Exchange of Carbon Dioxide in the Pregnant Rhesus Monkey: Multicompartmental Analysis of Carbon Dioxide Kinetics

KOTARO SUZUKI, ALBERT A. PLENTL,* and KARLIS ADAMSONS

From the Department of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons, New York 10032

ABSTRACT The exchange of carbon dioxide in the pregnant rhesus monkey has been studied quantitatively using sodium bicarbonate-^{14}C and applying the model of a system of seven compartments. The transfer rates among the various compartments, compartment sizes, and the rate of production of carbon dioxide by fetus and mother were determined with a computer programmed to fit the theoretical model to the data by adjusting the parameter values of the model until a "best fit" was obtained. It was confirmed that the exchange of carbon dioxide between fetal and maternal blood across the placenta is rapid, that between fetal blood and amniotic fluid is slow, and that there is no appreciable exchange between maternal blood and amniotic fluid. The mean net production of CO_2 by fetus was 0.476 ±0.0402 mmoles/kg·min, and that by mother was 0.373 ±0.0279 mmoles/kg·min.

INTRODUCTION

Since the carbohydrates are the principal source of energy of the fetus (1), carbon dioxide is the most important end product of fetal energy metabolism. Thus, the amount of carbon dioxide transferred from the fetus to the mother can be used as an indicator of its metabolic activity. The standard methods of determining the metabolic rate of the fetus are based on measurements of oxygen consumption. This requires quantitation of umbilical or uterine blood flow and difference in oxygen content between artery and vein. Accurate measurement of these variables without disturbing the normal state is difficult, if not impossible. The principal objective of this study was to determine CO_2 output of the fetus (net transfer of CO_2 from fetus to mother) without the knowledge of flow rates and arteriovenous differences by means of a ^{14}C distribution technique. Furthermore, it was anticipated that knowledge of CO_2 exchange between the various maternal and fetal compartments would be of value in the assessment of the acid-base state of the fetus during labor.

CO_2 discussed here denotes the total CO_2 which exists in various forms in the animal, such as the physically dissolved CO_2, carbonic acid, bicarbonate, and carbamino compounds, and which can be measured as acid-volatile CO_2.

METHODS

Materials. Two nonpregnant adult and four pregnant rhesus monkeys (*Macaca mulatta*) near term were used in this study. The nonpregnant animals were used in order to estimate the minimal number of compartments regarding the distribution of carbon dioxide. The rhesus monkey was chosen because of the similarity of placental structure and the relative size of body compartments of the fetus and mother with those of the human. In addition, the presence of a secondary placenta enables one to obtain fetal blood from interplacental vessels without rupturing the amnion. In the rhesus monkey the umbilical vessels reaching the chorionic plate divide into numerous branches supplying the primary placenta. One or more trunks consisting usually of two arteries and one vein bypass it to reach the secondary lobe. The interplacental vessels course in the potential space between amnion and chorion, and can be readilly isolated. This morphologic arrangement has been exploited in various animals, and was first applied to the rhesus monkey by Reynolds, Paul, and Huggett (2). Table I gives details of materials studied.

Experimental design. The mothers were anesthetized with Sernylan[1] (2 mg/kg of body weight intramuscularly) about 30 min before the operative procedure. Body temperature was maintained at 37°C by a heat lamp. In the nonpregnant animals a catheter was inserted into a calf vein of each leg with the tip of the catheter threaded upward into the inferior vena cava, one with the tip above renal veins for injection of the isotope and the other with the tip below renal veins for sampling of blood. In the pregnant monkeys, after bilateral

* Deceased.

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[1] 1-(1-phenylcyclohexyl) piperidine hydrochloride. Parke, Davis & Co., Detroit, Mich.

TABLE I
Vital Statistics

Experiment No.	Monkey No.	Gestation	Weight
		days	*kg*
	406	Nonpregnant	4.6
	397	Nonpregnant	5.3
I	319	152	8.5
II	408	153	5.6
III	428	157	6.8
IV	408	146	6.18
	Mean of 4 pregnant monkeys	152	6.8

catheterization of a calf vein with the tips of catheters threaded upward into the inferior vena cava in the same fashion, a midline incision was made and the uterus was delivered through the abdominal incision. Placental margins and interplacental vessels were located by transillumination and were marked on the uterine serosa with silver nitrate. A small incision was made over the interplacental vessels and either the artery or the vein was isolated. A Silastic T-shaped catheter was inserted into the vessel and secured with silver rings (3). The myometrial incision was then approximated with interrupted sutures and the uterus was returned to the abdominal cavity. Whenever possible, the interplacental artery was chosen because of greater facility to obtain blood samples. In experiment IV in which the tracer was injected into the fetal circulation, two interplacental vessels at two different sites, one artery and one vein, were catheterized (Fig. 1). In all preparations a separate catheter was introduced into the amniotic cavity.

The isotope used was a ^{14}C-labeled sodium bicarbonate with an activity of 20 mCi/mmole. The total dose employed in each experiment was 1.0 mCi dissolved in a volume of 1.0 ml of normal saline. This amount of ^{14}C was chosen to provide a readily measurable quantity of the isotope in a 1 to 10^6 dilution.

In the two experiments with nonpregnant monkeys the isotope was injected into vena cava above renal veins in less than 30 sec, and serial blood samples were collected from vena cava below renal veins over the subsequent 4 hr, at first every few minutes and then at longer intervals.

In the experiments with pregnant monkeys, in three instances (experiments I, II, III) the tracer was injected into the maternal vena cava above renal veins and in one experiment (experiment IV) it was injected directly into the fetal circulation (interplacental vein). Samples of maternal blood from vena cava below renal veins, fetal blood from interplacental artery, and amniotic fluid were collected in the same fashion as above over 4–5.5 hr. In each collection of either blood or amniotic fluid the initial portion of the sample was withdrawn in a separate syringe which was not used for determination of the isotope but was saved for replacement. A fresh 1 ml disposable syringe was then attached and 0.5 ml of sample collected. The original 1 ml of blood or amniotic fluid which had been withdrawn to clear dead space was then reinjected. The sample was transferred under oil into a 2 ml heparinized tube containing sodium fluoride. For the collection of amniotic fluid, a nonheparinized tube was used.

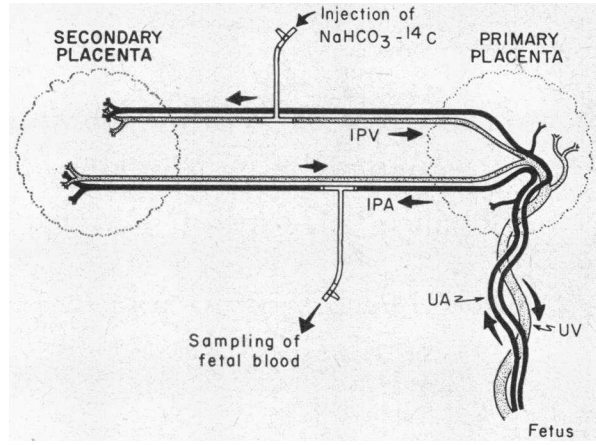


FIGURE 1 Schematic presentation of the vascular structures of the placentas in the rhesus monkey and the location of catheters in the fetal vessels. IPV, interplacental vein; IPA, interplacental artery; UV, umbilical vein; UA, umbilical artery; →, direction of blood flow. Only IPA catheter was used for sampling the fetal blood in experiments I, II, and III.

Before the determination of the radioactivity of the sample, the material had to be transformed into carbon dioxide gas. The total carbon dioxide content of each sample, 0.2 ml of whole blood or amniotic fluid, was determined according to the procedures described by Peters and Van Slyke (4). Collection of $^{14}CO_2$ for scintillation counting was done according to the procedure of Van Slyke as modified by Weyman, Williams, and Plentl (5), using phenethylamine as absorbent. Radioactivity of ^{14}C was measured by the use of liquid scintillation counter (Nuclear-Chicago 720 series), at 4°C against a background of 20–22 cpm. Each sample was counted three times, each time either for 80 min or up to one million counts, whichever occurred first. The counting error was less than 1%. The value was corrected for counting efficiency (about 60%) which was obtained from the channels ratio; thus the average result could be expressed as disintegrations per minute per 0.2 ml of sample. Knowing the total CO_2 content in the sample the specific activity was expressed as disintegrations per minute per millimole of CO_2. The fractional amount of the tracer in each compartment was expressed as a function of time from the specific activity. This was derived by dividing the product of CO_2 content and each specific activity in a compartment by the number of disintegrations per minute of the injected tracer (in this case 1 mCi = 2.2×10^9 dpm). Maternal plasma volume was determined by dilution method using Evans blue (T-1824) as an indicator, and a Beckman model DU spectrophotometer for measurement of absorbance. With the knowledge of the concentration of red blood cells the maternal blood volume was calculated. Fetal blood volume was estimated to be 10% of fetal weight and 30% of placental weight. Amniotic fluid volume was determined by dilution method using 1 ml of radioactive iodinated serum albumin (RISA) (6) of low activity (approximately 0.5 uCi/ml) which was injected into amniotic fluid. The withdrawn specimen of amniotic fluid was counted in a Nuclear-Chicago well-type scintillation counter equipped with a model 186 decade scaler. The theory of Berman and Schoenfeld (7) was applied for analysis of data taking advantage of a general purpose computer program developed by Berman (8) (SAAM-22; Simulation, Analysis and Modeling).

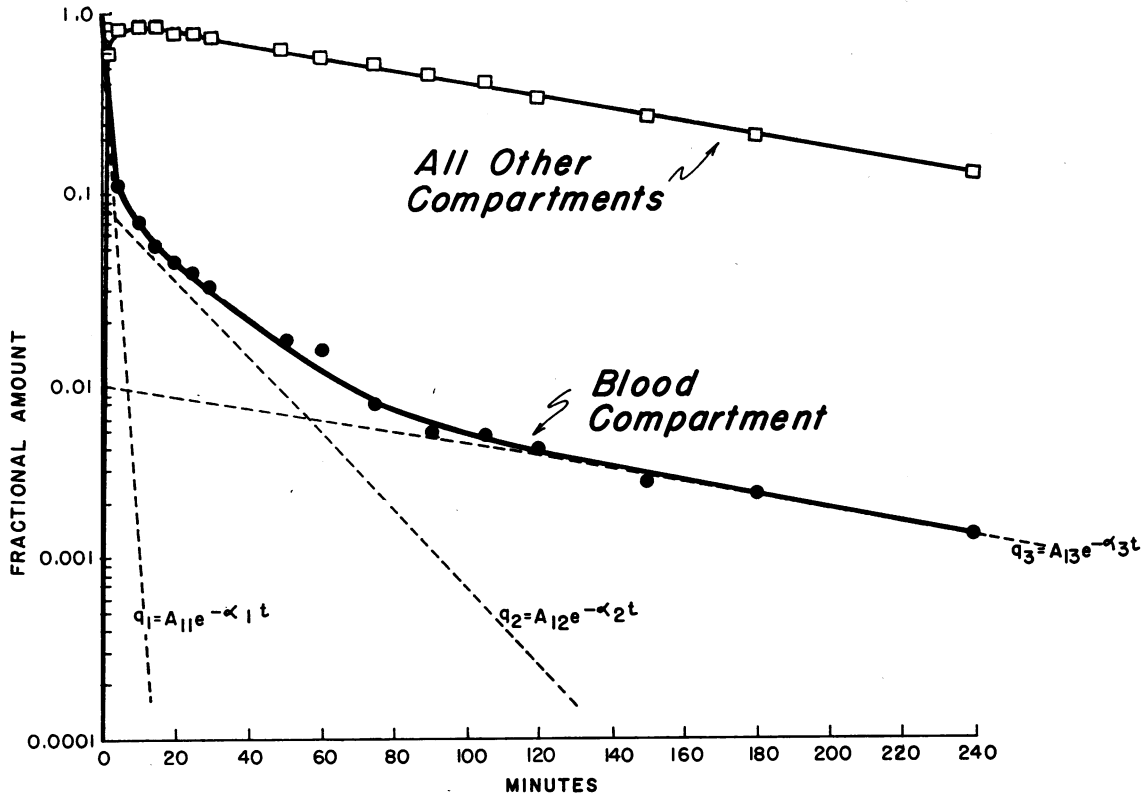


FIGURE 2 Fractional amount of ^{14}C in blood and all other body compartments of a nonpregnant monkey as function of time. Three principal compartments are identified (q_1, q_2, and q_3). The coefficients A_{11}, A_{12}, and A_{13} (intercepts) and the exponential constants α_1, α_2, and α_3 (slopes) have been determined by the "peeling"technique.

RESULTS

Observed data. The curves obtained in the two experiments with nonpregnant monkeys were nearly identical. One of them is reproduced in Fig. 2, where the theoretic isotope content for ^{14}C at time zero (t_0) is arbitrarily set equal to unity, and the fractional amount in the blood compartment and all other compartments in the rest of the body are expressed as a function of time on a semilogarithmic scale. The data are also given in Appendix I. The curves obtained in the four experiments with pregnant animals in which the isotope was injected into either maternal or fetal blood stream were also nearly identical; two of them are reproduced in Fig. 3 A and B and Fig. 4 A and B. The concentration of ^{14}C is given as a function of time and is expressed as specific activity (Figs. 3 A and 4 A) and as fractional amount (Figs. 3 B and 4 B). The specific activity in the primary compartment at t_0 is estimated by extrapolation based on the knowledge of the blood volume and the amount of tracer injected. As an example the data of fractional amount and its statistical weight of experiment I is given in Appendix II.

Theoretical considerations. There are two basic assumptions inherent in the present analysis: first, that CO_2 pool in each animal is in a steady state, and second, that the system is compartmentalized regarding the constituents of the CO_2 system. The first assumption is reasonable since the studies were carried out while the animal was under light sedation at least 1 hr after the completion of the surgical procedure; furthermore, there were no significant changes in the total CO_2 in each sample of maternal and fetal blood and amniotic fluid throughout the study period. The second assumption also appears valid for the following reasons. First, there are several reports which give evidence that "compartmentalized system" can be assumed regarding the distribution of CO_2 and its homologues. Steele (9) described a method for calculating the distribution of bicarbonate-^{14}C measured as total acid-volatile $^{14}CO_2$ in an idealized "three compartment" cat and found a satisfactory agreement between the observed values and those predicted for such a model. He assumed a central compartment representing the circulating blood and two other discrete peripheral compartments consisting of the solid carbonate of bone and the bicarbonate of the soft

tissues. Singer et al. (10) showed that in man equilibrium in the distribution of $NaHCO_3$ between blood and interstitial fluid possibly including collagen was reached in about 30 min, whereas that between interstitial fluid and intracellular fluid required a much longer time interval. Certainly the infused bicarbonate must have been distributed homogeneously in the primary blood compartment within the first few minutes after the completion of infusion. Their studies indicate the existence of at least three compartments regarding the distribution of the bicarbonate. The previous reports from this laboratory by Friedman et al. (11) and Plentl and Friedman (12) have given evidence that in the pregnant animal there are several distinct compartments such as maternal blood, maternal extravascular pool, fetal blood, fetal extravascular pool, and amniotic fluid with regard to the distribution of acid-volatile $^{14}CO_2$ after the injection of bicarbonate-^{14}C. Furthermore such an assumption is consistent with the present data which can be clearly broken down into at least three exponential components (Figs. 2, 3, and 4). This indicates that the compartmental reflection of data is quite adequate

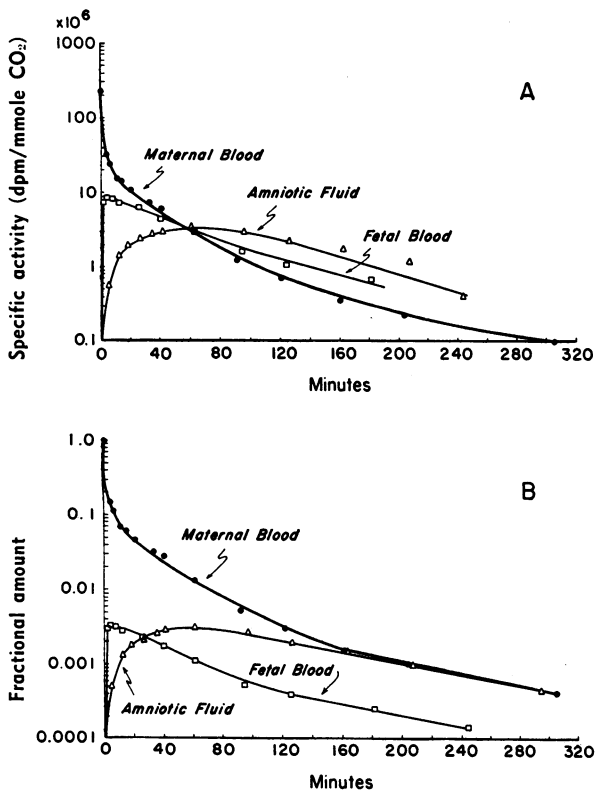


FIGURE 3 A Specific activity of ^{14}C of maternal and fetal blood and amniotic fluid as function of time after injection of 1 mCi of $NaHCO_3$-^{14}C into the maternal vein. B. Fractional amount of ^{14}C in maternal and fetal blood and amniotic fluid as function of time after injection of 1 mCi of $NaHCO_3$-^{14}C into the maternal vein.

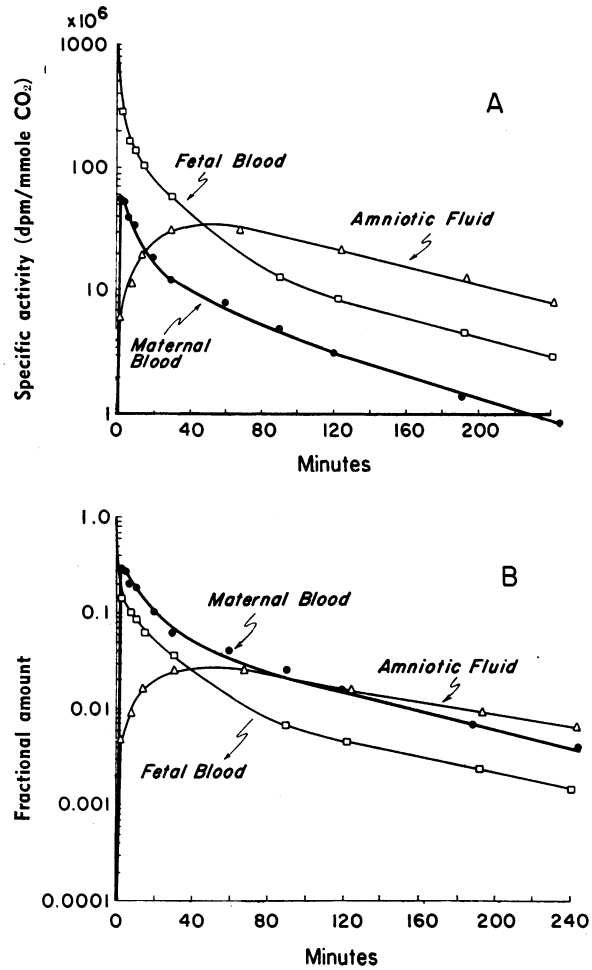


FIGURE 4 A Specific activity of ^{14}C of fetal and maternal blood and amniotic fluid as function of time after injection of 1 mCi of $NaHCO_3$-^{14}C into the interplacental vein (fetal blood stream). B. Fractional amount of ^{14}C in fetal and maternal blood and amniotic fluid as function of time after injection of 1 mCi of $NaHCO_3$-^{14}C into the interplacental vein (fetal blood stream).

(7). Because of the high diffusibility the gaseous component of the CO_2 family is obviously not compartmentalized in the above terms. Since it represents only about 5% of the total it does not, however, invalidate the assumption of compartmentalization.

The concept of a compartmentalized system applies only as an approximation for biologic system, because variations in physical distributions, inhomogeneity of the media, and diffusion processes are all interrelated with chemical changes. Therefore, a compartment in this context is a space of relatively large dimensions within which distribution of the specific substance under non-steady-state conditions, i.e. tracer, is very rapid compared to distribution between this space and others. Under the assumption that the system is in a steady state

and consists of n compartments, a given set of time-activity curves can be expressed as a sum of the respective exponential equations

$$q_k(t) = \sum_{i=1}^{n} A_{ki}e^{-\alpha_i t} \quad (1)$$

where $q_k(t)$ is the amount or fraction of the labeled CO_2 in the k^{th} compartment at time t. Each exponential term on the right of this equation represents a straight line in a semilogarithmic coordinate system. Using the "peeling" technique, the exponential constants (slope α's) and the coefficients (intercepts A's) can be determined. Theoretically, the number of exponential terms should correspond to the number of compartments for the system under consideration. Then, the rate of change with time will be expressed as

$$\frac{dq_k(t)}{dt} = \sum_{i=1}^{n} - \alpha_i A_{ki}e^{-\alpha_i t} \quad (2)$$

In a steady state where the amounts of labeled and nonlabeled CO_2 entering and leaving each compartment are equal, the rate of change of the amount of labeled CO_2 in each compartment will be given by

$$\frac{dq_i(t)}{dt} = -\lambda_{ii}q_i(t) + \sum_{\substack{j=1 \\ j\neq i}}^{n} \lambda_{ij}q_j(t) \quad (i = 1, 2, \ldots, n) \quad (3)$$

where $q_i(t)$ = the amount of the labeled CO_2 in the i^{th} compartment at time t; $q_j(t)$ = the amount of the labeled CO_2 in the j^{th} compartment at time t; λ_{ii} = the total turnover rate in the i^{th} compartment; and λ_{ij} = the fractional amount of CO_2 (labeled and nonlabeled) in the j^{th} compartment transferred to the i^{th} compartment per unit of time, i.e., a fractional transfer rate of CO_2 from compartment j to compartment i.

From equations 1, 2, and 3 and with the use of algebra of matrices, we can obtain

$$[\lambda] = [A][\alpha][A]^{-1} \quad (4)$$

where $[\lambda]$ and $[A]$ are $n \times n$ matrices and $[\alpha]$ is a diagonal matrix. The transfer rates λ's are thus dependent on the values of α's and A's, a relation first established by Hart (13).

From a knowledge of A's, α's, and the boundary conditions, the number of degrees of freedom can then be deduced which gives an indication of the feasibility of solving the equation 4. When the tracer is injected into one compartment only, the sum of coefficients for that compartment is

$$\sum A_{1j} = 1.0$$

and for all others

$$\sum A_{kj} = 0.0 \ (k \neq 1).$$

In physiologic systems containing multiple compartments all the necessary α's and A's are rarely known, since some of the compartments are not accessible. Thus it becomes necessary to use a transformation matrix to map the system within the configuration based upon the available information. The transformed $[\lambda]$ becomes

$$[\lambda'] = [P][\lambda][P]^{-1} \quad (5)$$

where $[\lambda']$ is a similar matrix of $[\lambda]$, and $[P]$ is a transformation matrix. By means of $[P]$, variables can be introduced according to the degrees of freedom.

The generating model (a lambda matrix) obtained by performing the operation in equation 4 need not correspond to a physically realizable set of λ's though some of them might be negative. Incorporating the constraints and the variables equal to the number of degrees of freedom, a similar matrix is obtained which represents all possible models. In this matrix the λ's are dependent on the new variables introduced or, if such dependence does not exist, some of them must be constant, i.e., invariants of the system. This circumstance can be used to define the realizable space, area or line according to number of degrees of freedom. This is done by setting

$$\lambda_{ij} \geqq 0$$

$$\lambda_{jj} \geqq \sum_{i=1}^{n} \lambda_{ij} \text{ where } i \neq j.$$

The region where these values overlap defines the limits for the fractional transfer rates λ's. This procedure of mapping, however, becomes complex when more than three compartments are involved or when pooling of compartments is not justified. Therefore, it is best to use the procedure only as a way to arrive at initial estimates of fractional transfer rates for the digital computer program SAAM-22 (Appendix III).

To determine the minimal number of compartments necessary for the model and obtain information necessary for building the model the data of the nonpregnant monkeys were analyzed. First, in Fig. 2 at least three straight lines could be drawn as shown by the "peeling" technique. This indicates that regarding the distribution of CO_2 there are at least three compartments in the nonpregnant animal. Further, all the data from a single study were fitted jointly to sums of exponentials, using the digital computer program SAAM-22 (8). Three exponentials were necessary and sufficient to satisfy the data from 5 min to 240 min. Hence it was assumed that the fetus has at least an equal number of compartments in addition to the physically well defined compartment of amniotic fluid. The system under consideration thus has a minimum of seven compartments.

The specific activity curve in primary compartment (maternal blood in Fig. 3 A, fetal blood in Fig. 4 A) showed a rapid decline during the first few minutes

which corresponded to a general distribution of CO_2 within the various maternal or fetal compartments. After a certain length of time, equilibration was established between them and the secondary compartments, except for that of amniotic fluid. Eventually the curve approached a straight line which presumably reflected the rate of production of endogenous carbon dioxide. In Fig. 3 A the time-activity curves for mother and fetus approached each other within the first few minutes and became almost identical thereafter. It indicated a rapid transfer from mother to fetus. When ^{14}C is injected into fetal blood (Fig. 4 A), the specific activity curve for maternal blood also rose rapidly but never reached the fetal level. The lower activity of ^{14}C in maternal blood is expected due to the larger size of the compartment and is not indicative of the low rate of transfer. This established a transfer from fetus to mother. Taken together, these two facts represent evidence for an exchange of CO_2 between the maternal and fetal blood.

The concentration of ^{14}C in amniotic fluid rose gradually but ultimately exceeded that of maternal and fetal blood; from then on the three curves declined at an identical slope. In Fig. 3 A the specific activity curve for amniotic fluid crossed maternal and fetal curves at about 60 min at which time it had reached its maximum. Since maternal and fetal curves are nearly identical at this time, both maternal and fetal blood can be regarded as sources of carbon dioxide in amniotic fluid. However, in Fig. 4 A the specific activity curve for amniotic fluid crossed the maternal curve at about 16 min and continued to rise and crossed the fetal curve at about 45 min at its maximum. This indicates that fetal blood is the sole source of the carbon dioxide in amniotic fluid. Friedman and his associates (11) and Seeds, Bissonnette, Lim, and Behrman (14) showed that bicarbonate infused into amniotic fluid was transferred mainly into fetal blood directly, indicating the insignificance of the pathway from amniotic fluid to mother. In this study placenta and fetal membranes were regarded as a part of fetal subcompartment 5 and maternal blood as well as fetal blood must have direct pathway of CO_2 exchange with this compartment. The simplest possible model of seven compartments which meets all the requirements stated above is given in Fig. 5. The transfers between the maternal blood, fetal blood, and amniotic fluid are of major interest whereas the exchange between the other maternal (2 and 3) and fetal (5 and 6) compartments, though dependent, are not of immediate significance. Since there are seven compartments, there are $7^2=49$ λ's to be represented by 7×7 matrix. All these values are interrelated, and these values and their limits within which the system can be described must be found. As noted above, the system can be expressed as a set of simultaneous differential equations which are linear and time dependent.

Computational procedures and derived results. Data fitting was accomplished by means of an iterative process using the computer program SAAM-22 (8). This pro-

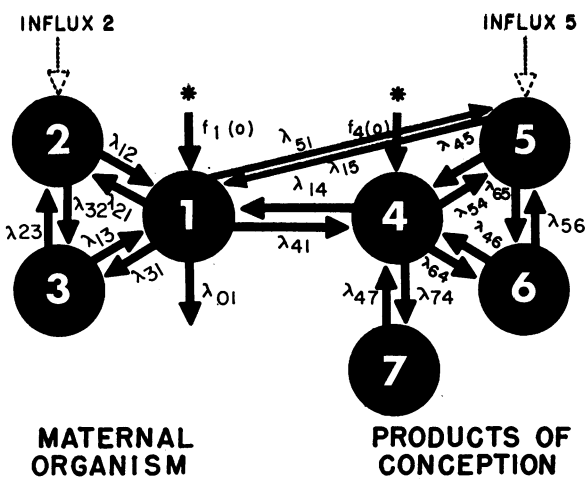


FIGURE 5 Schematic presentation of the seven-compartment model used for the estimation of the transfer rates of carbon dioxide between mother and fetus in the rhesus monkey. Compartments 1, 4, and 7 are the accessible compartments, i.e., maternal and fetal blood and amniotic fluid respectively. Compartments 2, 3 and 5, 6 are maternal and fetal subcompartments respectively. Both subsystems have their own characteristic influx rate (production rate of CO_2). λ_{ij} denotes fractional transfer rate from compartment j to compartment i. The * indicates the initial condition in compartment 1 or 4 at $t=0$. $f_1(0)$ or $f_4(0)$ is the amount of tracer present in compartment 1 or 4 at the beginning of the measurement. λ_{0i} denotes loss of CO_2 from compartment 1 to outside the system.

TABLE II

Comparison of the Initial Estimates for the Independent Adjustable Lambda Parameters with the Final Values Obtained after 10 Iterations (Experiment I)

Lambda		Initial estimate			Final values	
To	From		Minima	Maxima	Value	Standard deviation
4	5	0.0935600	0.0500	0.2000	0.08865183	±0.02489358
4	7	0.0157917	0.0120	0.0170	0.01621956	±0.00060146
2	1	0.5870000	0.5000	0.7000	0.60281230	±0.05765683
1	2	0.0696000	0.0400	0.0800	0.07336304	±0.00882026
3	2	0.0273800	0.0200	0.0400	0.02742380	±0.00723806
0	2	0.0100000	0.0050	0.0200	0.01004614	±0.00642462
0	3	0.0081400	0.0050	0.0200	0.00772685	±0.00277876
0	1	0.0302700	0.0000	0.1500	0.03128194	±0.04987118
1	3	0.0005460	0.0000	0.1000	0.00041189	±0.00033172
5	4	0.7109000	0.2000	1.0000	0.70986840	±0.3741805
6	4	0.0311000	0.0100	0.0900	0.03152570	±0.04009691
6	5	0.0291200	0.0100	0.1000	0.02087857	±0.00495305
4	6	0.0104600	0.0000	0.1000	0.01136830	±0.00189480
4	1	0.0055000	0.0015	0.0100	0.00530514	±0.00103321
1	4	0.7819900	0.5000	0.9000	0.81094570	±0.17963710
5	1	0.0360000	0.0100	0.0500	0.03365489	±0.00832059
7	4	0.0389423	0.0200	0.0400	0.03979820	±0.00153615

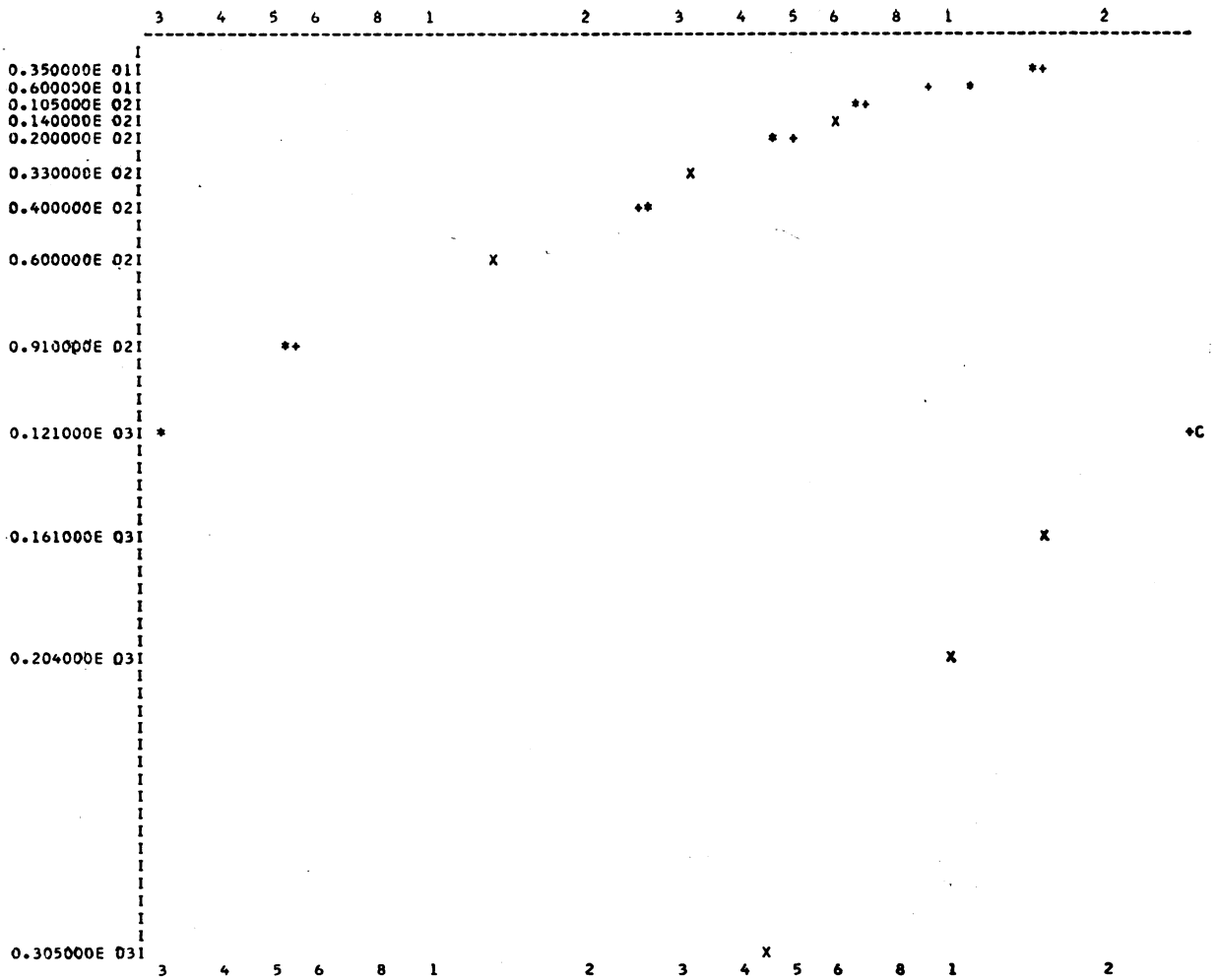


FIGURE 6 Final computer output showing calculated (+) and observed (*) values on a semi-logarithmic coordinate system. (X) denotes identical values. Time is on ordinate (E denotes 10, last two columns 01 to 03 indicate power to 10). Fractional amount of the tracer in the maternal blood compartment is on abscissa.

gram is designed to fit physical or mathematical models to data by adjusting the parameter values of the model repeatedly until a "best fit" is obtained. Of the many model types available for routine use in this program, the solution of linear differential equations with constant coefficients (model code 1) was used as it was best suited for the present problem.

All data from a single study were fitted simultaneously to a single set of model parameters. The data included fractional amounts of $^{14}CO_2$ of maternal and fetal blood and amniotic fluid.

By trial and error using only changes in selected parameters λ's over a fairly wide but reasonable range determined by a similarity transformation and mapping, the estimates of the parameters were brought within the limits where the program could be asked to iterate to adjust the parameter values until a specified degree of convergence was reached between the calculated values and data. The final values for each parameter λ (fractional transfer rate) and their standard deviations were then printed out, and the calculated and observed values were compared numerically and graphically.

As an example, one of the experiments (experiment I) where the data obtained after injection of 1 mCi of ^{14}C-labeled bicarbonate into the maternal vein (Fig. 3 A and B) is considered here. The fractional amounts as a function of time in three accessible compartments (maternal and fetal blood and amniotic fluid) represent the needed data, and the constraints must now be set. The initial conditions are given by the fact that at time zero compartment 1 contains all the tracer and none exists in the others. A number of possible λ's can be ex-

cluded for physical reasons, since no physical relation exists between a number of maternal and fetal compartments (Fig. 5). The fetus produces CO_2 (influx 5) and eliminates it by transport to the mother. Since there is no other access to the outside, all the fractional amount of CO_2 leaving the fetal compartments (λ_{0i}'s, i = 4, 5, 6) must be zero and the total amount of CO_2 transferred from fetus to mother per unit of time ρ_{MF} must be greater than that from mother to fetus per unit of time ρ_{FM} ($\rho_{MF} > \rho_{FM}$).

After a number of trials taking into consideration the various possibilities for the values of the parameters λ's (maxima and minima), the best estimates for the lambdas were then submitted. The number of iterations were set to nine which when completed gave a new set of λ's. Table II shows these values for comparison with original estimates. The solution using the iterated values for the transfer rates was an excellent match for the observed and calculated points as given in Figs. 6, 7, and 8. The computed final lambda matrix is shown in Table III (experiment I) where fractional transfer rates among various compartments are expressed in fractions per mintue. In this experiment the maternal organism and the products of gestation must have their own characteristic influx rates (CO_2 production) which may, but need not, be proportional to their weights. The model is so designed that the influx into compartment 5 must be equal to the net transfer (the total amount ρ not fractional transfer rate λ) between fetus and mother. A set of lambdas which are best fit for the experimental data can now be used to calculate the influx into compartments 2 and 5 as well as the ratio of all compartments by using the equation:

$$[\lambda][C] = [I] \tag{6}$$

where [C] is a column matrix of the compartment sizes and [I] is a column matrix of the influx rates.

The λ matrix (Table III, experiment I) has 49 elements, 28 of which are zero. The sizes of three compartments (maternal and fetal blood and amniotic fluid) are known, leaving four unknown sizes of compartments. Only two influx rates which are positive are unknown, five others being zero. Therefore, there are seven equations and six unknowns, a redundance which per-

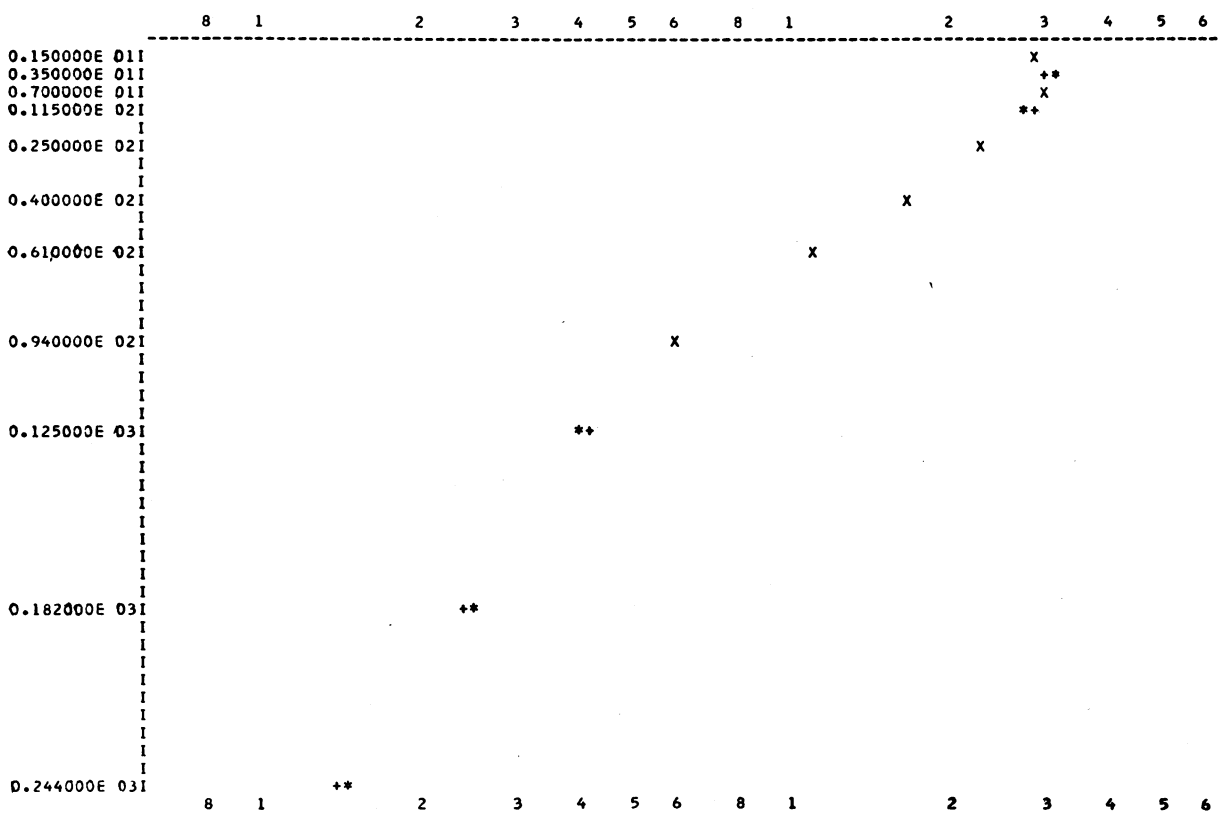


FIGURE 7 The final computer output showing calculated (+) and observed (*) values on a semilogarithmic coordinate system. (X) denotes identical values. Time is on ordinate (E denotes 10, last two columns 01 to 03 indicate power to 10). Fractional amount of the tracer in the fetal blood compartment is on abscissa.

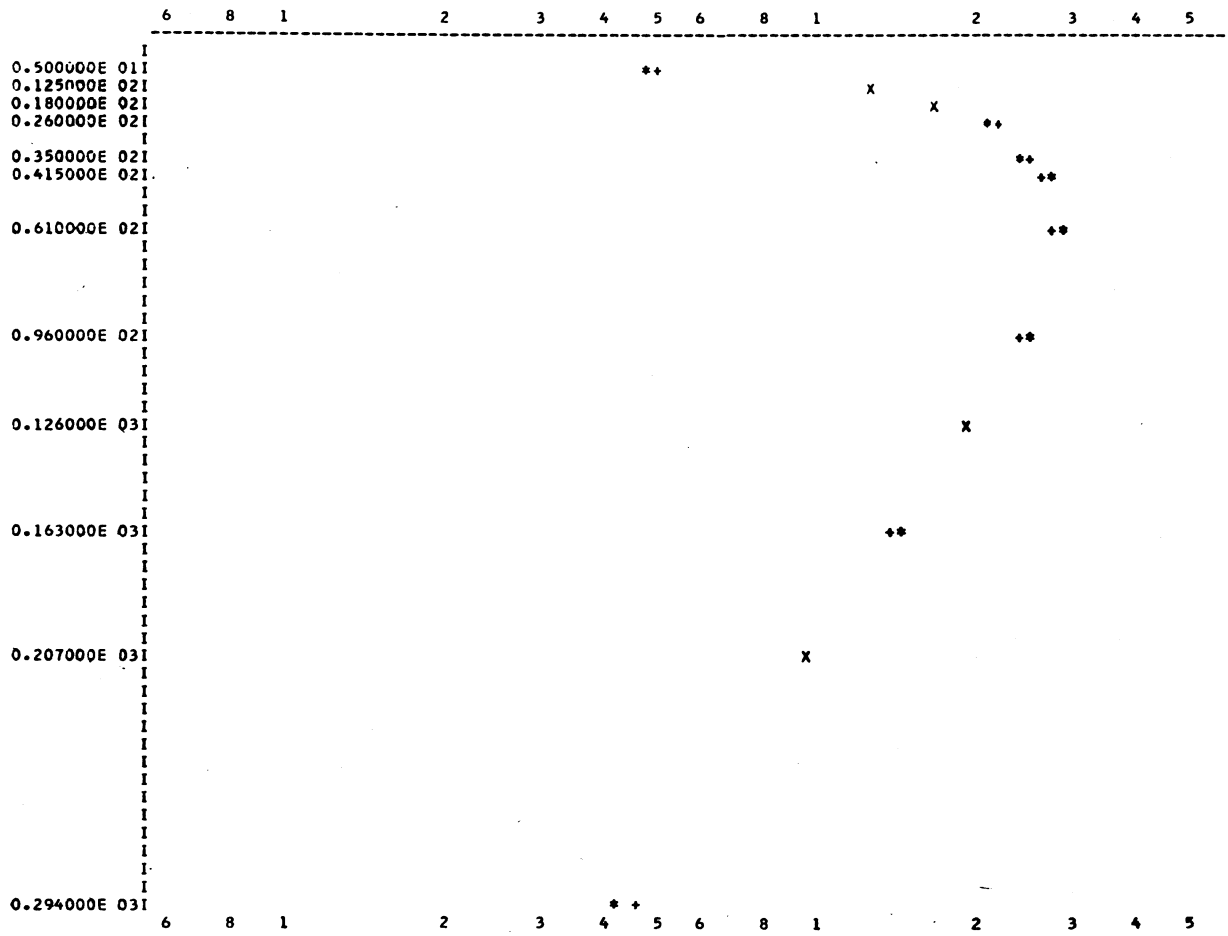


FIGURE 8 The final computer output showing calculated (+) and observed (*) values on a semilogarithmic coordinate system. (X) denotes identical values. Time is on ordinate (E denotes 10, last two columns 01 to 03 indicate power to 10). Fractional amount of the tracer in the amniotic fluid compartment is on abscissa.

mits one to select the most reliable values of the known compartment sizes. Thus choosing compartments 1 and 7 (maternal blood and amniotic fluid compartments), rearranging the equation, and solving by the subroutine MATINV (matrix inversion), the values given in Table IV, experiment I, are obtained. The total amount of CO_2 transferred from one compartment j to another i ($\rho_{ij} = \lambda_{ij} \cdot C_j$) is also calculated by the computer (Table V, experiment I). This animal weighed 8.500 kg; the fetus, placenta, and membranes, and amniotic fluid weighed 0.480 kg, 0.080 kg, and 0.129 kg respectively, i.e., 0.689 kg totally, leaving a net weight of 7.811 kg for the mother. The influx rate for the mother was 2.8133 mmoles CO_2/min (Table IV, experiment 1), hence the loss of CO_2 from the mother was 2.8133/7.811 = 0.3602 mmoles/kg·min. If we assume that only fetus and placenta and membranes are responsible for the influx of nonlabeled CO_2 in compartment 5, the rate of CO_2 production for the products of conception would be 0.28639/(0.480 + 0.080) = 0.5114 mmoles/kg·min. This value is, or should be, identical with the net transfer of CO_2 from the products of conception to the mother. After one additional iteration the limits were further narrowed. The net transfer of CO_2 from fetus to mother was found to be 0.2864 ±0.0215 mmoles/min or 0.5114 ±0.0384 mmoles/kg·min or about 11.5 ±0.9 ml of CO_2/kg·min.

Similar computations were performed on three other experiments. Fractional transfer rates are shown in Table III and compartment sizes and influx rates given in Table IV. The pertinent data for each are listed in Table V and the final values given in Table VI. Average results of data analysis for our seven-compartment model are shown in Fig. 9.

DISCUSSION

In this study it was assumed that $^{14}CO_2$ met the following fundamental requirements inherent to a tracer study; first, it was biologically indistinguishable from CO_2 and therefore subject to the same physiological changes; secondly, it was administered in such a small, though detectable, amount as not to disturb the equilibrium of the system as a whole; and thirdly, it was distributed evenly and instantaneously in the compartment into which it was injected, and it exhibited quantitative changes in distribution into other compartments as a representative of CO_2 of the system. First assumption is reasonable since $^{14}CO_2$ has been used successfully as a tracer for studies of the kinetics of CO_2 in biological system by many investigators without isotope effects. Second assumption is also reasonable because the total amount of $NaH^{14}CO_3$ infused was 0.05 mmoles. Such a small amount of tracer should not disturb the equilibrium of the whole system. Third assumption has some limitations since some small fractions of $^{14}CO_2$ would leave the blood stream through the lungs before a single circulation is completed when the $NaH^{14}CO_3$ is injected into maternal circulation. However, considering the blood flow and the amount of CO_2 in the inferior vena cava where

TABLE III

The Computed Lambda Matrix for the 7-Compartment System Schematically Shown in Fig. 5

Compartment No.	1	2	3	4	5	6	7
Experiment I							
1	−0.67305427	0.07336304	0.00041189	0.81094570*	0.00000000	0.00000000	0.00000000
2	0.60281230	−0.11083290	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
3	0.00000000	0.02742380	−0.00813874	0.00000000	0.00000000	0.00000000	0.00000000
4	0.00530514	0.00000000	0.00000000	−1.59213801	0.08865180	0.01136830	0.01621960
5	0.03365489	0.00000000	0.00000000	0.70986840	−0.10953040	0.00000000	0.00000000
6	0.00000000	0.00000000	0.00000000	0.03152570	0.02087857	−0.01136800	0.00000000
7	0.00000000	0.00000000	0.00000000	0.03979820	0.00000000	0.00000000	−0.01621960
Experiment II							
1	−0.89885311	0.15832010	0.00107011	0.71635400	0.00000000	0.00000000	0.00000000
2	0.67224750	−0.16073750	0.00019415	0.00000000	0.00000000	0.00000000	0.00000000
3	0.10953540	0.00044294	−0.01439515	0.00000000	0.00000000	0.00000000	0.00000000
4	0.01661677	0.00000000	0.00000000	−1.21995901	0.16625120	0.00797480	0.02534700
5	0.04714632	0.00000000	0.00000000	0.43437150	−0.17686780	0.00000000	0.00000000
6	0.00000000	0.00000000	0.00000000	0.05777160	0.01061659	−0.00797487	0.00000000
7	0.00000000	0.00000000	0.00000000	0.01146190	0.00000000	0.00000000	−0.02534700
Experiment III							
1	−0.76518621	0.09692449	0.00045539	0.68890770	0.01806671	0.00000000	0.00000000
2	0.66039530	−0.12332999	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
3	0.00000000	0.02640550	−0.00533008	0.00000000	0.00000000	0.00000000	0.00000000
4	0.01534630	0.00000000	0.00000000	−0.97000926	0.13732730	0.00718314	0.00761140
5	0.04260063	0.00000000	0.00000000	0.25568880	−0.16770592	0.00000000	0.00000000
6	0.00000000	0.00000000	0.00000000	0.01996572	0.01231191	−0.00718314	0.00000000
7	0.00000000	0.00000000	0.00000000	0.00544704	0.00000000	0.00000000	−0.00761140
Experiment IV							
1	−0.18746794	0.01630126	0.00000000	0.24767230	0.00000000	0.00000000	0.00000000
2	0.09729273	−0.02968570	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
3	0.05949312	0.00001514	−0.07650742	0.00000000	0.00000000	0.00000000	0.00000000
4	0.00958070	0.00000000	0.00000000	−1.27173601	0.25985820	0.01633928	0.00985296
5	0.00000000	0.00000000	0.00000000	0.92611670	−0.25985820	0.00000000	0.00000000
6	0.00000000	0.00000000	0.00000000	0.08801362	0.00000000	−0.01633928	0.00000000
7	0.00000000	0.00000000	0.00000000	0.00993340	0.00000000	0.00000000	−0.00985296

The values are fractional transfer rates in fractions per minute from which the compartment sizes, influx rates, and total amount of CO_2 transferred from one compartment to another are to be calculated.

* λ_{ij} (the value i^{th} row and j^{th} column) denotes the fractional transfer rate from compartment j to compartment i, e.g., λ_{14} (0.81094570) in Experiment I is the fractional transfer rate from compartment 4 (fetal blood) to compartment 1 (maternal blood), expressed in fractions per minute.

TABLE IV

Compartment Sizes and Influx Rates Calculated from the Lambda Matrix in Table III

	Compartment No.	Experiment I		Experiment II		Experiment III		Experiment IV	
		Compartment size	Influx rate	Compartment size	Influx rate	Compartment size	Influx rate	Compartment size	Influx rate
		mmoles CO_2	*mmoles CO_2/min*	*mmoles CO_2*	*mmoles CO_2/min*	*mmoles CO_2*	*mmoles CO_2/min*	*mmoles CO_2*	*mmoles CO_2/min*
Maternal blood	1	9.59800	0.00000	12.37000	0.00000	11.51000	0.00000	11.99000	0.00000
Other maternal compartments	2	77.58620	2.81331	63.06670	1.80283	80.12384	2.28052	117.86258	2.33229
	3	261.42972	0.00000	96.06622	0.00000	396.93779	0.00000	9.34690	0.00000
Fetal blood	4	0.81428	0.00000	1.43963	0.00000	1.09831	0.00000	1.31800	0.00000
Other fetal compartments	5*	10.84118	0.28639	8.20427	0.24254	5.75268	0.19360	5.51139	0.21156
	6	22.16916	0.00000	21.35096	0.00000	12.91290	0.00000	7.09957	0.00000
Amniotic fluid	7	1.99800	0.00000	0.65100	0.00000	0.78600	0.00000	1.32876	0.00000

Compartments 1 and 7 were determined by independent means. Influx is assumed to occur only into compartments 2 and 5.
* Compartment 5 contains placenta and fetal membranes.

the tracer was injected, the rate of elimination of CO_2 through the lungs, and the circulation time, the amount of the tracer lost through the lungs in a single circulation is negligible, possibly less than 1% of the amount of the tracer injected. The difference between the assumed and actually observed rate of homogenous distribution is so small that the introduced error is much smaller than those incurred by the analytical processes and the computational procedures of iterative process to get a least square fit to the data. Therefore, even if some fractions of the injected tracer is lost before a single circulation, this would not affect this assumption significantly.

Although various studies on placental transfer of CO_2 in vivo have been conducted, mainly in sheep (15, 16), goats (15, 17, 18), monkeys (19), and man (20), the experimental approach was entirely different from the tracer distribution method. These traditional studies were mainly concerned with determination of

TABLE V

Pertinent Data for Four Experiments Performed on Four Pregnant Rhesus Monkeys

		^{14}C injected into maternal blood			^{14}C injected into fetal blood
		Experiment I	Experiment II	Experiment III	Experiment IV
Maternal weight, g		8500	5600	6800	6180
Fetal weight, *g*		480	375	350	370
Placenta and membranes, *g*		80	100	88	110
Amniotic fluid volume, *ml*		129	30	44.9	79
Maternal blood compartment, mmoles CO_2		9.598	12.370	11.510	11.990
Fetal blood compartment, mmoles CO_2		0.814	1.440	1.098	1.318
Amniotic fluid compartment, mmoles CO_2		1.998	0.651	0.786	1.329
Total amount of CO_2 transferred*					
To	From				
				mmoles CO_2/min	
Fetal blood	Maternal blood	0.0509 (1.6)‡	0.2055 (12.5)	0.1766 (29.4)	0.1149 (8.8)
Maternal blood	Fetal blood	0.6603 (20.4)	1.0313 (62.5)	0.7566 (126.1)	0.3264 (24.9)
Amniotic fluid	Fetal blood	0.0324 (1)	0.0165 (1)	0.0060 (1)	0.0131 (1)
Fetal blood	Amniotic fluid	0.0324 (1)	0.0165 (1)	0.0060 (1)	0.0131 (1)

* Total amount of CO_2 transferred from compartment i to j can be calculated from the fractional transfer rate (λ_{ji}) and compartment size (C_i) as $\rho_{ji} = \lambda_{ji} \cdot C_i$.
‡ The figures in the brackets denote the ratio in each experiment.

the difference in concentration of CO_2 between the fetal and maternal blood and (or) of arteriovenous difference in CO_2 concentration on maternal side of placenta.

However, there exists a number of almost insurmountable obstacles in applying this approach to the placenta. It is difficult to determine the mean values of functional diffusion distance and area available for diffusion (21). Furthermore, CO_2 concentration in uterine vein or artery and in umbilical vein or artery does not allow one to calculate the concentration at interphase with precision because of the presence of shunts (22), and the heterogeneity in composition within the intervillous space (23) makes the sampling of the representative maternal blood quite difficult. Moreover, small difference in concentration of any solute makes the use of Fick's equation unsatisfactory.

Another conventional way to calculate the quantity of CO_2 produced by the fetus is by determination of arteriovenous difference of CO_2 concentration on the fetal side of the placenta. This, however, requires an accurate measure of the umbilical blood flow which is difficult to obtain in a relatively undisturbed preparation.

A study of the carbon dioxide transfer using ^{14}C in the rhesus monkey has been previously conducted in this laboratory (11, 12). Although it yielded only qualitative interpretation of the time-activity curves, the study indicated that the isotope distribution technique was perhaps the most promising in the quantitation of CO_2 transfer across the placenta. It was found that there was a rapid exchange of CO_2 across the placenta and a slower exchange between fetal blood and amniotic fluid and little or no exchange between mother and amniotic fluid. This was confirmed and quantitated in the present study. In Table V the rate of transfer of the total amount of CO_2 from fetal blood to maternal blood was 20–126 times as high as that between fetal blood and amniotic fluid. The rate from maternal blood to fetal blood was lower than that exchanged in the opposite direction, yet up to nearly 30 times as high as that between fetal blood and amniotic fluid.

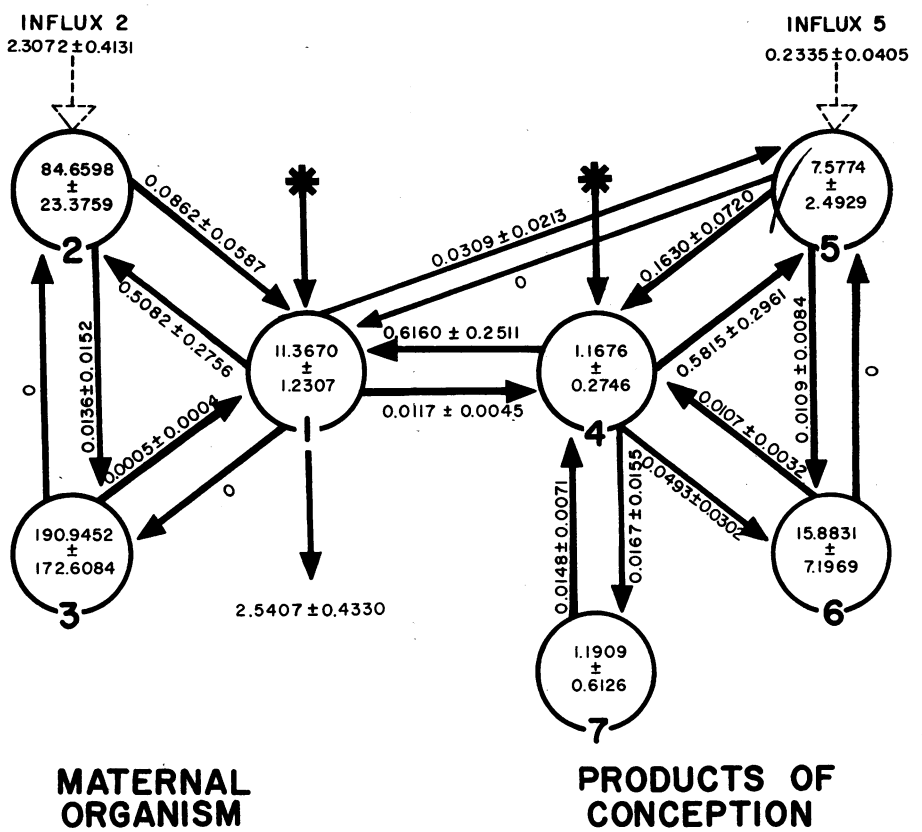


FIGURE 9 Average results of data analysis for the compartmental model. All values represent mean ±1 SD for four pregnant monkeys. To obtain the uncertainties of the mean values, divide the standard deviations by $\sqrt{4} = 2$. Numbers along the arrows are fractional transfer rates, in fractions per minute. Numbers within circles are compartment sizes, in mmoles CO_2. Influx into compartments 2 and 5 and loss of CO_2 from compartment 1 are expressed in mmoles CO_2 per minute.

TABLE VI

Final Values of CO_2 Transfer Between Fetus and Mother in Rhesus Monkeys

	Experiment I	Experiment II	Experiment III	Experiment IV	Mean ±SD
Influx rate into mother (I_2), *mmoles/min*	2.8133	1.8028	2.2805	2.3323	2.3072 ±0.4131
Influx rate into fetus (I_5), *mmoles/min*	0.2864	0.2425	0.1936	0.2116	0.2335 ±0.0405
Loss of CO_2 from mother (rate of CO_2 production by mother), *mmoles/kg·min*	0.3602	0.3564	0.3610	0.4149	0.3731 ±0.02792*
Net transfer of CO_2 from fetus to mother (rate of CO_2 production by fetus), *mmoles/kg·min*	0.5114	0.5105	0.4420	0.4408	0.4762 ±0.04016*

* Significantly different ($P < 0.01$) (Student's t test).

It was also found that CO_2 was transferred quite rapidly from maternal blood to fetal subcompartment 5 which contains placenta and membranes as portions. In fact, the transfer rate was about 2.5 times as fast as that from maternal blood to fetal blood. We could calculate the transfer rate of CO_2 with sufficient accuracy among seven compartments designed in the pregnant monkeys by analyzing the experimental data quantitatively using the computer program SAAM 22. The determination of transfer rates enabled us to calculate the compartment sizes and the influx rates into fetus and mother, thus the net transfer of CO_2 from fetus to mother and the loss of CO_2 from mother.

As physiologic measurements are generally expected to have limitations which exert their influence on the analysis of the data, the concept of a compartmentalized system in a complex biologic system is only an approximation. Although our preparation had in actuality a nearly infinite number of physically distinct compartments, regarding CO_2 distribution the seven-compartment model appeared to be satisfactory.

The present study showed that the primate fetus near term has a higher rate of the CO_2 production per unit body mass than its mother. As seen in Table VI the mean value of the net transfer of CO_2 from fetus to mother (the rate of CO_2 production per kg by the fetus) is 0.4762 ±0.04016 mmoles/kg·min whereas that of loss of CO_2 from mother (the rate of CO_2 production per kg by mother) is 0.3731 ±0.02792 mmoles/kg·min. The difference between the two mean values is statistically significant ($P < 0.01$). It should be noted that in the estimation of CO_2 production by the fetus the placenta and fetal membranes are viewed as an integral part of fetal body. Since the rate of CO_2 production can be taken as an indicator of the rate of the energy metabolism, it is suggested that the primate fetus near term has a higher metabolic rate per unit mass than its mother. This appears to be justified because under relatively constant conditions the rate of CO_2 fixation by either the fetal or maternal tissue would be insignificantly low compared to the rate of CO_2 production.

Recently, estimates have been provided of O_2 consumption of the mature fetus in the sheep and human. Dawes, Mott, and Widdicombe (24), Acheson, Dawes, and Mott (25), and Dawes and Mott (26) obtained a mean value of 4–5 ml/kg·min in anesthetized mature fetal lambs. Romney, Reid, Metcalfe, and Burwell (27) reported O_2 consumption of the human fetus at term of about 5.0 ml/kg·min. Since the respiratory quotient of the fetus is assumed to be near unity these values would be almost equivalent to those of CO_2. The CO_2 production rate we obtained for the mature monkey fetus in this study, however, was much higher (average 10.7 ±0.90 ml/kg·min). Dawes and Mott (26) and Romney et al. (27) concluded that O_2 consumption per kilogram of the mature fetus in sheep and human was similar to that of its mother. We found the primate fetus had a higher metabolic rate than its mother near term. Their method of calculating the O_2 consumption of the fetus depends on the measurement of the blood flow rate of the umbilical vessel or the uterine vessel and arteriovenous O_2 difference. The accurate measurement of the blood flow of the umbilical or uterine vessel is quite difficult and the reported values must be viewed as approximations only. The determination of the difference of O_2 concentration between artery and vein is also likely to invite errors. Species difference and differences in anesthesia and in surgical techniques must also be factors causing the different results in the fetal metabolic rate. However, Dawes, Jacobson, Mott, and Shelley (28) observed five newborn rhesus monkeys at an environmental temperature of 35°C and found the rate of O_2 consumption to be 9.3–12.5 ml/kg·min. This figure matches well that of the rate of CO_2 production of the fetus in our study. This agreement in two studies in the rhesus monkey seems to indicate that species difference is the most likely cause of different results from other studies in sheep.

Judging from the findings that the metabolic rate based upon the rate of CO_2 production by the fetal monkey in our study is similar to that based upon O_2 consumption by the newborn monkey found by Dawes et al. which

was measured quite accurately because of the technical feasibility in the newborn animal, and that all the fetuses in our study survived with normal heart rates for 5–14 days after the experiment, we believe that the condition of our fetuses was within the physiological limit. However, our values are also only approximations based upon the simplified model and limited number of experiments. Further investigations would be necessary to clarify this problem.

APPENDIX

I. Fractional amount of ^{14}C in blood and all other body compartments of a nonpregnant monkey No. 397 as function of time.

Time (min)	Blood compartment, fractional amount	Statistical normalized weight
5	0.1071	0.00236074
10	0.0710	0.00537167
15	0.0542	0.00921781
20	0.0427	0.0148515
25	0.0359	0.0210105
30	0.0312	0.0278174
50	0.0157	0.109857
60	0.0140	0.138156
75	0.0069	0.568759
90	0.0050	0.704009
105	0.00475	1.20016
120	0.00400	1.69241
150	0.00263	3.91485
180	0.00217	5.75052
240	0.00132	15.5410

Time (min)	All other compartments, fractional amount	Statistical normalized weight
5	0.8329	0.0000390338
10	0.8290	0.0000394019
15	0.8058	0.0000397634
20	0.7773	0.0000448176
25	0.7541	0.0000476177
30	0.7288	0.0000509811
50	0.6243	0.0000694768
60	0.5660	0.0000845266
75	0.5131	0.000102854
90	0.4550	0.000130799
105	0.3953	0.000173290
120	0.3460	0.000226190
150	0.2674	0.000378707
180	0.2078	0.000627098
240	0.1287	0.00163482

Weight is calculated as $W = 1/SD^2$. After weights are assigned to all data, they are normalized so that the sum of the weights equals the number of data points having nonzero weights.

II. Fractional amount of ^{14}C in maternal and fetal blood and amniotic fluid of a pregnant monkey No. 319 (experiment I) as function of time.

Time (min)	Maternal blood, fractional amount	Statistical normalized weight
3.5	0.146071	0.0000171360
6.0	0.108758	0.0000309111
10.5	0.0675262	0.0000801842
14.0	0.0606013	0.0000995567
20.0	0.0468996	0.000166224
33.0	0.0316106	0.000365906
40.0	0.0271074	0.000497576
60.0	0.0134210	0.00202984
91.0	0.00521010	0.0134692
121.0	0.00304887	0.0393329
161.0	0.00151761	0.158751
204.0	0.000995941	0.368609
305.0	0.000436488	1.91906

Time (min)	Fetal blood, fractional amount	Statistical normalized weight
1.5	0.00281485	0.0461450
3.5	0.00310182	0.0380015
7.0	0.00296787	0.0415091
11.5	0.00266348	0.0515388
25.0	0.00228805	0.0698397
40.0	0.00160390	0.142127
61.0	0.00107127	0.318595
94.0	0.000589070	1.05366
125.0	0.000389040	2.41571
182.0	0.000244075	6.13746
244.0	0.000140528	18.5144

Time (min)	Amniotic fluid, fractional amount	Statistical normalized weight
5.0	0.000500030	1.46232
12.5	0.00126871	0.227149
18.0	0.00173660	0.121236
26.0	0.00213514	0.0802011
35.0	0.00247976	0.0594585
41.5	0.00280010	0.0466324
61.0	0.00294943	0.0420297
96.0	0.00259041	0.0544876
126.0	0.00192165	0.0990119
163.0	0.00148269	0.166315
207.0	0.000997704	0.367308
294.0	0.000433861	1.94237

III. Data in an open three-compartment system in a nonpregnant and a pregnant monkey for initial estimates of transfer rates in an open seven-compartment system.

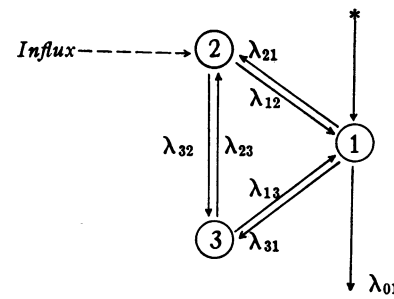
From Fig. 2:

$$A_{11} = 0.90000 \quad A_{12} = 0.09000 \quad A_{13} = 0.01000$$
$$\alpha_1 = 0.69300 \quad \alpha_2 = 0.04620 \quad \alpha_3 = 0.00866$$

The experimental data are not very precise or numerous, and the constants were to be regarded as the best estimates. However, the information is sufficient to tell us that there are minimum of three compartments. The blood compartment is then described by the equation

$$q(t) = 0.90000\, e^{-0.69300\, t} + 0.09000\, e^{-0.04620\, t} + 0.01000\, e^{-0.00866\, t}$$

Since we know all compartments exchange with each other, we set up the model as the following one,



where compartment 1 is blood compartment and 2 and 3 are other compartments. Influx (production of CO_2) is assumed to occur in compartment 2. The * indicates the initial condition in compartment 1 at t = 0. λ_{01} denotes loss of CO_2 from compartment 1 to outside the system. λ_{ij} indicates fractional transfer rate from compartment j to i.

The coefficients for compartment 2 and 3 are unknown, and their sum must be equal to zero at t = 0. In order to proceed with the mapping it is only necessary to start with a matrix for the coefficients that meets the conditions.

$$[A] = \begin{bmatrix} A_{11} & A_{12} & A_{13} \\ -(A_{22}+A_{23}) & A_{22} & A_{23} \\ (A_{22}+A_{23}) - A_{11} & -A_{12}-A_{22} & 1-A_{23}-A_{13} \end{bmatrix}$$

An arbitrary selection for A_{22} and A_{23} will give a generating model (a lambda matrix) that can be used to find physically realizable models compatible with the data.

Using the values derived from the graph and setting $A_{22} = -0.30000$ and $A_{23} = 0.50000$, and A matrix will be

$$[A] = \begin{bmatrix} 0.90000 & 0.09000 & 0.01000 \\ -0.20000 & -0.30000 & 0.50000 \\ -0.70000 & 0.21000 & 0.49000 \end{bmatrix}$$

and α matrix will be

$$[\alpha] = \begin{bmatrix} 0.69300 & 0.00000 & 0.00000 \\ 0.00000 & 0.04620 & 0.00000 \\ 0.00000 & 0.00000 & 0.00866 \end{bmatrix}$$

A lambda matrix (generating model) calculated according to equation 4 in the text will be

$$[\lambda] = \begin{bmatrix} 0.62794 & 0.09664 & -0.11126 \\ -0.14813 & 0.00587 & 0.00587 \\ -0.47115 & -0.09385 & 0.11405 \end{bmatrix}$$

Here the values for λ_{12} and λ_{23} are negative hence the model is purely theoretical and not directly applicable. However we can use this generating model for mapping purposes.

In this case the number of degrees of freedom is $3^2 = 9$; we know A_{11}, A_{12}, A_{13} for compartment 1 and three α's, and $\sum_{i=1}^{3} A_{2i} = 0$, $\sum_{i=1}^{3} A_{3i} = 0$, thus the system is defined within two degrees of freedom. Selecting a suitable transformation matrix [P] containing two variables β and γ,

$$[P] = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \beta+\gamma & \beta \\ 0 & 1-(\beta+\gamma) & 1-\beta \end{bmatrix}$$

A new similar matrix $[\lambda']$ is obtained according to equation 5 in the text and a new $[\lambda']$ is expressed as a function of β and γ with the use of values of a generating model. Each new λ' is set equal to or greater than zero. This yields a plot of β vs. γ. An area limited by lines $\lambda'_{13} = 0$, $\lambda'_{31} = 0$, $\lambda'_{23} = 0$, and $\lambda'_{32} = 0$ meets the requirements. Any λ' obtained from β and corresponding γ within this area will yield a physically realizable values corresponding to the experimental data. All these calculations can be done by a computer program SAAM-22 (8). The theoretical limits for the fractional transfer rates of physically realizable models for three-compartment open system are, (in fractions per minute)

	Maximum	Minimum
λ_{12}	0.10003	0.06153
λ_{13}	0.00257	0.00000
λ_{21}	0.61928	0.37463
λ_{23}	0.00417	0.00000
λ_{31}	0.24465	0.00000
λ_{32}	0.03850	0.00000

The ratio of the compartment sizes for all λ's in the defined area can be calculated according to equation 6 in the text and they are,

Compartments:	1	2		3	
		Maximum	Minimum	Maximum	Minimum
Ratio:	1.00000	10.19858	3.44199	168.79845	21.22984
Actual sizes: (mmoles CO_2)	9.25	94.35	31.82	1561.4	196.38

The influx rate to compartment 2 calculated from equation 6 in the text has the range of 0.19964–0.35109 in fractions/min, or 1.85–3.25 mmoles CO_2/min. This represents the total elimination of CO_2 from the system, which includes all mechanisms, such as respiration, metabolic processes, and urinary excretion.

Then all the data are fed into the computer program SAAM-22 (8) and data fitting is accomplished by means of an iterative process. Those values of λ's obtained above serve as initial estimates for parameters λ's in this program. Final values for λ's are (fractions per minute):

$\lambda_{12} =$ 0.09082884 ±0.006164936
$\lambda_{13} =$ 0.00252647 ±0.001081707
$\lambda_{21} =$ 0.4121689 ±0.03638044
$\lambda_{23} =$ 0.0000104619 ±0.00118815
$\lambda_{31} =$ 0.2042536 ±0.03023778
$\lambda_{32} =$ 0.009234226 ±0.006287368

The same process of calculation for an open three-compartment system as stated above is carried out in each data of the pregnant monkeys in order to obtain proper initial estimates for λ's. Thus, from Fig. 3 B in the experiment I we obtain:

$$[A] = \begin{bmatrix} 0.90970 & 0.08530 & 0.00500 \\ -0.20000 & -0.30000 & 0.50000 \\ -0.70970 & 0.21470 & 0.49500 \end{bmatrix}$$

$$[\alpha] = \begin{bmatrix} 0.69300 & 0.00000 & 0.00000 \\ 0.00000 & 0.03647 & 0.00000 \\ 0.00000 & 0.00000 & 0.00815 \end{bmatrix}$$

and a generating model of lambda matrix calculated according to equation 4 in the text,

$$[\lambda] = \begin{bmatrix} 0.63357 & 0.09592 & -0.10320 \\ -0.14546 & 0.00119 & 0.00850 \\ -0.47996 & -0.08896 & 0.10286 \end{bmatrix}$$

Then a similarity transformation and mapping are done. The theoretical limits for λ's of physically realizable models for three-compartment open system are (in fractions per minute):

	Maximum	Minimum
λ_{12}	0.08662	0.05689
λ_{13}	0.00112	0.00000
λ_{21}	0.62544	0.40794
λ_{23}	0.00170	0.00000
λ_{31}	0.21748	0.00001
λ_{32}	0.02974	0.00000

The compartment sizes are calculated from equation 6 in the text and they are:

Compartments:	1	2		3	
		Maximum	Minimum	Maximum	Minimum
Ratio:	1.00000	11.14165	6.48945	97.32467	23.15836
Actual sizes: (mmoles CO_2)	9.598	106.938	62.286	934.122	222.274

The influx rate to compartment 2 calculated from equation 6 in the text has the range of 0.17404–0.36774 in fractions per minute, or 1.670–3.530 mmoles CO_2/min.

Then all the data are fed into the computer program SAAM-22 (8) and data fitting is carried out by means of an iterative process. Those values obtained above serve as initial estimates for λ's in this program. Final values for λ's obtained through this iteration can be used for initial estimates for λ's in the 7-compartment open system model, which are listed in Table II.

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