The Effect of Metabolic Acidosis on the CO_2 Titration Curve

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Carbon dioxide titration curves were measured *in vivo* in anesthetized dogs and *in vitro* in dog blood with normal and diminished [HCO_3^-]. Bicarbonate concentrations were lowered with HCl. The recovery from the infusion of hydrochloric acid was also studied *in vivo*.

It was demonstrated that *in vitro*, in the presence of a constant buffer power (β), the slope of the CO_2 titration curve was diminished by lowering [HCO_3^-]. *In vivo*, the slope of the CO_2 titration curve remained constant in the presence of a diminished [HCO_3^-]; this was accompanied by a rise in buffer power (β).

It was concluded that the rise in β, which occurred in the presence of a fall in [HCO_3^-] *in vivo*, represented a homeostatic mechanism for dealing with the concurrence of respiratory and metabolic acidosis.

THE SLOPE of the carbon dioxide titration curve of blood (P_{CO_2} versus [HCO_3^-]) is dependent upon the concentrations of hemoglobin, protein and phosphate buffers which take up or give off hydrogen ions in response to variations in P_{CO_2}. The change in bicarbonate concentration which accompanies a rise or fall in P_{CO_2} merely reflects the amount of hydrogen ion taken up or given off by these respiratory buffers.[1] The slope of the CO_2 titration curve *in vivo* is less than that of blood *in vitro* because of the dilutional effect of interstitial fluid in which the concentration of respiratory buffers is low.[2-4]

The slope of the CO_2 titration curve is also determined by the initial concentration of bicarbonate; the lower the concentration, the less the slope. This relationship is determined by the effect of the concentration of bicarbonate on the mass action equation for the dissociation of carbonic acid and not upon direct buffering by bicarbonate.

Both determinants of the slope of the CO_2 titration curve, (1) the concentrations of respiratory buffers and (2) initial bicarbonate, can vary in disease, *e.g.*, the former in anemia, the latter in metabolic acidosis. It was the purpose of this study to examine the interaction between these two variables *in vivo* and *in vitro*.

Methods

In Vivo

Carbon dioxide titration curves were measured in anesthetized dogs before and after infusion of hydrochloric acid.

Seven mongrel dogs weighing 13–24 kg (mean 21 kg) were anesthetized with pentobarbital (Nembutal®) (26 mg/kg iv), paralyzed with succinylcholine (Anectine®) (40 mg im) and ventilated through a tracheal cannula by a constant-volume pump set to maintain P_{aCO_2} at approximately 40 mm Hg. The carotid artery and femoral vein were cannulated, and all blood samples were obtained anerobically from the carotid artery. A blood sample was drawn during 100 per cent O_2 breathing, following which gas mixtures containing 2.2, 6 and 8.8 per cent CO_2 in oxygen were breathed successively for ten minutes. At the end of each period, an arterial sample was drawn and analyzed at the dog's rectal temperature for *p*H, P_{O_2} and P_{CO_2} by

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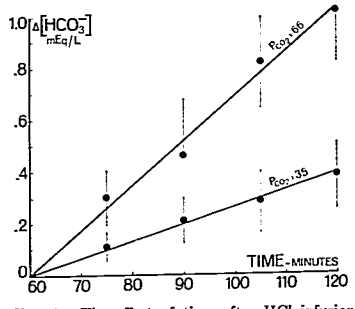

FIG. 1. The effect of time after HCl infusion (3 mEq/kg) on the recovery of plasma $[HCO_3^-]$ at P_{aco_2} 35 and 66 mm Hg. The vertical bars represent the standard error of the mean Δ plasma $[HCO_3^-]$.

electrodes*; plasma CO_2 content was determined in duplicate by Van Slyke manometric apparatus and hematocrit by capillary method.

Hydrochloric acid was then given intravenously over a ten-minute period in a dose of 3 mEq/kg in a volume of 10 ml/kg. The animal was allowed to recover for 60 minutes after completion of the acid infusion. Carbon dioxide titration was then repeated, using the same CO_2 mixtures. A ten-minute period of recovery, breathing 100 per cent O_2, was followed by a second and, in some instances, a third infusion of hydrochloric acid.

By this method, CO_2 titration curves were obtained at the following initial levels of bicarbonate concentration when P_{aco_2} was 40 mm Hg ($[HCO_3^-]_{40}$): 21 mEq/l (six dogs), 16 mEq/l (two dogs), 13 mEq/l (two dogs), 12 mEq/l (two dogs) and 9 mEq/l (five dogs).

The recovery of plasma bicarbonate concentration, following HCl infusion, was studied in another series of dogs similarly prepared. In ten such studies (four dogs), the ventilation was set to establish a control P_{aco_2} of 40 mm Hg. Hydrochloric acid was infused in the dose and manner described, after which plasma bicarbonate concentration, pH_a, P_{aco_2}, P_{ao_2} and hematocrit were measured at intervals up to three hours after infusion. Seven similar studies (three dogs) were performed in animals in whom the control ventilation during O_2 breathing was decreased to produce a P_{aco_2} of 65 mm Hg.

IN VITRO

CO_2 titration was performed *in vitro* at various initial bicarbonate concentrations, mean values being $[HCO_3^-]_{40}$ = 19.3, 15.4, 10.6, 6.5 and 3.1 mEq/l. Venous blood from eight dogs was drawn in heparinized syringes and stored in ice. The $[HCO_3^-]_{40}$ was adjusted by the addition to 7 ml of blood to 1 ml of HCl of varying concentration or 1 ml of saline solution. The mixtures were then equilibrated in 250-ml flasks at 37 C for ten minutes with 2.8, 5.7 and 12.3 per cent CO_2 in O_2.

The *p*H was measured in triplicate at 37 C† this value, with the known P_{co_2} of the equilibrating mixture, was used to calculate the $[HCO_3^-]$ by the Henderson-Hasselbalch equation.

Results

IN VIVO

One hour following the infusion of 3 mEq/kg of 0.3N HCl, the bicarbonate concentration rose in a linear fashion for the next 60 minutes at a rate directly proportional to P_{aco_2} (figure 1). From the data shown in figure 1 and the known linear relationship between P_{aco_2} and renal hydrogen ion excretion,[5,6] a recovery rate of $[HCO_3^-]$/min/mm P_{co_2} (between 30 and 90 mm) was derived:

Increase in $[HCO_3^-]$/min in mEq/l = .0036 (P_{aco_2} − 30) + .04.

The $[HCO_3^-]$ at the end of a period of CO_2 breathing was corrected according to the P_{aco_2} and the length of time of CO_2 breathing.

From the experiments shown by figure 1 a rate of loss of hydrogen ion was established as 0.1 nEq/l/min. The measured $[H^+]$ was then corrected according to length of time of CO_2 breathing, using this value. The correction applied at all levels of P_{aco_2} studied since $[HCO_3^-]$ changed proportionately to P_{aco_2}.

The measured and corrected bicarbonate or hydrogen ion concentrations for each experiment were plotted against measured P_{aco_2} for each *in vivo* CO_2 titration. In all experiments, it was possible to construct a smooth

* Manufactured by Instrumentation Laboratory, Inc., Watertown, Massachusetts.

† Beckman Research *p*H Meter, Model 1019, Beckman Instruments, Inc., Fullerton, California.

curve for $[HCO_3^-]$; or a straight line in the case of $[H^+]$. From these plots, values were taken at 10-mm Hg intervals of P_{CO_2} and used to construct figures 2 and 3.

The slopes of the *in vivo* CO_2 titration curves between P_{CO_2} 40 and 80 mm Hg were approximately the same, regardless of the initial $[HCO_3^-]$, as shown by the slope of each curve in figure 2. The calculated buffer power[1] ($\beta = \Delta[HCO_3^-]/\Delta pH$) is also indicated. With increasing degrees of bicarbonate displacement, the value for β increased from 16 to 23.

For a given increase in P_{aCO_2}, there was a greater rise in $[H^+]$ the lower the level of initial bicarbonate ($[HCO_3^-]_{40}$) as shown by the slopes in figure 3. At all levels, the relationship between P_{aCO_2} and $[H^+]$ was linear.

During *in vivo* CO_2 titration, the hemotocrit rose progressively from 46 to 52 per cent.

In Vitro

The results of *in vitro* CO_2 titration with varying $[HCO_3^-]_{40}$, produced by addition of HCl, are shown in table 1. The slope of the CO_2 titration curve ($\Delta[HCO_3^-]/\Delta P_{CO_2}$) became less with increasing degrees of bicarbonate displacement. The buffer power (β) of the solution remained constant, as did the hematocrit.

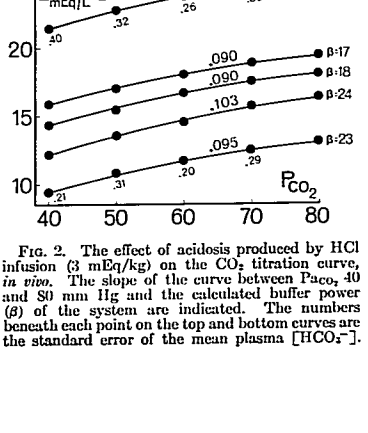

FIG. 2. The effect of acidosis produced by HCl infusion (3 mEq/kg) on the CO_2 titration curve, *in vivo*. The slope of the curve between P_{aCO_2} 40 and 80 mm Hg and the calculated buffer power (β) of the system are indicated. The numbers beneath each point on the top and bottom curves are the standard error of the mean plasma $[HCO_3^-]$.

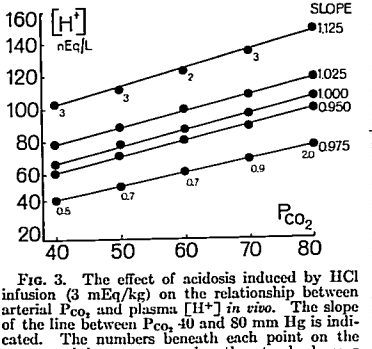

FIG. 3. The effect of acidosis induced by HCl infusion (3 mEq/kg) on the relationship between arterial P_{CO_2} and plasma $[H^+]$ *in vivo*. The slope of the line between P_{CO_2} 40 and 80 mm Hg is indicated. The numbers beneath each point on the upper and lower curves give the standard error of the mean plasma $[H^+]$.

Discussion

If the partial pressure of carbon dioxide is raised from 40 to 80 mm Hg in a pure solution of sodium bicarbonate containing 24 mEq/l, the hydrogen ion concentration will increase from 40 to 80 nEq/l (*p*H from 7.4 to 7.1). The bicarbonate concentration will likewise increase by the same number of nanoequivalents, a change which is too small to measure. The result can be described by the following reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

If, however, respiratory buffers are present in the solution (primarily hemoglobin and serum proteins in the case of blood), they will take up hydrogen ions and allow the reaction to proceed to the right. The resultant measurable rise in bicarbonate concentration will be equal to the quantity of hydrogen ions taken up by the buffers in mEq/l. The final hydrogen ion concentration of the solution will be less (*p*H higher) than had the buffers not been present. The change in bicarbonate concentration which occurs per unit *p*H change is, therefore, a measure of the buffer power (β) of the solution; thus:

$$\beta = \frac{\Delta[HCO_3^-]}{\Delta pH} \quad (2)$$

In vitro, the magnitude of β depends primarily upon the hemoglobin concentration,

TABLE 1. The Effect of Bicarbonate Displacement by HCl on CO_2 Titration *in Vitro* (mean values and standard error of the mean are given)

P_{CO_2} 19 (mm Hg)		P_{CO_2} 39 (mm Hg)		P_{CO_2} 84 (mm Hg)		β†	Slope of CO_2 Titration Curve*		
H^+(nEq/l) (pH)	Calc. $[HCO_3^-]_p$ (mEq/l)	H^+(nEq/l) (pH)	Calc. $[HCO_3^-]_p$ (mEq/l)	H^+(nEq/l) (pH)	Calc. $[HCO_3^-]_p$ (mEq/l)		P_{CO_2} 19–39 (mm Hg)	P_{CO_2} 39–84 (mm Hg)	P_{CO_2} 19–84 (mm Hg)
30±0.4 (7.52)	15.2±0.2	49±0.4 (7.31)	19.3±0.2	82±2 (7.09)	24.8±0.6	22	0.205	0.122	0.148
43±4 (7.37)	11.1±0.8	62± 2 (7.21)	15.4±0.6	98± 4 (7.01)	20.7±0.8	27	0.210	0.118	0.148
64± 5 (7.20)	7.4±0.5	90± 4 (7.05)	10.6±0.4	128± 5 (6.90)	15.9±0.7	28	0.160	0.116	0.129
117± 8 (6.93)	4.0±0.3	146± 7 (6.84)	6.5±0.3	205±12 (6.69)	10.0±0.5	25	0.125	0.078	0.092
281±23 (6.55)	1.7±0.1	298±45 (6.53)	3.1±0.2	390±29 (6.41)	5.4±0.4	26	0.070	0.051	0.057

* Calculated slope $= \Delta[HCO_3^-]/\Delta P_{CO_2}$.
† β = buffer power ($\Delta[HCO_3^-]/\Delta$ pH).

each gram of hemoglobin contributing 1.8 units,[7] yielding a β of approximately 27 in blood with Hgb of 15 g per cent. *In vivo*, this value decreases, since the interstitial fluid with which CO_2 must also equilibrate has a low buffer power ($\beta = 3$). The net dilutional effect of hemoglobin by interstitial fluid yields an *in vivo* value for β of approximately 12.

In addition to the buffer power, the slope of the CO_2 titration curve is dependent upon the initial concentration of bicarbonate. The $[HCO_3^-]$ influences the curve not through buffering but through its mass action effect on the dissociation of carbonic acid as expressed by Equation 1. Using the appropriate constants, the equation can be written:

$$[H^+]\ \text{nEq/l} = 24\,\frac{P_{CO_2}\ \text{mm Hg}}{[HCO_3^-]\ \text{mEq/l}} \quad (3)$$

If the P_{CO_2} is raised the same amount in two systems, one with a low $[HCO_3^-]_{40}$ and the other with high $[HCO_3^-]_{40}$, the absolute rise in $[H^+]$ will be larger in the presence of a low $[HCO_3^-]_{40}$ for the same fall in pH, despite the same β of the system. This is inherent in the logarithmic nature of the pH designation, since at low pH the change in $[H^+]$ for a given change in pH is greater than at high pH. The large change in $[H^+]$ for a given rise in P_{CO_2} results in less of an increase in $[HCO_3^-]$ by virtue of the mass action effect (Equation 3) and, hence, the slope of the CO_2 titration curve is diminished. The slope, therefore, depends upon the interaction of the value of β with the initial level of $[HCO_3^-]$.

Bicarbonate concentration ($[HCO_3^-]_{40}$) can vary with disease states. It increases with metabolic alkalosis and decreases with metabolic acidosis. Under these circumstances, one would predict a change in the slope of the CO_2 titration curve, depending upon the interaction between the buffer power and the influence of bicarbonate concentration on the mass action equation. The interaction can be calculated as follows:

$$\Delta[HCO_3^-] = \Delta p\text{H} \times \beta \text{ (see Equation 2)}$$

$$p\text{H} = 6.1 + \log\frac{[HCO_3^-]}{0.0301\ P_{CO_2}}$$

$$p\text{H} + \Delta p\text{H} = 6.1 + \log\frac{[HCO_3^-]_{40} + (\Delta p\text{H}\cdot\beta)}{0.0301\ P_{CO_2}} \quad (4)$$

This equation can be solved for the P_{CO_2} that would exist for any change in pH, assuming any initial bicarbonate concentration and a value for β. The results of such calculations for $[HCO_3^-]_{40}$ of 48, 36, 24, 12 and 6 mEq/l and values for β of 10, 20 and 30 are shown in figure 4.

Two important interactions between buffer power and mass action effect are illustrated:

1. The higher the initial bicarbonate concentration, the greater is the effect of the buffer power (β) on the slope of the CO_2 titration curve (fig. 4).

2. With small rises in P_{CO_2}, the value for β makes very little absolute difference in the amount of HCO_3^- generated except at very high initial bicarbonate concentrations.

The reason for these interactions is shown in figure 5, which is an enlargement of part of the curves of figure 4 with $[HCO_3^-]_{40}$ of 24 and 6 mEq between P_{CO_2} 40 and 50 mm Hg. When $[HCO_3^-]_{40}$ is 24 mEq/l (fig. 5A), the difference between $\beta = 30$ and $\beta = 10$ with a 10 mm rise in P_{CO_2} is 2 nEq/l of $[H^+]$ and 1 mEq/l of HCO_3^-. When $[HCO_3^-]_{40} = 6$ mEq/l (fig. 5B), these same two values of β result in an 8-nEq/l difference in $[H^+]$ and, because of the mass action effect, a smaller difference in $[HCO_3^-]$ of 0.3 mEq/l. The relationships shown in figures 5A and B explain why the difference between *in vivo* and *in vitro* CO_2 titration curves is not readily apparent except with large rises in P_{CO_2} or at high values for $[HCO_3^-]_{40}$.

The *in vitro* studies demonstrated the effect of lowering the $[HCO_3^-]_{40}$ while maintaining

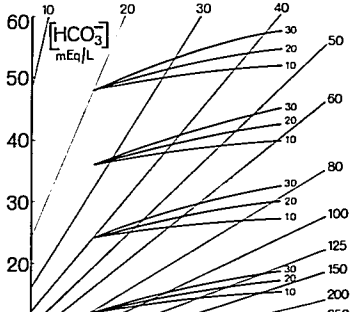

FIG. 4. The calculated CO_2 titration curves of blood *in vitro* at values for β of 30, 20 and 10 at varying levels of plasma $[HCO_3^-]_{40}$. The isopleths represent plasma $[H^+]$ in nEq/l.

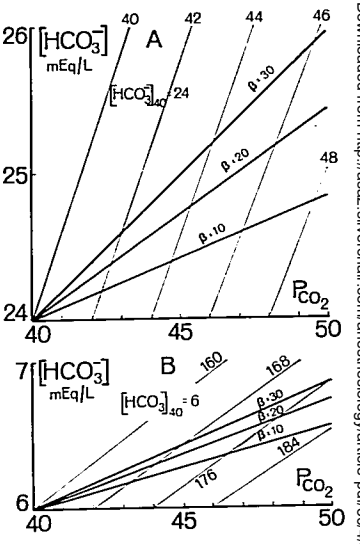

FIG. 5. The calculated effect of increasing P_{CO_2} from 40 to 50 mm Hg on the $[HCO_3^-]$ with β 30, 20 and 10 and plasma $[HCO_3^-]_{40}$ of (A) 24 mEq/l and (B) $[HCO_3^-]_{40}$ 6 mEq/l. The isopleths represent plasma $[H^+]$ in nEq/l.

constant β in the system. Table 1 shows that, as the $[HCO_3^-]_{40}$ was lowered, the slope of the CO_2 titration curve ($\Delta HCO_3^-/\Delta P_{CO_2}$) fell.

In vivo, the slopes of the CO_2 titration curves with decreasing $[HCO_3^-]_{40}$ do not become progressively less. The finding of undiminished slope implies an increase in the buffer power of the system. The calculated values for β shown in figure 2 bear this out.

There are two possible mechanisms for this *in vivo* rise in buffer value which occurred consequent to the acidemia resulting from HCl infusion—the absolute amount of nonbicarbonate buffer (Hgb and/or serum proteins) increased or the volume of the interstitial fluid, which diluted the buffer power of blood, diminished. The latter mechanism seems unlikely, since it has been shown by others[8,9] that there is an increase in extracellular fluid volume with hydrochloric acid infusion.

By calculation, the rise in hematocrit from 40 to 52 per cent with increasing degrees of induced acidemia could account for part, but not all, of the rise in β. However, it must be noted that precise calculation of the increase in hemoglobin concentration or decrease in interstitial fluid that would have been required to increase β from 16 to 23, though possible from the available data, is inappropriate. These calculations are profoundly influenced by small errors in measurement of $[HCO_3^-]$ and *p*H. For example, in figure 4, a P_{CO_2} rise from 40 to 80 mm Hg with $[HCO_3^-]_{40}=9$ mEq/l was accompanied by a fall in *p*H of 0.14 units while the $[HCO_3^-]$ rose 2.8 mEq/l; β was, therefore, 20. However, if an error in measurement of $[HCO_3^-]$ of 0.2 mEq had been made ($HCO_3^- = 2.6$) and *p*H had been overestimated by 0.01, the calculated β would have been 17.

Conclusions

It is only appropriate to conclude that, with progressive acidemia induced by HCl infusion, the CO_2 titration curve did not decrease in slope. This lack of change in slope was the result of an *in vivo* increase in buffer power. This increase represents an interesting homeostatic defense against changes in hydrogen ion concentration when respiratory acidosis is superimposed upon diminished bicarbonate concentration.

It has been reported previously that, when respiratory acidosis is produced by hypoventilation while breathing air, the resultant hypoxia induces superimposed metabolic acidosis through lactic acid accumulation.[10] The *in vivo* CO_2 titration curve is displaced downward from that found when hypoxia is prevented during respiratory acidosis by oxygen breathing. It was observed that the bicarbonate displacement from a theoretical curve was greater than could be accounted for by the lactic acid concentration. The present studies suggest an explanation for the discrepancy. The bicarbonate displacement arising from superimposed metabolic acidosis would, by the mechanisms described above, be expected to diminish the slope of the CO_2 titration curve to a greater extent than could be accounted for by the concentration of displacing acid.

Acidemia produced by HCl infusion differs from that which results from lactic acid accumulation[11] and other forms of metabolic acidosis induced by disease; nonetheless, this homeostatic mechanism demonstrated in these experiments no doubt has a clinical counterpart when respiratory and metabolic acidosis occur together.

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