The manometric technique of Van Slyke and Neill (1) for the combined
determination of oxygen and carbon dioxide in blood cannot be applied
without modifications if ethyl ether in anesthetic concentrations is present
in the blood. Part of the ether introduced with the blood, or serum, into
the chamber of the Van Slyke apparatus will vaporize and add to the vol-
ume of extracted gases. If the amount of ether in the gas phase remained
unchanged throughout the procedure, an interference with the correct
blood gas analysis might not be encountered. Owing to the high solubility
coefficient of ether in water, a considerable portion of the extracted ether
is reabsorbed when the sodium hydroxide solution is added for the absorp-
tion of carbon dioxide, and is absent in the final measurement (2). This
reabsorption of ether, according to Austin (2), may result in values up to
15 per cent in excess of the true carbon dioxide values. Shaw and Downing (3)
reported errors of a similar magnitude in oxygen determinations in
the presence of ether.

Austin (2) introduced a modification of the Van Slyke-Neill procedure
for the determination of carbon dioxide in serum in the presence of ether.
Essentially, his modification consists of reextraction of the alkaline solu-
tion after absorption of carbon dioxide, and the use of an empirical correc-
tion factor to compensate for the increase of ether in the gas phase over
the alkaline solution. Fuss and Derra (4) applied Austin’s principle to
the simultaneous determination of blood oxygen and carbon dioxide in the
presence of ether. They provided no experimental data to prove the
validity of their procedure. Shaw and Downing (3) were unable to obtain
correct values for oxygen, using the modification of Fuss and Derra.
Shaw and Downing (3) described another modification of the Van Slyke-
Neill technique for the determination of blood oxygen in the presence of
ether. Their method requires the use of a modified Hempel pipette to
which the extracted gases are temporarily transferred, allowing for the
cleaning of the chamber of the ether-containing blood-reagent mixture.
The method produces satisfactory results, and with the use of a correction.
factor is practically free from systematic errors; it is, however, time-con-
suming and cumbersome. Whether the technique of Shaw and Downing
can also be used for the determination of carbon dioxide, or for the com-
bined determination of oxygen and carbon dioxide, has not been reported.

A simple modification of the Van Slyke-Neill technique for the combined
determination of oxygen and carbon dioxide in the presence of ether was
needed in an investigation of the effects of controlled breathing anesthesia
on the blood oxygen saturation and carbon dioxide tensions of anesthetized
patients.1 The concentration of ether in the blood of patients anesthetized
when using the Crafoord (5) or Mautz (6) machines for controlled respi-
ration was found never to exceed 0.7 mg. per ml. in any of the twenty-three
patients studied. The modification which is described below is directly
applicable to blood containing ether in concentrations not exceeding 0.7
mg. per ml.

Method

The reagents and apparatus are the same as the ones used in the un-
modified procedure of Van Slyke and Neill (1, 7). The Van Slyke-Neill
procedure is followed without changes up to the completion of the gas ex-
traction and the taking of the first reading, p1. The sodium hydroxide is
then added, the cup sealed, and the mercury in the chamber lowered to the
50 ml. mark, and the chamber shaken for 1 minute, timed with a stop-
watch. A reading, p2 (I), is then taken. Immediately thereafter the mer-
curry is again lowered to the 50 ml. mark, the chamber is shaken for another
minute, and another reading, p2 (II), is taken. The value for p2 cor-
rected is obtained by using the formula, p2 corrected equals p2 (II) +
(p2 (I) - p2 (II)). The carbon dioxide content is calculated by means of
the formula (p1 - p2 cor. - c) × f. The values c and f are the same as those
used in the ordinary Van Slyke-Neill procedure. The oxygen absorbent
is then introduced and the solution shaken for 2 minutes under lowered
pressure. Reading p3 is taken. The oxygen content is calculated accord-
ing to the formula (p2 cor. - p3 - c) × f.

EXPERIMENTAL

On repeated extractions of the blood-reagent mixture after the addition
of sodium hydroxide, successively lower manometric readings are ob-
tained (Table I). Van Slyke and Stadie (8) noted, in connection with
the alkaline ferricyanide methods of oxygen determination used by earlier
workers, that the alkaline reaction favored some oxidative processes by
which part of the oxygen freed is slowly consumed. Hence, lower values
for oxygen were obtained with these methods. Reducing substances in

1 Stayman, J. W., Jr., Gibbon, J. H., Jr., and Allbritten, F. F., Jr., unpublished.
blood corpuscles (9) and plasma (10) were held responsible for the loss of oxygen in the alkaline ferricyanide methods. Yet this explanation could not account for lower oxygen capacity values of fresh hemoglobin solutions found with Haldane’s alkaline ferricyanide method, as compared with values produced by the Van Slyke technique (11).

In the course of the present investigation it appeared that the oxygen consumption that apparently occurs when the alkaline blood-reagent mixture is shaken is at least to a large extent caused by the oxidation of caprylic alcohol and also saponin by ferricyanide. This oxidation apparently involves elementary oxygen. When a mixture of ferricyanide solution, sodium hydroxide, a few drops of caprylic alcohol, and air was admitted into the chamber of the Van Slyke apparatus, successively lower

<table>
<thead>
<tr>
<th>Reading No.</th>
<th>Time of shaking</th>
<th>Manometric reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before addition of NaOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>305.5</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>305.5</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>186.5</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>184.0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>182.2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>180.0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>178.5</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>177.8</td>
</tr>
</tbody>
</table>

In Table I, manometric readings were obtained on repeated extractions (Table II). The mixture was then collected in a test-tube and a few drops of ferric chloride solution were added. The typical color of ferric ferrocyanide appeared, proof of the presence in solution of (potassium) ferrocyanide. Less, but still significant, oxygen consumption was observed when saponin, in amounts used for a single blood gas determination, was shaken with an alkaline ferricyanide solution. The addition of ferric chloride to the ferricyanide solution before shaking with caprylic alcohol, or saponin, did not produce the Prussian blue reaction. The oxygen consumption that occurred on shaking the Van Slyke reagents, including sodium hydroxide, with air, was comparable in magnitude to the oxygen consumption of the alkaline blood-reagent mixture. No oxygen consumption occurred when caprylic alcohol, or saponin, was shaken with ferricyanide in neutral or acid solution.

Austin, who introduced the principle of reextraction, used it only for
carbon dioxide determinations in which no potassium ferricyanide is employed. If reextraction is used for oxygen determinations, compensation has to be made for the oxygen consumption occurring during the period of shaking after the addition of sodium hydroxide. Such compensation has apparently been neglected by Fuss and Derra (4). The rate of oxidation can, for practical purposes, be assumed to remain constant within the two 1 minute shaking periods employed here. (A slight decrease in the oxidation rate actually occurs as the oxidizable substances are being used up.) Therefore, the difference in the readings, $p_2 (\text{m}) - p_2 (\text{r})$, is added to $p_2 (\text{r})$ in order to obtain the hypothetical reading $p_2$ that would have been obtained without reextraction if no reabsorption of ether occurred. No correction for the $p_3$ reading taken after 2 minutes shaking need be applied.

### Results

Twenty experiments, involving over 120 gas determinations, were performed to test the validity of the method. The procedure in these test experiments was as follows: 30 ml. of blood were aerated for 20 minutes. 10 ml. samples of the aerated blood were transferred, with minimum exposure, to each of two oiled 10 ml. Luer lock syringes, to one of which ethyl ether in amounts ranging between 3 and 30 mg. was added with a micro pipette. The syringes were immediately capped with soldered needle hubs and kept on ice until analysis. A drop of mercury was added to facilitate proper mixing of the blood. Oxygen and carbon dioxide determinations were performed, as a rule in triplicate, on the ether-free control samples by the Van Slyke-Neill technique, and on the ether-containing samples ("ether" samples) with the modification here described. At the conclusion of each experiment, the hematocrit of both samples was

<table>
<thead>
<tr>
<th>Reading No.</th>
<th>Manometric reading (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before addition of NaOH</td>
<td>1 327.8, 2 327.8, 3 324.0, 4 320.1, 5 317.1, 6 313.5</td>
</tr>
<tr>
<td>After addition of 1 ml. 1 N NaOH</td>
<td></td>
</tr>
</tbody>
</table>
determined as a check of the relative identity of the samples. Generally good agreement between the gas content of the control and "ether" samples was found, when the concentration of ether did not exceed 0.7 mg. per ml. The deviation from the control values in these instances was always

### TABLE III

Comparison of Oxygen and Carbon Dioxide Values Found in Two Samples of Same Blood, One of Which Contained 0.6 Mg. of Ethyl Ether per Ml.

The determinations were performed in triplicate with the Van Slyke-Neill technique on a control sample, with the authors' modified technique on the "ether" sample.

<table>
<thead>
<tr>
<th>Manometric readings</th>
<th>CO\textsubscript{2} content\textsuperscript{c}</th>
<th>O\textsubscript{2} content\textsuperscript{c}</th>
<th>Manometric readings</th>
<th>CO\textsubscript{2} content\textsuperscript{c}</th>
<th>O\textsubscript{2} content\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p_1) 202.1</td>
<td>32.4</td>
<td>8.25</td>
<td>(p_1) 214.1</td>
<td>57.8</td>
<td>8.27</td>
</tr>
<tr>
<td>(p_2) 169.7</td>
<td>57.6</td>
<td>13.68</td>
<td>(p_2) (cor.) 180.6</td>
<td>8.23</td>
<td>13.72</td>
</tr>
<tr>
<td>(p_3) 112.1</td>
<td></td>
<td></td>
<td>(p_3) (cor.) 180.2</td>
<td>57.8</td>
<td>13.72</td>
</tr>
</tbody>
</table>

Within the accepted limits of error of the Van Slyke-Neill technique; \textit{i.e.}, 0.2 volume per cent for oxygen and 0.5 volume per cent for carbon dioxide, but remained generally much lower. In ten experiments in which the ether concentration ranged between 0.3 and 0.7 mg. per ml. the mean difference between values found in the control and "ether" samples was \(-0.03\) volume per cent for oxygen and \(-0.29\) volume per cent for carbon dioxide. In almost all individual experiments the mean deviations be-
DETERMINATION OF \(O_2\) AND \(CO_2\)

tween control and "ether" values were lower than the maximum deviations on triplicate determination of the same sample. Table III shows the result of a typical experiment. The error in carbon dioxide values, if calculated in per cent, would appear to be excessively high because the carbon dioxide content of the aerated blood was very low. Proof was obtained, however, that the magnitude of the error depends mainly on the concentration of ether and not on the concentration of blood gases. The direction of the error was toward lower rather than true values for both oxygen and carbon dioxide. This is due to the higher solubility of ether in acid solution than in alkaline, as explained by Austin (2). No correction was used, since the error with low anesthetic concentrations as encountered in our clinical investigation was negligible. With higher concentrations of ether, Austin's correction formula could probably be used, but was not tried here. The method has been successfully used in the previously mentioned clinical study on twenty-three patients. Duplicate checks are easily obtained if proper care is exerted, consisting mainly in accurate timing of the extractions and smooth and even timed reducing of the gas volume after each extraction.

SUMMARY

A modification of the Van Slyke-Neill technique for the combined determination of blood oxygen and carbon dioxide, to be used in the presence of ethyl ether, is described. Data are presented to prove the validity of the method for low anesthetic concentrations of ether.

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