Inhibition of Erythropoietin Production in Unanesthetized Rabbits Exposed to an Acute Hypoxic-Hypercapnic Environment

By Joachim Wolf-Priessnitz, John C. Schooley, and Lynn J. Mahlmann

Major factors affecting changes in the arterial Hb-O₂ affinity (P₅₀) were examined in relation to the initiation of erythropoietin (ESF) production in unanesthetized New Zealand white male rabbits. They were exposed to an isobaric hypoxic environment (8.8% O₂) with and without CO₂ (5.6% or 10%). During 5 hr exposure samples of arterial blood were collected for measurements of (1) plasma ESF titers, (2) whole blood pH, Pₒ₂, P₅₀, SO₂, lactate, pyruvate, and P₅₀, and (3) intraerythrocytic 2,3-DPG, ATP, and ADP. Exposure to 8.8% O₂ alone stimulated ESF production and caused a leftward shift in the P₅₀ in vivo (P₅₀(i)); the addition of CO₂ significantly inhibited ESF production and caused a leftward shift in the P₅₀ in vivo (P₅₀(i)). The shifts in P₅₀(i) generally followed changes in pH; possibly a lesser influence was exerted by changes in the ADP concentration. The data suggest that increased oxygenation of the whole-body tissues occurs with exposure to 8.8% O₂ + CO₂, as reflected by lower whole-blood “excess lactate” accumulation. We conclude that the suppression of ESF production is a result of this increased oxygenation. Increased tissue oxygenation with the hypoxic-hypercapnic exposures is probably the result of increased pulmonary ventilation coupled with facilitated oxygen unloading to the tissues as a result of a relatively right-shifted P₅₀(i).

In 1967 it was discovered that organic phosphates decrease the affinity of hemoglobin for oxygen. Since then, many investigators have studied the influence of shifts in the P₅₀ (oxygen pressure necessary to 50% saturate Hb with oxygen) on oxygen delivery to the body tissues during hypoxic stress. During high-altitude exposure, the P₅₀ increases in vitro (hereafter designated P₅₀(i)). This decreased Hb-O₂ affinity occurs in response to increases in red cell 2,3-diphosphoglycerate (2,3-DPG). Whether such increases in P₅₀(i) have an impact on the P₅₀ in vivo (expressed as P₅₀(i)) sufficient to partially offset tissue hypoxia appears to be dependent on the degree and duration of the hypoxic stress to which different animal species are subjected.

It is well established that conditions that lead to inadequate tissue oxygenation are frequently associated with increased plasma erythropoietin (ESF) levels. A number of recent reports suggest that shifts in P₅₀(i) can sufficiently alter oxygen delivery to the tissues and thus stimulate ESF production. It has been shown in the rat, following the administration of cobalt or acute bleeding, that a decrease in the P₅₀(i) occurs before ESF production is detectable. During short-term high-altitude exposure a similar pattern is observed in man, but when the leftward P₅₀(i) shift is blocked by simultaneous administration of acetazolamide (a carbonic anhydrase inhibitor), ESF production is suppressed.
Using acetazolamide, Schooley and Mahlmann also observed partial blocking of the ESF response in the rat exposed to acute simulated high altitudes. In all these investigations, the authors speculated that the increase in Hb-O_2 affinity sufficiently decreases oxygen unloading to the tissues to cause tissue hypoxia, which in turn triggers ESF production. Blocking this increase in Hb-O_2 affinity should therefore increase oxygen unloading to the tissues, thus alleviating tissue hypoxia. Therefore the stimulus for ESF production should be reduced.

Some experimental observations are not consistent with this hypothesis. The administration of cyanate (NaOCN) to rats causes a leftward shift in the P_{50}, but does not trigger an ESF response, nor is ESF production suppressed in the rat by uremic acidosis during exposure to simulated high altitudes, even though the acidosis is probably severe enough to prevent the leftward P_{50} shift normally observed with acute high-altitude exposure.

Schooley and Mahlmann observed nearly complete suppression of ESF production in rats breathing a 10% O_2 + 10% CO_2 gas mixture when compared to controls breathing 10% O_2 alone, but physiologic events modifying ESF production were not examined. It was the purpose of this study to determine the effect of CO_2 inhalation on ESF production in the unanesthetized rabbit during a brief hypoxic exposure. Particular emphasis is given to the factors contributing to the P_{50} shifts, as well as the interrelationships between such P_{50} shifts and changes in tissue oxygenation, as reflected by “excess lactate” (XL) accumulation.

**MATERIALS AND METHODS**

Male New Zealand white rabbits (3-4.5 kg) were exposed for 5 hr to isobaric gas mixtures with the following compositions: air; 20% O_2 + 5.6% CO_2; 8.8% O_2; 8.8% O_2 + 5.6% CO_2; or 8.8% O_2 + 10% CO_2. Mixtures other than air were prepared in nitrogen. For convenience, gas mixtures containing 8.8% O_2 are termed “hypoxic” or “hypoxic + CO_2.” Unanesthetized rabbits were placed in a standard restraining device. The auricular artery was cannulated with a 19-gauge needle attached to polyethylene tubing. Samples of arterial blood were drawn anaerobically without stasis into heparinized glass syringes at 30 min and 5 hr exposure. Two-hour samples were also drawn with the hypoxic and hypoxic + 5.6% CO_2 exposures.

The exposure chamber used for all experiments (including the exposure to air) was a lucite box with 4.8 cu ft capacity. The gas flow rate was adjusted to 30 cu ft/hr. Thirty minutes were allowed for chamber equilibration before the timed experiment began, and only gas mixtures within ±0.2% of the required percentage composition were used.

Concentrations of ESF in the plasma of the experimental rabbits were estimated by the 72-hr percentage ^59Fe uptake in plethoric LAF_1/JAX female mice, as previously described by Schooley and Mahlmann. Samples of test plasma expected to contain high concentrations of ESF were first diluted with normal rabbit plasma; in all cases a total of 1 ml plasma was injected subcutaneously. Conversion to units of ESF was made by relating the ^59Fe uptake to a standard curve prepared by using the International Reference Preparation (IRP).

Whole-blood lactate and pyruvate concentrations were estimated by the enzymatic assay methods described in the 1968 Sigma Technical Bulletins Nos. 726-UV and 826-UV (Sigma Chemical, St. Louis, Mo.). Whole-blood XL accumulation was calculated according to the method of Huckabee.

Intraerythrocytic concentrations of 2,3-DPG and adenosine-5'-triphosphate (ATP) were estimated by enzymatic methods described in Sigma Technical Bulletins Nos. 24-UV (1971) and 366 (1973), respectively. Red cell adenosine-5'-diphosphate (ADP) concentrations were determined by the enzymatic assay method of Lowry et al., as modified by Sigma Chemicals (personal communication). All reagents and enzyme preparations were purchased from Sigma.

P_{02}, P_{CO_2}, and pH were determined in vivo in triplicate at 37°C within 2 hr of the time of sampling. Measurements were made with the Blood Micro System, Type BMS 3b (Radiometer,
MECHANISM OF ERYTHROPOIETIN PRODUCTION

Copenhagen, Denmark) and values monitored with the Digital Acid-Base Analyzer, Type PHM72c (Radiometer). The oxygen saturation (SO2) was measured in quadruplicate by the freeze-thaw method15 with a dual beam type OSM1 oxygen saturation meter (Radiometer) calibrated with normal rabbit blood. The hematocrit (Hct) and Hb concentration were measured in duplicate, the former by the standard micromethod, the latter by the cyanmethemoglobin colorimetric method.

Hemoglobin-oxygen affinity (P02). A 5-ml sample of whole blood was equilibrated for 20-30 min at two PO2 levels with the IL 237 Tonometer (Instrumentation Laboratory, Lexington, Mass.). The PO2 was controlled with the IL 208-01 gas-mixing module and IL 208-02 oxygen monitor (Instrumentation Laboratory). The Hb-oxygen affinity was determined from the position of the Hb-oxygen dissociation curve, the position being defined by the PO2 (torr) necessary to half saturate the Hb with oxygen; it was expressed as P02(7.4) when determined in vitro under standard conditions (37°C; pH 7.4; PCO2 40 torr). The P02(7.4) was interpolated from the plot of the corrected PO2 versus SO2; for this purpose, a Bohr factor of -0.45, given by Hilpert et al.16 for rabbit blood, was used. The P02(7.4) was calculated from the P02(7.4) by correcting to the pH and base excess (BE) observed in vivo. The equation

\[
\log P02(7.4) = \log 26.6 + 0.5(MCHC - 33) + 0.69(DPG - 14.5) + 0.013BE + 0.48(7.4 - \text{pH}) + 0.024(\text{Temp} - 37)
\]

given by Bellingham et al.17 for calculation of P02(7.4) for human blood was modified to give an approximation of the P02(7.4) for rabbit blood:

\[
\log P02(7.4) = \log P02(7.4) + 0.013BE + 0.45(7.4 - \text{pH})
\]

This simplified expression for P02(7.4) eliminates the MCHC and 2,3-DPG terms, since they are already incorporated in the experimentally determined P02(7.4). Equation (1) was further modified by replacing the Bohr factor of 0.48 with that for the rabbit (0.45) and by deleting the temperature factor. It was assumed that the rabbit’s body temperature did not change significantly during exposure to the isobaric gas mixtures at room temperature (23°C). The BE was estimated with the blood gas calculator of Severinghaus18 by using the pH, PCO2, and Hb values in vivo.

The standard Student’s t test was used to determine the significance levels of all measurements.

RESULTS

Exposure of rabbits to 8.8% O2 for 2 hr resulted in a small increase in plasma ESF levels; by 5 hr the ESF level had risen to 0.59 IRP units/ml plasma. Figure
Table 1. Arterial Lactate, Pyruvate, and Calculated Excess Lactate Concentrations in Whole Blood During 5-hr Exposure to Hypoxia With and Without CO2

<table>
<thead>
<tr>
<th>Gas mixture flowing (through number)</th>
<th>30 Min</th>
<th>2 Hrs</th>
<th>5 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate</td>
<td>Pyruvate</td>
<td>Excess Lactate</td>
</tr>
<tr>
<td>All</td>
<td>(μmoles/ml whole blood)</td>
<td>(μmoles/ml whole blood)</td>
<td>(μmoles/ml whole blood)</td>
</tr>
<tr>
<td>20% O2</td>
<td>1.22 ± 0.06</td>
<td>0.29 ± 0.02</td>
<td>1.51 ± 0.08</td>
</tr>
<tr>
<td>5% O2 (100%)</td>
<td>3.46 ± 0.15</td>
<td>0.29 ± 0.02</td>
<td>3.75 ± 0.17</td>
</tr>
<tr>
<td>8% O2 (100%)</td>
<td>2.45 ± 0.15</td>
<td>0.29 ± 0.02</td>
<td>2.74 ± 0.17</td>
</tr>
<tr>
<td>10% O2 (100%)</td>
<td>3.90 ± 0.15</td>
<td>0.29 ± 0.02</td>
<td>4.20 ± 0.17</td>
</tr>
</tbody>
</table>

Fig. 2. P50 (A) in vitro and (B) in vivo in arterial blood during 5 hr exposure to hypoxia with and without CO2. Means ± 1 SEM. A: pre-exposure, n = 14; experimental, n = 6–9. B: pre-exposure, n = 13; experimental, n = 6–9.
MECHANISM OF ERYTHROPOIETIN PRODUCTION

157
to the hypoxic gas mixture apparently alleviated tissue hypoxia because XL accumulation during the 5-hr exposure period was not significantly different from the preexposure or air control values.

The mean P_{so} values are presented in Fig. 2. The preexposure P_{so(74)} was 31.9 torr, similar to the value of 31 torr reported by Bartels for the normal rabbit. No significant change was observed with exposure to air. After 2 hr of hypoxia a large increase in P_{so(74)} to 34.2 torr, was observed; this value was maintained for 5 hr. Addition of CO_{2} to the hypoxic gas mixture caused a significant decrease of the P_{so(74)} to about 30 torr within 30 min; no further decrease was observed at 5 hr. The normal P_{so(v)} for the rabbit has been reported to be approximately 33 torr. This is higher than the preexposure value of 30.8 torr observed in the present experiments. A significant decrease to 27.2 torr occurred after 5 hr exposure to air. Exposure to normoxia or hypoxia with 5.6% CO_{2} caused no significant changes. A dramatic decrease in the P_{so(v)} to 26.6 torr, occurred after 30 min hypoxic exposure; by 2 hr partial recovery to 27.8 torr was apparent; at 5 hr the P_{so(v)} was indistinguishable from the preexposure value. Exposure to hypoxia + 10% CO_{2} for 30 min caused a rapid increase to 33.8 torr, which was maintained at 5 hr.

The mean P_{o2} and S_{o2} values for arterial whole blood are presented in Fig. 3. The preexposure P_{o2} was 75.6 torr, with an S_{o2} of 94.4%. Addition of 5.6% CO_{2} to the normoxic gas mixture increased the P_{o2} 15% above the preexposure value with no major shift in the S_{o2}. With exposure to hypoxia, the P_{o2} decreased to 33.8 torr by 30 min and remained at that level for 5 hr; the S_{o2} grad-

---

**Fig. 3.** Arterial oxygen saturation (%) and P_{o2} in whole blood during 5 hr exposure to hypoxia with and without CO_{2}. Means ± 1 SEM. Preexposure, n = 19; experimental, n = 7–12.
Table 2. Arterial pH, \( P_{CO_2} \), and Calculated Base Excess in Whole Blood During 5-hr Exposure to Hypoxia With and Without CO2

<table>
<thead>
<tr>
<th>Gas mixture flowing through chamber</th>
<th>EXPOSURE TIME</th>
<th>Pre Exposure</th>
<th>30 min</th>
<th>2 Hours</th>
<th>5 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>( P_{CO_2} )</td>
<td>Calculated Base Excess</td>
<td>pH</td>
<td>( P_{CO_2} )</td>
</tr>
<tr>
<td>AIR</td>
<td>7.46</td>
<td>30.2</td>
<td>2</td>
<td>0.3</td>
<td>7.50</td>
</tr>
<tr>
<td>20% O(_2), 5% CO(_2)</td>
<td>7.37</td>
<td>43.0</td>
<td>0</td>
<td>0.4</td>
<td>7.36</td>
</tr>
<tr>
<td>8.8% O(_2), 5% CO(_2)</td>
<td>7.65</td>
<td>17.1</td>
<td>0</td>
<td>1.2</td>
<td>7.58</td>
</tr>
<tr>
<td>8.8% O(_2), 10% CO(_2)</td>
<td>7.42</td>
<td>41.8</td>
<td>2</td>
<td>0.4</td>
<td>7.39</td>
</tr>
</tbody>
</table>

\( p < 0.001 \)

usually decreased from 71.9\(_{\circ}\) at 30 min to 63.7\(_{\circ}\) at 5 hr. Addition of 5.6\(_{\circ}\) or 10\(_{\circ}\) CO\(_2\) to the hypoxic gas mixture partially prevented this decrease in \( P_{O_2} \), resulting in \( P_{O_2} \) values 22\(_{\circ}\) or 34\(_{\circ}\), respectively, above the value for hypoxia alone. The \( S_{O_2} \) values for the exposures to hypoxia + CO\(_2\) were indistinguishable at 30 min from the value for hypoxia alone; at 5 hr the \( S_{O_2} \) value for the exposure to hypoxia + 5.6\(_{\circ}\) CO\(_2\) was the same as the 5-hr hypoxic value, while that for hypoxia + 10\(_{\circ}\) CO\(_2\) was slightly higher.

The hematocrit and hemoglobin values did not change significantly during exposure to any of the gas mixtures. However, the MCHC increased from a preexposure value of 33.4 ± 0.1 to 34.7 ± 0.3 \( (p < 0.001) \) after 5 hr exposure to 8.8\(_{\circ}\) O\(_2\) and decreased to 32.1 ± 0.5 \( (p < 0.001) \) after 5 hr exposure to 8.8\(_{\circ}\) O\(_2\) + 10\(_{\circ}\) CO\(_2\).

Table 2 presents values for the pH, \( P_{CO_2} \), and BE in arterial whole blood. Exposure to air for 5 hr caused a slight respiratory alkalosis; the pH increased to 7.50 and the \( P_{CO_2} \) decreased to 22.0 torr from the preexposure values of 7.45 and 33.4 torr, respectively. A pronounced respiratory alkalosis was induced at 30 min by the hypoxic exposure (pH 7.65, \( P_{CO_2} \) 17.1 torr); partial recovery of the pH to 7.51 was observed after 5 hr with no change in \( P_{CO_2} \). This recovery occurred presumably as a result of increases in fixed acids as reflected by a BE of −8.5 meq/liter. As seen in Table 1, elevated lactate (approximately 9 meq/liter) apparently accounted for this phenomenon. Addition of 5.6\(_{\circ}\) CO\(_2\) to the hypoxic gas mixture effectively blocked the respiratory alkalosis and decrease in BE. Hypoxia + 10\(_{\circ}\) CO\(_2\) caused a respiratory acidosis by 30 min \( (pH \ 7.30, \ P_{CO_2} \ 68.6 \ \text{torr}) \) that persisted for 5 hr.

Table 3 gives the mean concentrations of organic phosphates in arterial erythrocytes. The preexposure concentration of 2,3-DPG was 27.58 \( \mu \text{moles/g Hb} \). A transient decrease at 30 min followed at 5 hr by a recovery approaching the preexposure level was observed with air and hypoxia + 5.6\(_{\circ}\) CO\(_2\). Exposure
Table 3. Arterial Intraerythrocytic Organic Phosphate Concentrations During 5-hr Exposure to Hypoxia With and Without CO2

<table>
<thead>
<tr>
<th>Gas mixture</th>
<th>50 min</th>
<th>2 hrs</th>
<th>5 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2,3-DPG</td>
<td>ATP</td>
<td>ADP</td>
</tr>
<tr>
<td>AIR</td>
<td>4.93 ± 2.79</td>
<td>2.09 ± 10</td>
<td>25.85 ± 1.22</td>
</tr>
<tr>
<td>20% O2</td>
<td>3.73 ± 0.45</td>
<td>1.66 ± 13</td>
<td>26.72 ± 86</td>
</tr>
<tr>
<td>5.6% CO2</td>
<td>4.26 ± 0.55</td>
<td>3.46 ± 51</td>
<td>29.24 ± 37</td>
</tr>
<tr>
<td>8.0% O2</td>
<td>4.67 ± 0.21</td>
<td>1.83 ± 12</td>
<td>25.44 ± 60</td>
</tr>
<tr>
<td>5.6% CO2</td>
<td>4.26 ± 0.25</td>
<td>2.16 ± 15</td>
<td>25.71 ± 84</td>
</tr>
<tr>
<td>10% CO2</td>
<td>4.30 ± 0.22</td>
<td>2.03 ± 09</td>
<td>25.97 ± 89</td>
</tr>
</tbody>
</table>

*P<.001

DISCUSSION

The present study partially resolves the problem presented by Krantz and Graber21 that “hypercapnea reduced the erythropoietic response to hypoxia, but that the mechanism of this effect is not known.” Evidence has been presented showing that the normal ESF response observed in the unanesthetized rabbit after a 5-hr hypoxic exposure is almost completely blocked by the addition of 5.6% CO2 or hypoxia + 5.6% CO2.

Inadequate tissue oxygenation is generally accepted as the primary cause of increased plasma ESF titers. By implication, enhanced tissue oxygenation is the probable cause of ESF inhibition. To prove this, it is necessary to show that tissue hypoxia is reduced in the presence of CO2. The measurement used to give an estimate of the degree of hypoxia to which body tissues are subjected is XL. The anesthetized dog and unanesthetized human accumulate XL within 30 min exposure to 10% O2.13 The degree of environmental hypoxia necessary to cause XL accumulation in the unanesthetized rat during the first 30 min of exposure appears to vary from 13%22 to 6.6%23 O2. Similar studies have not been reported for the rabbit.
The present study shows that XL is detectable in the unanesthetized rabbit after 30 min exposure to 8.8% O<sub>2</sub>. This shift to anaerobic metabolism continues for at least 5 hr. Addition of CO<sub>2</sub> to 8.8% O<sub>2</sub> alleviates average tissue hypoxia as reflected by XL accumulation, and this is the probable cause of the suppression of ESF production. It must be stressed that whole-blood XL represents the average XL accumulation of all body tissues. It is not possible to conclude that XL accumulation is a reflection of oxygen availability to the renal cells, which are presumably the primary site for ESF production.

Evidence supports the concept that inadequate tissue oxygenation is the primary stimulus for ESF production, not merely a trigger for an all-or-none release of stored hormone. If one accepts this hypothesis, it follows that the hypoxic stimulus must precede ESF production as seen at 2 and 5 hr with 8.8% O<sub>2</sub>. Addition of 5.6% CO<sub>2</sub> blocks the 5-hr response dramatically and the 2-hr response totally. If this difference in ESF production as seen at 2 hr is attributable to differences in tissue oxygenation caused by changes in arterial P<sub>O<sub>2</sub></sub> and/or P<sub>(S<sub>O<sub>2</sub></sub>)</sub>, such changes should be operative prior to 2 hr of exposure. For this reason, the following discussion will focus primarily on the changes in the arterial P<sub>O<sub>2</sub></sub> and P<sub>(S<sub>O<sub>2</sub></sub>)</sub> observed at the end of the 30-min exposure period.

It is well established that brief inhalation of gas mixtures either low in oxygen or high in carbon dioxide stimulates pulmonary ventilation; a combination of low O<sub>2</sub> with high CO<sub>2</sub> enhances this effect. Such hyperventilation raises the arterial P<sub>O<sub>2</sub></sub> unless other variables affecting pulmonary gas exchange counteract this effect. The present study shows that the addition of CO<sub>2</sub> to the hypoxic gas mixture resulted in a higher P<sub>O<sub>2</sub></sub> than with hypoxia alone. In spite of the fact that the P<sub>O<sub>2</sub></sub> was significantly higher than that observed in rabbits breathing 8.8% O<sub>2</sub> alone, the arterial S<sub>O<sub>2</sub></sub> remained constant at about 70% after 30 min exposure. The present study with hypoxia + CO<sub>2</sub> suggests that the increase in arterial P<sub>O<sub>2</sub></sub> balances the decrease in Hb-O<sub>2</sub> affinity, resulting in a constant arterial S<sub>O<sub>2</sub></sub>. Thus oxygen loading in the lungs is maintained by the simultaneous increase in pulmonary ventilation despite the decrease of the Hb-O<sub>2</sub> affinity. This same decrease in Hb-O<sub>2</sub> affinity probably facilitates oxygen unloading to the tissues. The net effect is that the tissue hypoxia occurring in rabbits breathing 8.8% O<sub>2</sub> alone is partially alleviated when CO<sub>2</sub> is added to the gas mixture.

The position of the P<sub>90</sub> relative to the degree of hypoxic stress coupled with the varying capacity of different animal species to increase oxygen delivery to the tissues through adjustments other than changes in P<sub>90</sub> determines whether or not oxygen requirements can be met during exposure to environmental hypoxia. It is not valid to generalize about the benefits of P<sub>90</sub> shifts, and the common practice of using the P<sub>50(U)</sub> instead of the P<sub>50(U,v)</sub> considerably increases the possibility of misinterpreting the effects of such shifts on oxygen delivery.

The P<sub>50(U)</sub> reflects the influence of changes in MCHC and organic phosphates on P<sub>50(U,v)</sub> shifts. Under conditions where the pH deviates from 7.4, the P<sub>50(U,v)</sub> and P<sub>50(U)</sub> need not be similar. In the present study, this is most clearly shown by the P<sub>90</sub> changes observed with exposure to 8.8% O<sub>2</sub>. By 30 min the P<sub>50(U,v)</sub> decreased very significantly, yet the P<sub>50(U)</sub> remained constant. Since the
MECHANISM OF ERYTHROPOIETIN PRODUCTION

change in BE was insignificant, this decrease in the $P_{50(74)}$ must have been caused by the increase in pH. By 2 hr the $P_{50(74)}$ partially recovered due to a decrease in pH resulting from fixed acid accumulation as well as the same factors that contributed to the rapid increase in the $P_{50(74)}$. By 5 hr the $P_{50(74)}$ recovered to the preexposure level primarily as a result of the continued decline of the pH.

The difference between the experimental and preexposure $P_{50(74)}$ is a function of changes in MCHC and organic phosphates. For the rabbit, a first approximation of this relationship can be expressed by the equation

$$\text{Calculated } P_{50(74)} = \text{Preexposure } P_{50(74)} + 0.5(\text{MCHC} - 33.4) + 0.69(\text{DPG} - 27.6). \quad (3)$$

Unlike the other gas mixtures, 8.8% $O_2$ causes a very significant increase in the experimentally determined $P_{50(74)}$ after 2 hr exposure. The $P_{50(74)}$ can also be calculated by substituting into Eq. (3) the preexposure $P_{50(74)}$ and the measured values for the MCHC and 2,3-DPG concentration. The calculated $P_{50(74)}$ values for the 2- or 5-hr hypoxic samples are lower by 1.9 or 0.8 torr, respectively, than those determined experimentally. This discrepancy could be due to the increase in intraerythrocytic ADP concentration, which is not incorporated into the above equation. Owing to the high 2,3-DPG levels in the red cell, the contribution of ATP and especially ADP to shifts in $P_50$ are frequently ignored.

The present experiments show that the concentrations of ATP and 2,3-DPG do not change significantly during hypoxic exposure, while that of ADP does, measuring 4 μmoles/g Hb above the preexposure value after 5 hr. This increase is almost three times greater than that of 2,3-DPG. Benesch and Benesch showed that at a concentration of $5 \times 10^{-3} \text{ M}$ the effects of the di- and triphosphates on Hb-O$_2$ affinity in vitro were similar. On the other hand, Perutz stated that “ATP, ADP and AMP have binding constants with haemoglobin lower than DPG by one, two and three orders of magnitude respectively.” In the absence of an alternative explanation for the discrepancy between the measured and calculated $P_{50(74)}$ in the present study, the increases in ADP observed with the hypoxic exposure suggest that a cause and effect relationship is plausible. More extensive experimentation would have to be completed to confirm such a relationship.

ACKNOWLEDGMENT

Special thanks to Dr. Mary Barker for her important contribution of scientific editing and to Joan Graham for her excellent secretarial assistance.

REFERENCES

4. Torrance JD, Lenfant C, Cruz J, Marti-


Inhibition of erythropoietin production in unanesthetized rabbits exposed to an acute hypoxic-hypercapnic environment

J Wolf-Priessnitz, JC Schooley and LJ Mahlmann