A Simple Microtonometric Method for Whole Blood Oxygen Dissociation Curve and a Critical Evaluation of the "Single Point" Procedure for Blood $P_{50}$

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Abstract We devised a rapid and simple method to obtain oxygen dissociation curve (ODC) for a small amount of blood with simple equipment. This system achieved the gas-blood equilibrium within 3 min. Oxygen saturation of the equilibrated blood was measured spectrophotometrically while the pH, $P_{CO_2}$, and $P_O_2$ was measured with a Radiometer blood gas analyzer system. Whole procedures to construct ODC from the 6 point measurements could be performed within 1 hr. The standard ODC for 25 normal Japanese adults showed a mean $P_{50}$ of 27.6±1.1 Torr (pH 7.40, $P_{CO_2}$ 40 Torr, 37°C), a slightly higher value than previously reported. The discrepancy, however, can be eliminated when corrected for a slightly lower $S_O_2$, by the present procedure. The standard curves for the adult blood at pH 7.20 and 7.60 and for the cord blood at pH 7.40 were also described. The "single point" procedure, a much quicker approach to measure the $P_{50}$ (ABERMAN et al., 1975), was scrutinized by comparing with the "whole curve" method. The $P_{50}$'s by the two methods were not significantly different, mean±S.D. of the differences being 0.4±2.5 Torr ($n=126$) for the adult blood at pH 7.40, $P_{CO_2}$, 40 Torr and 37°C. Similar results were obtained for the adult blood at pH 7.20 and 7.60 and for the cord blood at 7.40. We concluded that the "single point" method was sufficiently accurate and reliable.

Key Words: human, blood, oxygen dissociation curve, $P_{50}$.

One of the most important tasks of respiration is to transport oxygen from ambient air to tissue cells. This transport process consists of diffusion and ventilatory and circulatory convection. These processes are closely interrelated with blood of which respiratory function is represented by an oxygen dissociation curve (ODC). Thus, ODC for whole blood offers valuable information on the
oxygen transport process. However, making the ODC is not easy, because classical gasometric methods require technical skills, experience, and much time, while recent automatic recording methods (Duvelleroy et al., 1970; Reeves, 1980) need expensive equipment.

In the present paper, we report a convenient and simple method to determine the ODC for a small amount of whole blood. Also, we introduced a procedure to obtain a \( P_0.5 \) for half oxygenation of the blood \((P_0.5)\) from a single measurement of pH, \( P_{O_2} \), and \( S_{O_2} \), using a 250 µl sample.

**MATERIALS AND METHODS**

1. **Blood sample.** Approximately 2 ml whole blood sample was obtained by the venipuncture from 25 healthy adult non-smokers. Blood was heparinized immediately and kept in ice until use. Fresh human cord blood was supplied from the Tocology Clinic, Nara Medical University.

2. **Microtonometry of blood.** An \( O_2-CO_2-N_2 \) gas mixture was prepared by two gas mixing pumps in series (Dainihonseiki, DNS-100 and -110). The exact composition of the gas mixture was checked by the Scholandar gas analyzer. The blood-gas equilibrium was attained with the Astrup-microtonometer (Radiometer, AMT 1). The blood sample (250 µl each) was placed in the equilibration tubes of the microtonometer and equilibrated with continuously flowing gas mixture by vibrating for 3 min at 37°C. The \( O_2 \) concentrations from 1 to 10% were employed for the equilibration and 6 to 7 points were determined under varied \( P_{O_2} \) and constant pH and \( P_{CO_2} \). The equilibrated blood was collected into two glass capillaries (0.5 x 75 mm, 0.5 x 200 mm), and the \( S_{O_2} \), pH, \( P_{O_2} \), and \( P_{CO_2} \) were promptly measured. The equilibration blood pH was controlled by varying the \( CO_2 \) concentration, i.e. \( CO_2 2.7, 5.4, \) and 10.8% for pH 7.60, 7.40, and 7.20.

3. **Measurement of pH, \( P_{CO_2}, \) \( P_{O_2}, \) and \( S_{O_2} \).**

   1) pH, \( P_{CO_2} \), and \( P_{O_2} \): pH, \( P_{CO_2} \), and \( P_{O_2} \) of the equilibrated blood in the glass capillary (0.5 x 200 mm) were measured at 37°C with a blood gas analyzer (Radiometer, BMS-Mk2). The measured \( P_{O_2} \) was corrected for a given pH value using the Bohr factor \(-0.48\) for the adult blood (Severinghaus, 1966) and \(-0.50\) for the cord blood (Enoki et al., 1972).

   2) \( S_{O_2} \): The glass capillary (0.5 x 75 mm) with the equilibrated blood was spun for 5 min at 12,000 rpm with a microhematocrit centrifuge (Kubota, KH 120), the packed cell portion was cut off, and hemolyzed by freezing and thawing. The \( S_{O_2} \) was measured spectrophotometrically in duplicate with an oxygen saturation meter (Radiometer, OSM I). The results by the photometric (OSM I) and gasometric methods (Van Slyke and Plazin, 1961) were shown to agree fairly well with each other (Fig. 1).

4. **Measurement of 2,3-diphosphoglycerate (2,3-DPG).** 2,3-DPG in the sample blood was measured by an enzymatic method (Maeda et al., 1971).
5. Assessment of blood $P_{50}$ by a “single point” procedure. Although the method described above requires much shorter time than the conventional gasometric methods for making ODC, it still takes 1 hr for the 6 point determinations at different $P_{O_2}$ under a constant pH. To overcome this drawback, we adopted a quicker way to determine the $P_{50}$ value using a single point procedure (Weiskopf and Severinghaus, 1972; Aberman et al., 1975). This procedure estimates the blood $P_{50}$ from pH, $P_{O_2}$, and $S_{O_2}$ of the single equilibration point, assuming that the position of ODC can shift but its shape remains constant under the physiological condition. Thus, the $P_{50}$ can be obtained from the following equation by a single measurement of pH, $P_{O_2}$, and $S_{O_2}$,

$$P_{50} = P_{50}' 	imes \frac{P_{O_2}^S}{P_{O_2}^m}.$$  

$P_{50}'$, $P_{50}$ value of the standard ODC (e.g. 27.6 Torr for normal adult blood at pH 7.40, $P_{CO_2}$ 40 Torr and 37.0°C); $P_{O_2}^S$, the measured $P_{O_2}$, corrected from the measured pH to a desired pH, say pH 7.40, according to the Bohr factor; $P_{O_2}^m$, the $P_{O_2}$ corresponding to the measured $S_{O_2}$ on the standard ODC. By the single point procedure, the $P_{50}$ values of the adult human blood (pH 7.60, 7.40, 7.20) and the cord blood (pH 7.40) were determined. We compared the $P_{50}$ values from this procedure with those from the whole curve, and evaluated the reliability and accuracy.

RESULTS

1. Standard ODC of normal human adult blood

Figure 2 shows the ODC by our present procedure of normal adult blood at
three different pH’s (7.40, 7.60, and 7.20) and at 37°C. The mean±S.D. of the $P_{50}$ was 27.6±1.1 Torr ($n=19$) at pH 7.40, $P_{CO_2}$ 40 Torr and 37°C. The values for pH 7.60 and 7.20 were 21.5±0.4 Torr ($n=3$) and 34.2±1.5 Torr ($n=5$), respectively. Hill’s exponent $n$ (Hill, 1910) for the standard curves was 2.64 in average. The Bohr coefficient at half oxygen saturation was $-0.54$ between pH 7.40 and 7.60, and $-0.47$ between pH 7.40 and 7.20. 2,3-DPG content in the 25 subjects was 5.71±0.7 mmol/liter red cells and DPG/Hb molar ratio was 0.97±0.1 (Table 1).

2. Standard ODC of the normal human cord blood

Figure 3 shows the ODC of 5 fresh human cord blood samples (pH 7.40, 37°C) with the $P_{50}$ of 22.4±0.4 Torr.

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Table 1. Parameters relevant to oxygenation of normal adult human blood.

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Age (years)</th>
<th>[Hb] (mm)</th>
<th>Ht (%)</th>
<th>[DPG] (mmol/liter RBC)</th>
<th>DPG/Hb molar ratio</th>
<th>$P_{50}$ (Torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>29.4±7.1</td>
<td>2.65±0.25</td>
<td>45.2±4.1</td>
<td>5.71±0.70</td>
<td>0.97±0.12</td>
<td>27.6±1.1</td>
</tr>
<tr>
<td>3</td>
<td>32.3</td>
<td>2.70</td>
<td>46.6</td>
<td>5.09</td>
<td>0.88</td>
<td>21.5</td>
</tr>
<tr>
<td>5</td>
<td>27.0</td>
<td>2.62</td>
<td>45.2</td>
<td>5.60</td>
<td>0.93</td>
<td>34.2</td>
</tr>
</tbody>
</table>

Values at 37°C for blood pH 7.40, 7.60, and 7.20.
Fig. 3. Oxygen dissociation curve for human cord blood by the present method (pH 7.40, 
$P_{CO_2}$ 40 Torr, 37°C). Dashed curve is the standard curve for the adult blood under 
comparative condition (cf. Fig. 2).

Fig. 4. Differences ($dP_{50}$) between the $P_{50}$ values by the single point method and those 
actually measured in wide range of oxygen saturation (S$_{O_2}$). Human adult blood (37°C). 
a, pH 7.20, $P_{CO_2}$ 80 Torr; b, pH 7.40, $P_{CO_2}$ 40 Torr; and c, pH 7.60, $P_{CO_2}$ 20 Torr.
3. *P*<sub>50</sub> values by the "single point" procedure compared to the actually measured values

Figure 4 shows a difference between the *P*<sub>50</sub> values calculated by the single point procedure from the data shown in Fig. 2 and those actually read from each curve. The differences were plotted against the measured *S*<sub>O</sub><sub>2</sub>. In the human adult blood data (pH 7.40), mean±S.D. of the differences was 0.4±2.5 Torr (n=126) throughout the whole measured *S*<sub>O</sub><sub>2</sub> range, 0.3±1.7 Torr (n=73) for *S*<sub>O</sub><sub>2</sub> ranging from 30 to 80%, -0.1±2.9 Torr (n=22) for *S*<sub>O</sub><sub>2</sub> less than 30% and 1.0±3.7 Torr (n=31) for *S*<sub>O</sub><sub>2</sub> higher than 80%. The mean differences for the whole *S*<sub>O</sub><sub>2</sub> range of adult blood at pH 7.60 and 7.20 were 0.8±2.1 Torr (n=20) and -0.1±1.8 Torr (n=35), respectively. For the cord blood (pH 7.40), the difference was 0.3±0.9 (n=31) (Fig. 5).

**DISCUSSION**

Determination of whole blood ODC is an important test relevant to various fields of respiration physiology. So far the routine practice has been limited for several reasons, such as the technical difficulties, time-consuming procedures or requirement of expensive equipment. In the present paper, we described a procedure for the ODC determination measuring directly pH, *P*<sub>O</sub><sub>2</sub>, *P*<sub>CO</sub><sub>2</sub>, and *S*<sub>O</sub><sub>2</sub> of the sample blood equilibrated by the Astrup-microtonometry. This procedure does not require expensive specified equipment or technical skills which can be obtained only after a patient exercise. It is easy to construct the whole curve of 6 points with 1.5 ml blood within 1 hr.

Table 2 shows the comparison of the standard ODC data (pH 7.40, *P*<sub>CO</sub><sub>2</sub> 40 Torr, 37°C) by our method with the previously published standard values (Bartels et al., 1961; Severinghaus, 1966). These values agree well generally.

Table 2.
but our value for the $P_{50}$ is a little higher (0.8–1.3 Torr) than those by DILL and FORBES (1941), BARTELS et al. (1961), and SEVERINGHAUS (1966). This difference may be caused by the technical errors inherent in the present procedure and/or by the actually higher $P_{50}$ possessed by the present 25 Japanese adults. DPG/Hb molar ratio and hematocrit (Ht) of the 25 subjects are 0.97 and 45.2% on average, respectively (Table 1) and fall within a normal range. Thus the latter possibility seems improbable. pH and $P_{O_2}$ electrode were calibrated prior to each measurement, thus errors in pH and $P_{O_2}$ measurement would be unlikely. As shown in Fig. 1, $S_{O_2}$ values measured by OSM I are slightly lower by 1.9% on average than those by the Van Slyke-Plazin micromanometric method. On behalf of this difference, the $P_{50}$ by the present method might be expected to be higher by 0.8 Torr than those by the gasometric procedure. Therefore, when corrected for this discrepancy the standard $P_{50}$ value by us must be 26.8 ± 1.1 Torr, which is almost identical with the previous values (DILL and FORBES, 1941; BARTELS et al., 1961; SEVERINGHAUS, 1966; ENOKI et al., 1972).

Hill's exponent, $n$ (HILL, 1910) and CO2 Bohr coefficient agree with the published values of BARTELS et al. (1961) and HLASTALA et al. (1977).

The ODC for adult blood at pH 7.60, 7.20 and cord blood at pH 7.40 conform to the previously reported one (BARTELS et al., 1961; ENOKI et al., 1972).

Figures 2 and 3 together with Tables 1 and 2 prove that our new method for ODC determination of whole blood is as accurate and reliable as other classical and modern methods.

Figure 4 indicates that it is the $S_{O_2}$ range of 30 to 80% where the smallest differences were found between the $P_{50}$ by the single point procedure and that by actual reading from the whole curve. The 95% confidence limits for the mean
of the differences are $-0.13 < \mu < 0.65$ Torr for $30 \leq S_{O_2} < 80\%$ range, $-1.37 < \mu < 1.21$ Torr in $S_{O_2} < 30\%$ and $-0.38 < \mu < 2.40$ Torr in $S_{O_2} \geq 80\%$ range. It should be noted that, with the $S_{O_2}$ of the middle range ($30 \leq S_{O_2} < 80\%$), the single point procedure can provide more accurate $P_{50}$ estimation. Furthermore, from the practical point, when a sample is obtained from the venous blood (at rest, $S_{O_2}$ ranging from 30 to 80\%) instead of the arterial blood, the $P_{50}$ value can be determined more accurately and reliably. ABERMAN et al. (1975) reported the observed variation in the calculated $P_{50}$ is smallest when the $S_{O_2}$ is between 20 and 90\%, and this agrees with our finding. Figures 4 and 5 indicate that the single point procedure can afford accurate and reliable estimation of the $P_{50}$ also in the adult blood at pH 7.60 and 7.20 and the cord blood at pH 7.40.

REFERENCES


