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KINETICS OF CO_2-HCO_3^- IN NORMAL ADULT MALES

H. S. Winchell, H. Stahelin, N. Kusubov, B. Slanger, M. Fish, M. Pollycove and J. H. Lawrence

Donner Laboratory, University of California, Berkeley, California

The importance of quantitating the interchange between various components of the body's CO_2-HCO_3^- pools is well appreciated by investigators studying *in vivo* metabolism by measuring the rate of expiration of $^{14}CO_2$ after the administration of ^{14}C-labeled materials. It is clear that the rate of $^{14}CO_2$ expiration in the breath following administration of ^{14}C-labeled materials is not only a function of the *in vivo* metabolism of the given material but also of the kinetics of the CO_2-HCO_3^- pools which the labeled CO_2 must traverse before its expiration in the breath (*1*). The present study was undertaken to provide quantitative information about the rates of interchange between various components of the body's CO_2-HCO_3^- pools in normal male subjects at rest.

MATERIALS AND METHODS

The apparatus for continuous measurement of the rate of $^{14}CO_2$ and CO_2 excretion in the breath is similar to that described previously (*2*). The ^{14}C-labeled sodium bicarbonate was obtained from New England Nuclear Corp. with a specific activity of 5-6 mCi/m*M*. Before use the labeled bicarbonate was dissolved in sterile saline solution made slightly alkaline by adding a small quantity of sodium hydroxide. In all studies (with the exception of Patient JJ) 1.4–6.3 μCi ^{14}C-labeled bicarbonate was injected rapidly as a single intravenous bolus while the patient was breathing normally into the breath analysis apparatus. The $^{14}CO_2$ and total CO_2 excretion in the breath were monitored continuously in each subject for a minimum of 2 hr following intravenous administration of the label.

The $^{14}CO_2$ excretion rate during any given time interval was divided by the total CO_2 excretion rate during the same time interval. This ratio was then multiplied by the average CO_2 excretion rate measured during the study to give a $^{14}CO_2$ excretion rate for the given time period which was corrected for minute-to-minute variations in total CO_2 excretion. The $^{14}CO_2$ excretion data obtained during the initial 3 min were discarded, and only the corrected $^{14}CO_2$ excretion rates obtained after the initial 3-min interval were analyzed. Such data were fit to a function consisting of sums of exponential terms where one of the exponential terms had a rate constant fixed at the value of the turnover rate previously measured for the $^{14}CO_2$ breath analyzer used in the study (k). Data values were weighted by a factor equal to the reciprocal of the data value. A variable metric minimization program in a CDC 6600 computer was used to fit the function to the data (*3*).

Solutions for the rate constants of the model shown in Fig. 1 were obtained by identifying the terms of the Laplace transforms of the differential equations describing this model with the terms of the Laplace transforms of the function used to fit the data. Taking the amount of radioisotope injected as equal to unity, the initial conditions were taken as: $N_{10} = 1 - 3\alpha_{11}$; $N_{20} = 3\alpha_{12}$; $N_{30} = \frac{\Sigma Ai}{k}$ where N_{10}, N_{20} and N_{30} are the fractions of administered radioactivity in compartments 1, 2 and 3, respectively, (Fig. 1) 3 min after i.v. administration of labeled bicarbonate, $\alpha_{11} = \alpha_{12} + \alpha_{13} + \alpha_{1e}$, Ai = constant coefficient of the ith exponential term, k = turnover rate of the $^{14}CO_2$ breath analysis device. The total CO_2-HCO_3^- content of Compartment 1 was taken as the average breath CO_2 excretion rate divided by α_{13}. If one assumes that none of the CO_2 produced in the body is introduced into Compartment 2, then the CO_2-HCO_3^- content of Compartment 2 is equal to the product of the CO_2-HCO_3^- content of Compartment 1 times α_{12}/α_{21}. If all of the CO_2 produced in the body is introduced into Compartment 2 then the CO_2-HCO_3^- content of Compartment 2 is equal to the value calculated above plus the total-body CO_2 excretion rate divided by α_{21}. These two values give a lower and upper limit to the CO_2-HCO_3^- content of Compartment 2.

RESULTS

Figure 2 presents an example of the pattern of $^{14}CO_2$ excretion in the breath of patient (JG) given a single i.v. bolus of ^{14}C-labeled bicarbonate. The

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For reprints contact: H. S. Winchell, Donner Laborator University of California, Berkeley, Calif. 94720.

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TABLE 1. PARAMETERS OF EXPONENTIAL FUNCTION USED TO FIT $^{14}CO_2$ EXCRETION DATA FOLLOWING $H^{14}CO_3$ ADMINISTRATION

Patient	Age	Wgt. (kg)	A_1	A_2	A_3	r_1	r_3	Average CO_2 excretion rate (mM/kg*/min)
[redacted]	[redacted]	86.4	0.01028	0.03687	0.00476	0.01426	0.201	0.1455
		78.4	0.00861	0.01382	0.01061	0.01138	0.1279	0.1356
		70.5	0.00634	0.01256	0.01361	0.0091	0.1119	0.1387
		79.5	0.00716	0.00989	0.01544	0.01022	0.1192	0.1286
		54.5	0.00853	0.01814	0.00149	0.01058	0.1496	0.1512
		75.0	0.00658	0.0226	0.00926	0.00972	0.1362	0.1312
		70.5	0.00605	0.01266	0.01310	0.0103	0.1121	0.1252
		81.8	0.00785	0.01944	0.00333	0.01058	0.164	0.1538
		80.9	0.00806	0.01594	0.01374	0.0116	0.105	0.1491
		76.2	0.01096	0.02431	0.00403	0.01636	0.1792	0.2021
		74.1	0.00594	0.01729	0.02492	0.00881	0.1322	0.1448
		63.2	0.00603	0.01933	0.01766	0.00915	0.1106	0.1687
		56.2	0.00529	0.01003	0.00778	0.01013	0.0973	0.1183

* kg = kilogram body weight.

TABLE 2. FRACTIONAL INTERCOMPARTMENTAL RATE CONSTANTS AND CO_2 CONTENT OF COMPARTMENTS SHOWN IN FIG. 1

Patient	α_{12}*	α_{21}*	α_{13}*	α_{10}*	N_1 (mM/kg†)	N_2‡ (mM/kg)
[redacted]	0.0521	0.0513	0.1081	0.0039	2.43	5.13– 7.96
	0.0232	0.0547	0.0580	0.0034	5.84	6.19– 8.67
	0.0195	0.0426	0.0545	0.0044	7.11	9.11–12.35
	0.0175	0.0548	0.0524	0.0053	7.35	7.03– 9.38
	0.028	0.0531	0.077	0.0021	5.40	7.83–10.68
	0.0306	0.0370	0.0731	0.0052	4.29	8.53–12.02
	0.0193	0.0423	0.0528	0.0079	6.49	8.10–11.06
	0.0289	0.0523	0.0891	0.0043	5.32	9.07–12.01
	0.0247	0.0421	0.0453	0.0042	6.04	8.04–10.04
	0.0433	0.0582	0.0870	0.0071	4.68	7.00–10.47
	0.0264	0.0367	0.0726	0.0053	5.49	10.85–14.81
	0.0281	0.0311	0.0561	0.0045	6.00	10.83–16.25
	0.0165	0.0383	0.0434	0.0092	4.21	4.73– 7.86
	0.0275	0.0457	0.0670	0.0051	5.48	7.88–11.09
	0.0098	0.0084	0.0187	0.0018	1.27	1.81– 2.39
	0.0028	0.0024	0.0054	0.0005	0.37	0.52– .69

* Units of all α's are min^{-1}.
† Kilogram body weight.
‡ The lower limit of N_2 is calculated assuming no CO_2 is introduced into Compartment 2, and the upper limit assumes all CO_2 produced is introduced into Compartment 2. In these patients studied at rest, most of the CO_2 produced in the body would be expected to arise in abdominal and thoracic viscera and little would be produced in muscle and skin. Thus the lower value for N_2 listed is probably closer to the actual value of CO_2-HCO_3^- in the body in this compartment in these subjects.

data points are shown as open circles. During the initial 3 min following intravenous administration of labeled bicarbonate, the breath $^{14}CO_2$ excretion rate rises to a maximum and then begins to diminish. The biological events occurring during this 3-min time interval are thought to be related primarily to mixing of the label in the initial pool of equilibration. After the initial 3-min period, the rate of $^{14}CO_2$ excretion in the breath diminishes in a way that can be adequately described by a function consisting of three exponential terms. The exponential coefficient of one of these terms is equal to the measured turnover rate of the $^{14}CO_2$ detection apparatus (*1*). The values for the fitting parameters A_1, A_2, A_3, r_1, r_2 and average total CO_2 excretion rate obtained in 13 normal male subjects are given in Table 1.

The compartmental model used to analyze the $^{14}CO_2$ excretion data is shown in Fig. 1. Since three exponential terms were required to fit the $^{14}CO_2$ excretion data after initial equilibration had occurred, it was clear that there must be at least three compartments in any compartmental model used to ana-

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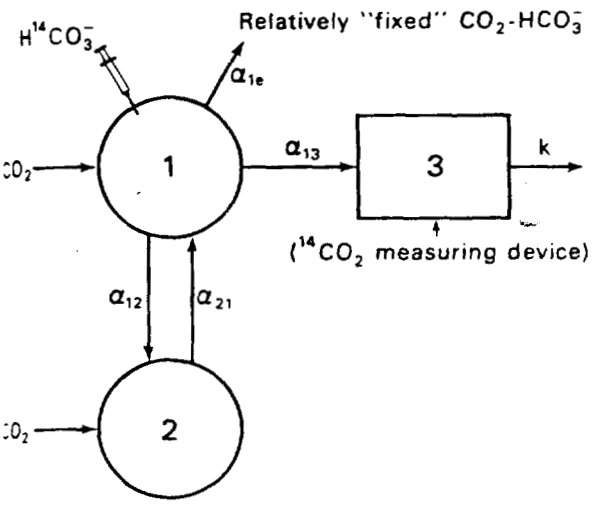

FIG. 1. Compartmental model used to analyze $^{14}CO_2$ excretion in breath before intravenous administration of ^{14}C-labeled bicarbonate. Compartment 1 represents those CO_2-HCO_3^- pools that have equilibrated with blood CO_2-HCO_3^- within first 3 min after intravenous administration of labeled bicarbonate. Compartment 2 represents those CO_2-HCO_3^- pools which show significantly delayed equilibration with blood CO_2-HCO_3^-. Compartment 3 represents ^{14}C activity in measuring device. Relatively "fixed" CO_2-HCO_3^- represents that labeled bicarbonate leaving Compartment 1 which is not significantly recycled back into Compartment 1 during time of study.

lize such data. One of these compartments is the measuring device with a turnover constant (k). One of the two remaining compartments must be that compartment in which the initial equilibration of the label occurred after its intravenous administration. As such it must include the CO_2-HCO_3^- contained within the blood, and thus the $^{14}CO_2$ excreted in the breath must arise directly from this compartment. The second compartment must be in equilibrium with the first. It is possible for $^{14}CO_2$ to be relatively fixed from both the first and the second CO_2-HCO_3^- compartments, but in the present analysis the fixation of $^{14}CO_2$ from the second compartment was considered to be negligible.

Table 2 presents the values for the intercompartmental rate constants for the model in Fig. 1 obtained by analysis of the excretion rates of $^{14}CO_2$ and total CO_2 in the breath of the 13 normal male subjects listed in Table 1. The CO_2-HCO_3^- content of Compartment 1 and the upper and lower limits of the CO_2-HCO_3^- content of Compartment 2 are also listed in Table 2.

DISCUSSION

It is current opinion that CO_2 rapidly passes across cell membranes. If mixing equilibrium between the intravascular CO_2-HCO_3^- pool and the CO_2-HCO_3^- pool of any given tissue is complete on one passage of blood through the capillary bed of the organ in question, then the major parameter which influences the rate of CO_2-HCO_3^- equilibration between CO_2-HCO_3^- contained within the blood and the CO_2-HCO_3^- in various tissue compartments throughout the body is the ratio of regional blood flow rate to regional CO_2-HCO_3^- content. Using similar reasoning, Farhi and Rahn estimated that the mean equilibration time of bicarbonate in certain viscera with a large blood perfusion rate is on the order of 1–2 min, while that due to the relatively small vascular perfusion rate of muscle and the large muscle mass of the body the mean turnover time of the CO_2-HCO_3^- pool in resting muscle is on the order of 30 min (*4*). In view of such estimated turnover times for the CO_2-HCO_3^- pool in various organs, one would anticipate that CO_2-HCO_3^- in organs with high vascular perfusion (heart, liver, kidneys, intestinal tract) would be a component of Compartment 1 in the present model. On the other hand, CO_2-HCO_3^- contained in organs with a low ratio of blood flow rate to CO_2-HCO_3^- content, such as muscle, skin and fat, would be a component of Compartment 2 in the present model. This argument is consistent with the comparable calculated mean turn-

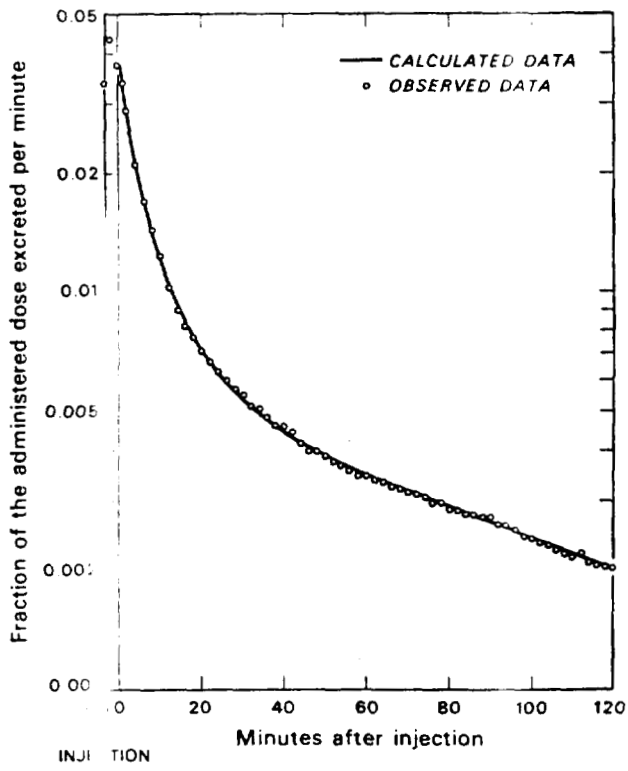

FIG. 2. $^{14}CO_2$ excretion rate in breath subsequent to intravenous administration of ^{14}C-labeled bicarbonate. Ordinate is expressed as fraction of administered dose excreted per minute. Abscissa is expressed as time in minutes minus 3 after intravenous administration of labeled bicarbonate. Measured $^{14}CO_2$ excretion rate is presented as open circles and solid line represents digital computer least squares best fit of three exponential function to data.

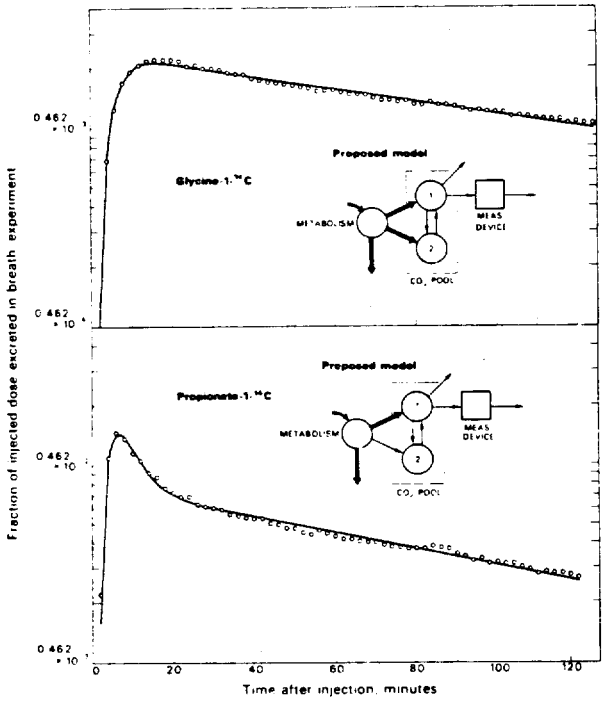

FIG. 3. $^{14}CO_2$ excretion rate in breath expressed as fraction of amount of radioactivity administered subsequent to intravenous administration of 1-^{14}C-labeled glycine (above) and 1-^{14}C-labeled propionate (below). Downslope of $^{14}CO_2$ excretion curves corresponding to turnover of $^{14}CO_2$ in body CO_2-HCO_3^- pools can be represented by single exponential function following administration of 1-^{14}C-glycine but requires two exponential terms following administration of 1-^{14}C-propionate.

over time of Compartment 2 (the average value for the 13 normal subjects in the present study is 22 min) and the mean turnover time of the CO_2-HCO_3^- pool in muscle estimated by Farhi and Rahn (30 min).

Although the brain is an organ with a low ratio of CO_2-HCO_3^- content to vascular perfusion rate, the presence of a blood brain barrier limiting the diffusion of bicarbonate into the brain may result in a delayed equilibration rate, similar to that calculated for muscle.

Blood CO_2-HCO_3^- is unquestionably in exchange with the bone bicarbonate. In a standard 70-kg man the bicarbonate content of bone is on the order of 5 moles (*4*). If the bone bicarbonate pool acts kinetically as a homogenous compartment, then labeled bicarbonate leaving the blood to enter this large bone bicarbonate pool would not be expected to significantly re-enter the blood during the time of the present study. Thus we may tentatively identify some of the CO_2-HCO_3^- leaving the blood destined for relatively "fixed" sites with bicarbonate entering bone. Further evidence that significant quantities of blood CO_2-HCO_3^- may be fixed in bone can be inferred from the studies of Myers and Hunter, in which they found significant bone fixation of ^{14}C given as bicarbonate in bones involved by tumor (*5*). Another possible site of trapping of body CO_2-HCO_3^- may be metabolic processes which result in incorporation of CO_2 into larger molecules (*6*). Other sites of bicarbonate loss in the body (feces, urine and sweat) probably account for only a small component of the bicarbonate entering relatively "fixed" sites from the blood when the subject is at rest.

When labeled bicarbonate enters Compartments 1 and 2 of the present model in such a way that the specific activity of $^{14}CO_2$ in the two compartments is roughly comparable at all times, then both of these body CO_2-HCO_3^- pools act as though they were a single compartment. In such a case, the apparent mean turnover time for the equilibrated Compartments 1 and 2 for the 13 normal subjects studied in the present communication would be 89–110 min (fractional turnover rate of 0.0113–0.0091/min). Such a situation is approximated when a ^{14}C-labeled organic material is metabolized widely throughout the body (e.g., by muscle as well as by viscera). An example of this type of material is glycine labeled with ^{14}C in the #1 position (see upper curve, Fig. 3). When such ^{14}C-labeled materials are oxidized to $^{14}CO_2$, the breath $^{14}CO_2$ excretion curve shows only one exponential term corresponding to the turnover rate of the equilibrated CO_2-HCO_3^- Compartments 1 and 2 of the model. When a ^{14}C-labeled metabolite is metabolized primarily in one of the viscera whose CO_2-HCO_3^- content is a component of Compartment 1, then the body CO_2-HCO_3^- pools act kinetically as though they consisted of two discrete compartments. An example of this is shown in the lower portion of Fig. 3 for 1-^{14}C-propionate. The #1 carbon atom of intravenously administered propionate appears to be metabolized largely in the liver, and the $^{14}CO_2$ resulting from this metabolism is liberated into the blood in a similar way to an intravenous administration of labeled bicarbonate. The breath $^{14}CO_2$ excretion curve shows a definite peak in this circumstance with two exponential components in the downslope related to internal equilibration of the $^{14}CO_2$, thus liberated within the body's CO_2-HCO_3^- pools.

SUMMARY

Intravenously administered ^{14}C-bicarbonate is felt to rapidly mix with the CO_2-HCO_3^- contained in the blood and tissues with a high ratio of blood flow rate to tissue CO_2-HCO_3^- content (abdominal and thoracic viscera, etc.), together having a total CO_2-HCO_3^- content of 5.5 m*M*/kg body weight. In 13 normal subjects, an average fraction of 0.067/min

of this first pool left to enter a second pool with a total CO_2-HCO_3^- content of 7.9–11.0 m*M*/kg body weight. This second pool is felt to consist of CO_2-HCO_3^- contained in tissues with a small ratio of blood flow rate to tissue CO_2-HCO_3^- content, such as muscle. In these subjects an average fraction of 0.046/min left this second CO_2-HCO_3^- pool to feed back to the first (corresponding to a mean turnover time of 22 min). An average fraction of 0.028/min of the first pool left to be expired in the breath, while an average fraction of 0.005/min of the first pool left to become "fixed" somewhere in the body. This "fixed" fraction is felt to be in part related to deposition in bone and possibly metabolic processes resulting in incorporation of CO_2 into larger molecules. Additionally, there is a small portion of the "fixed" fraction which is lost from the body by excretion in urine, feces and sweat. The relative rates of internal equilibration of ^{14}C-labeled HCO_3^- within the body's CO_2-HCO_3^- pools appears to depend largely on relative vascular perfusion rates of different tissues, and the kinetic values herein obtained in 13 normal male subjects would be expected to change in nonresting or pathological states.

Analysis of the kinetics of the body's CO_2-HCO_3^- pools should aid in understanding patterns of $^{14}CO_2$ excretion in the breath following administration of various ^{14}C-labeled substrates.

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