

# Effects of Inhibition of RBC $\text{HCO}_3^-/\text{Cl}^-$ Exchange on $\text{CO}_2$ Excretion and Downstream pH Disequilibrium in Isolated Rat Lungs

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**ABSTRACT** To determine the effects of the speed of the erythrocyte membrane chloride shift on pulmonary gas transfer,  $\text{CO}_2$  exchange and the kinetics of pH equilibration were measured with isolated rat lungs perfused with 20% suspensions of human erythrocytes. The lungs were ventilated with room air, and the inflowing perfusate blood gases were similar to those in mixed venous blood in vivo. All experiments were performed at 37°C. Rates of  $\text{CO}_2$  excretion were determined by measuring the fraction of  $\text{CO}_2$  in mixed expired gas in a steady state. The time-course of extracellular pH equilibration in the effluent perfusate was measured in a downstream stopflow pH electrode apparatus.  $\text{CO}_2$  excretion was reduced by ~16% when the lungs were perfused with suspensions containing erythrocytes whose  $\text{HCO}_3^-/\text{Cl}^-$  exchange rate was inhibited, compared with  $\text{CO}_2$  excretion when the lungs were perfused with normal erythrocyte suspensions. A fall of 0.06 in effluent perfusate extracellular pH was noted during perfusion with inhibited erythrocyte suspensions, in contrast to no observable downstream pH change during perfusion with normal erythrocyte suspensions. These results are in close agreement with the predictions of a theoretical model. Our observations suggest that  $\text{CO}_2$  transfer in capillary beds will be adversely affected in vivo when the rate of the erythrocyte  $\text{HCO}_3^-/\text{Cl}^-$  exchange is abnormally low. Since a number of commonly used drugs are known to inhibit the chloride shift in human erythrocytes, these findings may have important clinical implications, especially in patients with impaired lung function.

## INTRODUCTION

A number of important kinetic events occur within blood during and after capillary  $\text{CO}_2$  transfer, one of which is  $\text{HCO}_3^-/\text{Cl}^-$  exchange across the erythrocyte

(RBC)<sup>1</sup> membrane (chloride shift). In the tissue capillaries, metabolically produced  $\text{CO}_2$  diffuses into RBC, where it is quickly converted to  $\text{H}_2\text{CO}_3$  under the influence of carbonic anhydrase (CA). The  $\text{H}_2\text{CO}_3$  instantaneously dissociates into  $\text{HCO}_3^-$  and  $\text{H}^+$ . The intracellular reaction ( $\text{CO}_2 \rightarrow \text{H}_2\text{CO}_3$ ) continues to be driven forward, in part owing to the very high buffering capacity (secondary to concentrated intracellular hemoglobin, [Hb]), which prevents  $\text{H}^+$  from accumulating inside the cell.  $\text{HCO}_3^-$  leaves the RBC down its electrochemical potential gradient in exchange for  $\text{Cl}^-$ , thereby promoting additional  $\text{CO}_2$  diffusion into the cell. The reverse sequence of processes takes place when  $\text{CO}_2$  is transferred from blood to alveolar gas in the pulmonary capillaries. These processes allow mobilization of the plasma  $\text{HCO}_3^-$  pool by giving it access to intracellular CA, permitting a larger fraction of total  $\text{CO}_2$  to be transferred from tissue to alveolar gas.

It has recently been shown that CA activity may be present on the endothelial surface of capillary beds, and thus readily accessible to the plasma (1-7). Even though the amount of vascular CA activity in any specific organ is not precisely known, it might appear at first glance that RBC  $\text{HCO}_3^-/\text{Cl}^-$  exchange is not necessary to ensure maximal  $\text{CO}_2$  transfer, since CA activity is present both inside and outside the RBC. It must be realized, however, that blood remains a two-compartment fluid despite vascular CA activity being accessible to plasma, since hemoglobin is confined to

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<sup>1</sup>Abbreviations used in this paper: ACTZ, acetazolamide; CA, carbonic anhydrase; DIDS, 4,4'-diisothiocyano-2,2'-disulfonic stilbene; KRB, Krebs-Ringer bicarbonate buffer; RBC, erythrocyte.

the interior of the RBC. The very high buffering capacity present inside the RBC relative to the plasma, and the intracellular Bohr shift, lead to a differential rate of reaction in the two compartments that necessitates the exchange of  $\text{HCO}_3^-$  and  $\text{Cl}^-$  across the RBC membrane for maximal  $\text{CO}_2$  transfer (see below).

Because the RBC membrane is highly permeable to  $\text{HCO}_3^-$ , the quantitative effects of the rate of the chloride shift on  $\text{CO}_2$  excretion in the lungs and  $\text{CO}_2$  uptake in the tissues has been largely ignored. Owing to the long RBC capillary transit time of 750 ms at rest compared to a  $t_{1/2}$  for  $\text{HCO}_3^-/\text{Cl}^-$  exchange at  $37^\circ\text{C}$  of  $\sim 100$  ms (8–10), it has usually been assumed that  $\text{HCO}_3^-/\text{Cl}^-$  exchange could not limit capillary  $\text{CO}_2$  transfer. If  $\text{HCO}_3^-/\text{Cl}^-$  exchange were significantly slowed (e.g., in abnormal RBC), it is likely that the exchange would become rate-limiting. It is already well known that a number of commonly used drugs can markedly inhibit RBC membrane anion transport (11–13), a finding that is not surprising in view of the fact that a specialized membrane protein-associated transport pathway for anion exchange is present in RBC (14, 15).

Recent theoretical investigations have shown that slowing of RBC  $\text{HCO}_3^-/\text{Cl}^-$  exchange rates can have a major influence on capillary  $\text{CO}_2$  transfer (16–18). It was predicted (18) that a decrease in the rate of the chloride shift by 90% leads to a decrease in  $\text{CO}_2$  elimination from blood in the lung by 30% for given values of mixed venous blood and alveolar gas tensions. It was also predicted (18) that slow downstream extracellular pH and  $\text{PCO}_2$  changes will occur within blood after gas exchange in the pulmonary capillaries even though a large amount of CA activity may be available to plasma in the capillaries.

In the present work, we perfused isolated rat lungs with RBC suspensions and measured  $\dot{V}\text{CO}_2$  and downstream extracellular pH ( $\text{pH}_o$ ) changes.  $\dot{V}\text{CO}_2$  is lower in lungs perfused with suspensions containing DIDS-treated (DIDS: 4,4'-diisothiocyano-2,2'-disulfonic stilbene) RBC (in which  $\text{HCO}_3^-/\text{Cl}^-$  exchange is inhibited), compared with  $\dot{V}\text{CO}_2$  with control RBC suspensions. Furthermore, large downstream  $\text{pH}_o$  changes are found in suspensions with DIDS-treated RBC, although none is found in control RBC suspensions. These findings imply that avoidance of inhibition of RBC  $\text{HCO}_3^-/\text{Cl}^-$  exchange may be of considerable importance for maintaining normal gas exchange *in vivo*.

## METHODS

**Isolated lung preparations.** Male Sprague-Dawley rats (250–360 g) were anesthetized with pentobarbital sodium (50 mg/kg *i.p.*). A tracheostomy was performed and the lungs were mechanically ventilated (model 680, Harvard Apparatus Co., Inc., S. Natick, Mass.) with room air at a tidal volume of 1.5

ml/stroke and a rate of 60 strokes/min. The lungs were periodically sighed. The abdomen and chest were opened, both ventricles of the heart were transected, and a cannula was inserted into the pulmonary artery through the right ventricle. Blood was then rapidly cleared from the pulmonary circulation by infusing a Krebs-Ringer bicarbonate buffer (KRB) solution ( $37^\circ\text{C}$ ) at 20 ml/min (pump model 7014, Cole-Parmer Instrument Co., Chicago, Ill.) through the pulmonary artery cannula. The lungs were removed from the thoracic cavity, a cannula placed in the left atrium, and the lungs hung in a water-jacketed chamber at  $37^\circ\text{C}$ . The left atrial cannula was connected to a T-tube, one arm of which was open to the atmosphere. The third arm of the T-tube was connected to a stop-flow pH electrode described in detail previously (1, 19). After the lung was hung ( $<20$  min after the start of surgery), perfusate was switched to a 20% human RBC suspension at the same flow rate of 20 ml/min. Perfusate was not recirculated. A schematic diagram of the experimental apparatus has been reported previously (1). Pressure transducers (P23, Statham Instruments, Inc., Oxnard, Calif.) were used to monitor tracheal airway pressure and pulmonary artery pressure. Fractional concentration of  $\text{CO}_2$  in mixed expired gas was measured on-line with a recording capnograph (type 17070, Godart-Statham, Bilthoven, Holland).

**Preparation of perfusion fluids.** Outdated human blood obtained from the UCLA blood bank was centrifuged (Sorvall RC2-B, Dupont Co., Instrument Products Div., Sorvall Biomedical Div., Claremont, Calif.) at 1,000 g for 10 min and the supernate (plasma and buffy coat) discarded. The RBC were suspended to 20% hematocrit in a saline solution (146.5 mM NaCl, 3.5 mM KCl), recentrifuged, and the supernate discarded. This washing procedure was repeated two more times. The RBC were then resuspended to 10% hematocrit in a solution of 146.5 mM NaCl, 20 mM Trizma buffer (Tris [hydroxymethyl] amino methane and hydrochloride) and 0.2% ethanol. This suspension was incubated for 10 min at  $37^\circ\text{C}$  with continuous shaking. After incubation, the suspension was recentrifuged and the supernate removed. The cells were then washed at 20% hematocrit, once in 146.5 mM NaCl plus 3.5 mM KCl, and twice in KRB solution (see below). After the last (sixth) wash, the cells were resuspended to 20% hematocrit in KRB solution. This suspension, henceforth called perfusate A, was subsequently maintained at  $37^\circ\text{C}$  in a water-jacketed glass reservoir.

Perfusate B was prepared by exactly the same procedure as that for perfusate A, except that the RBC were treated with the anion exchange inhibitor, DIDS, between the third and fourth washes. After the third wash, the cells were incubated at 10% hematocrit and  $37^\circ\text{C}$  for 10 min in 146.5 mM NaCl, 20 mM Trizma buffer, 0.2% ethanol, and 0.1 mM DIDS. The cells were then rewashed three times as described above, suspended to 20% hematocrit in KRB solution, and maintained at  $37^\circ\text{C}$  in a separate water-jacketed glass reservoir. Both perfusates A and B were gently bubbled with a water-saturated nominal 89%  $\text{N}_2$ , 5%  $\text{O}_2$ , and 6%  $\text{CO}_2$  gas mixture, until the blood gases of the two perfusates were  $\sim \text{pH}_o = 7.4$ ,  $\text{PO}_2 = 36$  torr, and  $\text{PCO}_2 = 36$  torr. The variability in the blood gases among experimental runs was small and random. Minimal foaming was encountered. The perfusates were subsequently maintained at these blood gases by passing the gas mixture over the surface of the suspensions. The suspensions were mechanically stirred throughout the experiments.

In four experiments, bovine CA (860 Wilbur-Anderson U/ml, Sigma Chemical Co., St. Louis, Mo.) was added to perfusate B. In three other experiments, 0.25 mg/ml of acetazolamide (ACTZ) (Lederle Laboratories, Div. American Cyanamid Co., Pearl River, N. Y.) was added to perfusate A

and/or perfusate B. The KRB solution consisted of 118.5 mM NaCl, 1.3 mM  $\text{CaCl}_2$ , 4.7 mM KCl, 1.2 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , and 22.2 mM  $\text{NaHCO}_3$ .

**Experimental procedure.** A typical experiment proceeded as follows. The lung was ventilated and perfused until a steady state was attained. At a given time thereafter, perfusate was withdrawn through the stop-flow electrode apparatus at 15 ml/min, care being taken that some flow always continued to exit from the open arm of the T-tube. Approximately 5 s elapse between the time blood leaves the pulmonary capillaries and the time it reaches the pH electrode (1). After 25–30 ml of perfusate had been withdrawn, the flow through the electrode was suddenly terminated. Temperature and  $\text{pH}_0$  of the suspension in the electrode chamber were monitored both before and after stopping flow, recorded on a storage oscilloscope screen (model 5103N, Tektronix, Inc., Beaverton, Oreg.), and photographed. Changes in pH were measurable to  $\pm 0.0005$  U and changes in temperature to  $\pm 0.05^\circ\text{C}$ . Shortly after each run, samples of inflowing and effluent perfusates were collected anaerobically for subsequent measurement of their equilibrated  $\text{pH}$ ,  $\text{PCO}_2$  and  $\text{PO}_2$  on a blood gas analyzer (model 168, Corning Medical, Corning Glass Works, Medfield, Mass.). During each run, mixed expired  $\text{CO}_2$  fraction, tracheal airway pressure, and pulmonary artery pressure were monitored.

In a typical series of experimental runs, an isolated lung was perfused first either with perfusate A or perfusate B. After a run was completed, the lung was perfused with the second perfusate (A or B) and the second run completed. These two runs constituted an experimental pair. This procedure was repeated 1–2 times for each lung. In four independent experiments, a run obtained with perfusate B was followed by a run with perfusate B to which CA had been added. In three other experiments, data obtained with perfusate B were compared with those obtained with perfusate B or perfusate A to which ACTZ had been added. All experiments were completed <55 min after starting surgery.

To determine whether the results of these experiments might be influenced by direct interactions of DIDS with lung tissue, three experiments were performed in which the lung was perfused with either KRB alone or KRB to which 0.1 mM DIDS was added. Downstream pH changes and  $\dot{V}\text{CO}_2$  were monitored. Furthermore, at the end of three experiments with cell suspension perfusates, the rate of  $\text{HCO}_3^-/\text{Cl}^-$  exchange across the DIDS-treated RBC membranes was compared with that for the control cells in a stop-flow rapid-reaction apparatus with the aid of an approach that has been well described previously (9, 15).

The effects of DIDS on CA activity was investigated to rule out enzyme inhibition as a factor in our experiments. We monitored the rate of the reaction of 20 mM  $\text{NaHCO}_3$  with 10 mM HCl in a stop-flow rapid reaction apparatus as described previously (20). The rate was measured in the presence of a given quantity of CA, with and without 0.1 mM DIDS added to the reaction mixture. Two experiments were performed with bovine CA and one experiment with human CA contained in the lysate from outdated normal human RBC. In addition, the rate of the test reaction above accelerated by human CA in lysate from normal RBC (without DIDS in the reaction mixture) was compared with that with the same quantity of human CA in lysate from DIDS-treated RBC.

Before each isolated lung experiment, the pH electrode and capnograph were calibrated according to known standards. The capnograph was calibrated for 0–2%  $\text{CO}_2$  full scale. The effluent extracellular fluid nonbicarbonate buffer capacity was determined automatically (TTT 60 Titrator with Autoburette ABU 13, Radiometer Co., Copenhagen,

Denmark). Hemoglobin concentration in the extracellular effluent fluid was measured spectrophotometrically (Coleman 124, Hitachi Perkin-Elmer Corp., Norwalk, Conn.) at 576 nm. Hematocrits were measured both before and during experimental runs for all RBC suspensions with standard Wintrobe tubes.

**Theoretical methods.** A theoretical model previously described for *in vivo* studies (18) was used to simulate several experimental runs from the present work. The input parameters to the model were the inflowing blood gases for typical pairs of experimental runs on the isolated lung. Alveolar gases were chosen as necessary to match the effluent blood gases and mixed expired  $\text{CO}_2$ . Other parameters used in the model have been given previously (18).

## RESULTS

Oscilloscope records from a typical isolated rat lung experiment are shown in Fig. 1. With perfusate A (Fig. 1a, control), there was no change in  $\text{pH}_0$  after perfusate left the lung. With perfusate B (Fig. 1b, DIDS-treated), however, a large slow  $\text{pH}_0$  fall ( $\sim 0.064$ ,  $t_{1/2} \sim 40$  s) was observed. In this particular experimental pair, the lung was perfused with perfusate B before perfusate A.  $\dot{V}\text{CO}_2$  for the runs shown in Fig. 1 were 1.09 ml BTPS/min for perfusate A and 0.97 ml BTPS/min for perfusate B.

A summary comparing data from experimental runs with perfusates A and B is given in Table I. In each lung, there was little or no change in  $\text{pH}_0$  of the effluent perfusate after stopping flow during perfusion with perfusate A, but a large fall in  $\text{pH}_0$  was always observed when perfusate B was used. The mean  $\Delta \text{pH}_0$  was 0.000 with perfusate A, and the mean  $\Delta \text{pH}_0$  was 0.057 ( $t_{1/2} = 44$  s) with perfusate B. Furthermore,  $\dot{V}\text{CO}_2$  averaged 16% lower when the lung was perfused with perfusate B than when it was perfused with perfusate A.

Fig. 2 shows  $\text{pH}_0$  time-courses in the effluent suspensions with perfusate B, and perfusate B with added CA, from the same lung. Both tracings reveal an almost identical large slow downstream fall in  $\text{pH}_0$ . For these experiments ( $n = 6$ ), the average  $\Delta \text{pH}_0$  was 0.071 ( $t_{1/2} = 40$  s) with perfusate B (Fig. 2a) and 0.072 ( $t_{1/2} = 39$  s) with perfusate B to which CA had been added (Fig. 2b).  $\dot{V}\text{CO}_2$  was 0.83 ml/min with both perfusate B and CA-containing perfusate B. None of these results differed significantly.

A  $\text{pH}_0$  time-course in effluent suspension with perfusate B is compared with those obtained with either perfusate B or perfusate A containing ACTZ in Fig. 3. These experiments ( $n = 3$ ) show a large slow fall in  $\Delta \text{pH}_0$  ( $\Delta \text{pH}_0 = 0.074$ ,  $t_{1/2} = 41$  s) with  $\dot{V}\text{CO}_2 = 0.89$  ml/min with perfusate B (Fig. 3a). When the lung was perfused with perfusate B or perfusate A, each containing ACTZ,  $\text{pH}_0$  time-courses after flow was stopped revealed biphasic behavior. In both curves, there was an initial rise in  $\text{pH}_0$ , followed by a large slow  $\text{pH}_0$

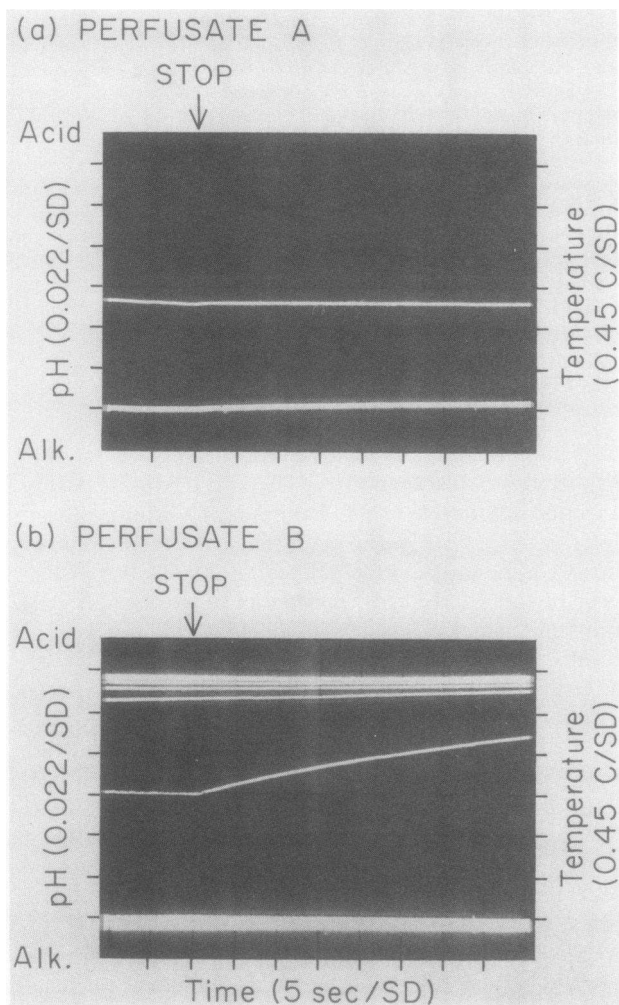


FIGURE 1 Oscilloscope records of effluent perfusate extracellular pH (upper traces) and temperature (lower traces) vs. time for lung 8, pair b. Perfusate was drawn through the electrode chamber at a constant rate of 15 ml/min until withdrawal was suddenly stopped (as indicated). Record (a) was obtained with perfusate A (control) and record (b) with perfusate B (DIDS-treated). Multiple sweeps across the oscilloscope screen are shown in record (b).

decrease. The portion of the biphasic curve during which  $pH_o$  is falling is much slower with perfusate B containing ACTZ ( $\Delta pH_o = 0.057$ ,  $t_{1/2} = 64$  s,  $\dot{V}CO_2 = 0.73$  ml/min) than that with perfusate A containing ACTZ ( $\Delta pH_o = 0.078$ ,  $t_{1/2} = 26$  s,  $\dot{V}CO_2 = 0.67$  ml/min).

Fig. 4 shows the predicted (solid curve) time-courses of extracellular pH, starting from the time perfusate enters the pulmonary capillaries in a typical experiment (Table I, lung 1, pair b). For comparison, the experimentally measured time-course of extracellular pH downstream from the lung is also indicated (dashed curve). These theoretical calculations use ex-

perimentally measured inflowing perfusate blood gas values and estimated alveolar gas tensions. No observable downstream  $pH_o$  change is predicted with perfusate A, whereas a large slow downstream decrease ( $\Delta pH_o = 0.080$ ,  $t_{1/2} = 45$  s) in effluent perfusate  $pH_o$  is predicted with perfusate B. These findings compare favorably with those measured experimentally ( $\Delta pH_o = 0.075$ ,  $t_{1/2} = 52$  s) for the runs with perfusate B. A decrement in  $\dot{V}CO_2$  of  $\sim 18\%$  with perfusate B relative to perfusate A is predicted, close to the 17.5% decrease in  $\dot{V}CO_2$  measured experimentally.

Theoretical (solid lines) and experimental (dashed lines) curves showing  $pH_o$  time-courses with perfusate B, perfusate B plus ACTZ, and perfusate A plus ACTZ, starting from the time the perfusate enters the pulmonary capillaries, are shown in Fig. 5. The experimental time-courses correspond to those shown in Fig. 3 and begin  $\sim 5$  s after the blood leaves the pulmonary capillaries. With perfusate B (top curve), extracellular pH is predicted to fall by  $\sim 0.080$  ( $t_{1/2} = 45$  s), which is in close agreement with the  $\Delta pH_o$  of 0.070 ( $t_{1/2} = 40$  s) measured experimentally. With perfusate B containing ACTZ (middle curve),  $pH_o$  is predicted to rise  $\sim 0.045$  over 9 s after leaving the lung, and then fall slowly by  $\sim 0.075$  ( $t_{1/2} = 52$  s). With perfusate A containing ACTZ (lower curve),  $pH_o$  is predicted to rise by  $\sim 0.075$  over 10 s after leaving the pulmonary capillaries, and then fall by  $\sim 0.080$  ( $t_{1/2} = 35$  s). The predictions for both suspensions containing ACTZ are in close agreement with experimentally measured changes in extracellular pH.

In the experiments that compared data obtained from the lungs perfused with KRB or KRB containing DIDS, no downstream pH change was found in either case, and no difference in  $\dot{V}CO_2$  was observed. The stop-flow rapid reaction experiments on the effluent RBC showed that the rate of  $HCO_3^-/Cl^-$  exchange for the DIDS-treated cells was only 2% (98% inhibition) that of the control cells. No difference was noted in the rates of the CA-accelerated test reaction (20 mM  $NaHCO_3$  plus 10 mM  $HCl$ ) in the presence and absence of 0.1 mM DIDS. Furthermore, there was also no difference observed in the rates of the test reaction accelerated with equal amounts of lysate from control RBC or from DIDS-treated RBC.

Peak tracheal airway pressure and mean pulmonary artery pressure during the perfusions were  $\sim 10$  and 12 cm  $H_2O$ , respectively. The hematocrits of the RBC suspensions taken both before and during the experimental runs were all found to be  $\sim 20\%$ . The mean concentration of hemoglobin in the extracellular fluid of the effluent perfusates was 70.87  $\mu M$  for perfusate A and 70.94  $\mu M$  for perfusate B. The extracellular non-bicarbonate buffer capacity was 1.29 mM  $H^+/pH$  for perfusate A and 0.87 mM  $H^+/pH$  for perfusate B.

TABLE I  
Summary of Stop Flow  $\Delta pH_o$  and  $\dot{V}CO_2$  Data from Isolated Rat Lungs

No.	Rat weight	Experimental pair	Perfusate A (control)			Perfusate B (DIDS-treated)		
			$\Delta pH_o$	$t_{1/2}$	$\dot{V}CO_2$	$\Delta pH_o$	$t_{1/2}$	$\dot{V}CO_2$
	g			s	ml BT/PS/min		s	ml BT/PS/min
1	359	a	0.001	2	0.69	0.077	50	0.67
		b	0.000	—	0.85	0.075	52	0.70
2	306	a	0.000	—	0.69	0.072	55	0.66
		b	0.004	3	0.71	0.066	52	0.63
3	251	a	0.000	—	0.98	0.057	49	0.85
		b	0.002	2	0.84	0.038	39	0.66
4	276	a	0.000	—	1.01	0.083	47	0.80
		b	0.000	—	0.52	0.026	39	0.39
5	276	a	0.000	—	1.21	0.073	42	0.91
6	304	a	0.000	—	0.62	0.040	45	0.55
7	348	a	0.000	—	1.09	0.063	41	0.80
		b	0.000	—	0.89	0.048	37	0.79
8	280	a	0.000	—	1.17	0.038	32	0.96
		b	0.000	—	1.09	0.064	40	0.97
		c	0.000	—	0.78	0.053	48	0.71
9	326	a	0.000	—	0.82	0.041	40	0.56
		b	0.000	—	0.84	0.056	51	0.78
10	303	a	0.000	—	0.92	0.049	32	0.85
		b	0.000	—	0.97	0.054	35	0.90
Mean*	303±11		0.000±0.000†	—	0.88±0.04§	0.057±0.004‡	44±1	0.74±0.04§

\* Mean±SEM.

† Significantly different ( $P < 0.0001$ ).

§ Significantly different ( $P < 0.0001$ ).

## DISCUSSION

The results shown in Fig. 1 and Table I reveal that a large slow downstream fall in  $pH_o$  occurs when isolated rat lungs are perfused with DIDS-treated RBC suspensions. This can be contrasted to no change in downstream  $pH_o$  when the same lungs are perfused with control RBC suspensions. Furthermore,  $CO_2$  transfer across the lung capillaries is significantly less using the DIDS-treated RBC suspensions. These findings are consistent with the data obtained from a theoretical model applied previously to an in vivo situation (18), in which a downstream pH disequilibrium and a decreased  $\dot{V}CO_2$  are predicted when RBC membrane  $HCO_3^-/Cl^-$  exchange rates are decreased below normal.

The mechanisms by which these observations occur are as follows. As the control RBC suspension passes through the pulmonary capillaries in our isolated rat

lungs, dehydration of  $H_2CO_3$  to  $CO_2$  catalyzed by CA occurs both extracellularly and intracellularly. This  $CO_2$  rapidly diffuses from the perfusate to the alveoli and is eliminated with expired gas. Extracellular CA activity is present owing both to hemolysis of perfusate RBC (estimated acceleration factor of 60), and to CA activity present in the pulmonary capillaries (1, 4, 7). Despite CA activity available both intra- and extracellularly, however, the dehydration reaction still is not able to proceed to the same extent in the two compartments while perfusate is in the capillary bed; rather, it goes farther inside the RBC. The reasons for this are twofold: (a) oxygenation of hemoglobin inside the RBC leads to production of Bohr protons, which drive the dehydration reaction; and (b) the lower buffer capacity in the extracellular fluid (1/60 that inside the RBC) allows a greater fall in extracellular  $[H^+]$ , which thereby slows the dehydration reaction. As a result of the difference between the rates

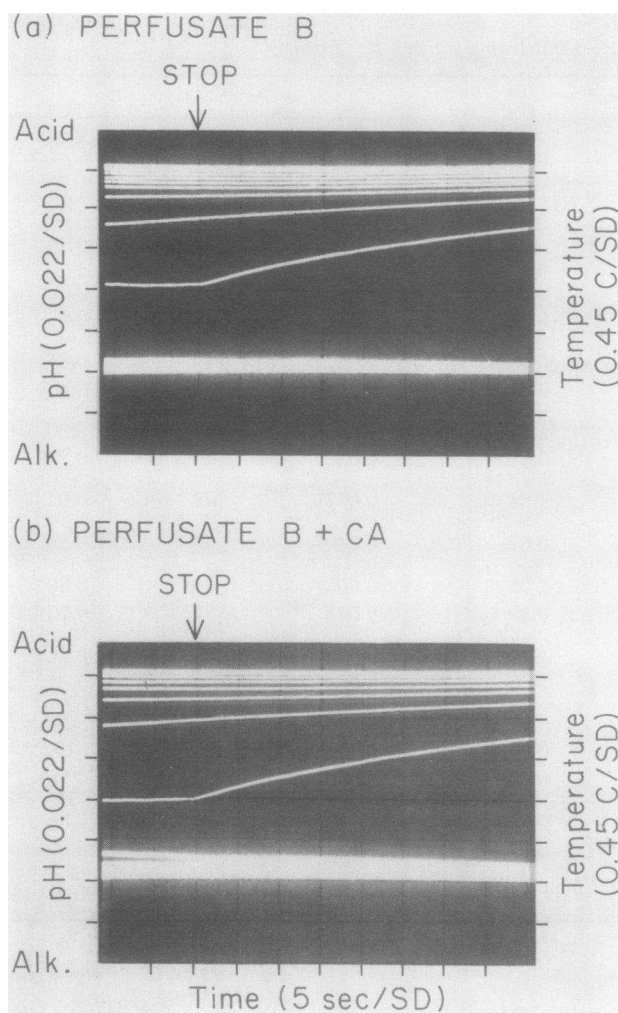


FIGURE 2 Oscilloscope records of effluent perfusate extracellular pH (upper traces) and temperature (lower traces) vs. time. Multiple sweeps across the oscilloscope screen are shown. Perfusate was drawn through the electrode chamber at a constant rate of 15 ml/min until withdrawal was suddenly stopped (as indicated). Record (a) was obtained with perfusate B (DIDS-treated) and record (b) with perfusate B containing added CA. The downstream total  $\Delta \text{pH}_0$  was 0.065 ( $t_{1/2} = 40$  s) for record (a), and 0.069 ( $t_{1/2} = 40$  s) for record (b). The mean expired  $\text{CO}_2$  ( $\dot{V}\text{CO}_2$ ) corresponding to both records was 0.85 ml/min.

of the dehydration reaction extra- and intracellularly,  $[\text{HCO}_3^-]$  inside the RBC falls farther than that outside, setting up a gradient that drives  $\text{HCO}_3^-$  into the cells in exchange for  $\text{Cl}^-$ . As extracellular  $\text{HCO}_3^-$  enters the RBC, it is rapidly converted to  $\text{CO}_2$  for excretion. It can thus be seen that, even in the presence of large amounts of extracellular CA activity, a rapid chloride shift remains necessary for mobilization of the extracellular  $\text{HCO}_3^-$  pool during  $\text{CO}_2$  transfer in capillary beds.

As a result of the events described above, it can be

noted that a disequilibrium for  $\text{H}^+$  will be present within the suspension between the RBC and extracellular fluid as the suspension leaves the pulmonary capillaries. Because of the greater depletion of extracellular  $\text{H}^+$ , intracellular  $\text{H}^+$  must be transferred to the extracellular fluid to reestablish electrochemical equilibrium. This is accomplished via the Jacobs-Stewart cycle (18, 19, 21), in which  $\text{HCl}$  is translocated from one compartment to another in cell suspensions. As a result, extracellular pH falls after the suspension leaves the lung. With control suspensions, however, this  $\text{pH}_0$  change will be completed very shortly ( $t_{1/2} \sim 0.5$  s) after the suspension exits from the capillaries, since the steps of the Jacobs-Stewart cycle ( $\text{CO}_2$  diffusion, hydration-dehydration reactions in extra- and intracellular fluid, and  $\text{HCO}_3^-/\text{Cl}^-$  exchange) are all very rapid (see Fig. 1a).

The entire sequence of events described above for control suspensions also takes place in the suspensions containing DIDS-treated RBC. Because  $\text{HCO}_3^-/\text{Cl}^-$  exchange rates are slowed down, however, two important consequences result. The first is that, within the lung capillaries, the extracellular  $\text{HCO}_3^-$  pool does not have rapid access to the RBC CA. As a result,  $\text{CO}_2$  transfer across the alveolocapillary barrier is decreased (by  $\sim 16\%$  under the conditions of our in vitro experiments) compared with  $\dot{V}\text{CO}_2$  in the control experiments. Secondly, the dissipation of the end-capillary  $\text{H}^+$  disequilibrium by the Jacobs-Stewart cycle is greatly delayed since the cycle becomes rate-limited by the inhibited  $\text{HCO}_3^-/\text{Cl}^-$  exchange. Consequently, a large slow fall in extracellular pH (total  $\Delta \text{pH}_0 = 0.057$ ,  $t_{1/2} = 44$  s) is observed downstream in our pH electrode apparatus (see Fig. 1b and Table I).

As can be appreciated from the above discussion, essentially identical results should be obtained whether or not further CA is purposely added to the DIDS-treated RBC suspensions. This is due to the fact that in DIDS-treated RBC perfusates,  $\text{HCO}_3^-/\text{Cl}^-$  exchange is the step that limits both the intracapillary chloride shift and the downstream Jacobs-Stewart cycle. Confirmation of these considerations is given in Fig. 2. There was no difference in  $\text{pH}_0$  vs. time, or in  $\dot{V}\text{CO}_2$ , when the lung was perfused with perfusate B or with perfusate B containing added CA. Our theoretical considerations suggest that the same observations would be made even if no CA activity were available to the extracellular fluid, but we were not able to confirm these predictions because of our inability to eliminate hemolysis or pulmonary vascular CA activity without inhibiting intracellular CA activity.

As seen in Fig. 3c, biphasic  $\text{pH}_0$  curves result when isolated rat lungs are perfused with control RBC suspensions containing ACTZ. ACTZ inhibits CA activity both inside and outside the RBC. The fall in  $\text{pH}_0$  in the second phase of the curve is effected by the Jacobs-Stewart cycle. Hemoglobin oxygenation in the

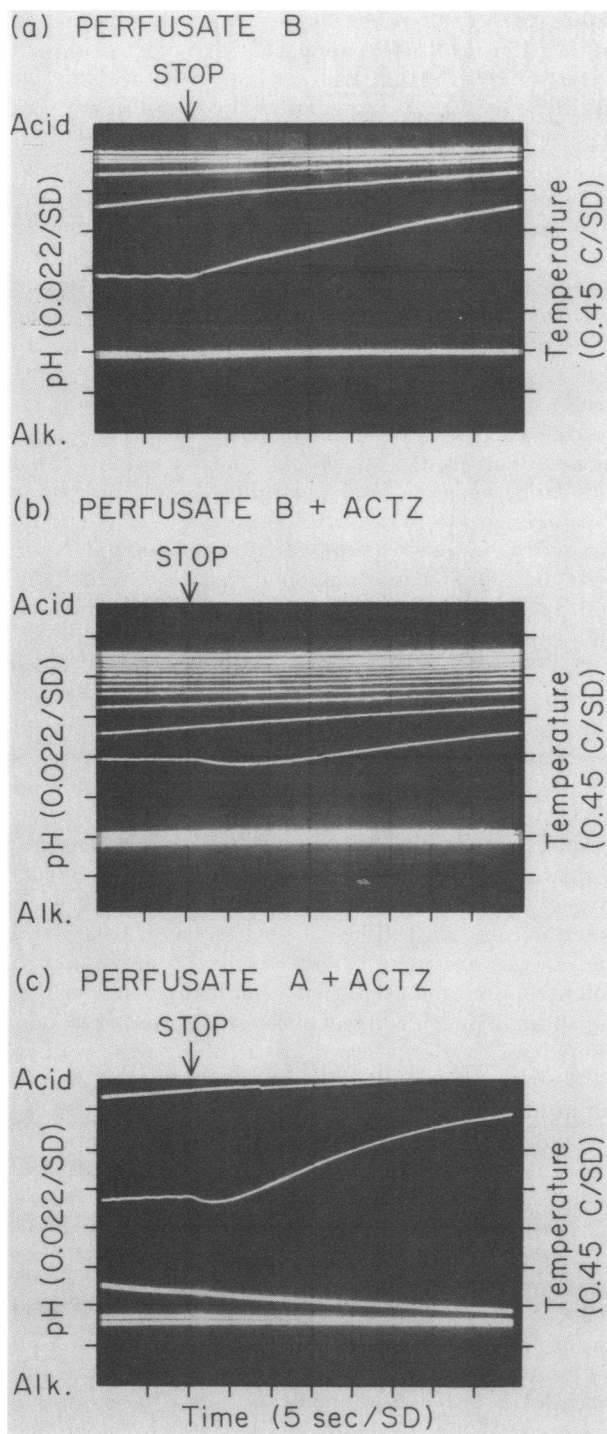


FIGURE 3 Oscilloscope records of effluent perfusate extracellular pH (upper traces) and temperature (lower traces) vs. time. Multiple sweeps across the oscilloscope screen are shown. Perfusate was drawn through the electrode chamber at a constant rate of 15 ml/min until withdrawal was suddenly stopped (as indicated). Record (a) was obtained with perfusate B (DIDS-treated), record (b) with perfusate B containing ACTZ, and record (c) with perfusate A (control) containing ACTZ. The total downstream  $\Delta \text{pH}_o$  for record (a)

RBC interior causes the release of a large quantity of Bohr protons. Since no process is present to change extracellular  $[\text{H}^+]$ , these Bohr protons must leave the RBC and enter extracellular fluid in order to reestablish electrochemical equilibrium. The Jacobs-Stewart cycle is the mechanism by which this equilibrium takes place, leading to an overall  $\Delta \text{pH}_o$  of 0.078 ( $t_{1/2} = 26$  s).

The first phase of the curve (Fig. 3c) is due to equilibration within the extracellular fluid of  $\text{HCO}_3^-$  and  $\text{CO}_2$ . Because CA is inhibited both intra- and extracellularly, equilibration of the extracellular hydration-dehydration reaction is slowed to the extent that we are able to observe the tail end of it in our pH electrode chamber. As equilibration occurs,  $\text{H}^+$  is depleted as it combines with  $\text{HCO}_3^-$  to form  $\text{CO}_2$ , the concentration of which fell rapidly in the lung capillaries. This extracellular reaction process and the Jacobs-Stewart cycle occur simultaneously, but the biphasic curve is seen owing to the fact that the latter cycle takes longer to reach equilibrium than does the former process. These results (biphasic curves) are remarkably similar to those obtained in previous *in vivo* studies using ACTZ-treated animals (19, 22). The  $\dot{V}\text{CO}_2$  obtained during perfusion with control RBC suspensions containing ACTZ (0.67 ml/min) was lower than that obtained with control RBC suspensions alone (0.88 ml/min).

Biphasic  $\text{pH}_o$  curves are also obtained when isolated rat lungs are perfused with DIDS-treated RBC suspensions containing ACTZ, as seen in Fig. 3b. Again, the second phase of the curve showing a fall in  $\text{pH}_o$  represents the time-course of the Jacobs-Stewart cycle. In this case, however, the cycle is limited by the very slow rate of the RBC  $\text{HCO}_3^-/\text{Cl}^-$  exchange, as opposed to the somewhat faster rates of the uncatalyzed hydration-dehydration reactions in the control plus ACTZ case. The mean total  $\text{pH}_o$  fall using perfusate B containing ACTZ is 0.057 with the very long  $t_{1/2}$  of 64 s.

The first phase of the curve (Fig. 3b) showing a rise in  $\text{pH}_o$  is due to nonequilibrium of extracellular  $\text{HCO}_3^-$  and  $\text{CO}_2$  as perfusate leaves the pulmonary capillaries (similar to the control plus ACTZ case), but the time to reach maximum  $\text{pH}_o$  in DIDS-treated perfusate containing ACTZ is longer because the process responsible for the second phase of the biphasic curve is much slower than it is in the control plus ACTZ case. The  $\dot{V}\text{CO}_2$  observed during perfusion of the lungs with DIDS-treated RBC suspensions containing ACTZ (0.73 ml/min) was lower than that in the paired experimental runs using only DIDS-treated RBC suspensions (0.89 ml/min).

was 0.071 ( $t_{1/2} = 39$  s). The total  $\Delta \text{pH}_o$  of the second phase of the curves in records (b) and (c) were 0.062 ( $t_{1/2} = 66$  s) and 0.078 ( $t_{1/2} = 26$  s), respectively. The mean expired  $\text{CO}_2$  ( $\dot{V}\text{CO}_2$ ) was 0.86 ml/min for record (a), 0.71 ml/min for record (b), and 0.67 ml/min for record (c).

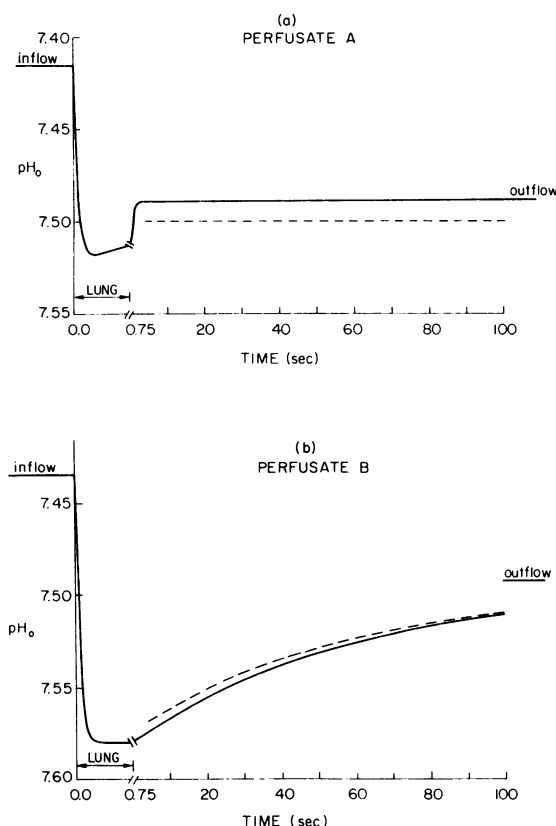


FIGURE 4 Predicted (solid curves) and actual (dashed curves) time-courses of perfusate extracellular pH during and after capillary transit for lung 1, pair b. The upper panel shows  $pH_0$  vs. time for perfusate A, while the lower panel shows  $pH_0$  vs. time for perfusate B. Inflowing perfusate blood gas values used in computing the time-courses were:  $pH_0 = 7.416$ ,  $PCO_2 = 33.0$  torr, and  $PO_2 = 43.4$  torr for perfusate A; and,  $pH_0 = 7.434$ ,  $PCO_2 = 31.7$  torr, and  $PO_2 = 42.7$  torr for perfusate B. Alveolar gas tensions used were  $PCO_2 = 25.5$  torr and  $PO_2 = 110$  torr for perfusate A, and  $PCO_2 = 22.5$  torr and  $PO_2 = 103$  torr for perfusate B. Extracellular buffer capacity was  $1.4 \text{ mM H}^+/\text{pH}$  for perfusate A and  $0.92 \text{ mM H}^+/\text{pH}$  for perfusate B. Extracellular catalyzing factors used in these computations were 100 for both perfusate B and perfusate A. Intracellular catalyzing factor was taken as 6,500 in both perfusates. Bicarbonate and chloride permeability of the RBC membrane was  $6.0 \text{ nm/s}$  for perfusate B, and  $1 \text{ } \mu\text{m/s}$  for perfusate A. Other parameter values used in these computations have been given previously (18).

FIG. 3a, which shows downstream  $pH_0$  vs. time for DIDS-treated RBC suspension without ACTZ, reveals only the monotonic fall in  $pH_0$ . The absence of the first phase of the biphasic curves which are seen in Fig. 3b and c is due to the rapid equilibration of extracellular  $HCO_3^-$  and  $CO_2$  secondary to the availability of CA activity there.

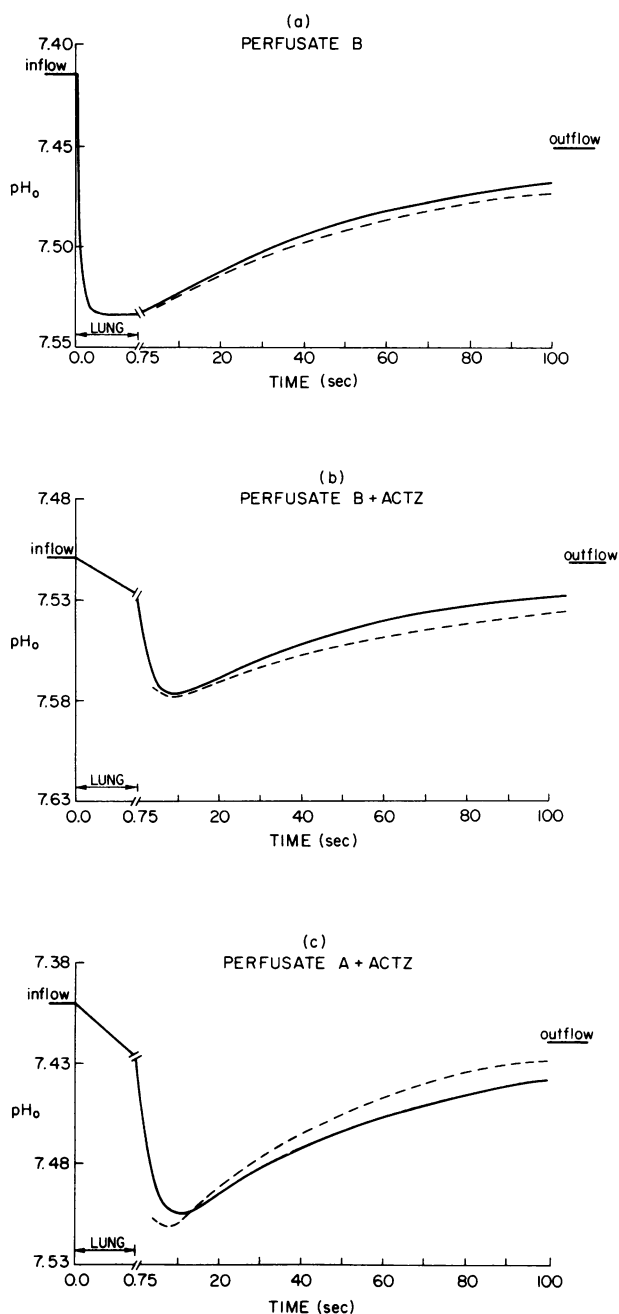
The decrease in  $\dot{V}CO_2$  in vivo when RBC  $HCO_3^-/Cl^-$  exchange was inhibited  $>90\%$  was previously predicted (18) to be  $\sim 25\text{--}30\%$ . In our isolated rat lung experiments, however, we found only a 16% decrement

in  $\dot{V}CO_2$ . The difference in the two results is due to the fact that in the theoretical in vivo calculations, it was assumed (18) that alveolar  $PCO_2$  was the same for all RBC  $HCO_3^-/Cl^-$  exchange rates. In the present experiments, however, minute ventilation was held constant when perfusing with perfusate A or perfusate B. Consequently, alveolar  $PCO_2$  was lower when perfusing with DIDS-treated RBC suspensions (compared with alveolar  $PCO_2$  when perfusing with the control suspensions), owing to the lower rate of transfer of  $CO_2$  from perfusate to alveolar gas. As a result, the  $CO_2$  gradient from the inflowing perfusate to the alveolar gas was greater for the DIDS-treated RBC suspension (compared with control), which favors increased  $CO_2$  transfer. The net result of the offsetting factors (decreased capillary  $CO_2$  transfer but increased inflowing perfusate-to-alveolar  $PCO_2$  gradient) was a 16% decrease in  $\dot{V}CO_2$  with perfusate B. When the theoretical model was applied to our isolated lungs with the actual experimental data, close agreement with the experimental results for both  $\dot{V}CO_2$  and  $pH_0$  time courses was obtained, as shown in Fig. 4.

The theoretical model was also used to simulate the experimental results with perfusates containing ACTZ. The theoretical and experimental results again are in good agreement (Fig. 5). These findings closely resemble previously reported in vivo experimental and theoretical data from animal studies (19, 22).

Although we used only lungs that were apparently normal in our experimental runs, as judged by visual inspection and low perfusion and ventilation pressures, deterioration with time of the isolated lung (and thereby gas exchange) was possible. To prevent such potential deterioration from influencing our results, the order in which the experimental runs were performed was varied. Two-thirds of the pairs given in Table I were run with DIDS-treated RBC suspension before control suspension. The other one-third were carried out in the reverse order. No significant differences were found between the two groups of pairs. In any case, deterioration in gas exchange would have tended to work against the observed fall in  $\dot{V}CO_2$  with perfusate B, since the DIDS-treated RBC perfusates were used first in more than half the pairs.

Because DIDS might have had an independent effect on the lung, we perfused rat lungs with either a KRB solution or a KRB solution containing DIDS, and found that DIDS did not influence either  $\dot{V}CO_2$  or downstream  $\Delta pH$ . Furthermore, since it is well known that DIDS binds irreversibly to the surface of the RBC (23), it is unlikely that the lung tissue was exposed to any more than a minute concentration of the inhibitor during the perfusion with DIDS-treated RBC suspensions. Irreversibility of DIDS binding to the RBC in our experiments was confirmed in the stop-flow rapid reaction apparatus, since the DIDS-treated RBC, even after the isolated rat lung experiment was com-



**FIGURE 5** Predicted (solid curves) and actual (dashed curves) time-courses of perfusate extracellular pH during and after capillary transit for isolated rat lung. The upper panel shows  $pH_o$  vs. time for perfusate B, the middle panel shows  $pH_o$  vs. time for perfusate B plus ACTZ, and the lower panel shows  $pH_o$  vs. time for perfusate A plus ACTZ. Inflowing perfusate blood gas values used in computing the time-courses were:  $pH_o = 7.415$ ,  $PCO_2 = 34.4$  torr, and  $PO_2 = 33.4$  torr for perfusate B;  $pH_o = 7.509$ ,  $PCO_2 = 29.4$  torr, and  $PO_2 = 35.1$  torr for perfusate B plus ACTZ;  $pH_o = 7.401$ ,  $PCO_2 = 35.7$  torr, and  $PO_2 = 47.8$  torr for perfusate A plus ACTZ. Alveolar gas tensions used in the computations were  $PCO_2 = 26$  torr and  $PO_2 = 106$  torr with perfusate B;  $PCO_2 = 23$  torr and  $PO_2 = 110$  torr with perfusate B plus ACTZ; and  $PCO_2$

pleted, had a  $HCO_3^-/Cl^-$  exchange rate that was only 2% of that of the control RBC.

Finding no change in the results from lungs perfused with KRB or KRB plus DIDS, we infer that DIDS has no inhibitory influence on rat lung vascular carbonic anhydrase. The experiments using the test reaction 20 mM  $NaHCO_3$  plus 10 mM HCl (see above) reveal that DIDS did not inhibit bovine or human CA activity. Our finding that acceleration of  $H_2CO_3$  dehydration was the same with lysates from either control or DIDS-treated RBC shows that incubation of RBC in DIDS has no effect on the intracellular CA activity. This latter observation is not surprising, since DIDS is known not to enter RBC (24, 25). These results, taken together, indicate that neither intracellular CA activity nor CA activity available to extracellular fluid was affected by the use of DIDS.

It has been reported that in isolated rabbit lungs some gas exchange takes place across the pleural surface (26). It is unlikely that this phenomenon is of importance in our experiments, since  $<10\%$  of  $\dot{V}CO_2$  is lost through the pleural surface, and our lungs were perfused at steady state in almost closed chambers, which allowed  $PCO_2$  in the chamber atmosphere to approach alveolar  $PCO_2$ .

We have now shown experimentally and theoretically that  $CO_2$  elimination from blood in the lung capillaries and  $CO_2$  uptake by blood in systemic capillaries are dependent on the speed of the RBC chloride shift. Reductions in the speed of the chloride shift may therefore have important clinical implications with respect to gas exchange and acid-base homeostasis. Decreased human RBC anion exchange rates have been observed in the presence of many commonly used drugs, including salicylates (11), furosemide (12), and anesthetics (13). It is also possible that individuals with abnormal RBC membranes (e.g., muscular dystrophy [27]) will turn out to have abnormal  $HCO_3^-/Cl^-$  exchange kinetics. Severe anemias may, in effect, reduce the overall rate of the chloride shift by reducing the total available RBC surface area (17, 28).

The specific clinical result of decreased RBC  $HCO_3^-/Cl^-$  exchange rates will depend upon the

$= 24$  torr and  $PO_2 = 110$  torr with perfusate A plus ACTZ. Extracellular buffer capacity was 0.92 mM  $H^+/pH$  for perfusate B and perfusate B plus ACTZ; and 1.2 mM  $H^+/pH$  for perfusate A plus ACTZ. Extracellular catalyzing factors used in these computations were 100 for perfusate B, and 1 for perfusate B plus ACTZ and for perfusate A plus ACTZ. Intracellular catalyzing factor was taken as 6,500 for perfusate B and as 1 for perfusate B plus ACTZ and for perfusate A plus ACTZ. Bicarbonate and chloride permeability of the RBC membrane were 6.0 nm/s each for perfusates B and B plus ACTZ, and 1  $\mu m/s$  for perfusate A plus ACTZ. Other parameter values used in these computations have been given previously (18).

clinical status of the patient in whom such an abnormality exists. Since both tissue and lung capillary  $\text{CO}_2$  transfer will be impaired, tissue  $\text{PCO}_2$  will tend to rise. Patients with normal lungs would increase alveolar ventilation slightly as required to decrease alveolar  $\text{PCO}_2$  to maintain normal  $\dot{V}\text{CO}_2$  by excreting an increased fraction of  $\text{CO}_2$  from the dissolved pool. Arterial blood gases would remain approximately normal and changes in the clinical status of the patient would not be noticeable. Since tissue (including brain and carotid body)  $\text{PCO}_2$  would be elevated, however, a variable degree of hyperventilation may result (29). It has been suggested that the hyperventilation associated with salicylate poisoning may be related to this phenomenon (16). In patients with impaired lung function, however, who may not be able to chronically increase alveolar ventilation to lower alveolar  $\text{PCO}_2$  (e.g., owing to chronic obstructive pulmonary disease), impaired lung capillary  $\text{CO}_2$  exchange could lead to  $\text{CO}_2$  retention and respiratory acidosis. Further work will be necessary to determine the wisdom of administration of drugs that impair RBC  $\text{HCO}_3^-/\text{Cl}^-$  exchange to certain patients, especially those with impaired lung function.

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