THE EFFECT OF TEMPERATURE ON THE ACID-BASE-PROTEIN EQUILIBRIUM AND ITS INFLUENCE ON THE CO₂ ABSORPTION CURVE OF WHOLE BLOOD, TRUE AND SEPARATED SERUM.

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The influence of change of temperature on the carbonic acid-base-protein equilibrium in blood or serum involves three factors: (1) Change in solubility of CO₂ in serum or blood. (2) Change in the equilibrium constants of the buffer systems in the blood. (3) Change in the equilibrium constant for the dissociation of water.

The fact that the solubility of CO₂ changes with temperature has naturally been fully appreciated. On the other hand, the physiological significance of the change in the dissociation constant of water is more or less obscure but has been discussed in a recent paper by Austin and Cullen (1925). These topics will not be considered here.

The relations of temperature to the equilibrium constants of the buffer systems of the blood were discussed by Stadie and Martin (1924) whose work was confined to whole blood. Further work from this laboratory by Cullen, Keeler, and Robinson (1925) together with that presented in this paper have enabled us to extend the equilibrium relations as affected by temperature so as to include true serum and separated serum as well as whole blood. In addition the relations developed lead to a theoretical expression for one of the factors in the C correction of Cullen used to convert [pH] of serum at 20°C. to pH at 38° which shows the limitations of the C correction as a constant.


Equilibrium Relations of the Buffers of the Blood.

The equilibrium constants of the buffer systems of serum may be written as follows:

\[
\begin{align*}
\text{pH} &= pK'_{[\text{H}_2\text{CO}_3]} + \log \frac{[\text{BHC}_1\text{O}_3]}{[\text{H}_2\text{CO}_3]} \\
&= pK'_{[\text{H}_2\text{PO}_4]} + \log \frac{[\text{B}_2\text{HPO}_4]}{[\text{BH}_2\text{PO}_4]} \\
&= pI + \frac{[\text{BPr}]}{\beta[\text{Pr}]} \\
&= pK'_{(x)} + \log \frac{\text{BX}}{\text{HX}}
\end{align*}
\]

The portions of the above expression relating to carbonates and phosphates are logarithmic forms of the mass law equation, the former being the familiar Henderson-Hasselbalch equation. The last portion of the expression is a similar expression for any additional hypothetical weakly dissociated acid that may occur in serum under pathological conditions. There is evidence against the existence of any such acid in normal serum in significant quantity. The expression

\[
\text{pH} = pI + \frac{[\text{BPr}]}{\beta[\text{Pr}]}
\]

is a rearrangement of Van Slyke's buffer equation

\[
[\text{BPr}] = \beta[\text{Pr}] (\text{pH} - pI)
\]

where \([\text{BPr}] = \) milli-equivalents of base bound by protein.

\(\beta = \) the buffer constant for the particular serum or blood; 
\(\alpha, \) the change in milli-equivalents of base bound by 
1 unit of \([\text{Pr}]\) for 1 unit \(\Delta\text{pH}\).

\([\text{Pr}] = \) the concentration of protein for the serum or blood. For 
serum protein the unit of \([\text{Pr}]\) is 1 gm. For hemoglobin 
it is 1 millimol.

\([pI] = \) an empirical constant that for a single protein is close to 
but not necessarily identical with the \(\text{pH}\) at the isoelectric point.
This equation (3) was first used by Van Slyke (1922) in his study of buffers and in his application of his general formula for buffers to the special case of hemoglobin and serum protein. It is used by Van Slyke, Wu, and McLean (1923) in their equations 54, 55, and 56. We rearrange it merely that it may be more readily compared with the other expressions in equations (1). This buffer equation of Van Slyke, applicable to any buffer system, happens to be especially convenient for the protein buffer of serum and whole blood over physiological pH range because of the fact observed by Van Slyke and his coworkers that over this range the base bound by protein $[BPr]$ in serum or in reduced blood is approximately a linear function of pH and hence $\beta[Pr]$ is a constant over this pH range. The values for $pI$ and $\beta$ are different for cells and for serum and hence for whole blood.

From the studies of Van Slyke, Hastings, and Neill (1922) and of Stadie and Martin (1924), it is clear that for oxygenated hemoglobin there is a deviation from the linear relation of $[BPr]$ to pH. However, one can calculate from Fig. 2 of Stadie and Martin that between pH of 7.0 and 8.0 for a blood of normal hemoglobin content the deviation from a mean straight line drawn through the true values does not exceed $\pm 0.6$ mM $[BPr]$ at any point. For the purpose of this paper then the linear relation for fully oxygenated as well as reduced blood is assumed as an approximation.

The base in the blood is partly bound by strongly dissociated acids which at all physiological pH values and temperatures exist as salts and bind a constant amount of base. The remainder is distributed between the various weakly dissociated acids of equations (1). This may be expressed as follows:

$$[B] = [BA] + [BHCO_3] + [BPr] + ([BH_2PO_4] + [B_2HPO_4] + [BX])$$

where A includes the anion of all strongly dissociated acids, chiefly $Cl^-$, and where the part in parenthesis is negligibly small in normal blood and serum as compared with the $[BHCO_3]$ and $[BPr]$. Under these conditions base bound as $[BHCO_3] + [BPr]$ may be considered a constant which we will call $[b]$ for a given sample of blood or separated serum regardless of changes within physiological limits of $[O_2]$, $[CO_2]$, pH, or $t^\circ$. This is indicated diagramatically in Fig. 1 where the distribution of base
CO₂ Absorption Curve

between Pr' and HCO₃', as it is equilibrated with varying tensions of CO₂ with consequently varying pH, is shown for the same separated serum at 20° and 38°.

Fig. 1 emphasizes the fact that the slope and location of the CO₂ absorption curve of a serum (and the same is true of whole

![Graph](image)

**Fig. 1.** Showing the relation of CO₂ absorption curve of a separated serum expressed as [BHCO₃] against pH to the curve of [BPr] against pH. [BHCO₃] + [BPr] = [b], a constant (approximately) for any given separated serum.

blood) is determined essentially by the constant value for [BHCO₃] + [BPr] and by the value of [BPr] at any pH. The location and slope of the CO₂ absorption curve either of whole blood or of separated serum is essentially determined by the location and slope of the curve for [BPr]. In other words the carbon dioxide dissociation curve of separated serum is determined by the concen-
stration of total CO$_2$, the concentration of protein, and its constants pI and $\beta$. The relation, therefore,

$$\Delta[BHCO_3] = -\Delta[BPr]$$  \hspace{1cm} (5)

under the limitations stated is necessarily true.

For true serum, subjected to changes in p$_{CO_2}$, pH, or temperature while still in contact with the cells, that is as a phase of whole blood, the relationship between $[BHCO_3]$ and $[BPr]$ is less simple. In true serum under these conditions with changes in $[BPr]$ of cells and serum there is migration of all anions including the quantitatively important anion Cl' through the cell membrane in amounts determined as shown by Van Slyke, Wu, and McLean by the total amount of each anion in the blood and the requirements of the Donnan equilibrium. Under these conditions $[BA]_{TS}$ is not constant and $\Delta(BHCO_3)_{TS}$ does not equal $-\Delta[BPr]_{TS}$. If we neglect changes in the relative volume of cells and serum and changes in the Donnan ratio of anions in cells and serum which are known to be small over narrow pH ranges, the ratio of concentration of $[BHCO_3]_{TS}$ to $[BHCO_3]_C$ and therefore to $[BHCO_3]_B$ will be constant, and as an approximation we may write

$$\Delta[BHCO_3]_{TS} = \Delta[BHCO_3]_B = -\Delta[BPr]_B$$  \hspace{1cm} (6)

This question will be discussed at greater length below. Therefore, while changes in $[BHCO_3]$ of separated serum are a measure of changes in $[BPr]$ of separated serum, changes in $[BHCO_3]$ of true serum at $38^\circ$C, and of whole blood are both a measure of changes in $[BPr]$ of whole blood. The quantitatively important change in $[BPr]$ of whole blood is the change in base bound by hemoglobin. The slope and location of the CO$_2$ absorption curve is determined therefore by equations (3) and (4) when the factors in the parenthesis of (4) are quantitatively negligible so that equation (5) holds. Since

$$pH = pI + \frac{[BPr]}{[Pr]}$$  \hspace{1cm} (2)

and if

$$[BHCO_3] + [BPr] = [b]$$  \hspace{1cm} (7)
where \( [b] \) is a constant for the particular separated serum or blood we can write

\[
pH = pI + \frac{[b] - [BHCO_3]}{\beta[Pr]} \tag{8}
\]

Equation (8) defines the location of the \( CO_2 \) absorption curve expressed as \([BHCO_3]\) against pH when the constants \( pI, [b] \) and \( \beta[Pr] \) are known. Conversely the curve of \([BHCO_3]\) against pH determines \( \beta[Pr] \); for

\[
pH - pH' = pI + \frac{[b] - [BHCO_3]}{\beta[Pr]} - \left( pI + \frac{[b] - [BHCO_3]'}{\beta[Pr]'} \right) \tag{9}
\]

or

\[
\beta[Pr] = \frac{[BHCO_3'] - [BHCO_3]}{pH - pH'} \tag{10}
\]

Further, if \( \beta[Pr] \) is constant at all temperatures, then it will be obvious from equation (2) that if we select points on the curves at two temperatures having the same value for \([BHCO_3]\) it will be true that:

\[
pH_t - pH_{t'} = pI_t - pI_{t'} \tag{11}
\]

and we can evaluate the change of \( pI \) with temperature.

The equation developed above may be used to evaluate experimental data relating temperature and \( CO_2 \) absorption curves and in the present paper we present additional data on whole blood, true serum, and separated serum of dog and sheep at 20°C. and 38°C.

**Methods.**

Blood was drawn from heart of the dog, or jugular vein of the sheep under oil or from the rat by instant decapitation. The blood was defibrinated by stirring under oil with a glass rod.

Equilibration of blood or serum was performed by the first or second saturation method of Austin, Cullen, Hastings, McLean, Peters, and Van Slyke (1922) and the technique there described for separation of true serum was employed.

Electrometric pH was done by the method and with the standardization described by Cullen, Keeler, and Robinson (1925).
Colorimetric pH at 20°C was read by the technique of Cullen (1922) and at 38°C by that of Hastings and Sendroy (1924) using for both the latters’ bicolor standards. Colorimetric readings are expressed with bracketed figures, \([\text{pH}]_{20}\) indicating the color given at 20°C by the phosphate buffer of corresponding pH at 20°C and \([\text{pH}]_{38}\) indicating the color given at 38°C by the phosphate buffer of corresponding pH at 38°C. Unbracketed pH figures indicate gasometric or electrometric pH. \([\text{CO}_2]\) analyses were performed by Van Slyke’s method. Gasometric pH was calculated by the Henderson-Hasselbalch equation from data on separated serum or true serum using Bohr’s \(\alpha = 0.975 \alpha_{\text{water}}\) and \(pK'_{38} = 6.10, pK'_{20} = 6.19\) (Cullen, Keeler, and Robinson).

**DISCUSSION.**

Our experimental data are shown in Table I. From these data \([\text{BHCO}_3]\) was plotted against pH. The resulting slopes are given in Table I in the columns headed \(\frac{\Delta[\text{BHCO}_3]}{\Delta \text{pH}}\) for each true serum, whole blood, and separated serum.

**Effect of Temperature on \(\beta[Pr]\).**

The effect of temperature on the slope of the CO₂ absorption curve of whole blood, i.e. upon the value of \(\beta[Pr]_B\), is not completely settled by the data of Table I. Two of the four experiments show a greater slope at 38°C than at 20°C, one the reverse outside the limits of experimental error. One gives within experimental limits the same slope. Such evidence neither proves nor disproves the assumption of Stadie and Martin that \(\beta[Pr]\) does not vary with temperature and furnishes no evidence for abandoning for the present the assumption. We tentatively assume, therefore, that at both 20°C and 38°C

\[
\Delta[\text{BHCO}_3]_B = - \Delta[\text{BPr}]_B
\]

(12)
total available base \([b]_B\) of blood being constant.

It will be seen from Table I that both at 20°C and 38°C the slope \(\frac{\Delta[\text{BHCO}_3]}{\Delta \text{pH}}\) is constantly steeper for true serum than for the corresponding blood. The ratio of the slopes is \(1.13 \pm 0.04\) with
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</table>
no striking difference in the dog between the slopes at the two
temperatures. Equation (12) as an approximation holds for
true serum but could be more accurately written

$$\Delta[BHCO_3]_TS = k \Delta[BHCO_3]_B = -k \Delta[BPr]_B$$

(13)

We may also write at constant pH but changing temperature

$$t - t' \Delta[BHCO_3]_TS = k (t - t' \Delta[BHCO_3]_B) = -k (t - t' \Delta[BPr]_B)$$

(14)

At present we may put $k = 1.1$.

That the slope of the true serum curve is greater than that for
whole blood is to be expected from the following considerations.

For whole blood, if $v$ is the volume of the cells we have

$$\begin{align*}
(1 - v) [BHCO_3]_TS + v[BHCO_3]_C &= [BHCO_3]_B \\
\end{align*}$$

Assuming $v$ constant a similar expression could be written for a
higher CO$_2$ tension so that for a small increase we may write

$$\begin{align*}
(1 - v) \Delta[BHCO_3]_TS + v \Delta[BHCO_3]_C &= \Delta[BHCO_3]_B \\
\end{align*}$$

or

$$\Delta[BHCO_3]_TS = \Delta[BHCO_3]_B + v(\Delta[BHCO_3]_TS - \Delta[BHCO_3]_C)$$

(17)

If the concentrations of BHCO$_3$ be referred to the water content
of cells and serum denoted by $w$ and $w'$ respectively, the Donnan
ratio can be written

$$\frac{[BHCO_3]_C}{[BHCO_3]_TS} \cdot \frac{w'}{w} = r$$

(18)

or for a small change at constant $r$, $w$ and $w'$

$$\Delta[BHCO_3]_TS = \frac{w'}{w} \Delta[BHCO_3]_C$$

(19)

The data on pp. 844 and 845 of the paper by Van Slyke, Wu,
and McLean (1923) show for horse blood that $\frac{w'}{w}$ is always greater
than 1.0 between pH 7.0 and 7.7. It follows then that
($\Delta[BHCO_3]_TS - \Delta[BHCO_3]_C$) is finite and positive and equation
(17) becomes

$$\Delta[BHCO_3]_TS = \Delta[BHCO_3]_B + x[BHCO_3]$$

where $x$ is positive; (20)
or as an approximation we may write

\[ \Delta [BHCO_3]_{TS} = k \Delta [BHCO_3]_B = -k \Delta [BPr]_B \]  

(21)

where \( k = 1.1 \) approximately.

It would, of course, be possible to pursue this matter further and elaborate a definite exact theoretical expression relating \([BHCO_3]_{TS}\) to \([BHCO_3]_B\) in terms of protein concentration, buffer slope, water content, pH, \( r \), and total anion concentration of serum and cells respectively. This would involve merely a rearrangement of the equations derived by Van Slyke, Wu, and McLean (1923). However, the establishment of the validity of this relation would be nothing more than an experimental confirmation of their hypotheses. Theoretically this would be no advance and practically for each blood the relation would be different because of variation in the constants. Peters, Bulger, and Eisenman's empirical solution of this problem is eminently satisfactory for practically all purposes.

In twenty-one determinations of the CO\(_2\) curve of whole blood and true plasma on human blood at 38°C., Peters, Bulger, and Eisenman (1924) found a greater slope for plasma in nineteen cases. The ratio of the slopes in these nineteen cases was 1.08 in close agreement with the value found here.

The approximate position of the CO\(_2\) absorption curve of true serum relative to the whole blood curve may be located if the value of \([BHCO_3]_{TS}\) at one point is known by drawing a line through this point on a \([BHCO_3] - pH\) plot having a slope 1.1 of the slope of the whole blood. Lacking the one point on the true serum curve the location of this line is more hazardous but may be approximated by the method outlined by Peters, Bulger, and Eisenman (1924). Employing the principles outlined here the true serum curve located at any one temperature may be approximately located at other temperatures.

For separated serum the one experiment shows within the limits of experimental error the same slope at 20° and 38°C.; i.e., \( \beta [Pr]_{SS} = \) constant, so that we may write at any temperature

\[ \Delta [BHCO_3]_{SS} = - \Delta [BPr]_{SS} \]  

(22)

total available base \([b]_{SS}\) of separated serum being constant.
From the data of Table I certain approximations as to the effect of temperature upon the acid-base equilibrium may be justifiably drawn. Assuming as an approximation a linear relationship of \([BHCO_3]\) against pH at both temperatures, we calculate from the data of Table I the changes with temperature of \([BHCO_3]\) at constant pH, at constant \([H_2CO_3]\), and at constant \(p_{CO_2}\) and also the changes of pH at constant \([BHCO_3]\), \([H_2CO_3]\), and \(p_{CO_2}\). These relations are tabulated in Table II.

It will be seen that a rather constant temperature effect is the \(20^\circ - 38^\circ \Delta \text{pH}\) at constant \([BHCO_3]\). This we have pointed out above, if the slope of \([BHCO_3]\) against pH be the same at the two temperatures, is a measure of \(20^\circ - 38^\circ \Delta \text{pI}\) of whole blood when the \(\Delta \text{pH}\) measured is that of true serum and of \(20^\circ - 38^\circ \Delta \text{pI}\) of separated serum when the \(\Delta \text{pH}\) measured is that of separated serum. The data suggest that the \(20^\circ - 38^\circ \Delta \text{pI}\) is for whole blood and separated serum approximately:

\[
\frac{\Delta \text{pI}}{\Delta t^\circ} = \frac{\Delta \text{pH}}{\Delta t^\circ} \text{[BHCO}_3\text{]} \text{constant} = -0.017 \text{ to } -0.023 \tag{23}
\]

If it is desired to approximate the position of a \([BHCO_3]\) curve of a serum or blood against pH at some other temperature (t') when the curve at t is known, it can be done conveniently by means of equation (23) and the assumption that the slope of the \([BHCO_3]\) curve against pH at t' is the same as at t.

In order to aid in visualizing the nature of these changes nomograms (Figs. 2 and 3) have been prepared of the relations in the true serum of Dog 33, June 4, and in the separated serum of Dog 27, January 14. It will be seen that while the interval between the lines is much greater in separated serum due to its smaller \(\beta[\text{Pr}]\) value and consequently flatter CO₂ absorption curve, the slope of the lines in physiological ranges is almost the same for true serum and for separated serum. These nomograms also bring out the fact, pointed out by Austin and Cullen (1925) that with change in temperature from \(38^\circ\) to \(20^\circ\) at constant \(p_{CO_2}\) the pH of serum varies very little. They pointed out, however, that there is a marked associated change in pOH.
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<th>Temperature°C</th>
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<th>pH</th>
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<th>[BHCO₃] and (\Delta pH) at constant [H₂CO₃].</th>
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<td>Dog 33.</td>
<td>June 4.</td>
<td>Blood.</td>
<td>19.7</td>
<td>38.0</td>
<td>15.2</td>
<td>7.61</td>
<td>7.30</td>
<td>0.38</td>
</tr>
<tr>
<td>--------</td>
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<td>------</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.7</td>
<td>38.0</td>
<td>20.0</td>
<td>7.37</td>
<td>7.30</td>
<td>0.40</td>
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<td>True serum.</td>
<td></td>
<td></td>
<td>19.7</td>
<td>38.0</td>
<td>18.9</td>
<td>7.60</td>
<td>7.28</td>
<td>0.34</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>19.7</td>
<td>38.0</td>
<td>23.5</td>
<td>7.38</td>
<td>7.28</td>
<td>0.31</td>
</tr>
<tr>
<td>Sheep.</td>
<td>May 20.</td>
<td>Blood.</td>
<td>20.2</td>
<td>38.0</td>
<td>15.5</td>
<td>7.62</td>
<td>7.28</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>20.2</td>
<td>38.0</td>
<td>20.0</td>
<td>7.39</td>
<td>7.28</td>
<td>0.31</td>
</tr>
<tr>
<td>True serum.</td>
<td></td>
<td></td>
<td>20.2</td>
<td>38.0</td>
<td>19.5</td>
<td>7.61</td>
<td>7.28</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.2</td>
<td>38.0</td>
<td>23.5</td>
<td>7.36</td>
<td>7.28</td>
<td>0.31</td>
</tr>
<tr>
<td>Dog 27.</td>
<td>Jan. 14.</td>
<td>Separated serum.</td>
<td>20.0</td>
<td>38.0</td>
<td>22.0</td>
<td>7.94</td>
<td>7.40</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.0</td>
<td>38.0</td>
<td>22.0</td>
<td>7.52</td>
<td>7.40</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.0</td>
<td>38.0</td>
<td>25.0</td>
<td>7.35</td>
<td>7.40</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.0</td>
<td>38.0</td>
<td>25.0</td>
<td>7.00</td>
<td>7.40</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Fig. 2. Nomogram of true serum Dog 33 (June 4) showing relations of
[BHCO₃]ₐ, pCO₂, [H₂CO₃]ₐ, pH, and t°C.

Fig. 3. Nomogram of separated serum Dog 27 (Jan. 14) showing relation of
[BHCO₃]ₐ, pCO₂, [H₂CO₃]ₐ, pH, and t°C.
Change of pH of Serum with Temperature at Constant Total CO2 and Constant Total Available Base: Calculation of \( t - \nu \Delta pH \).

Separated serum equilibrated under known conditions at a given temperature, \( t^\circ \), or true serum separated from cells under equilibrium conditions and kept so that no loss or gain of CO2 may occur (under oil or in mercury receivers) will on change of temperature assume a new equilibrium with consequent change of pH. The same thing is necessarily true for diluted serum. This change of pH enters into the C correction of Cullen's (1922) colorimetric method for pH of serum and plasma since the conditions defined are exactly those employed by Cullen in reading at \( 20^\circ \)C. the [pH] of a serum equilibrated at \( 38^\circ \)C. Austin, Stadie, and Robinson (1925) have shown elsewhere, however, that this pH change is not necessarily the most important cause of variation in the C correction. Disregarding this for the moment we will proceed to show the factors involved in the change of pH of serum with change of temperature from \( 38^\circ \)C. to \( 20^\circ \)C.; i.e., \( t - \nu \Delta pH \) and its possible variations. If we accept the evidence that with change in temperature the equilibrium of serum protein-carbonic acid systems is determined by change in pI of equation (3) and of pK' and \( \alpha \) of the Henderson-Hassellbalch equation, it is possible to calculate the \( t - \nu \Delta pH \) under the above conditions as follows.

The amount of base in equivalents bound by serum protein at \( t^\circ \) is given by the linear relation, equation (3)

\[
[BPr] = \beta [Pr] (pH_t - pI_t)
\]

where [Pr] = protein concentration gm. per liter
\( \beta \) = protein buffer change in equivalents of base bound per gm. of protein per 1 unit \( \Delta pH \).
\( pI_t \) = pH close to but not necessarily identical with the isoelectric point at \( t^\circ \).C.

The equivalents of base bound by HCO3 at \( t^\circ \) are given by the familiar equation

\[
[BHCO_3] = \frac{k'_t}{k'_t + [H^+]\text{t}} [CO_2]
\]

At equilibrium the sum of the base \([BP] + [BHCO_3]\) is \([b]\)

\[
[b] = \beta [Pr] (pH_t - pI_t) + \frac{k'_t}{k'_t + [H^+]\text{t}} [CO_2]
\]
At $t\,^\circ C$, if no loss of CO$_2$ or change of protein concentration occurs

$$[b] = \beta(Pr) (pH_{t'} - PI_{t'}) + \frac{k'_t}{k'_t + [H^+]} [CO_2]$$

Equating, and assuming the total available base $[BP] + [BHCO_3]$ which we call $[b]$ unchanged, we have

$$(t - t' \Delta pH - t - t' \Delta PI) \beta(Pr) = \left( \frac{k'_t}{k'_t + [H^+]} - \frac{k'_t}{k'_t + [H^+]} \right) [CO_2]$$

Equation (27) shows that $t - t' \Delta pH$ in serum (and likewise in diluted serum) at constant [CO$_2$] and [b] is dependent on the relation of the total [CO$_2$] to $\beta(Pr)$, the magnitude of $\Delta PI$ with temperature, the change in $k'$ (the dissociation of carbonic acid) with temperature and also upon the initial value of [H$^+$] or pH.

The relation of $k'_t$ and $k'_t$ of equation (27) to the Henderson-Hasselbalch equation is as follows:

$$\log \frac{k'_t}{k'_t} = pK'_t - pK'_t = 0.09$$

between 38°C. and 20°C.

Evidence that $\beta$ for hemoglobin is independent of temperature was presented by Stadie and Martin and as already pointed out our data furnish no evidence for change of $\beta(Pr)$ of serum protein or whole blood with temperature. A graphical solution of equation (27) yields the graphs shown in Fig. 4, where abscissa are initial pH$_{38}$, ordinates are $t - t' \Delta pH = pH_{20} - pH_{38}$. It is at once evident that $t - t' \Delta pH$ is not a constant. Variations in initial pH from 7.0 to 8.0 cause variations as large as 0.16 in $t - t' \Delta pH$. Changes in protein buffer of serum well within the limits of variations observed in different species cause considerable change in the values of $t - t' \Delta pH$ as well as in the importance of change in initial pH. Thus for a serum with [CO$_2$] = 20 mm, initial pH = 7.4 and $\beta(Pr) = 6$, we find $20 - 38 \Delta pH = 0.34$. A change in total [CO$_2$] of 10 mm causes change in $20 - 38 \Delta pH$ (at same initial pH) of 0.04 to 0.05 pH.

It should be pointed out that here over narrow physiological
ranges, 7.30 to 7.50, the changes in \( 20 - 38 \Delta p\text{H} \) are small (0.04 to 0.05 pH). However, the use of a constant value for Cullen's C correction (which involves \( 20 - 38 \Delta p\text{H} \)) in experiments on separated serum saturated at different \( \text{CO}_2 \) tensions is associated with greater variations of \( t - 38 \Delta p\text{H} \) because lower initial pH is associated with higher \([\text{CO}_2]\). From Fig. 4 it will be seen that lowering initial pH\(_{38}\) and increasing \([\text{CO}_2]\) both tend to diminish \( 20 - 38 \Delta p\text{H} \); we have here, therefore, a summation of effects. For true serum the \( \Delta [\text{CO}_2] \) at different initial pH\(_t\) values is still greater with greater alteration in \( 20 - 38 \Delta p\text{H} \) at different initial pH values.

The use of a constant Cullen's C correction therefore within physiological ranges introduces the possibility of errors due to variation in \( t - 38 \Delta p\text{H} \) of from 0.06 to 0.09 pH units between 20°C and 38°C. In experimental work on blood or serum over wider pH ranges still larger errors would be introduced as pointed out above.

Equation (27) must be valid, as already pointed out, if equation (3) is valid and if change in temperature affects only the constants \( pK' \) and \( \alpha \) of the Henderson-Hasselbalch equation and \( pI \) while \( \beta[\text{Pr}] \) remains constant independent of temperature.

However, since the special interest of this aspect of the paper lies in the \( 20 - 38 \Delta p\text{H} \) of serum under conditions of constant total \([\text{CO}_2]\) and \([b]\), experiments were done to test equation (27) under these special conditions. The data are given in Table III.

The experiments are on both separated and true serum and
CO₂ Absorption Curve

divide themselves into two groups, Nos. 1 to 12 and Nos. 13 to 19. The sample of serum was equilibrated at 38° and its gasometric pH₃₈ calculated from the Henderson-Hasselbalch equation.

TABLE III.
Effect of Temperature on pH of Serum at Constant [CO₂].

<table>
<thead>
<tr>
<th>Source</th>
<th>Experiment No.</th>
<th>[CO₂] [mm]</th>
<th>β[Pr] [mm]</th>
<th>pH 20°</th>
<th>pH 38°</th>
<th>ΔpH</th>
<th>Calculated ΔpH</th>
<th>Difference observed - calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 27</td>
<td>1</td>
<td>27.0</td>
<td>4.0</td>
<td>7.32</td>
<td>7.08</td>
<td>0.24</td>
<td>0.22</td>
<td>+0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24.5</td>
<td>4.0</td>
<td>7.65</td>
<td>7.40</td>
<td>0.25</td>
<td>0.29</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22.4</td>
<td>4.0</td>
<td>7.80</td>
<td>7.58</td>
<td>0.22</td>
<td>0.33</td>
<td>-0.11</td>
</tr>
<tr>
<td>Sheep 1</td>
<td>4</td>
<td>23.2</td>
<td>2.3</td>
<td>7.17</td>
<td>6.98</td>
<td>0.19</td>
<td>0.15</td>
<td>+0.04</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>21.6</td>
<td>2.3</td>
<td>7.57</td>
<td>7.30</td>
<td>0.27</td>
<td>0.23</td>
<td>+0.04</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>19.4</td>
<td>2.3</td>
<td>7.90</td>
<td>7.60</td>
<td>0.30</td>
<td>0.32</td>
<td>+0.02</td>
</tr>
<tr>
<td>Same with added NaHCO₃</td>
<td>7</td>
<td>46.2</td>
<td>2.3</td>
<td>7.22</td>
<td>7.08</td>
<td>0.14</td>
<td>0.10</td>
<td>+0.04</td>
</tr>
<tr>
<td>Dog 33, true serum.</td>
<td>9</td>
<td>25.5</td>
<td>4.0*</td>
<td>7.28</td>
<td>7.08</td>
<td>0.20</td>
<td>0.22</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>19.9</td>
<td>4.0*</td>
<td>7.54</td>
<td>7.27</td>
<td>0.27</td>
<td>0.29</td>
<td>-0.02</td>
</tr>
<tr>
<td>Sheep 1, true serum.</td>
<td>11</td>
<td>25.3</td>
<td>2.3*</td>
<td>7.30</td>
<td>7.06</td>
<td>0.24</td>
<td>0.16</td>
<td>+0.08</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>19.9</td>
<td>2.3*</td>
<td>7.57</td>
<td>7.26</td>
<td>0.31</td>
<td>0.24</td>
<td>+0.07</td>
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<td>31</td>
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<td>[7.22]</td>
<td>[7.10]</td>
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<td>14</td>
<td>24</td>
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<td>&gt;[7.95]</td>
<td>[7.75]</td>
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<td>Rat pooled serum.</td>
<td>15</td>
<td>26</td>
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<td>[7.37]</td>
<td>[7.21]</td>
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<td>16</td>
<td>24</td>
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<td>[7.67]</td>
<td>[7.47]</td>
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</tr>
<tr>
<td>Dog 27</td>
<td>17</td>
<td>25</td>
<td></td>
<td>[7.22]</td>
<td>[7.05]</td>
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<tr>
<td></td>
<td>18</td>
<td>19</td>
<td></td>
<td>[7.61]</td>
<td>[7.40]</td>
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<td></td>
</tr>
<tr>
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<td>19</td>
<td>17</td>
<td></td>
<td>[7.87]</td>
<td>[7.63]</td>
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</table>

* Assumed.

The color reading [pH]₂₀ at 20°C. of each sample was determined colorimetrically. The gasometric pH₂₀ and the correction ([pH]₂₀ - pH₂₀) were determined independently on samples of the same serum by equilibration at 20°C. over a range of pH. These
(\text{pH}_{20} - \text{pH}_{38}) \text{ corrections were used to correct the other } 20^\circ \text{ colorimetric readings on the same serum equilibrated at } 38^\circ \text{ so that the values given as pH}_{38} \text{ in Table III are essentially gasometric pH}_{38} \text{ values. } \beta[\text{Pr}] \text{ was determined from the slope of the serum CO}_2 \text{ absorption curves. The "calculated } 20 - 38 \Delta \text{pH} \text{" values are obtained from Fig. 4.}

In the second group of observations (experiments 13 to 19) no \((\text{pH}_{20} - \text{pH}_{38}) \text{ corrections were determined so that only the colorimetric readings of the diluted serum at } 20^\circ \text{ and } 38^\circ\text{C. are given. Direct comparison therefore cannot be made with the calculated } 20 - 38 \Delta \text{pH} \text{ (see Austin, Stadie, and Robinson, 1925). The experimental data with the exception of No. 3 are in complete qualitative as well as quantitative agreement with the calculated values. } \Delta \text{pH} \text{ as observed is not constant but regularly (except No. 3) increases with pH. Increasing total CO}_2 \text{ (Nos. 4 to 8) decreases } 20 - 38 \Delta \text{pH} \text{ roughly about } 0.04 \text{ per } 10 \text{ mm increase of CO}_2 \text{ per liter. The calculated } 20 - 38 \Delta \text{pH} \text{ is in agreement with the observed for most of the observations. In Nos. 11 and 12 the difference is outside the experimental error but calculated and observed } 20 - 38 \Delta \text{pH} \text{ vary with change in } [\text{CO}_2] \text{ and with initial pH}_{38} \text{ in the same direction and to the same extent. No reasons for the discrepancies in Nos. 3, 11, and 12 are known.}

\text{SUMMARY.}

1. The dependence of the CO}_2 \text{ absorption curve of serum or blood upon the base bound by protein at varying pH is discussed and the relation stated in equations (8) and (10).

2. The change in CO}_2 \text{ absorption curve between } 38^\circ \text{ and } 20^\circ\text{C. is determined experimentally for whole blood, true serum, and separated serum in dog and sheep.}

3. Our data furnish no evidence that the value of } \beta[\text{Pr}] \text{ is altered consistently by this change of temperature.

4. On the assumption that } \beta[\text{Pr}] \text{ is the same at both temperatures one can evaluate the change of pI of equations (3) or (8) with temperature.

5. It is found that approximately for whole blood, true serum, and separated serum

\[
\frac{\Delta \text{pI}}{\Delta t^\circ} = \frac{\Delta \text{pH}}{\Delta \text{pH}_{\text{constant}}} = -0.017 \text{ to } -0.023.
\]
6. When it is desired to approximate the position of a [BHCO₃] curve against pH for either separated serum, true serum, or whole blood at another temperature, t', when the curve at t is known, it can best be done by means of the equation in conclusion 5 and the assumption that the slope of the [BHCO₃] curve against pH at t' is the same as at t.

7. The slope of the true serum curve is found to be higher than that of the corresponding blood both at 20°C. and 38°C. The reason for this is discussed and an empirical relation

\[ \Delta [BHCO₃]_{TS} = 1.1 \Delta [BHCO₃]_R \]

given relating the two slopes at any temperature.

8. The change in pH of a separated serum or whole blood undergoing change in temperature at constant [CO₂] is calculated and the influence of initial pH, initial [CO₂], and initial value for β[Pr] is shown.

9. This change in pH (which enters into the C correction of Cullen's colorimetric method) may vary 0.09 pH units within physiological ranges with change in temperature from 20° to 38°, and is a factor therefore in the variation of the C correction.

BIBLIOGRAPHY.


THE EFFECT OF TEMPERATURE ON THE ACID-BASE-PROTEIN EQUILIBRIUM AND ITS INFLUENCE ON THE CO₂ ABSORPTION CURVE OF WHOLE BLOOD, TRUE AND SEPARATED SERUM

William C. Stadie, J. Harold Austin and Howard W. Robinson

J. Biol. Chem. 1925, 66:901-920.