between $Q_t$ and $Q_{ts}$ for the rest condition and by a range of 121% for moderate exercise (Table 3 and Fig. 4).

Similarly, the effect on the total $CO_2$ content of increased $CO_2$ production with exercise can be bounded but not calculated precisely. This is shown diagrammatically in Fig. 4B, where $Q_t$ and $Q_{ts}$ have been plotted as functions of metabolic rate. For each metabolic rate, $Q_t$ represents the minimum and $Q_{ts}$ the maximum estimate of total exchangeable $CO_2$ in the three pools. Depending on the hypothesized source of endogenous $CO_2$, increases of 1% ($Q_{ts}$ at rest to $Q_t$ for moderate exercise, dashed horizontal line in Fig. 4B) up to 337% ($Q_t$, rest to $Q_{ts}$ for moderate exercise, solid vertical line) can be derived for the increase in body $CO_2$ with exercise. Independent estimates of the increase in $CO_2$ stores with exercise would shed considerable light on this current uncertainty.

Recovery of injected or infused labeled bicarbonate as $CO_2$ in the breath has been reported to range from 50 to 90%, whether the species is man (1, 6, 19–21, 31, 34, 35), dog (11), or cat (23). We found that recovery increased during mild exercise but did not rise further when metabolic rate was increased with moderate exercise. Similarly, Van Aerde et al. (33) found in newborn infants a variable ($r = 0.64$) but significant ($P < 0.01$) increase in recovery of $^{13}CO_2$ (from 70 to 84%), which was proportional to resting metabolic rate over a small range, from 5 to 7.5 ml·kg$^{-1}$·min$^{-1}$. Slanger et al. (31) found that recovery fell, on average, from 83% at rest to 73% with very mild exercise (60% increase in $V_{CO_2}$), but this was not statistically significant (our analysis).

Unaccounted loss of labeled bicarbonate ($k_{ls}$) was assumed by us and others (19, 31, 35) to occur from the central pool; this meant that all of the model rate constants ($k_{ls}$ values) were identifiable. If loss occurred from compartments 2 and/or 3 only upper and lower bounds rather than explicit values for the rate constants associated with that pool can be found (8, 25). Physiological processes that may represent effective loss of labeled bicarbonate over the course of an experiment include: 1) loss of labeled $CO_2$ in the breath with the first pass of venous blood through the lungs (12), 2) true, irreversible loss of bicarbonate directly into the urine, sweat, or urea (23), and/or 2) transfer into alternate pools whose turnover is so slow as to represent effectively unidirectional flux over the course of the experiment, such as incorporation into bone (23) or macromolecules (17). Loss of labeled $CO_2$ with the first pass through the lungs is equivalent to a reduction in the injected dose by that amount of label. Kornberg et al. (23) found in the resting, anesthetized cat that 6% of injected $[^{14}C]bicarbonate label was found in bone after 5 h, while 3% remained as urea. Irving et al. (19) estimated that bone sequestering of bicarbonate label could be as high as 13% over 4 h of rest in humans. While incorporation of labeled bicarbonate specifically into blood glucose is low (1%; 17), accumulation of label by other moieties of intermediary metabolism could be quantitatively important.

We have shown here that across subjects and a wide range of metabolic rates without metabolic acidosis, labeled $CO_2$ washout dynamics generally exhibit triexponential decay. In addition, recovery of label increased with a modest increase in metabolic rate during mild exercise but did not change further during moderate exercise. We also found that metabolic rate as $V_{CO_2}$ could be accurately predicted from the washout curve for $^{14}CO_2$. Finally, the three-compartment mammillary model constructed from the washout curve allowed us to evaluate the effects of increased metabolic rate with exercise on $CO_2$ pool dynamics within the body. Characterization of the washout of labeled bicarbonate thus yields substantial information regarding the bicarbonate storage/transport system.

We thank Joe Ruth and Eleanor Dent for analysis of the $^{14}CO_2$ samples.

This work was supported in part by National Heart, Lung, and Blood Institute Grant HL-11907. T. J. Barstow was supported by National Institutes of Health Grant T32-DK-07461-04. E. M. Landau was supported in part by National Cancer Institute Grant CA-16042. D. M. Cooper is the recipient of the Clinician Scientist Award of the American Heart Association, Greater Los Angeles Affiliate, and the Career Investigator Award of the American and California Lung Associations.

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Received 8 February 1989; accepted in final form 6 March 1990.

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