

# The In-Vitro and In-Vivo Carbon Dioxide Dissociation Curves of True Plasma

## A Theoretical Analysis

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A theoretical study was made of the carbon dioxide dissociation curve of true plasma, both under *in-vitro* and *in-vivo* conditions. Dissociation curves were derived by calculation at a variety of normal and pathologic blood and body fluid compositions and volumes. The nature of the difference between *in-vitro* and *in-vivo* curves was analyzed. The difference between the course of mixed venous and of arterial plasma composition during *in-vivo* CO<sub>2</sub> titration was stressed; and it was pointed out that only mixed venous sampling can be expected to yield accurate *in-vivo* CO<sub>2</sub> titration curves. It was shown that, when blood CO<sub>2</sub> tension is changed *in vivo*, the conventionally derived "true plasma standard bicarbonate" and "blood buffer base" do not remain unchanged, in contrast with their behavior in *in-vitro* CO<sub>2</sub> titration. Methods were given to correct these two indices, so that they become invariant with the CO<sub>2</sub> tension at which the sample is withdrawn, and thus can serve their function as measures of fixed acid excess or deficit.

THE CLASSICAL analysis of the Acid-Base status of the blood has resulted in a rather complete picture of the steady state electrolyte distribution between red cells and plasma, both under normal and abnormal conditions. The blood used for such studies was removed from

the vascular bed, and then submitted, *in vitro*, to various modifying conditions. In the living organism, however, blood is in contact, across the capillary walls, with the large interstitial fluid space; this space, in turn, can exchange ions with the intracellular fluid. Under such *in-vivo* conditions the changes in ionic concentrations of plasma and red blood cells, resulting from imposed alterations in CO<sub>2</sub> tension or in fixed acid content, are different from the changes which occur when *in-vitro* blood is exposed to these alterations. It is the purpose of this paper to analyze the nature of these differences, as well as their magnitude.

The difference between the *in-vitro* and *in-vivo* behavior of blood, which was first demonstrated by Shaw and Messer,<sup>1</sup> can be clarified by comparing the changes in acid-base balance when a sample of mixed venous blood is equilibrated *in vitro* to a different P<sub>CO<sub>2</sub></sub>, with the changes occurring when this same mixed venous blood is brought *in vivo* to the same new CO<sub>2</sub> tension. The *in-vivo* equilibration can be accomplished by changing either alveolar ventilation or inspired P<sub>CO<sub>2</sub></sub>. The changes in plasma (and also in red cell) bicarbonate concentration and pH which accompany a particular change in P<sub>CO<sub>2</sub></sub> are determined by the buffering capacity of the medium. This buffering capacity is, in turn, a function mainly of hemoglobin and plasma protein concentrations. The greater the concentrations of these substances, the greater will be the change in bicarbonate concentration, and the less that in pH, resulting from a particular change in CO<sub>2</sub> tension. In an *in-*

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*in vitro* blood sample the pertinent concentrations of the buffer substances refer, of course, to the blood by itself. When CO<sub>2</sub> equilibration is accomplished *in vivo*, not only the blood volume, but also the interstitial fluid volume is titrated with CO<sub>2</sub>. Since the interstitial fluid normally contains no hemoglobin and much less protein than does plasma, the average concentrations of these buffer substances in the mixed "pool" (blood volume plus interstitial fluid volume) are considerably lower than those in blood alone. It can therefore be expected that the same change in P<sub>CO<sub>2</sub></sub> results in a smaller change in bicarbonate concentration, and in a larger change in pH of the pool, and thus of blood plasma, under *in vivo* conditions than in *in vitro*. A more rigorous analysis of *in vivo* equilibration can be found in the Appendix.

Not only the blood and interstitial fluid, but also the interior of the fixed cells is brought to a new P<sub>CO<sub>2</sub></sub> by *in vivo* equilibration. We shall assume, until stated otherwise, that no net exchanges of any ions occur across the walls of the tissue cells. It will also be assumed that the new steady state of acid-base balance has been maintained for a sufficiently short length of time so that changes in renal electrolyte excretion will have had no significant effect on the ionic composition of the extracellular compartment.

Compare now the two titration curves of true plasma (*i.e.*, plasma kept in contact with its red cells during the process of equilibration): one obtained by *in vitro* equilibration of a sample of mixed venous blood at different CO<sub>2</sub> tensions, the other by *in vivo* exposure of this blood, together with the interstitial compartment, to different CO<sub>2</sub> tensions by means of ventilatory maneuvers. We have selected mixed venous, rather than arterial blood, because the electrolyte composition and CO<sub>2</sub> tension of its plasma water, in the steady state of respiration, circulation and metabolism, should be nearly equal to those of the water in the interstitial fluid compartment. For simplicity's sake we shall assume, for the time being, full oxygenation of the mixed venous blood. The *in vitro* titration with CO<sub>2</sub> would lead to a curve such as Aa in figure 1. When CO<sub>2</sub> titration is accomplished *in vivo*, a titration curve such as AB would be obtained.

TABLE 1. Concentrations of Nonbicarbonate Buffer Substances in Blood and Pool\*, and Slopes of *In-Vitro* and *In-Vivo* CO<sub>2</sub> Titration Lines of Oxygenated Mixed Venous Plasma, at Four Values of Blood Hematocrit (V<sub>c</sub>) and Corresponding Values of Pool Hematocrit (V<sub>c</sub>').

V <sub>c</sub>	0.15	0.30	0.45	0.60
Hb conc. (mM/l. blood)	3.0	6.0	9.0	12.0
Pl. Prot. conc. (g./l. blood)	61.2	56.4	39.6	28.8
<i>In-vitro</i> slope at [BBB]=49	12.8	19.3	26.9	36.6
V <sub>c</sub> '	0.057	0.115	0.172	0.229
Hb conc. (mM/l. pool)	1.15	2.29	3.44	4.58
Protein conc. (g./l. pool)	36.4	32.3	28.1	24.0
<i>In-vitro</i> slope at [PBB]=38	5.9	7.9	10.1	12.4

\* Blood Volume + interstitial fluid volume. Slopes at average normal blood buffer base [BBB] of 49 and pool buffer base [PBB] of 38.

[BBB] and [PBB] do not include carbamino-CO<sub>2</sub>. Its concentration amounts to 0.33, 0.66, 1.0, and 1.33 mEq/liter oxygenated blood at blood hematocrit 0.15, 0.30, 0.45, and 0.60, respectively; and 0.13, 0.26, 0.38, and 0.51 mEq/liter oxygenated pool at the corresponding values of pool hematocrit.

Blood volume at all hematocrits 5.5 l., interstitial fluid volume (including lymph) 8.9 l., with protein concentration of 21 g./l. Red cell hemoglobin concentration 20 mM/l.; plasma protein concentration 72 g./l.

Both are straight line relations between bicarbonate concentration of true plasma and plasma pH; but the *in vitro* line is steeper than the *in vivo* one. The linearity is a reflection of the nearly constant buffering powers of both oxyhemoglobin and plasma proteins over the relatively narrow range of physiologic pH (6.8-7.8); buffering power being defined as the amount of strong acid (or base) required to change pH by one unit. It is this linearity which makes the plotting of bicarbonate concentration against pH so convenient.\* The difference in slopes ( $\Delta[\text{HCO}_3^-]_v/\Delta\text{pH}_v$ ) of the two titration lines is due to the presence of greater concentrations of oxyhemoglobin and non-hemoglobin proteins in the blood than in the pool (blood volume plus interstitial fluid volume); the CO<sub>2</sub> titration slope is a quantitative index of the buffering power of these two fluids.

The *in vitro* titration slope of true plasma of normal blood (hematocrit 0.45, plasma proteins 72 g./liter) amounts to 26.9, while at normal blood volume (5.5 liters) and normal interstitial fluid volume (8.9 liters) and composition (protein content 21 g./liter) the *in vivo* slope of the true plasma of this same

\* Surveys of acid-base balance which employ this graphic method are those of Davenport<sup>2</sup> and of Woodbury.<sup>7</sup>

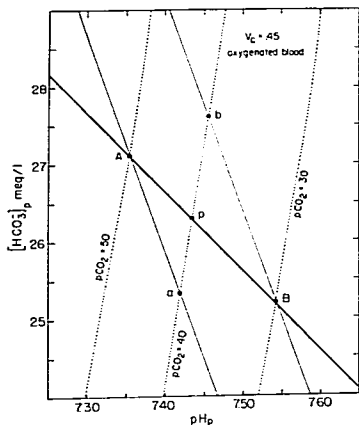


FIG. 1. Normal *in-vitro* CO<sub>2</sub> titration curve of true oxygenated mixed venous plasma (AB), and two *in-vitro* curves (Aa and Bb), obtained on mixed venous blood withdrawn at P<sub>CO<sub>2</sub></sub> = 50 (A) and 30 (B), respectively. For details see text.

blood is only 10.1, less than half the *in-vitro* value (table 1). The derivation of the slope values is given in the Appendix.

The *in-vitro* titration line in figure 1 has been positioned to include the point which represents average normal mixed venous plasma at 100 per cent oxygen saturation: pH = 7.35, [HCO<sub>3</sub><sup>-</sup>]<sub>p</sub> = 27.2, P<sub>CO<sub>2</sub></sub> = 51 (table 2). Point A, the point common to both lines, represents the plasma composition (P<sub>CO<sub>2</sub></sub> = 50), prevailing when the sample was removed from the circulation for *in-vitro* equilibration. If the subject had been hyperventilated to reduce his mixed venous P<sub>CO<sub>2</sub></sub> to 30 (point B), the results of *in-vitro* equilibration of a sample withdrawn at this lower P<sub>CO<sub>2</sub></sub> would be represented by the straight line Bb. This line has a slope nearly equal to that of Aa because the blood withdrawn at B has nearly the same buffering power as that withdrawn at A.

The CO<sub>2</sub> tension at any point of a titration line can be derived from the Henderson-Hasselbalch equation: pH<sub>p</sub> = 6.11 + log ([HCO<sub>3</sub><sup>-</sup>]<sub>p</sub>/0.0311 P<sub>CO<sub>2</sub></sub>), where 6.11 is the pK<sub>a</sub>' of carbonic acid in plasma, and 0.0311

the solubility factor of CO<sub>2</sub> in plasma. For the purpose of orientation, a family of curves can be added to the diagram, which represent the relation between plasma bicarbonate concentration and pH at fixed values of CO<sub>2</sub> tension. For instance, at P<sub>CO<sub>2</sub></sub> = 40 this relation can be expressed by pH<sub>p</sub> = 6.11 + log ([HCO<sub>3</sub><sup>-</sup>]<sub>p</sub>/0.0311 × 40), and thus log [HCO<sub>3</sub><sup>-</sup>]<sub>p</sub> = pH<sub>p</sub> - 6.015. Figure 1 shows 3 such logarithmic curves, at P<sub>CO<sub>2</sub></sub> values of 30, 40 and 50 mm. of mercury.

The relation between the values for "standard bicarbonate" under the three conditions can now be obtained. As will be recalled, standard bicarbonate, a measure of fixed acid or base excess, is defined as the bicarbonate concentration of true plasma at P<sub>CO<sub>2</sub></sub> = 40 mm. of mercury; the hemoglobin being fully oxygenated. When oxygenated mixed venous blood whose *in-vitro* titration line is represented by AB in figure 1 is brought *in vivo* to this P<sub>CO<sub>2</sub></sub>, the composition of its plasma is represented by point p. On the other hand, when mixed venous blood, withdrawn at P<sub>CO<sub>2</sub></sub> higher than 40 (A), is equilibrated at 40 in the conventional way, that is *in vitro*, its standard bicarbonate is less (a), while blood withdrawn at P<sub>CO<sub>2</sub></sub> less than 40 (B), has an *in-vitro* standard bicarbonate value greater (b) than that under *in-vitro* conditions. Thus the *in-vitro* obtained standard bicarbonate varies with the CO<sub>2</sub> tension at which the blood was withdrawn. The example in figure 1 shows a normal value for *in-vitro* standard bicarbonate of 26.3 mEq./liter, and *in-vitro* values of 25.3 and 27.5 mEq./liter when the mixed venous blood is sampled at 50 and 30 mm. of mercury P<sub>CO<sub>2</sub></sub> respectively.

A second commonly used measure of fixed acid or base excess is the "blood buffer base," defined as the sum of the blood buffer anion concentrations: Hb<sup>-</sup>, plasma proteins<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, and carbamino CO<sub>2</sub><sup>-</sup>, all expressed per liter blood; *i.e.*, the difference between total blood cation concentration and that of the fixed anions (mainly Cl<sup>-</sup>). Since these two concentrations remain unchanged in an *in-vitro* blood sample, equilibrated at various CO<sub>2</sub> tensions, *in-vitro* blood buffer base is also constant, that is, invariant with CO<sub>2</sub> tension.

However, *in vivo*, it is the buffer base of the pool which remains unchanged at varying

$\text{CO}_2$  tensions for similar reasons; the buffer base of the blood component of the pool will fall with increasing  $\text{CO}_2$  tension. This latter point can be appreciated from figure 1. Let mixed venous blood, withdrawn at B, be equilibrated *in vitro* to a higher  $\text{CO}_2$  tension, so that pH falls; then compare this blood with mixed venous blood brought *in vivo* from B to the same pH by raising  $\text{CO}_2$  tension. Both bloods contain the same concentrations of plasma proteins<sup>-</sup> and  $\text{Hb}^-$ , since these ionic concentrations are a unique function of plasma pH (see Appendix). Carbamino  $\text{CO}_2$  is also the same, since its concentration, in the physiologic  $\text{P}_{\text{CO}_2}$  range, is almost solely determined by oxygen saturation. But, as figure 1 shows, the *in-vivo* equilibrated blood contains a lower concentration of plasma bicarbonate, and thus of blood bicarbonate, than the sample equilibrated *in vitro*. Therefore, the blood buffer base of the *in-vivo* blood is less than that of the *in-vitro* sample at the same pH, and the *in-vivo* blood buffer base has fallen with increased  $\text{CO}_2$  tension. At normal hematocrit, blood buffer base of fully oxygenated mixed venous blood falls by 2.5 mEq./liter when  $\text{P}_{\text{CO}_2}$  is raised *in vivo* from 30 to 50 mm. of mercury.

We shall now examine the relation between the acid-base status of mixed venous and of arterial plasma under various conditions. Again, both bloods are assumed to be fully oxygenated. In the steady state of respiration, circulation, and metabolism there is no net change with time in the composition of interstitial fluid. Therefore the changes which the arterial blood undergoes in passage through the capillaries, are identical with those which it would undergo, if it were exposed *in vitro* to the  $\text{CO}_2$  tension prevailing in the interstitial fluid. Thus the arterial and mixed venous points will fall on a line with *in-vitro* slope (26.9) (figure 2,  $\bar{v}_1 a_1$ ). For the same reason, all venous points, representing the plasma bicarbonate and pH values of venous blood from each of the various tissue regions, will fall on this *in-vitro* titration line; again assuming full oxygenation throughout. It follows that the *in-vitro* standard bicarbonate, and also blood buffer base, will be the same for all samples of blood drawn from all regions of the vascular bed during the steady state.

Let us now reduce cardiac output, while maintaining constant metabolic  $\text{CO}_2$  production, as well as arterial  $\text{CO}_2$  tension (figure 2). The mixed venous blood plus interstitial fluid will be titrated to a higher  $\text{CO}_2$  tension, and the point representing its new steady state ( $\bar{v}_2$ ) will fall on the *in-vitro* line (slope 10.1) through the original mixed venous point ( $\bar{v}_1$ ). This new mixed venous point and the corresponding arterial point ( $a_2$ ) will once again fall on a line with *in-vitro* slope; but not on the original line. The new line ( $\bar{v}_2 a_2$ ) is located below the original one. This means that the arterial plasma composition has not remained unchanged even though its  $\text{CO}_2$  tension had been kept fixed at 40 mm. of mercury: as a result of the reduction in cardiac output arterial plasma bicarbonate concentration has fallen, as has pH. Figure 2 illustrates an example, in which cardiac output is reduced to half of its normal value; this leads to a fall in arterial plasma bicarbonate concentration of 1 mEq./liter, and a fall in pH of 0.02. Details of arterial and mixed venous blood composi-

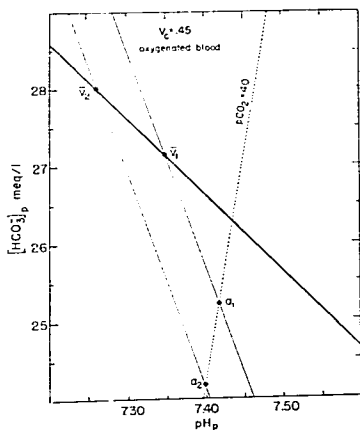


FIG. 2. Normal *in-vitro*  $\text{CO}_2$  titration curve of true oxygenated mixed venous plasma ( $\bar{v}_1 \bar{v}_2$ ).  $a_1$  and  $\bar{v}_1$  represent plasma compositions of, respectively, arterial and mixed venous blood at control conditions;  $a_2$  and  $\bar{v}_2$  plasma compositions after reduction of cardiac output to half normal. The detailed blood compositions are given in table 2. For discussion see text.

TABLE 2. Composition of Arterial and Mixed Venous Blood (Assuming Full Oxygen Saturation) at Normal Circulatory, Ventilatory and Metabolic rates (Control), After Reduction of Cardiac Output to Half Normal (Condition 1), and After Increase in Arterial CO<sub>2</sub> Tension to 58.2 mm. of Mercury, Accompanied by Increase in Cardiac Output of 77 Per Cent (Condition 2).

	Control		Condition 1		Condition 2	
	a	$\bar{v}$	a	$\bar{v}$	a	$\bar{v}$
O <sub>2</sub> saturation (%)	100.0	100.0	100.0	100.0	100.0	100.0
P <sub>CO<sub>2</sub></sub> (mm. Hg)	40.0	50.5	40.0	63.5	58.2	65.6
pH <sub>p</sub>	7.420	7.348	7.399	7.262	7.287	7.250
pH <sub>e</sub>	7.200	7.150	7.190	7.091	7.108	7.083
[HCO <sub>3</sub> <sup>-</sup> ] <sub>p</sub> (mEq./l. plasma)	25.20	27.17	24.20	28.04	27.20	28.17
[HCO <sub>3</sub> <sup>-</sup> ] <sub>e</sub> (mEq./l. cells)	12.89	14.50	12.60	15.93	15.18	16.15
[carbamino CO <sub>2</sub> ] <sub>e</sub> (mEq./l. blood)	1.0	1.0	1.0	1.0	1.0	1.0
[total CO <sub>2</sub> ] <sub>e</sub> (mEq./l. blood)	21.82	23.93	21.14	25.43	24.47	25.66
$\Delta$ [total CO <sub>2</sub> ] <sub><math>\bar{v}</math>-a</sub> (mEq./l. blood)		2.11		4.29		1.19

Hematocrit 0.45. For details see text.

pH<sub>e</sub> was obtained from equation (9) (Appendix), [HCO<sub>3</sub><sup>-</sup>]<sub>e</sub> from the Henderson-Hasselbalch equation applied to red cells (CO<sub>2</sub> solubility factor 0.0262).

tions are given in table 2. In figure 2 arterial CO<sub>2</sub> tension was maintained at 40 mm. of mercury, but the results would of course be similar at any other fixed value for arterial CO<sub>2</sub> tension. Therefore, an arterial (or, for that matter, any other) blood sample withdrawn under the initial conditions and equilibrated *in vitro* to a CO<sub>2</sub> tension of 40 mm. of mercury will yield a higher value for standard bicarbonate than a sample withdrawn at the same arterial P<sub>CO<sub>2</sub></sub>, but after cardiac output has been reduced. An increase in cardiac output will have the reverse effect.

The effect of an increase in arterial CO<sub>2</sub> tension, such as would be the consequence of a reduction in alveolar ventilation, on arterial and mixed venous plasma compositions will now be examined (figure 3). The new mixed venous point ( $\bar{v}_2$ ) must of course lie on the *in-vitro* titration line through the original mixed venous point ( $\bar{v}_1$ ). The new arterial point ( $a_2$ ) will fall on the *in-vitro* titration line through the new mixed venous point. It should be noted that there is no predictable relation between the location of the two arterial points,  $a_1$  and  $a_2$ : in general the line joining them will have neither *in-vitro* slope nor *in-vitro* slope. If the increase in CO<sub>2</sub> tension is associated with an increase in cardiac output, the slope of  $a_1a_2$  will be steeper than that of the *in-vitro* titration line; this situation is depicted in figure 3, when an increase in

cardiac output of 77 per cent was assumed as a consequence of an increase in arterial P<sub>CO<sub>2</sub></sub> from 40 to 58.2 mm. of mercury (see table 2 for details). If a fall in cardiac output accompanies the rise in CO<sub>2</sub> tension, the slope of  $a_1a_2$  will generally be less than that of the *in-vitro* line. Only when the reduction in cardiac output happens to be such that the new venous-arterial plasma bicarbonate difference is identical with the original one, will the two arterial points fall on a line with *in-vitro* slope, and thus parallel to  $\bar{v}_1\bar{v}_2$ .

Figure 3 also illustrates the course of arterial and mixed venous plasma compositions during the attainment of a new steady state. Thus the mixed venous point will slide up along the *in-vitro* line from its original position (heavy dashed line). If we assume that the arterial CO<sub>2</sub> tension had been raised instantaneously to the new value, the arterial point will instantaneously rise along the *in-vitro* line to a new position (m), characterized by the new CO<sub>2</sub> tension (58.2 mm. of mercury), after which it will slide down the 58.2 isobar to the new steady state,  $a_2$  (heavy line). The diagram demonstrates the possible development of a negative  $\bar{v}$ -a plasma bicarbonate difference during the transition period. Woodbury<sup>3</sup> has given a comparable presentation of the transition.

These examples illustrate the contrast between the courses of the mixed venous and

arterial plasma compositions. The mixed venous point always moves along the *in-vivo* titration line, either as a consequence of a change in alveolar  $P_{CO_2}$ , a change in cardiac output, or a combination of the two. The course of the arterial point is not so predictable; all that can be said is that after a new steady state has been accomplished, it is located, once again, with the new mixed venous point on a titration line with *in-vitro* slope. Therefore, arterial samples obtained at different levels of alveolar  $P_{CO_2}$  will, in general, yield an erroneous value for the slope of the *in-vivo*  $CO_2$  titration line.

If the blood samples are obtained from a peripheral vein, for instance an arm vein, their pH and bicarbonate values will yield the correct *in-vivo* slope only when these values differ by a constant amount from the corresponding mixed venous values. Since such a constant difference would be a coincidence, peripheral venous samples will generally also not give an accurate measure of the *in-vivo* titration slope, even if the regional circulatory rate were to remain constant.

Thus far it has been assumed that hemoglobin is fully saturated with oxygen throughout the vascular bed. While this is approximately correct for normal arterial blood, it is of course not the case for venous blood. The  $CO_2$  titration line at a particular degree of oxygen desaturation is located above the line for full saturation, and runs nearly parallel to it. The displacement is due to the lesser acidity of reduced hemoglobin, as compared to oxyhemoglobin; the near-parallel course to the fact that in the physiologic pH range the buffering powers of the two compounds are nearly identical. The maximal displacement of the normal *in-vitro* true plasma titration line, due to complete reduction of fully oxygenated hemoglobin at hematocrit 0.45, amounts to 0.10–0.13 pH unit,<sup>4,5</sup> corresponding to a vertical displacement of about 2.7 mEq.  $HCO_3^-$  per liter plasma; the displacement is proportionate to the degree of desaturation. The maximal displacement of the *in-vivo* true plasma titration line is much less, since the hemoglobin concentration in the *in-vivo* "pool" is only 0.38 of that in blood (table 1); it can be calculated to amount to about 0.8 mEq.  $HCO_3^-$  per liter plasma.

Therefore the relatively small changes in mixed venous oxygen saturation which accompany alterations in cardiac output will affect the position of the *in-vivo* true plasma titration line of mixed venous blood only very slightly.

We are now in a position to examine the changes in mixed venous and arterial blood compositions resulting from breathing  $CO_2$ -enriched air (figure 4). Start with the condition found in normal resting man at sea level. Arterial and mixed venous plasma values are plotted as  $a_1$  and  $\bar{v}_1$ . Note that  $a_1$  and  $\bar{v}_1$  fall on different (but parallel) *in-vitro* titration lines (slope 26.9), 0.02 pH unit apart, since their oxygen saturations differ by 20 per cent. Arterial  $P_{CO_2}$  is now raised from 40 to 58.2 mm. of mercury, and the arbitrary

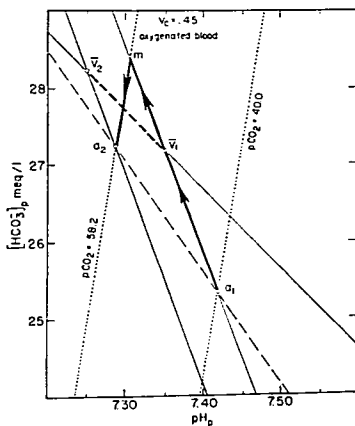


FIG. 3. Normal *in-vitro*  $CO_2$  titration curve of true oxygenated mixed venous plasma ( $\bar{v}_1\bar{v}_2$ ).  $a_1$  and  $\bar{v}_1$  represent plasma compositions of, respectively, arterial and mixed venous blood at control conditions;  $a_2$  and  $\bar{v}_2$  plasma compositions after increase in arterial  $P_{CO_2}$  from 40 to 58.2 mm. of mercury, accompanied by increase in cardiac output of 77 per cent. The detailed blood compositions are given in table 2. Assuming instantaneous rise of arterial  $P_{CO_2}$  to the new value, the course of arterial plasma composition is given by the heavy continuous line  $a_1\bar{v}_1a_2a_2$ , that of mixed venous plasma by the heavy interrupted line  $\bar{v}_1\bar{v}_2$ . The interrupted line  $a_2a_2$  represents the erroneous *in-vitro* curve derived from arterial sampling. For discussion see text.

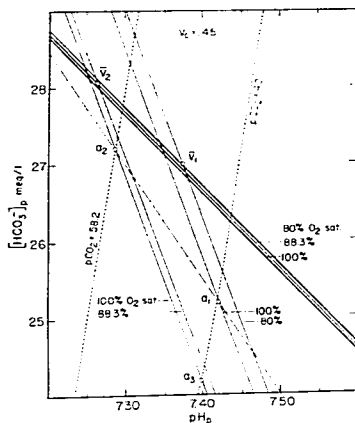


FIG. 4. *In-vitro* CO<sub>2</sub> titration curves of true mixed venous plasma, at 3 levels of oxygenation (3 heavy parallel lines).  $a_1$  and  $\bar{v}_1$  represent plasma compositions of, respectively, arterial and mixed venous blood under normal resting conditions;  $a_2$  and  $\bar{v}_2$  plasma compositions after increase in arterial  $P_{O_2}$  from 40 to 58.2 mm. of mercury, accompanied by increase in cardiac output of 71 per cent. The detailed blood compositions are given in table 3. The interrupted line  $a_2\bar{v}_2$  represents the erroneous *in-vitro* curve derived from arterial sampling. The ordinates of  $a_1$  and  $a_2$  represent the standard bicarbonate concentrations under the two conditions. For discussion see text.

but reasonable " assumption is made that this is accompanied by an increase in cardiac output of 71 per cent; respiratory quotient and metabolism remaining unchanged. Mixed venous oxygen saturation will increase to 88.3 per cent; the new plasma composition will be represented by  $\bar{v}_2$  in figure 4, which lies nearly on the same *in-vitro* line as  $\bar{v}_1$  (slope 10.1). The arterial point will move to  $a_2$ . Details of the blood compositions at the two conditions are given in table 3. Standard bicarbonate (as determined conventionally *in vitro*) will fall with increasing CO<sub>2</sub> tension from 25.20 ( $a_1$ ) to 24.20 ( $a_2$ ); each of these values of course pertaining to arterial, mixed venous or any peripheral venous blood, withdrawn during the particular steady state. Blood buffer base will also fall (from 49.05 to 47.72). These values similarly pertain to

blood from any region; this can be appreciated when it is recalled that in the steady state the changes undergone by the blood in passage through the capillaries are equivalent to those resulting from *in-vitro* equilibration, and that blood buffer base is quantitatively equal to the difference between total cation and fixed anion concentrations, both of which are invariant with  $P_{O_2}$  and  $P_{CO_2}$  under *in-vitro* conditions.

If arterial samples were taken at the 2 conditions depicted in figure 4, they would lead to an erroneous *in-vitro* titration slope of 15.0 ( $a_1a_2$ ), 50 per cent steeper than the correct one of 10.1 ( $\bar{v}_1\bar{v}_2$ ). Of course the error will vary with the magnitude of the circulatory response. It can be calculated that only if the cardiac output would fall by 15 per cent as a consequence of the increase in arterial CO<sub>2</sub> tension from 40 to 58.2 mm. of mercury, would arterial sampling yield a faithful value for the *in-vitro* titration slope.

This sensitivity to changes in circulatory rate places a limit on the usefulness of arterial sampling as a means of constructing accurate *in-vitro* CO<sub>2</sub> titration curves. Since the circulatory response to changes in CO<sub>2</sub> tension varies even among normal subjects,<sup>6</sup> the "normal" *in-vitro* CO<sub>2</sub> titration curve based on the composition of arterial plasma samples will show corresponding variation. Moreover, arterial curves obtained from individuals suffering from circulatory disorders, or from patients under general anesthesia may fall outside of the normal range solely because their circulatory response to CO<sub>2</sub> deviates from normal.

Brackett *et al.*<sup>7</sup> exposed 7 normal resting volunteers to room air and to mixtures of 7 or 10 per cent CO<sub>2</sub> with 21 per cent O<sub>2</sub> for 15-90 minutes. Arterial CO<sub>2</sub> tensions ranged from 34 to 90 mm. of mercury. From the measurements on arterial true plasma, an *in-vitro* CO<sub>2</sub> titration slope can be derived of 11.6 (standard deviation of the slope 1.4). This slope is 15 per cent greater than the average normal true (mixed venous) slope calculated by us, probably because of some increase in cardiac output in response to the elevated CO<sub>2</sub> tensions.

Considerably greater *in-vitro* slopes (15.0 and 16.2) were obtained by Michel *et al.*<sup>8</sup> on 2 normal subjects in whom arterialized

blood was sampled from a superficial vein of the forearm. The subjects breathed 2, 5 or 6 per cent  $\text{CO}_2$  in air for 15 to 80 minutes, or voluntarily hyperventilated on air for 40-50 minutes at a rate of 10 liters per minute. Blood  $\text{CO}_2$  tensions ranged from about 25 to 50 mm. of mercury. A 70 per cent increase in cardiac output with hypercapnia would account for the discrepancy between these experimental slopes and the theoretical one, as was demonstrated above; however, some increase in circulation might also have occurred with hypocapnic hyperventilation<sup>6</sup> thus tending to reduce the slope.

Even if mixed venous sampling were routinely feasible, the experimental *in-vivo*  $\text{CO}_2$  titration curve will be affected by a number of variables which must now be briefly examined.

**Volume and Composition of Blood and Interstitial Fluid.** Our calculations assumed an interstitial fluid space (including lymph) of 8.9 liters, containing protein in an average concentration of 21 g./liter and with a buffering power, equal to that of plasma proteins. Blood volume was taken as 5.5 liters, hematocrit 0.45 and plasma protein concentration 72 g./liter. (For details see Appendix.) Even in normal man, and certainly under pathologic conditions, significant deviations from these normal average values can occur. Variations in protein concentrations of plasma or of interstitial fluid affect the titration slopes to only a small extent, since these proteins are re-

sponsible for relatively small fractions of the total non-bicarbonate buffering of blood. Thus, reducing plasma protein concentration to half the normal value (35 g./liter) changes the *in-vivo* slope only from 10.1 to 9.2, the *in-vitro* slope from 26.9 to 23.3; halving interstitial protein concentration (to 10.5 g./liter) reduces the *in-vivo* slope to 9.3, while the *in-vitro* slope of course remains unchanged. Changes in the volumes of the spaces have more pronounced effects: doubling of the interstitial space to 17.8 liters, while maintaining its protein concentration unchanged, reduces the *in-vivo* slope to 6.9; reduction of the space to half of its normal volume (4.45 liters) increases to *in-vivo* slope to 14.6. A reduction of blood volume by 25 per cent (1.4 liters) by itself leads to an *in-vitro* titration slope of 8.6. Changes in blood hematocrit have a significant effect both on *in-vivo* and on *in-vitro* slopes, as is shown in table 1.

Under clinical conditions both volume and protein content of the extracellular spaces might deviate from normal. A few examples follow. In a severe case of congestive heart failure the interstitial space may be doubled, and contain only negligible amounts of protein, while blood volume and its protein content may be normal. The *in-vivo* slope will then be reduced to 4.9. If in addition the plasma protein concentration is half the normal value, such as might be the case in severe hypoproteinemia, the *in-vivo* slope is further lowered to 4.5. After a severe blood loss of 1.4 liters

TABLE 3. Composition of Arterial and Mixed Venous Blood (Hematocrit 0.45) at Normal Circulatory, Ventilatory and Metabolic Rates (Control) and After Increase in Arterial  $\text{CO}_2$  Tension to 58.2 mm. of Mercury, Accompanied by Increase in Cardiac Output of 71 Per Cent (Condition 1).

	Control		Condition 1	
	a	$\bar{v}$	a	$\bar{v}$
$\text{O}_2$ saturation (%)	100.0	80.0	100.0	88.3
$\text{PCO}_2$ (mm. Hg)	40.0	47.0	58.2	63.5
$\text{pH}_p$	7.420	7.374	7.287	7.263
$\text{pH}_i$	7.200	7.170	7.108	7.092
$[\text{HCO}_3^-]_p$ (mEq./l. plasma)	25.20	26.86	27.20	28.07
$[\text{HCO}_3^-]_i$ (mEq./l. cells)	12.89	14.13	15.18	15.96
[carbamino $\text{CO}_2$ ] <sub>b</sub> (mEq./l. blood)	1.00	1.50	1.00	1.28
[total $\text{CO}_2$ ] <sub>b</sub> (mEq./l. blood)	21.82	23.99	24.47	25.74
$\Delta$ [total $\text{CO}_2$ ] <sub>b-a</sub> (mEq./l. blood)		2.17		1.27

For details see text.



compensatory fluid shifts may lead to restoration of normal blood volume (5.5 liters), with resulting lowering of hematocrit to 0.34 and of plasma protein concentration to 45 g./liter. Interstitial fluid volume and protein concentration may not have been significantly affected. The slope of the *in-vivo* titration curve would fall to 7.7, that of the *in-vitro* curve to 18.3.

**Time Required for Steady State.** Our calculations assumed that the exposures to abnormal  $\text{CO}_2$  tensions were of relatively short duration, though long enough to reach a new steady state. This time depends to a great extent on the blood flow through the muscles, both because they form about half of the total body mass, and because under resting conditions their perfusion rate (per unit mass) is very much less than that of the remaining tissues.<sup>9</sup> In conscious normal resting man an equilibration period of 20 minutes is probably sufficient to at least approach the steady state. The kinetics of  $\text{CO}_2$  uptake are discussed by Woodbury,<sup>2</sup> who points out that the drastic reduction in muscle flow which may occur during general anesthesia can be expected to prolong the time required for the new steady state to be reached.

**Intracellular-Extracellular Ionic Movements.** Our calculations of the *in-vivo* titration curve

are predicated on a closed extracellular pool, except with respect to  $\text{CO}_2$  and  $\text{O}_2$ . In exposure to abnormal  $\text{CO}_2$  tensions for not longer than 1½ hours (as in the above two studies) there are probably no significant contributions of renal adjustments to extracellular electrolyte composition. The possibility that ionic exchanges between extracellular and intracellular fluid might affect the extracellular electrolyte composition must still be examined. Some elevation in blood lactate was found in the quoted experiments of Michel *et al.*<sup>8</sup> as a consequence of hypocapnia; lactate was unchanged during hypercapnia. In the studies of Brackett *et al.*: plasma phosphate, Na and K slightly increased with 10 per cent  $\text{CO}_2$  breathing, while blood lactate and pyruvate did not change. These are the only two such studies carried out in man, and seem to indicate that at least in hypercapnia of relatively short duration, ionic shifts across fixed cell membranes are of only minor importance, and should not affect the *in-vivo*  $\text{CO}_2$  titration slope to a significant extent. However, it is well known that their effect will increase with time; after 24 or more hours of exposure to each elevated  $\text{CO}_2$  level, the net result of transmembrane exchanges and renal adjustments is an *in-vivo*  $\text{CO}_2$  titration slope which,

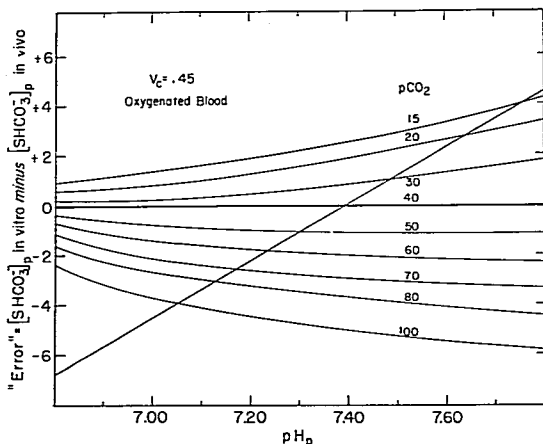
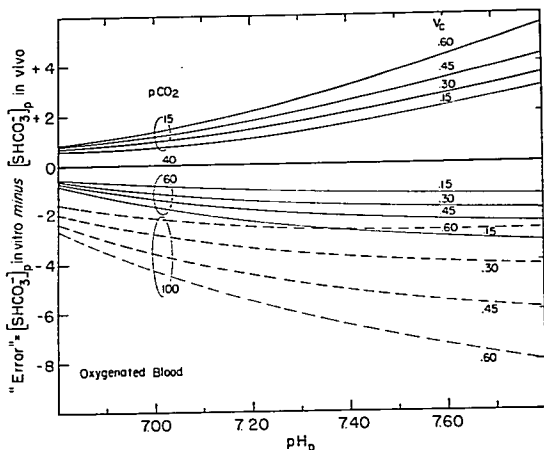


FIG. 5. "Error" in conventionally determined (*in vitro*) standard bicarbonate concentrations, for oxygenated blood samples of various  $\text{pH}_p$  and  $\text{P}_{\text{CO}_2}$ , at hematocrit 0.45. The "error" is defined as standard bicarbonate *in vitro* minus that *in vivo*; that is, subtraction of the "error" from the *in-vitro* value will give in *in-vivo* value. As an example, at  $\text{pH}_p = 7.00$  and  $\text{P}_{\text{CO}_2} = 70$ ,  $-2.2$  mEq. should be subtracted, *i.e.*,  $2.2$  mEq. added to the conventional value for standard bicarbonate concentration. The diagonal curve connects all plasma compositions with *in-vitro* standard bicarbonate concentration of 24 mEq./liter; the points to the left of the curve represent metabolic acidosis, to the right metabolic alkalosis.

FIG. 6. "Error" in conventionally determined (*in vitro*) standard bicarbonate concentration, for oxygenated blood samples of various hematocrit,  $pH_p$  and  $P_{CO_2}$ . The use of this figure is similar to that of figure 5.



in the dog, even exceeds the normal *in-vitro* value.<sup>10</sup>

### Practical Considerations

As much as half a century ago, it was felt to be desirable to separate the blood acid-base deviations due to abnormal  $CO_2$  tension from those due to accumulation of fixed acids or bases. Early studies proposed the use of a single  $P_{CO_2}$ -invariant parameter to serve as a quantitative measure of the fixed acid excess or deficit. Hasselbalch<sup>11</sup> suggested the measurement of plasma  $pH$  after whole blood at  $38^\circ C$ . had been equilibrated with carbon dioxide at 40 mm. of mercury. Van Slyke and Cullen<sup>12</sup> introduced plasma  $CO_2$  combining power, defined as bicarbonate concentration of separated plasma, equilibrated at room temperature with air containing 5.5 per cent  $CO_2$ . Most clinical workers since then have indorsed the usefulness of such a  $CO_2$ -invariant parameter, and at the present time two indices of fixed acid excess or deficit are in wide use: whole blood buffer base concentration<sup>13</sup> and true plasma standard bicarbonate concentration.<sup>14</sup> The accumulation of detailed knowledge of the acid-base relations of blood has made it even unnecessary to actually measure the index experimentally: from the composi-

tion of native blood it can be derived by means of a nomogram or an appropriate family of graphs.<sup>13, 15</sup>

Recently the suggestion has been made to refer blood composition to the "normal" arterial (*in vivo*)  $CO_2$  titration curve of true plasma, and to discontinue the use of derived indices.<sup>7</sup> This curve is affected by the circulatory changes in response to  $CO_2$ , as was explained above; moreover it is quite sensitive to hematocrit (see table 1), so that a family of reference curves would actually have to be used. In view of the fact that fifty years of clinical experience have vindicated the practical usefulness of a fixed acid index, both for diagnosis and for treatment, we feel that its continued use is fully justified. However a complication is introduced by the fact that the fixed acid indices refer to *in-vitro* blood, rather than to blood contained in the vascular bed. The extent to which this affects the usefulness of the indices will now be examined.

**Standard Bicarbonate.** Earlier in this paper it was pointed out that the conventionally obtained (*in vitro*) standard bicarbonate is affected by the  $P_{CO_2}$  of the blood sample (figure 1). This  $P_{CO_2}$ -dependence would in principle vitiate its use as a fixed acid index. Since the *in-vitro* standard bicarbonate is in-

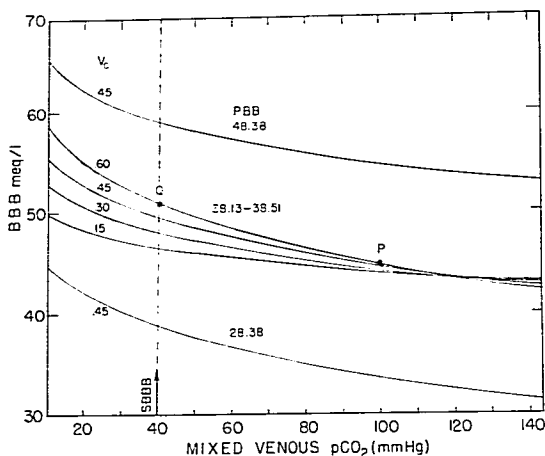


FIG. 7. Blood buffer base (BBB) as a function of *in-vivo*  $P_{CO_2}$  of oxygenated mixed venous blood. Pool buffer base (PBB) of the four clustered curves is 38.13, 38.26, 38.38, and 38.51 at blood hematocrit of 0.15, 0.30, 0.45 and 0.60, respectively; pool buffer base and blood hematocrit of the topmost curve are 48.38 and 0.45, respectively; those of the bottom curve 28.38 and 0.15. SBBB = standard blood buffer base, i.e., blood buffer base at *in-vivo* mixed venous  $P_{CO_2}$  = 40 mm. of mercury. For discussion and interpretation of points P and Q see text.

variant with  $P_{CO_2}$ , the *in-vitro* minus *in-vivo* value may be called the "error" in *in-vitro* standard bicarbonate. In other words, subtraction of the "error" from the *in-vitro* value will give the *in-vivo* value. This "error" has been calculated for a wide range of blood compositions; its magnitude as a function of  $pH_p$ ,  $P_{CO_2}$ , and hematocrit is shown in figures 5 and 6. The calculations were made as graphically shown in figure 1, using the theoretical *in-vitro* and *in-vitro*  $CO_2$  titration slopes derived in the Appendix. Hemoglobin was assumed to be fully saturated with oxygen. As an example, figure 5 shows that at  $P_{CO_2}$  = 70 and plasma  $pH$  = 7.00, - 2.2 mEq. should be subtracted, that is, 2.2 mEq. added to the conventionally obtained *in-vitro* value for standard bicarbonate, in order to find the *in-vitro* value. The diagonal curve in figure 5 connects all plasma compositions whose *in-vitro* standard bicarbonate is 24 mEq./liter; all points to the left of the curve represent fixed acid excess (metabolic acidosis), all points to the right represent fixed acid deficit (metabolic alkalosis). The two figures show that the "error" falls within  $\pm 4$  mEq. for the vast majority of clinical abnormalities, the largest "errors" of course occurring at very high and very low

$CO_2$  tensions. At moderately abnormal values for  $P_{CO_2}$ , the conventional index can still be looked upon as a valid clinical guide to the amount of fixed acid present in the blood. If greater accuracy is desired, or at extreme values of  $P_{CO_2}$ , figures 5 and 6 can be used for correction. The figures are predicated on normal blood and interstitial volumes, but deviations of these volumes by as much as 25 to 30 per cent affect the "error" by 0.1-0.3 mEq. at the most.

Figures 5 and 6 refer to oxygenated mixed venous blood; however, they can be applied to obtain the "error" in arterial *in-vitro* standard bicarbonate as well, as long as it is kept in mind that the theoretical value for arterial *in-vitro* standard bicarbonate, used in the calculation of the "error," may not be identical with the experimental one, which is susceptible to the circulatory changes accompanying the change in  $P_{CO_2}$ .

The effect of oxygen desaturation on the "error" has been calculated. For 50 per cent desaturation and hematocrit of 0.45, about 0.8 mEq. should be added to the absolute magnitude of the "error" shown in figure 5 when  $CO_2$  tension is greater than 40, and subtracted when  $CO_2$  tension is less than 40.

This correction, which is due to the shift of the *in-vitro* titration line, is proportionate to the degree of desaturation, and thus amounts to only a few tenths of a mEq. for most arterial samples; it might be neglected for practical purposes.

**Blood Buffer Base.** It will be recalled that an increase in  $P_{CO_2}$  of the "pool" (blood plus interstitial fluid) leaves pool buffer base unchanged, while reducing blood buffer base. Figure 7 shows this fall in BBB as a function of  $P_{CO_2}$  of mixed venous blood. The curves, assuming full oxygenation, were theoretically derived as described in the Appendix. Pool buffer base of the four clustered curves is within normal range, namely 38.13, 38.26, 38.38, and 38.51 at hematocrit of 0.15, 0.30, 0.45, and 0.60, respectively. The fall in blood buffer base can be seen to increase with hematocrit, a consequence of the increasing difference between buffering powers of pool and of blood. Pool buffer base of the topmost curve is 48.38 (moderate fixed acid deficit), that of the lowermost one 28.38 (moderate fixed acid excess); both curves refer to hematocrit of 0.45.† The fall in blood buffer base with  $P_{CO_2}$  is found to be practically independent of oxygen saturation, due to the nearly equal buffering powers of oxygenated and reduced hemoglobin.

As in the case of *in-vitro* standard bicarbonate, the  $P_{CO_2}$ -dependence of blood buffer base impairs its practical usefulness as a fixed acid index, especially at very high and very low  $CO_2$  tensions. We, therefore, propose to substitute for the BBB of native blood the value which would be obtained if the blood  $P_{CO_2}$  were brought, *in vivo*, to 40 mm. of mercury. This parameter might properly be named Standard Blood Buffer Base (SBBB); its magnitude is affected by the amount of fixed acid present, but not by the  $P_{CO_2}$  of the native sample. Thus SBBB for all blood compositions which fall on the topmost curve in figure 7 is 59 mEq./liter blood; that for the bloods of the lowermost curve 38.6. It would be possible

to construct correction curves to convert BBB into SBBB, similar to those shown for standard bicarbonate; but we prefer to employ the Singer-Hastings nomogram<sup>22</sup> to derive SBBB simultaneously with the conventional BBB.

To illustrate this new application of the nomogram, assume a fully oxygenated mixed venous sample of the following composition: hematocrit 0.60,  $P_{CO_2}$  100 mm. of mercury,  $pH_p$ , 7.11. A straight line through the last two values on the nomogram, not only yields the BBB of 44.9 at blood hematocrit 0.60, but also a buffer base of 46.2 at hematocrit 0.23. This is the pool hematocrit (table 1), and thus 46.2 might be considered the approximate buffer base value of the pool. Now a line drawn through this "pool point" and the point 40 on the  $P_{CO_2}$  scale yields a BBB of 51.0 at hematocrit 0.60. This is the BBB that would have been obtained if the mixed venous blood had been brought, *in-vivo*, to 40 mm. of mercury; it therefore represents the SBBB. The value of 51.0 closely agrees with the theoretically derived one of 50.7; in spite of the actual buffer base of the pool being 38.5 rather than 46.2. (This difference is due to the interstitial fluid—plasma mean protein concentration being 31 g./liter, rather

TABLE 4. Blood Buffer Base (BBB) and Standard Blood Buffer Base (SBBB) at Various Compositions of Fully Oxygenated Mixed Venous Blood.

V <sub>r</sub>	P <sub>CO<sub>2</sub></sub>	pH <sub>p</sub>	BBB	SBBB	
				Actual	Nom.
0.30	20	7.69	50.4	48.0	48.8
0.30	100	7.09	44.4	48.0	47.5
0.45	20	7.49	42.0	38.6	39.3
0.45	20	7.67	52.5	49.4	50.2
0.45	20	7.81	61.9	59.0	59.9
0.45	20	7.92	70.6	68.0	68.8
0.45	100	6.76	33.7	38.6	38.2
0.45	100	7.10	44.7	49.4	49.0
0.45	100	7.21	54.8	59.0	58.8
0.45	100	7.28	64.4	68.0	67.8
0.60	20	7.67	54.6	50.7	52.2
0.60	100	7.11	44.9	50.7	51.0

For details see text.

BBB and "Nom." SBBB graphically derived from the Singer-Hastings nomogram; "Actual" SBBB obtained from figure 7, which was calculated as described in Appendix. Both BBB and SBBB include carbamino- $CO_2$ .

† The decimal fractions of the pool buffer base values represent the concentrations of carbamino  $CO_2$  per liter pool; these values are nearly independent of  $P_{CO_2}$ . Carbamino  $CO_2$  concentration was assumed to be unaffected by metabolic acidosis and alkalosis; this assumption may not be entirely justified.

than 72 g./liter, the plasma protein concentration.) The compositions of the native blood and of the blood after *in-vivo* equilibration at  $P_{CO_2} = 40$  are shown in figure 7 by points P and Q, respectively.

The agreement between the theoretical values for SBBB (figure 7) and those derived nomographically is quite acceptable for clinical purposes, as shown in table 4 for a number of blood compositions. The table also shows that the error between the two is considerably less than the inaccuracy that would result from omitting the derivation of SBBB and only using the conventional BBB.

Although theoretically SBBB should be derived from mixed venous composition, arterial blood can be used in the same way with nearly the same results. Assume, for example, a  $CO_2$  tension of 92 of the arterial blood obtained simultaneously with the mixed venous blood in the above example. BBB is the same for both bloods, namely 44.9. After lining up these two parameters,  $P_{CO_2}$  and BBB, on the nomogram and going through the steps outlined above, SBBB is found to be 50.2, again quite close to the theoretical mixed venous value (50.7).

In the presence of a significant degree of oxygen desaturation, values for BBB and SBBB can be derived in the usual way, as if the blood were fully oxygenated; their difference (SBBB minus BBB) should then be added to the corrected BBB, obtained as described by Singer and Hastings.<sup>13</sup>

Deviations of blood and interstitial fluid volumes by as much as 30 per cent have only an insignificant effect on the value of SBBB.

However, *greater* deviations in these volumes, and also marked abnormalities in their protein content, will affect both SBBB and *in-vivo* standard bicarbonate. This must be kept in mind in the evaluation of these two parameters under severely abnormal conditions.

In conclusion, the classical analysis and graphic representation based on *in-vitro* blood can, with some modification, continue to serve their useful function in the clinical evaluation of the acid-base balance.

## APPENDIX

A. Calculation of *In-Vitro*  $CO_2$  Titration Curves of Oxygenated True Plasma.

Symbols:

[ $\bar{c}$ ]	= concentration in red cells (/liter cells)
[ $\bar{c}$ ] <sub>p</sub>	= concentration in plasma (/liter plasma)
[ $\bar{c}$ ] <sub>b</sub>	= concentration in blood (/liter blood)
$V_c$	= hematocrit of blood
[BBB]	= blood buffer base concentration, not including carbamino $CO_2$ (meg./liter blood)
[ $P^-$ ]	= plasma protein charge concentration (mEq./liter blood)
[Hb $^-$ ]	= hemoglobin charge concentration (mEq./liter blood)
$r_{HCO_3^-}$	= $\frac{[HCO_3^-]_{red\ cell\ water}}{[HCO_3^-]_{plasma\ water}}$
$r_H$	= $\frac{[H^+]_{plasma\ water}}{[H^+]_{red\ cell\ water}}$

By definition

$$\frac{[HCO_3^-]_c}{[HCO_3^-]_p} \cdot \frac{[H_2O]_p}{[H_2O]_c} = r_{HCO_3} \quad (1)$$

The most recent studies on steady state electrolyte distribution between red cells and plasma indicate that "as the data are improved, they increasingly uphold the validity of the Gibbs-Donnan principle, as governing the relative distribution of  $H^+$ ,  $Cl^-$  and  $HCO_3^-$  ions between cells and serum."<sup>14</sup> Thus

$$r_{HCO_3} = r_H \quad (2)$$

Now

$$\frac{[HCO_3^-]_b}{[HCO_3^-]_p} = \frac{(1 - V_c)[HCO_3^-]_p + V_c[HCO_3^-]_c}{[HCO_3^-]_p} \\ = (1 - V_c) + V_c \frac{[HCO_3^-]_c}{[HCO_3^-]_p} \quad (3)$$

Substituting (1) and (2) into (3) we get

$$\frac{[HCO_3^-]_b}{[HCO_3^-]_p} = (1 - V_c) + V_c r_H \frac{[H_2O]_c}{[H_2O]_p},$$

or

$$[HCO_3^-]_b = \frac{[HCO_3^-]_p}{(1 - V_c) + V_c r_H ([H_2O]_c/[H_2O]_p)}, \quad (4)$$

By definition [BBB] =  $[HCO_3^-]_b + [P^-] + [Hb^-]$ ,

or

$$[HCO_3^-]_b = [BBB] - ([P^-] + [Hb^-]). \quad (5)$$

Substituting (5) into (4) we get

$$[HCO_3^-]_b = \frac{[BBB] - ([P^-] + [Hb^-])}{(1 - V_c) + V_c r_H ([H_2O]_c/[H_2O]_p)} \quad (6)$$

In this equation  $[P^-]$  is a function of plasma protein concentration, plasma pH ( $pH_p$ ) and  $V_c$ .

Plasma protein concentration was taken as 72 g./liter plasma; the  $[P] - p\text{H}$ , relation was taken from the data listed by Singer and Hastings<sup>13</sup> in their table 4. These data are based on the classical titration experiments of normal human blood and plasma by Dill *et al.*<sup>2</sup>; they can be expressed by the equation:

$$\text{protein charge conc. (i.e., mEq./g. protein)} \\ = 0.103 p\text{H}_p - 0.521 \quad (7)$$

$[\text{Hb}^-]$  in equation (6) is a function of red cell hemoglobin concentration, red cell  $p\text{H}$  ( $p\text{H}_r$ ) and  $V_r$ . Red cell hemoglobin concentration was taken as 20 mM/liter cells; the  $[\text{Hb}^-] - p\text{H}_r$  relation was taken from the same table. For oxygenated cells this relation is:

$$\text{Hemoglobin charge conc. (i.e., mEq./mM Hb)} \\ = 3.567 p\text{H}_r - 23.596 \quad (8)$$

$n_{\text{H}}$  in equation (6) is a function of plasma (or red cell)  $p\text{H}$ . For oxygenated blood this function, based on the Singer-Hastings table, is:

$$n_{\text{H}} = -0.466 p\text{H}_p + 4.080 \quad (9)$$

This equation is nearly identical with that given by Fitzsimons and Sendroy<sup>20</sup> on the basis of their own experiments. It differs significantly from that theoretically derived by Lloyd and Michel<sup>12</sup> from the titration curves of hemoglobin of Rossi and Roughton<sup>21</sup>; over the  $p\text{H}_p$  range 7.2-7.6 the Lloyd-Michel equation<sup>13</sup> yields values for  $p\text{H}_e$ , 0.07 unit higher than those derived from (9).

Finally the ratio  $[\text{H}_2\text{O}]/[\text{H}_2\text{O}]_p$  in equation (6) was taken as 0.720/0.940 = 0.766, thus assuming constant red cell water content. This is justified since it changes by less than 3 per cent over the  $p\text{H}_p$  range 6.8-7.8.<sup>2</sup>

Thus for any value of  $[\text{BBB}]$ ,  $V_e$  and  $p\text{H}_p$ , equation (6) can be solved for  $[\text{HCO}_3^-]_p$ . When this is done at various values of  $p\text{H}_p$ , while keeping  $[\text{BBB}]$  and  $V_e$  unchanged, the resulting relationship between  $[\text{HCO}_3^-]_p$  and  $p\text{H}_p$  represents an *in-vitro*  $\text{CO}_2$  titration curve of true plasma in the presence of oxygenated hemoglobin, since both  $[\text{BBB}]$  and  $V_e$  are unaffected by varying  $\text{CO}_2$  tension.

These  $\text{CO}_2$  titration curves turn out to be straight lines, the slopes of which are slightly affected by the magnitude of  $[\text{BBB}]$ . For instance, at  $V_e = 0.45$  the slope  $(\Delta[\text{HCO}_3^-]_p/\Delta p\text{H}_p)$  is 26.9 at  $[\text{BBB}] = 49$ , and 29.5 at  $[\text{BBB}] = 39$ . This increase of slope with metabolic acidosis is somewhat greater than that found experimentally by Siggaard-Anderson and Engel<sup>22</sup>; their data have been plotted by Lloyd and Michel.<sup>12</sup> Table I lists the slope values at the average normal  $[\text{BBB}]$  of 49 for four hematocrits.

Our titration lines are identical with those derived at the same  $[\text{BBB}]$  by the method of Singer and Hastings.<sup>13</sup> Whereas they use the ratio of plasma over blood  $\text{CO}_2$  content at various values of  $V_e$  and  $p\text{H}_p$ , taken from measurements by Van

Slyke and Sendroy,<sup>20</sup> we have employed the theoretical Gibbs-Donnan relation (equation 2). The agreement does not necessarily validate equation (2), since an error in  $n_{\text{HCO}_3^-}$  of as much as 10 per cent affects  $[\text{HCO}_3^-]_p$  (equation 6) at  $V_e = 0.60$  by only 4 per cent; at lower  $V_e$  the difference is even less.

### B. Calculation of In-Vitro $\text{CO}_2$ Titration Curves of Oxygenated True Mixed Venous Plasma.

Additional symbols:	$[ ]_m$ = concentration in total plasma + interstitial fluid mixture (/liter mixture)
	$V_e'$ = hematocrit of pool (blood volume + interstitial fluid volume)
	$[\text{PBB}]$ = pool buffer base concentration, not including carbamino $\text{CO}_2$ (mEq./liter pool)
	$[\text{P}^-]$ = protein charge concentration of total plasma + interstitial fluid mixture (mEq./liter pool)
	$[\text{Hb}^*]$ = hemoglobin charge concentration (mEq./liter pool)

In order to clarify the derivation of the *in-vitro* mixed venous titration curve, assume a steady state of respiration, circulation and metabolism at a particular  $P_{\text{CO}_2}$  of fully oxygenated mixed venous blood (partial desaturation would not affect the basic reasoning).  $\text{CO}_2$  tension and ionic concentrations in interstitial water are taken to be equal to those in mixed venous plasma water.<sup>†</sup> These concentrations can be obtained from an arterial sample by its *in-vitro* equilibration at mixed venous  $\text{CO}_2$  tension. The two plasmas, arterial and mixed venous, are thus located on an *in-vitro*  $\text{CO}_2$  titration line, as was explained in the body of this paper. We now move to a new *in-vitro* steady state, characterized by a higher mixed venous  $P_{\text{CO}_2}$ , and reached via three imaginary steps, during which all vital functions are supposed to be temporarily suspended. (1) the above arterial equilibration is carried out on the entire mass of arterial blood, while it remains in the arterial bed. The entire plasma volume will then have the ionic composition of the mixed venous plasma, and will thus be in ionic equilibrium with the interstitial fluid which has not been affected. (2)  $P_{\text{CO}_2}$  everywhere in the extracellular fluid (including blood and interstitial fluid) is raised to a new value (*in-tracellular*  $P_{\text{CO}_2}$  is, of course, raised concomitantly).

<sup>†</sup> In other words, a Donnan distribution ratio of 1.0 is assumed. This is not exactly correct: the monovalent anionic venous plasma water/interstitial water ratio is a few per cent less than unity, the exact value being a function of  $p\text{H}_p$  and of protein concentrations of plasma and interstitial fluid.

and a new ionic equilibrium is once more allowed to be established between total blood volume (of homogeneous composition) and interstitial fluid. (We may, in our imagination, have to stir the blood to speed up this process!) Equilibrium of bicarbonate ions will have been reached by a net flux of these ions from plasma into interstitial fluid, since on account of the superior buffering power of blood the rise in plasma  $[\text{HCO}_3^-]$  will, initially, have been greater than that in interstitial fluid. (It is this disappearance of some bicarbonate ions from the blood, which in older publications led to the conclusion that respiratory acidosis is always associated with metabolic acidosis.) It should be emphasized that bicarbonate ions will equally disappear from the blood in the arterial and the venous beds; everything else being equal, the reduction in plasma  $[\text{HCO}_3^-]$  will be less, the larger the total blood volume. (3)  $P_{\text{CO}_2}$  of the mass of arterial blood in its bed is now lowered; mixed venous and interstitial compositions are left unchanged. Arterial and mixed venous plasmas will again fall on an *in-vitro* titration curve, but not on the same one as the initial steady state; the disappearance of some bicarbonate ions from the two plasmas has shifted the curve downward.

After these three imaginary steps, circulation, respiration and metabolism are re-established. With proper selection of their relative rates, the ionic distributions and extracellular  $\text{CO}_2$  tensions prevailing immediately after (3) can be maintained indefinitely; we have reached the new steady state.

This analysis shows that the mixed venous compositions in the two steady states are those of the entire blood volume, in equilibrium with the interstitial fluid (steps (1) and (2)). Therefore, in order to derive mixed venous composition at any value of mixed venous  $P_{\text{CO}_2}$  (or, for that matter,  $p\text{H}$  or  $[\text{HCO}_3^-]_v$ ), the entire blood volume (and interstitial fluid volume) must be considered to be endowed with this  $P_{\text{CO}_2}$  (or  $p\text{H}$  or  $[\text{HCO}_3^-]_v$ ), and not just the mixed venous blood. The derivation of the *in-vitro* mixed venous  $\text{CO}_2$  titration curve thus becomes entirely analogous with that of the *in-vitro* curve. The only difference is, that the pool (interstitial fluid + total blood volume), rather than the blood by itself, it now titrated; and that buffer base of pool, rather than that of blood, is now invariant with  $\text{CO}_2$  tension.

The following expression for bicarbonate concentration of the plasma + interstitial fluid mixture is comparable to equation (6):

$$[\text{HCO}_3^-]_m = \frac{[\text{PBB}]_v - ([\text{P}^*]_v + [\text{Hb}^*]_v)}{(1 - V_e) + V_e r_{\text{H}} ([\text{H}_2\text{O}]_v / [\text{H}_2\text{O}]_m)} \quad (10)$$

As explained, the equation also defines the bicarbonate concentration of the *in-vitro* mixed venous plasma, except that the plasma values are 3 per cent less, due to the water content of the plasma

+ interstitial fluid mixture being slightly greater than that of plasma.

Equations (7), (8) and (9) are also used for the solution of (10). Hemoglobin and protein concentrations per liter pool were calculated from blood volume (5.5 liters), interstitial fluid volume (8.9 liters, based on a 70 kg. man with inulin space of 170 ml./kg.), and mean protein concentration of interstitial fluid, including lymph (21 g./liter, with buffering power equal to that of plasma proteins<sup>21</sup>). These pool concentrations are listed in table 1.  $[\text{H}_2\text{O}]_v / [\text{H}_2\text{O}]_m$  in equation (10) was taken as  $0.720/0.970 = 0.742$ ; the ratio is practically independent of hematocrit.

For any value of  $[\text{PBB}]_v$ ,  $V_e$  and  $p\text{H}_v$  (same as  $p\text{H}_m$ ), equation (10) can now be solved for  $[\text{HCO}_3^-]_m$ , and thus for *in-vitro* mixed venous  $[\text{HCO}_3^-]_v$ . When this is done at various values of  $p\text{H}_v$ , while keeping  $[\text{PBB}]_v$  and  $V_e$  unchanged, a straight line relation between mixed venous  $[\text{HCO}_3^-]_v$  and  $p\text{H}_v$  is obtained. This represents the *in-vitro*  $\text{CO}_2$  titration curve of oxygenated mixed venous plasma, since both  $[\text{PBB}]_v$  and  $V_e$  are invariant with  $P_{\text{CO}_2}$ . The titration lines at different  $[\text{PBB}]_v$  only slightly converge toward the acid side. For example, at  $V_e = 0.172$  (i.e.,  $V_e = 0.45$ ) the slope ( $\Delta[\text{HCO}_3^-]_v / \Delta p\text{H}_v$ ) is 10.1 at  $[\text{PBB}]_v = 38$ , and 10.7 at  $[\text{PBB}]_v = 28$ . The slope values listed in table 1 are all calculated on the basis of  $[\text{PBB}]_v = 38$ , an average normal value.

One aspect of *in-vitro* equilibration still deserves mention. The vascular bed actually consists of a large number of parallel circuits, rather than a single one as has been assumed. Each of these circuits is in contact with its own interstitial space, and each terminates in a vein containing blood of different composition. We therefore calculated the titration curve for a system of seven parallel circuits (corresponding to the major organs). An average normal mixed venous-arterial  $\text{CO}_2$  difference was assumed, and on the basis of published data and some estimates, reasonable *v-a*  $\text{CO}_2$  differences and interstitial volumes and protein concentrations were assigned to each of these pathways. At a given total pool buffer base, the *in-vitro* mixed venous titration curve was found to be nearly the same for the seven circuit model as for a single circuit with the same total interstitial volume and protein content. The single circuit model therefore provides an acceptable basis for the calculation of the *in-vitro* mixed venous titration curve.

#### C. Calculation of In-Vivo Relation Between Blood Buffer Base Concentration and $P_{\text{CO}_2}$ of Oxygenated Mixed Venous Blood.

In the preceding section values were derived for *in-vitro* mixed venous  $[\text{HCO}_3^-]_v$  at different values for  $p\text{H}_v$ , obtained by varying  $\text{CO}_2$  tension, while  $[\text{PBB}]_v$  and  $V_e$  were kept unchanged at arbitrary values. The Henderson-Hasselbalch equation can be used to calculate these tensions from the other

two plasma parameters. It is also a simple matter to derive [BBB] from equation (6) at the various *in-civo* combinations of mixed venous  $[HCO_3^-]$ , and  $pH_p$ . To this [BBB] carbamino- $CO_2$  has to be added, in order to obtain total blood buffer base. Carbamino- $CO_2$  concentration (which is independent of  $P_{CO_2}$  in the physiologic range) amounts to 0.33, 0.66, 1.0, and 1.33 mEq./liter oxygenated blood at hematocrit 0.15, 0.30, 0.45, and 0.60, respectively.<sup>19</sup> Total blood buffer base can now be plotted against mixed venous  $P_{CO_2}$ ; this has been done in figure 7 at several values of [PBB] and  $V_e$ . While blood buffer base *in-vitro* does not change with  $P_{CO_2}$ , it can be seen to fall under *in-civo* conditions with increasing  $P_{CO_2}$ . This is a consequence of the progressive disappearance of bicarbonate ions from the blood into the interstitial space, as has been discussed above. Since in the steady state blood buffer base is the same throughout the vascular bed, the ordinate of figure 7 pertains equally to arterial and to any venous blood.

When the curves in figure 7 are replotted on logarithmic coordinates, they become straight lines, the slopes of which represent the ratio  $\Delta \log BBB / \Delta \log P_{CO_2}$ . The relation between this ratio and blood hematocrit ( $V_e$ ) at normal pool buffer base (38.13-38.51) is described by the following equation:

$$-\Delta \log BBB / \Delta \log P_{CO_2} = 0.156V_e + 0.0333.$$

### References

- Shaw, L. A., and Messer, A. C.: The transfer of bicarbonate between the blood and tissues caused by alterations of the carbon dioxide concentration in the lungs, *Amer. J. Physiol.* 100: 122, 1932.
- Davenport, H. W.: *The ABC of Acid-Base Chemistry*, ed. 4. Chicago, University of Chicago Press, 1958.
- Woodbury, J. W.: Regulation of pH, chapter 46 in *Physiology and Biophysics*, ed. 19. Edited by T. C. Ruch and H. D. Patton. Philadelphia, W. B. Saunders Co., 1965.
- Bock, A. V., Field, H., Jr., and Adair, G. S.: The oxygen and carbon dioxide dissociation curves of human blood, *J. Biol. Chem.* 59: 353, 1924.
- Dill, D. B., Edwards, H. T., and Consolazio, W. V.: Blood as a physicochemical system. XI. Man at rest, *J. Biol. Chem.* 118: 635, 1937.
- McGregor, M., Donevan, R. E., and Anderson, N. M.: Influence of carbon dioxide and hyperventilation on cardiac output in man, *J. Appl. Physiol.* 17: 933, 1962.
- Brackett, N. C., Jr., Cohen, J. J., and Schwartz, W. B.: Carbon dioxide titration curve of normal man, *New Eng. J. Med.* 272: 6, 1965.
- Michel, C. C., Lloyd, B. B., and Cunningham, D. J. C.: The *in vivo* carbon dioxide dissociation curve of true plasma, *Resp. Physiol.* 1: 121, 1966.
- Farhi, L. E., and Rahn, H.: Dynamics of changes in carbon dioxide stores, *ANESTHESIOLOGY* 21: 604, 1960.
- Polak, A. G., Haynie, G. D., Hays, R. M., and Schwartz, W. B.: Effects of chronic hypercapnia on electrolyte and acid-base equilibrium. I. Adaptation, *J. Clin. Invest.* 40: 1223, 1961.
- Hasselbalch, K. A.: Die "reduzierte" und die "regulierte" Wasserstoffzahl des Blutes, *Biochem. Z.* 74: 56, 1916.
- Van Slyke, D. D., and Cullen, G. E.: Studies of acidosis. I. The bicarbonate concentration of the blood plasma; its significance and its determination as a measure of acidosis, *J. Biol. Chem.* 30: 289, 1917.
- Singer, R. B., and Hastings, A. B.: An improved clinical method for the estimation of disturbances of the acid-base balance of human blood, *Medicine* 27: 223, 1948.
- Jørgensen, K., and Astrup, P.: Standard bicarbonate, its clinical significance, and a new method for its determination, *Scand. J. Clin. Lab. Invest.* 9: 122, 1957.
- Siggaard-Andersen, O.: *The Acid-Base Status of the Blood*, ed. 2. Baltimore, Williams & Wilkins Co., 1964.
- Fitzsimons, E. J., and Sendroy, J., Jr.: Distribution of electrolytes in human blood, *J. Biol. Chem.* 236: 1595, 1961.
- Lloyd, B. B., and Michel, C. C.: A theoretical treatment of the carbon dioxide dissociation curve of true plasma *in vitro*, *Resp. Physiol.* 1: 107, 1966.
- Roughton, F. J. W.: Transport of oxygen and carbon dioxide, *In: Handbook of Physiology*, Section 3 (Respiration). Edited by W. O. Fenn and H. Rahn. Washington, Amer. Physiol. Society, 1964, Vol. 1, Ch. 31.
- Siggaard-Andersen, O., and Engel, K.: A new acid-base nomogram, *Scand. J. Clin. Lab. Invest.* 12: 177, 1960.
- Van Slyke, D. D., and Sendroy, J., Jr.: Studies of gas and electrolyte equilibria in blood. XV. Line charts for graphic calculations by the Henderson-Hasselbalch equation, and for calculating plasma carbon dioxide content from whole blood content, *J. Biol. Chem.* 79: 781, 1928.
- Landis, E. M., and Pappenheimer, J. R.: Exchange of substances through the capillary walls, *In: Handbook of Physiology*, Section 2 (Circulation). Edited by W. F. Hamilton and P. Dow. Washington, Amer. Physiol. Society, 1963, Vol. 2, Ch. 29.