POLAROGRAPHIC DETERMINATION OF OXYGEN TENSION OF WHOLE BLOOD*

BY MAKEPEACE U. TSAO AND CHARLES H. SLOAN

WITH THE CLINICAL ASSISTANCE OF JEANA LEVINTHAL

(From the Department of Pediatrics and Communicable Diseases, Medical School, University of Michigan, Ann Arbor, Michigan)

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The purpose of this study is to establish a procedure for the direct determination of oxygen tension of whole blood that requires a smaller sample and simpler manipulation than methods previously reported (1). A procedure has been worked out for determining dissolved plasma oxygen which proved satisfactory in our investigations. Since the success of the application of the proposed polarographic methods depends upon the proper construction of the apparatus and upon the technique, these aspects of our work are described in detail. Statistical analysis of the precision of the method is also reported.

**Apparatus**

The complete apparatus, consisting of five units, is shown schematically in Fig. 1. The designation and function of these units is as follows: (A) polarograph for the automatic recording of the diffusion current due to dissolved oxygen; (B) electrodes and sample cell for the application of electric potential to the dropping mercury electrode in the plasma without contact with room air; (C) tonometer box for the transfer of the blood sample from syringe to sample cell and the equilibration of the sample with the standard gas (gas of known oxygen concentration) at the constant temperature of 37°; (D) water bath for heating the standard gas while bubbling through gas washers; and (E) centrifuge box for the maintenance of constant temperature while the sample cell is being centrifuged to separate blood cells from plasma.

(A) **Polarograph**—A Leeds and Northrup electrochemograph type E is used to record the diffusion current from −0.4 to −0.6 volt with respect to the calomel half cell. The use of this automatic recording device not only releases the operator from the difficult task of taking current readings

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which change rapidly, but also gives instant visual indication on the chart as to whether the electrical system is working properly.

(B) Electrodes and Sample Cell—One of the leads from the polarograph is connected to a platinum electrode on the top of the mercury leveling bulb. The electrode, being in contact with the mercury in the bulb, is electrically connected to the mercury drops that form at the bottom of the capillary dropping electrode located directly below the mercury column. The other wire from the polarograph is attached to the calomel half cell, as shown in Fig. 2, F.

The dropping mercury electrode (Fig. 2, D) is made from capillary tubing (5 mm. outside diameter, 0.2 mm. bore) drawn out after heating in a hot flame to approximately 1 mm. outside diameter. The capillary section

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**Fig. 1**

Schematic diagram of the apparatus for polarographic determination of dissolved oxygen in blood plasma. A, recording polarograph; B, dropping mercury electrode and accessories; C, tonometer box; D, water bath; E, centrifuge box; F, tank of analyzed gas mixture.

**Fig. 2**

Electrodes and sample cell. A, sample cell in salt bath (blood cells and plasma not shown); B, curved agar bridge; C, platinum wire loop; D, capillary of the dropping mercury electrode; E, ball and socket joint (clamp not shown); F, connecting clamp of the lead from the polarograph; G, calomel half cell; H, water jacket of the saturated potassium chloride bath.
is cut to about 4 to 5 cm. so that the tip of the capillary reaches the center of the upper compartment of the sample cell (Fig. 2, A), while the shoulder nearly closes off the opening of the neck of the sample cell. The bore of the capillary is coated with silicone (Desicote, Beckman Instruments, Inc.) and dried after rinsing with distilled water.

The calomel half cell (Fig. 2, G), to be used immersed, has a side arm with a rubber cap for filling the cell with the chemical constituents and a fritted glass window of fine porosity. Behind the window (9 mm. diameter) is an agar plug. When the sample cell and the calomel half cell are both immersed in the saturated potassium chloride bath, electrical contact between the plasma in the sample cell and the platinum lead of the calomel half cell is achieved by a curved agar bridge (Fig. 2, B) which hangs onto the lip of the socket part on top of the sample cell and dips into the salt bath. The upper end (not shown) of the glass enclosure of the agar bridge is shaped to curve half way around the capillary electrode so that a small amount of overflow plasma (when the electrode is inserted into the sample cell) is sufficient for good electrical contact. The bridge is kept in a moist vessel when not in use.

The saturated potassium chloride solution (salt bath) is maintained at 37° by circulating water through the water jacket (Fig. 2, H) pumped from a 38° water bath (Fig. 1, D). The salt bath is supported inside a large beaker which can be raised or lowered by a rack and pinion-controlled platform (Fig. 1, B). The stand, which supports the mercury column and the leveling bulb as well as the adjustable platform, in turn rests on a vibration-reduced stand. The vibration due to the circulating pump as transmitted through the connecting Tygon tubes does not seem to affect the polarogram, and therefore the water circulation may be maintained throughout the procedure.

The sample cell (Fig. 2, A) is constructed in such a manner that the plasma from a blood sample, once the blood is introduced into the cell, may be separated from the cells anaerobically and made ready for polarography without the need for transfer into another vessel. The glass part of the sample cell is made from a ball and socket joint (standard taper 12/2). The capillary of the socket joint is thickened and two compartments blown to the contour shown in Fig. 2. The total length is approximately 9 cm. The pear-shaped upper compartment has no projections to detain any blood cells during centrifugation. The volume ratio of the upper compartment to the lower compartment with the glass valve is approximately 1:2, which allows collection in the upper compartment of plasma free from cells. The glass valve, which is ground to fit the constricted part of the sample cell, is maintained in the "closed" position (as shown) by a steel spring. During centrifugation of the sample cell, however, the valve is opened by cen-
trifugal force to allow the sedimentation of cells into the lower part and later returns to the "closed" position when the centrifuge slows down to stop. The steel spring is held against the glass valve by a rubber cap made from a serum bottle cap. This cap may be removed easily for cleaning the sample cell. The stem of a ball joint (standard taper 12/2) is completely removed, and the cut is ground flat to insure a good fit between the bore of the ball joint and the needle hub during the transfer of the blood sample. The ball joint (not shown) is lightly greased to fit the socket so that the sample cell may be sealed after the introduction of the blood sample by simple rotation of the ball joint; it is removed from the socket just before the dropping mercury electrode is inserted into the sample cell.

(C) Tonometer Box—As shown in Fig. 1, C, this unit consists of a deep wooden cover with armholes on the front panel and a wooden tray beneath (Fig. 3). The top of the wooden cover is made of double sheets of Plexiglas with 2 cm. of air space between the sheets as insulation. The armholes are just large enough to admit hands into the constant temperature box for such manipulations as transfer of blood sample from the sampling syringe into the sample cell. It might be pointed out here that control of the temperature at 37° is essential throughout the procedure to prevent the redistribution of oxygen between plasma and erythrocytes. The air stream which maintains the temperature of the box is heated by passage over a heating tape wound on a glass core 1.5 × 70 cm., enclosed in an outer glass tube of 5 cm. diameter (not illustrated). There are rubber stoppers at both ends, through one of which is an air inlet and through the other an outlet. The heating tape used is of the uninsulated type, 2.5 × 90 cm., and has a power rating of 50 watts at 110 volts. Compressed air flows through the system at the rate of 100 liters per minute. A similar air-heating unit for the centrifuge is illustrated in Fig. 4, E. Where the heated air enters the tray (Fig. 3, B), there is a thermoswitch (Fenwal,
Ashland, Massachusetts) nearby (Fig. 3, A) which is adjusted to maintain the temperature inside the tonometer box at 37° ± 0.1.

The standard gas, saturated with water vapor and to be used for calibration, passes from the gas washer in the water bath (Fig. 1, D) to the tonometer box through insulated Tygon tubing (Fig. 3, C). Through a hypodermic needle (Fig. 3, D) the gas flows on past a standard tapered joint 7/12 (Fig. 3, G) held by a thick walled rubber tube (Fig. 3, F) which is clamped on a wooden support. The inner joint is the gas inlet of the glass tonometer, as illustrated in Fig. 3. The interior of the tonometer has been coated with silicone to reduce the possibility of hemolysis when blood is rotated in it. Rotation of the tonometer is provided by connecting the other end of the tonometer to the drive shaft (Fig. 3, I) by a short piece of Tygon tubing. An electric motor turns the drive shaft at approximately 60 r.p.m. The tonometer box is supported at an angle of 7° to the horizontal (Fig. 1, C) so that the blood sample cannot be blown out of the tonometer by the equilibrating gas.

(D) Water Bath—The water bath is maintained at 38° to provide water for the water jacket of the saturated potassium chloride bath and to heat the standard gas bubbling through the gas washers (Fig. 1, D). Two 125 ml. gas washers, each filled two-thirds full with distilled water, are connected in series. By passing through the gas washers, the standard gas becomes saturated with water vapor and will not alter the water content of the blood during the equilibration. Physiological saline, which seemed to be the logical phase for the equilibration, has been found unsatisfactory
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owing to the apparent drying effect of the equilibrated standard gas on the sample in the tonometer.

(E) Centrifuge Box—A centrifuge (International clinical centrifuge) with its housing removed (Fig. 4, A) is maintained at 37° in an insulated box (Fig. 4, H). In one of the shields (Fig. 4, B, of the size for a 50 ml. centrifuge tube) is to be placed a Lucite cylinder, the cross-sectional view of which is shown in Fig. 4, I. A counterweight in the other shield exactly balances the cylinder when the filled sample cell is inside the cylinder. The cylinder is 9 cm. long, with an inside diameter of 1.5 cm. and an outside diameter of 3 cm.; it serves as the insulation for the sample cell during centrifugation when the air temperature in the box might rise 0.5–1.0°. Greater rise of temperature is prevented by an air stream of constant temperature, i.e. 37°, flowing through the box at 200 liters per minute. The air-heating unit (Fig. 4, E) is similar to that unit described in the tonometer box section, except that the heating tape is 5.1 X 122 cm. and has a power rating of 125 watts at 110 volts. The temperature of the air stream is regulated by a thermoswitch (Fig. 4, F) and indicated by a thermometer inside a glass tube (Fig. 4, C). Another thermometer (not shown) which registers air temperature of the box is also read through the cover. Through the armhole in the Transite roof of the box, the Lucite cylinder holding the sample cell can be placed in the shield. A 25 watt tungsten light bulb (Fig. 4, G) maintains the box above room temperature and so the box temperature may be brought up to 37° relatively soon after the heated air stream is turned on. Under the above conditions the temperature of the sample cell inside the Lucite cylinder will not rise more than 0.1° during centrifugation.

Procedure

Sampling—The sampling syringe (5 or 10 ml.) is lightly lubricated with mineral oil, and a porcelain disk cut from a crucible is placed in the barrel. The syringe is then sterilized. Slightly more than enough sterile heparin solution (10 gm. per cent) to fill the dead space is drawn into the syringe. A hypodermic needle (No. 21), or a similar needle with its shaft elongated by a 20 cm. length of polyethylene tubing, is sealed onto the syringe with collodion to prevent air leakage. Air in the needle is replaced by the heparin solution from the syringe. The syringe is then placed in a Lucite jacket which serves as insulation during sampling. The assembled sampling device is warmed up to and maintained at 37°. Sterile technique is used up to this point. As soon as a sufficient amount (5 ml.) of blood has been drawn into the syringe, the latter is put in the tonometer box and the Lucite jacket is removed. About 0.01 volume (0.05 ml.) of fluoride solution (40 gm. per cent of sodium fluoride and 1.2 gm. per cent of potassium fluoride) is drawn through the needle into the syringe. The needle tip is stuck
into a rubber stopper. The fluoride solution and the blood are mixed thoroughly by allowing the porcelain disk to travel by gravity from one end to the other inside the syringe through inversions. The needle is removed from the syringe and replaced by a hypodermic needle (No. 19, 3 inches) with blunted tip. After the air is expelled from this needle, it is inserted downward into the sample cell, which has been previously filled with mercury. (This was accomplished by injecting mercury into the sample cell with the aid of a syringe and a long needle and then applying suction with a vacuum pump to remove the trapped air bubbles. The glass valve of the sample cell was kept open only during the evacuation with a thin stainless steel wire. The mercury-filled sample cell was capped with the greased ball joint and equilibrated at 37°C before use.) Now the blood sample with inhibitor added is injected into the sample cell with the latter in an inverted position so that a thin stream of mercury flows between the ground opening of the ball joint and the shoulder of the needle. The displacement of mercury by blood permits anaerobic filling of the sample cell. When the cell has been completely filled, it is righted and the needle is slowly withdrawn while the needle space is replaced with blood. The ball joint is then rotated sidewise to seal off the sample cell, and the excess blood in the bore of the ball joint is removed by aspiration through the needle. The last trace of blood in the bore is wiped out with a wick. The sample cell is placed in a Lucite cylinder and immediately transferred to the centrifuge.

Centrifugation and Polarography—The sample cell is placed in the counterbalanced centrifuge through the armhole (Fig. 4, D) on the top of the box (maintained at 37°C for 30 minutes prior to centrifugation) and centrifuged for 4 minutes at 2200 r.p.m. Deceleration is hastened by braking the spindle with a cork. The sample cell is removed from the centrifuge. The ball joint of the sample cell is removed with a stick inserted into the bore, and the sample cell is then dropped through the platinum wire loop (Fig. 2, C) into the 37°C saturated potassium chloride bath. The wire loop serves to support the sample cell in an upright position, yet allows considerable flexibility for positioning of the cell with regard to the capillary dropping mercury electrode. The electrode should be wiped only shortly before its introduction into the sample cell; otherwise irregular dropping time will persist for several seconds until the plasma has wetted the tip of the capillary. While the potassium chloride bath is being positioned in the horizontal plane with one hand, the platform on which the bath rests is raised with the rack and pinion by the other until the socket joint of the sample cell touches the shoulder of the capillary electrode. At this point enough plasma is displaced from the sample cell to form a small pool in the socket joint, and the circuit is closed by dropping one end of the agar bridge into the plasma pool and the other into the salt bath.
As soon as the circuit is closed, the zero point of the polarograph is set near one end of the recording chart; then the potential is turned to exactly \(-0.4\) volt, and the automatic voltage change is set in motion at the moment the recording pen is crossing a marker line on the chart. A reading is taken at \(-0.5\) volt. The current range setting has been selected to give the highest possible reading of current on the chart within the voltage range \(-0.4\) to \(-0.6\). After each recording of the polarogram the potential is turned back from \(-0.6\) to \(0.0\) volt and a reading of the zero point taken. The zero point reading is subtracted from the \(-0.5\) volt reading, and the difference multiplied by the factor corresponding to the current range used yields the diffusion current in microamperes. Two more polarograms are recorded to serve as a check for the validity of the first. After the last polarogram the platform is lowered and the dropping mercury electrode rinsed and wiped clean. The tip of the electrode is then immersed and left in a beaker of distilled water between runs. The mercury leveling bulb is lowered only after the determination has been completed to a point below the side tube of the mercury column, and the flow of mercury gradually stops.

It is necessary to record the time of each step during the procedure so that the calibration run can be timed to simulate closely the sample run. The dropping time of the mercury can be readily calculated from the polarogram. Barometric pressure during the experiment is also recorded.

**Calibration**—Judging from the color of the blood, a rough estimation of its oxygen saturation may be made and, on the basis of saturation, the approximate range of the oxygen tension. Thus, 5, 10, or 15 per cent oxygen in a gas mixture of 5 per cent carbon dioxide and the remainder nitrogen is used to equilibrate with the blood sample. The equilibrating gas is first bubbled through distilled water in gas washers maintained at \(37^\circ\) in a water bath. The blood sample remaining in the sampling syringe is injected into the tonometer through its gas outlet hole; slightly more than that is required to fill the sample cell (2 ml.) is thus introduced with a long needle. After 1 hour of equilibration the blood in the tonometer is taken up in a 2 ml. syringe which has been lubricated with mineral oil and heated to \(37^\circ\). The dead space of the syringe and needle has been filled with the gas mixture in equilibrium with the blood. The blood is then transferred into the sample cell and the remainder of the procedure is exactly as described for the original sample.

**Calculation**—The formula for the calculation of oxygen tension of the blood sample is as follows:

\[
O_2 \text{ tension of sample in mm. Hg} = \frac{O_2 \text{ tension of standard gas} - (\text{sample microampere}) - (\text{standard microampere})}{\text{slope of calibration curve}}
\]
O₂ tension of the standard gas is obtained by multiplying the oxygen concentration of the standard gas in volumes per cent by the barometric pressure, corrected for water vapor pressure. Sample microampere and standard microampere refer to the diffusion current values from the polarograms. The determination of the slope of the calibration curve, (diffusion current)/(oxygen tension), will be described below.

**EXPERIMENTAL**

Experiments were carried out to ascertain (a) the linearity of diffusion current versus oxygen tension curve for pooled plasma, (b) the reproducibility of results when the proposed procedure is applied to blood samples, and (c) the slope (diffusion current)/(oxygen tension).

The linearity of the diffusion current versus oxygen tension curve described by previous investigators was confirmed with pooled plasma equilibrated with nitrogen containing 6.0, 10.3, 15.6, and 20.5 per cent O₂, respectively, and 5 per cent CO₂. A special tonometer and sample cell were constructed for this study which allow direct transfer of equilibrated plasma from the tonometer to the sample cell for polarography, but details of the apparatus will be omitted here. As will be seen later, the presence of cells in blood samples influences the actual diffusion current readings to the extent that no prediction can be made on the basis of the curve for pooled plasma as to the nature of the curve for blood. However, this study does indicate that the diffusion current is directly proportionate to the oxygen concentration of the plasma in the range examined, indicating the absence of complicating factors.

The reproducibility of results was established by two series of experiments. First, the diffusion current of a blood sample equilibrated with a gas of known oxygen tension was compared with the current of the plasma, obtained from the same equilibrated blood and reequilibrated under identical conditions. Second, comparison was made on blood samples divided into two portions and equilibrated with the same gas. Fluoride solution was added to the fresh venous blood samples before equilibration, and gases of different ranges of oxygen tensions were used for equilibration.

The results of the first series are reported in Table I; they demonstrate that no significant difference exists between the plasma and blood sample equilibrated with the same gas.

It was found that fresh blood kept at a given oxygen tension and later equilibrated under the same tension gave progressively lower values for diffusion current. This may explain the slightly lower diffusion current for the plasma sample. For this reason only duplicate runs in quick succession were made on blood samples in the second series of experiments (Table II). Since only one oxygen tension determination can be made on the fresh blood sample, there is no way to reduce the standard deviation of
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the result by duplicate or triplicate analysis. However, the standard deviation of a single determination is not excessive for physiological studies.

The standard gases used for the equilibration were analyzed with the Scholander micro gas analyzer (2), and the oxygen content of the tanks was found to be the same for at least 2 weeks. With a gas stream of constant oxygen content, there is no need to analyze the gas in the tonometer.

The constancy of the relationship between the diffusion current and the oxygen tension was determined within the range of application. With pooled plasma, there is a linear relation for these factors, as previously shown. Blood was equilibrated with gases of various oxygen tension and the diffusion current determined to discover whether the relation was sim-

**Table I**

Comparison of Diffusion Current of Blood and Plasma Equilibrated with Same Gas

<table>
<thead>
<tr>
<th>Oxygen tension (mm. Hg)</th>
<th>Diffusion current (µA)</th>
<th>Blood</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.5</td>
<td>1.432</td>
<td>1.410</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.114</td>
<td>1.090</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.136</td>
<td>1.104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.172</td>
<td>1.184</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.182</td>
<td>1.182</td>
<td></td>
</tr>
<tr>
<td>77.0</td>
<td>2.092</td>
<td>2.070</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.253</td>
<td>2.202</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.980</td>
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<tr>
<td></td>
<td>2.082</td>
<td>2.052</td>
<td></td>
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<td>106</td>
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<td></td>
<td>2.781</td>
<td>2.709</td>
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</tr>
<tr>
<td></td>
<td>2.712</td>
<td>2.754</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.541</td>
<td>2.565</td>
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</tr>
</tbody>
</table>

**Table II**

Reproducibility of Results in Various Ranges of Oxygen Tension

<table>
<thead>
<tr>
<th>Oxygen tension (mm. Hg)</th>
<th>No. of duplicate runs</th>
<th>Σ(per cent Δ)^2</th>
<th>Standard deviation (per cent)</th>
<th>Standard deviation (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>8</td>
<td>123.8</td>
<td>2.8</td>
<td>0.8</td>
</tr>
<tr>
<td>75</td>
<td>9</td>
<td>52.1</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>140</td>
<td>9</td>
<td>35.2</td>
<td>1.4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Σ(per cent Δ)^2 = the sum of the squares of per cent difference between duplicate diffusion current determinations.
ilarly linear for whole blood. Each blood sample was equilibrated with two gases, with a minimal time lag (1 hour) between determinations. The slopes, \((\text{diffusion current})/(\text{oxygen tension})\), for the ranges 35 to 70, 70 to 100, 100 to 140 mm. of Hg were determined and tabulated (Table III), and from the range of values for the slope it seems that the diffusion current-oxygen tension curve is not a straight line for whole blood. Hence, one of the four standard gases with respective oxygen partial pressures of 35, 70, 100, and 140 mm. of Hg is needed for analyzing a blood sample of any normal physiological oxygen saturation. If one wishes to reduce the contribution of error due to the value of the slope, the standard gas oxygen tension should be as close as possible to the tension of the sample.

It is obvious that the value of the slope is characteristic of the dropping mercury electrode and the cell used. In case of breakage of either item, a new value for the slope must be determined under the identical conditions employed for the analysis of blood samples. Since the electrode is protected by the flexible joints and the highly adaptable support of the sample cell, namely the platinum wire loop, the chances of its breakage are small.

**DISCUSSION**

Wiesinger’s experience has been of great value in our work (1). We have eliminated interference by hemoglobin by keeping the sample free from hemolysis. With a few experiments in which hemolysis occurred, the slope of the calibration curve was unpredictable. Therefore the calibration for hemolyzed plasma suggested by Wiesinger would seem inaccurate, and it is preferable to discard hemolyzed samples.

There are two important periods in the procedure when the oxygen tension may drop: (a) from the time of sampling until the addition of the metabolic inhibitor, while the leucocytes continue to consume oxygen, and (b) during the separation of cells from plasma until the reading of the diffusion current, when the plasma oxygen is no longer maintained constant by equilibration with the cells. The second period is controlled by use of

**Table III**

<table>
<thead>
<tr>
<th>Range of oxygen tension</th>
<th>Slope, (10^{-4}) ampere per mm. Hg</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm. Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35–70</td>
<td>17.6, 18.2, 18.6, 18.8, 17.5</td>
<td>18.1</td>
</tr>
<tr>
<td>70–100</td>
<td>16.7, 16.7, 16.4, 16.8, 18.4</td>
<td>16.8</td>
</tr>
<tr>
<td>100–140</td>
<td>18.5, 15.2, 15.1, 17.4, 16.6, 16.0</td>
<td>16.5</td>
</tr>
</tbody>
</table>
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our modified sample cell which shortens this crucial period (3). The total procedural time is 12 to 13 minutes. With the use of heparin instead of oxalate as an anticoagulant, it is possible to add a greater concentration of fluoride to suppress the respiration of leucocytes of the blood sample without causing hemolyzing hypertonicity. In order to keep the volume of the fluoride solution minimal, so that the effect of the dissolved oxygen in the solution on the plasma oxygen tension would be negligible, a highly concentrated suspension of sodium fluoride was finally used. Since sodium fluoride goes into solution rather slowly, while potassium fluoride dissolves in water readily, the latter was added to effect quickly a high fluoride concentration in the blood sample. A final concentration of approximately 2 mmoles of potassium fluoride and 100 mmoles of sodium fluoride was found to be effective without causing any hemolysis. This required the addition of 1 volume of a mixture of 40 gm. per cent of sodium fluoride and 1.2 gm. per cent of potassium fluoride to 100 volumes of blood. It was observed that the blood invariably turns a considerably brighter red with the addition of fluorides, and this change of color indicates the distribution of the inhibitor in the sample. It was also found that the use of mercury to facilitate the mixing of the inhibitor invariably caused lower results; this phenomenon has been explained by Wiesinger on the basis of chemical reaction between mercury and chloride ions and casts doubts on the oxygen tension values in previous studies in which prolonged contact of the blood sample and mercury (and especially finely divided mercury) was permitted. To eliminate this source of error a porcelain disk was placed in the sampling syringe for mixing purposes prior to the displacement of air in the dead space with heparin solution.

It seemed feasible to set up a calibration curve so that the oxygen tension of a blood sample could be read off the curve once the diffusion current was determined. That has been the procedure used by all previous workers. Wiesinger attributed the variation of the absolute values of the diffusion current of human plasma to inadequate temperature control prior to the complete separation of erythrocytes from the plasma. This might very well be a major factor when one is dealing with blood samples. However, when plasma samples were obtained from the blood of human subjects and then equilibrated with gas of the same composition, the absolute diffusion currents were of considerably different values, as shown in Table I. These plasma samples are all free from detectable hemolysis, thus eliminating the contribution of hemoglobin on the diffusion current. We have further made the observation that the absolute value of the diffusion current decreased gradually in the course of several hours in spite of continuous equilibration of the blood with the same gas current. One is forced to conclude
that under the experimental conditions described in this report the assumption of a constant residual current for plasma of different individuals is not valid. In other words, even though the slope of the calibration curve may be assumed to be constant, the intercept of the curve is not constant for different blood samples. It follows then that for each blood sample a reference point must be obtained so that one may fix the intercept of the calibration curve. Further precision is gained by placing the reference point as close as possible to the point representing the sample. Therefore the proposed procedure seems to offer the simplest and yet most reliable method of calibration. When only the relative blood oxygen tension in the same subject during an experiment is of interest, one may omit the calibration for reference point.

SUMMARY

A polarographic procedure for the direct determination of oxygen tension on 5 ml. of saturated or unsaturated blood has been described. The precision of the method has been determined; the standard deviations of a single analysis for the oxygen tension ranges 30, 75, and 140 mm. of Hg are 0.8, 1.4, and 2.0 mm. of Hg, respectively.

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