In Vivo CO_2 Buffer Curves of Arterial and Mixed Venous Blood[1] (35552)

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In recent years the difference between *in vitro* and *in vivo* buffering of CO_2 and some of the factors that influence these differences have been described (1–4). The principal reason for the lower *in vivo* buffer value in the initial period following a step change in CO_2 tension is distribution of HCO^-_3 through the largely unbuffered interstitial fluid (5). As a further complication Roos and Thomas (6), and Michel (7), have demonstrated, on theoretical grounds, that determinations made on arterial blood may give a false value if the arteriovenous CO_2 content difference (*a–v* CO_2) changes when the step change in P_{CO_2} is made. Such an *a–v* CO_2 change could be produced by a change in cardiac output or a change in metabolic rate. It is also recognized that the slope of the CO_2 buffer curve changes as a function of time after an abrupt change in CO_2 tension (8, 9).

In addition to the factors which influence *in vivo* CO_2 buffer curves in hypercapnia, the increasing concentrations of blood lactate and pyruvate with hyperventilation, even over short intervals of time (10–12), make it very difficult to establish values for the slope of the *in vivo* CO_2 buffer curve in hypocapnia. It was the purpose of this investigation to compare the *in vitro* CO_2 buffer curves of arterial and mixed venous blood in dogs after either abruptly increasing $P_{a_{CO_2}}$ to greater than 70 Torr, or lowering $P_{a_{CO_2}}$ to less than 15 Torr, and to determine the change in slope over intervals of 6 and 2 hr, respectively, in the two groups.

Experimental Procedure. Experiments were carried out on 12 sodium pentobarbital-anesthetized dogs divided into two groups of six each. After anesthetization, catheters were placed in the abdominal aorta by way of the femoral artery, and in the right ventricle by way of the right external jugular vein. A cuffed endotracheal tube was inserted and the dog breathed 100% oxygen unassisted. The oxygen source was then connected through a positive pressure pump by means of which tidal volume and respiratory rate could be adjusted. Pulmonary ventilation was abruptly increased to a level which reduced end-tidal P_{CO_2} to less than 15 Torr as monitored by an infrared CO_2 analyzer. Blood samples were drawn at 10 min, 1 hr, and 2 hr after beginning hyperventilation. At this point the respirator was disconnected and the dog again breathed 100% oxygen unassisted for 1 hr of recovery. Blood samples were drawn at 10 and 60 min during the recovery period.

In the group of animals in which P_{CO_2} was increased, 1.5 mg of tubocurarine chloride was injected intravenously every 2 hr and the dogs were ventilated by means of a positive pressure pump. During the 1-hr control period on 100% oxygen the pump was adjusted to maintain $P_{a_{CO_2}}$ at approximately 35 Torr. Blood samples were drawn and 10% CO_2–90% O_2 was substituted for 100% oxygen. Frequency and tidal volume were regulated to maintain $P_{a_{CO_2}}$ near 70 Torr. Blood samples were drawn at 15 min, 1, 2, 3, 4, and 6 hr during the 10% CO_2 breathing. The animals then breathed 100% oxygen for a 15-min recovery period, at which time the final blood sample was drawn

Analytical Methods. Blood P_{O_2}, P_{CO_2} and pH were determined at 37.5° with appropri-

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TABLE I. Blood and Plasma Values from Which CO_2 Buffer Slopes Were Calculated Following Step Changes in P_{CO_2}.

	pH	P_{CO_2} (Torr)	$(HCO_3^-)_P$ (mmoles/liter)	$(O_2)_B$ (vol %)	$a\text{–}v$ O_2 (vol %)	Hb (g/100 ml)	(mmoles/liter)		CO_2 buffer slope (slykes)		
							Lactate	Pyruvate	Observed	Corrected	Calculated
$N = 6$					Control						
Arterial	7.34	37.9	20.6	19.1		14.2	1.0	0.08			
	0.02	3.4	0.9	0.9		0.6	0.2	0.02			
Venous	7.31	43.9	22.2	14.1	5.0	14.1	1.0	0.07			
	0.02	2.5	0.8	1.1	0.9	0.6	0.2	0.02			
					Hyperventilation						
10 min											
Arterial	7.61	16.7	16.6	19.8		14.8	1.7	0.10	14.8	12.2	
	0.03	1.4	1.3	0.9		0.6	0.2	0.03	3.8		
Venous	7.54	23.2	19.6	14.5	5.3	15.0	1.6	0.09	11.2	8.3	11.4
	0.02	1.6	1.0	1.4	1.0	0.5	0.3	0.02	2.7		
2 hr											
Arterial	7.58	14.9	13.9	20.5		15.4	2.9	0.18			
	0.03	0.8	1.3	0.9		0.6	0.7	0.04			
Venous	7.53	20.3	17.0	13.1	7.4	15.4	2.9	0.20			
	0.02	0.7	1.1	1.5	0.7	0.6	0.7	0.03			
					Recovery						
10 min											
Arterial	7.31	34.7	17.7	20.8		15.6	2.7	0.18	14.3	14.0	
	0.02	3.3	1.3	1.0		0.5	0.7	0.05	2.7		
Venous	7.29	40.5	19.8	14.1	6.7	15.5	2.0	0.19	11.6	11.0	11.8
	0.02	2.4	0.7	1.6	0.6	0.6	0.6	0.05	2.8		

TABLE I (continued).

	pH	P_{CO_2} (Torr)	$(HCO_3^-)_P$ (mmoles/liter)	$(O_2)_B$ (vol %)	a–v O_2 (vol %)	Hb (g/100 ml)	(mmoles/liter)		CO_2 buffer slope (slykes)		
							Lactate	Pyruvate	Observed	Corrected	Calculated
					Control						
$N = 6$											
Arterial	7.38	34.8	21.3	18.3		13.6	1.3	0.09			
	0.01	2.3	1.4	1.5		1.1	0.1	0.02			
Venous	7.33	42.4	22.4	13.4	4.9	13.4					
	0.03	0.9	1.4	1.8	0.6	1.0					
					10% CO_2						
15 min											
Arterial	7.16	68.0	23.9	19.0		14.0	1.2	0.08	11.7	11.7	
	0.02	1.2	0.8	1.4		1.0	0.1	0.01	1.3		
Venous	7.16	76.0	24.6	14.6	4.4	13.9			12.9	13.4	11.1
	0.04	2.2	1.2	1.4	0.5	1.0			3.3		
2 hr											
Arterial	7.12	72.8	22.4	23.8		18.5	1.2	0.07			
	0.04	1.6	2.4	2.8		2.1	0.2	0.01			
Venous	7.04	92.6	23.8	14.8	9.0	18.4					
	0.04	6.2	0.5	2.1	1.3	2.2					
					Recovery						
15 min											
Arterial	7.23	40.8	19.0	25.8		20.0	1.4	0.06	29.3	27.7	
	0.04	6.6	2.9	2.8		2.2	0.3	0.01	4.9		
Venous	7.16	63.5	21.3	14.0	11.8	19.9			18.2	17.4	14.6
	0.03	5.1	1.1	2.1	1.8	2.2			4.5		

ate electrodes. The Hb concentrations and O_2 saturation were determined spectrophotometrically with the Co-Oximeter. Plasma bicarbonate concentration was calculated from pH and P_{CO_2} with appropriate temperature corrections (13), and corrected value for pK' according to Austin *et al.* (14), using the Henderson-Hasselbalch equation. Blood oxygen content was calculated from Hb concentration, O_2 saturation, and O_2 tension. Blood lactate and pyruvate were determined by the enzymatic technique. Buffer value was calculated as $\Delta(HCO^-_3)p/\Delta pH$ and designated as slykes (9).

Results. Mean values and their standard errors on blood samples used for calculating buffer slopes are presented in Table I. With a step decrease in P_{CO_2} from 37.9 to 16.7 Torr in arterial blood, plasma bicarbonate decreased by 4 mmoles/liter and pH increased to 7.61. With only 10 min of hyperventilation the sum of lactate and pyruvate increased from 1.07 to 1.80 mmoles/liter. Similar changes were observed in venous blood. A slight increase in the calculated *a–v* oxygen content difference (*a–v* O_2) indicating a fall in cardiac output or an increase in O_2 consumption, appeared during the first 10 min of hyperventilation. Over the next 1 hr and 50 min, both P_{CO_2} and (HCO^-_3)p decreased slightly with very little change in pH. On discontinuing the hyperventilation, P_{CO_2} and bicarbonate concentration increased and pH returned toward normal within 10 min. During this 10 min the sum of lactate and pyruvate decreased to 2.88 and *a–v* O_2 decreased.

Increasing $P_{a_{CO_2}}$ to 70 Torr produced the expected increase in plasma bicarbonate concentration, and decrease in pH. The *a–v* O_2 decreased somewhat during the first 15 min of 10% CO_2 breathing. After 6 hr of 10% CO_2 breathing under the conditions of these experiments, *a–v* O_2 had increased 83% over control value suggesting that a marked fall in cardiac output had taken place following the initial increase. Further increase in *a–v* O_2 after 10 min of 100% O_2 breathing suggested that a further reduction in cardiac output accompanied the change from 10% CO_2 to 100% O_2.

Mean value for buffer slope and its standard error were calculated from slopes of individual experiments, and the value may not agree precisely with that calculated from mean pH and bicarbonate values. Buffer slope calculated from arterial blood values was larger than venous buffer slope with both decrease in P_{CO_2} and increase in P_{CO_2} on the hyperventilated dogs. Arterial and venous slopes differed very little with the step increase in P_{CO_2} produced by breathing 10% CO_2. Reducing CO_2 tension after 6 hr of breathing 10% CO_2 gave buffer values for arterial blood which were higher than those for venous blood. The difference between arterial and venous slope however was not of sufficient magnitude and consistency in direction to make the difference statistically significant in any of the four changes.

If it is assumed that the change in the sum of lactate and pyruvate is reflected mole for mole in an opposite change in plasma bicarbonate concentration, and if we make the slight correction necessary for the Haldane shift due to changes in oxygen saturation, we can calculate corrected slopes which differ only slightly from the uncorrected value except with the step decrease in P_{CO_2} in the hyperventilated animals. If we make an assumption of a normal value for the ratio of blood water to total extracellular water, and for plasma protein concentration, we can calculate *in vivo* buffer slopes for the different Hb concentrations (8) as indicated in the last column.

Discussion. We have previously demonstrated that cardiac output changes in the same direction as CO_2 tension in the anesthetized dog (15, 16). In the present series of experiments, we did not measure O_2 consumption rate but the changes in *a–v* O_2 with step changes in P_{CO_2} are consistent with this concept. Although the changes in *a–v* O_2 in the first 10 min of hyperventilation or recovery were small (6 and 9%, respectively) the direction of change was consistent and the differences were significant ($P < .05$) in both cases. The changes in *a–v* O_2 with onset and discontinuance of 10% CO_2 breathing were not significant.

It has been pointed out by Roos and Thomas (6) and by Michel (7) that when an increase in cardiac output accompanies a step increase in P_{CO_2}, the *in vivo* CO_2 buffer slope of arterial blood will be steeper than the true slope obtained on mixed venous blood corrected for any minor shift due to the Haldane effect. When a decrease in cardiac output accompanies a step decrease in P_{CO_2}, arterial blood will again give a falsely high CO_2 buffer slope. Although the differences in slope between arterial and venous blood obtained in these experiments are not significant, they are in the direction that would be expected from the predicted change in cardiac output in three of the four cases; the exception being the change that occurred when a step increase in P_{CO_2} was produced by breathing 10% CO_2.

Other available data are consistent with the hypothesis that cardiac output increases with increasing CO_2 tension and that the *in vivo* CO_2 buffer value on arterial blood under these circumstances will be falsely high compared with mixed venous blood. In previous studies on 9 dogs breathing 30% CO_2 we found mean values of 12.2 sl for arterial blood and 9.3 sl for venous blood (17). Prys-Roberts (18) obtained mean values of 20.9 ± 5.0 sl and 15.9 ± 3.1 sl on arterial and mixed venous blood, respectively, on 13 anesthetized patients. Average hematocrit was 46 and mean *in vitro* slope was 28.8 sl. Theoretical *in vivo* slope for these patients would be approximately 12 sl. Values usually obtained on arterial blood in humans are larger than those predicted from the equation for *in vivo* buffering of extracellular fluid (8).

Evaluating CO_2 buffer slopes by determination of P_{CO_2} and pH with subsequent calculation of plasma bicarbonate concentrations is subject to the errors of these determinations. If, for example, the change in calculated bicarbonate concentration with the step change in P_{CO_2} is 2 mmoles/liter with an accompanying change in pH of 0.20 pH units, (slope = 10 sl) an error of 0.01 pH in each of determinations from which the ΔpH is calculated could make a change in the calculated buffer slope of 10%. With normal values for P_{CO_2} and pH, an error of 0.01 pH combined with an error of 1 Torr P_{CO_2} could give an error of 1.2 mmoles/liter bicarbonate concentration. Thus, with step changes in P_{CO_2} which produce plasma bicarbonate changes of no more than 2 or 3 mmoles/liter, the precision of calculated buffer slopes is poor.

Assuming that distribution of HCO^-_3 and Cl^- between blood and interstitial fluid is essentially complete in 10 to 15 min, and that only minimal changes in other components have taken place in this time, the most reliable value to compare with the theoretical value for *in vivo* buffering would be that obtained from blood values immediately before and within 15 min after the step increase or decrease in P_{CO_2}. Even in this short interval, which we have called the, "Chemical buffering and distribution phase," (8, 17) with a step decrease in P_{CO_2} to values below normal, blood lactate may increase sufficiently to decrease plasma HCO^-_3, and increase the apparent buffer value (12). Certainly over longer intervals, and after physiological adjustments alter the original acid-base state, use of the term "buffering" for the *in vivo* situation is very questionable (19, 20).

In the experiments reported here the "apparent *in vivo* buffer values" during hyperventilation were 15, 22, and 28 sl in arterial blood and 11, 19, and 27 sl in mixed venous blood at 10 min, 1 hr, and 2 hr, respectively. In the dogs breathing 10% CO_2 the values were 12, 10, 13, 10, 5, and 4 sl in arterial blood and 13, 10, 22, 20, 14, and 6 sl in mixed venous blood at 15 min, 1, 2, 3, 4, and 6 hr, respectively. The increase in value of $\Delta HCO^-_3/\Delta pH$ in the early hours of hyperventilation is undoubtedly related to the increase in lactate (10, 12) although this may not be the whole story. In the early hours of hypercapnia, extracellular HCO^-_3 may be increasing by virtue of electrolyte transfers between intracellular and extracellular fluids (8) as well as by renal action.

In the experiments reported here the agreement between theoretical and mixed venous buffer values at 10 or 15 min after the step change in P_{CO_2} were quite acceptable, and the only disparity of consequence with arterial blood was in the step decrease with discontinuance of 10% CO_2 breathing. There was considerable variation in the buffer value obtained in this series as indicated by the large standard error.

Given that arterial blood values may be falsely high, is the difference of clinical importance in distinguishing between respiratory and metabolic components in acid-base disturbances? Variations in slope between 7 and 15 sl over the range of 20 to 80 Torr P_{CO_2} could produce a maximum error of 2 mmoles/ liter in the plasma bicarbonate value projected from a change in P_{CO_2} alone (21). Unless marked changes in O_2 consumption rate or cardiac output take place as a concomitant of the change in CO_2 tension, it is unlikely that the difference in CO_2 buffer slope between arterial and mixed venous blood will be clinically significant.

Summary. Six dogs were hyperventilated to an arterial P_{CO_2} of less than 15 Torr for 2 hr and then allowed to breathe spontaneously on 100% oxygen. A second group of 6 dogs breathed 10% CO_2–90% O_2 for 6 hr and then returned to breathing 100% oxygen. Arterial and mixed venous blood samples were drawn for determination of pH and P_{CO_2}. From these values plasma bicarbonate concentrations were calculated. CO_2 buffer slopes calculated as $\Delta HCO^-_3/\Delta pH$ were determined by comparing bloods drawn before and 10 or 15 min after a step change in P_{CO_2}. In three of the four cases the buffer slope of mixed venous blood was between 8.3 and 14.0 sl with the predicted value, calculated from Hb concentration and assumed values for other parameters, between 11 and 12 sl. Difference between arterial and venous slopes over the range of P_{CO_2} change utilized in these studies was neither statistically significant nor of a degree to be of practical importance in evaluating acid-base disturbances clinically.

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