Kinetics of CO₂ excretion and intravascular pH disequilibria during carbonic anhydrase inhibition

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Cardenas, Victor, J. R., Thomas A. Heming, and Akhil Bidani. Kinetics of CO₂ excretion and intravascular pH disequilibria during carbonic anhydrase inhibition. J. Appl. Physiol. 84(2): 683–694, 1998.—Inhibition of carbonic anhydrase (CA) activity (activity in red blood cells and activity available on capillary endothelium) results in decrements in CO₂ excretion (VCO₂) and plasma-erythrocyte CO₂-HCO₃⁻ disequilibrium as blood travels around the circulation. To investigate the kinetics of changes in blood PCO₂ and pH during progressive CA inhibition, we used our previously detailed mathematical model of capillary gas exchange to analyze experimental data of VCO₂ and blood-gas/pH parameters obtained from anesthetized, paralyzed, and mechanically ventilated dogs after treatment with acetazolamide (Actz, 0–100 mg/kg iv). Arterial and mixed venous blood samples were collected via indwelling femoral and pulmonary arterial catheters, respectively. Cardiac output was measured by thermodilution. End-tidal PCO₂, as a measure of alveolar PCO₂, was obtained from continuous records of airway PCO₂ above the carina. Experimental results were analyzed with the aid of a mathematical model of lung and tissue-gas exchange. Progressive CA inhibition was associated with stepwise increments in the equilibrated mixed venous-alveolar PCO₂ gradient (9, 19, and 26 Torr at 5, 20, and 100 mg/kg Actz, respectively). The maximum decrements in VCO₂ were 10, 24, and 26% with 5, 20, and 100 mg/kg Actz, respectively, without full recovery of VCO₂ at 1 h postinfusion. Equilibrated arterial PCO₂ overestimated alveolar PCO₂, and tissue PCO₂ was underestimated by the measured equilibrated mixed venous blood PCO₂. Mathematical model computations predicted hysteresis loops of the instantaneous CO₂-HCO₃⁻-H⁺ relationship and in vivo blood PCO₂-pH relationship due to the finite reaction times for CO₂-HCO₃⁻-H⁺ reactions. The shape of the hysteresis loops was affected by the extent of Actaz inhibition of CA in red blood cells and plasma.

gas exchange; carbonic anhydrase; acetazolamide

Molecular CO₂ has a high solubility-diffusivity product (~20 times that for O₂), and on that basis it has been tacitly assumed that equilibration of CO₂ between alveolar gas and capillary blood occurs almost instantaneously. CO₂ is only moderately soluble in aqueous media, and the transport of CO₂ in physical solution alone is inadequate to keep pace with metabolic CO₂ production (6, 21–23). CO₂, produced in metabolically active cells as a by-product of fuel utilization or lipogenesis, diffuses freely through tissues on the basis of its small molecular size and relatively high lipid solubility and behaves as a weak acid that hydrates to yield HCO₃⁻ and H⁺ via the following reaction: CO₂ + H₂O ⇌ H₂CO₃ ⇌ HCO₃⁻ + H⁺ (pKₐ 6.1). Under physiological conditions (pH 7.4), the majority of CO₂ is in the form of HCO₃⁻. CO₂ excretion (VCO₂) in the lungs requires transformation of intravascular HCO₃⁻ stores to molecular CO₂ via the Jacobs-Stewart cycle (18). The rate-limiting step in the reaction is the hydration/dehydration of CO₂/HCO₃⁻, which when catalyzed by carbonic anhydrase (CA) is reduced from a half time (t₁/₂) of 5–8 s to 5–10 ms (6, 22, 23). In addition, HCO₃⁻/Cl⁻ exchange is important in facilitating transfer of HCO₃⁻ from the extracellular to intraerythrocyte space for conversion to molecular CO₂ and subsequent excretion (tₑ = 125 ms) (11, 17, 19, 20). Therefore, VCO₂ in vivo requires efficient catalysis of HCO₃⁻ dehydration by red cell CA during pulmonary capillary transit (6, 22, 26). Under normal physiological conditions, there is sufficient red cell CA activity to accelerate the dehydration reaction by ~6,500-fold, and VCO₂ reaches completion during the early phase of pulmonary capillary transit time (750 ms) (6, 23). Inhibition of CA activity should affect the kinetics of intracapillary gas exchange and the kinetics of intravascular pH equilibration (5, 6) but not the steady-state VCO₂. In an earlier study, Bidani and Crandall (3) measured postcapillary pH disequilibria during differential grades of CA inhibition. The study indicated complex postcapillary pH disequilibria depending on the activities of red cell and intravascular CA (3, 6). Subsequently, Bidani (2) used a detailed mathematical analysis to quantify the contributions of red cell anion exchange and vascular and red cell CA activities in maintaining VCO₂ during rest and exercise. On the basis of a calculated transfer capacity for CO₂ (TₐCO₂), estimates were obtained for the alterations in alveolar ventilation and cardiac output necessary to maintain VCO₂ when red cell anion exchange and/or CA activities are inhibited (2). Swenson et al. (25) reported the quantitative effects of red cell anion exchange and CA inhibitors on VCO₂ in anesthetized, paralyzed, and mechanically ventilated dogs. Although the experimental results were in general agreement with the previous predictions (2), Swenson et al. estimated the effects of anion exchange and CA inhibitors on single-pass VCO₂ but not on steady-state excretion.

Taki et al. (29) evaluated the effects of progressive CA inhibition on VCO₂ in anesthetized and paralyzed dogs. Their experimental protocol consisted of measurements of end-tidal (ETCO₂), arterial (PACO₂), and mixed venous blood PCO₂ (PVCO₂) after the administration of 5 mg/kg acetazolamide (Actz) at 10-min intervals up to a cumulative dose of 20 mg/kg. No significant decrements in VCO₂ were reported. The authors did note a gradual increment in PACO₂ from ~33 to 52 Torr, whereas PVCO₂ increased from ~43 to 58 Torr at the end of the experimental protocol. The "estimated" alveolar PCO₂ (PACO₂) fell from 32 to 27 Torr after the first dose of 5
mg/kg Actz, but no change was noted with three successive doses of 5 mg/kg Actz. The authors used the electrometric method of Wilbur and Anderson (30) to estimate the extent of red cell CA inhibition after each successive dose of 5 mg/kg Actz. The maximum degree of red cell CA inhibition was estimated to be ~73% at the end of the experimental protocol (cumulative dose of 20 mg/kg Actz). A significant problem with the study of Taki et al. is the lack of an adequate time to reach steady state. The lack of any significant transient decrement in VCO₂ after Actz administration does not agree with the previous study of Berthelsen and Dich-Nielson (1). It also is difficult to reconcile the estimates of Taki et al. on the extent of red cell CA inhibition with earlier estimates of Swenson and Maren (26) and Wistrand (31), who reported that no significant differences in alveolar-blood PCO₂ gradient would be expected for CA inhibition <95%. Maren noted that a dose of 5 mg/kg Actz, as was utilized by Taki et al., should inhibit ~98% of red cell CA activity. It is interesting that a recent study by Taki et al. (28) found significant widening of the alveolar-arterial blood PCO₂ gradient [Δ(A-a)PCO₂] at 5 mg/kg Actz in anesthetized dogs. This PCO₂ gradient increased progressively with the dose of Actz up to a total of 30 mg/kg.

Reestablishment of a steady state for VCO₂ after CA inhibition can be mediated by alterations in cardiac output, alveolar ventilation, and/or increments in tissue PCO₂ (2, 9, 10). Our present study was designed to quantify the transient and quasi-steady-state effects of progressive CA inhibition in vivo using intravenous Actz administration on VCO₂ in anesthetized mechanically ventilated dogs. A few experiments also were performed with the weakly permeant CA inhibitor benzolamide. The goal was to evaluate the veracity of previous predictions of the extent of mixed venous blood-alveolar PCO₂ gradient [Δ(V-A)PCO₂] necessary to maintain VCO₂ for different levels of CA inhibition. Additionally, we have utilized our previous mathematical model (2, 7) to predict the kinetics of intravascular PCO₂ and pH equilibration as blood travels around the circulation during different levels of CA inhibition, after reestablishment of a steady state. The model computations indicate hysteresis loops of the instantaneous CO₂-HCO₃⁻-H⁺ relationship and in vivo blood PCO₂-pH relationship due to the finite reaction times for the CO₂-HCO₃⁻-H⁺ reactions. The shape of the hysteresis loops is affected by the extent of Actz inhibition of CA in red blood cells and plasma.

Methods

Animal preparation. Five unconditioned mixed-breed dogs of either sex (20–24 kg body mass) underwent anesthetic induction with an intravenous bolus injection of 30 mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories, N. Chicago, IL). A midline tracheostomy was performed, and the animal was placed on a volume-cycled ventilator (Harvard Apparatus, Millis, MA). Pancuronium bromide, a nondepolarizing paralyzing agent (Pavulon, Organon), was administered via intravenous bolus of 2 mg every 1–2 h to eliminate spontaneous respirations. Tidal volume was set at 15 ml/kg, and rate was adjusted to obtain a baseline PACO₂ of 36 ± 2 Torr. Depth of anesthesia was determined utilizing pupillary light reflex, faciulation, and salivation as markers and was maintained using intermittent intravenous pentobarbital at 5–10 mg/kg every 1–2 h. Cannulas were placed in a femoral artery (via arteriotomy) and the contralateral femoral vein (percutaneous). An 8-F introducer was inserted into the external jugular vein (venotomy), and an oximetric pulmonary arterial catheter (Abbott Laboratories) was floated into position under waveform guidance. Core temperature was monitored utilizing the pulmonary arterial catheter thermistor and maintained at 37.5 ± 1.5°C by the use of an external heating/cooling blanket. After completion of surgery, the animal was given an intravenous bolus of 2,000 U of heparin sodium and placed on a constant infusion of 1,000 U/h to minimize intravascular hemolysis.

Airway CO₂ was measured using a side-stream monitor (Datex Instruments), with the sampling cannula placed at the level of the main carina. The monitor was calibrated before each experiment using two gas mixtures with different CO₂ concentrations (medical-grade gas standard, Liquid Carbonic). Systemic arterial blood pressure was monitored at the femoral artery (Hewlett-Packard transducer and amplifier). Both parameters (airway CO₂ and systemic arterial blood pressure) were recorded continuously on a chart recorder (model 2600, Gould, Cleveland, OH). At intervals, expired gas was collected in Douglas bags over 1-min periods, and the fractional concentrations of CO₂, N₂, and O₂ were determined using mass spectroscopy (model MGA 1100, Perkin-Elmer, Pomona, CA). Blood-gas samples were obtained at intervals from the arterial, femoral venous, and distal pulmonary arterial catheter ports, stored on ice, and measured within 2 h. Blood PCO₂, pH, and PO₂ were determined on a blood-gas analyzer (model ABL 330, Radiometer, Copenhagen, Denmark) at 37°C. Blood hemoglobin content and O₂ saturation (SO₂) were determined using the accompanying CO-oximeter (OEM 3 hemoximeter) adjusted for canine blood. In vivo mixed venous SO₂ was determined at the fiber-optic pulmonary arterial catheter tip and processed in the accompanying SO₂/CO computer module (Oximetric 3 SO₂/CO computer, Abbott Laboratories) using three-wavelength spectrophotometry. Calibration was performed using the in vivo technique of adjusting the displayed value to match a standard in vitro determination of mixed venous blood (Radiometer CO-oximeter). Cardiac output was determined by the modilution technique using 10-ml injectates of iced or room-temperature saline, with each recorded value the average of two to three determinations. Pulmonary arterial pressures were not monitored continuously, inasmuch as the distal port of the pulmonary arterial catheter was used primarily to obtain samples of central mixed venous blood. Blood samples for pre- and postexperimental hematocrit and plasma hemoglobin were obtained from the femoral vein. Hematocrit was determined by the microhematocrit technique. Plasma hemoglobin was determined by the 3,3',5,5'-tetramethylbenzidine method (3, 13).

After surgical interventions were complete, a 30- to 60-min period followed under stable conditions to allow the establishment of a steady-state condition. The CA inhibitor Actz (Diamox, Lederle) was prepared in a saline solution on the day of the experiment and separated into three incremental doses to provide cumulative doses of 5, 20, and 100 mg/kg for each animal. The doses of Actz were chosen to induce the following conditions: 1) complete inhibition of extravascular CA with significant residual red cell CA activity, 2) near-complete inhibition of red cell CA, and 3) complete inhibition of red cell CA. Because 5 mg/kg Actz is expected to inhibit substantial red cell CA activity (3), a few experiments were...
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performed with low-dose benzolamide (2 mg/kg) to simulate the situation where all endothelial/intravascular CA activity is inhibited with minimal inhibition of red cell CA (13).

At time 0, arterial, femoral venous, and mixed venous blood samples were obtained. Expired gas was collected, core temperature, 
ET CO2, and mixed venous So2 were recorded, and cardiac output was determined. Actz (5 mg/kg) then was administered via slow injection into the femoral vein. The samples and readings were repeated at 5 min, 15 min, and 1 h postinfusion. The process was repeated serially for 20 and 100 mg/kg Actz. One animal received a sham infusion of alkalized saline of similar pH to the Actz solution (pH 9.5) under conditions of no CA inhibition and complete inhibition (100 mg/kg Actz). The parameters were monitored as described above. The experimental protocol was approved by the Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines regarding animal research.

Calculations. 

\[ \dot{V}_{CO2} = F_{E_{CO2}} (\dot{V}_i) (F_{IN2}/F_{EN2}) \]

where \( \dot{V}_i \) is the inspired minute volume and the gas fractions are the values as determined by mass spectroscopy of mixed expired \( \dot{V}_{CO2} \) and inspired \( \dot{V}_{E_{CO2}} \) and expired \( \dot{V}_{N2} \) \( (F_{IN2} \) and \( F_{EN2} \), respectively). Expired minute ventilation \( \dot{V}_E \) was estimated from

\[ \dot{V}_{E} = \dot{V}_i (F_{IN2}/F_{EN2}) \]

ET CO2 was obtained from the continuous airway CO2 tracings as recorded with the chart recorder. Dead space ratios \( (VD/VT) \) were calculated using the equation

\[ VD/VT = (\dot{V}_{CO2}/\dot{V}_E) (Pb/P_{ACO2}) \]

where \( VD/VT \) was used as an approximation of \( P_{ACO2} \) and \( Pb \) is barometric pressure.

Statistical analyses. Values are means \( \pm \) SE. Significance was determined using the method of sum of least squares with \( P < 0.05 \).

Mathematical model computations. The experimental data were analyzed using a previously described model of capillary gas exchange (2, 7). Several simplifying assumptions are incorporated into the mathematical model. Alveolar gas is assumed to be well mixed and uniform throughout the lung. Alveolar and tissue-gas tensions are assumed to be time invariant. Blood is considered to consist of two well-mixed compartments (plasma and erythrocyte). The residence time of blood in the pulmonary capillaries is taken as 1 s and that in the tissue capillaries as 2 s. Blood flow in the capillaries is assumed to be constant and uniform, and axial and radial diffusion are considered negligible.

A kinetic mathematical description of pulmonary and tissue capillary \( O_2 \) and CO2 exchange was obtained by deriving mass balance equations for each of the relevant chemical species: \( CO_2 \), \( O_2 \), water (or volume), hemoglobin-carbonate (oxygenated and reduced), \( HCO_3^- \), \( Cl^- \), and \( H^+ \). Because the latter three exist at different concentrations in the plasma and red cell compartments, the behavior of 11 variables must be described as a function of time (from the time blood enters the pulmonary or tissue capillaries). The mass balances describing the time rates of change of the 11 variables took into consideration the rate of consumption or production of that species by chemical reaction within its compartment and net transport of the species into or out of its compartment. All tissues were lumped into a single compartment, and the time delay between lung-to-tissue and tissue-to-lung transit was assumed to be fixed.

Processes included in the quantitative analysis are 1) \( CO_2 \)-\( HCO_3^- \)-\( H^+ \) reactions in plasma and red blood cells, 2) \( CO_2 \) binding to hemoglobin, 3) \( O_2 \) binding to hemoglobin and the release of Bohr protons, 4) intra- and extracellular buffering of \( H^+ \) by hemoglobin and plasma proteins, respectively, 5) \( HCO_3^-\)/\( Cl^- \) exchange across the red cell membrane mediated by band 3 protein, 6) transcellular movement of water in response to changes in osmolality, and 7) diffusion of \( O_2 \) and \( CO_2 \) between alveolar gas (or tissues) and capillary blood. Bohr and Haldane effects are included. Ion and water fluxes are described assuming passive diffusion down their respective electrochemical potential and osmotic gradients. Red cell anion exchange is described in terms of a "phenomenological permeability coefficient (P \( \text{HCO}_3^- \))." Catalysis of \( CO_2\)-\( HCO_3^- \)-\( H^+ \) reactions is described using dimensionless catalytic factors for plasma (\( A_p \)) and red cell reactions (\( A_r \)).

The mathematical model consists of a set of 22 simultaneous nonlinear ordinary differential equations. The only difference between capillary and postcapillary equations is that net \( O_2 \) and \( CO_2 \) movements can take place into or out of the blood while blood is in the capillaries, but after blood enters the postcapillary vessels it becomes a closed system and total \( O_2 \) and \( CO_2 \) contents remain constant. Numerical integration of the model differential equations was implemented using the Gear algorithm (16), ideally suited for stiff differential systems.

Parameter values for the mathematical model. Input parameters for the model included kinetic parameters, estimated alveolar gas tensions (assumed equal to the measured endtidal values), metabolic rates (based on measurements during the control period), hematocrit, cardiac output, and appropriate catalytic factors (\( A_p \) and \( A_r \)). The kinetic parameters have been provided previously (2, 7, 12). The adjustable parameters for steady-state simulation of each experimental condition were the tissue \( Po_2 \) and tissue \( PCO_2 \) needed to match the measured \( O_2 \) consumption (\( V_{O2} \)) and \( V_{CO2} \) as well as the measured \( Pa_{O2} \), arterial \( Po_2 \) (\( Pa_{AO2} \)), arterial pH (\( pH_A \)), \( P_{ACO2} \), central mixed venous \( Po_2 \) (\( P_{VM} \)), and central mixed venous pH (\( pH_V \)).

For control calculations, CA activity was assumed to be available to the blood plasma during capillary transit via CA associated with the capillary endothelium. This was incorporated in our model calculations using an \( Ao \) of 100 on the basis of our previous estimate of the extent of CA activity available on pulmonary capillary endothelium (8). It was assumed that a similar level of CA activity was available on the endothelial cells of tissue capillaries. In our control experiments we noted significant red cell hemolysis similar to that in previous studies (3, 13). On the basis of an average plasma hemoglobin concentration of 5 mg/100 ml, we used an \( Ao \) of 2 for plasma \( CO_2\)-\( HCO_3^- \)-\( H^+ \) reactions in postcapillary blood under control conditions. As in our previous work (2, 7), the catalysis factor for red cell \( CO_2\)-\( HCO_3^- \)-\( H^+ \) reactions (\( A_r \)) was taken as 6,500. For computations corresponding to 60 min after the administration of 5 mg/kg Actz, we assumed complete inhibition of capillary endothelial and free CA activity in plasma (\( Ao = 1 \)) and partial (98.3%) inhibition of red cell CA activity (\( A_r = 110 \)) (3). For computations corresponding to 60 min after the administration of 100 mg/kg Actz, we assumed near-complete (99.98%) inhibition (3) of red cell CA activity (\( A_r = 2.3 \)) as well as complete inhibition of CA activity associated with capillary endothelium and that in plasma due to red cell hemolysis (\( Ao = 1 \)). The sensitivity of the model computations to the selected values of \( A_r \) was evaluated (see below).
We assumed a capillary transit time of 1 s for the pulmonary capillaries and 2 s for the tissue capillaries. An overall transit time of 40 s for the entire circulatory loop was assumed, partitioned as 14 s for lung-to-tissue transit and 23 s for tissue-to-lung transit. The sensitivity of model computations to the assumed values of capillary transit times also was evaluated (see below).

**RESULTS**

A summary of the experimental data on the quasi-steady-state mixed venous and arterial blood gas parameters for all experimental groups is provided in Table 1. Measurements of hematocrit, \(V_I\), \(ET_{CO_2}\), and cardiac output as well as the measured values for \(VO_2\) and \(VCO_2\) are provided in Table 2. Pulmonary arterial pressures were not monitored continuously. Intermittent measurements of pulmonary arterial systolic pressure were 18–24 mmHg, and pulmonary arterial diastolic pressure ranged from 12 to 16 mmHg. The pulmonary capillary wedge pressure, also sampled intermittently, was 9–13 mmHg.

The effect of progressive CA inhibition on \(VCO_2\) is shown in Fig. 1A, with \(VCO_2\) expressed as percentage of baseline value (i.e., time 0) and the line fitted by eye. After 5 mg/kg Actz, there was a slow monotonic decrement in \(VCO_2\) to 90% of control, with no significant recovery over 1 h. After an additional 15 mg/kg Actz, \(VCO_2\) fell rapidly to 80% of baseline and recovered to 85% over 1 h. Subsequent administration of 80 mg/kg Actz caused a fall in \(VCO_2\) to 75% of baseline, with a gradual return to 80% of control over 1 h. Thus, for all levels of CA inhibition, there was significant decrement in \(VCO_2\) followed by incomplete recovery over 1 h. This also is reflected by the significant reduction in the respiratory exchange ratio (R; Table 2). The time course of changes in \(ET_{CO_2}\) (Fig. 1B) roughly paralleled the changes in \(VCO_2\). Complete inhibition of extracellular CA (5 mg/kg Actz) resulted in a fall of \(ET_{CO_2}\) to 82% of baseline (t = 5 min), with minimal recovery to 85% over 1 h. Moderate inhibition of red cell CA (20 mg/kg Actz) resulted in a further decline of \(ET_{CO_2}\) to 63% of control, with a t of <1 min, followed by recovery to 78% of control with t of 10 min. “Complete” inhibition of all CA activity (100 mg/kg Actz) resulted in a fall in \(ET_{CO_2}\) to 64% of control, with a t of 1 min, followed by recovery to 73% of baseline. A few experiments were performed with the slowly permeable CA inhibitor benzoalidamide. \(VCO_2\) fell an average of 10% from control values at 10 min after 2 mg/kg benzolalidamide (n = 3).

Figure 2A shows the measured time course of changes in \(\Delta (V-A)PCO_2\), where \(ET_{CO_2}\) was used to approximate \(PA_{CO_2}\). A stepwise increase in \(\Delta (V-A)PCO_2\) to the effective driving force for \(VCO_2\), was noted for each level of CA inhibition. The average \(\Delta (V-A)PCO_2\) increased from ~4 Torr at time 0 to 9 Torr (5 mg/kg Actz), 22 Torr (20 mg/kg Actz), and 27 Torr (100 mg/kg Actz). The corresponding time course of changes in the arterial-alveolar \(PCO_2\) gradient \(\Delta (A-a)PCO_2\) is shown in Fig. 2B. Because of the \(CO_2\)-H\(_2\)O-H\(^+\) disequilibrium associated with progressive CA inhibition, there is a progressive widening of \(\Delta (A-a)PCO_2\) from 2 Torr at baseline to 20 Torr at 60 min after a cumulative dose of 100 mg/kg Actz.

\(V_I\) was maintained constant throughout the duration of each experiment (Table 2). Measured blood hematocrit declined somewhat because of blood sampling over the duration of the experiments, more in certain animals than in others. No red cell transfusions were given. Cardiac output remained stable at 4 l/min, except in the last 60-min experimental period, when it declined ~15–20%. Indirect evidence for a slight worsening of ventilation-perfusion mismatch with progressive CA inhibition can be deduced from the estimated alveolar-arterial \(PCO_2\) gradient \(\Delta (A-a)PCO_2\) using the simplified alveolar gas equation and the measured R values (Table 2), with \(ET_{CO_2}\) used in place of \(PA_{CO_2}\). \(\Delta (A-a)PCO_2\) increased from a control value of 6.3 Torr to 8.9 Torr for 5 mg/kg Actz and to 10.5 Torr at 100 mg/kg Actz.

Figures 3–5 show the steady-state computed time courses of blood \(PCO_2\), \(CO_2\) content ([\(CO_2\)]), and plasma pH as blood travels around the entire (“closed-loop”)
circulation for a representative animal under control conditions, 60 min after 5 mg/kg Actz, and 60 min after 100 mg/kg Actz. Computed results for the case of 20 mg/kg Actz were intermediate between those for 5 and 100 mg/kg and are not shown. For the control condition (Fig. 3) before the administration of any Actz, as blood arrives in the pulmonary capillaries, blood PCO₂ falls almost instantaneously from that in the central mixed venous blood (37 Torr) to that in the alveolar gas (33 Torr) and remains at that level during pulmonary capillary transit. Associated with this very rapid fall in blood PCO₂ is a rapid fall in [CO₂] as blood enters the pulmonary capillaries. For the remainder of pulmonary capillary transit, there is a small fall in [CO₂] due to the continued dehydration of plasma and red cell HCO₃⁻ to molecular CO₂. Because sufficient CA activity is available on the pulmonary capillary endothelium (A₀ = 100) and because of the low non-HCO₃⁻ buffer capacity (~6 mM/pH unit), plasma pH rises from 7.39 in central mixed venous blood to 7.43 early during pulmonary capillary transit. By the time blood leaves the pulmonary capillaries, there is net depletion of plasma H⁺ concentration ([H⁺]) relative to that in the red blood cell. This disequilibrium of H⁺ across the red cell membrane is dissipated in postcapillary blood via the Jacobs-Stewart cycle (5, 18), wherein the “excess” red cell H⁺ combine with intraerythrocytic HCO₃⁻ to generate molecular CO₂, which diffuses into plasma and is dehydrated there to generate H⁺ and HCO₃⁻. Simultaneously, the newly generated plasma HCO₃⁻ is transported into the red blood cell in exchange for Cl⁻ by band 3-mediated anion exchange. As a result, plasma pH falls by 0.01 unit (in postcapillary blood) and blood Pco₂ rises very slightly. The time course of these internal changes in blood is determined by the CA activity available to postcapillary blood (via that due to red cell hemolysis, A₀ = 2) and the rate of the red cell HCO₃⁻/Cl⁻ exchange. For the control condition, the tᵢ is ~1 s. Analogous changes, but opposite in direction, occur in the tissue capillaries. Blood Pco₂ falls and plasma pH rises in the posttissue capillaries (Fig. 3).

For the steady-state condition after 5 mg/kg Actz (Fig. 4), central mixed venous blood (not internally at equilibrium) arrives at the lung with a Pco₂ of 37 Torr
and, soon after entering the pulmonary capillaries, falls to the alveolar level of 27 Torr and remains at that level during pulmonary capillary transit. An initial rapid decrement in $[\text{CO}_2]$ is associated with the fall in blood PCO$_2$. For the remainder of the pulmonary capillary transit, there is a small fall in $[\text{CO}_2]$ due to the continued slow dehydration of plasma and red cell HCO$_3^-$ to molecular CO$_2$. Plasma pH rises from 7.365 in central mixed venous blood to 7.370 during pulmonary capillary transit. Because no CA activity is available to capillary blood plasma under these conditions (complete inhibition of endothelium-associated and free CA activity in plasma, $A_o = 1$), as blood leaves the pulmonary capillaries, plasma HCO$_3^-$ concentration ($[\text{HCO}_3^-] \times [\text{H}^+]/[\text{CO}_3^-]$, and consequently blood PCO$_2$ rises in postcapillary blood from the end-capillary (= alveolar) level of 27 to 32 Torr (Fig. 4). Additionally, by the time blood leaves the pulmonary capillaries, there is net depletion of red cell $[\text{H}^+]$ relative to that in the plasma. This disequilibrium of $\text{H}^+$ across the red cell membrane is dissipated in postcapillary blood via the Jacobs-Stewart cycle (5, 18), as described above. As a result, plasma pH rises by 0.01 unit (in postcapillary blood). However, before a new electrochemical equilib-


central mixed venous blood arrives at the lung under closed-loop conditions. For open-loop conditions where central mixed venous blood is followed to equilibrium, there is good agreement between the predicted and measured central mixed venous blood gas/pH parameters (measured $\text{PaO}_2 = 45.0 \text{Torr}$, $\text{PaCO}_2 = 38.4 \text{Torr}$, $\text{pH}_a = 7.34$; calculated $\text{PaO}_2 = 42.3 \text{Torr}$, $\text{PaCO}_2 = 37.2 \text{Torr}$, $\text{pH}_a = 7.36$).

For the steady-state condition after 100 mg/kg Actz (Fig. 5), central mixed venous blood (not internally at equilibrium) arrives at the lung with a $\text{PCO}_2$ of 47 Torr and, soon after entering the pulmonary capillaries, falls to the alveolar level of 22 Torr and remains at that level during pulmonary capillary transit. After the initial rapid decrement in blood $[\text{CO}_2]$ associated with the fall in blood $\text{PCO}_2$, there is minimal change for the remainder of the pulmonary capillary transit due to the very slow dehydration of plasma and red cell $\text{HCO}_3^-$ to molecular $\text{CO}_2$. Plasma pH rises minimally from 7.25 in central mixed venous blood to 7.26 during pulmonary capillary transit. Because no CA activity is available to capillary blood plasma and very minimal residual activity is present in the red blood cells ($A_i = 2.3$), as blood leaves the pulmonary capillaries, plasma $[\text{HCO}_3^-] \times [\text{H}^+] \Rightarrow [\text{CO}_2]$, and consequently blood $\text{PCO}_2$ rises slowly in postcapillary blood from the end-capillary (= alveolar) level of 23 to 39 Torr during lung-to-tissue transit. Associated with this rise in blood $\text{PCO}_2$, there is a slow rise in plasma pH from 7.27 to 7.35 in postcapillary blood. Additionally, by the time blood leaves the pulmonary capillaries, there is net depletion of plasma $[\text{H}^+]$ relative to that in the red blood cell. This disequilibrium of $\text{H}^+$ across the red cell membrane would be dissipated in postcapillary blood via the Jacob-Stewart cycle and ensuing plasma $\text{CO}_2$-$\text{HCO}_3^-$-$\text{H}^+$ reactions (5, 18), as described above. However, because of the longer time required for this $\text{H}^+$ equilibrium ($t = 15 \text{ s}$) and the limited lung-to-tissue transit time ($14 \text{ s}$), these changes do not take place and blood arriving at the tissue capillaries is in a state of significant electrochemical disequilibrium. Analogous changes, but opposite in direction, occur in the tissue capillaries (Fig. 5). Blood $\text{PCO}_2$ rises rapidly to the tissue level (60 Torr) and falls in posttissue capillaries. Because of the longer tissue-to-lung transit time, the predicted biphasic changes in plasma pH are manifested. A fall in plasma pH due to the $\text{CO}_2$ hydration-dehydration equilibration in plasma and red blood cells occurs first, and subsequently the initial phase of $\text{H}^+$ equilibration across the red cell membrane via the Jacob-Stewart cycle (18) results in a slow rise in plasma pH. Despite the somewhat longer tissue-to-lung transit time, electrochemical equilibrium is not established as central mixed venous blood arrives at the lung. For open-loop conditions where arterial and central mixed venous blood are followed to equilibrium, there is good agreement between the predicted and measured arterial and mixed venous blood gas/pH parameters (measured $\text{PaO}_2 = 112.8 \text{Torr}$, $\text{PaCO}_2 = 38.9 \text{Torr}$, $\text{pH}_a = 7.29$, $\text{PaO}_2 = 45.2 \text{Torr}$, $\text{PaCO}_2 = 46.3 \text{Torr}$, $\text{pH}_a = 7.27$; cal-

Fig. 5. Computed steady-state time course of blood $\text{PCO}_2$ (A), $[\text{CO}_2]$ (B), and $\text{pH}$ (C) as blood travels around entire circulation for a typical animal given 100 mg/kg (cumulative) Actz. Input parameters for model calculations are as follows: hematocrit = 31%, cardiac output = 3.0 l/min, $\text{V}^\dot{O}_2 = 22.8 \text{Torr}$, $\text{PAO}_2 = 110.0 \text{Torr}$, tissue $\text{PaCO}_2 = 60.0 \text{Torr}$, tissue $\text{VO}_2 = 46.3 \text{Torr}$, $\text{A}_i = 2.3$, $\text{A}_o = 1$ for blood in capillary bed and for postcapillary blood. Measured values for this simulation are as follows: $\text{V}^\dot{CO}_2 = 72.0 \text{ml/min}$, $\text{VO}_2 = 104.0 \text{ml/min}$, $\text{PaO}_2 = 112.8 \text{Torr}$, $\text{PaCO}_2 = 38.9 \text{Torr}$, $\text{pH}_a = 7.29$, $\text{PaO}_2 = 45.2 \text{Torr}$, $\text{PaCO}_2 = 46.3 \text{Torr}$, $\text{pH}_a = 7.27$. Model-computed values are as follows: $\text{V}^\dot{CO}_2 = 67.0 \text{ml/min}$, $\text{VO}_2 = 94.0 \text{ml/min}$, $\text{PaO}_2 = 113.6 \text{Torr}$, $\text{PaCO}_2 = 42.0 \text{Torr}$, $\text{pH}_a = 7.29$, $\text{PaO}_2 = 46.2 \text{Torr}$, $\text{PaCO}_2 = 45.6 \text{Torr}$, $\text{pH}_a = 7.27$. 

rrium can be established between plasma and red blood cells, blood arrives at the tissue and is in a state of electrochemical disequilibrium (Fig. 4). For open-loop conditions where arterial blood is followed to equilibrium, there is good agreement between the predicted and measured arterial blood-gas/pH parameters (measured $\text{PaO}_2 = 94.0 \text{Torr}$, $\text{PaCO}_2 = 33.3 \text{Torr}$, $\text{pH}_a = 7.37$; calculated $\text{PaO}_2 = 100.7 \text{Torr}$, $\text{PaCO}_2 = 32.2 \text{Torr}$, $\text{pH}_a = 7.39$). Analogous changes, but opposite in direction, occur in the tissue capillaries and are shown in Fig. 4. Blood $\text{PCO}_2$ and plasma pH fall in the posttissue capillaries, but because of the limited tissue-to-lung transit time, electrochemical equilibrium is not established as
culated $P_{O_2} = 113.6$ Torr, $P_{CO_2} = 42.0$ Torr, $pH_a = 7.29$, $P_{VCO_2} = 46.2$ Torr, $P_{VCO_2} = 45.6$ Torr, $pH_t = 7.27$).

No significant changes in $V_{CO_2}$, $V_{O_2}$, or arterial and mixed venous blood-gas parameters were noted in the one animal that was given a sham infusion of alkalinized saline with pH similar to the Actz solution (pH 9.5) under conditions of no CA inhibition and complete (100 mg/kg Actz) inhibition (data not shown).

**DISCUSSION**

On the basis of our computations and the experimental results, the following conclusions can be made. 1) In the face of fixed $V_I$, compensatory recovery of $V_{CO_2}$ after CA inhibition occurs primarily by an increase in the $\Delta(p-H\alpha)PCO_2$ with minimal changes in cardiac output. 2) During significant CA inhibition, in vivo $P_{CO_2}$ is overestimated and in vivo $P_{VCO_2}$ is underestimated by use of in vitro equilibrated blood measurements. 3) Even in the presence of plasma CA activity, the instantaneous relationship between blood ($CO_2$) and $PCO_2$ is different for $CO_2$ uptake in tissues vs. that for $V_{CO_2}$ in the lung. This results in a hysteresis loop. 4) The shape of the in vivo blood $PCO_2$-$pH$ relationship is complex and depends on the CA activity available to plasma and in red blood cells. Under control conditions, CA activity is available to plasma during capillary transit (via that associated with capillary endothelium) and to plasma in postcapillary blood (via that released in plasma from hemolysis of red blood cells), and a great excess of endogenous CA activity exists in the red blood cells. After administration of 5 mg/kg Actz, CA activity on the capillary endothelium, as well as that in plasma due to red cell hemolysis, is inhibited ($A_o = 1$). Despite substantial inhibition of red cell CA with this dose of Actz (3), there is significant residual CA activity ($A_i = 110$). Sixty minutes after the administration of 100 mg/kg Actz, there is essentially complete (99.98%) inhibition of red cell CA ($A_i = 2.3$).

Quantitative comparison of previous model predictions and present results. Previous calculations (2) utilized the concept of a transfer capacity for $CO_2$, i.e., $T_{CO_2} = V_{CO_2}/\Delta(p-H\alpha)PCO_2$, to estimate the efficiency of $V_{CO_2}$. At rest, it was estimated that $T_{CO_2}$ in normal humans is 25.2 ml $CO_2$·min$^{-1}$·Torr$^{-1}$ when capillary endothelium-associated CA activity is intact but no CA is available in postcapillary plasma. In the absence of CA inhibition, we estimate a $T_{CO_2}$ of 27.2 ml $CO_2$·min$^{-1}$·Torr$^{-1}$ on the basis of our present results. However, the transfer coefficient $K_{CO_2} (= T_{CO_2}/Q$, where $Q$ is blood flow rate) in the present study of 6.8 ml $CO_2$·Torr$^{-1}$·l$^{-1}$ is significantly higher than that predicted previously ($K_{CO_2} = 4.6$ ml $CO_2$·Torr$^{-1}$·l$^{-1}$) (2). We ascribe this difference to the extra CA activity available in plasma due to red cell hemolysis, which was not considered in the previous calculations (2). When red cell CA activity is intact but endothelial CA activity is inhibited, it was predicted that $K_{CO_2} = 4.1$ ml $CO_2$·Torr$^{-1}$·l$^{-1}$ (2), which is considerably higher than the value obtained by us in this study of 2.5 ml $CO_2$·Torr$^{-1}$·l$^{-1}$ after 5 mg/kg Actz (Tables 1 and 2). We ascribe this difference to the significant red cell CA inhibition associated with 5 mg/kg Actz (98.5%). For the case where all CA activity is inhibited, our estimate of $K_{CO_2}$ (0.94 ml $CO_2$·Torr$^{-1}$·l$^{-1}$) is comparable to that predicted earlier (0.8 ml $CO_2$·Torr$^{-1}$·l$^{-1}$) (2). The slightly higher value in our present study is likely due to the lower hematocrit (~45%; Table 2) than in previous calculations (45%) (2).

On the basis of previous estimates of $K_{CO_2}$ and $T_{CO_2}$ (2), it was predicted that, in the absence of all CA activity, preservation of steady-state $V_{CO_2}$ would require an increment in the $\Delta(p-H\alpha)PCO_2$ from 6 to 39 Torr (i.e., ~550% increment). Our present study indicates that the average $\Delta(p-H\alpha)PCO_2$ increased from 3.6 to 26.8 Torr (~640% increment). We ascribe the slightly higher $\Delta(p-H\alpha)PCO_2$ in our present study to the lower hematocrit than that used in the previous calculations (2).

In vitro $CO_2$ dissociation curves vs. in vivo relationships. The instantaneous (dynamic) in vivo blood $[CO_2]$-$PCO_2$ relationship differs significantly from the "equilibrium" or "static" $CO_2$ dissociation curve and forms hysteresis loops around the in vitro linearized $CO_2$ dissociation curve. The width of these hysteresis loops is dependent on the available CA activity (Fig. 6). During control conditions, as blood passes through the pulmonary capillaries, blood $PCO_2$ falls very rapidly relative to the total change in blood $[CO_2]$. Later in the
capillary, blood $P_{CO_2}$ changes very little while blood [CO$_2$] continues to change slowly because of ongoing mobilization of plasma HCO$_3^-$ via anion exchange across the red cell membrane and subsequent HCO$_3^-$ dehydration within the red blood cell. In postpulmonary capillaries, total blood [CO$_2$] remains constant while internal electrochemical equilibrium between $P_{CO_2}$, [HCO$_3^-$], and [H$^+$] is established. Even when CA activity is available in plasma and via capillary endothelium, a hysteresis loop is present (Fig. 6A), rather than a symmetrical change in blood [CO$_2$] and $P_{CO_2}$ along the in vitro line, equilibrated central venous blood (VBG) = equilibrated arterial blood (ABG) (dashed line in Fig. 6). These differences become significantly larger as CA activity is progressively inhibited. For 5 mg/kg Actz, the in vitro line, VBG = ABG, has a much higher slope ("capacitance coefficient") than the in vivo relationship across the lung (mv → a), where mv is mixed venous blood entering the pulmonary capillaries and a is blood leaving the pulmonary capillaries) or that across the tissues (at → vt, where at is arterial blood entering the tissue capillaries and vt is venous blood leaving the tissue capillaries). The largest differences occur when CA activity is completely inhibited. The hysteresis loop is much wider and the in vitro capacitance coefficient is much higher than the instantaneous dynamic in vivo capacitance coefficient.

In vitro vs. in vivo acid-base relationships. As a consequence of CO$_2$-HCO$_3^-$-H$^+$ disequilibrium, the in vivo plasma pH-blood $P_{CO_2}$ relationship is nonlinear and is manifested as a hysteresis loop around the in vitro $P_{CO_2}$-pH relationships across the lung (mv → a) and that across the tissues (at → vt; Fig. 7). The two in vitro lines are parallel but slightly displaced.

A useful way to examine in vivo pH-$P_{CO_2}$ relationships during CA inhibition is via use of the buffer line on a plot of log($P_{CO_2}$) vs. pH, popularized by Siggard-Andersen (24). The slope of the buffer line on such a plot provides important information about the buffering power of the solution ($\beta$). A slope of -1 signifies a constant [HCO$_3^-$] and thereby minimal buffering power. On the other hand, a slope of $\infty$ represents a perfect buffer. Blood yields a buffer line with slope intermediate between these two extremes. For example, for values approximating that for a normal human ($P_{CO_2 \text{ABG}} = 46.0$ Torr, $pH_a = 7.37$, $P_{CO_2 \text{mv}} = 40.0$ Torr, $pH_a = 7.40$), a line with slope of -2 is obtained. The points on this line are based on in vitro measurements and represent equilibrium (or static) conditions. It is useful to compare this relationship with the in vivo dynamic acid-base relationship. This is akin to the approach in respiratory mechanics wherein lung volume vs. transpulmonary pressure is approximated as linear, with the slope representing static lung compliance. During tidal breathing, the volume-pressure relationship forms a loop around the static line. The width of the hysteresis loop represents the dynamic compliance, and the difference between the static and dynamic curves is a measure of airway resistance.

We have utilized this approach to examine the effects of CA inhibition on the in vitro vs. in vivo pH-$P_{CO_2}$ relationships. These computed results are shown in Fig. 7. For control conditions (Fig. 7A), the in vitro buffer line (ABG = VBG) has a slope of $\beta = -1.8$. This can be compared with the in vivo "linearized" pH-$P_{CO_2}$ line for tissue CO$_2$ uptake ($\beta_{at\rightarrow vt}$) of -1.7 and for pulmonary VCO$_2$ ($\beta_{mv\rightarrow a}$) of -1.6. Thus, for control conditions, despite very similar values for the linearized in vivo slopes for CO$_2$ uptake and VCO$_2$ and that based on in vitro equilibrated values, there is still a hysteresis loop around the in vitro buffer line. For the quasi-steady-state condition after 5 mg/kg Actz, the computed in vitro and in vivo $P_{CO_2}$-pH relationships are shown in Fig. 7B. There is a large discrepancy between the slope of the in vitro buffer line and the in vivo path of blood $P_{CO_2}$-pH as blood travels around the closed-loop circulation. The computed in vitro $\beta$ ($\beta_{\text{ABG}=\text{VBG}} = -1.7$) is very similar to that for control conditions but is substantially different from the linearized slopes of the in vivo pH-$P_{CO_2}$ lines ($\beta_{mv\rightarrow a} = -24.0$ and $\beta_{at\rightarrow vt} = -12.6$). There is a significant difference between the in vivo $\beta$ for VCO$_2$ and CO$_2$ uptake because of differences in
the pulmonary and systemic capillary transit times. For the quasi-steady-state condition after 100 mg/kg Actz, the computed in vitro and in vivo Pco$_2$-pH relationship is shown in Fig. 7C. The hysteresis loop is much broader. Again, the computed values of the in vitro buffer line ($\beta_{ABG-VBG} = -2.0$) is similar to that for control conditions but is substantially different from the linearized in vivo slopes of the pH-Pco$_2$ lines for Vco$_2$ and CO$_2$ uptake ($\beta_{V-t} = -19.4$ and $\beta_{V-t} = -6.9$). Thus, as for 5 mg/kg Actz, there is a major difference between the slopes of the in vitro and in vivo pH-Pco$_2$ relationships. It is reasonable to conclude from these calculations that the in vitro buffer line is not useful in deciphering the in vivo dynamic instantaneous acid-base relationships, just as static compliance is not helpful in estimating dynamic compliance.

Sensitivity of model predictions to input parameters. In our mathematical simulation we assumed that 5 mg/kg Actz resulted in 98.3% inhibition of red cell CA activity. Thus an $A_i$ of 110 was chosen for these conditions on the basis of an acceleration factor of 6,500 for "normal" red cell CA activity (2, 7). Computations using $A_i$ of 33, which corresponds to 99.5% inhibition of red cell CA, are shown in Fig. 8 and compared with those using the nominal value of $A_i$ of 110. Although the qualitative trends are preserved, a higher tissue Pco$_2$ of 53.2 Torr (compared with a tissue Pco$_2$ of 39.8 Torr used when $A_i = 110$) is required to ensure adequate CO$_2$ uptake during tissue capillary transit. Additionally, there is a significant discrepancy between the measured and calculated PaCO$_2$ (measured = 40.0 Torr and calculated = 43.2 Torr) and PVCO$_2$ (measured = 38.4 Torr, calculated = 42.3 Torr).

Fig. 8. Computed hysteresis loops of instantaneous blood Pco$_2$ vs. pH$_a$ as blood recirculates between lungs and tissues corresponding to 5 mg/kg Actz and with $A_i = 110$ ("nominal value" corresponding to 99.5% inhibition of red cell CA activity) or $A_i = 33$ (corresponding to 99.95% inhibition of red cell CA activity). L, T, a, at, vt, mv, ABG, and VBG have same meaning as in Fig. 6 and correspond to results with $A_i = 110$ (same as Fig. 7B). L’, T’, (a’), (at’), (vt’), (mv’), (ABG’), and (VBG’) represent analogous values for results obtained using $A_i = 33$. See Fig. 4 legend for values of input parameters, measured and calculated Vco$_2$ and Vo$_2$, and measured and calculated equilibrated arterial and mixed venous blood gas/pH parameters for $A_i = 110$. For $A_i = 33$, values are as follows: PVco$_2 = 26.6$ Torr, PaO$_2 = 100.0$ Torr, tissue Pco$_2 = 53.2$ Torr, tissue Po$_2 = 42.0$ Torr. Measured values for this simulation are as follows: Vco$_2 = 92.0$ ml/min, Vo$_2 = 119.0$ ml/min, PaO$_2 = 94.0$ Torr, PaCO$_2 = 33.3$ Torr, pH$_a = 7.37$, PV$_O_2 = 45.0$ Torr, PVCO$_2 = 38.4$ Torr, pH$_a = 7.34$. Model-computed values for $A_i = 110$ are as follows: Vco$_2 = 22.8$ Torr, PaO$_2 = 110.0$ Torr, tissue Pco$_2 = 62.3$ Torr, tissue Po$_2 = 45.3$ Torr. Measured values for this simulation are as follows: Vco$_2 = 72.0$ ml/min, Vo$_2 = 104.0$ ml/min, PaO$_2 = 112.8$ Torr, PaCO$_2 = 38.9$ Torr, pH$_a = 7.29$, PV$_O_2 = 45.2$ Torr, PVCO$_2 = 46.3$ Torr, pH$_a = 7.27$. Model-computed values for $A_i = 1$ are as follows: Vco$_2 = 67.5$ ml/min, Vo$_2 = 95.0$ ml/min, PaO$_2 = 110.8$ Torr, PaCO$_2 = 44.6$ Torr, pH$_a = 7.26$, PV$_O_2 = 45.1$ Torr, PVCO$_2 = 48.5$ Torr, pH$_a = 7.24$.

For mathematical simulation of experiments with 100 mg/kg Actz, we utilized an $A_i$ of 2.3, which corresponds to a 99.95% inhibition of red cell CA activity. Computations using $A_i$ of 1, which correspond to complete red cell CA inhibition, are shown in Fig. 9. The predicted Pco$_2$-pH relationship shares many qualitative features with the nominal calculations; however, a slightly higher tissue Pco$_2$ of 62.3 Torr (compared with a tissue Pco$_2$ of 60.0 Torr) is required to match the measured Vco$_2$ for this experiment. Furthermore, this result in a greater disparity between the measured and calculated Pco$_2$ (measured = 38.9 Torr, calculated = 44.6 Torr), pH$_a$ (measured = 7.29, calculated = 7.26), PV$_O_2$ (measured = 46.3 Torr, calculated = 48.5 Torr), and pH$_a$ (measured = 7.27, calculated = 7.24).

Our assumed values for the pulmonary capillary and tissue capillary transit times of 1 and 2 s, respectively, are based on previous estimates (2, 7). Calculations using much smaller capillary transit times (0.5 s for pulmonary capillary transit and 1 s for tissue capillary transit) result in a decreased efficiency of Vco$_2$ in the lung and CO$_2$ uptake in tissues (results not shown). As a consequence, for example, with 5 mg/kg Actz, tissue Pco$_2$ is predicted to be significantly higher to match the measured Vco$_2$. The time course of computed changes in blood-gas/pH parameters is quite similar to those shown in Fig. 8, where a greater degree of red cell CA inhibition was assumed than in the nominal calculations. Limitations of experimental methods and mathematical analyses. Quasi-steady-state Vco$_2$ at the end of the experimental period (generally 4 h), 60 min after a cumulative dose of 100 mg/kg Actz, was ~10–15% lower than at the initiation of the experiments (Table 2). This could be due to inadequate time to reach a true
steady state or a global reduction in metabolic CO₂ production. The latter could be due to pentobarbital anesthesia (3), a slight lowering of animal core temperature, or a depressive effect of systemic acidosis on glycolysis. Cardiac output generally remained constant throughout the experimental period, except during the last 60 min, when there tended to be a decrement of 10–15%.

We utilized ET CO₂ as a measure of PA CO₂ in these experiments. Although this is true for the "idealized uniform" lung, ventilation-perfusion inhomogeneities would cause a heterogeneity of PCO₂ within the lung, invalidating the equality between ET CO₂ and true "alveolar" PCO₂.

The mathematical model has many inherent assumptions, summarized in methods, that need to be examined. The assumption of a lumped alveolar compartment and idealized pulmonary capillary ignores regional ventilation-perfusion differences and the microvascular implications of two-phase flow (15). Increased ventilation-perfusion mismatch, secondary to Actz administration, as has been described by Swenson et al. (27), is ignored. Use of a lumped tissue compartment is a gross simplification, since there are significant differences in organ blood flow-to-metabolic rate ratios. Thus the use of a single lumped tissue compartment with a single tissue PCO₂ is clearly a major limitation of the model. During transient conditions of an imbalance between tissue CO₂ production and CO₂ removal by blood flow from tissues, metabolically produced CO₂ is stored extravascularly, predominantly as molecular CO₂ and HCO₃⁻. The rate of CO₂ dehydration to HCO₃⁻ is dependent on the available CA activity as well as the non-HCO₃⁻ buffer capacity, which is dependent on the local concentration of proteins and phosphate, among others. Within each tissue compartment, there are regional differences between the extracellular and intracellular compartments. There might exist specialized transport mechanisms for HCO₃⁻ and H⁺ influx or efflux at the cell membrane. Thus there are complex pathways and mechanisms within each tissue that determine the dynamic CO₂ capacitance. Our model does not include tissue CO₂ stores and is thus limited to simulation of steady states. The transient changes in CO₂ stores when CA activity is inhibited, as well as the time to reach a new steady state, cannot be estimated with our present model. We simply assume that 60 min is an adequate time period for a new steady state to be established after differential CA inhibition. Under these steady-state conditions, tissue PCO₂ is taken as a global driving force for CO₂ uptake by blood.

Use of time-variant lung-to-tissue and tissue-to-lung transit times is another major limitation of our current model. Regional changes in blood flow during graded CA inhibition are ignored. We have assumed that CA activity is present on the endothelium of all tissue capillaries, which is not likely to be true (14).

The mathematical computations presented are for a typical animal and are representative of different levels of CA inhibition. We have chosen to present computed results for experimental runs where the cardiac output remained constant to ease the comparison between animals with different levels of CA inhibition. Our control conditions do not entirely mimic the in vivo situation, where CA activity is available to blood on the vascular endothelial cells as blood travels through the pulmonary and systemic capillaries but no CA activity is available after blood leaves the capillary bed. Because of inherent red cell hemolysis associated with instrumentation and surgical manipulations, CA activity is available in postcapillary vessels in our control experiments. For the control animals, finite postcapillary pH and PCO₂ disequilibria are predicted that are rapidly dissipated in postcapillary vessels. The in vivo relationship of CO₂ content to blood PCO₂, as blood travels around the circulation, is nonlinear and forms a hysteresis loop. The width of the hysteresis loop reflects the extent of CO₂-HCO₃⁻-H⁺ disequilibrium as it exists in vivo. These predictions are consistent with our previous calculations that indicate a persistence of postcapillary pH disequilibria even when CA activity is available to the plasma. Do such pH disequilibria exist in vivo in humans? Because of the inherent experimental limitations of associated red cell hemolysis and the small magnitude of the predicted pH changes, there remains considerable disagreement about this phenomenon in the literature (5, 6, 21).

Use of 5 mg/kg Actz is not an adequate model to simulate selective inhibition of vascular endothelial CA activity. As noted above, a dose of 5 mg/kg Actz probably was associated with significant red cell CA inhibition. Thus the arterial and central venous blood-gas/pH values measured under these conditions do not accurately reflect those that might be expected under conditions of selective vascular CA inhibition. Our estimate of ~10% contribution of vascular endothelial CA to VCO₂ agrees with that observed with "selective" CA inhibition in our limited series of experiments with 2 mg/kg benzolamide. The lack of adequate blood-gas/pH data for these latter experiments prevents mathematical model verification of "minimal" red cell CA inhibition.

Summary. Progressive inhibition of vascular plus red cell CA activity was associated with stepwise increments in the equilibrated Δ(Θ-Λ)PCO₂. The maximum decrements in VCO₂ were 10% at 5 mg/kg Actz, 24% at 20 mg/kg Actz, and 26% at 300 mg/kg Actz, without full recovery of VCO₂ at 1 h postinfusion. Equilibrated PA CO₂ overestimated PA CO₂, and tissue PCO₂ was underestimated by the measured PV CO₂. Model computations, used to predict the kinetics of steady-state intravascular PCO₂ and pH equilibration as blood travels around the circulation during different levels of CA inhibition, indicate hysteresis loops of the instantaneous CO₂-HCO₃⁻-H⁺ relationship and in vivo blood PCO₂-pH relationship due to the finite reaction times for the CO₂-HCO₃⁻-H⁺ reactions. The shape of the hysteresis loops is affected by the extent of Actz inhibition of CA in red blood cells and plasma.

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