The Use of Carbon Monoxide and T-1824 for Determining Blood Volume

L. G. Myhre,* D. K. Brown,* F. G. Hall,† and D. B. Dill

Blood volume was determined with both T-1824 (Evans blue) and carbon monoxide (CO) for 2 young women, 2 young men, and 2 men ages 70 and 75. In the CO method a precise volume of CO was administered from a closed system, and the CO concentrations in blood were determined on the Van Slyke-Neill apparatus before and at the end of a 10-min. rebreathing period. The manometric technic is presented in detail and possible sources of errors are discussed. Comparisons were made during the spring in Indiana, in the hot desert, and at an altitude of 3800 m. Blood volume was taken as the sum of plasma volume (by T-1824 method) and red cell volume (by CO method); from these values body hematocrit was calculated. The ratios of body hematocrit to observed hematocrit ranged from 0.89 to 1.05 in 16 observations, with a median value of 0.93. In 12 of the 16, they ranged from 0.89 to 0.95, also with a median value of 0.93. Blood volumes determined by the CO method, using the 0.93 ratio, were within −1 to +4% of the blood volumes as determined above in 12 of 16 subjects. The other four differed by −7 to −13%.

Pugh’s experience on Mount Everest with the carbon monoxide (CO) method for determining blood volume proved its value: under primitive conditions in a laboratory at 15,000 ft., he successfully analyzed blood samples collected at much greater altitudes (2). The principle of the method is simple: a measured volume of CO is inhaled from a closed system, and the concentration in the blood is determined on samples drawn before and 10 min. after starting the rebreathing procedure. The quantity of CO used produces no subjective symptoms; daily determinations can be made without ill effects under conditions that militate against intravenous procedures. We needed such a method in studies

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of adaptation to high altitude of men and women with an age range of 20–75 years. After acquiring the necessary equipment and technics, we decided to validate the CO method against the method using T-1824 (Evans blue). Such a validation had not been made at high altitude.

**Methods**

The apparatus used for inhaling a precise volume of CO is illustrated in Fig. 1. It was designed for use either at barometric pressures of 690–760 mm. Hg or at about 585 mm. Hg. The two water-jacketed vessels were calibrated to contain about 100 and 200 ml., the latter for use at high altitudes. The subjects, the environments, and the schedule

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**Fig. 1. Apparatus for administering precise volume of CO.**

A. To source of CO—C. P. grade, 99.5%, Mathieson; No. 3 cylinder fitted with needle valve. B. to waste bag for collecting CO used for flushing connection. C. To source of oxygen—cylinder fitted with needle valve. D. Water-jacketed, calibrated CO vessel containing about 100 ml. E. Second CO vessel containing about 200 ml.; thermometer extends into each jacket. F. Leveling bulb—250 ml. with Tygon tubing and T-tube permitting connection to either CO vessel. G. Soda lime vessel—about 250 ml. H1. Copper elbow, 18 mm. I.D., with a side outlet, 5 mm. I.D., to connect with Tygon tubing to sources of O2 and CO. H2. Copper elbow, 18 mm. I.D. I. Anesthesia bag, capacity about 4 L. J1 and J2. One-way valves (Collins P-304). K. Two-way breathing valve with mouthpiece outlet and side outlet (Collins P-323). Except for mercury line, glass stopcocks and connecting glass tubes have bore of about 1 mm.; nonkinkable plastic tubing, 1/8 in. I.D. (Collins P-500) is used beyond soda lime container; molded ends (Collins P-503) are cemented to plastic tubing.
of exercise tests have been described (2). The two methods for blood volume were compared on 5 subjects at Bloomington, Ind.—near sea level in the spring; on 6 subjects, on eight occasions in the summer in the desert; and on 3 subjects at high altitude. The subjects were always recumbent and fasting. The first blood sample was drawn into a heparinized syringe of 12-ml capacity. A part was used for determining control CO concentration; the remainder was centrifuged for hemato-
crit level and T-1824 concentration in plasma. The subject then breathed through the closed system for 10 min. after introduction of the CO. Then, while he was still breathing through the system, a second blood sample was obtained for the same observations. The T-1824 method for plasma volume offers no problem, but the determination of CO in blood on the Van Slyke-Neill apparatus has many pitfalls. As others have had similar experiences, our procedure is described in detail. It is based on the procedure described by Peters and Van Slyke (3), a version of the Horvath-Roughton method (4) including changes subsequently recommended by Roughton.

Reagents

Sodium dithionite, powdered, Na₂S₂O₄ (also called sodium hyposulfite, sodium hydrosulfite, and lycopon)  Caution: this reagent deteriorates if exposed to moist air; keep tightly covered.

Potassium hydroxide solution, 0.1 N  A 1-L. stock solution is kept in a rubber-stoppered Pyrex bottle. Each day 40 ml. is rendered air-
free by extraction in the Van Slyke apparatus. It is transferred to a
50-ml. buret containing about 5 ml. of paraffin oil. Attached to the
buret is a rubber tube closed with a pinch clamp and a glass tube fitted with a rubber tip (AHT* 8203C). This permits transfer of the air-free solution from the extraction chamber to the buret.

Saponin-borax solution  Saponin, 1 gm., and borax (Na₁₂B₄O₇·10H₂O), 3 gm., are dissolved in 100 ml. of water. This and the following
two reagents are stored in 200-ml. glass-stoppered Pyrex bottles.

Potassium ferricyanide solution  K₃Fe(CN)₆, 32 gm., is dissolved in hot water, cooled to room temperature, made up to 100 ml., and
shaken to stabilize its content of dissolved gases.

Acetate buffer, pH about 6.0  Sodium acetate (NaC₂H₄O₂·3H₂O), 75 gm., is dissolved in 100 ml. of water; 15 ml. glacial acetic acid is added. This solution also is shaken to stabilize its content of dissolved gases.

Capryl alcohol (2-octanol)  Dispense from a dropping bottle.

Distilled water Stored in a wide-mouth 2-L. bottle on top of the Van Slyke apparatus; a siphon glass tube leads to a pinch-clamp closure fitted with a glass tube with a tip long enough to reach into the cup of the extraction chamber.

Apparatus

Van Slyke-Neil manometric apparatus
Ostwald-Van Slyke pipets, 2 ml. (AHT 8203A)
Glass rods, 3 × 120 mm.
Glass or plastic spoon This should hold 35 ± 5 mg. sodium dithionite powder.
Ostwald-Folin pipets; 1, 3, and 4 ml.; to deliver (AHT 8200)
Vacuum source with interposed safety bottle and suction line running to cup of extraction chamber

Procedure

1. With the extraction chamber and the bores of the stopcock filled with mercury, add three drops of capryl alcohol to the cup; introduce most of it. Follow with a few drops of mercury in the cup and introduce 2 ml. of blood using the Ostwald-Van Slyke pipet with rubber tip. This step is best done by using the lower stopcock.

2. Cover the extraction chamber with aluminum foil and add 4 ml. saponin-borate to the cup, followed by about 35 mg. dithionite, using the calibrated spoon; mix with a glass rod quickly; introduce all but one or two drops of the mixture. Seal with mercury and rinse the cup thoroughly with distilled water. Evacuate until mercury is at the 50-ml. mark; shake 3 min. to absorb oxygen and free dissolved nitrogen. Release the vacuum smoothly and eject the nitrogen bubble without loss of solution, as follows: First close the stopcock leading to the leveling bulb when the gas pressure in the chamber is slightly above atmospheric. Place the leveling bulb in the upper ring and open the cock at the top of the chamber. Open the lower stopcock cautiously until the solution from the chamber has filled the capillary above the cock. Guard against nitrogen bubbles that sometimes are trapped below the mercury in the cup.

3. To extract CO and CO₂, use pipets to add to the cup: 1 ml. acetate buffer and 3 ml. 32% ferricyanide; stir with a glass rod. Draw the blood down until the gas volume is 5 or 6 ml. Admit 1.5 ml. of the acetate-ferricyanide solution, seal with mercury, and rinse the cup thoroughly with distilled water. Lower the mercury level 1 cm. below the 50-ml. mark. Shake vigorously 2 min., then stop long enough to run the mercury
up 1 cm. above the mark to ensure complete mixing. Lower the mercury to the same level as before and shake 2 min. more, *vigorously*. Admit mercury from the leveling bulb until the gas volume is reduced to 5 or 6 ml. Add 3 ml. of air-free 0.1 N KOH to the cup. Introduce 1.0 ml. during 1 min. Release the vacuum and add another 0.5 ml. KOH. Seal and draw gently below the 2-ml. mark. Allow 1 min. for drainage, note the jacket temperature, adjust precisely to 2.0 ml., and read: this is $P_1$. Preferably, reading is done with a light shining away from the extraction chamber onto a white background.

4. Eject the gas, as in Step 2. Do not seal with mercury, since this involves the hazard of reintroducing gas. Gently evacuate below the 2-ml. mark and, after drainage, adjust to 2 ml. and read. This is $P_2$.

**Calculation**

Table 30 (p. 325) of Peters and Van Slyke (3) is used to calculate from $(P_1 - P_2)$ the volumes of CO per 100 ml. In blood volume determination two samples obtained as described above are analyzed. The initial determination includes the $c$ correction and the little CO that is always present. A separate $c$ correction with water is unnecessary. In these determinations one requires only the difference, $\Delta$ CO, between the CO contents of the final and initial blood samples, so the $c$ correction is not employed except when one is interested in the amount of CO in the control sample. The CO inhaled, milliliters at STPD (5), divided by CO in milliliters per liter of blood gives the volume of blood in liters, uncorrected for extravascular loss.

**Comment**

The most frustrating aspect of the procedure is apt to be the cleaning of the chamber between analyses. The following method is recommended: After ejecting the residue to the waste bottle, admit about 10 ml. of 0.1 N KOH, lower to about 5 or 6 ml. in the gas phase, shake *slowly* 1 min., and eject. Admit about 6 ml. of 0.1 N KOH and about 15 ml. of water. Lower the mercury slightly below the 50-ml. mark, shake *vigorously* for 1 min., and eject. Admit 20–30 ml. of water, lower the mercury slightly below the 50-ml. mark, and shake vigorously for 1 min. Stop shaking and, with the stopcock open, raise the water to about the 5- or 6-ml. level, shake *slowly* for another minute, and eject. Rinse the chamber with about 5 ml. of 0.1 N lactic acid plus about 20 ml. of water. Eject and rinse finally with distilled water. The chamber is then ready for the next analysis. The stopcock bores are sealed with mercury, the chamber is evacuated, and the mercury allowed to rise slowly. The residual water is ejected, and the bores sealed. At the end
Table 1. Plasma Volume Using T = 1824; Red Cell Volume Using CO

<table>
<thead>
<tr>
<th>Col. 1</th>
<th>Col. 2</th>
<th>Col. 3</th>
<th>Col. 4 Plasma vol. by T = 1824 (ml.)</th>
<th>Col. 5 RBC vol. by CO using Hct (ml.)</th>
<th>Col. 6 Blood vol. Col. 4 + Col. 5 (ml.)</th>
<th>Col. 7 Hct</th>
<th>Col. 8 Hct</th>
<th>Col. 9 Ht ratio Col. 7 + Col. 8</th>
<th>Col. 10 Blood vol. by CO using Hgb (ml.)</th>
<th>Col. 11 Col. 2 − Col. 10 + 100</th>
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<td>0.421</td>
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<td>2310</td>
<td>5610</td>
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<td>BAR</td>
<td>3120</td>
<td>2320</td>
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<td>0.449</td>
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* BLM indicates Bloomington, Ind.; BC, Boulder City (desert), and BAR, 3800 m. above sea level.
of the day clean as above and fill with 45% (w/v) urea solution. By morning the remaining coagulum is loosened and can be washed out. It is important not to shake vigorously when the chamber is nearly filled with mercury. The strain can break the chamber.

What analytic error is to be expected? The method involves the same analytic procedure on the two blood samples, one before and another after administering CO. Hence the maximum error will not often exceed twice the mean difference between duplicate analyses. In 60 duplicate analyses in 1966 the mean blood CO concentration was 2.81 ± a mean difference of 0.03 volume per 100 ml. In the last 16 analyses of that year, the mean difference was 0.02. There was continued improvement: In 1967–1968 the mean difference in 25 duplicate analyses was 0.015 volume per 100 ml. We estimate that our error in blood volume arising from analytic errors does not exceed 3% with present technics. To illustrate the errors which can be expected, on March 30 the blood volume of one of us (D.B.D.) was 5230 ml. as determined by CO. An error of ±0.03 volume per 100 ml. in the analysis of blood CO corresponds to an error of less than ±2% in blood volume. Some errors may have been twice that in 1966, but certainly this did not happen often. The average error in blood volume attributable to an analytic error probably was not more than 2%.

Results

Sixteen comparisons of the two methods are found in Table 1. Plasma volume by the T-1824 method plus red cell volume by the CO method, using the venous hematocrit (Hct), resulted in the blood volume (Table 1, Column 6). No correction was made for extravascular loss of CO or for trapped plasma. Red cell volume divided by blood volume, as defined above, gave body hematocrit (Table 1, Column 7). The ratios of observed venous hematocrit to the calculated body hematocrit are shown in Table 1, Column 9.

Discussion

There are other reliable methods for determining blood volume with CO. Pugh (1), at 15,000 ft., used the Scholander-Roughton technic for blood analysis (6). A method for estimating total circulating hemoglobin used by Swedish investigators has been used by Cardus et al. (7). It depends on measuring the pCO and pO2 in air equilibrated in vivo with oxygenated blood after the subject has rebreathed air containing a high pO2 and a known amount of CO. The CO concentration in the air is measured with an infrared analyzer. Root et al. (8), using
a combination of the Scholander-Roughton microgasometric technic (6) with the Horvath-Roughton modification of the Van Slyke technic (6), reported a mean difference between duplicates of 0.02 volumes per 100 ml. We decided to use the Horvath-Roughton method chiefly because we had had much experience with the Van Slyke-Neill apparatus in other analyses. Evidently, Root et al. had learned many refinements of technic that were not fully described in their original report. Eventually we learned most of these same technics and decided to save time for others by describing the procedure in detail.

Only brief reference will be made to the many pertinent studies, partly because the discussion of the problem by Root et al. (8) is so comprehensive. Also Lawson's excellent review (9) covers the literature up to 1964.

The ratio of body hematocrit to venous hematocrit found by Root et al. ranged from 0.90 to 1.08. Lawson reported a consensus that the ratio lies between 0.89 and 0.94. Our own ratios ranged from 0.89 to 1.05, but 12 of the 16 ranged from 0.89 to 0.95. The median value was 0.93 for the set of 12 values, as well as for all 16. On the strength of our evidence we now employ the ratio 0.93 for calculating blood volume by the CO method.

Blood volumes calculated by the CO method using the observed venous hematocrit multiplied by 0.93 are shown in Table 1, Column 10. The results are not surprising: since 12 of the 16 ratios found were within the range 0.89-0.95, there must be close agreement between blood volume calculated in the two ways, from red blood cell (RBC) volume by the CO method using the venous hematocrit plus plasma volume by the T-1824 method, and from RBC volume by the CO method using the venous hematocrit multiplied by 0.93. In the 12 cases the differences ranged from -1 to +4%.

The four ratios ranging from 1.0 to 1.05 deserve attention. Two of these are for one of us (F.G.H.)—the only two comparisons made for him. Other measurements for him using CO and a later measurement using 18I gave higher plasma volumes and much higher red cell volumes than on the two occasions when T-1824 was used. The blood volumes on those two occasions were about 15% less than on other occasions. In this case there seems to have been errors in both methods when they were used simultaneously. A third high ratio was for L.G.M.; here also there seem to have been errors with both methods, giving too low a plasma volume with T-1824 and too high an RBC volume with CO. There was a difference of 1700 ml. in blood volume. This evidence leads us to believe that at least three of our four high ratios were erroneous.

Our findings convince us that the CO method is valid for assessing
changes in red cell and plasma volumes in the same individual as environmental conditions change. However, one must admit doubt about the absolute value for RBC volume determined by the CO method. Most investigators find that cells tagged with labeled chromium or iron indicate a red cell volume about 15% greater than the CO method. Then, in studies of CO production, distribution, and metabolism in the body, Coburn and Luomanmäki (10) report that in dogs about 23% of body CO is extravascular. The apparent discrepancy awaits resolution.

References