# CONSIDERATIONS IN ANALYSIS OF BREATH $^{14}CO_2$ DATA

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The rate at which $^{14}CO_2$ is measured in expired air after intravenous administration of $^{14}C$-labeled materials ($^{14}C$-R) is a function of the physical parameters involved in delivery and removal of the material to and from metabolic sites, the kinetics of the metabolism of the material, the delays related to passage of $^{14}CO_2$ from its production site across the body's $CO_2$-$HCO_3^-$ pools before its expiration in the breath and the delays inherent in the measuring device from the time $^{14}CO_2$ actually appears in the breath and the time it is recorded (Fig. 1). Each of these factors is discussed separately in this paper.

## DELAYS IN $^{14}CO_2$ MEASUREMENT INHERENT IN MEASURING DEVICE

When a constant infusion of $^{14}CO_2$ was delivered into the patient helmet or through the animal-holding chamber of devices used for continuous measurement of $^{14}CO_2$ exhalation in man (*1*) or rodents (*2,3*), the readings of the vibrating reed electrometer increased according to the function $C(1-e^{-kt})$ in which C and k are constants and t is units of time.

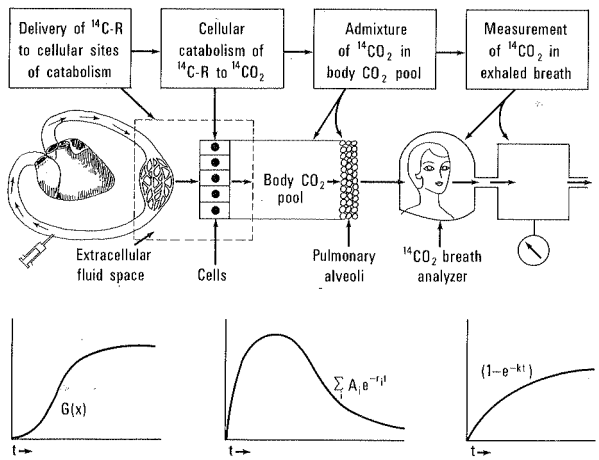


**FIG. 1.** Schematic representation of physical, biochemical and technical factors involved in measurement of $^{14}CO_2$ in breath subsequent to i.v. administration of $^{14}C$-labeled material ($^{14}C$-R). Description of processes involved are noted in upper portion of figure and corresponding processes are diagramatically presented in center of figure. Estimated form of isolated mathematical functions describing each component of model are shown in lower portion of figure. For discussion of specific mathematical forms consult text of paper.

This fact suggested that changes introduced in the measurement of $^{14}CO_2$ excretion by delays inherent in the measuring devices could be accounted for by considering the measuring devices as a compartment with a fractional turnover rate of k ($time^{-1}$) (right side of Fig. 1). If we let F(t) be the rate of expiration of $^{14}CO_2$ by the experimental subject then, N(t), the amount of $^{14}CO_2$ in the measuring apparatus, is given by the equation

$$N(t) = e^{-kt} \int_0^t e^{kt} F(t)dt. \qquad (1)$$

## METABOLISM OF $^{14}C$-LABELED MATERIAL ($^{14}C$-R) TO $^{14}CO_2$ AND ITS PASSAGE ACROSS THE $CO_2$-$HCO_3^-$ SPACE

If we wish to apply compartmental theory to the analysis of the metabolism of $^{14}C$-R to $^{14}CO_2$ and its subsequent passage across the $CO_2$-$HCO_3^-$ space, we can approximate these processes by the function $\sum_i A_i e^{-r_i t}$ in which $A_i$ and $r_i$ are constants and t is time after introduction of $^{14}C$-R. Since at $t = 0$ there is no production of $^{14}CO_2$, $\sum_i A_i = 0$. There are thus $2i - 1$ parameters in this function, and the maximum number of rate constants which can be defined in any compartmental model generating this function is likewise $2i - 1$ (*4*). This function is a measure of both the metabolism of $^{14}C$-R to $^{14}CO_2$ and the passage of the $^{14}CO_2$ across the body's $CO_2$-$HCO_3^-$ space. Individual kinetic analysis of the body's $CO_2$-$HCO_3^-$ pools is the subject of subsequent papers (*5,6*).

## ANALYSIS OF PHYSICAL PARAMETERS INVOLVED IN DELIVERY AND REMOVAL OF $^{14}C$-MATERIAL TO AND FROM METABOLIC SITES

Subsequent to the rapid intravenous administration of $^{14}C$-R, there is a definite delay before the radioisotope is "homogeneously" mixed in the initial meta-

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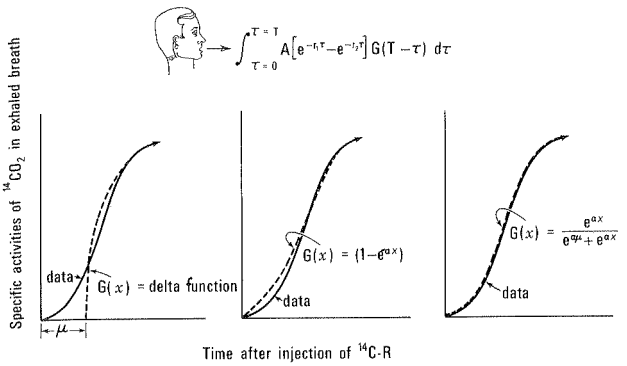


**FIG. 2.** Three solutions for G(x), function corresponding for digression of observed data from ideal compartmental behavior. Solid lines represent actual data values obtained in study of $^{14}CO_2$ expiration in breath of human subject given histidine (imidazole-2-$^{14}$C). After first 5 min, metabolism of material during 2–3 hr following its i.v. administration is described well by two-compartment system. Broken lines represent least squares best fit to early portion of curves for forms of F(t) generated using form of G(x) shown for each curve.

bolic pool involved in its metabolism. The tracer material must traverse the intravascular space until the capillaries are reached; it must then traverse the capillary endothelium and mix in the extravascular-extracellular fluid space. It must subsequently cross the cell membrane and enter the intracellular site associated with its subsequent metabolism. These processes are not instantaneous, and some function must be introduced to account for their presence.

Let G(x) be the fractional rate of introduction of $^{14}$C-R into the primary metabolic pool (x) times units after the administration of $^{14}$C-R. If we consider the actual metabolism of $^{14}$C-R to be adequately described by a compartmental system, we can write the following form for F(t), the rate of appearance of $^{14}CO_2$ in the breath (*7*)

$$F(t) = \int_{\tau=0}^{\tau=t} \left(\sum_i A_i e^{-r_i\tau}\right) G(t-\tau)d\tau. \qquad (2)$$

G(x) primarily influences the early portion of the $^{14}CO_2$ breath curve. The physiologic functions determining its form are complex, and it is difficult to derive its form from first principles. We have thus used arbitrary but convenient forms for G(x) in the present analysis. In Fig. 2 results are presented for three forms of G(x). In this example, actual data describing the measured rate of appearance of $^{14}CO_2$ in the measuring apparatus following a single intravenous administration of histidine (imidazole-2-$^{14}$C) were fit to Eq. 1. For this we used the two-compartmental solution of Eq. 2 (i = 2) given in the upper portion of this figure as F(t), utilizing a variable metric minimization program in a CDC 6600 computer (*8*). Three forms of G(x) are presented. G(x) = delta function is comparable to assuming a single unique delay time, $\mu$, as characteristic of the delay between i.v. injection of $^{14}$C-R and its final equilibrium in the primary metabolic pool. $G(x) = (1 - e^{-ax})$ is comparable to assuming that the delay between administration of $^{14}$C-R and its final equilibrium in the primary metabolic pool is characterized by the introduction of an initial mixing "compartment." $G(x) = e^{ax}/(e^{a\mu} + e^{ax})$ is comparable to assuming that delay administration of $^{14}$C-R and its final equilibrium in the primary metabolic pool is characterized by a Verhulst-Pearl growth curve (*9*). This latter is similar to the assumption that the probability of delivering $^{14}$C-R from its site of injection to the primary metabolic pool is a Gaussian function with mean transit time $\mu$ and $\sigma = 1.74/a$ (*10*). While this last form of G(x) gives the best fit of the fitting function to the data, the exact determination of a and $\mu$ requires that very exact data be available during the first 5 min of the study. In practice when the total radioisotopic dose of $^{14}$C-R is minimized the $^{14}CO_2$ data during the first 5 min of a study are poorly defined, and it is convenient to use the simplest form of G(x) = delta function.

## ESTIMATION OF NUMBER OF COMPARTMENTS IN "METABOLIC" SYSTEM

To estimate the number of compartments or pools in any metabolic model which may be defined by the data, the following empirical approach may be tried. In the final form of Eq. 1 let i take on integer values from 1 to "n," generating "n" solutions for the equation. Determine the least squares best fit for

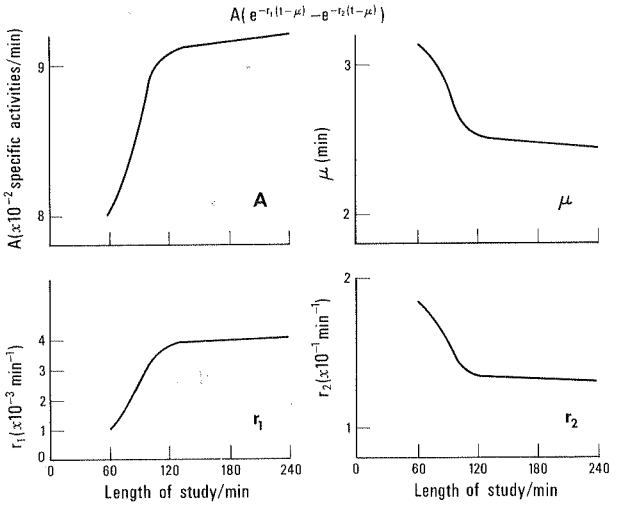


**FIG. 3.** Duration of data collection required to define parameters of a given model describing $^{14}CO_2$ production from $^{14}$C-2-histidineimidazole. (See text of paper for explanation.) Values for fitting parameters A, $\mu$, $r_1$ and $r_2$, calculated by computer for data limited to interval $0 \leq \tau \leq T$, are plotted on ordinates, and corresponding values of T are plotted on abscissa.

each solution and record the value of chi squared ($\chi^2$). For each solution corresponding to larger values of i, the $\chi^2$ will become smaller. However, the difference in $\chi^2$ becomes small between the solutions of i to i + 1 as i becomes large. The value of i beyond which the corresponding $\chi^2$ does not change significantly* is equal to the number of exponential terms which can be uniquely defined by the data and also the necessary number of compartments in the metabolic model proposed to explain the data.

## DETERMINATION OF MINIMUM DURATION OF DATA COLLECTION TO DEFINE MODEL PARAMETERS

For any given model it is necessary to know the minimum duration of data collection to define the parameters of the model. An example of how this may be estimated is given in the following discussion of Fig. 3.

In this example $^{14}CO_2$ excretion in the breath was measured for 240 min following the i.v. administration of histidine (imidazole-2-$^{14}$C), and the data were offered to the computer for the time interval $0 \leq \tau \leq T$ (where T varied from 60 to 240 min). The computer was programmed to determine the least squares best fit of the data to a fitting function derived from a two-compartmental model incorporating a delay time, $\mu$, [G(x) = delta function]. (The actual fitting function was the solution of Eq. 1 using $(F(t) = A\,[e^{-r_1(t-\mu)} - e^{-r_2(t-\mu)}])$. It should be noted that variable values of A, $\mu$, $r_1$ and $r_2$ are obtained for studies of different duration within the range of 60–120 min of study. However, for studies longer than 120 min, little difference between these fitting parameters is noted between studies. One can thus state that for the data and model in question data must be collected for at least 120 min after the injection of $^{14}$C-R in order to define the fitting parameters. Similar maneuvers must be performed for each model and set of data to determine the minimal duration of the study required to define the fitting parameters (an alternate approach is to use "real-time programming").

## SUMMARY

Previous experience with graphical analysis of breath $^{14}CO_2$ curves suggested that the late portion of the curve was well fit by a series of exponential terms ($\sum_i A_i e^{-r_i t}$). In the present paper one of these terms is identified with the turnover rate of $^{14}CO_2$ in the measuring device employed while the remaining terms are identified with compartments involved in the metabolism of $^{14}$C-labeled material being studied and the kinetics of the $CO_2$-$HCO_3^-$ pools which the labeled $CO_2$ must traverse before its expiration in the breath. To correct for digression of the observed data from idealized compartmental behavior, a function G(x) is defined. Examples are presented for G(x) = (delta function), $\sum_j A_j e^{-r_j x}$, $e^{ax}/(C + e^{ax})$. A method is presented for estimating the minimum length of time data must be collected to define the fitting parameters of a given model and for estimating the number of compartments which must be postulated to explain a set of $^{14}CO_2$ data.

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* The "significance" of any change in $\chi^2$ will depend on the errors inherent in the measurements and the biological variability of the data, etc., and must be evaluated for each use.

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