Red Cell 2,3-Diphosphoglycerate and Intracellular Arterial pH in Acidosis and Alkalosis

By Jane F. Desforges and Philip Slawsky

With the use of $^{14}$C-DMO ($^{14}$C-5,5-dimethyl-2,3-oxazolidinedione), a weak organic acid, we measured the intraerythrocytic hydrogen ion concentration in 16 acidotic and alkalotic patients. Whole blood pH, red cell 2,3-diphosphoglycerate, hemoglobin, oxyhemoglobin, plasma pCO₂, and plasma bicarbonate were measured simultaneously on heparinized arterial blood. The results show: (1) hydrogen ion concentration in the red cell varies directly with that of whole blood, (2) red cell concentration of 2,3-diphosphoglycerate varies inversely with the whole blood hydrogen ion concentration, and (3) red cell 2,3-diphosphoglycerate concentration also varies inversely with the intracellular hydrogen ion concentration. There were no significant relationships between the arterial total hemoglobin or oxyhemoglobin and intracellular or whole blood pH, nor was there any relationship between plasma pCO₂ or plasma bicarbonate and intracellular or whole blood pH. We concluded that in a number of clinical conditions in which the hydrogen ion concentration is altered, the cellular pH parallels that of the whole blood and that the 2,3-diphosphoglycerate concentration varies with the hydrogen ion concentration.

INTRAERYTHROCYTIC METABOLISM normally takes place in a slightly alkaline milieu. However, its metabolism in clinical states inducing extracellular changes in hydrogen ion concentration is not well studied. The amount of one of the glycolytic substrates in the Rapoport-Leubering shunt, 2,3-diphosphoglycerate (2,3-DPG), is affected in situations in which the hydrogen ion concentration changes. It is the purpose of this paper to show that in abnormal clinical states: (1) $^{14}$C-DMO can be used to measure the red cell hydrogen ion concentration, (2) the amount of 2,3-DPG in red cells is related to intracellular hydrogen ion concentration, and (3) both the intracellular hydrogen ion concentration and the amount of 2,3-DPG fluctuate with the whole blood hydrogen ion concentration.

MATERIALS AND METHODS

Sixteen patients were selected from the Tufts medical wards at the Boston City Hospital. All patients were acutely ill, and none had a hemoglobin value less than 12 or a hematocrit under 35. There was no blood loss except for that drawn in the course of treatment and diagnosis. No patient was taking thyroid medication, nor was bicarbonate or oxygen...
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being administered at the time the blood was drawn for studies. All patients had been in the hospital for at least 8 hr. Heparinized arterial blood was used for all determinations, with the exception of an occasional venous specimen for 2,3-diphosphoglycerate determination. Venous samples were used primarily to measure the cellular and plasma water content. We have found that venous and arterial plasma water content differ by less than 1% and cellular water differs by less than 0.5%. Nine patients were alkalotic and seven were acidotic.

Hemoglobin, hematocrit, and red cell counts were performed in the usual manner. The hemoglobin and per cent oxygen saturation were measured in a I.L. cooximeter 182, and the oxyhemoglobin concentration was calculated. Whole blood pH and pCO₂ were measured on a pH meter, and the bicarbonate was calculated. The accuracy of the pH reading is within 0.005 pH units. The red cell 2,3-diphosphoglycerate concentration was determined spectrophotometrically by a modification of Krimsky's technique. Three milliliters of heparinized blood were added to 6 ml of cold 10% trichloroacetic acid (TCA) at room temperature. The supernatant fluid was immediately adjusted to pH 7.4 with potassium hydroxide. An aliquot of this extract was then added to the appropriate reagents, and the change in optical density associated with the degradation of phosphoenolpyruvate was measured spectrophotometrically at 240 μm.

The intracellular hydrogen ion concentration was obtained by using ¹⁴C-DMO (¹⁴C-5,5-dimethyl-2,4-oxazolidinedione) as an indicator of acidity. Subsequent calculations were performed from the dissociation characteristics of DMO as a weak organic acid. The procedure is as follows: From 3 to 5 ml of stoppered, heparinized arterial blood were incubated at 37°C for 5-10 min with a small volume of saline-diluted ¹⁴C-DMO. In six experiments with tubes tightly stoppered, the pH remained stable between 5 and 30 min. Measurements under 5 min and over 30 min were not made. After 5 min 1.0 ml of the plasma was separated at room temperature. One milliliter of plasma and 1.0 ml of whole blood were then each added to 2.0 ml of 10% TCA. One milliliter of each supernate was then added to 9.0 ml Aquafior, and the β-irradiation was counted in a liquid scintillation counter. The supernate and the counting fluid (Aquafior) were clear and colorless at the time of counting.

An attempt was also made to determine if ¹⁴C-DMO binds to the lipoprotein of cell membranes. Red cell membranes were prepared by hemolyzing cells in distilled water and allowing the membranes to settle in an acetate buffer, pH 4.5, overnight in the cold. After centrifuging and washing, the procedure for incubation with ¹⁴C-DMO, TCA extraction, and counting was carried out. The results showed that no radioactivity was present in the membrane fraction.

Internal quenching curves for TCA extracts of whole blood and plasma were similar and obviated the necessity to account for hemoglobin as an additional quenching agent. The calculation of intraerythrocytic pH (pH₁) is then based on the Henderson-Hasselbalch equation for the dissociation of weak organic acids. For the calculations it was necessary to determine the water content of plasma and red blood cells. This was done by weighing 1.0 ml samples of blood and plasma before and after heating at 100-105°C for 30 min. Proper adjustments in the weights and counts were made for trapped plasma calculated according to the hematocrit. The concentration of ¹⁴C-DMO in red cells (DMO₁) is then equal to cpm/g cell water, and the concentration of ¹⁴C-DMO in plasma (DMO₂) is equal to cpm/g plasma water. The final mathematical form is:

\[
\text{pH₁} = \text{pK$_{\text{DMO}}$} + \log \left( \frac{\text{pH₀} - \text{pK$_{\text{DMO}}$}}{(DMO₁) \left( \frac{10^{(DMO₁)}}{1} - (DMO₂) \right)} \right)
\]

in which: pH₀ = red cell pH, pH₀ = whole blood pH, DMO₁ = cpm/g red cell water, DMO₂ = cpm/g plasma water, and pK$_{\text{DMO}}$ = dissociation constant for ¹⁴C-DMO at 37°C = 6.13,5-4
RESULTS

The data are summarized in Tables 1 and 2. Of the nine patients with alkalosis, as defined by a whole blood pH of greater than 7.42, two of them (Nos. 6 and 7) had significant CO₂ retention. In these particular cases this retention was compensatory for metabolic alkalosis, and the difference between the pHₑ and pHᵢ was greater than in the other seven patients with alkalosis. The erythrocyte 2,3-diphosphoglycerate concentration in this series tended to be high. The two patients with CO₂ retention had values similar to others in this group.

Of the seven patients with acidosis, as defined by a whole blood pH of less than 7.38, the differences between pHₑ and pHᵢ varied more than in the alka- lotic group. In patients 11 and 12 the bicarbonate retention was associated with a smaller difference between the pHₑ and pHᵢ than in most of the others in the group. However, in case 14 the pH difference was low in the presence of a low bicarbonate. Noteworthy is the low 2,3-DPG concentration in the red cells of most, but again not all, of the patients in this group.

As revealed in Table 2, despite the deviations mentioned above, there was a significant parallel between the intracellular and whole blood hydrogen ion concentrations. There was no significant correlation between either the intra-

Table 1. Comparison of Parameters Measured

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>pHₑ</th>
<th>pHᵢ</th>
<th>pHₑ-pHᵢ</th>
<th>pCO₂</th>
<th>HCO₃</th>
<th>2.3-DPG</th>
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<tbody>
<tr>
<td>1</td>
<td>Respiratory alkalosis</td>
<td>7.48</td>
<td>7.32</td>
<td>0.16</td>
<td>34</td>
<td>25</td>
<td>4.55</td>
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<tr>
<td>2</td>
<td>Respiratory alkalosis</td>
<td>7.51</td>
<td>7.40</td>
<td>0.11</td>
<td>28</td>
<td>22</td>
<td>3.28</td>
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<tr>
<td>3</td>
<td>Respiratory alkalosis</td>
<td>7.47</td>
<td>7.29</td>
<td>0.18</td>
<td>36</td>
<td>25</td>
<td>2.59</td>
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<tr>
<td>4</td>
<td>Respiratory alkalosis</td>
<td>7.44</td>
<td>7.31</td>
<td>0.13</td>
<td>36</td>
<td>24</td>
<td>4.28</td>
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<tr>
<td>5</td>
<td>Respiratory alkalosis and metabolic alkalosis</td>
<td>7.52</td>
<td>7.26</td>
<td>0.26</td>
<td>34</td>
<td>27</td>
<td>3.54</td>
</tr>
<tr>
<td>6</td>
<td>Metabolic alkalosis and respiratory acidosis</td>
<td>7.44</td>
<td>7.12</td>
<td>0.32</td>
<td>52</td>
<td>34</td>
<td>3.50</td>
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<tr>
<td>7</td>
<td>Metabolic alkalosis and respiratory acidosis</td>
<td>7.47</td>
<td>7.14</td>
<td>0.33</td>
<td>59</td>
<td>42</td>
<td>5.76</td>
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<td>8</td>
<td>Metabolic alkalosis</td>
<td>7.64</td>
<td>7.45</td>
<td>0.19</td>
<td>39</td>
<td>42</td>
<td>3.89</td>
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<td>9</td>
<td>Metabolic alkalosis</td>
<td>7.52</td>
<td>7.41</td>
<td>0.11</td>
<td>38</td>
<td>29</td>
<td>4.36</td>
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<tr>
<td>10</td>
<td>Respiratory acidosis and metabolic alkalosis</td>
<td>7.04</td>
<td>6.73</td>
<td>0.31</td>
<td>108</td>
<td>27</td>
<td>1.11</td>
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<td>11</td>
<td>Respiratory acidosis and metabolic alkalosis</td>
<td>7.30</td>
<td>7.19</td>
<td>0.11</td>
<td>91</td>
<td>43</td>
<td>2.52</td>
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<td>12</td>
<td>Respiratory acidosis and metabolic alkalosis</td>
<td>7.27</td>
<td>7.10</td>
<td>0.17</td>
<td>70</td>
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<td>13</td>
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<td>2.92</td>
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<td>14</td>
<td>Metabolic acidosis</td>
<td>7.12</td>
<td>7.04</td>
<td>0.08</td>
<td>38</td>
<td>10</td>
<td>2.94</td>
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<tr>
<td>15</td>
<td>Metabolic acidosis</td>
<td>7.24</td>
<td>6.84</td>
<td>0.40</td>
<td>39</td>
<td>15</td>
<td>1.67</td>
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<tr>
<td>16</td>
<td>Metabolic acidosis and respiratory alkalosis</td>
<td>7.34</td>
<td>7.12</td>
<td>0.22</td>
<td>36</td>
<td>19</td>
<td>2.42</td>
</tr>
</tbody>
</table>

Normal values: pHₑ (whole blood pH), 7.38–7.42; pHᵢ (intracellular pH), about 7.1–7.2; pCO₂ (partial pressure of carbon dioxide in plasma), 40±2 mm Hg; HCO₃ (bicarbonate), 23–25 meq/liter; 2,3-DPG (diphosphoglycerate), 3.2±0.4 umole/10¹⁰ RBC.
cellular or whole blood hydrogen ion concentration, and plasma bicarbonate, plasma pCO₂, or hemoglobin. There was a lack of a correlation between the intracellular hydrogen ion concentration and the mean corpuscular oxyhemoglobin concentration [MC (Hb02)C], a value obtained by measuring the absolute hemoglobin in g/100 ml multiplying by the per cent oxygen saturation as measured by an oximeter, and dividing by the packed red cell volume. This also showed no correlation with red cell 2,3-DPG.

The concentration of red cell 2,3-DPG correlated with both intracellular and whole blood hydrogen ion concentration. However, in one alkalotic patient (No. 3) the 2,3-DPG concentration was lower than normal, and in two others (Nos. 13 and 14) this value was normal in the presence of significant acidosis.

DISCUSSION

The methods used heretofore to measure the hydrogen ion concentration in cells have been three: CO₂ equilibration, freeze-thaw, and spectrophotometric assay of DMO distribution between cells and extracellular fluid. All three methods have been tried to determine the hydrogen ion concentration of the human red blood cell. Depending on the technique, the acceptable range for normal arterial red cells seems to be between 7.1 and 7.2.

Since 1959, this compound has been used to determine the hydrogen ion concentration of the human red blood cell in venous, but only rarely in normal arterial, blood samples. As a matter of fact, the normal arterial red cell pH has not been extensively studied by any method.

In 1959, Waddell and Butler first used DMO to determine the acidity and pH of dog skeletal muscle. This compound is a substituted trimethadione with a pK of 6.13 at 37°C. It has the following formula:

```
O
/ \CH₃
0   C
\   /CH₃
H - N ----- C = O
```

The physical and chemical characteristics of DMO are such that it can be used to measure the hydrogen ion concentration in arterial or venous red cells. In our experiments ¹⁴C-DMO, a radioactive form of DMO, was used. Our studies confirm the fact that this substance does not bind to red cell membranes. DMO equilibrates across red cell and other cell membranes according to the hydrogen ion concentration of the intracellular and extracellular fluid, its distribution being unaffected by the quantity of substance used.
Table 2. Comparative Statistical Values

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-DPG and $H^+_e$</td>
<td>-0.6</td>
<td>2.84</td>
<td>0.02</td>
</tr>
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<td>2,3-DPG and $pH_e$</td>
<td>0.66</td>
<td>3.25</td>
<td>0.01</td>
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<tr>
<td>2,3-DPG and $H^+_i$</td>
<td>-0.58</td>
<td>-2.70</td>
<td>0.02</td>
</tr>
<tr>
<td>2,3-DPG and $pH_i$</td>
<td>0.61</td>
<td>2.88</td>
<td>0.02</td>
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<tr>
<td>$H^+_e$ and $H^+_i$</td>
<td>0.90</td>
<td>7.75</td>
<td>0.001</td>
</tr>
<tr>
<td>$pH_e$ and $pH_i$</td>
<td>0.89</td>
<td>7.65</td>
<td>0.001</td>
</tr>
<tr>
<td>$H^+_i$ and MCHC</td>
<td>0.20</td>
<td>0.79</td>
<td>Insignificant</td>
</tr>
<tr>
<td>$H^+_i$ and MC (HbO2) C</td>
<td>0.11</td>
<td>0.40</td>
<td>Insignificant</td>
</tr>
<tr>
<td>$pH_i$ and HCO3</td>
<td>0.25</td>
<td>0.81</td>
<td>Insignificant</td>
</tr>
<tr>
<td>$pH_i$ and pCO2</td>
<td>0.32</td>
<td>1.20</td>
<td>Insignificant</td>
</tr>
<tr>
<td>$H^+_i$ and Hb</td>
<td>0.12</td>
<td>0.45</td>
<td>Insignificant</td>
</tr>
</tbody>
</table>

2,3-DPG, 2,3-diphosphoglycerate; $H^+_e$, extracellular hydrogen ion concentration; $pH_e$, extracellular $pH$; $H^+_i$, intracellular hydrogen ion concentration; $pH_i$, intracellular $pH$; MCHC, mean corpuscular hemoglobin concentration; MC (HbO2) C, mean corpuscular oxyhemoglobin concentration; Hb, total hemoglobin in g/100 ml blood.

Bromberg et al. have found this technique, using nonradioactive material, to give an intraerythrocyte pH value about 0.1 U lower than that calculated on the basis of Cl— distribution. Use of radioactive material to measure intraerythrocytic pH has not previously been reported.

Our results indicate that the arterial intraerythrocytic hydrogen ion concentration parallels the fluctuations in whole blood in abnormal clinical conditions. In addition, the correlation of intracellular hydrogen ion concentration with red cell 2,3-DPG levels was significant. The studies also confirm previous observations between this metabolite and whole blood hydrogen ion concentration. Such relationships have been studied by Chillar et al. and Astrup et al. The amount of red cell 2,3-DPG fluctuates with the clinical condition. For example, it is decreased in diabetic acidosis and the acidosis of shock: it may rise in respiratory alkalosis and it may vary with conditions in which serum inorganic phosphate changes. Astrup et al. has reported a relation between red cell pH and 2,3-DPG in patients with acid-base disturbances. However, most reports of the association of this substance with intracellular hydrogen ion concentration of red cells in clinical conditions have been speculative, and most of the experimental work, until very recently, refers to whole blood pH and red cell 2,3-DPG. In vitro studies by Asakura et al. have revealed a significant rise in red cell 2,3-DPG when cells are incubated in an alkaline buffer and a decrease when incubated in an acid buffer, both buffers containing fixed amounts of inorganic phosphate.

In 1965, Battaglia et al. studying pH showed that in acute respiratory alkalosis and acidosis the red cell pH followed closely the whole blood pH. 2,3-DPG concentrations were not measured. Furthermore, the use of peripheral venous blood for this study limits the interpretation of results, since the variability of tissue oxygen extraction and CO2 and lactic acid formation are complicating factors. In vivo studies by Astrup et al. have revealed elevations
of red cell 2,3-DPG concentration when the red cell pH rises in subjects made alkalotic and a decrease in subjects made acidotic. Also noted by Astrup is a significant relationship between red cell 2,3-DPG concentration and plasma bicarbonate. In the most recent study, Bellingham et al.13 have shown, in arterialized venous blood in which the oxygen saturation of hemoglobin is over 80%, that red cell pH increases and red cell 2,3-DPG levels rise in subjects made alkalotic; similarly, there is a fall in red cell pH and 2,3-DPG concentration when acidosis is induced. Although one might expect if the red cell oxyhemoglobin decreased resulting in a decrease in red cell hydrogen ion concentration (oxyhemoglobin being a stronger acid than reduced hemoglobin) that the red cell 2,3-DPG concentration might be increased,14 we could find no such relationship between these two variables. The presence of increased amounts of 2,3-DPG has been shown by Battaglia et al. to be associated with an increase in difference between intra- and extraerythrocyte pH, presumably on the basis of its action as an intracellular anion increasing the ratio of intra- and extracellular H+ via the Donnan equilibrium.15 Deuticke et al. have calculated the effect of an increase in 2,3-DPG in lowering intracellular pH.16 Thus, the inverse correlation between H+ and 2,3-DPG cannot be explained by a direct effect of the latter molecule.

Our results in abnormal clinical states, produced by respiratory or metabolic abnormalities or both, compare favorably with the experimental studies simulating clinical conditions. We did not find a significant relationship, as had been reported by Astrup, between the red cell hydrogen ion concentration and plasma bicarbonate. However, in our patients, the bicarbonate was not the only variable. The clinical conditions under which our particular studies were obtained were relatively acute, extremely unstable, and changing rapidly as acid-base problems so often do.

In summary, we found that in 16 patients studied with a method used to measure hydrogen ion concentration in red cells, the intracellular hydrogen ion concentration varies closely and directly with whole blood hydrogen ion concentration. This essentially agrees with others using different techniques. We found no correlation between hydrogen ion concentration and plasma pCO2 or between hydrogen ion concentration and plasma bicarbonate. The red cell 2,3-DPG concentration varied significantly with the intraerythrocytic and whole blood changes in hydrogen ion concentration and with the pH equivalents.

ACKNOWLEDGMENT

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REFERENCES


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