A SIMPLE, ACCURATE METHOD OF ESTIMATING CARBON MONOXIDE IN BLOOD

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There are two chief manometric methods for estimating CO in blood; viz., that due to Van Slyke and Neill (4) and that due to Sendroy and Liu (3). In both of these the first stage is the liberation of all the O₂, CO₂, CO, and N₂ of the blood by shaking in vacuo with acid ferricyanide. In the Van Slyke-Neill method the O₂ and CO₂ are then absorbed with alkaline hyposulfite, leaving a residue of CO and N₂. The amount of N₂ is calculated from its solubility in blood and thence by subtraction the CO content is obtained. Owing to the blank correction for dissolved N₂ and also for the slight reabsorption of CO by the reduced hemoglobin, which is formed after the addition of the hyposulfite, the method is only accurate to 0.2 volume per cent CO, but this is good enough for many purposes.

In the Sendroy-Liu method the extracted gas is transferred to a Hempel pipette containing alkaline pyrogallol and the O₂ and CO₂ therein are absorbed. The gas is then returned to the usual Van Slyke-Neill chamber and the CO estimated by absorption with cuprous chloride. The method is more difficult and much more elaborate than the Van Slyke-Neill method, but gives as high an accuracy as is ever likely to be needed.

The method now to be described is as simple as the Van Slyke-Neill method but approaches the Sendroy-Liu method in accuracy. It has a further advantage, not possessed by any previous method; namely, that it can be used in Barcroft differential manometers as well as in the Van Slyke-Neill manometers, and so may be of service in laboratories which happen to be better equipped with the former.
Determination of Blood CO

CO Estimation in Van Slyke-Neill Manometer

Principle of Method—The blood is laked and shaken in the Van Slyke-Neill chamber with a sodium glycinate-hyposulfite (pH about 10) mixture in the dark for 3 minutes. The O\textsubscript{2} and CO\textsubscript{2} remain in solution chemically bound, but practically all the dissolved N\textsubscript{2} is extracted into the vacuum. The affinity of CO for reduced hemoglobin is, under these conditions, so high that no appreciable amount is normally liberated.\textsuperscript{1} The extracted N\textsubscript{2} is quantitatively ejected and excess K\textsubscript{3}FeC\textsubscript{y} drawn into the chamber, which is then shaken until the whole of the COHb is dissociated. The only gas evolved is CO, which can therefore be measured accurately without use of cuprous chloride or other special absorbing reagents. The manipulative procedure is thus exceptionally easy.

Reagents Required—

Saponin solution. 1 gm. of Merck's saponin is dissolved in 100 cc. of water.

Glycinate-hyposulfite solution. This is a 1.0 M solution of glycine in 0.9 M NaOH, containing 2 per cent hyposulfite. 50 cc. of it are enough for a day's work and are prepared freshly as follows: 3.75 gm. of glycine (analytical reagent) are dissolved in 45 cc. of carbonate-free 1 N NaOH in a 50 cc. stoppered cylinder. 1 gm. of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} is added, the stopper inverted, and the solid dissolved with minimal shaking. The volume is then made up to 50 cc. and the solution at once transferred to a burette under paraffin oil. It should only be used on the day of preparation.

Potassium ferricyanide solution, 32 per cent. This solution is deaerated in a vacuous Barcroft tonometer rather than in the Van Slyke-Neill chamber, since strong K\textsubscript{3}FeC\textsubscript{y} oxidizes mercury rather readily. The solution is stored in a burette.

\textsuperscript{1} The following considerations indicate that no significant proportion of CO from the COHb will escape into the gas phase during the 3 minute extraction. At least an hour's shaking would be needed for equilibrium between the CO in the liquid and gas phases and even then inspection of the COHb dissociation curve shows that the volume of CO liberated into the gas phase should not exceed 0.5 c.mm., if the percentage saturation of the hemoglobin lies within the range for which the present method is intended; \textit{viz.}, 0 to 40 per cent. In 3 minutes the amount of CO liberated should thus be utterly negligible.
Procedure

4 drops of octyl alcohol are drawn into the Van Slyke-Neill chamber and 2 cc. of saponin solution are then placed in the cup. 2 cc. of the blood are then drawn into the chamber, followed by the 2 cc. of saponin. The blood and saponin are mixed in the chamber, and, after 1 minute for laking to be complete, 1.5 cc. of the glycinate-hyposulfite solution are drawn into the chamber which is then covered by black paper (or tin-foil). The mercury is lowered to the 50 cc. mark and the mixed solution shaken in vacuo for 3 minutes. The black paper is then removed and the extracted \( N_2 \) is quantitatively ejected. 0.5 cc. of deaerated ferricyanide is drawn into the chamber, the mercury lowered to the 50 cc. mark, and the chamber shaken for 10 minutes. The pressure is measured at the 2.0 or 0.5 cc. mark. The solution is then reevacuated and shaken for a further 5 minute period to check that the end-point has been reached (pressure reading \( p_2 \)). This is so if \( p_2 - p_1 \geq 0.3 \) mm. of Hg, as should be the case if the temperature is 22° or over.

The gas is then quantitatively ejected and a final pressure reading taken, \( p_3 \).

The CO content of the blood = \( (p_3 - p_2 - c) \times \text{constant} \).

The \( c \) correction is obtained by a blank with water in place of blood. It should not exceed 0.5 mm. of Hg, being mainly due to dissolved \( N_2 \) which is not quite completely extracted during the first shaking. The value of the constant, correct to within 0.1 per cent, is given by the most right-hand column of Table 30 of Peters and Van Slyke (2). Thus at 23° the constant = 0.1215, and if \( (p_3 - p_2 - c) = 15 \) mm. of Hg, the CO content = 1.83 volumes per cent. The whole procedure takes about 30 minutes, but in nearly half of this time the observer is free to undertake some other task. At temperatures below 20° longer shaking periods are necessary, owing to the high temperature coefficient of the rate of dissociation of COHb. The method has only been used over the range 0 to 30 per cent COHb saturation, since this is of most practical importance in blood volume determinations and in estimations of relatively small degrees of CO poisoning. It gives equally good results with human, pig, and ox blood, and in the presence of heparin or oxalate as anticoagulant. It works
also with defibrinated blood with or without 1 per cent boric acid as preservative.

Accuracy—The maximum discrepancy between duplicate values of \((p_3 - p_2 - c)\) was found to be 0.6 mm. of Hg, while the average was 0.4 mm. of Hg. The latter is near to the limits of accuracy of the Van Slyke-Neill apparatus, and with 2 cc. blood samples corresponds to a figure of 0.05 volume per cent CO or to 0.25 per cent saturation with CO. This precision is of the same order as is claimed in the Sendroy-Liu method, and is as great as is needed for practical purposes. For this reason no tests have yet been made with 5 cc. blood samples, though presumably the same proportionate increase in precision would be obtained as is found in the Sendroy-Liu method.

A further check on the accuracy of the method is provided by the comparisons with other methods which are summarized in Table II. This will be discussed later.

**CO Estimation in Barcroft Manometer**

**Principle of Method and Reagents Required**—The principle is exactly the same as in the Van Slyke-Neill manometer except that the CO is liberated into a gas phase consisting of \(N_2\) at atmospheric pressure instead of into a vacuum. The only additional solution required is 1 per cent \(NaCl\), the other reagents being identical with those used in the Van Slyke method, though there is no need in this case to deaerate the ferricyanide. A tank of compressed \(N_2\) and a 5 liter pressure-equalizing bottle are also required.

**Procedure**

2 cc. of saponin solution and 2 cc. of 1 per cent \(NaCl\) are run into the left-hand bottle, Bottle L, of the Barcroft apparatus; then 2 cc. of saponin solution and 2 cc. of the blood sample are run into Bottle R, the right-hand bottle. Bottle R is then mixed by two quick shakes, no appreciable COHb dissociation being caused thereby. 1.5 cc. of the glycine-hyposulfite solution are next run below the surface of the liquid in Bottle L, and then similarly in Bottle R. A Keilin dangling tube containing 0.5 cc. of 32 per cent ferricyanide is then loaded into Bottles L and R respectively and the two bottles are at once fixed to the Barcroft manometer, the
taps and joints of which must be thoroughly gas-tight. The manometer is connected to a water filter pump and the whole apparatus evacuated to 0.1 atmosphere and washed thrice with \( N_2 \) gas. Finally the bottles and their connections to the gage are left filled with \( N_2 \) at atmospheric pressure. The manometer is then at once mounted in a bath and shaken at about 120 round trips per minute, temperature 20-25\(^\circ\). The whole filling process should take about 6 minutes and care should be taken not to expose the hyposulfite unduly to the air, nor to allow the \( N_2 \) to enter the manometers too suddenly after the pressure in them has been reduced, as otherwise the gage liquid may be blown over. After 10 to 15 minutes shaking, the gage readings generally become steady, temperature equilibration and absorption of traces of \( O_2 \) in the commercial \( N_2 \) both being complete in this time. The manometer is then quickly lifted out of its stand, and the Keilin cups dislodged by a sharp tap so that the ferricyanide is mixed with the solutions in each bottle. The manometer is at once returned to the shaker and shaking continued until there is no further evolution of gas. The time required for this is usually 25 to 30 minutes at 22\(^\circ\). The CO content of the blood is then calculated with the aid of the constant of the apparatus determined by the mercury weighing method as described by Dixon (1). Table I shows a typical protocol of quadruplicate determinations on a sample of ox blood containing 15 per cent COHb.

Each pair of columns gives the readings of the left and right limbs of the manometer, the serial number of which is at the head. The letter \( K \) shows the stage at which the contents of the Keilin cups are shaken into the blood. In the case of Manometer BR the shaking was continued for 45 minutes after the end-point had been reached, in order to make sure there was no drift. In a few cases shaking was continued as long as 24 hours, and even then drifts of no more than 1.0 mm. were observed, which might very well be attributed to decomposition of the solutions or to very slight leakage. The only instance in which a satisfactory end-point was not obtained was when the hyposulfite used appeared to contain some sulfide. Here the results were worthless, for much less than the expected volume of CO was evolved, and then followed a steady absorption of some gas. Care must therefore
be taken to use a good sample of \( \text{Na}_2\text{S}_2\text{O}_4 \). That used in the present work was supplied by The British Drug Houses, Ltd., and the solid was kept out of contact with air as far as possible.

**Table I**  
*Barcroft Method for Estimating CO in Blood*

A 2 cc. sample of defibrinated ox blood treated with 1 per cent boric acid as preservative.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Manometer BR</th>
<th>Manometer B55</th>
<th>Manometer B54</th>
<th>Manometer B66</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>0</td>
<td>101.2</td>
<td>100.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>101.5</td>
<td>100.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>101.8</td>
<td>100.7</td>
<td>100.9</td>
<td>100.9</td>
</tr>
<tr>
<td>15</td>
<td>101.9</td>
<td>100.7</td>
<td>100.8</td>
<td>101.7</td>
</tr>
</tbody>
</table>

\( K^* \)

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Manometer BR</th>
<th>Manometer B55</th>
<th>Manometer B54</th>
<th>Manometer B66</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>20</td>
<td>93.9</td>
<td>117.0</td>
<td>100.6</td>
<td>101.3</td>
</tr>
</tbody>
</table>

**Average** = 2.90 volumes per cent.

CO content by Van Slyke method = 2.91 volumes per cent.

*\( K^* \) represents the stage at which the contents of the Keilin cups are shaken into the blood.
The method has been tested under the same range of conditions as the Van Slyke method (see above).

Accuracy and Applicability—In some thirty duplicates, there was only one case in which the discrepancy was greater than 0.2 volume per cent CO (i.e., 1 per cent COHb saturation). The average discrepancy was 0.12 volume per cent CO (i.e., 0.6 per cent COHb). The method is thus only about one-third as accurate as the Van Slyke method described above. Since no Barcroft method has hitherto been available for CO estimations in blood, the present one was thoroughly tested against the Van Slyke method of this paper. The maximum discrepancy between these two methods was 0.14 volume per cent CO (= 0.7 per cent COHb), and the average discrepancy 0.06 volume per cent CO (= 0.3 per cent COHb). The mean of several Barcroft determinations on one sample agrees extraordinarily closely with the results obtained by the Van Slyke method (Table II).

The time taken for a single determination is 45 to 50 minutes, but most of this time is spent in shaking; in a series of determinations the method is much more expeditious, for as Table I shows it is possible to load the contents of the Barcroft apparatus into the shaker at the rate of one per 10 minutes and to complete ten determinations within a space of 2 hours. The Barcroft method is thus amply accurate for measuring small amounts of CO in the blood of subjects whose occupation renders them liable to CO poisoning. It is indeed particularly suitable for application on a large scale, since the contents of twelve or more Barcroft manometers can easily be shaken simultaneously and data on a large number of individuals subject to a given condition can thus be quickly and readily secured. For blood volume determinations with small amounts of CO it is perhaps, at best, only just exact enough, though even here it offers one compensatory advantage; namely, that if the blood from the subject before breathing CO is placed in Bottle L (in place of 1 per cent NaCl) and the blood after breathing CO is placed in Bottle R, the manometer reading gives directly the increase in CO content of the blood as a result of inspiring the measured volume of CO. With the Van Slyke methods two separate CO determinations are necessary, and the CO increase of the blood obtained therefrom by subtracting the first from the second thus leads to some loss in accuracy.
Determination of Blood CO

The success of the method with the Barcroft manometers suggests that it should be equally feasible with the Warburg manometric technique, but owing to lack of time this has not yet been tried.

Comparison with Other Methods—Table II summarizes the comparisons of the methods of the present paper with each other and with previous methods. The results are expressed in volumes per cent of CO.

In the mixture method 4.7 cc. of CO-saturated blood were mixed with 32.6 cc. of aerated blood, and the CO content of the mixture calculated from the combining capacity of the blood and the CO content of the aerated blood.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Comparison of Volumes Per Cent of CO by Present and Previous Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Slyke method of this paper</td>
<td>Barcroft method of this paper</td>
</tr>
<tr>
<td>2.08(1)</td>
<td>2.05(9)</td>
</tr>
<tr>
<td>2.90(1)</td>
<td>2.90(4)</td>
</tr>
<tr>
<td>3.09(1)</td>
<td>3.08(7)</td>
</tr>
<tr>
<td>4.50(1)</td>
<td>4.60(1)</td>
</tr>
<tr>
<td>4.68(1)</td>
<td>4.82(1)</td>
</tr>
<tr>
<td>4.52(1)</td>
<td>4.58(1)</td>
</tr>
<tr>
<td>2.66(1)</td>
<td>2.25(1)</td>
</tr>
<tr>
<td>7.10</td>
<td></td>
</tr>
</tbody>
</table>

The figures in parentheses represent the number of determinations.
* This sample of blood was first freed of dissolved N2 before being treated with CO, thus eliminating the uncertainty in the Van Slyke-Neill method due to the correction for dissolved N2.

The results of the present paper not only agree satisfactorily with one another, but also, within experimental error, with the results of the previous methods.

SUMMARY

Normally blood contains four gases; viz., O2, CO2, N2, and CO. The principle of the method is to bind the O2 and CO2 by mixing the blood solutions with an alkaline glycinate-hyposulfite mixture, and at the same time to evolve the N2 into the gas phase. The only remaining gas in solution is then CO, which is subsequently liberated by shaking with neutral ferricyanide solution. The
technique can be carried out both in the Van Slyke-Neill apparatus and in the Barcroft differential manometer. The two apparatus give extremely concordant results both with each other and with alternative methods of estimating CO in blood.

In the Van Slyke-Neill apparatus the method gives results of the same order of precision as the Sendroy-Liu method (viz. ±0.025 volume per cent of CO with a 2 cc. blood sample); it is, however, very much simpler, being indeed easier than the Van Slyke-Neill method, since no special gas-absorbing agents are required.

The results with the Barcroft apparatus are not quite so accurate, but this technique is especially convenient when a large number of estimations are required in the course of 1 day.

My thanks are due to Dr. M. Dixon and Professor D. Keilin for help with, and loan of, the Barcroft apparatus. I am also grateful to Professor C. Lovatt Evans and to Professor H. C. Bazett for courtesies extended to me in their respective laboratories.

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