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Acidosis oxygen dissociation curve
bicarbonate pH
hemoglobin THAM

The Effect of THAM and Sodium Bicarbonate on the Oxygen Dissociation Curve and pH Difference Across the Red Cell

F.C. BATTAGLIA[8], E.L. MAKOWSKI, G. MESCHIA and M.M. NIERNBERG

Departments of Obstetrics and Gynecology, Pediatrics, Physiology, and Computer Service, University of Colorado Medical Center, Denver, Colorado, USA

Extract

This report presents the difference (Δ [H^+]), and ratio (Δ pH), of the hydrogen ion activity between the inside and outside of the red cell for THAM-buffered and $NaHCO_3$-buffered ACD blood of man. Figure 2 shows that there was a significant difference in Δ pH and Δ [H^+] between bloods buffered with THAM or sodium bicarbonate, this difference being due to the fact that normal Δ pH was reduced in THAM-buffered blood to a Δ pH of 0.072 (see table I). The oxygen dissociation curves were constructed at a variety of whole blood pHs for THAM-buffered and sodium bicarbonate-buffered ACD blood. As predicted from the measured difference in Δ pH, the oxygen dissociation curves were different for these two bloods. Table II presents the appropriate constants for the general equation $\log pO_2 = k_1 + k_2 (7.4 - pH) + k_3 \log (\frac{s}{100-s})$. With the use of a computer program and Calcomp plot routine, the $\log \frac{s}{100-s}$/$\log pO_2$ plot for THAM-buffered (fig. 3) and sodium bicarbonate-buffered ACD blood (fig. 4), together with their 95% confidence limits, were constructed.

Speculation

Comparison of Δ pH measurements with oxygen dissociation curves should help in distinguishing between direct effect of acids and bases upon the oxygen affinity of hemoglobin and their indirect effect on oxygen affinity through an alteration in Δ pH across the red cell membrane. Secondly, the use of THAM to increase the oxygen affinity of whole blood may be of help to those studying fetal physiology and interested in determining whether fetal oxygen consumption is limited by an increased oxygen affinity in fetal blood.

Introduction

The factors which determine the difference in pH between the inside and outside of the red cell (Δ pH), have not been clearly established. In addition, the extent to which changes in Δ pH across the red cell account for changes in the oxygen dissociation curve of whole blood under various conditions has not been described; however, the suggestion has often been made that changes in Δ pH might be responsible for observed changes in the oxygen dissociation curve of whole blood.

Since the two buffers most commonly used for the titration of blood stored in acid-citrate-dextrose (ACD) to a pH within the normal physiologic range are tris [hydroxymethyl] aminomethane (THAM) and so-

dium bicarbonate ($NaHCO_3$), it seemed worthwhile to study their effect upon Δ pH and upon the oxygen dissociation curve of ACD blood titrated with these buffers to a pH of approximately 7.40. The suggestion has been made that such blood be used for exchange transfusions in newborns when heparinized whole blood is not available, thus avoiding the large acid load of unbuffered ACD blood. This report compares the effect of THAM *vs* $NaHCO_3$ on Δ pH and upon the oxygen dissociation curve of the whole blood.

Methods

Part I: Measurement of Δ pH

Blood samples were collected from normal adults into syringes containing ACD solution in the proportion of one to four by volume of ACD to whole blood. After mixing, such blood has a pH in the range of 6.9 to 6.7. The blood was divided into two aliquots. To each aliquot, sufficient buffer was added to bring the extracellular pH to approximately 7.6 in air. One aliquot was buffered by the addition of 0.3 M THAM and the other by the addition of 0.6 M $NaHCO_3$. The buffers were added slowly to the blood while the blood was mixed with a magnetic stirrer. Two ml aliquots of the buffered blood were then added to tonometers containing varying CO_2 concentrations in room air. As shown in figure 1, a new Δ $[H^+]$ across the red cell was established rapidly after the addition of buffer, and this pH difference remained fairly constant thereafter. On all samples, equilibration was carried out for 30 minutes at 38° after which the blood was removed from the tonometers anaerobically into syringes. The extracellular and intracellular pHs were then determined in duplicate as described previously [2].

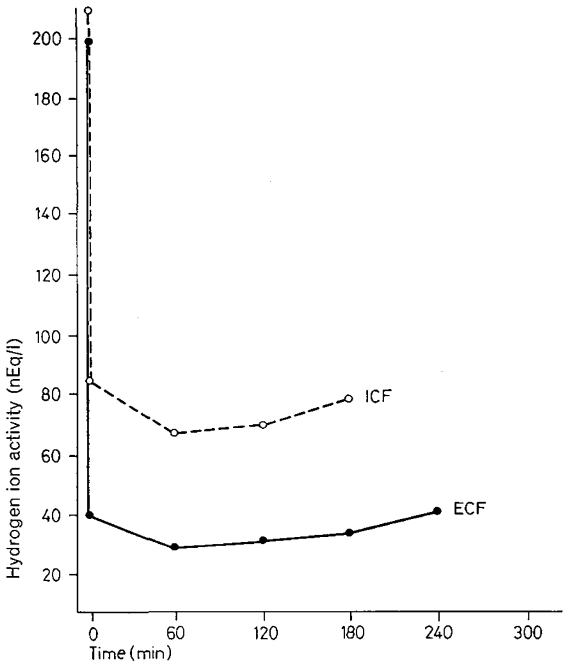


Fig. 1. Addition of 0.6 M $NaHCO_3$ to ACD whole blood, showing rapidity with which a new Δ $[H^+]$ is established across the red cell membrane.

Part II: Construction of Oxygen Dissociation Curves

The technique used in this report for the construction of oxygen dissociation curves at several extracellular pHs is similar to that described by ASTRUP *et al.* [1] with some minor modifications. One ml of blood was exposed for 15 minutes at 38° to 50 ml of gas in a glass tonometer which had been connected by a pyrex ball and socket joint to a manifold for establishing appropriate pCO_2 and pO_2 tensions. Thus, the pH of THAM-buffered and $NaHCO_3$-buffered bloods was varied by changes in pCO_2 in the gas phase. Previous studies by others have shown the effect of pCO_2 independent of pH to be negligible [1, 5]. After rotation for 15 minutes a 1 ml blood sample was removed from the tonometer anaerobically into a 2 ml oiled glass syringe. Oxygen tensions were measured polarographically with a Radiometer microelectrode, pH with a Radiometer model No. 4 meter, and oxygen saturations with a Radiometer oximeter on hemolyzed packed red cells. All determinations were done in duplicate on each sample. A computer program, together with Calcomp plot routines designed for use with computer model CDC 6400, was written which provides for the computation of the appropriate constants for equation No. 1 (k_1, k_2, k_3), together with the variance of each constant and the plot $\log \frac{s}{100-s}/\log pO_2$ with its 95 % confidence limits [6].

Results

Part I: pH and Hydrogen Ion Activity Differences

Table I presents the mean values obtained for Δ pH and Δ $[H^+]$ across the red cell for both THAM-buffered and $NaHCO_3$-buffered ACD blood. Figure 2 presents the THAM and $NaHCO_3$ intracellular hydrogen ion activities over a wide range of extracellular hydrogen ion activities. It is clear that there was a highly significant difference between the two types of buffered blood in terms of both the Δ $[H^+]$ across the red cell, and the intracellular $[H^+]$ extracellular $[H^+]$ ratio as reflected by the Δ pH differences. Thus, the hemoglobin

Table I. Comparison of THAM *vs* $NaHCO_3$ on ΔpH and Δ $[H^+]$ across the red cell

	$NaHCO_3$ (42)	THAM (31)	$NaHCO_3$-THAM
Δ $[H^+]$	31.7±1.0	8.6±0.8	23.1[1]
Δ pH	0.266±0.018	0.072±0.005	0.194[1]

Δ $[H^+]$ = ICF $[H^+]$–ECF $[H^+]$ in nEq/l.
Δ pH = ECF pH–ICF pH.
Each number in the first two columns represent the mean ±1 S.E.M. The numbers in parentheses are total number of observations.
[1] $p < 0.001$.

Table II. Physical constants for blood

	k_1[1]	k_2	k_3
THAM	1.382±0.007	0.502±0.045	0.385±0.022
$NaHCO_3$	1.424±0.005	0.421±0.030	0.381±0.018

There were a total of 34 observations in each group.
[1] Difference of k_1 for THAM and $NaHCO_3$ significant at $p < 0.001$.

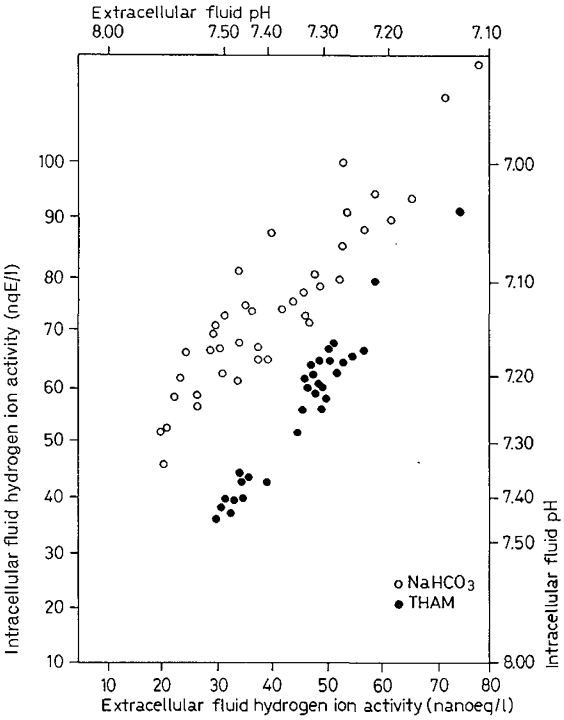


Fig. 2. Points represent data obtained on ACD blood, buffered with either 0.6 M $NaHCO_3$ or 0.3 M THAM.

in THAM-buffered blood was exposed to a higher intracellular pH at any given extracellular pH than it was $NaHCO_3$-buffered blood. It is interesting that the mean Δ $[H^+]$ and ΔpH differences for $NaHCO_3$-buffered ACD blood (31.7 nEq/l and 0.266 respectively) are close to the values previously reported for fresh heparinized adult blood of man (27.0 nEq/l and 0.209) [2].

Part II: Oxygen Dissociation Curves

Oxygen tension, saturation and pH were measured in 34 THAM-buffered ACD blood samples and in 34 $NaHCO_3$-buffered ACD blood samples. The relation among the three variables, pO_2, O_2 saturation, and pH, at 38° has been expressed in the form of the general equation given below. In this form, 'k_1' is equal to the log pO_2 at 50 % saturation and pH 7.4.

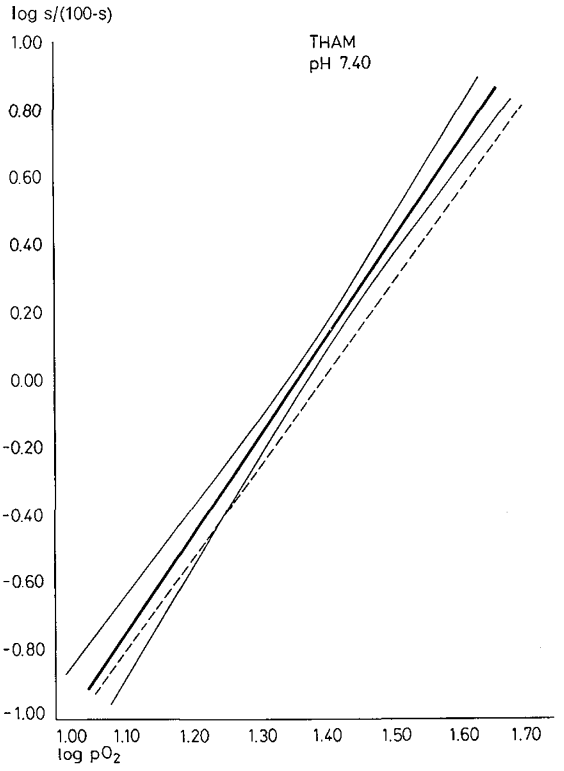


Fig. 3. Heavy solid line represents regression line of log $\frac{s}{100-s}$/log pO_2 for THAM-buffered ACD blood. Fine solid lines represent its 95 % confidence limits. The dotted line represents similar regression line for $NaHCO_3$-buffered ACD blood.

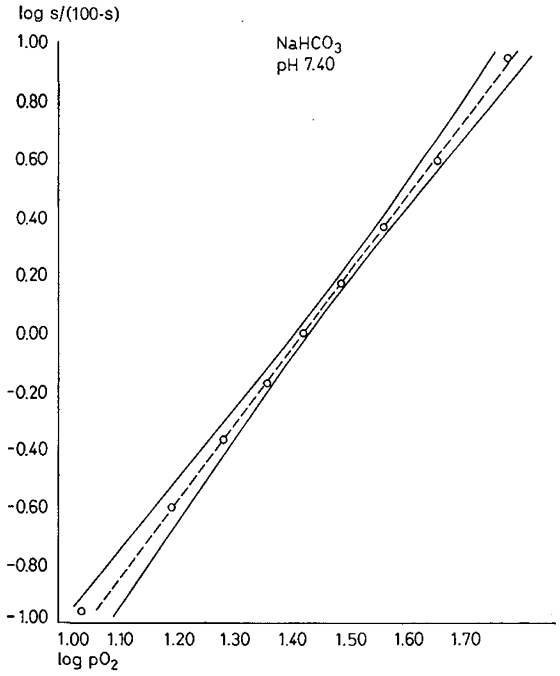
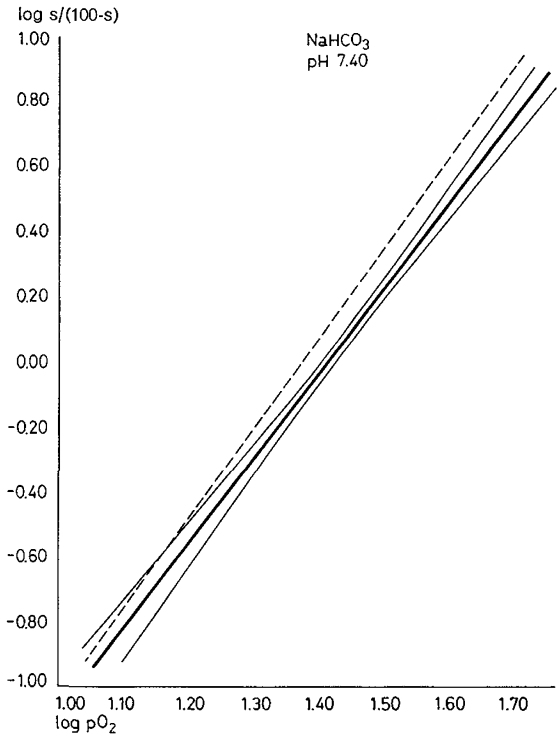


Fig. 4. Solid lines represent regression line of log $\frac{s}{100-s}$/ log pO_2 for $NaHCO_3$-buffered blood. The dotted line represents the regression line for THAM-buffered blood.



(1) $\log pO_2 = k_1 + k_2\,(7.4 - pH) + k_3 \log \left(\frac{s}{100-s}\right)$

The values of k_1, k_2, and k_3, together with the standard deviations of each constant, are shown in table II for both THAM- and $NaHCO_3$-buffered ACD blood. The log pO_2 (50 %, 7.4) was significantly different for the two bloods.

Figures 3 and 4 present the log $\frac{s}{100-s}$/log pO_2 plots of THAM- and $NaHCO_3$-buffered ACD bloods at pH 7.4, together with their 95 % confidence limits. For comparison, the regression of the paired buffered blood is shown as well. It is clear that the mean log $\frac{s}{100-s}$/log pO_2 plot for each blood is outside the 95 % confidence limits over an oxygen saturation range of approximately 25 % to 90 %.

The difference in log pO_2 between THAM- and $NaHCO_3$-buffered blood at 50 % saturation and extracellular pH 7.4 is 0.042. On the basis of this difference, the intracellular pH_i of THAM-buffered blood should be approximately 0.10 pH units more alkaline, compared to the measured values of 0.19.

Discussion

This report has pointed out a physiologic difference of blood buffered with THAM *vs* blood buffered with $NaHCO_3$. To our knowledge, THAM represents the first compound which, when added to whole blood, produces a shift in the oxygen dissociation curve of that blood. It is of interest that this change in the oxygen dissociation curve was predicted from a change in the measured pH difference across the red cells. Since the measurement of the pH difference across the red cells can be done easily and rapidly on small samples of blood, it provides one method of screening the effect on the oxygen dissociation curve of various acids and bases of physiologic interest. It should be emphasized that, as with all methods of measuring intracellular pH, the limits of error cannot be precisely defined. However, the correlation between changes in this measurement of intracellular pH with changes in oxygen affinity of the whole blood support the internal consistency of the method. The computer program used in this study provides a treatment of the data very similar to that produced by ROSSING and CAIN [5], with the exception that the confidence limits are expressed in terms of the linear

Fig. 5. Points represent data of ASTRUP *et al.* [1] plotted in relation to regression line of log $\frac{s}{100-s}$/log pO_2 for $NaHCO_3$-buffered blood from the present study.

$\log \frac{s}{100-s}$/log pO_2 plot rather than the percent saturation/pO_2 plot used in their report.

Such a treatment of oxygen tension, saturation, and pH data should help considerably in the interpretation of differences of blood or hemoglobin solution oxygen dissociation curves. Whatever the merits and limitations of such a program, it should be pointed out that the significance of many differences among dissociation curves claimed in the past was not adequately documented.

In figure 5, the data of ASTRUP *et al.* for heparinized blood of man have been superimposed on our data for $NaHCO_3$-buffered blood. It is encouraging to see the good agreement between the two studies using somewhat different techniques. This agreement between the two studies points out that the addition of $NaHCO_3$ to whole blood does not alter the position of the oxygen dissociation curve, despite the fact that the $NaHCO_3$-buffered blood must, of necessity, be quite hypertonic. Thus, it would seem that wide variation in intracellular total solute concentration within the physiologic range has no effect upon the oxygen affinity of whole blood. Furthermore, the slope of the $\log \frac{s}{100-s}$/log pO_2 plot shows excellent agreement between the two studies, lending further support for a standard oxygen dissociation curve at pH 7.40, 38° as proposed earlier [1].

It should be pointed out that in both the studies of ASTRUP *et al.* and in the present report, neither potassium oxalate nor sodium fluoride was added to the blood. Yet, these data are in accord with previous measurements on blood drawn into a potassium oxalate-sodium fluoride mixture [3]. This suggests that the preparation of blood for oxygen dissociation curves with potassium oxalate or sodium fluoride produces no major alteration in the oxygen dissociation curve. This conclusion is at variance with that of KIRSCHBAUM *et al.* [4], who used as a basis for their report data with very high variability.

Summary

The pH difference across the red cell membrane and oxygen dissociation curves of THAM- and $NaHCO_3$-buffered ACD blood have been measured. THAM lowers the pH difference across the red cell and, consistent with this, shifts the oxygen dissociation curve of blood to the left. $NaHCO_3$ as a buffer does not alter the normal pH difference across the red cell nor the oxygen dissociation curve of the blood.

References and Notes

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6. Further information on this program may be obtained by writing Mr. MICHAEL NIERNBERG, Senior Scientific Programmer, Computer Services, University of Colorado Medical Center, 4200 East 9th Ave., Denver, Colo. 80220 (USA).
7. This work was supported by USPHS Grants HD 00781-03 and HD 02348-02.
8. Requests for reprints should be addressed to: F.C. BATTAGLIA, M.D., Department of Pediatrics, University of Colorado Medical Center, 4200 East 9th Ave., Denver, Colo. 80220 (USA).