Effect of Smoking on Tissue Oxygen Supply

By Arthur L. Sagone, Jr., Thomas Lawrence, and Stanley P. Balcerzak

The purpose of this study was to determine if chronic exposure to low levels of carbon monoxide (CO) in man results in tissue hypoxia. For this reason, nine smokers (more than one pack of cigarettes per day) were studied. The presence of hypoxia was assessed by measurement of red cell mass (RCM). The effect of CO on intraerythrocytic factors involved with oxygen delivery was determined by measurement of oxygen-hemoglobin affinity (P50) and of red cell 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP). Values were compared to those of 18 nonsmokers of similar age, sex, and race. Values for carboxyhemoglobin (COHb), RCM, hemoglobin, hematocrit, and red cell count were significantly higher in the smokers. DPG levels were unaltered, while P50 and ATP levels were significantly lower in smokers. These data suggest that chronic exposure to low levels of CO results in tissue hypoxia, probably as a result of decreased blood oxygen carrying capacity and increased blood-O2 affinity. Adaption is reflected in an increased RCM and not by intraerythrocytic changes. The response in RCM may be to levels seen in polycythemia vera, as evidenced by a value of 37.6 in one smoker whose RCM fell to normal after discontinuing cigarettes. This study indicates that smoking causes mild erythrocytosis comparable to that seen in spurious or stress polycythemia. It also suggests that chronic exposure to low levels of CO may further embarrass tissue oxygen supply in patients with anemia, heart disease, chronic lung disease, and cerebral vascular disease in whom oxygen delivery to tissues is already marginal.

CHRONIC EXPOSURE to carbon monoxide (CO) is occurring increasingly in man as a result of industrial wastes, automobile exhaust, and smoking.1-3 Although such exposure produces relatively low levels of carboxyhemoglobin (COHb) with only a minor decrease in oxygen carrying capacity, CO also enhances oxygen-hemoglobin affinity.4 These two abnormalities in oxygen delivery could combine to interfere significantly with tissue oxygen supply and to provoke adaptive responses, such as an increased red cell mass.

Reports of increased hematocrits in smokers5,6 and in those exposed through their occupation1,2 to low levels of CO suggest that erythrocytosis does, in fact, occur in these settings. However, these increased hematocrits could result from a decreased plasma volume, rather than from an increased red cell mass. The present study demonstrates that the red cell mass in smokers is actually increased. Although the increase presumably is in response
to tissue hypoxia, evidence of intraerythrocytic adaptation to hypoxia, i.e., 2,3-diphosphoglycerate (DPG), was not found. This lack of intraerythrocytic adaptation may exaggerate the erythropoietic response. These findings suggest that smoking may be one cause of spurious erythrocytosis or Gaisböck's syndrome.

MATERIALS AND METHODS

Subjects

Twenty-seven healthy white men between the ages of 20 and 32 participated in this study. Nine men who smoked one or more packs of cigarettes per day constituted the study group; the remaining 18 men were nonsmokers and served as controls. No subject had donated blood during the 6 mo prior to study.

Procedure

The following studies were done on the same venous blood sample collected from each subject on the morning of the study: blood counts, red cell adenosine triphosphate (ATP), DPG, carboxyhemoglobin (COHb) levels, and oxygen-hemoglobin curves. An aliquot of blood collected in ACD and tagged with Na$_{51}$CrO$_4$ was used for measurement of red cell mass.

Specific methods. (1) Hemoglobins, hematocrits, reticulocyte counts, red cell counts, and platelet counts were done by standard techniques. (2) COHb levels were measured directly with an IL Model 182 CO-oximeter. (3) Red cell mass was determined by the Na$_{51}$CrO$_4$ method. Values were expressed as milliliters per kilogram of body weight, per centimeter of height, and per square meter of body surface. No correction was made for plasma trapping. (4) Erythrocyte DPG was determined enzymatically and was expressed as μmoles/g of hemoglobin. (5) Erythrocyte ATP was determined fluorometrically by the firefly method and was expressed as μmoles/g hemoglobin. (6) Arterial blood gases were measured with a radiometer blood gas system on brachial artery blood collected anaerobically in heparin. (7) Oxygen-hemoglobin dissociation curves were determined by a spectrophotometric method, employing a tonometer cuvette in which a dilute suspension of whole blood in phosphate-buffered saline (pH 7.4) is used to determine oxygen-hemoglobin affinity at 37°C.

Deoxygenation was accomplished by a short exposure of the suspended red cells to a vacuum. Bubbling was minimized by cooling the suspension in an ice bath prior to brief evacuations, with further degassing accomplished by rewarming the solution. Known quantities of air were then added to the tonometer, and the spectrum of the solution was determined at several oxygen tensions on a Cary model 15 recording spectrophotometer. The technique of air addition and the calculation of oxygen saturation and tension were identical to those described by Benesch et al. An oxygen-hemoglobin curve was determined on the basis of at least five points. Oxygen affinity was expressed as the partial pressure of oxygen at 50% saturation.

The reliability of this method to determine the effect of COHb on oxygen-hemoglobin affinity was tested in three ways. (1) The effect of levels of COHb from 5%-20% on oxygen-hemoglobin affinity was determined on intact human red cells. Venous blood was collected in heparin from a normal nonsmoking man and was centrifuged to remove the plasma; the red cells were washed twice with normal saline. The cells were resuspended in normal saline to a hematocrit of 40%-50%. An aliquot of these cells was exposed to CO for 15 min. The treated suspension was then diluted with untreated red cells to make a suspension of red cells with COHb levels from 5% to 20%. COHb levels were determined on the blood, and oxygen-hemoglobin affinity was measured. A 1 mm Hg shift in the $P_{50}$ was found per 5% COHb in the range from 5% to 20%. This is similar to results reported by other investigators.
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Fig. 1. Percentage carboxyhemoglobin (COHb) in smokers and nonsmokers.

Fig. 2. Hematocrit and red cell mass values in smokers and nonsmokers.

(2) Suspensions of red cells with 100% COHb had the same absorption spectra before and after the vacuum extraction, indicating that no loss of CO occurs during this part of the determination.

(3) COHb levels of red cells were measured after completion of the procedure and were always found to be greater than 80% of the initial value.

RESULTS

Carboxyhemoglobin Levels

The COHb levels in both the smokers and nonsmokers can be seen in Fig. 1. In eight of nine smokers, the morning levels of COHb were between 4% and 6.8%. These individuals smoked between one and two packs of cigarettes per day. One individual who smoked two to three packs of cigarettes per day had a value of 9.2%. Since these values were determined on morning blood samples, they represent minimum COHb levels. The mean value of 6.2% in the smoking group is significantly higher than the 1.0% value of the nonsmoking group.

Peripheral Blood Counts

Smokers had significantly higher hematocrit values than nonsmokers (Fig. 2). The mean value in smokers was 47.4%, as compared to 45% in nonsmokers. Similarly, the mean hemoglobin level of 16.6 g/100 ml and red cell count of $5.50 \times 10^6$/cu mm in smokers were significantly higher than the control group values of 15.7 g/100 ml and $5.08 \times 10^6$/cu mm. Platelet and reticulocyte counts were similar in both groups. White counts were higher in smokers than in nonsmokers. Respective mean values were $7457 \pm 1119$ and $5838 \pm 1076$/cu mm.

Red Cell Mass

The mean red cell mass in the smokers was significantly greater than in the nonsmokers (Table 1). This difference in red cells mass would be even
Table 1. Mean Red Cell Mass Values in Smokers and Nonsmokers

<table>
<thead>
<tr>
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<th>RCM/kg</th>
<th>RCM/sq in</th>
<th>RCM/cm</th>
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<td>NONSMOKERS</td>
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<td>± 2.9</td>
<td>± 91</td>
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<td>10.0-13.6</td>
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<tr>
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<td>1321</td>
<td>14.9</td>
</tr>
<tr>
<td>SD</td>
<td>± 3.2</td>
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<td>± 1.4</td>
</tr>
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Values for nonsmokers are similar to those reported for normal men by other investigators.23-25

more striking if the red cell mass were expressed in terms of lean body mass. The mean weight of the smokers was 82.3 kg and was significantly higher than the control group mean value of 73.6 kg, in spite of the fact that the mean height in the smokers was 176.7 cm and was slightly lower than the control group mean height of 178.5 cm. Individual red cell mass values per sq m can be seen in Fig. 2. Six of nine smokers had red cell mass values greater than 1300 ml/sq m, as compared with 1 of 18 in the nonsmoking group. Four smokers had values that were outside the range of the controls in this study.

In addition to the fact that all the subjects in this study were healthy young men without evidence of pulmonary disease, blood gases (pH, PCO₂ and PO₂) were measured in the three smokers with the highest red cell masses and were found to be normal, indicating that the erythrocytosis in these persons was not the result of hypoxemia. The subject who smoked two to three packs per day and had a COHb level of 9.2% had the highest red cell mass value of 1581 ml/sq m (37.6 ml/kg), a level similar to that seen in polycythemia rubra vera. Three months after cessation of smoking, this subject’s red cell mass had decreased to 1381 ml/sq m (33 ml/kg), with a corresponding decrease in hematocrit from 50% to 47%. Six months after he stopped smoking, his hematocrit was 45%. During this period his weight remained stable at 86 kg.

**Oxygen-Hemoglobin Affinity and Red Cell Organic Phosphate Levels**

As seen in Fig. 3, the mean P₅₀ value was 23.1 mm Hg in the smokers, significantly lower than the control mean value of 24.2 mm Hg. Red cell ATP was significantly lower in smokers (4.50 μmoles/g Hb) than in nonsmokers (5.15 μmoles/g Hb) (Fig. 4). The mean value for DPG in both groups was 12.7 μmoles/g Hb (Fig. 5).

**DISCUSSION**

This study indicates that those who smoke more than one pack of cigarettes per day have an increased red cell mass when compared to an identical group of nonsmokers. This increased red cell mass accounts for the elevated hematocrit values found in smokers in this and other studies.5,6
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Fig. 3. Red cell oxygen affinity in smokers and nonsmokers. Values are expressed in terms of oxygen tension required to half saturate hemoglobin with oxygen (P50).

Fig. 4. Red cell 2,3-diphosphoglycerate (DPG) values in smokers and nonsmokers.

Fig. 5. Red cell adenosine triphosphate (ATP) values in smokers and nonsmokers.
increase in red cell mass is best explained as a response to impaired tissue oxygen supply. Impaired tissue oxygen supply results from decreased oxygen carrying capacity and increased oxygen-hemoglobin affinity caused by COHb. The erythrocytosis in these smokers could not be readily explained on the basis of hypoxemia, because all smokers were healthy young men and the three with the highest red cell masses had normal blood gases. Further evidence against hypoxemia as the cause of erythrocytosis in smokers is given in a recent report by Isager and Hagerup, who show that the increased hematocrit level in smokers could not be correlated with altered pulmonary function.6

One smoker in our study had a red cell mass comparable to levels seen in polycythemia rubra vera. This suggests that some cases of unexplained erythrocytosis might be primarily the result of cigarette smoking. Eisen and Hammond reported that hematocrits greater than 52% may occur in some smokers.5 Furthermore, 21 of 25 patients with unexplained high hematocrits reported by Russell and Conley as cases of Gaisböck’s syndrome were known to be smokers.10 Although smoking eventually may cause pulmonary disease followed by hypoxemia and erythrocytosis, results presented herein indicate that erythrocytosis may occur on the basis of COHb alone. Further studies are necessary to determine the importance of smoking as a primary cause of spurious erythrocytosis.

Decreased oxygen-hemoglobin affinity as a result of increased red cell organic phosphate levels has been described in patients who are hypoxic as a result of high altitude,17 anemia,18 chronic pulmonary disease,19 and heart disease.20 Our data indicate that no significant intraerythrocytic adaptation occurs to the hypoxia associated with chronic CO exposure. In fact, a significantly lower mean red cell ATP level was found in smokers. This finding and the unaltered DPG level suggest that chronic exposure to CO as a result of smoking may impair this adaptive response in those already hypoxic as a result of their primary disease. Kjeldsen has reported that CO may impair this adaptive response in subjects acutely exposed to high altitude.51

Benesch and Benesch postulate that the main mechanism for increased red cell DPG and ATP levels occurring in most hypoxic states is increased binding of these organic phosphates to reduced hemoglobins.22 Since the binding of DPG for COHb is similar to that for oxyhemoglobin22 and the hypoxia produced as a result of CO is not associated with increased red cell reduced hemoglobin, no alteration of red cell organic phosphate would be expected.

ACKNOWLEDGMENT

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