Special Article

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_2 titration was stressed; and it was pointed out that only mixed venous sampling can be expected to yield accurate *in-vivo* CO_2 titration curves. It was shown that, when blood CO_2 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_2 titration. Methods were given to correct these two indices, so that they become invariant with the CO_2 tension at which the sample is withdrawn, and thus can serve their function as measures of fixed acid excess or deficit.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_2 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,[1] 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_{CO_2}, with the changes occurring when this same mixed venous blood is brought *in vivo* to the same new CO_2 tension. The *in-vivo* equilibration can be accomplished by changing either alveolar ventilation or inspired P_{CO_2}. The changes in plasma (and also in red cell) bicarbonate concentration and *p*H which accompany a particular change in P_{CO_2} 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 *p*H, resulting from a particular change in CO_2 tension. In an *in-*

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vitro blood sample the pertinent concentrations of the buffer substances refer, of course, to the blood by itself. When CO_2 equilibration is accomplished *in vivo,* not only the blood volume, but also the interstitial fluid volume is titrated with CO_2. 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_{CO_2} results in a smaller change in bicarbonate concentration, and in a larger change in *p*H of the pool, and thus of blood plasma, under *in-vivo* conditions than *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_{CO_2} 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_2 tensions, the other by *in-vivo* exposure of this blood, together with the interstitial compartment, to different CO_2 tensions by means of ventilatory maneuvers. We have selected mixed venous, rather than arterial blood, because the electrolyte composition and CO_2 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_2 would lead to a curve such as Aa in figure 1. When CO_2 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_2 Titration Lines of Oxygenated Mixed Venous Plasma, at Four Values of Blood Hematocrit (V_c) and Corresponding Values of Pool Hematocrit (V_c').

V_c	0.15	0.30	0.45	0.60
Hb conc. (m*M*/l. blood)	3.0	6.0	9.0	12.0
Pl. Prot. conc. (g./l. blood)	61.2	50.4	39.6	28.8
In-vitro slope at [BBB] = 49	12.8	19.3	26.9	36.6
V_c'	0.057	0.115	0.172	0.229
Hb conc. (m*M*/l. pool)	1.15	2.29	3.44	4.58
Protein conc. (g./l. pool)	36.4	32.3	28.1	24.0
In-vivo 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_2. 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 *p*H; 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 *p*H (6.8–7.8); buffering power being defined as the amount of strong acid (or base) required to change *p*H by one unit. It is this linearity which makes the plotting of bicarbonate concentration against *p*H so convenient.° The difference in slopes ($\Delta[HCO_3^-]_p / \Delta\, pH_p$) 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_2 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[2] and of Woodbury.[3]

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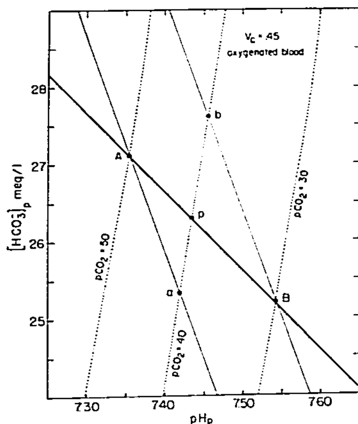

FIG. 1. Normal *in-vivo* CO_2 titration curve of true oxygenated mixed venous plasma (AB), and two *in-vitro* curves (Aa and Bb), obtained on mixed venous blood withdrawn at $P_{CO_2} = 50$ (A) and 30 (B), respectively. For details see text.

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-vivo* 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: $pH=7.35$, $[HCO_3^-]_p = 27.2$, $P_{CO_2} = 51$ (table 2). Point A, the point common to both lines, represents the plasma composition ($P_{CO_2} = 50$), 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_{CO_2} to 30 (point B), the results of *in-vitro* equilibration of a sample withdrawn at this lower P_{CO_2} 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_2 tension at any point of a titration line can be derived from the Henderson-Hasselbalch equation: $pH_p = 6.11 + \log ([HCO_3^-]_p/0.0311\ P_{CO_2})$, where 6.11 is the pK_1' of carbonic acid in plasma, and 0.0311 the solubility factor of CO_2 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_2 tension. For instance, at $P_{CO_2} = 40$ this relation can be expressed by $pH_p = 6.11 + \log ([HCO_3^-]_p/0.0311 \times 40)$, and thus $\log [HCO_3^-]_p = pH_p - 6.015$. Figure 1 shows 3 such logarithmic curves, at P_{CO_2} 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 $P_{CO_2} = 40$ mm. of mercury; the hemoglobin being fully oxygenated. When oxygenated mixed venous blood whose *in-vivo* titration line is represented by AB in figure 1 is brought *in vivo* to this P_{CO_2}, the composition of its plasma is represented by point p. On the other hand, when mixed venous blood, withdrawn at P_{CO_2} 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_{CO_2} less than 40 (B), has an *in-vitro* standard bicarbonate value greater (b) than that under *in-vivo* conditions. Thus the *in-vitro* obtained standard bi-carbonate varies with the CO_2 tension at which the blood was withdrawn. The example in figure 1 shows a normal value for in-vivo 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_{CO_2}, 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^-, plasma proteins $^-$, HCO_3^-, and carbamino CO_2^-, all expressed per liter blood; *i.e.*, the difference between total blood cation concentration and that of the fixed anions (mainly Cl^-). Since these two concentrations remain unchanged in an *in-vitro* blood sample, equilibrated at various CO_2 tensions, *in-vitro* blood buffer base is also constant, that is, invariant with CO_2 tension.

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

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CO_2 tensions for similar reasons; the buffer base of the blood component of the pool will fall with increasing 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 CO_2 tension, so that *p*H falls; then compare this blood with mixed venous blood brought *in vivo* from B to the same *p*H by raising CO_2 tension. Both bloods contain the same concentrations of plasma proteins$^-$ and Hb^-, since these ionic concentrations are a unique function of plasma *p*H (see Appendix). Carbamino CO_2 is also the same, since its concentration, in the physiologic P_{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 *p*H, and the *in-vivo* blood buffer base has fallen with increased CO_2 tension. At normal hemotocrit, blood buffer base of fully oxygenated mixed venous blood falls by 2.5 mEq./liter when P_{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 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, $\bar{v}_1a_1$). For the same reason, all venous points, representing the plasma bicarbonate and *p*H values of venous blood from each of the various tissue regious, 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 CO_2 production, as well as arterial CO_2 tension (figure 2). The mixed venous blood plus interstitial fluid will be titrated to a higher CO_2 tension, and the point representing its new steady state ($\bar{v}_2$) will fall on the *in-vivo* 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 ($\bar{v}_2a_2$) is located below the original one. This means that the arterial plasma composition has not remained unchanged even though its 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 *p*H. 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 *p*H of 0.02. Details of arterial and mixed venous blood composi-

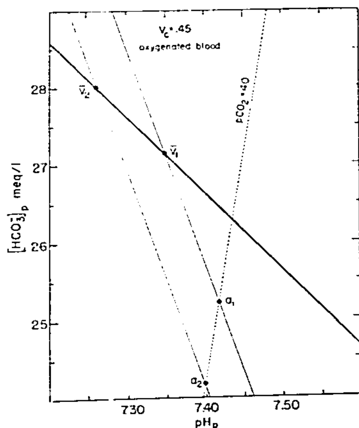

FIG. 2. Normal *in-vivo* CO_2 titration curve of true oxygenated mixed venous plasma ($\bar{v}_1\bar{v}_2$). a_1 and $\bar{v}_1$ represent plasma compositions of, respectively, arterial and mixed venous blood at control conditions; a_2 and $\bar{v}_2$ plasma compositions after reduction of cardiac output to half normal. The detailed blood compositions are given in table 2. For discussion see text.

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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_2 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_2 saturation (%)	100.0	100.0	100.0	100.0	100.0	100.0
P_{CO_2} (mm.Hg)	40.0	50.5	40.0	63.5	58.2	65.6
pH_p	7.420	7.348	7.399	7.262	7.287	7.250
pH_c	7.200	7.150	7.190	7.091	7.108	7.083
$[HCO_3^-]_p$ (mEq./l. plasma)	25.20	27.17	24.20	28.04	27.20	28.17
$[HCO_3^-]_c$ (mEq./l. cells)	12.89	14.50	12.60	15.93	15.18	16.15
[carbamino CO_2]$_b$ (mEq./l. blood)	1.0	1.0	1.0	1.0	1.0	1.0
[total CO_2]$_b$ (mEq./l. blood)	21.82	23.93	21.14	25.43	24.47	25.66
Δ[total CO_2]$_{\bar{v}-a}$ (mEq./l. blood)	2.11		4.29		1.19	

Hematocrit 0.45. For details see text.

pH_c was obtained from equation (9) (Appendix), $[HCO_3^-]_c$ from the Henderson-Hasselbalch equation applied to red cells (CO_2 solubility factor 0.0262).

tions are given in table 2. In figure 2 arterial CO_2 tension was maintained at 40 mm. of mercury, but the results would of course be similar at any other fixed value for arterial CO_2 tension. Therefore, an arterial (or, for that matter, any other) blood sample withdrawn under the initial conditions and equilibrated *in vitro* to a CO_2 tension of 40 mm. of mercury will yield a higher value for standard bicarbonate than a sample withdrawn at the same arterial P_{CO_2}, 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_2 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-vivo* 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-vivo* slope. If the increase in CO_2 tension is associated with an increase in cardiac output, the slope of a_1a_2 will be steeper than that of the *in-vivo* 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_{CO_2} from 40 to 58.2 mm. of mercury (see table 2 for details). If a fall in cardiac output accompanies the rise in CO_2 tension, the slope of a_1a_2 will generally be less than that of the *in-vivo 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-vivo* 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-vivo* line from its original position (heavy dashed line). If we assume that the arterial CO_2 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_2 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 [3] 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 *p*H 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 *p*H 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 *p*H unit,[4,5] 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 *p*H 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

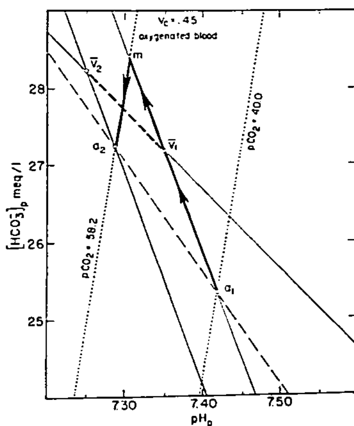

FIG. 3. Normal *in-vivo* CO_2 titration curve of true oxygenated mixed venous plasma ($\bar{v}_1\bar{v}_2$). a_1 and $\bar{v}_1$ represent plasma compositions of, respectively, arterial and mixed venous blood at control conditions; a_2 and $\bar{v}_2$ plasma compositions after increase in arterial P_{CO_2} 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 $a_1\bar{v}_1ma_2$, that of mixed venous plasma by the heavy interrupted line $\bar{v}_1\bar{v}_2$. The interrupted line a_1a_2 represents the erroneous *in-vivo* curve derived from arterial sampling. For discussion see text.

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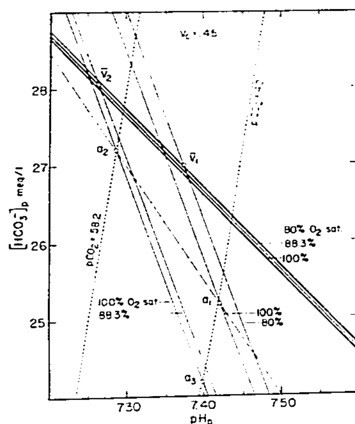

FIG. 4. *In-vivo* CO_2 titration curves of true mixed venous plasma, at 3 levels of oxygenation (3 heavy parallel lines). a_1 and $\bar{v}_1$ represent plasma compositions of, respectively, arterial and mixed venous blood under normal resting conditions; a_2 and $\bar{v}_2$ plasma compositions after increase in arterial P_{CO_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_1a_2 represents the erroneous *in-vivo* curve derived from arterial sampling. The ordinates of a_1 and a_3 represent the standard bicarbonate concentrations under the two conditions. For discussion see text.

but reasonable[6] 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-vivo* 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_2 tension from 25.20 (a_1) to 24.20 (a_3); 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-vivo* 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_2 tension from 40 to 58.2 mm. of mercury, would arterial sampling yield a faithful value for the *in-vivo* 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-vivo* CO_2 titration curves. Since the circulatory response to changes in CO_2 tension varies even among normal subjects,[6] the "normal" *in-vivo* CO_2 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_2 deviates from normal.

Brackett *et al.*[7] exposed 7 normal resting volunteers to room air and to mixtures of 7 or 10 per cent CO_2 with 21 per cent O_2 for 15–90 minutes. Arterial CO_2 tensions ranged from 34 to 90 mm. of mercury. From the measurements on arterial true plasma, an *in-vivo* CO_2 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_2 tensions.

Considerably greater *in-vivo* slopes (15.0 and 16.2) were obtained by Michel *et al.*[8] on 2 normal subjects in whom arterialized

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blood was sampled form a superficial vein of the forearm. The subjects breathed 2, 5 or 6 per cent 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 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 [6] thus tending to reduce the slope.

Even if mixed venous sampling were routinely feasible, the experimental *in-vivo* 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 responsible 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-vivo* 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 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	$\bar{v}$
O_2 saturation (%)	100.0	80.0	100.0	88.3
P_{CO_2} (mm. Hg)	40.0	47.0	58.2	63.5
pH_p	7.420	7.374	7.287	7.263
pH_c	7.200	7.170	7.108	7.092
$[HCO_3^-]_p$ (mEq./l. plasma)	25.20	26.86	27.20	28.07
$[HCO_3^-]_c$ (mEq./l. cells)	12.89	14.13	15.18	15.96
$[\text{carbamino } CO_2]_b$ (mEq./l. blood)	1.00	1.50	1.00	1.28
$[\text{total } CO_2]_b$ (mEq./l. blood)	21.82	23.99	24.47	25.74
$\Delta[\text{total } CO_2]_{\bar{v}-a}$ (mEq./l. blood)	2.17		1.27	

For details see text.

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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 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.[9] In conscious normal resting man an equilibration period of 20 minutes is probably sufficient to at least approach the steady state. The kinetics of CO_2 uptake are discussed by Woodbury,[2] 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 CO_2 and O_2. In exposure to abnormal 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.*[8] as a consequence of hypocapnia; lactate was unchanged during hypercapnia. In the studies of Brackett *et al.*[7] plasma phosphate, Na and K slightly increased with 10 per cent 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* 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 CO_2 level, the net result of transmembrane exchanges and renal adjustments is an *in-vivo* CO_2 titration slope which,

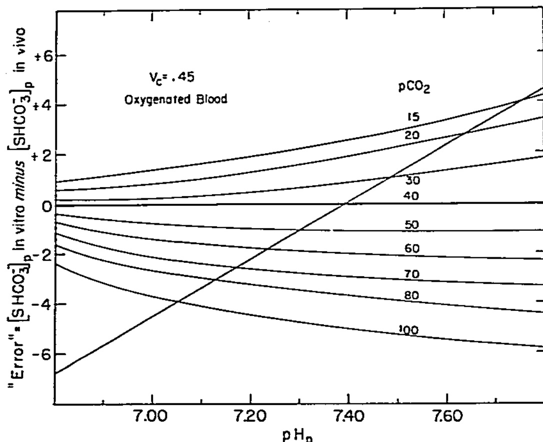

FIG. 5. "Error" in conventionally determined (*in vitro*) standard bicarbonate concentrations, for oxygenated blood samples of various pH_p and P_{CO_2}, at hematocrit 0.45. The "error" is defined as standard bicarbonate *in vitro* minus that *in vivo;* that is, subtraction of the "error" from the *in-vitro* value will give in *in-vivo* value. As an example, at $pH_p = 7.00$ and $P_{CO_2} = 70$, -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-vivo* standard bicarbonate concentration of 24 mEq./liter; points to the left of the curve represent metabolic acidosis, to the right metabolic alkalosis.

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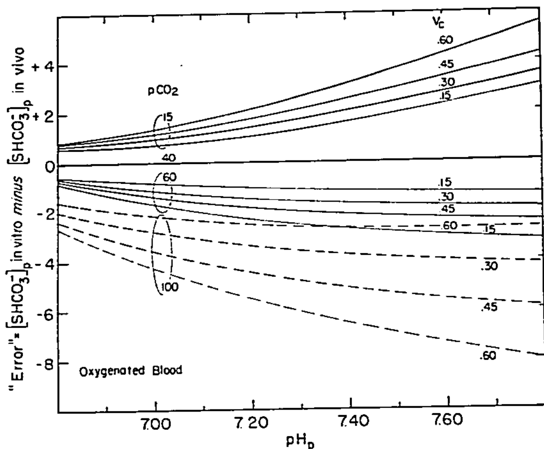

FIG. 6. "Error" in conventionally determined (*in vitro*) standard bicarbonate concentration, for oxygenated blood samples of various hematocrit, pH_p and P_{CO_2}. The use of this figure is similar to that of figure 5.

in the dog, even exceeds the normal *in-vitro* value.[10]

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 [11] suggested the measurement of plasma *p*H after whole blood at 38° C. had been equilibrated with carbon dioxide at 40 mm. of mercury. Van Slyke and Cullen [12] 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 [13] and true plasma standard bicarbonate concentration.[14] 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 composition of native blood it can be derived by means of a nomogram or an appropriate family of graphs.[13, 15]

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.[7] 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 if 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-vivo* standard bicarbonate is in-

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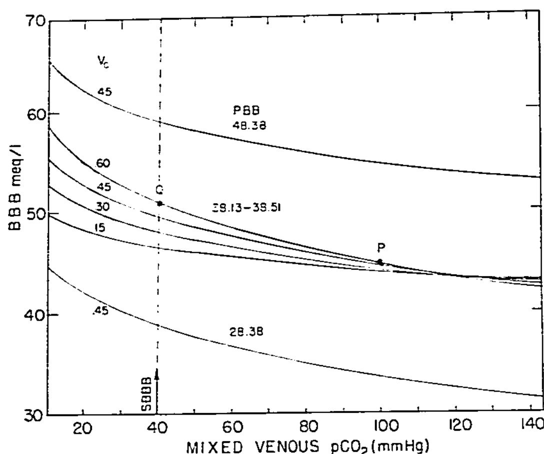

FIG. 7. Blood buffer base (BBB) as a function of *in-vivo* P_{CO_2} of oxygenated mixed venous blood. Pool buffer base (PBB) of the four clustered curves is 38.13, 38.26, 38.38, and 38.51 at blood hematocrit of 0.15, 0.30, 0.45 and 0.60, respectively; pool buffer base and blood hematocrit of the topmost curve are 48.38 and 0.45, respectively, those of the bottom curve 28.38 and 0.45. 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-vivo* 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-vivo* value. The diagonal curve in figure 5 connects all plasma compositions whose *in-vivo* 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-vivo* 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.

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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 [12] 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_c	P_{CO_2}	pH_p	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[12]; "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}.[18] Carbamino CO_2 concentration was assumed to be unaffected by metabolic acidosis and alkalosis; this assumption may not be entirely justified.

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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.[13]

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:

$[\ ]_c$ = concentration in red cells (/liter cells)

$[\ ]_p$ = concentration in plasma (/liter plasma)

$[\ ]_b$ = concentration in blood (/liter blood)

V_c = hematocrit of blood

$[BBB]$ = blood buffer base concentration, not including carbamino CO_2 (meq/liter *blood*)

$[P^-]$ = plasma protein charge concentration (mEq/liter *blood*)

$[Hb^-]$ = hemoglobin charge concentration (mEq/liter *blood*)

$$r_{HCO_3} = \frac{[HCO_3^-] \text{ red cell water}}{[HCO_3^-] \text{ plasma water}}$$

$$r_H = \frac{[H^+] \text{ plasma water}}{[H^+] \text{ red cell water}}$$

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."[16] 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

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

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

or

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

Substituting (5) into (4) we get

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

In this equation $[P^-]$ is a function of plasma protein concentration, plasma pH ($p\mathrm{H}_p$) and V_c.

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Plasma protein concentration was taken as 72 g./liter plasma; the $[P^-] - pH_p$ relation was taken from the data listed by Singer and Hastings [13] in their table 4. These data are based on the classical titration experiments of normal human blood and plasma by Dill *et al.*[5]; they can be expressed by the equation:

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

$[Hb^-]$ in equation (6) is a function of red cell hemoglobin concentration, red cell pH (pH_c) and V_c. Red cell hemoglobin concentration was taken as 20 mM/liter cells; the $[Hb^-] - pH_c$ relation was taken from the same table. For oxygenated cells this relation is:

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

r_H in equation (6) is a function of plasma (or red cell) pH. For oxygenated blood this function, based on the Singer-Hastings table, is:

$$r_H = -0.466\ pH_p + 4.080 \quad (9)$$

This equation is nearly identical with that given by Fitzsimons and Sendroy [14] on the basis of their own experiments. It differs significantly from that theoretically derived by Lloyd and Michel [17] from the titration curves of hemoglobin of Rossi and Roughton [15]: over the pH_p range 7.2–7.6 the Lloyd-Michel equation 13 [17] yields values for pH_c, 0.07 unit higher than those derived from (9).

Finally the ratio $[H_2O]_c/[H_2O]_p$ 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 pH_p range 6.8–7.8.[5]

Thus for any value of [BBB], V_c and pH_p, equation (6) can be solved for $[HCO_3^-]_p$. When this is done at various values of pH_p, while keeping [BBB] and V_c unchanged, the resulting relationship between $[HCO_3^-]_p$ and pH_p represents an *in-vitro* CO_2 titration curve of true plasma in the presence of oxygenated hemoglobin, since both [BBB] and V_c are unaffected by varying CO_2 tension.

These CO_2 titration curves turn out to be straight lines, the slopes of which are slightly affected by the magnitude of [BBB]. For instance, at $V_c = 0.45$ the slope ($\Delta[HCO_3^-]_p/\Delta pH_p$) is 26.9 at [BBB] = 49, and 29.5 at [BBB] = 39. This increase of slope with metabolic acidosis is somewhat greater than that found experimentally by Siggaard-Anderson and Engel [19]; their data have been plotted by Lloyd and Michel.[17] Table 1 lists the slope values at the average normal [BBB] of 49 for four hematocrits.

Our titration lines are identical with those derived at the same [BBB] by the method of Singer and Hastings.[13] Whereas they use the ratio of plasma over blood CO_2 content at various values of V_c and pH_p, taken from measurements by Van Slyke and Sendroy,[20] we have employed the theoretical Gibbs-Donnan relation (equation 2). The agreement does not necessarily validate equation (2), since an error in r_{HCO_3} of as much as 10 per cent affects $[HCO_3^-]_p$ (equation 6) at $V_c = 0.60$ by only 4 per cent; at lower V_c the difference is even less.

B. *Calculation of In-Vivo CO_2 Titration Curves of Oxygenated True Mixed Venous Plasma.*

Additional symbols:

- $[\ ]_m$ = concentration in total plasma + interstitial fluid mixture (/liter mixture)
- V_c' = hematocrit of pool (blood volume + interstitial fluid volume)
- [PBB] = pool buffer base concentration, not including carbamino CO_2 (mEq./liter *pool*)
- $[P'^-]$ = protein charge concentration of total plasma + interstitial fluid mixture (mEq/liter *pool*)
- $[Hb'^-]$ = hemoglobin charge concentration (mEq./liter *pool*)

In order to clarify the derivation of the *in-vivo* mixed venous titration curve, assume a steady state of respiration, circulation and metabolism at a particular P_{CO_2} of fully oxygenated mixed venous blood (partial desaturation would not affect the basic reasoning). CO_2 tension and ionic concentrations in interstitial water are taken to be equal to those in mixed venous plasma water.‡ These concentrations can be obtained from an arterial sample by its *in-vitro* equilibration at mixed venous CO_2 tension. The two plasmas, arterial and mixed venous, are thus located on an *in-vitro* CO_2 titration line, as was explained in the body of this paper. We now move to a new *in-vivo* steady state, characterized by a higher mixed venous P_{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_{CO_2} everywhere in the extracellular fluid (including blood and interstitial fluid) is raised to a new value (intracellular P_{CO_2} is, of course, raised concomitantly),

‡ 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 pH_p and of protein concentrations of plasma and interstitial fluid.

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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 $[HCO_3^-]$ 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 $[HCO_3^-]$ will be less, the larger the *total* blood volume. (3) P_{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 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_{CO_2} (or, for that matter, pH or $[HCO_3]_p$), the *entire* blood volume (and interstitial fluid volume) must be considered to be endowed with this P_{CO_2} (or pH or $[HCO_3]_p$), and not just the mixed venous blood. The derivation of the *in-vivo* mixed venous 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 CO_2 tension.

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

$$[HCO_3^-]_m = \frac{[PBB] - ([P'^-] + [Hb'^-])}{(1 - V_e') + V_e' r_H ([H_2O]_e/[H_2O]_m)} \quad (10)$$

As explained, the equation also defines the bicarbonate concentration of *in-vivo* 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 [31]). These pool concentrations are listed in table 1. $[H_2O]_e/[H_2O]_m$ in equation (10) was taken as $0.720/0.970 = 0.742$; the ratio is practically independent of hematocrit.

For any value of [PBB], V_e' and pH_p (same as pH_m), equation (10) can now be solved for $[HCO_3^-]_m$, and thus for *in-vivo* mixed venous $[HCO_3^-]_p$. When this is done at various values of pH_p, while keeping [PBB] and V_e' unchanged, a straight line relation between mixed venous $[HCO_3^-]_p$ and pH_p is obtained. This represents the *in-vivo* CO_2 titration curve of oxygenated mixed venous plasma, since both [PBB] and V_e' are invariant with P_{CO_2}. The titration lines at different [PBB] only slightly converge toward the acid side. For example, at $V_e' = 0.172$ (*i.e.*, $V_e = 0.45$) the slope $(\Delta[HCO_3^-]_p/\Delta pH_p)$ is 10.1 at $[PBB] = 38$, and 10.7 at $[PBB] = 28$. The slope values listed in table 1 are all calculated on the basis of $[PBB] = 38$, an average normal value.

One aspect of *in-vivo* 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 CO_2 difference was assumed, and on the basis of published data and some estimates, reasonable v-a 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-vivo* 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-vivo* mixed venous titration curve.

C. Calculation of In-Vivo Relation Between Blood Buffer Base Concentration and P_{CO_2} of Oxygenated Mixed Venous Blood.

In the preceding section values were derived for *in-vivo* mixed venous $[HCO_3^-]_p$ at different values for pH_p, obtained by varying CO_2 tension, while [PBB] and V_e were kept unchanged at arbitrary values. The Henderson-Hasselbalch equation can be used to calculate these tensions from the other

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two plasma parameters. It is also a simple matter to derive [BBB] from equation (6) at the various *in-vivo* combinations of mixed venous $[HCO_3^-]_p$ 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.[18] 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_c. While blood buffer base *in-vitro* does not change with P_{CO_2}, it can be seen to fall under *in-vivo* 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_c) at normal pool buffer base (38.13–38.51) is described by the following equation:

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

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