THE DETERMINATION OF THE GASES OF THE BLOOD.

BY DONALD D. VAN SLYKE AND WILLIAM C. STADIE.
(From the Hospital of The Rockefeller Institute for Medical Research.)
(Received for publication, December 30, 1920.)

CONTENTS.

A modified form of blood gas apparatus for especially accurate work. 1
Magnification of small gas volumes by reduction of pressure .......... 5
The nitrogen gas content of blood, and its effect on the calculation of results of other blood gases ...................................... 6
Determination of oxygen ............................................. 10
Determination of carbon dioxide in whole blood and plasma .......... 22
Determination of carbon monoxide ................................... 32
Determination of methemoglobin .................................... 32
Determination of all the gases in one blood sample .................. 33
Examples of calculations ............................................. 39

During several years of use certain improvements have developed in the technique for using the apparatus designed by one of us for extracting from the blood and measuring the carbon dioxide (Van Slyke, 1917), oxygen (Van Slyke, 1918), and carbon monoxide (Van Slyke and Salvesen, 1919). A technique has furthermore been developed whereby all the blood gases may be determined in one sample of 1 cc. of blood. The purpose of the present paper is to present these developments.

A Modified Form of Blood Gas Apparatus for Especially Accurate Work.1

For analyses in which it is desirable to reduce the error in reading the gas volumes to less than 0.005 cc. an apparatus modified in one respect from the original macro form (Van Slyke, 1917) is now used. The upper, graduated stem of the apparatus has been reduced in bore from 4 mm. to 2.7 mm., so that its

1 The apparatus, with the water jacket and mechanical shaking device, may be obtained from Emil Greiner, 55 Fulton Street, New York.

THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. XLIX, NO. 1
cross-section is halved. Consequently the length of tube holding 1 cc. of gas is lengthened from 75 to 80 mm. to 150 to 160 mm. and it is divided into 100 instead of 50 divisions. In the finer tube gas volumes can be estimated to 0.1 of a division, or 0.001 cc. Also, because of its narrowness the column of liquid can be easily seen through and the meniscus read sharply, even when the solution is deeply colored, as in whole blood analyses.

The only difference in manipulation necessitated by the change in construction is in the manner in which the apparatus is shaken in extracting the gases. The extraction is accomplished by whirling the blood mixture about the wall of the chamber, rather than by the repeated inversion of the apparatus. The measuring tube is of such small diameter that liquid caused to lodge in it by inverting the apparatus is dislodged with some difficulty. The mechanical shaker described by Stadie (1921) is well adapted for use with this form of the apparatus.

In the narrow tube CO₂ and O₂ may be absorbed by admission of alkali and pyrogallol solutions as readily as in the wider tube of the original apparatus. It is desirable, however, in admitting the absorbing solution from the cup at the top of the apparatus to open the cock so slightly that the solution trickles down only one side of the narrow measuring tube below. If the solution enters so rapidly that it forms a solid column in the measuring tube it is dislodged by tapping the tube with the finger, or by running in mercury from the cup. When a viscous absorbing solution, like pyrogallol, is used, it is followed by a little water to clear the inner wall of the measuring tube. Even after careful admission of water or absorbing solution it is the rule for a slight but measurable amount to stick in the upper end of the measuring capillary, just below the stop-cock. A few gentle taps with the finger, or the admission of a drop of mercury from above, suffice for dislodgment.

After the admission of any fluid from the cup into the chamber, time should be allowed for drainage before the gas volume is read. A few seconds suffice if only 0.1 cc. of gas remains to be measured, but 2 minutes are required if the volume approaches 1 cc.

In order to obtain the benefit of the accuracy obtainable with the fine bore apparatus, it is necessary to use it in a room in which the temperature does not vary by more than 1°C., or to provide
FIG. 1. Fine bore apparatus with water jacket and shaking device. A Stadie scale for use in oxygen determination is shown marked on the side of the supporting board. The stick projecting from the levelling bulb is for measuring 500 mm. reduction in pressure. It is well to insert a glass air trap between the apparatus and levelling bulb, as described for the micro-apparatus (Van Slyke, 1917, p. 363).
Blood Gases

It with a water jacket such as is shown in Fig. 1. The apparatus may be shaken by hand, or may be attached to a hinged board, as shown in Fig. 1, so arranged that the shaking may be done by a motor.

The manipulation of the apparatus with the finer bore requires slightly more time and care than that of the original apparatus, and the latter is adequate for most purposes, such as the determination of the oxygen capacity of blood as a measure of the hemoglobin, and of the carbon dioxide as a measure of the alkaline reserve, both values being capable of determination by the original apparatus to within 1 per cent of the amounts normally measured. For some purposes, however, such as the determination of all the gases in 1 cc. of blood, the determination of carbon monoxide, or of the slight amount of nitrogen gas in blood, or when it is essential to keep the error in CO₂ determinations below 0.5 volume per cent, or in oxygen determinations below 0.25, the fine bore apparatus is desirable.

In calibrating the apparatus, we attach to the bottom, either by sealing or by joining glass on glass with a heavy-walled piece of suction tubing, a glass tube bearing a fine-bore stop-cock and drawn out into a fine capillary. Through the latter water is drawn up into the apparatus by means of suction applied to the outlet above the upper cock. When the entire apparatus and the upper outlet are filled with water, the cock of the capillary attached below is closed, and the suction is discontinued. The upper cock of the apparatus is then turned, so that the chamber is connected with the empty cup. The water is now delivered through the cock of the attached capillary, 0.1 cc. at a time. The drops are caught in a weighing bottle containing a layer several mm. thick of paraffin oil. After the delivery of each drop, the tip of the capillary is touched to the surface of the oil, to detach all adhering water except a uniform minimum. The drops of water sink beneath the oil, and loss of weight by evaporation is prevented with completeness that is not obtainable by merely using a covered weighing bottle, which must be opened to receive each addition of water. The weighings are made to the nearest milligram.

The calibration may also be performed with mercury, as described for the micro-apparatus. In this case sealing the

delivery capillary to the apparatus is necessary, as the pressure is sufficient to expand appreciably a rubber tube.

The constancy of results which may be obtained with the fine bore apparatus is indicated by Table I, which shows the duplicate readings obtained in a series of analyses performed by Dr. J. P. Peters, Jr., in determining the CO₂ absorption curve of a specimen of blood.

### Table I.

**Results of Series of Duplicate Determinations of CO₂ in Whole Blood with the Fine Bore Apparatus.**

1 cc. of blood was used for each analysis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Total volume of gas extracted.</th>
<th>V - CO₂</th>
<th>CO₂ By difference.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>1</td>
<td>0.735</td>
<td>0.042</td>
<td>0.693</td>
</tr>
<tr>
<td></td>
<td>0.725</td>
<td>0.033</td>
<td>0.692</td>
</tr>
<tr>
<td>2</td>
<td>0.604</td>
<td>0.130</td>
<td>0.474</td>
</tr>
<tr>
<td></td>
<td>0.603</td>
<td>0.130</td>
<td>0.472</td>
</tr>
<tr>
<td>3</td>
<td>0.704</td>
<td>0.128</td>
<td>0.576</td>
</tr>
<tr>
<td></td>
<td>0.696</td>
<td>0.122</td>
<td>0.574</td>
</tr>
<tr>
<td>4</td>
<td>0.703</td>
<td>0.126</td>
<td>0.637</td>
</tr>
<tr>
<td></td>
<td>0.764</td>
<td>0.126</td>
<td>0.633</td>
</tr>
</tbody>
</table>

**Magnification of Small Gas Volumes by Reduction of Pressure.**

When an accurate measurement of very small gas volumes (e.g., less than 0.05 cc.) is desired, as may be the case in determining the N₂ or CO content of blood, it is desirable for measurement to increase the gas volume by reducing the pressure. The reduction is accomplished by holding the levelling bulb lower, by the desired distance, than it would be placed in order to put the gas in the apparatus under atmospheric pressure. We have found 500 mm. of mercury to be a convenient reduction in pressure for this purpose. For readily locating this level we have used the light rod with the marker attached near its top, shown projecting up out of the levelling bulb in Fig. 1. The rod is a pine stick 2.5 mm. in diameter and a little over 500 mm. long.
The marker is a piece of stiff paper glued on 501 mm. from the bottom. The stick passes down into the levelling bulb through a piece of glass tubing about 10 cm. long, which is held upright in a rubber stopper and serves to keep the stick in a vertical position during readings. At its lower end, within the levelling bulb, the stick terminates in a cork, which floats on the mercury. It dips 1 mm. below the surface in the case of our apparatus (the exact distance would, of course, vary for different sticks and corks) and to correct for this depth of immersion the distance from the bottom of the cork to the marker is made 501 instead of 500 mm.

The measurement is made merely by placing the bottom of the marker at the level, in relation to the mercury and water levels inside the apparatus, at which one would place the level of the mercury itself in the bulb in order to obtain atmospheric pressure within the apparatus.

The volume of gas read is reduced to atmospheric pressure by multiplying by the factor \( \frac{B - H - w}{B - w} \), \( B \) being the atmospheric pressure, \( H \) the height from the mercury in the levelling bulb to the marker on the stick (e.g., 500 mm.), and \( w \) the vapor tension of the water at the temperature at which the reading is taken.

For example, a gas volume is read as 0.133 cc. at 25°, and 765 - 500 = 265 mm. pressure. The vapor tension of water at 25° is 23.6 mm. Hence, the volume of gas measured moist at room temperature and pressure would be \( 0.133 \times \frac{265 - 23.6}{765 - 23.6} = 0.0433 \). This is reduced to 0°, 760 mm. measured dry by the usual factor given in Table XIII, which in this case is \( 0.888 \times \frac{765}{760} = 0.894 \). The consequent volume at 0°, 760 mm. is therefore \( 0.0433 \times 0.894 = 0.0387 \) cc.

The Nitrogen Gas Content of Blood, and its Effect on the Calculation of Results of Other Blood Gases.

In the original paper on the determination of oxygen, the oxygen and nitrogen were extracted from the blood and measured together, and the oxygen was estimated by deducting the
relatively small amount of nitrogen, which was calculated from Bohr's determinations (1905) of the solubilities of gases in blood and water. The solubility coefficient of nitrogen gas in water (the volume of gas reduced to 0°, 760 mm., which 1 volume of water dissolves when in equilibrium with the gas at 760 mm. tension) at 38° is 0.0122; in blood Bohr found the solubility coefficients of chemically inert gases to be 92 per cent as great as in water. The volume of nitrogen calculated to be held in solution by 100 cc. of water at body temperature and 760 mm. barometric pressure (diminished by 48 mm. vapor tension of water) in equilibrium with the atmosphere is, therefore, $100 \times 0.0122 \times \frac{760 - 48}{760} = 0.90$ cc. For blood, the calculated amount would be 0.92 as great, or 0.83 cc.

Earlier analysts had found as much as 4 to 5 volumes per cent of nitrogen gas in the blood, and attributed the excess above that which water would dissolve as due to leakage of air into the evacuated apparatus used. Bohr and Henriques (1897) by more accurate methods determined the nitrogen gas, along with the carbon dioxide and oxygen, in both arterial and venous bloods of a number of dogs (twenty-two analyses). They found that the nitrogen contents varied from the theoretically expected 0.8 volume per cent to 1.7 volumes per cent, the average and most of the individual figures being in the neighborhood of 1.2 volumes per cent. In a paper published at nearly the same time Bohr (1897) found that when shaken with air at room temperature blood of the ox and dog, and likewise 10 to 12 per cent solutions of oxyhemoglobin, absorbed 1.65 to 1.98 volumes per cent of nitrogen. Water under the same conditions absorbed only 1.30 volumes per cent. Peculiarly enough, oxygen-free nitrogen was absorbed in no greater proportion by blood than by water. For the fact that blood in contact with air absorbs more nitrogen than does water, there appears to be as yet no well grounded explanation, although Bohr suggested that some easily dissociated oxide of nitrogen might be formed when nitrogen and oxygen meet in the presence of dissolved hemoglobin.

In Bohr's later paper (1905) on the absorption coefficients of gases in blood and water the bearing of these results on the absorption coefficient of nitrogen in blood is not considered, and the coefficient is given as 92 per cent of that in water.

When the original paper on the determination of oxygen in the blood was published (Van Slyke, 1918) Bohr's earlier data (1897) were overlooked, and the corrections for nitrogen gas were based on the solubility coefficient given in his 1905 publication. Van Slyke and Salvesen (1919), in later analyses in which the oxygen and nitrogen were measured separately, the oxygen being absorbed in the apparatus by pyrogallol, found that the
nitrogen gas in rabbit blood approximated 1.2 volumes per cent, a figure which agrees with the average found in dog blood by Bohr and Henriques (1897). Smith, Dawson, and Cohen (1919-20) have recently reported further analyses showing a nitrogen content of blood higher than that estimated from the solubility coefficient.

We have performed a number of determinations of the nitrogen gas content of blood, both as drawn from the veins and after equilibration with air at room temperature. It was found that the same results were obtained when no reagents were added as when ferricyanide was used to release the oxygen, or acid to release carbon dioxide. Consequently in the series here reported the nitrogen was determined by extraction in a vacuum without the addition of reagents. The apparatus employed was the model with the finer bore measuring tube described at the beginning of this paper. The technique used was the following:

3 cc. of water and a few drops of octyl alcohol were freed completely from air by vacuum extraction in the apparatus in the usual manner. The extraction was always repeated in order to make certain that not even 0.001 cc. of air was left. 1 to 2 cc. of the water were run up into the cup, and 5 cc. of fresh blood, which had been drawn under paraffin oil without exposure to air, were run under the water and into the chamber of the apparatus. (The fact that the water during its brief contact with air in the cup absorbed no measurable amounts of air was proved by controls.) The blood-water mixture was evacuated in the apparatus and the gases were shaken out by hand for 3 minutes; longer shaking was found not to increase the yield of nitrogen gas. After release of the vacuum the oxygen and carbon dioxide were absorbed by allowing a few drops of alkaline pyrogallol solution to run slowly from the cup down the measuring tube. When absorption was complete, as shown by shrinkage of the gas to a constant volume, the somewhat viscous pyrogallol solution was washed from the inner walls of the tube by means of a little water, and the volume of unabsorbed gas was measured as nitrogen. The results are given in Table II.

The results in Table II confirm those of Bohr in showing that the blood, both in the veins and after aerating at room temperature, contains about 0.5 volume per cent more nitrogen gas than calculated from the solubility coefficient of the gas in water. Our results, perhaps because the simplicity of our method has reduced the chance of error, vary over a smaller range than Bohr's. In venous blood the total range is $1.36 \pm 0.11$ volumes per cent of
nitrogen, while in blood aerated at 23–30° in vitro it is $1.52 \pm 0.2$ volumes per cent.

The results in Table II, as well as those of Bohr above referred to, include as "nitrogen" all the gas extracted by evacuating blood, and left after absorption of oxygen and carbon dioxide. Regnard and Schlössing (1897) found besides nitrogen 0.04 vol-

<table>
<thead>
<tr>
<th>No.</th>
<th>Subject..</th>
<th>Duplicates.</th>
<th>Mean.</th>
<th>Temperature.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sol. per cent</td>
<td>sol. per cent</td>
<td>sol. per cent</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1.36</td>
<td>1.42</td>
<td>1.39</td>
</tr>
<tr>
<td>2</td>
<td>St.</td>
<td>1.30</td>
<td>1.39</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>M. L.</td>
<td>1.63</td>
<td>1.30</td>
<td>1.47</td>
</tr>
<tr>
<td>4</td>
<td>Kel.</td>
<td>1.33</td>
<td></td>
<td>1.33</td>
</tr>
<tr>
<td>5</td>
<td>Schu.</td>
<td>1.36</td>
<td></td>
<td>1.36</td>
</tr>
<tr>
<td>6</td>
<td>M. S.</td>
<td>1.30</td>
<td></td>
<td>1.30</td>
</tr>
<tr>
<td>7</td>
<td>Koh.</td>
<td>1.43</td>
<td></td>
<td>1.43</td>
</tr>
<tr>
<td>8</td>
<td>Dog 1.</td>
<td>1.44</td>
<td>1.26</td>
<td>1.35</td>
</tr>
<tr>
<td>9</td>
<td>&quot; 2.</td>
<td>1.31</td>
<td>1.40</td>
<td>1.36</td>
</tr>
<tr>
<td>10</td>
<td>Dr. B.</td>
<td>1.25</td>
<td></td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>E.</td>
<td>1.78</td>
<td>1.63</td>
<td>1.71</td>
</tr>
<tr>
<td>2</td>
<td>St.</td>
<td>1.47</td>
<td>1.64</td>
<td>1.56</td>
</tr>
<tr>
<td>3</td>
<td>Schu.</td>
<td>1.50</td>
<td>1.31</td>
<td>1.51</td>
</tr>
<tr>
<td>4</td>
<td>Koh.</td>
<td>1.52</td>
<td></td>
<td>1.52</td>
</tr>
<tr>
<td>5</td>
<td>V. S.</td>
<td>1.60</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td>6</td>
<td>Dog 2.</td>
<td>1.40</td>
<td>1.41</td>
<td>1.41</td>
</tr>
<tr>
<td>7</td>
<td>Ox.</td>
<td>1.55</td>
<td></td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>1.52</td>
</tr>
</tbody>
</table>

Whether it actually is all nitrogen, or a mixture of nitrogen and gases, such as methane, hydrogen, etc., not yet identified, is still uncertain. Harttridge (1919–20) has found as much as 1 volume per cent of carbon monoxide in the blood of a heavy

---

Table II: Nitrogen Gas in Whole Blood.

Venous blood as drawn.

<table>
<thead>
<tr>
<th>No.</th>
<th>Subject..</th>
<th>Duplicates.</th>
<th>Mean.</th>
<th>Temperature.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sol. per cent</td>
<td>sol. per cent</td>
<td>sol. per cent</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1.36</td>
<td>1.42</td>
<td>1.39</td>
</tr>
<tr>
<td>2</td>
<td>St.</td>
<td>1.30</td>
<td>1.39</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>M. L.</td>
<td>1.63</td>
<td>1.30</td>
<td>1.47</td>
</tr>
<tr>
<td>4</td>
<td>Kel.</td>
<td>1.33</td>
<td></td>
<td>1.33</td>
</tr>
<tr>
<td>5</td>
<td>Schu.</td>
<td>1.36</td>
<td></td>
<td>1.36</td>
</tr>
<tr>
<td>6</td>
<td>M. S.</td>
<td>1.30</td>
<td></td>
<td>1.30</td>
</tr>
<tr>
<td>7</td>
<td>Koh.</td>
<td>1.43</td>
<td></td>
<td>1.43</td>
</tr>
<tr>
<td>8</td>
<td>Dog 1.</td>
<td>1.44</td>
<td>1.26</td>
<td>1.35</td>
</tr>
<tr>
<td>9</td>
<td>&quot; 2.</td>
<td>1.31</td>
<td>1.40</td>
<td>1.36</td>
</tr>
<tr>
<td>10</td>
<td>Dr. B.</td>
<td>1.25</td>
<td></td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>1.36</td>
</tr>
</tbody>
</table>

Venous blood saturated with air at room temperature.

<table>
<thead>
<tr>
<th>No.</th>
<th>Subject..</th>
<th>Duplicates.</th>
<th>Mean.</th>
<th>Temperature.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sol. per cent</td>
<td>sol. per cent</td>
<td>sol. per cent</td>
</tr>
<tr>
<td>1</td>
<td>E.</td>
<td>1.78</td>
<td>1.63</td>
<td>1.71</td>
</tr>
<tr>
<td>2</td>
<td>St.</td>
<td>1.47</td>
<td>1.64</td>
<td>1.56</td>
</tr>
<tr>
<td>3</td>
<td>Schu.</td>
<td>1.50</td>
<td>1.31</td>
<td>1.51</td>
</tr>
<tr>
<td>4</td>
<td>Koh.</td>
<td>1.52</td>
<td></td>
<td>1.52</td>
</tr>
<tr>
<td>5</td>
<td>V. S.</td>
<td>1.60</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td>6</td>
<td>Dog 2.</td>
<td>1.40</td>
<td>1.41</td>
<td>1.41</td>
</tr>
<tr>
<td>7</td>
<td>Ox.</td>
<td>1.55</td>
<td></td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>1.52</td>
</tr>
</tbody>
</table>
tobacco smoker, but there appears to be no reason to believe that carbon monoxide forms any part of the unexplained "nitrogen" ordinarily present in normal blood. The probability that the residual gas is actually nitrogen is strengthened by the fact that it is increased by aerating the blood at room temperature. The reverse would be expected if any considerable proportion of the residual gas were other than nitrogen.

It is evident from the above that when the nitrogen and oxygen, or nitrogen and carbon monoxide, of the blood are measured together, the volume of the gas accompanying the nitrogen cannot be estimated by deducting the volume of nitrogen estimated (as 0.83 volume per cent of the blood) from its solubility coefficient, without introducing an error of approximately plus 0.5 volume per cent into the calculation of the oxygen or carbon monoxide.

There are two alternatives. (1) The average \( N_2 \) content of blood as empirically determined (Table II) may be subtracted from the \( N_2 + O_2 \) or from the \( N_2 + CO \) in order to estimate the \( O_2 \) or the \( CO \). (2) The gas measured with the nitrogen may be determined by absorption with a proper reagent (e.g., pyrogallol for oxygen). The details of the calculations, from analyses made by both alternatives, of oxygen content, oxygen capacity, carbon monoxide content, and of methemoglobin, the determination of which is based on oxygen capacity determinations, will be taken up in the following pages in connection with the discussions of these determinations.

**Determination of Oxygen.**

1. **Changes in Technique.**

   **A. Reduction in Amount of Ferricyanide Used.**—We have reduced the amount of potassium ferricyanide per cc. of blood from 60 or 70 mg. (0.4 cc. of saturated solution for 2 cc. of blood) to 10 mg., which, as shown in Table III, is still twice the minimum necessary amount. The ferricyanide solution used contains 20 gm. per 100 cc. Of this 0.1 cc., containing 20 mg. of the salt, is used for 2 cc. of blood.

   The smaller amount of ferricyanide causes as rapid and complete an evolution of oxygen as do larger amounts, and it has two
advantages over the latter. First, the smaller amount does not appreciably retard laking, and it is consequently unnecessary to wait for laking to become complete in the apparatus before the ferricyanide is introduced. Also, the possibility of error from incomplete laking is avoided. Second, reduction in the amount of ferricyanide reduces the formerly somewhat annoying amount of precipitate formed by interaction with the mercury in the apparatus.

B. Use of Water Instead of Ammonia for Laking Blood.—In the original procedure (Van Slyke, 1918) the blood, before addition of ferricyanide, was laked in the apparatus with saponin in a dilute ammonia solution, the proportions of blood and ammonia being those previously adopted by Haldane and by Barcroft in their well known methods for determination of blood oxygen.

We have found that the use of such a mixture may introduce two errors, both usually small but frequently measurable by our present technique: (1) the alkalinity of the mixture is not sufficient entirely to prevent in all cases the escape of CO₂, particularly when the room temperature is high, and (2) the alkaline reaction accelerates some oxidative process by which part of the oxygen freed is slowly consumed. Hence, as shown by Table V, when the oxygen is freed in too alkaline a solution, the entire amount is not obtained.

With the dilute ammonia formerly used, the slight alkalinity caused but little oxygen consumption, sometimes none (Table VI) and the average error from this source was approximately

---

TABLE III.


<table>
<thead>
<tr>
<th>Ferricyanide added to 2 cc. of blood.</th>
<th>O₂ + N₂ measured at 20°, 765 mm.</th>
<th>Calculated O₂ content of blood.</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td>cc.</td>
<td>vol. per cent</td>
</tr>
<tr>
<td>0.005</td>
<td>0.41</td>
<td>17.3</td>
</tr>
<tr>
<td>0.010</td>
<td>0.53</td>
<td>22.8</td>
</tr>
<tr>
<td>0.020</td>
<td>0.53</td>
<td>22.8</td>
</tr>
<tr>
<td>0.100</td>
<td>0.53</td>
<td>22.8</td>
</tr>
</tbody>
</table>

* 1.36 volumes per cent of N₂ being subtracted from O₂ + N₂ after reduction of the total volume to 0°, 760 mm.
balanced by the error in the opposite direction due to the small amount of CO₂ set free. Consequently the normal figure for the oxygen capacity of human blood determined by the former technique is not affected by present changes.

At a temperature of 20°, and with ammonia of at least 0.06 N concentration (obtained by diluting 1 cc. of ordinary concentrated ammonia to 200 cc.), the CO₂ tension is usually too near zero to affect measurably the volume of the extracted gases (no decrease observed when the gases are washed with NaOH solution). If the temperature rises to 25 or 30°, however, or if by reason of standing or preparation from a weak stock solution the ammonia is of less than usual concentration, enough CO₂ may escape with the O₂ to increase the calculated volume per cent of the latter by several tenths, and occasionally more. There is also a difference in bloods, some freeing measurable amounts of CO₂ under conditions where others do not. Presumably bloods richer in hemoglobin, which acts as a weak acid, require more alkali to reduce their CO₂ tension to zero.

The obvious way to prevent mixture of CO₂ with the O₂ + N₂ would be to increase the alkali added to the blood to such an extent that there would in all cases be a sufficient excess. Such addition, however, increases the error due to oxygen consumption.

The latter error, like that due to CO₂ escape, may be nil or slight within the 2 minutes that usually suffice to extract all the oxygen. It is not uncommon, however, on extracting for a third minute, to observe a decrease of a few thousandths of a cc., amounting to 0.1 to 0.3 volume per cent of the blood.

At present, therefore, we use water for laking the blood, which seems to obviate the error due to oxygen consumption. The 10 to 20 volumes per cent of CO₂ that accompany the oxygen are removed after the extraction by absorption with NaOH solution, before the O₂ + N₂ volume is measured. Consequently the danger of error from admixture of CO₂ with the O₂ + N₂ is also avoided.

The avoidance of ammonia has an additional advantage in reducing the insoluble black precipitate which forms in its presence by the interaction of ferricyanide and mercury. With water as diluent and the lesser amounts of ferricyanide now used the precipitate is reduced to practically nil.
C. Details of Present Oxygen Determination.—The apparatus is washed out twice before each analysis with water, in the rapid manner described later in connection with the CO₂ determination (p. 22), in order to remove the alkali used to absorb CO₂ in any previous analysis. For 2 cc. of blood 6 cc. of water, 0.3 cc. of 1 per cent saponin (Merck) solution, and 2 or 3 drops of caprylic alcohol are introduced into the apparatus and freed of air, as described in the original paper (Van Slyke, 1918), the extraction being repeated until no more air is obtained.

Nearly the entire 6 cc. is then forced up into the cup of the apparatus. The blood is stirred to assure even distribution of corpuscles, and drawn into a pipette calibrated to deliver 2 cc. between two marks, of which the lower is 3 or 4 cm. from the tip. The pipette is introduced under the water solution in the cup, so that the tip rests on the bottom near the capillary. As the blood flows out of the pipette held in the left hand, the stop-cock is partially opened with the right, so that the blood accompanied by some of the water, flows at once on into the chamber of the apparatus. The layer of blood need never rise more than 1 or 2 mm. above the bottom of the cup, and the slight amount adhering is washed completely into the chamber by the water which follows after all the blood has been delivered.

Before the last cc. of water is readmitted, 0.10 to 0.12 cc. of a solution, containing 20 gm. of potassium ferricyanide per 100 cc., is added and thereby introduced into the chamber after the blood. (The ferricyanide may be measured with sufficient accuracy as 3 drops from a dropper which delivers 1 cc. in 25 to 30 drops.) A mercury seal is made, and the apparatus is evacuated and shaken for 3 minutes. Usually 2 minutes, frequently less, are sufficient, but repeated trials have shown that 3 minutes are sometimes required before the last traces of oxygen are extracted. Owing to the continued slow evolution of CO₂ from the unalkalinized mixture, it is not possible to check the completeness of the O₂ + N₂ extraction by repeated measurements of the O₂ + N₂ + CO₂ volume; so it is desirable to continue the extraction in each case for a sufficient period to insure its completion. Extraction being complete, the vacuum is released by opening the lower cock, and the mercury together with the reaction mixture is allowed to run up into the chamber of the
Blood Gases

The gases obtained from 2 cc. of blood are normally about 0.50 cc. of O₂, 0.015 cc. of N₂, and 0.2 cc. of CO₂, about one-fifth of the total blood CO₂ being evolved under the conditions of the analysis. In order to absorb the CO₂ the levelling bulb is placed at such a height that the mercury in it is slightly below the level of the mercury in the apparatus, so that a slight negative pressure is maintained in the latter. 0.5 cc. of 0.5 N NaOH solution, previously saturated with air or oxygen, is admitted from the cup of the apparatus and allowed to trickle slowly down the inner wall to absorb the CO₂ from the gas mixture. If, as is frequently the case with the fine bore apparatus, the latter part of the solution enters as a solid column instead of running down the walls, it is dislodged by letting a little mercury pass down through it in a fine stream from the cup above. Since the mercury droplets are coated with alkali solution, they rapidly complete the absorption of CO₂ as they pass the gas column.

Even when all the alkali solution appears to flow down the sides of the gas pipette a little usually remains just below the stop-cock. If neglected, this amount introduces an error of from 0.005 to 0.010 cc. in the gas volume reading, but it can be easily dislodged by a drop of mercury run in as above described.

After absorption of CO₂ is complete time for drainage of the alkali solution must be allowed before the reading is taken (see p. 2).

For the reading the height of the levelling bulb is regulated by the scale described by Stadie (1921) in the accompanying paper, and shown in Fig. 1 of this paper. The use of the scale may be obviated by trapping the extracted solution in the bulb below the lower cock, as in the determination of CO₂ (Van Slyke, 1917), before the vacuum is released.

2. Calculations Connected with Oxygen Determinations.

A. Calculation of Total Oxygen Content of Blood.—The total oxygen content of blood includes both the oxygen chemically bound by hemoglobin and that held in physical solution by the blood fluids.

When the oxygen and nitrogen are extracted and measured together, as described in the original paper, the calculation is made as there
D. D. Van Slyke and W. C. Stadie 15

described, except that instead of subtracting the theoretically estimated 0.9 volume per cent of nitrogen from the $O_2 + N_2$ content the empirically determined $1.36$ volumes per cent are subtracted.

When from the mixture of $O_2 + N_2$ the $O_2$ is directly determined by absorption with pyrogallol, as described by Van Slyke and Salvesen (1919), the empirical correction for nitrogen is, of course, avoided. Since absorption is completed in 1 minute it adds but little to the time required for an analysis. It is a useful check whenever there is reason to suspect leakage of air into the apparatus, as such an occurrence greatly increases the residual nitrogen gas.

B. Calculation of Oxygen Combined with Hemoglobin in Blood as Drawn.—The gases obtained together as described by extraction of arterial or venous blood collected out of contact with air are: (1) oxygen chemically combined with hemoglobin, (2) oxygen physically dissolved in the blood, and (3) nitrogen physically dissolved. For dissolved nitrogen the value as empirically determined above is $1.36$ cc. per 100 cc. of blood. For oxygen the volume physically dissolved depends upon the partial pressure of oxygen with which the blood is in equilibrium. Arterial blood normally is in approximate though not complete equilibrium (Krogh and Krogh, 1910) with the alveolar air, which varies in its oxygen content from 14.5 to 17.5 per cent. Assuming a mean value of 16 per cent, the oxygen dissolved at $38^\circ$C. is approximately $100 \times 0.022 \times 0.16 \times \frac{760 - 49}{760} = 0.33$ volume per cent. In venous blood, however, and in partially saturated arterial blood, the amount of physically dissolved oxygen varies with the varying oxygen tension, which is approximately indicated by the percentage of complete saturation of the blood with oxygen. An average value for the percentage of saturation of venous blood is 65 per cent, which corresponds to an oxygen tension of 35 mm. when the CO$_2$ tension is normal (40 mm.). There is, therefore, $100 \times 0.022 \times \frac{35}{760} = 0.10$ volume per cent of oxygen physically dissolved in such a sample.

Therefore when the total $O_2$ is determined by extraction and absorption with pyrogallol, the $O_2$ combined with hemoglobin
may be approximately estimated by subtracting from the total O₂ 0.3 volume per cent for arterial blood and 0.1 for venous.

When N₂ and O₂ are measured together, the corrections for dissolved N₂ + O₂ which are subtracted in order to estimate the combined O₂ are 1.36 + 0.3 = 1.66 volumes per cent for arterial, and 1.36 + 0.10 = 1.46 for venous blood. Discarding the last decimals, the corrections are 1.7 volumes per cent for arterial blood, 1.5 for venous.

The above corrections are estimated from the average oxygen tensions of arterial and venous blood. The corrections due to dissolved oxygen are so minute that fluctuations from the average can exceed the experimental error only in most refined work. If, however, a greater degree of accuracy is desired the values in Table IV may be used. They are calculated from the curve of Barcroft (1914).

C. Oxygen Combined with Hemoglobin in Blood Artificially Saturated with Air (Oxygen Capacity of Blood).—In determining the oxygen capacity (usually as a measure of the hemoglobin content) blood is saturated with air at room temperature, as described in the original paper (Van Slyke, 1918). From the total N₂ + O₂ obtained on extraction of blood thus saturated, it is necessary to deduct the N₂ and the physically dissolved O₂. According to Bohr (1905) the solubility coefficient of oxygen in blood is 0.031 at 15°, 0.022 at 38°. For the usual room temperature range of 15–25°, the coefficient varies from 0.031 to 0.027, with a mean of 0.029 at 20°. The volume per cent of oxygen physically dissolved by blood equilibrated with atmospheric air at 20°, 760 mm., may therefore be estimated as $100 \times 0.029 \times 0.209 \times \frac{760 - 17}{760} = 0.59$. At 25° the figure would be 0.55, at 15° it would be 0.63. The figure thus calculated from the solubility coefficient may, as in the case of nitrogen, not be exact, but it does not appear probable that the error, if there is one, is large enough to be significant. If we add the figure for oxygen dissolved at 20° to the empirically determined nitrogen content of blood saturated with air at room temperature we have $0.59 + 1.52 = 2.11$ volumes per cent of physically dissolved O₂ + N₂ to subtract from the total O₂ + N₂ content in order to obtain the oxygen combined with hemoglobin.
When the total $O_2$ of the saturated blood is determined directly, by absorption with pyrogallol, correction is to be made only for the 0.59 volume per cent of physically dissolved oxygen.

In Table I of the original article (Van Slyke, 1918) the volume of "dissolved air" to be subtracted from blood shaken at 20° was calculated from the solubility coefficient of air to be 0.034 cc. This corresponds to 0.031 cc. measured at 0°, or 1.55 volumes per cent of the 2 cc. of blood analyzed, a figure 0.55 volume per cent below that based on the nitrogen content which we find by actual analysis. Consequently oxygen capacities calculated as outlined in the original paper have been approximately 0.55 volume per cent too high. This corresponds to an error of plus 0.41 gm. of hemoglobin per 100 cc. of blood, or plus 3.0 per cent of the average normal hemoglobin content.

**D. Calculation of the Oxygen Unsaturation of Blood.**—The oxygen unsaturation of blood was first defined by Lundsgaard (1918)
as the difference between the oxygen content of blood and the oxygen capacity. In determining the oxygen unsaturation Lundsgaard and others, including the authors, have estimated the oxygen capacity as the oxygen content of blood saturated with air at room temperature. At average atmospheric conditions (20° and 143 mm. oxygen tension) blood containing sufficient hemoglobin to bind 20.00 volumes per cent of oxygen would contain 20.58 volumes per cent of total oxygen, the extra 0.58 being the amount held in physical solution. However, at 38° the same blood would likewise bind 20.00 volumes per cent of oxygen with its hemoglobin, but would dissolve physically only 0.32 volumes per cent, making a total of 20.32 volumes per cent. The oxygen unsaturation of such a blood would be calculated according to the above mentioned method as 20.58 - 20.32 = 0.26 volume per cent, instead of zero, which it obviously is. The error of calculation is barely within the limit of experimental error of the determinations, and is altogether too slight to affect significantly any results thus far published, but it seems nevertheless worth while to correct it in the future by basing the calculations on figures representing oxygen combined with hemoglobin rather than on total oxygen. The example given at the end of the paper indicates the preferred mode of calculation.

Table XIII at the end of this paper will be found of convenience in making the above calculations.


A. Measurement of Blood Sample.—The accuracy with which samples of blood can be delivered from an Ostwald pipette of the type described by Van Slyke and Cullen (1914, p. 215) is indicated by the following successive weighings. The blood was well stirred before each sample was drawn. Nine successive weighings from a 2 cc. pipette gave 2.1072, 2.1090, 2.1100, 2.1076, 2.1093, 2.1071, 2.1072, 2.1051, and 2.1069 gm. of blood, the maximum variation being from 2.1101 to 2.105, or 2.1075 ± 0.0025 gm., the maximum deviation, 0.0025 gm. being 0.11 per cent, the average 0.05 per cent of the amount measured.

B. Reading of Gas Volume in Fine Bore Apparatus with 0.1 to 0.2 Cc. of Water above Mercury. Levelling Bulb Adjusted with
Eye. No Side Arm or Levelling Scale. A series of measurements on the same portion of gas under the above conditions gave, when reduced to 760 mm., 0°, eight successive readings between 0.729 and 0.732 cc., the maximum deviation being, therefore, 0.0015 cc. from the mean, or 0.2 per cent of the gas volume measured.

C. Reading of Gas Volume in Fine Bore Apparatus with 8.5 Cc. of Water above Mercury. Side Arm on Levelling Bulb. New Levelling Scale (Stadie, 1921) Used in Adjusting Levelling Bulb.—Nine successive readings on a given gas sample varied between 0.884 and 0.882 cc., the maximum deviation being, therefore, 0.001 cc. from the mean, or 0.1 per cent of the gas volume measured. Without the Stadie levelling scale, a maximum variation of ±0.004 cc., or four times as great, was encountered.

D. Reabsorption of Air by Reaction Mixture During Delivery of Blood.—When, after being freed of air, the reaction mixture is forced up into the cup it is exposed to the atmosphere for a period of 1 to 4 minutes while the blood is being stirred and pipetted into the apparatus. Repeated tests have shown that no measurable amounts of air are absorbed by the reaction mixture during even longer periods of quiet exposure to the atmosphere.

E. Constancy of Results Obtainable.—With the use of the levelling scale and the technique described above the volumes of O₂ + N₂, reduced to 0°, 760 mm., obtained from 2 cc. samples of blood were the following:

Blood 1, 0.555, 0.555, 0.555, and 0.556 cc.
Blood 2, 0.436, 0.436, 0.442, 0.433, and 0.436 cc.

The first series represents somewhat exceptional constancy, but the second, with a maximum variation of 0.437 ± 0.005 cc., or ±0.25 volume per cent of oxygen, indicates rather greater variations than are usually to be expected.


The fact that a sufficient grade of alkalinity diminishes the yield of oxygen, and that acid may to a less extent cause a similar diminution is indicated by the data in Table V. The analyses were performed as described on page 13 of this paper, except that instead of 6 cc. of water added to the 2 cc. sample of blood,
an equal volume of a solution containing the indicated amounts
of acid or alkali was added. The extracted gas was tested with
NaOH solution to absorb CO₂ after use of the alkaline as well
as the acid and neutral solutions. An amount of CO₂ equivalent
to 0.5 volume per cent of the blood was obtained from the blood +
ammonia mixture, and to 0.65 from the carbonate.

TABLE V.

Oxygen Content of Blood Determined after Laking in Alkali, Water, and Acid,
Respectively.

<table>
<thead>
<tr>
<th>Alkali or acid added</th>
<th>Amount of acid or alkali per cc. of blood</th>
<th>O₂ + N₂</th>
<th>CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂CO₃</td>
<td>0.28 (millimols)</td>
<td>25.15</td>
<td>93.6</td>
</tr>
<tr>
<td>NH₃</td>
<td>0.15</td>
<td>26.35</td>
<td>98.1</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.04</td>
<td>21.35</td>
<td>79.3</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.02</td>
<td>26.45</td>
<td>98.3</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.01</td>
<td>26.85</td>
<td>100.0</td>
</tr>
<tr>
<td>None</td>
<td>0.00</td>
<td>26.80</td>
<td>99.8</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.01</td>
<td>26.80</td>
<td>100.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.06</td>
<td>26.65</td>
<td>99.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.10</td>
<td>26.35</td>
<td>98.1</td>
</tr>
</tbody>
</table>

The results with this blood seem to indicate that the maximum
yield of oxygen is obtained when water with negligible amounts
of acid or alkali is used. That other bloods do not consistently
yield higher oxygen contents when no acid or alkali is added is
indicated by Table VI, however.

That the variations in results with the different reagents were
not due to analytical errors appears to have been excluded by
the constancy with which duplicates with each solution agreed.
Blood 3 was controlled with particular care. One sample
each was analyzed in ammonia, water, lactic acid, and sodium
carbonate solution in the order mentioned, then the entire series
was repeated three times in the same order. The possibility of
a change in the oxygen content of the blood, such as might have
been conceived had all the ammonia solutions been done together,
then all the water solutions, etc., was excluded. The results
obtained were, nevertheless, in cc. of gas reduced to 0°, 760 mm.,
from 2 cc. of blood, the following: ammonia, 0.431, 0.431, 0.435, 0.433, average 0.433; water, 0.430, 0.430, 0.430, 0.431, average 0.430; lactic acid, 0.415, 0.408, 0.409, 0.417, average 0.410; carbonate, 0.409, 0.410, 0.410, average 0.410.

The variations might be explainable on the basis of the previously mentioned oxygen reabsorption if the alkaline solutions were regularly lower, but they are not. All that one can conclude at present is that the maximum, or nearly maximum amount of oxygen is yielded by blood treated with ferricyanide in water solution; and that markedly increasing the alkalinity greatly reduces the yield. The amounts of ammonia hitherto used, 0.15

TABLE VI.

Oxygen Contents of a Series of Bloods Determined after Laking in Alkali, Water, and Acid, Respectively.

<table>
<thead>
<tr>
<th>Blood No.</th>
<th>Species</th>
<th>O2 + N2 content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ammonia 0.225 millimol per cc. of blood vol. per cent</td>
</tr>
<tr>
<td>1</td>
<td>Horse ++</td>
<td>17.58</td>
</tr>
<tr>
<td>2</td>
<td>Man ++</td>
<td>20.73</td>
</tr>
<tr>
<td>3</td>
<td>Dog ++++</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>Dog ++</td>
<td>21.5</td>
</tr>
<tr>
<td>5</td>
<td>Man ++</td>
<td>22.2</td>
</tr>
</tbody>
</table>

++ Two agreeing determinations with each reagent.
++++ Four " " " " "

5. Comparison of Results of Oxygen Capacity Determinations by Present Method and Haldane Method.

Comparative determinations were carried out in a series of five bloods by our method as described in this paper, with water to lake the blood, and by Haldane’s recent modification of his method. The results given in Table VII are each the average of
two or more determinations. The results by Haldane's method average 1 volume per cent lower than those by ours. It appears probable that the relatively low results by Haldane's method are due to the difference in the laking solutions used, rather than to the difference in apparatus employed to measure the oxygen evolved, since when instead of water we used 1 per cent Na$_2$CO$_3$ solution (as in the Haldane method) to lake the blood in our own apparatus we encountered similarly low results.

**TABLE VII.**

Oxygen Capacities of a Series of Bloods Determined by the Authors' and by Haldane's Method.

<table>
<thead>
<tr>
<th>No.</th>
<th>Authors' method.</th>
<th>Haldane method.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vol. per cent O$_2$</td>
<td>vol. per cent O$_2$</td>
</tr>
<tr>
<td>1</td>
<td>21.78</td>
<td>19.80</td>
</tr>
<tr>
<td>2</td>
<td>21.84</td>
<td>20.84</td>
</tr>
<tr>
<td>3</td>
<td>19.37</td>
<td>19.62</td>
</tr>
<tr>
<td>4</td>
<td>19.62</td>
<td>18.75</td>
</tr>
<tr>
<td>5</td>
<td>15.29</td>
<td>14.30</td>
</tr>
<tr>
<td></td>
<td>Average.</td>
<td></td>
</tr>
</tbody>
</table>

Determination of Carbon Dioxide in Whole Blood and Plasma.

The following changes have been made in the original methods.

1. **Transfer of Blood into Apparatus.**—The 1 per cent ammonia solution used for washing out the cup of the apparatus before each determination has been dispensed with. Unless kept with especial precautions it gradually absorbs carbon dioxide from the air, and consequently must be continually tested. Otherwise a few drops left in the apparatus may carry enough carbon dioxide to make a measurable plus error in the results.

At present we merely rinse the cup out with water before each determination, and then run into it 1 cc. of distilled water. The blood or plasma is run under this layer of water. Even if the latter has an acid reaction it affords an efficient mechanical hindrance to loss of carbon dioxide from the blood in the short time that the latter is in the cup. A drop of octyl alcohol is

added, and the blood or plasma, followed by the layer of water, is run into the chamber of the apparatus. The layer of water, thus following the blood, serves to wash down the walls of the cup. In the case of whole blood, some corpuscles settle on the bottom of the cup. These are suspended in the water by stirring them up with the last 0.5 cc. of water after the first 0.5 cc. has passed into the chamber.

After the blood or plasma, water, and octyl alcohol in successive layers have run into the apparatus, leaving only the drop of alcohol in the capillary above the cock, the 0.5 cc. of acid is measured into the cup and run through into the chamber.

2. Lactic Acid Instead of Sulfuric for Decomposing the Blood Bicarbonate.—With plasma, sulfuric acid is entirely convenient, but when sulfuric acid is added to whole blood it causes a heavy protein precipitate, which adds some difficulty to accurate reading and subsequent cleaning of the apparatus. L. L. Van Slyke and Baker (1919) encountered a similar difficulty with casein precipitates in using the apparatus to determine carbon dioxide in milk, and obviated it by substituting lactic acid for sulfuric, the lactic holding the protein in solution instead of precipitating it. We have found that a similar advantage is gained by substituting \( N \) lactic acid for \( N \) sulfuric in the analysis of whole blood. The hemoglobin forms a clear burgundy solution with a smooth meniscus to read, and after the analysis is completed the apparatus may be cleaned by simply rinsing with water. Lactic acid is made up with sufficient accuracy for the purpose by diluting 1 volume of concentrated acid (specific gravity 1.20) to 10 volumes with water.

3. Absorption of CO\(_2\) in Analysis of Whole Blood.—For absorption of the CO\(_2\) in the mixture of gases obtained after extraction of whole blood we find 0.5 \( N \) NaOH preferable to the 10 per cent KOH recommended previously. The more dilute alkali is of such light specific gravity that it floats on top of the blood, and makes a clear meniscus for reading the final volume. It should be admitted into the chamber with slight negative pressure, the mercury in the levelling bulb being held a few cm. below that in the chamber. If it is held much lower a slight amount of air may be extracted from the NaOH solution.

4. Measurement of CO₂ without Removal of Blood Solution from the Measuring Chamber.—When the manipulations are properly performed, it is possible to leave out one step in the analysis; viz., running the solution out of the main chamber into the lower bulb before releasing the vacuum. The vacuum may be released and the gas volume read with all the solution in the chamber, and yet without increased absorption of CO₂, if the following procedure is followed. After extraction of the gas the lower cock is opened, admitting the mercury into the extraction chamber rapidly until the meniscus of the water solution reaches the contracted upper portion of the chamber. At this moment the lower cock is partially closed, and the remainder of the mercury is admitted at a rate sufficiently retarded to prevent oscillation of the water column in the calibrated portion of the apparatus, when pressure equilibrium is reached. The pressure is then adjusted by placing the mercury surface in the levelling bulb above the mercury meniscus in the chamber by a height equal to 1/13 that of the water column, in order to balance the latter. After some practice controlled by a centimeter rule, one can estimate this level with the eye to within 2 mm. of mercury, which is sufficiently accurate for many purposes. A more accurate practice, however, is to use the levelling scale described by Stadie (1921) and avoid the possibility of any error whatever in regulating the pressure. When the pressure has been adjusted the lower cock of the apparatus is closed. The gas volume may then be read at leisure.

5. Cleaning Apparatus after Determination of CO₂ in Whole Blood.—When, as in most plasma analyses, the CO₂ is not re-absorbed no washing of the apparatus is necessary before using it for another determination, since the acid solution which wets the walls of the chamber contains a negligible amount of CO₂. When the CO₂ is reabsorbed by alkali solution, however, as in analysis of whole blood, the solution retains all the absorbed CO₂ and must be completely washed out before another analysis is performed. The washing is conveniently carried out as follows: The upper cock of the apparatus is closed, and the levelling bulb dropped to its lowest level. While the mercury in the apparatus is falling, the 6 cc. cup at the top is filled with water. By this time the apparatus is evacuated and nearly all the water from the cup is let in, washing the entire chamber. The water is ejected,
and the rinsing repeated once more, 1 cc. of water being left in
the cup to start the next analysis. Washing the apparatus out
in this manner consumes less than a minute.

6. Correction for CO₂ Reabsorbed After Release of Vacuum.

While the mercury is rising in the apparatus during release of
the vacuum after extraction of CO₂, the layer of water over the
mercury surface has an opportunity to reabsorb some of the
CO₂ gas. In the original method (Van Slyke, 1917) such reab-
sorption was guarded against by withdrawing the water solution
as completely as possible into the bulb below the lower cock
before the vacuum was released. By this expedient the volume
of the water layer left on the mercury was reduced to 0.01 to
0.02 cc. Control analyses with standard solutions of Na₂CO₃
gave theoretical results, so that it seemed that the error due to
reabsorption had been made negligible.

We have found, however, that although small, it is not negli-
gible. The film of water as it rises in the apparatus reabsorbs
1.5 to 2 per cent of the total amount of CO₂ present. The fact
that in the former control analyses 100 per cent instead of 98 to
98.5 per cent of the theoretical amount of CO₂ was obtained
from standard Na₂CO₃ solutions was presumably due to the
presence of a slight excess of CO₂ absorbed from the laboratory
air by the strongly alkaline standard stock solution during the
interval between its preparation and the analyses. Such absorp-
tion may occur more rapidly than at the time was realized.

Concerning the reabsorption of CO₂ in the apparatus after
release of the vacuum, we have ascertained the following.

a. The amount of CO₂ reabsorbed is a constant proportion of
the amount present.

b. The reabsorption occurs almost entirely during the last part
of the compression, while the gas volume is undergoing reduction
from 5 cc. to its final volume.

c. It is independent of the volume of water solution on the
surface of the mercury. The absorption apparently is confined so
nearly to the surface during the short period involved that depth
of layer has relatively slight influence. The volume of water
solution may be varied from 0.10 to 2.5 cc. without varying the
volume of CO₂ reabsorbed.
d. Variations within wide limits (e.g. 5 and 40 seconds) in the period during which the mercury is allowed to rise have no appreciable influence on the volume of CO₂ reabsorbed.

e. The one factor that may markedly increase the proportion of CO₂ reabsorbed, is the manner in which the meniscus of water over mercury is brought to rest at atmospheric pressure in the measuring tube. If, as the meniscus approaches the narrower upper part of the apparatus, its rise is retarded, and the surface is allowed to come gently to the point of equilibrium with no oscillation, a minimum and constant proportion of CO₂ (1.7 per cent of the total) is reabsorbed. On the other hand, if the final rise is permitted to be violent, and the mercury with the water above it in consequence oscillates several times to a distance of several mm. above and below the point corresponding to atmospheric pressure before it comes to rest, 0.01 to 0.02 cc. of CO₂ may be reabsorbed in the process.

Certain of the above points are demonstrated by the following experiments.

**Analyses of Standard Na₂CO₃ Solutions.**—Na₂CO₃ was prepared from Merck's "Reagent" NaHCO₃ by heating for several hours in an oven at 250°C. The Na₂CO₃ thus prepared was kept in bottles with glass stoppers that had been rendered tight by regrinding and greasing.

A 0.03 molecular solution of Na₂CO₃ (3.180 gm. Na₂CO₃ per liter) was prepared by weight, and checked by titrating against 0.1 N HCl. 20 cc. neutralized to methyl orange 11.97 and 12.04 cc. of 0.1 N HCl, after subtraction of 0.10 cc. of 0.1 N HCl required to give the chosen end-point in a control water solution. Calculated 12.00 cc.

The CO₂ content was determined with an apparatus of the usual size (50 cc. chamber, 1 cc. measuring scale), but designed like the micro-apparatus described by Van Slyke (1917, p. 363), so that there was no chance for reabsorption of CO₂ during release of the vacuum. Found, 67.4, 67.0, and 67.2 volumes per cent CO₂. Calculated 67.2. Found = 100.0 per cent of calculated.

The CO₂ content of this solution was redetermined on 1 cc. samples with the ordinary macro-apparatus (Van Slyke, 1917, p. 349) with a single extraction. Found, 65.5, 66.2, 65.9, 66.2,
A 0.015 M solution was prepared by dilution of the above 0.03 M solution with CO₂-free water, and the CO₂ was determined by a single extraction in the same apparatus. Found, 33.1, 33.1, 33.1, and 33.1 volumes per cent of CO₂. Calculated 33.6. Found = 98.5 per cent of calculated.

Direct Determination of Reabsorbed CO₂.—The determinations were carried out in the fine bore macro-type apparatus (Fig. 1) and were performed in the usual manner up to the point at which the vacuum is released. The mercury was allowed to rise to the desired point. At this point the outlet capillary, a, was connected with a suction pump. Then the supernatant atmosphere was freed of CO₂ by applying suction, letting in air, and applying suction again, by revolving the upper cock. This operation was performed in a few seconds, during which the solution in the chamber was kept quiet, in order to avoid reextracting the CO₂ that it held in solution. The air was then driven out through the cup at the top, the solution being driven after it into the cup, where it remained for a few seconds while the mercury seal of outlet, a, was reestablished. The solution was then readmitted into the chamber, and its CO₂ was extracted and measured. The measurements of the small volumes of gas were made after reducing the pressure by 500 mm. of mercury, as described in this paper, and the purity of the CO₂ was controlled by absorption with NaOH. The results are given in Table VIII.

From the preceding analyses of standard carbonate solutions we have shown that one extraction by the usual technique yields 98.3 to 98.5 per cent of the calculated CO₂ volume, while from the analysis, in Table VIII, of the untrapped solution remaining in the chamber we have recovered 1.5 to 2.2, on the average 1.7 per cent of CO₂ reabsorbed during the rise of the mercury to the point of establishment of atmospheric pressure (Determinations 9 to 15). It is therefore demonstrated in two ways that reabsorption diminishes by a quite constant proportion, viz. 1.7 per cent, the amount of CO₂ obtained by the single extraction method used as originally described by Van Slyke. The constancy of the correction justifies its introduction into the calculations by
multiplying the theoretically established factors by \( \frac{1}{0.983} = 1.017 \).

This we have done in Table XIII.

Alternatives to using this correction factor are: (1) To re-extract the untrapped water solution after each analysis, as described in the experiments reported in Table VIII, and (2) to use

**TABLE VIII.**

<table>
<thead>
<tr>
<th>No.</th>
<th>CO(_2) in Na(_2)CO(_3) solution analyzed.</th>
<th>Volume of free space left in chamber over H(_2) when CO(_2) was replaced by air.</th>
<th>Volume of untrapped solution left in chamber.</th>
<th>CO(_2) obtained from 2nd extraction of untrapped solution.</th>
<th>CO(_2) estimated left in untrapped solution after 1st extraction.*</th>
<th>CO(_2) reabsorbed after 1st extraction</th>
<th>(100 \frac{c - d}{a}) per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.672 cc.</td>
<td>50 cc.</td>
<td>0.17 cc.</td>
<td>0.002 cc.</td>
<td>0.002 cc.</td>
<td>0.000 cc.</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.672 cc.</td>
<td>50 cc.</td>
<td>0.25 cc.</td>
<td>0.004 cc.</td>
<td>0.003 cc.</td>
<td>0.001 cc.</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.672 cc.</td>
<td>25 cc.</td>
<td>0.29 cc.</td>
<td>0.004 cc.</td>
<td>0.004 cc.</td>
<td>0.000 cc.</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.672 cc.</td>
<td>5 cc.</td>
<td>0.20 cc.</td>
<td>0.005 cc.</td>
<td>0.003 cc.</td>
<td>0.002 cc.</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>0.672 cc.</td>
<td>5 cc.</td>
<td>2.50 cc.</td>
<td>0.033 cc.</td>
<td>0.030 cc.</td>
<td>0.008 cc.</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>0.672 cc.</td>
<td>2 cc.</td>
<td>0.20 cc.</td>
<td>0.007 cc.</td>
<td>0.002 cc.</td>
<td>0.005 cc.</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>0.672 cc.</td>
<td>1 cc.</td>
<td>0.16 cc.</td>
<td>0.012 cc.</td>
<td>0.002 cc.</td>
<td>0.010 cc.</td>
<td>1.5</td>
</tr>
<tr>
<td>8</td>
<td>0.672 cc.</td>
<td>1 cc.</td>
<td>0.10 cc.</td>
<td>0.012 cc.</td>
<td>0.001 cc.</td>
<td>0.011 cc.</td>
<td>1.6</td>
</tr>
<tr>
<td>9</td>
<td>0.672 cc.</td>
<td>0.78 cc.</td>
<td>0.18 cc.</td>
<td>0.014 cc.</td>
<td>0.003 cc.</td>
<td>0.011 cc.</td>
<td>1.6</td>
</tr>
<tr>
<td>10</td>
<td>0.672 cc.</td>
<td>0.78 cc.</td>
<td>0.20 cc.</td>
<td>0.014 cc.</td>
<td>0.003 cc.</td>
<td>0.011 cc.</td>
<td>1.6</td>
</tr>
<tr>
<td>11</td>
<td>0.672 cc.</td>
<td>0.74 cc.</td>
<td>0.18 cc.</td>
<td>0.013 cc.</td>
<td>0.003 cc.</td>
<td>0.010 cc.</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>0.336 cc.</td>
<td>0.40 cc.</td>
<td>0.10 cc.</td>
<td>0.007 cc.</td>
<td>0.001 cc.</td>
<td>0.006 cc.</td>
<td>1.8</td>
</tr>
<tr>
<td>13</td>
<td>0.336 cc.</td>
<td>0.40 cc.</td>
<td>0.20 cc.</td>
<td>0.009 cc.</td>
<td>0.0016 cc.</td>
<td>0.0084 cc.</td>
<td>2.2</td>
</tr>
<tr>
<td>14</td>
<td>0.336 cc.</td>
<td>0.40 cc.</td>
<td>2.50 cc.</td>
<td>0.024 cc.</td>
<td>0.018 cc.</td>
<td>0.006 cc.</td>
<td>1.8</td>
</tr>
<tr>
<td>15</td>
<td>0.336 cc.</td>
<td>0.40 cc.</td>
<td>2.50 cc.</td>
<td>0.023 cc.</td>
<td>0.018 cc.</td>
<td>0.005 cc.</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Calculated as \( a \frac{\alpha_{CO_2}'}{A + (\alpha_{CO_2}' - 1) S} \)

the micro-apparatus (Van Slyke, 1917) in the original or an enlarged form. In such an apparatus the water solution may be entirely separated from the gas phase before the vacuum is released, and reabsorption of CO\(_2\) therefore made impossible.

We have found, however, that with care to prevent oscillation of the mercury-water layer after atmospheric pressure is reached, such constant results are obtained with the simpler, usual apparatus and technique (see Table I), that we prefer to continue its use, merely introducing the factor 1.017 to correct for the reabsorp-
tion. This correction involves a change of about 1 volume per cent in the results of an ordinary blood or plasma CO₂ determination.

7. Calculation of CO₂ Results.—In deriving the formula by which results were calculated in the original paper (Van Slyke, 1917, p. 358) the factor \( \frac{S}{50} \alpha'_{\text{CO₂}} \) was used to calculate the fraction of the gas remaining dissolved in the water when equilibrium was reached in extraction. \((S = \text{cc. of water solution, } 50 = \text{cc. volume of extraction chamber, } \alpha'_{\text{CO₂}} = \text{cc. of CO₂, measured at the prevailing temperature, dissolved by 1 cc. of water in equilibrium with CO₂ gas at 760 mm. tension.})\) This factor represents an approximation that is entirely exact only when \( \alpha'_{\text{CO₂}} = 1 \), which occurs at 18°. The deviation between the above approximate solubility correction factor and the exact factor increases, (1) as \( \alpha'_{\text{CO₂}} \) becomes greater or less than 1, and (2) as the ratio \( \frac{S}{50} \) increases. For the conditions under which the approximate factor was used, however, (temperature range 15 - 30°, \( \frac{S}{50} = 0.05 \)) the error introduced does not exceed 1 part per 1,000. Consequently the numerical factors arrived at by the original approximate equation do not require correction for the sake of accuracy.

It seems, however, desirable to present the exact general equation which expresses the relationships between gas and liquid phases under conditions such as those prevailing in the apparatus.

\[
\begin{align*}
V_i &= \text{volume of CO₂ obtained by one extraction and measured at atmospheric conditions of } t \degree \text{ temperature and } B \text{ mm. barometric pressure.} \\
V_{0^\circ, 760} &= \text{total volume of CO₂, reduced to } 0^\circ, 760 \text{ mm., in the solution analyzed.} \\
S &= \text{volume of water solution in apparatus.} \\
A &= \text{volume of chamber occupied by gas and solution during extraction (50 cc. in our apparatus).} \\
A - S &= \text{volume of gas phase during extraction.} \\
T &= \text{absolute temperature, } = t + 273. \\
\alpha_{\text{CO₂}} &= \text{solubility coefficient of CO₂ in water, the cc. of CO₂ measured at } 0^\circ, 760 \text{ mm., dissolved by 1 cc. of water in equilibrium with CO₂ under 760 mm. tension.}
\end{align*}
\]
\[ \alpha'_{CO_2} = \alpha_{CO_2} \times \frac{T}{273} \] is the distribution coefficient of CO₂ between gas and water = cc. of CO₂ measured at \( T^\circ \), B mm. dissolved by 1 cc. of water in equilibrium with pure CO₂ at \( T^\circ \), B mm. (\( \alpha'_{CO_2} \) was referred to as \( \alpha_{CO_2} \) in the original paper.)

\( w = \) vapor tension of water.

\( p = \) partial pressure of CO₂ in apparatus when equilibrium is reached in the extraction.

\( x' = \) volume of CO₂ gas, measured at \( 0^\circ, 760 \) mm., held in solution when equilibrium is reached.

The total CO₂ content of the solution analyzed is obtained by reducing the volume of CO₂ extracted to standard conditions by multiplication with the usual factor \( \frac{(B - w) 273}{760 T} \), and adding to the volume thus corrected the volume \( x \) of CO₂ remaining in solution. Thus:

\[ V_{0', 760} = V_t \frac{B - w}{760} \times \frac{273}{T} + x \]  

Since the volume of gas dissolved is proportional to its partial pressure, solubility, and the volume of the solvent, we have

\[ x = \frac{p}{760} S_{CO_2} \]  

Since pressure varies inversely as gas volume

\[ \frac{p}{B - w} = \frac{V}{A - S} \quad \text{or} \]  

\[ p = (B - w) \frac{V}{A - S} \]

Substituting this value for \( p \) in (2), and the value thereby found for \( x \) in (1), we have

\[ V_{0', 760} = V_t \frac{B - w}{760} \times \frac{273}{T} + V \frac{B - w}{760} \times \frac{S_{CO_2}}{A - S} \quad \text{or} \]  

\[ V_{0', 760} = V_t \frac{B - w}{760} \left( \frac{273}{T} + \frac{S_{CO_2}}{A - S} \right) \]
Since \( \alpha_{\text{CO}_2} = \alpha'_{\text{CO}_2} \times \frac{273}{T} \), equation (6) may be expressed as

\[
V^{o, 760} = V_i \frac{B - w}{760} \times \frac{273}{T} \left( 1 + \frac{S}{A - S} \alpha'_{\text{CO}_2} \right), \text{ or}
\]

\[
V^{o, 760} = V_i \frac{B - w}{760 (1 + 0.00367 t)} \left( 1 + \frac{S}{A - S} \alpha'_{\text{CO}_2} \right)
\]

Factor correcting for atmospheric pressure and temperature. Factor correcting for unextracted CO\(_2\).

In the approximate equation used in the original paper the factor correcting for unextracted CO\(_2\) was

\[
1 - \frac{S}{A - S} \alpha_{\text{CO}_2}
\]

\( \alpha'_{\text{CO}_2} = 1 \) (at 18\(^\circ\)) this becomes identical with 

\[
1 + \frac{S}{A - S} \alpha'_{\text{CO}_2}
\]

both factors then reducing to \( \frac{A}{A - S} \).

The values of the combined factor \( \frac{B - w}{760 (1 + 0.00367 t)} \left( 1 + \frac{S}{A - S} \alpha'_{\text{CO}_2} \right) \), multiplied by 1.017 to correct for reabsorption of CO\(_2\) during release of the vacuum, are given in Table XIII at the end of this paper for the calculation of results. Expressing \( \frac{B - w}{760 (1 + 0.00367 t)} \) as \( f \), we have given its values for \( B = 760 \), to be multiplied by \( \frac{B}{760} \) for other barometric pressures. Because the vapor tension, \( w \), is not quite the same fraction of \( B - w \) when \( B \) is other than 760, this usage is not absolutely exact. At a barometric pressure \( B = 740 \) it introduces an error of plus 0.1 per cent, at 700 mm., an error of plus 0.4 per cent. For barometric pressures outside the range 740 to 780 mm., therefore, one must use the customary tables for values of \( \frac{B - w}{760 (1 + 0.00367 t)} \) in order to avoid errors exceeding 1 part per 1,000. For work at ordinary altitudes, however, the factors as given in Table XIII are sufficiently exact.
Determination of Carbon Monoxide.

In the method of Van Slyke and Salvesen (1919) it appears slightly more accurate to use 1.36 volumes per cent as the correction for nitrogen gas rather than the 1.2 volumes per cent correction found by the above authors. Still more accurate results may be obtainable by absorption of the CO with ammoniacal, cuprous chloride solution as recently described by O'Brien and Parker (1921).

In the determination of carbon monoxide it is advisable to use the finer bore apparatus and to magnify the gas volumes by reducing the pressure as described at the beginning of this paper, since the amounts of gas measured are likely to be much smaller than those measured when carbon dioxide or oxygen is determined.

The blood should be trapped in the lower bulb of the apparatus before releasing the vacuum, as it is undesirable to mix blood with pyrogallol and cuprous chloride.

Determination of Methemoglobin.

In the determination of methemoglobin (Stadie, 1920) the total blood pigment is determined colorimetrically, and the methemoglobin is estimated by subtracting from the total pigment the oxyhemoglobin. The latter is determined by the oxygen capacity method. The oxygen capacity portion of the methemoglobin determination therefore requires revision in its method of calculation, as outlined above. Instead of utilizing Table I of Stadie's paper the oxygen capacity of the blood is determined and calculated as described above, to make complete allowance for the nitrogen gas content of the blood. The volume per cent of oxygen is then multiplied by 0.746, the number of gm. of hemoglobin that combines with 1 cc. of oxygen. The result is the number of gm. of hemoglobin per 100 cc. of blood.

The change in the mode of calculation affords sufficient increase in accuracy to be justified, but the error involved in the former method is too slight to invalidate any results that have been obtained by it. The absolute error is 0.5 volume per cent in oxygen capacity (equivalent to 0.37 gm. of hemoglobin per 100 cc. of blood) or about 2.5 per cent of the amount normally present. In the methemoglobin calculation this error is partly neutralized by the fact that the same percentage error is intro-
duced into the colorimetric estimation of total pigment by making the standard methemoglobin solution from blood in which the hemoglobin had been estimated by the same oxygen capacity method. For example, in Table IX the model calculation given on page 240 of Stadie's paper (1920) is reproduced together with the calculation as corrected in this paper. It is seen that the change in methemoglobin calculated is but 0.1 gm. per 100 cc. of blood.

**TABLE IX.**

**Methemoglobin Calculation.**

<table>
<thead>
<tr>
<th>Hemoglobin strength of standard blood calculated from oxygen capacity</th>
<th>Calculated as described in this paper.</th>
<th>Calculated as described in original paper (Stadie, 1920).</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>Hemoglobin determined by oxygen capacity</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Total blood pigment colorimetrically determined = $\frac{1}{2}$ of standard</td>
<td>10.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Hemoglobin determined by difference</td>
<td>12.5</td>
<td>12.2</td>
</tr>
<tr>
<td>Hemoglobin per 100 cc. of blood</td>
<td>15.0</td>
<td>14.6</td>
</tr>
</tbody>
</table>

**Determination of All the Gases in One Blood Sample.**

Both carbon dioxide and oxygen, as well as the residual nitrogen, may be determined in one sample of blood with but little more expenditure of time and effort than is required to determine any one of the gases alone. For liberation of the gases both acid and ferricyanide are added to the blood in the apparatus, and all the gases are extracted and measured together. The CO$_2$ is then absorbed by dilute alkali, leaving O$_2$ and N$_2$. The O$_2$ may then be determined by absorption with pyrogallol, or estimated by subtracting the average N$_2$ content of blood from the sum of O$_2$ + N$_2$.

The essential point in determining the gases together was found to lie in the use of proper amounts of the reagents employed to set free the carbon dioxide and oxygen, particularly in the use of a minimum amount of acid. If potassium ferricyanide and a large excess of acid are added to blood, both oxygen and carbon dioxide are quantitatively freed, but the ferricyanide forms such a heavy precipitate with the blood proteins that it is inconven-
ient, although possible, to handle the resulting suspension in the apparatus. The precipitation of the proteins by ferricyanide occurs, however, only when the reaction is strongly acid. If but the minimum amount of lactic acid necessary to free the CO₂ with certainty is added, the ferricyanide-protein precipitate formed is so small in amount and so finely divided that it does not interfere with the determination. Furthermore, the acid can be trapped in the lower bulb of the apparatus before the blood is admitted, and can then be mixed with the blood after the apparatus has been evacuated. The blood is then in the lower part of the evacuation chamber, and such precipitate as does form does not touch the measuring tube at the top, which remains clean and clear. The precipitate, unlike the black deposit formed by action of ferricyanide on the mercury in the ammoniacal solution used in the original oxygen determination, is instantly soluble in 0.1 N alkali. Consequently any particles that adhere to the walls of the apparatus are removed as a rule by a single washing with dilute alkali solution.

The minimum amount of acid that will entirely free the CO₂ of whole blood for extraction under the conditions of our analyses was determined by preliminary experiments, some of which are recorded in Table X. The amount needed was found to be 1.0 cc. of 0.1 N lactic acid for 1 cc. of whole blood. Half as much fails to set the CO₂ completely free.

The minimum amount of ferricyanide required in the presence of acid is the same as in ammoniacal solution; viz., 5 mg. of potassium ferricyanide per 1 cc. of normal blood (Table II). In avoiding protein precipitation, however, the amount of ferricyanide present appears to be of much less importance than the

<table>
<thead>
<tr>
<th>Lactic acid added to 1 cc. of blood.</th>
<th>CO₂ found in blood.</th>
</tr>
</thead>
<tbody>
<tr>
<td>millimols</td>
<td>vol. per cent</td>
</tr>
<tr>
<td>0.05</td>
<td>41.5</td>
</tr>
<tr>
<td>0.10</td>
<td>52.8</td>
</tr>
<tr>
<td>0.10</td>
<td>53.3</td>
</tr>
<tr>
<td>0.20</td>
<td>52.8</td>
</tr>
</tbody>
</table>
acidity of the mixture; if the acidity is too high, the minimum necessary amount of ferricyanide causes an inconvenient precipitate, while if the acidity is not too high an excess of ferricyanide may be used without such inconvenience. We consequently employ 10 mg. of ferricyanide per cc. of blood, twice the necessary amount.

The analysis is performed as follows:

Apparatus Used.—In order to obtain with 1 cc. of blood results for carbon dioxide and oxygen accurate to within 1 per cent of the amounts measured, it is desirable to use the fine bore type of apparatus described at the beginning of this paper, although, with care, results sufficiently accurate for many purposes may be obtained with the ordinary apparatus.

Details of Determination.—First 2 cc. of 0.05 N lactic acid are admitted into the chamber of the apparatus. The acid is freed of air by evacuation and shaking in the usual manner. The air-free acid is then trapped in the bulb below the lower cock of the apparatus, and 1.9 cc. of water, with a drop of octyl alcohol, are similarly introduced and freed of air. The blood is now stirred and a sample drawn into an Ostwald pipette calibrated between two marks to deliver 1 cc. About 1.5 cc. of the air-free water in the gas analysis apparatus are run into the cup at the top, and the blood sample is at once run beneath it. We usually slightly open the cock below the cup while the pipette is draining, so that most of the blood flows directly into the chamber of the apparatus, the layer in the cup at no time being more than 2 to 3 mm. deep. The layer of water, even though it be somewhat acidified, prevents, because of the relatively slow rate of diffusion through it, the loss of CO₂ from the blood. All the blood, followed by the water layer above it, is now admitted into the chamber of the apparatus. When about half the water layer has been run in, the half remaining in the cup is stirred with a rod in order to get into suspension a few corpuscles that have lodged on the bottom of the cup. 0.05 cc. of solution containing 20 gm. of potassium ferricyanide per 100 cc. is added to this last portion of water, which is then admitted into the chamber. The 0.05 cc. of ferricyanide solution may be conveniently measured as 1 drop from a pipette, which has been found by trial to deliver thus 0.05 to 0.06 cc. of the solution.
The chamber is evacuated until the mercury has fallen to the 50 cc. mark. The 2 cc. of 0.05 N lactic acid trapped in the lower bulb of the apparatus are now admitted and mixed with the rest of the solution. At this moment a small amount of brown precipitate forms, but not enough to interfere with the subsequent manipulations. The oxygen and carbon dioxide (and carbon monoxide if present) are extracted by whirling the solution about the wall of the evacuated chamber until, when the vacuum is released and the gas measured, no increase in volume is observed. Complete extraction is usually attained in 1 minute when the apparatus is shaken by hand. When the mechanical shaker devised by Stadie (1921) has been used, 2 minutes shaking after the first evacuation, followed by 30 seconds shaking for the check evacuations, has been our usual routine. As a rule no increase occurs after the first evacuation. Increase after the second indicates a leak in the apparatus, due usually to improper lubrication of the upper cock.

### TABLE XI.

*Comparison of Carbon Dioxide and Oxygen Contents of Normal Venous Blood Determined Separately by Former Methods, and Together by the Present Combined Method, Respectively.*

Blood kept under paraffin oil. All determinations made within 3 hours after blood was drawn.

<table>
<thead>
<tr>
<th>Determination</th>
<th>Method</th>
<th>Volume of blood for analysis</th>
<th>CO₂</th>
<th>O₂ + N₂</th>
<th>O₂</th>
<th>Potassium ferricyanide used</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ alone.</td>
<td>Van Slyke, 1918.</td>
<td>2 cc.</td>
<td>21.1</td>
<td>19.6</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>O₂ “</td>
<td>Van Slyke, 1918.</td>
<td>2 cc.</td>
<td>20.9</td>
<td>19.4</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Van Slyke, 1917.</td>
<td>1 cc.</td>
<td>65.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Van Slyke, 1917.</td>
<td>1 cc.</td>
<td>65.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Present</td>
<td>2 cc.</td>
<td>64.4</td>
<td>20.5</td>
<td>19.0</td>
<td>10</td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Present</td>
<td>2 cc.</td>
<td>65.0</td>
<td>20.9</td>
<td>19.4</td>
<td>10</td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Present</td>
<td>2 cc.</td>
<td>63.5</td>
<td>20.9</td>
<td>19.4</td>
<td>15</td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Present</td>
<td>2 cc.</td>
<td>64.4</td>
<td>20.9</td>
<td>19.4</td>
<td>30</td>
</tr>
<tr>
<td>O₂ + Co₂ “</td>
<td>Present</td>
<td>2 cc.</td>
<td>64.2</td>
<td>20.9</td>
<td>19.4</td>
<td>70</td>
</tr>
</tbody>
</table>

* The N₂ was determined and found to be 1.5 volumes per cent. It is deducted and subtracted from the O₂ + N₂ to estimate the O₂.
When extraction of the gases is complete the water solution is trapped below the lower cock, as in the original CO\textsubscript{2} determination, and the gases are measured at atmospheric pressure.

The gases measured are CO\textsubscript{2}, O\textsubscript{2}, N\textsubscript{2}, and sometimes CO. The CO\textsubscript{2} is absorbed by running 0.5 N NaOH from the cup down the side of the measuring tube until no further shrinkage of the gas volume occurs; about 0.5 cc. of the hydroxide solution suffices.

**TABLE XII.**

Comparison of Carbon Dioxide and Oxygen Contents Determined Separately by Former Methods, and Together by the Present Combined Method.

All determinations made on 1 cc. samples in a fine bore apparatus.

<table>
<thead>
<tr>
<th></th>
<th>Oxygen.</th>
<th>CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vol. per cent</td>
<td>vol. per cent</td>
</tr>
<tr>
<td>Blood aerated with alveolar air.............</td>
<td>18.1</td>
<td>17.9</td>
</tr>
<tr>
<td>&quot; diluted with saline......................</td>
<td>0.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Venous blood about 60 per cent satu-.........</td>
<td>13.6</td>
<td>13.9</td>
</tr>
<tr>
<td>rated with O\textsubscript{2}..............</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02025 M Na\textsubscript{2}CO\textsubscript{3} (calculated 58.8 volumes per cent CO\textsubscript{2})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As the volume, \( S \), of solution in the apparatus is twice as great as in the method as originally described, the factors used for calculation of carbon dioxide are different. For the present conditions, with 5 cc. of total water solution extracted in the apparatus, the factors are given in Table XIII.

If no CO is present, the residual mixture left after absorption of CO\textsubscript{2} may be measured as O\textsubscript{2} + N\textsubscript{2}, and from the O\textsubscript{2} + N\textsubscript{2} content reduced to 0\textdegree, 760 mm. (by Table XIII), the average N\textsubscript{2} content of blood, viz. 1.36 volumes per cent, may be subtracted in order to estimate the oxygen; or the oxygen may be measured directly by absorption, as described by Van Slyke and Salvesen. In the latter case about 0.5 cc. of pyrogallol solution is run in from the cup, and is followed by a little water to clean the tube and give a clear meniscus for reading.
<table>
<thead>
<tr>
<th>Temperature</th>
<th>$f = \frac{B - t}{760 (1 + 0.00367 t)}$</th>
<th>Air, measured at room temperature and pressure, dissolved by $\alpha' \text{co}_2$ factor by which the volume of CO$_2$ obtained after 1 extraction is multiplied in order to obtain the volume of CO$_2$, reduced to 0°, 760 mm., contained in the solution analyzed.</th>
<th>2.5 cc. H$_2$O</th>
<th>5 cc. H$_2$O</th>
<th>$S = 2.5 \text{ cc.}$</th>
<th>$S = 5.0 \text{ cc.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C.</td>
<td>$0.932 \times \frac{B}{760}$</td>
<td>$1.075 \times 0.052 \times 0.105$</td>
<td>$1.002 \times \frac{B}{760}$</td>
<td>$1.061 \times \frac{B}{760}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.928</td>
<td>1.043</td>
<td>0.995</td>
<td>1.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.924</td>
<td>1.015</td>
<td>0.989</td>
<td>1.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.919</td>
<td>0.989</td>
<td>0.983</td>
<td>1.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.915</td>
<td>0.966</td>
<td>0.978</td>
<td>1.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.910</td>
<td>0.942</td>
<td>0.972</td>
<td>1.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.906</td>
<td>0.919</td>
<td>0.966</td>
<td>1.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.901</td>
<td>0.896</td>
<td>0.960</td>
<td>1.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0.897</td>
<td>0.873</td>
<td>0.954</td>
<td>1.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0.892</td>
<td>0.850</td>
<td>0.948</td>
<td>0.993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.888</td>
<td>0.828</td>
<td>0.942</td>
<td>0.986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.883</td>
<td>0.808</td>
<td>0.936</td>
<td>0.978</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0.878</td>
<td>0.789</td>
<td>0.931</td>
<td>0.971</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0.873</td>
<td>0.772</td>
<td>0.924</td>
<td>0.964</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.868</td>
<td>0.755</td>
<td>0.918</td>
<td>0.957</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>0.863</td>
<td>0.738</td>
<td>0.912</td>
<td>0.950</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.863</td>
<td>0.738</td>
<td>0.912</td>
<td>0.950</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* To calculate O$_2$ or hemoglobin when O$_2$ + N$_2$ volume is measured, multiply gas volume by $f$, to reduce to 0°, 760 mm., and by such factor as is necessary (100 when 1 cc. of blood is used, 50 when 2 cc. are used) to bring results to volume per cent basis. Then for:

a. O$_2$ content, subtract...... 1.36 vol. per cent N$_2$

b. O$_2$ bound by hemoglobin
   in venous blood,
   subtract............... 1.5 " " " N$_2$ + dissolved O$_2$

c. O$_2$ bound by hemoglobin
   in arterial blood,
   subtract............... 1.7 " " " " " " "

d. O$_2$ bound by hemoglobin
   in blood saturated
   with air at 20°,
   subtract............ 2.1 " " " " " " "

...continued...
TABLE XIII—Concluded.

Per cent of normal hemoglobin (Haldane scale) = \( \frac{100}{18.5} d = 5.41 d \).

Grams of hemoglobin per 100 cc. of blood = 0.746 \( d \).

Per cent of total hemoglobin saturated with \( O_2 = \frac{100 b}{d} \) or \( \frac{100 c}{d} \).

Volumes per cent \( O_2 \) unsaturation = \( d - c \) or \( d - b \).

\( b \) and \( c \) may be determined with slightly greater accuracy with the aid of Table IV. The values for \( f \) given in the second column are for barometric readings corrected for temperature (see remarks on p. 31). The values for \( \alpha'_\text{co}_2 \) are obtained by multiplying by \( 1 + 0.00367 t \) the values for \( \alpha'_\text{co}_2 \) given by Bohr and Bock (1891).

† The dissolved air is given as measured at room temperature. It is subtracted from the air + \( \text{CO}_2 \) volume, measured after one extraction of plasma or aqueous carbonate solution, in order to obtain the \( \text{CO}_2 \), which is then multiplied by 1.017 \( f \left( 1 + \frac{S}{50} \alpha'_\text{co}_2 \right) \) in order to obtain the total volumes per cent of \( \text{CO}_2 \) in the solution analyzed. When whole blood is analyzed, the air correction cannot be used, because of the \( O_2 \) present, and the \( \text{CO}_2 \) must be determined by absorption with \( \text{NaOH} \) solution. The volume of gas absorbed is then multiplied by the above factor.

The factor 1.017, being empirical (see p. 27), may vary slightly for different apparatus.

If carbon monoxide is present, the oxygen must, of course, be absorbed by pyrogallol. The residual gas is the \( \text{CO} + 1.36 \) volumes per cent of \( \text{N}_2 \). The \( \text{CO} \) is calculated by reducing the mixture of \( \text{CO} \) and \( \text{N}_2 \) to \( 0^\circ, 760 \text{ mm.} \) (Table XIII), and subtracting 1.36 from the result in volumes per cent, or the \( \text{CO} \) may be absorbed by cuprous chloride (O’Brien and Parker, 1921).

The results of some determinations are given in Table XI and XII.

Examples of Calculations.

1. Total Oxygen Content of Venous or Arterial Blood.

a. From \( \text{O}_2 \) and \( \text{N}_2 \) Measured Together.

Blood sample.......... 2.00 cc.
\( \text{O}_2 + \text{N}_2 \) measured..... 0.405 " at \( 25^\circ, 750 \text{ mm.} \)

\[
0.405 \times 0.888^* \times \frac{750}{760} = \text{O}_2 + \text{N}_2 \quad \text{2.00 cc.} \]

\[0.3534^* \times 50 = \text{O}_2 + \text{N}_2 \text{ per 100 cc. blood} \]

\[
\text{N}_2 \text{ subtracted} \quad 1.36 \quad \text{at} \quad 0^\circ, 760 \text{ "} \]

\[
\text{O}_2 \text{ by difference} \quad 16.37 \quad \text{at} \quad 0^\circ, 760 \text{ "} \]

* Factor from second column of Table XIII.
b. From O₂ Determined by Absorption with Pyrogallol.

Blood sample........... 2.00 cc.
O₂ + N₂ measured...... 0.405 * 25°, 750 mm.
N₂ after absorption
of O₂.................. 0.031 * 25°, 750 mm.
O₂ by difference...... 0.374 * 25°, 750 mm.

0.374 \times 50 = O₂ per 100 cc. blood. 18.70 * 25°, 750 mm

18.70 \times 0.888^* \times \frac{750}{760} = O₂ " 100 " " 16.37 " " 0°, 760 "

2. Oxygen Combined with Hemoglobin in Venous or Arterial Blood as Drawn.

a. Venous Blood.

Total O₂, calculated as above................. 16.37 cc. at 0°, 760 mm.
O₂ in physical solution....................... 0.10† " 0°, 760 "
O₂ combined with hemoglobin............... 16.27 " 0°, 760 "

b. Arterial Blood.

Total O₂, calculated as above................. 16.37 cc. at 0°, 760 mm.
O₂ in physical solution....................... 0.32† " 0°, 760 "
O₂ combined with hemoglobin............... 16.05 " 0°, 760 "

3. Oxygen Combined with Hemoglobin in Blood Saturated with Air at Room Temperature (Oxygen Capacity).

a. From O₂ and N₂ Measured Together.

Blood sample........... 2.00 cc.
O₂ + N₂ measured...... 0.495 * 20°, 767 mm.

0.495 \times 0.910^* \times \frac{767}{760} = O₂ + N₂ .............. 0.455 " 0°, 760 "

50 \times 0.455 = O₂ + N₂ per 100 cc.

Physically dissolved O₂ + N₂ per 100 cc.

blood.................... 22.75 " 0°, 760 "

Combined O₂ per 100 cc. blood........... 20.65 " 0°, 760 "

b. From O₂ Determined by Absorption with Pyrogallol.

Blood sample........... 2.00 cc.
O₂ + N₂ measured...... 0.495 * 20°, 767 mm.
N₂ after absorption
of O₂.................... 0.033 * 20°, 767 mm.
O₂ by difference...... 0.462 " 20°, 767 "

* Factor from second column of Table XIII.
† These figures are approximate and are the average for arterial and venous bloods. For the accurate values of oxygen in physical solution see Table III.
D. D. Van Slyke and W. C. Stadie

\[ 0.462 \times 0.910^* \times \frac{767}{760} \times 50 = \text{Total O}_2 \text{ per 100 cc. blood.} \]

Physically dissolved:
\[ \text{O}_2 \text{ per 100 cc. blood.} = 0.58 \times 0^\circ, 760 \text{ mm.} \]
Combined \text{O}_2 \text{ per 100 cc. blood.} = 20.64 \times 0^\circ, 760 \text{ mm.}

4. Calculation of Hemoglobin Content from Oxygen Capacity.

Combined \text{O}_2 \text{ per 100 cc. blood, calculated as above, 20.64 cc. at 0\(^\circ\), 760 mm.}

\[ 20.64 \times 0.746 = \text{hemoglobin per 100 cc. blood.} \]
\[ = 15.40 \text{ gm. hemoglobin.} \]

20.64 18 \times 100 = \text{per cent of Haldane's average normal hemoglobin.} = 112 \text{ per cent.}


Oxygen combined with hemoglobin per 100 cc. of blood after saturation of blood with air at room temperature (oxygen capacity) (Calculation 3, b) = 20.64 cc. at 0\(^\circ\), 760 mm.

Oxygen combined with hemoglobin of blood as drawn (Calculation 2, a) = 16.27 " " 0\(^\circ\), 760 "

\[ \text{Oxygen unsaturation} = \frac{4.37}{20.64} \times 100 = 21.2 \text{ per cent.} \]

6. Calculation of Total Gas Content from Analysis of 1 Cc. of Blood as Described in This Paper.

Blood sample = 1.00 cc.

a. \text{CO}_2 + \text{O}_2 + \text{N}_2 \text{ measured.} = 0.845 " at 25\(^\circ\), 757 mm.
b. \text{O}_2 + \text{N}_2 \text{ measured after CO}_2 \text{ absorption.} = 0.248 " 25\(^\circ\), 757 "
c. \text{CO}_2 \text{ by difference (a - b).} = 0.597 " 25\(^\circ\), 757 "
\text{O}_2 \text{ by difference (b - c).} = 0.231 " 25\(^\circ\), 757 "

\[ 0.597 \times 0.986^\dagger \times \frac{757}{760} \times 100 = \text{CO}_2 \text{ per 100 cc. blood.} \]
\[ = 58.6 \times 0^\circ, 760 \text{ "} \]
\[ 0.231 \times 0.888 \times \frac{757}{760} \times 100 = \text{O}_2 \text{ per 100 cc. blood.} \]
\[ = 20.4 \times 0^\circ, 760 \text{ "} \]
\[ 0.017 \times 0.888 \times \frac{757}{760} \times 100 = \text{N}_2 \text{ per 100 cc. blood.} \]
\[ = 1.5 \times 0^\circ, 760 \text{ "} \]

\[ ^* \text{Factor from second column of Table XIII.} \]
\[ ^\dagger \text{Factor from last column of Table XIII.} \]
BIBLIOGRAPHY.

Bohr, C., and Henriques, V., Arch. physiol., 1897, ix, series 5, 819.
Haldane, J. S., J. Path. and Bact., 1920, xxiii, 443.
Lundsgaard, C., J. Biol. Chem., 1918, xxxiii, 133.
Stadie, W. C., J. Biol. Chem., 1920, xli, 237; 1921, xlix, 43.
THE DETERMINATION OF THE GASES OF THE BLOOD
Donald D. Van Slyke and William C. Stadie

Access the most updated version of this article at http://www.jbc.org/content/49/1/1.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/49/1/1.citation.full.html#ref-list-1