Oxygen Affinity of Hemoglobin in Persons with Acute Myocardial Infarction and in Smokers

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The oxygen affinity of hemoglobin (Bohr effect) may vary in different subjects. Under conditions of organ ischemia, these variations may be of significance.

A study was undertaken to assess the affinity of hemoglobin for oxygen among patients in various states. Included in the study were patients with typical acute myocardial infarction, subjects with clinical and electrocardiographic signs of myocardial ischemia but with normal coronary arteries angiographically, and control subjects.

Patients Studied

Group I ("Normals")

Twelve "normal" men and women served as controls. Two of the 12 were modest cigarette smokers (no more than eight cigarettes per day for less than 5 years).

Group II (Myocardial Infarction)

The eight patients in this group had proven acute (within 72 hours) myocardial infarction (five subendocardial and three transmural). All infarctions were proven by electrocardiography, enzyme studies, and in the three fatal instances by necropsy. Three were moderate cigarette smokers (1 to 2 packs per day for 20 or more years).

Group III (Myocardial Ischemia; Normal Coronary Arteries)

In this group were three premenopausal women between the ages of 30 and 40 years having the clinical symptoms of angina pectoris, normal resting but positive exercise electrocardiogram (positive Master's test), and "normal" selective coronary arteriograms. One was a heavy smoker (3 to 4 packs per day for more than 10 years).

Group IV (Smokers Without Detectable Cardiovascular Disease)

Of the two patients in this group one was a moderate smoker (2 packs a day for 25 years); the other was a heavy smoker (5 packs a day for 20 years). Neither of these individuals gave evidence for clinical coronary artery disease. Selective coronary arteriography was not performed in these two individuals.

Method

Five milliliters of blood was drawn by syringe from an antecubital vein of each patient. Blood pH determinations were made in each instance. Each sample was then immediately added to 50 mg of sodium citrate dissolved in 0.5 ml of H₂O. The blood was filtered through glass wool and washed four times with 0.9% NaCl, and the erythrocytes were collected each time by centrifugation. Finally, the erythrocytes were hemolyzed by pouring them into 10 volumes of cold deionized water. After hemolysis was completed (10 to 12 hours), the hemoglobin was purified in the following manner: Sephadex G-100 was soaked overnight in deionized water and washed several times. It was then packed in a column measuring 2.5 cm in diameter and 50 cm in length and was equilibrated in a cold room. About 100 mg of the hemoglobin sample was fractionated in this column collecting 4 ml in each tube. The purified hemoglobin was then dialyzed against the desired buffer solutions. In most instances 0.05 M tris-HCl was used at various pH values.

The oxygen dissociation studies were performed spectrophotometrically with a Cary 11 spectrophotometer using the apparatus illustrated in figure 1. The essential feature of this apparatus is the half-way bored stopcock (H) which permits the transfer of a known increment of oxygen from the reservoir (R) containing a known pressure and volume of oxygen to cham.
ber (C) where the oxygen and hemoglobin solution undergo equilibration.

A Pyrex 10-mm optical cell is fused to the lower part of the equilibration chamber (C). An aliquot of solution (concentration of 0.5 to 0.7 mg\% 10^{-5} M) is then transferred into C and sealed to the upper part of the cell with ground-glass joints. For higher concentrations of solution, a spacer is placed in the Pyrex cell. The entire cell is then connected to a vacuum line and gently evacuated while the solution is kept cool with an ice-water bath. Evacuation is continued for 20 to 30 minutes until complete deoxygenation is confirmed spectrophotometrically. The stopcock (B) is closed and gas reservoir (R) is filled with a known pressure of oxygen. After the stopcock (A) is closed, the apparatus is removed from the vacuum line and the entire cell is mounted in a housing with thermostatic control. The latter is made so that it will fit into a Cary 11 spectrophotometer. It is then mounted on a motor-driven shaker, and gently shaken for a period of 15 minutes to equilibrate the system. The visible spectrum (505 to 650 m\textmu) is then taken without adding oxygen. The housing is removed from the spectrophotometer and the half-way bored stopcock is turned 180° so that a stopcock full of oxygen is transferred from the reservoir to the reaction cell. The system is gently shaken for 15 minutes before the second spectrum is recorded. This process is repeated as necessary until the stopcock (B) is opened to complete the oxygenation of hemoglobin.

Knowing the pressure of oxygen in R, the volume of R and the half-way bored stopcock, and the volume of the gas phase in C, the partial pressure of oxygen after each addition of the stopcock full of oxygen can be calculated.

The percentage of oxyhemoglobin is determined from the ratio of change of absorption after each addition of gas against the total change at 578 m\textmu (or at any other wavelength).

The experimental errors which might have resulted from repeated mounting and dismounting onto the spectrophotometer are easily checked by examining the isosbestic points of all the spectra. The presence of other than oxyhemoglobin (methemoglobin for instance) was excluded spectrophotometrically.

As mentioned, the percentage of oxyhemoglobin was calculated at each added increment of partial pressure of oxygen using the absorbance at 577 and 541 m\textmu and plotted on a graph. Hill's constant “n”2 was obtained from the slope of the plot of log [Y/(1-Y)] against log pO2, where Y is the fraction of oxyhemoglobin and pO2 is the partial pressure of oxygen. This constant is a rough measure of the sigmoid nature of the hemoglobin-oxygen dissociation curve. A decrease in “n” indicates a tendency for less heme-heme interaction. The P50 (the partial pressure of gas when equal amounts of oxyhemoglobin and deoxyhemoglobin are present) was plotted against the respective pH values of the solutions to de-

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**Figure 1**

Diagrammatic representation of the apparatus employed in the oxygen dissociation studies reported herein. The essential feature is the half-way bored stopcock (H). This permits successive transfers of known increments of gas from reservoir (R), wherein pO2 is known to the equilibration chamber (C). There, the oxygen and buffered hemoglobin solution undergo equilibration. An optical cuvette designed for use with the Cary 11 Spectrophotometer extends below reservoir (C). The detailed method including the function of stopcocks (A and B) is outlined in the text.
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termine the Bohr effect. The Bohr effect is the empirical observation that hemoglobin-oxygen affinity decreases as the pH decreases.

The hemoglobin of normal controls (group I) and the suspected abnormal patients (groups II, III, and IV) were also analyzed with Canalco Disc electrophoresis at pH 9.1. The latter analysis was carried out to determine whether electrophoretically abnormal forms of hemoglobin were present.

**Figure 2**

Hemoglobin-oxygen affinity in the normal person decreases as the pH decreases. A comparable experiment performed in 0.05 M phosphate buffer at pH 7.0 is included for comparison. The oxygen affinity in phosphate buffer is decreased by comparison to 0.05 M tris-HCl at the same pH.

**Figure 3**

Hemoglobin-oxygen affinity in patient with myocardial ischemia but normal coronary arteriogram is shown. The oxygen affinity of the hemoglobin decreases as the pH decreases. (For comparison, see figure 2).

Results

Separation of the hemolysates using a Sephadex G-100 column revealed at least three peaks. The first was due primarily to the "ghosts" of the erythocytes; the second was a single hemoglobin peak; the third was a peak which represented some proteins other than hemoglobin. Hemoglobin used for the experiments was collected from the tubes having more than one half of the maximum absorbance of the hemoglobin effluent.

**Group I (Clinical "Normals," Twelve Subjects)**

Oxygen dissociation curves of the "normal" hemoglobin at various pH values were performed in tris buffer solutions (fig. 2). An experiment performed in 0.05 M phosphate buffer at pH 7.0 is included for comparison (fig. 2). The hemoglobin of two patients was studied at various pH values. The results of one of the patients are shown in figure 3. The rest of the samples were examined at pH 7.0, or at both pH 7.0 and 7.5 (figure 4).

The vertical lines (fig. 4) indicate standard deviations among 12 normals. At pH 7.0 the oxygen affinity of hemoglobin in tris buffer was found to be higher than that of hemoglobin in phosphate buffer (P50 = 8.0 mm Hg). This was true despite the fact that the
ionic strength of both solutions was approximately the same. Hill's constant “n” was slightly greater in the tris buffer (n = 3.0) than in the phosphate buffer (n = 2.7).

Hill's constant “n” in all pH values studied in this buffer was 3.0 with a standard deviation of 0.1 (table 1). The P50 of the 12 normals was 3.1 with a standard deviation of 0.2.

**Group II (Acute Myocardial Infarction, Eight Patients)**

A. Transmural

The hemoglobin of the three patients with transmural myocardial infarction showed gross abnormalities in the P50 and Hill's constant n at pH 7.0 (table 1). The pure hemoglobin of one of the patients in this group (table 1, patient 3) showed the largest P50 and the smallest n thus far observed, by us or others, to our knowledge. This is the lowest hemoglobin-oxygen affinity in this study. One patient (table 1, patient 1) showed the least degree of abnormality of P50 recorded (3.7 mm Hg).

B. Subendocardial

The hemoglobin of all patients having subendocardial infarction showed a markedly abnormal P50 and Hill's constant “n” (table 1).

**Group III (Myocardial Ischemia but “Normal” Coronary Arteries)**

The hemoglobin of all three patients demonstrated abnormal P50 of varying degree and a low Hill's constant n. It is noteworthy that all patients studied having myocardial infarction as well as those having myocardial ischemia but “normal” coronary arteries showed the same abnormality, that is, a high P50 value and a low Hill's constant n (table 1).

**Table 1**

*The Hemoglobin-Oxygen Dissociation Characteristics of Thirteen Patients and Twelve Normal Controls (in 0.05 M Tris HCl at 20 C at pH 7.0)*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number</th>
<th>P50*</th>
<th>“n”†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Normals</td>
<td>12</td>
<td>3.1 ± 0.2</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Group II:</td>
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<td></td>
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</tr>
<tr>
<td>Acute myocardial infarcts</td>
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<td></td>
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</tr>
<tr>
<td>Transmural</td>
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<td></td>
</tr>
<tr>
<td>Case 1</td>
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<td>3.7</td>
<td>—</td>
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<tr>
<td>Case 2</td>
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<td>4.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Case 3</td>
<td>3</td>
<td>7.2</td>
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<td>Subendocardial</td>
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<td>Case 4</td>
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<tr>
<td>Case 7</td>
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<tr>
<td>Case 8</td>
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<tr>
<td>Case 11</td>
<td>3</td>
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<tr>
<td>Group IV:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 packs daily for 20 yr</td>
<td>2</td>
<td>4.7</td>
<td>2.5</td>
</tr>
<tr>
<td>2 packs daily for 25 yr</td>
<td>2</td>
<td>4.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*P50 = % oxygen saturation point of hemoglobin (in above buffer solution normal = 3.1 ± 0.2).

†“n” = Hill's constant (normal = 2.9 to 3.1).
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Group IV (Smokers Without Detectable Cardiovascular Disease)

The hemoglobin of the heaviest smoker (5 packs per day for 20 years) showed a greater abnormality of P50 and Hill's constant n than did that of the more moderate smoker (2 packs per day for 25 years) (table 1).

No electrophoretic abnormality beyond that of the average constituents of minor components was noted in the hemoglobin of any individual in this study. Blood pH values were regularly within normal limits. Blood arterial oxygen saturations were also normal.

Comment

Abnormal hemoglobin-oxygen affinity was demonstrated among several individuals manifesting myocardial ischemia or necrosis and in some moderate-to-heavy smokers without known cardiovascular disease. That the hemoglobin abnormality is responsible for myocardial ischemia or necrosis in each or any instance cannot be ascertained by this study. Further, others have shown as well that the oxygen-binding characteristics of hemoglobin are remarkably sensitive to minor structural and environmental changes. It might, therefore, be difficult to duplicate these studies with whole blood owing to many added and uncontrollable influential plasma and red cell factors, control versus sample. It is noteworthy that Astrup has recently reported increased hemoglobin-oxygen affinity and decrease of Hill's constant n in the whole blood of patients with thromboangiitis obliterans. The cause is not yet known.

The authors are unaware of previous reports of altered hemoglobin-oxygen affinity in: (1) hemoglobin from patients with myocardial ischemia but without demonstrable associated coronary disease, (2) from patients with acute myocardial infarction, or (3) from heavy smokers without clinical evidence for coronary disease or peripheral vascular disease.

It remains to be shown whether the hemoglobin affinity for oxygen in vivo parallels that of the in vitro studies reported herein and elsewhere. If it does, certain physiological features bear mention.

The “abnormal” hemoglobin herein reported should accept oxygen readily at normal alveolar pO2 levels. Thus, arterial oxygen saturation should be normal as was demonstrated by the patients in this study.

As is shown, in a buffer solution, the amount of oxygen released from oxyhemoglobin is related to the decrease in the partial pressure of oxygen. In vivo, where cellular respiration is in progress, carbon dioxide is released into the circulation. Hemoglobin is acidified by the protons released from carbonic acid. This acidification of hemoglobin takes place while the pH of the blood is maintained nearly constant. Such acid hemoglobin has a reduced affinity for oxygen and consequently releases more oxygen to the circulation. The amount of oxygen released from oxyhemoglobin, in vivo, therefore, may be dependent not only on the partial pressure of the surrounding oxygen but also on the amount of hemoglobin in the more acidic form. Assuming that the log of P50 indicates the effectiveness of the oxygen-carrying capacity of a given hemoglobin, the abnormal hemoglobin, then, must release a lesser amount of oxygen as the pH shifts toward the acid, compared to that of normal (fig. 4).

The heart being the organ with highest oxygen extraction rate might be expected to be the most sensitive to decreased hemoglobin-oxygen release. Paradoxically, the myocardium might receive too little oxygen though supplied through patent coronary arteries with normally saturated arterial blood.

Summary

The hemoglobin-oxygen affinity of pure hemoglobin was studied in 11 patients with signs of myocardial ischemia or acute necrosis and in 12 controls. Additionally, the hemoglobin of a moderate and a heavy cigarette smoker without evidence of cardiovascular disease was studied. Under physiological pH conditions, the hemoglobin gave up oxygen in an abnormal manner in all except the 12 controls.

In all control subjects and patients, the arterial saturation was normal and blood pH
values were within normal limits. Electrophoretic abnormality was not found in any of the subjects studied. It is thus demonstrated that hemoglobin may be physicochemically abnormal without electrophoretic abnormality. From a functional point of view, the hemoglobin’s abnormal response to changes in oxygenation may result from altered proton-binding properties of the hemoglobin molecule. That such “abnormal” hemoglobin could contribute to the paradox of myocardial ischemia or necrosis occurring in the presence of full arterial saturation and patent coronary arteries is not certain from these studies.

Further studies investigating the role of abnormal hemoglobin-oxygen affinity in the pathophysiology of myocardial ischemia or necrosis appear justified and necessary.

References
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