DISPLACEMENT OF OXYGEN DISSOCIATION CURVES AND RED CELL CATION EXCHANGE IN CHRONIC HYPERCAPNIA  

by  
Karl E. Schaefer, Arthur A. Messier, Carolyn C. Morgan  

Bureau of Medicine and Surgery, Navy Department  
Research Work Unit MF12.524.006-9028.03  

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SUMMARY PAGE

PROBLEM

To investigate the oxygen transport in chronic hypercapnia to obtain a better understanding of metabolic adaptations to CO₂.

FINDINGS

It was found that the oxygen dissociation curve, which is known to shift to the right with acute decreases in pH, was displaced to the left at six hours and one day exposure (uncompensated phase of respiratory acidosis) and shifted back to the right again at three and seven days of exposure (compensated phase of respiratory acidosis) without fully reaching control values.

The displacement of the oxygen dissociation curve to the left facilitates oxygen uptake in the lungs but decreases the delivery of oxygen to the tissue. However, a simultaneous increase in the Bohr effect (Δ log P₀₂/Δ pH) was found to increase oxygen delivery to the tissues during the acute uncompensated phase of respiratory acidosis.

The intracellular pH changes are considered the primary cause of the changes in oxygen affinity in chronic hypercapnia. Red cell cation exchange plays a role in the mechanism involved in the alteration of oxygen affinity in chronic hypercapnia. The ratio of red cell cation/Hb was found to exhibit a close correlation to the changes in the half-saturation pressure (P₅₀) in chronic hypercapnia.

APPLICATION

Findings are of importance for scientists and submarine medical officers in providing new information to evaluate effects of prolonged exposure to CO₂.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Work Unit MF12.524.006-9028—Time-Concentration Exposure Limits of Carbon Dioxide. The present report is No. 3 on this Work Unit. The manuscript was approved for publication on 15 October 1969, and designated as Submarine Medical Research Laboratory Report No. 698.
Guinea pigs were exposed to 15% CO₂ in 21% O₂ for periods up to seven days. Two separate oxygen dissociation curves were determined at a PCO₂ of 40 mm Hg and a PCO₂ of 115 mm Hg on pooled blood samples. Red cell pH and cation concentrations were also measured. P₅₀ fell from 27.8 mm Hg to 18.6 at one day exposure and rose again to 24.7 after seven days of exposure to 15% CO₂. Blood oxygen capacity decreased significantly with the return shift of the oxygen dissociation curve. In spite of the increase in oxygen affinity during the uncompensated phase of respiratory acidosis, delivery of oxygen to the tissues was not reduced due an increase in the Bohr effect, resulting in a higher oxygen delivery to the tissues than at normal conditions.

Commensurate with the increase and decrease in oxygen affinity, cation concentrations of the red cells exhibited corresponding decreases and increases. The potassium concentrations showed a larger fall, while red cell content increased slightly during the uncompensated phase of respiratory acidosis. These changes were reversed during the compensated phase of respiratory acidosis without returning to control levels. The ratio of red cell cations/Hb showed a close correlation with the changes in P₅₀ values.

The pH gradients across the red cell were found to decrease during the uncompensated phase of respiratory acidosis and increase again during the compensated phase of respiratory acidosis.
DISPLACEMENT OF OXYGEN DISSOCIATION CURVES AND RED CELL CATION EXCHANGE IN CHRONIC HYPERCAPNIA

INTRODUCTION

It has been known for a long time that the characteristic "S" shaped form of the oxygen dissociation curve is influenced by the medium (Barcroft and Camis, 1909). More recently, Rossi-Fanelli and collaborators (1961) reinvestigated the influence of electrolytes on the oxygen dissociation curve varying ionic strength and salt composition of the medium over a narrow range. They observed that the affinity for oxygen increases as the ionic strength decreases associated with a change in the shape of the oxygen dissociation curve indicating decreased heme-heme interaction. Moreover, the same workers demonstrated that the magnitude of the Bohr effect decreases as the ionic strength increases to high levels (Antonini, Wyman, Rossi-Farnelli and Caputo, 1962). Waldeck and Zander (1968) found a linear relationship between the P$_{50}$ values and the quotient of the total cation concentration and hemoglobin concentration in the cells. The P$_{50}$ decreased with decreasing ratios. They did not find this relationship to hold to the same degree for the sum of sodium and potassium values which had been observed by Sommerkamp et al. (1961).

We had previously reported marked changes in erythrocyte cation concentrations in chronic hypercapnia (Schaefer et al., 1964) resulting in a decreased ratio of total cations/hemoglobin and we followed this up with an investigation of the oxygen dissociation curve in chronic hypercapnia which showed displacement to the left during the uncompensated phase of respiratory acidosis and a return nearly to normal during the compensated phase of respiratory acidosis associated with changes in the red cell cations and hemoglobin.

METHODS

Studies were performed on male guinea pigs weighing between 400-600 grams exposed for various periods of time to 15% CO$_2$ in 21% O$_2$. The animals were anesthetized with pentobarbital (40 mg/kg) and blood was taken from the abdominal aorta while the animal was still breathing the same CO$_2$ mixture to which it was previously exposed. Potassium oxalate-sodium fluoride was used to stop glycolysis.

Blood obtained from individual animals was cautiously transferred into a 50 ml siliconized plastic syringe and stored at 4°C until the completion of the analysis which usually took six to eight hours. Equilibration and analysis was performed according to the procedure of Bartels et al. (1959) with slight modifications. For each pooled sample two separate oxygen dissociation curves were determined at a P$_{CO_2}$ of 40 mm Hg (pH 7.40) and a P$_{CO_2}$ of 115 mm Hg (pH 7.07). The oxygen tensions of the equilibration gas mixtures were approximately 180, 80, 60, 40, and 20 mm Hg. Aliquots of 2.5 ml blood were equilibrated for 15-20 minutes prior to analysis. Following equilibration, 1 ml of blood was used for the oxygen and carbon dioxide content measurement in the van Slyke manometric apparatus. Hematocrit and pH (electrometric) were determined on another 1 ml blood sample immediately following withdrawal of blood from the animal using the Instrumentation Laboratory Model 113 pH Analyzer.

Dill's factor $\Delta \log P_{O_2} = -0.48 \Delta \text{pH}$ was used for the calculation of oxygen saturation in both sets of oxygen-dissociation curves at a pH of 7.4 and a pH of 7.07 and a temperature of 37°C. The oxygen half-saturation pressure (P$_{50}$) was graphically determined from a plot of the data using Hill's expression $\log S_{O_2}/100-S_{O_2} = n \log P_{O_2}$, according to Bartels et al. (1959). Hill's number "n" (Hill, 1910) was obtained from the slope of the curve.

Results of studies on the Bohr effect in chronic hypercapnia are reported in a separate communication (Schaefer and Messier, 1970).

The oxygen capacity was established from
the measurements of O₂ content in the samples equilibrated with a PO₂ of approximately 180 mm Hg.

Hemoglobin was determined spectrophotometrically after conversion to cyanmethemoglobin. The mean corpuscular hemoglobin concentration (MCHC) was estimated by the ratio hemoglobin/hematocrit.

Prior to the pooling of the blood, the individual samples were analyzed for pH, PCO₂, Hct, and one portion was centrifuged for the measurement of plasma and intracellular electrolytes and water. Measured pH was corrected to the body temperature of the animal determined prior to sacrifice. After centrifugation at room temperature for 50 minutes at 3000 revolutions per minute and taking off the plasma, 2 ml of red cell mass was transferred to a weighing flask and weighed on an analytic balance. Eight ml of triple distilled water was added and the flasks re-weighed. Sodium and potassium content of plasma and hemolysate were determined with a flame photometer.

Red cell pH was measured using the procedure of Hilpert et al. (1963) on hemolyzed separated erythrocytes which had been frozen in polyvinyl tubing avoiding contact with air.

Computer programs were used in the calculations of P₅₀ values. For statistical evaluations of differences between experimental values and control data, Student's "t" test was employed.

RESULTS

Data on whole blood pH, intracellular pH, electrolytes, and hemoglobin, found during chronic hypercapnia in guinea pigs, are presented in Table I. Following exposure to 15% CO₂, whole blood and intracellular pH show a rapid fall after one hour, reach the lowest point after six hours and begin to rise at one day, and continue to increase during the rest of exposure. The periods of fall and rise of the pH distinguish the two phases of respiratory acidosis. Since the pH in whole blood and the intracellular pH still rise 0.114 and 0.089 pH units respectively from the third to the seventh day of exposure, it is difficult to state at which time the respiratory acidosis can be considered to be compensated. However, on the basis of extensive previously reported studies of the effect of chronic exposure of guinea pigs to 15% CO₂, which demonstrated that the changes in lung functions and stress parameters produced by the respiratory acidosis subsided by the third day of exposure (Schaefer et al., 1964; Schaefer et al., 1968), we have for practical purposes considered the respiratory acidosis induced by exposure to 15% CO₂ to be compensated from the third day on, although the pH is still rising at a slow rate during the subsequent period of exposure.

The pH of the cells was found to be 0.16 pH units more acid than that of blood at a pH of 7.37 in control guinea pigs which is somewhat lower than the pH difference of 0.20 units reported for humans (Hilpert et al., 1963).

Since the extracellular pH falls much more than the intracellular pH following exposure to 15% CO₂, the pH gradient across the red cell membrane decreases drastically during the first part of exposure. It increases again during the later part of the exposure.

Data on intracellular electrolytes during chronic hypercapnia in guinea pigs show a significant increase in intracellular potassium during the uncompensated phase of the respiratory acidosis. During the compensated phase of respiratory acidosis (three days and seven days of exposure) the changes are reversed. However, neither of the cation concentrations quite returns to control levels after seven days of exposure. Sum of intracellular sodium and potassium, as well as the ratio of intracellular cations/hemoglobin decreases during the uncompensated phase without reaching control values.

The oxygen dissociation curves obtained from blood of guinea pigs exposed for one day and seven days to 15% CO₂ and equilibrated at a PCO₂ of 40 mm Hg are shown in Figure 1. After one day exposure to CO₂, corresponding to the uncompensated phase of respiratory acidosis, the oxygen dissociation curve is shifted to the left. The P₅₀ value decreased from 27.8 to 18.6. After seven days of exposure (corresponding to the compensated phase of respiratory acido-
TABLE I. EFFECT OF CHRONIC HYPERCAPNIA ON WHOLE BLOOD pH, RED CELL pH,
PH GRADIENT, RED CELL CATIONS, HEMOGLOBIN AND RATIO CELL Na+/K/Hb

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH Whole Blood</th>
<th>pH Red Cell</th>
<th>Hemolysate</th>
<th>pPH</th>
<th>Na+</th>
<th>K+</th>
<th>Na++K+</th>
<th>Hb (g%)</th>
<th>MCHC (%)</th>
<th>Ratio Cell Na+/K</th>
<th>Hb</th>
<th>Ratio Cell Na+/K</th>
<th>Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mean, S.E.M.</td>
<td>7.370</td>
<td>7.214</td>
<td>.156</td>
<td>10.1</td>
<td>96.4</td>
<td>196.4</td>
<td>12.82</td>
<td>31.5</td>
<td>3.4</td>
<td>8.5</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% CO₂</td>
<td>7.021*</td>
<td>6.966*</td>
<td>.055</td>
<td>12.1*</td>
<td>81.68*</td>
<td>103.5</td>
<td>13.18</td>
<td>31.0</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Hour Mean, S.E.M.</td>
<td>.014</td>
<td>.019</td>
<td>.51</td>
<td>1.44</td>
<td>1.56</td>
<td>.18</td>
<td>.07</td>
<td>.07</td>
<td>.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% CO₂</td>
<td>6.969*</td>
<td>6.945*</td>
<td>.024</td>
<td>13.84*</td>
<td>81.40*</td>
<td>95.24*</td>
<td>13.93*</td>
<td>31.3</td>
<td>3.0*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Hours Mean, S.E.M.</td>
<td>.017</td>
<td>.022</td>
<td>.89</td>
<td>1.24</td>
<td>.18</td>
<td>.48</td>
<td>.07</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day Mean, S.E.M.</td>
<td>7.020*</td>
<td>6.984*</td>
<td>.036</td>
<td>14.82*</td>
<td>72.49*</td>
<td>87.5*</td>
<td>13.09</td>
<td>34.3*</td>
<td>2.6*</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3 days Mean, S.E.M.</td>
<td>7.111*</td>
<td>7.056*</td>
<td>.061</td>
<td>13.1*</td>
<td>87.59*</td>
<td>100.3*</td>
<td>12.60</td>
<td>33.0</td>
<td>3.0*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days Mean, S.E.M.</td>
<td>7.235*</td>
<td>7.078*</td>
<td>.139</td>
<td>12.95*</td>
<td>80.29*</td>
<td>102.2*</td>
<td>12.14</td>
<td>31.4</td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significantly different from control levels at the 5% level and better.
pH values corrected to body temperature of animal measured prior to sacrifice.

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TABLE II. O₂ TRANSPORT IN CHRONIC HYPERCAPNIA (GUINEA PIGS)

<table>
<thead>
<tr>
<th>Condition</th>
<th>( P_{50} ) (7.40) mm Hg</th>
<th>( P_{50} ) (7.07) mm Hg</th>
<th>O₂ Capacity Vol%</th>
<th>( n^+ ) pH (7.40)</th>
<th>( n^+ ) pH (7.07)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mean, S.D.</td>
<td>27.8</td>
<td>38.2</td>
<td>16.8</td>
<td>2.874</td>
<td>2.532</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.8</td>
<td>1.1</td>
<td>.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15% CO₂</td>
<td>25.1</td>
<td>38.3</td>
<td>16.2</td>
<td>2.857</td>
<td>2.454</td>
</tr>
<tr>
<td>1 Hour Mean, S.D.</td>
<td>1.4</td>
<td>2.0</td>
<td>.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Hours Mean, S.D.</td>
<td>22.0*</td>
<td>32.2*</td>
<td>17.6</td>
<td>2.392</td>
<td>2.258</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.6</td>
<td>1.3</td>
<td>.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Hours Mean, S.D.</td>
<td>18.6*</td>
<td>27.4*</td>
<td>16.2</td>
<td>2.464</td>
<td>2.532</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.4</td>
<td>3.0</td>
<td>.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days Mean, S.D.</td>
<td>23.2*</td>
<td>33.0*</td>
<td>14.8°</td>
<td>2.592</td>
<td>2.532</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.7</td>
<td>3.1</td>
<td>.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Days Mean, S.D.</td>
<td>24.7</td>
<td>35.9</td>
<td>14.8°</td>
<td>2.641</td>
<td>2.636</td>
</tr>
<tr>
<td>S.D.</td>
<td>2.2</td>
<td>3.3</td>
<td>.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( n^+ \) = Hill’s value

* Statistically different from controls at the 5% level
Dissociation Curves in Chronic Hypercapnia

a $P_{CO_2}$ level of 115 mm Hg corresponding to inspired $CO_2$ tension during chronic hypercapnia induced by prolonged exposure to 15% $CO_2$ are shown in Figure 2.

The same shift to the left occurs after one day exposure, the $P_{50}$ decreased from 38.2 to 27.4. After seven days of exposure the oxygen dissociation curve returns nearly to the control position. For comparison, the oxygen dissociation curve of blood from control animals, equilibrated at a $P_{CO_2}$ of 40 mm Hg, is also exhibited.

The changes in oxygen delivery produced by the changes in the position of the oxygen dissociation curve in chronic hypercapnia are indicated by drawing vertical lines at an arterial pressure of 85 mm Hg (which is the average value we determined in our laboratory in guinea pigs) and 40 mm Hg corresponding to a typical mixed venous oxygen pressure. Disregarding the influence of the Bohr effect for this estimation, oxygen delivery appears to decrease during hypercapnia. Thirty-seven percent of the oxygen capacity are given up to the tissues under normal conditions, declining to 24% during the uncompensated phase of respiratory acidosis and arising again to 35% during the compensated phase of respiratory acidosis.

However, the observed change in oxygen affinity does not necessarily result in a tissue hypoxia because the decrease of oxygen supply, due to the changes in the position of the oxygen dissociation curve, is apparently compensated by an increase in the Bohr effect delta log $P_{50}$/delta pH (Schaefer and Messier, 1970) and an increase in blood volume reported elsewhere, Baker and Schaefer (1970).

The time course of changes in oxygen half-saturation pressure ($P_{50}$) calculated for blood pH as well as for red cell pH (Figure 3) clearly reflects the uncompensated and compensated phase of respiratory acidosis in the fall and subsequent rise of the $P_{50}$ values to a new equilibrium which remains below the control level.
The relationship between oxygen affinity (P50 values) and the sum of intracellular cations during chronic hypercapnia is presented in Figure 4. The values for one hour and six hours of exposure fall outside to the left. The best fit, a nearly linear relationship, exists for the plot of P50 data versus the ratio of erythrocyte sodium and potassium/hemoglobin (Figure 5).

No correlation was found to exist between oxygen affinity and base excess in chronic hypercapnia, when plotted in a manner corresponding to Figures 4 and 5.

Fig. 3. Time course of red cell pH and half-saturation pressure (P50) of guinea pigs during chronic hypercapnia (15% CO2). Blood samples were equilibrated with gas mixtures of varying oxygen tensions but constant PCO2 of 40 mm Hg. Red cell P50 was calculated using Dill's factor and the pH difference between whole blood and red cell hemolysate.

Fig. 4. Oxygen half-saturation pressure (P50) vs. sum of erythrocyte Na + K. Points marked by X—uncompensated phase of respiratory acidosis. Points marked by X—compensated phase of respiratory acidosis.

Fig. 5. Oxygen half-saturation pressure (P50) vs. ratio of erythrocyte \( \frac{(Na + K)}{Hb} \), points marked by X—compensated phase of respiratory acidosis.

DISCUSSION

The objective of this investigation was to establish whether there is a shift in the oxygen dissociation curve in chronic hypercapnia different from that observed in acute hypercapnia. Although many studies have been done on the acute effect of CO2 on oxygen transport there are, to our knowledge, no reports in the literature on the effects of chronic exposure to CO2 on oxygen dissociation curves.

The major finding of this study is the displacement of the oxygen dissociation curve to the left during the uncompensated phase of respiratory acidosis and a return to near control levels during the compensated phase of respiratory acidosis. This dependence of the shifts in the oxygen dissociation curves on the acid base status clearly indicates that the primary cause of the shift must be the changes in intracellular pH, since the arterial CO2 tension remains rather constant through-
out the exposure. The time course of intracellular pH changes (Figure 3) and P50 values parallel each other, with the exception of the changes observed during the first hour of exposure.

Differences in pH gradients across the red cell membranes have been thought to be responsible for the changes in oxygen affinity found in fetal and adult blood. The higher oxygen affinity of the fetal blood was found to be associated with a decreased pH gradient (Hilpert, et al., 1963; Steen and Turitzin, 1968).

Although the biphasic changes in the pH gradient across the red cells observed in chronic hypercapnia correspond, in general, with the biphasic changes in oxygen affinity, additional factors might influence oxygen affinity. Rooth, Sommerkamp, and Bartels (1962) observed a close correlation between base excess and affinity for oxygen, which was not found in our experiment.

Intracellular cations show biphasic changes in chronic hypercapnia which are associated with the changes in intracellular pH. Our observations of a marked potassium loss and an increase in sodium in red cells during the uncompensated phase of respiratory acidosis are in line with findings of Murphy (1963) on pH effects on cation transport in erythrocytes. He demonstrated that incubation of human erythrocytes at a pH of 7.0 resulted in a decrease in potassium and an increase in sodium when compared with an incubation at a pH of 7.5. These changes in cation exchange produced by lowering of the pH were found to be related to an inhibition of active transport of sodium and potassium associated with a 50% decrease in glucose utilization.

During the compensated phase of respiratory acidosis, in which red cell pH increases again, the alterations of intracellular cations produced by acute hypercapnia are reversed but do not reach initial levels after seven days of exposure to 15% CO2.

In patients with respiratory acidosis, who were treated with mechanical ventilation for eight hours, Kilburn (1965) found erythrocyte red cell potassium significantly lower during acidosis and the erythrocyte cation content was also decreased without reaching statistical significance.

In chronic hypercapnia produced by prolonged exposure of human subjects to 1.5% CO2 for 42 days a significant decrease in red cell potassium and an increase in erythrocyte sodium was observed. After four weeks of recovery all values had returned to control level (Schaefer, et al., 1964). Moreover, trained divers, who demonstrated a high CO2 tolerance, were also found to exhibit the same erythrocyte cation concentration, with a decrease in potassium and slight increase in erythrocyte sodium (Schaefer, 1965).

The changes in intracellular cations have been demonstrated to be one of the mechanisms causing changes in oxygen affinity. Sommerkamp et al. (1961) found a correlation between the affinity for oxygen and the sum of the cations Na+ and K+ in the cell by increasing the cation concentrations of the erythrocytes in in vitro experiments. Waldeck and Zander (1968) obtained a closer correlation with the ratio of the sum of intracellular cations/hemoglobin. Our findings show a decrease of P50 with a fall in the sum of cations and vice versa (Figure 4). A closer correlation is obtained with the ratio of the sum of cations/hemoglobin (Figure 5), which is in agreement with the results of Waldeck and Zander (1968).

The observed linear relationship between red cell cations and P50 (oxygen affinity) does not prove a direct casual relationship between the two functions. There is another mechanism which affects both the hemoglobin affinity to oxygen and the red cell cation exchange.

Benesch and Benesch (1967 and 1968) have demonstrated in vitro that an increase in 2,3 diphosphoglycerate (2,3 DPG) results in a decrease in oxygen affinity and vice versa. Since an increase in pH has been found to cause an increase in 2,3 DPG (Guest and Rappoport, 1939) and lowering of the pH by a few tenths of a unit has been shown to result in a decrease of 2,3 DPG in red cells by Rappoport and Guest (1939), it is most likely that the biphasic changes in pH and oxygen affinity, observed in chronic hypercapnia, are associated with a decrease and subsequent increase in 2,3 DPG.
The postulated biphasic changes in the concentrations of 2,3 DPG would imply an association with the biphasic changes of the red cell cations observed in chronic hypercapnia, which is in line with the recent proposal by Benesch and Benesch (1968) that binding of 2,3 DPG by deoxygenated hemoglobin may influence cation permeability. Gardon (1967) obtained some evidence supporting the notion that the concentration of 2,3 DPG in the red cell influences the permeability to potassium ions. He observed an accelerated loss of potassium ions in the iodoacetate treated cells following exhaustion of the 2,3 DPG stores in the cells.

The shift to the left of the oxygen dissociation curve during the uncompensated phase of the respiratory acidosis facilitates the uptake of oxygen in the lungs during a period in which the lungs of guinea pigs exhibit marked edema and hyaline membranes (Schaefer, Avery and Benesch, 1964). The adaptive value of this change in oxygen affinity is further enhanced by an enlarged Bohr effect reported in a separate communication (Schaefer and Messier, 1970) which makes it possible that more oxygen is delivered to the tissues under unfavorable conditions.

If one considers the oxygen transport in chronic hypercapnia, one has to take into account possible changes in red cell volume. In separate investigations, the red cell volume and plasma volume was determined in guinea pigs under the same conditions during prolonged exposure to 15% CO₂ using a combined method of Cr⁵¹ and I¹²⁵ (Baker and Schaefer, 1969). While the plasma volume did not change, the red cell volume and therefore total blood volume was found to be increased both during the uncompensated and compensated phase of respiratory acidosis.

Both factors, increased Bohr effect and increased red cell volume observed in chronic hypercapnia, contribute to an improved oxygen supply to the tissues. This conclusion is borne out by findings of van Liew (1964), who observed elevated tissue oxygen tension in subcutaneous gas pockets in rats exposed for 24 hours to 16.5% CO₂ in 21% O₂. We found, in rats, after 24 hours of exposure to 15% CO₂ in 21% O₂, similar shifts of the oxygen dissociation curve to the left and an increased Bohr effect as in guinea pigs.

Aging in erythrocytes is known to be associated with an increase in red cell sodium and decrease in potassium and decrease in 2,3 DPG (Bernstein, 1959). Moreover, “old” erythrocytes, separated from “young” erythrocytes in normal human blood by ultracentrifugation, showed an increased oxygen affinity and decreased heme-heme interaction (Hill's values) as compared with “young” erythrocytes (Edwards and Rigs, 1967). The question might be raised whether the biphasic changes in oxygen affinity observed in chronic hypercapnia reflect changes in the spectrum of young and old erythrocytes in the circulating blood. If the proportion of “old” erythrocytes is greatly increased during the epinephrine-induced discharge of blood stores during the compensated phase of respiratory acidosis and the number of “young” erythrocytes rises markedly in the subsequent period, oxygen affinity should first increase and then decrease which corresponds to our findings. Further studies would have to clarify to what extent the changes in oxygen affinity observed during chronic hypercapnia are influenced by a change in the spectrum of old and young erythrocytes in the circulating blood.

The observed biphasic changes in oxygen affinity during chronic hypercapnia have similarities with those found during pre-natal and post-natal life. Oxygen affinity is higher before birth and lower after birth. The high oxygen affinity before birth is apparently needed to improve the oxygen uptake from the mother. With the onset of lung respiration, the decrease in oxygen affinity facilitates the oxygen delivery to the tissues. Bartels (1964) has emphasized that the post-natal shift in the oxygen dissociation curve to higher P₅₀ values is always closely linked with a decrease in oxygen capacity.

In chronic hypercapnia the shift from higher oxygen affinity of the uncompensated phase of respiratory acidosis to lower oxygen affinity of the compensated phase of respiratory acidosis is also associated with a decrease in oxygen capacity.

Since increased carbon dioxide levels in the
atmosphere are frequently associated with decreased oxygen levels (closed space environment), it is of interest to compare the effects of hypercapnia and hypoxia on oxygen affinity. Hypoxemia has been found to cause a shift of the oxygen dissociation curve to the right associated with an increase in the level of organic phosphates (2,3 DPG) which is completed within two days after arrival at altitude (Lenfant, et al., 1968). Since the shift of the oxygen dissociation curve observed during the uncompensated phase of respiratory acidosis and that caused by hypoxia go in opposite directions, one would have to expect that they cancel each other out, if acute hypoxia and hypercapnia are combined. However, chronic hypercapnia and chronic hypoxemia combined. However, chronic hypercapnia and chronic hypoxemia produce the same effects, decreasing oxygen affinity, thereby facilitating oxygen transport from blood to tissues.

In chronic obstructive lung diseases with elevated arterial CO$_2$ tension and decreased arterial oxygen tension, Lenfant, et al., (1969) observed a shift to the right in the oxygen dissociation curve when the hemoglobin concentration was increased. The improved delivery of oxygen to the tissues resulting from the shift might account for the findings that chronic hypoxemia in patients with pulmonary insufficiency does not cause cellular hypoxia as indicated by normal plasma lactate and pyruvate levels (Huckabee, 1965).

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Guinea pigs were exposed to 15% CO₂ in 21% O₂ for periods up to seven days. Two separate oxygen dissociation curves were determined at a Pcco₂ of 40 mm Hg and a Pcco₂ of 115 mm Hg on pooled blood samples. Red cell pH and cation concentrations were also measured. P50 fell from 27.8 mm Hg to 18.6 at one day exposure and rose again to 24.7 after seven days of exposure to 15% CO₂. Blood oxygen capacity decreased significantly with the return shift of the oxygen dissociation curve. In spite of the increase in oxygen affinity during the uncompensated phase of respiratory acidosis, delivery of oxygen to the tissues was not reduced due to an increase in the Bohr effect, resulting in a higher oxygen delivery to the tissues than at normal conditions.

Commensurate with the increase and decrease in oxygen affinity, cation concentrations of the red cells exhibited corresponding decreases and increases. The potassium concentrations showed a larger fall, while red cell content increased slightly during the uncompensated phase of respiratory acidosis. These changes were reversed during the compensated phase of respiratory acidosis without returning to control levels. The ratio of red cell cations/Hb showed a close correlation with the changes in P50 values. The pH gradients across the red cell were found to decrease during the uncompensated phase of respiratory acidosis and increase again during the compensated phase of respiratory acidosis.
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