Variability of the Homeostatic Response to Altered $p_{50}$

By S. Charache, S. Achuff, R. Winslow, J. Adamson, and P. Chervenick

Blood from carriers of hemoglobin Osler (Hb Osler) had almost the same oxygen affinity as that of carriers of Hb McKees Rocks (Hb MR) ($p_{50}$ 10–11 mm Hg), but Hb concentrations were higher in male carriers of the former (21.6 versus 17.2 g/dl). Two carriers of each Hb were studied to compare their adaptations to altered oxygen affinity and their responses to phlebotomy. All four were healthy, and all excreted normal amounts of erythropoietin. Carriers of Hb MR had somewhat lower mixed venous $p_{O_2}$ than carriers of Hb Osler. There was no suggestion that phlebotomy impaired ability to exercise in either group of patients.

Measurements of erythropoietin excretion in healthy carriers of high- and low-affinity hemoglobins have yielded results within the normal range,$^1,^3$ suggesting that oxygen transport is normal and equal to oxygen consumption, despite the stress imposed by a shifted dissociation curve. This could be accomplished by changes in cardiac output, Hb concentration, and/or tissue $p_{O_2}$; anemic patients increase their cardiac output,$^4$ some hypoxic patients become polycythemic,$^5$ and we all lower our femoral venous $p_{O_2}$ when we exercise our legs.$^6$ In the case of hemoglobins with altered oxygen affinity, a strong argument has been made that adjustments are almost entirely on the basis of altered hemoglobin concentration, that cardiac output changes only slightly, and that mixed venous $p_{O_2}$ remains normal.$^7,^8$ More recent measurements have cast some doubt upon this concept, since mixed venous $p_{O_2}$ was at or below the lower limit of normal in six of eight carriers of abnormal hemoglobins in whom oxygen transport was evaluated (Table 1). Some of these patients probably were not in a stable state at the time of study (see Comments, Table I), but the data are of considerable interest when compared with those reported here.

We recently had an opportunity to study healthy carriers of hemoglobins Osler and McKees Rocks. Oxygen affinity was almost the same in the two families ($p_{50}$ 10–12 mm Hg, normal 27.5), but hemoglobin concentrations in the men of one kindred were higher than those in the other (Fig. 1). We assumed that adjustment to altered $p_{50}$ was accomplished by changes in cardiac output and tissue $p_{O_2}$ as well as erythropoiesis, and we hypothesized that differences in hemoglobin concentration were the result of different combinations...
HOMEOSTATIC RESPONSE TO ALTERED $p_{50}$

![Graph showing hematocrits of carriers of Hb Osler (△) and Hb McKees Rocks (□).]

Fig. 1. Hematocrits of carriers of Hb Osler (△) and Hb McKees Rocks (□).

of these adaptations. Hemodynamic studies were carried out to try to clarify the nature of those differences and to evaluate the effects of phlebotomy in patients with secondary polycythemia.

MATERIALS AND METHODS

Clinical summaries. Carriers of hemoglobins Osler--OSL-1 and 2 (the proband's sons in ref. 16, age 22 and 23 yr at the time of this study)--and McKees Rocks--MR-1 and 2 (II-1 and II-5 in ref. 17, ages 39 and 25 yr)--were healthy and free of symptoms referable to oxygen transport. All four patients were tobacco smokers; OSL-1 and 2 had mild restrictive ventilatory defects and MR-1 had a mild obstructive defect. MR-2, who was partially deaf and had mild diabetes, had normal pulmonary function. His serum haptoglobin was only 72 mg/dl and his reticulocyte count was somewhat increased (Table 2), but his hemoglobin and hematocrit did not differ significantly from those of his brother.

Laboratory studies. Analyses of the structure of hemoglobins Osler and McKees Rocks have been published. Oxygen affinity of blood was measured by the method of Rossi-Bernardi et al., using a modification that differed mainly in the degree of automation and the facility of data handling. Urinary erythropoietin was measured by $^{59}$Fe incorporation into hemoglobin of exphoxic polycythemic mice.

Clinical studies. Patients were hospitalized in the Clinical Research Unit. Patients gave formal consent for cardiac catheterization in the presence of an impartial witness, having had the procedure explained at least twice previously; the experimental methods, and the process of obtaining informed consent, were approved by the Clinical Investigation Committee of The Johns Hopkins Medical Institutions. Catheterization was carried out (for evaluation of the effect of phlebotomy) after baseline collections of urine for measurement of erythropoietin. Cardiac output and $P_{O_2}$ were measured at rest and during exercise, approximately 1000 cc blood was removed and replaced

| Table 1. Oxygen Transport in Carriers of Abnormal Hemoglobins |
|------------------|------------------|------------------|------------------|------------------|
| Hemoglobin       | Hb Concentration | $P_{50}$         | Cardiac Index    | Mixed Venous $pO_2$ | Ref. | Remarks               |
|                  | (g/dl)           | (mm Hg)          | (liters/min/m²)  | (mm Hg)           |      |                      |
| Creteil          | 16.6             | 14.5             | 5.0              | 33               | 9    | Postphlebotomy        |
| Hammersmith      | 8.7              | 34.5             | 5.3              | 40               | 10   | Hemolytic anemia      |
| Heathrow         | 18               | 10.9             | 3.7              | 41               | 11   | Postphlebotomy, $p_{50}$ corrected* |
| Köln             | 12.6, 13.5       | 23, 23           | 3.2, 2.8         | 31, 29           | 12   | Hemolytic anemia      |
| Little Rock      | 23.8             | 12.2             | 7.2 (liter/min)  | 36               | 13   | $p_{50}$ corrected*   |
| Yokima           | 18.6, 17.3       | 12               | 2.6, 2.3         | 34, 35           | 14   | Prior treatment with $^{32}$P, $pO_2$ calculated |
| McKees Rocks     | 17.1, 17.3       | 10.3             | 3.9, 6.4         | 26, 27           | 15   | This paper            |
| Osler            | 22.1, 21.1       | 11               | 5.3, 6.0         | 33, 33           |      | This paper            |
| Normal           | 15               | 26.6             | 3.7 ± 0.3        | 34-49            | 15   |                      |

*Corrected to a $p_{50}$ of 26.6 mm Hg for normal blood.
with plasma protein fraction (Plasmanate), and measurements at rest and during exercise were then repeated within 10-15 min. There were no complications of these studies.

Patients exercised by pedaling while lying supine for 5 min. The work done could not be quantified but was reproducible as judged by oxygen consumption before and after phlebotomy. Measurements of oxygen consumption and cardiac output were made during the last minute of the exercise period.

Cardiac output was measured by the direct Fick technique after catheterization of the pulmonary artery. Cardiac index (cardiac output/m2) was calculated from nomograms giving surface area as a function of height and weight. Oxygen pressures were measured with O2 electrodes in the Special Hematology Laboratory (Radiometer, BMS 3 Mk 2) and in the clinical Chemistry Laboratory (Corning Laboratories, Model 165); values agreed within 1 mm Hg. Oxygen contents were measured by the van Slyke technique, and saturations were calculated from O2 content and capacity or measured with an Instrumentation Laboratories Cooximeter. Oxygen consumption was calculated from the volume and oxygen content of expired air, the latter being measured with the Scholander technique. Oxygen transport was calculated as the product of cardiac output (dl/min) and the arteriovenous difference in oxygen content (ml O2/dl blood). Red cell mass was measured with 51Cr-labeled autologous red cells; samples were taken at 30, 60, and 90 min and the results averaged unless there was a clear upward trend, in which case the last value was used.

RESULTS

Oxygen dissociation curves of whole blood are shown in Fig. 2. Oxygen affinity was very high in both families, but the presence of the abnormal component was somewhat more evident in the carrier of Hb Osler. The curves were almost superimposable at oxygen saturations in the range of mixed venous

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hct (%)</th>
<th>Retic (%)</th>
<th>RBC Mass (ml/kg)</th>
<th>Hb Ep (g/dl) (U/24 hr)</th>
<th>Hb Peak EPO (g/dl) (U/24 hr)</th>
<th>P50 (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-1</td>
<td>53.0</td>
<td>3.8</td>
<td>41</td>
<td>17.1</td>
<td>5.33</td>
<td>14.6</td>
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<tr>
<td>MR-2</td>
<td>50.3</td>
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<td>—</td>
<td>17.3</td>
<td>1.6-3.0</td>
<td>14.9</td>
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<tr>
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<td>1.2</td>
<td>57.6</td>
<td>22.1</td>
<td>4</td>
<td>18.3</td>
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<tr>
<td>OSL-2</td>
<td>62.5</td>
<td>1.3</td>
<td>49.6</td>
<td>21.1</td>
<td>—</td>
<td>19.0</td>
</tr>
<tr>
<td>Normal range (male)</td>
<td>41-51</td>
<td>0.8-2.5</td>
<td>25-35</td>
<td>14-18</td>
<td>2-6</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Oxygen dissociation curves of blood from carriers of Hb Osler and McKees Rocks, 37°C, pH 7.4, pCO2 40 mm Hg. Y, percentage oxygen saturation.
CARDIAC INDEX (dl/min/m²)

OXYGEN CONSUMPTION (ml/min/m²)

PRE POST PHLEBOTOMY

A - V O₂ DIFFERENCE (ml/dl)

MIXED VENOUS P₀₂ (mm Hg)

Fig. 3. Assessment of oxygen transport in carriers of Hb McKees Rocks (■, ●) and Hb Osler (▲, ▼) before and after removal of 1000 cc blood, at rest. See text for further description. Normal ranges indicated by vertical bars.

blood (>60% saturation). The curve for Hb Osler is similar to those published for other families with this abnormality.24,25

Erythropoietin excretion was within the normal range in the carriers of high-affinity hemoglobins before phlebotomy and rose after the carriers were bled to near-normal hemoglobin levels (Table 2). In carriers of Hb Osler, augmented excretion occurred despite postphlebotomy hemoglobin concentrations of >18 g/dl.

Oxygen transport. Data obtained during cardiac catheterization (Table 1, Fig. 3) showed that in general oxygen consumption was normal, cardiac index was high, and mixed venous pO₂ and A-V oxygen difference were low or low-normal. The only consistent difference between carriers of Hb Osler and Hb MR was a slightly lower mixed venous pO₂ in the latter patients. During exercise (Fig. 4) even this difference disappeared. Particularly after exercise, there was no suggestion that phlebotomy increased cardiac work.

DISCUSSION

These studies were undertaken because male carriers of Hb MR had lower hemoglobin concentrations than male carriers of Hb Osler; they also had lower hematocrits than did female carriers in their own kindred (Fig. 1), although all had very similar p₅₀. The difference in mean hemoglobin level between the males was statistically significant (p < 0.005), but with such small samples and known normal variation, conclusions drawn must be circumspect.
These data were collected to determine if different combinations of adaptive mechanisms were used by the two groups of men. Their adaptations were effective, since erythropoietin excretion was normal, suggesting that oxygen consumption and transport were in balance, at least in the patients' kidneys. Carriers of Hb MR had somewhat lower mixed venous Po2 (Fig. 3), suggesting that more oxygen was extracted from blood in some or all of their tissues. In animals (and probably in man) residing at high altitudes, increased oxygen extraction is accomplished by altered tissue capillarity and metabolism, and such changes may be more pronounced in carriers of Hb MR. It would have been most interesting to assess oxygen transport in female carriers of Hb MR, since their hemoglobins are higher than those of the male carriers (Fig. 1). Unfortunately, these measurements could not be done.

Another interesting comparison can be drawn between our patients and persons living at high altitudes. Hematocrit levels are higher, at equivalent altitudes, among the Quechua of Peru than among the Sherpa of Nepal. The Sherpa are reported to have normal rather than decreased oxygen affinity. Their adjustment to very high altitude is better in both respects, perhaps because man has lived in the Himalayas longer than in the Andes. The medical ramifications of the difference is important because chronic mountain sickness is a problem in Peru but not in Nepal. Differences in the erythropoietic responses to hypoxic stress may be due to genetic or environmental factors or may be due only to random variation. We cannot be sure of the latter in our patients, because of the small size of our sample, but it seems unlikely as a cause of differences among residents of high altitudes, where many more persons have been studied.
We wished not only to study mechanisms of adaptation in our patients but also to evaluate the effects of phlebotomy in secondary polycythemia. The four men were bled because of concern over the effects of elevated blood viscosity, but indications for such treatment are vague in disorders of oxygen transport. There was no suggestion that phlebotomy was harmful: none of the men noted dyspnea or decreased exercise tolerance after the phlebotomy, and there was little difference between pre- and postphlebotomy measurements made during moderate exercise (Fig. 4). Our measurements were not designed to show phlebotomy-induced improvement in either hemodynamics or exercise tolerance, and none of the patients had symptoms that could have been alleviated. We concluded that phlebotomy was safe, and potentially useful, in the patients with very high hematocrits. Both OSL-l and OSL-2 declined such treatment, but their 45-yr-old mother has been bled regularly to keep her hematocrit below 55. We have not attempted to keep her hematocrit in the normal range, and unpleasant symptoms were reported in a carrier of Hb Creteil with equally high affinity when he was bled to a normal hematocrit (Poyart C: Personal communication).

Others have used hemodynamic studies as a guide to phlebotomy in more common types of secondary polycythemia. The goals are to improve blood flow and decrease cardiac work. Serial noninvasive measurements of cardiac work and exercise capacity might be an effective way to monitor such therapy in patients with cardiopulmonary disease as well as in those with abnormal hemoglobins.

ACKNOWLEDGMENT

Dr. E. A. Murphy provided valuable advice, Jean Scott, R. N., assisted in the care of the patients, and Patricia Hathaway and Mollie Jessop performed many of the laboratory studies.

REFERENCES

12. Woodson RD, Heywood JD, Lenfant C: For personal use only. on October 29, 2017. For personal use only.


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