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The Determination of Small Amounts of Carbon Monoxide in Air

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by

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Vita

Edward J. Fitzsimons was born in Chicago, Illinois, July 31, 1918.

He was graduated from Saint Ita Grammar School, Chicago, 1932; from Lane Technical High School, Chicago, 1936; and from Wright Junior College, Chicago, 1938. In June, 1940, he received the degree of B.S. in Chemistry, from the University of Illinois.

Since 1940, he has served as Technician, and as Assistant in Chemistry, under Dr. Sendroy, in the Department of Experimental Medicine, at the Loyola University School of Medicine. During the past two years, he has devoted his free time to graduate study in biochemistry.

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LIST OF TABLES

In 1924, when Van Slyke and Neill first described their manometric blood gas apparatus, they pointed out that it could be used for air analyses (1). Since then, details for the adaptation of this apparatus to the analyses of different gas mixtures encountered in biological investigations have been developed. Among these is Sendroy's method for the determination of carbon monoxide by absorption with blood (2). By his procedure, carbon monoxide in air, in concentrations of from 0.05 to 0.3 volume per cent, can be determined with an error of ± 2.4 (maximum ± 5) per cent of the amount present.

The gas sample, from which carbon dioxide and oxygen are first removed by absorption in alkaline hyposulfite, is equilibrated with reduced ox blood in the chamber of the Van Slyke-Neill apparatus. The carbon monoxide thus absorbed and bound as the hemoglobin compound is then determined by the method of Sendroy and Liu (3) for the gasometric determination of oxygen and carbon monoxide in blood.

In the latter method (3), accuracy of measurement of the CO, which is released from combination with hemoglobin by the addition of acidified potassium ferricyanide,

$$HbCO + K_3\overset{+++}{Fe}(CN)_6 \rightarrow K_4\overset{++}{Fe}(CN)_6 + MeHb + \uparrow CO$$

is assured by its selective absorption in Winkler's solution (ammoniacal cuprous chloride). Prior to this step, however, it is necessary to transfer the tiny bubble of evolved gas (mainly

a mixture of CO and N_2) to a vessel outside of the Van Slyke apparatus, and to return it back again, after the latter has been cleaned.

More recently, Roughton (4,5) has called attention to the fact that when it is required to analyze blood only for CO, the refinement and elaborate technique of Sendroy and Liu is not always necessary. It will suffice merely to apply the technique of Van Slyke and Hiller (14) for the CO capacity of blood as a measure of total hemoglobin, to the analysis of blood CO content. Thus, since no oxygen is liberated from blood previously reduced with alkaline hyposulfite, one need only extract dissolved nitrogen in vacuo and eject this gas prior to the release of CO by the addition of potassium ferricyanide. What little CO_2 is released at that time is absorbed by alkali and the remaining gas measured is carbon monoxide.

In this paper a method will be described for the determination of carbon monoxide in air, in concentrations of from 0.05 to 0.8 volume per cent, employing, with suitable modifications, the absorption with blood as used by Sendroy (2), and the subsequent determination of the carbon monoxide by the technique of Van Slyke and Hiller for CO capacity (14), as suggested by Roughton's (5, 10) application to CO content. This improved technique results in a saving of time and labor, and reduces the margin of error of Sendroy's original method (2) to ± 1.2 per cent.

Reagents

Sodium hyposulfite solution — 15 gm. of $Na_2S_2O_4$ are dissolved in 100 cc. of 2N NaOH. This solution is prepared fresh daily, and is kept under oil (1, p.534). The solution is used both for absorption of oxygen and carbon dioxide from the gas sample, and for blood reduction.

Neutral ferricyanide reagent — 20 gm. of potassium ferricyanide and 8 gm. of saponin are dissolved in water to make 100 cc. of solution. If kept in a glass-stoppered bottle, the solution may be used for two weeks.

Air-free 1N sodium hydroxide solution — Approximately 40 gm. of NaOH are dissolved in water to make 1 liter of solution. This is extracted air-free and kept over mercury (7) in a Sendroy vessel (8).

Glycerol-salt solution — One volume of glycerol is mixed with three volumes of saturated sodium chloride solution (13).

Caprylic alcohol — This is used to prevent foaming.

Fresh beef blood is reduced as described in the following.[1]

Procedure

In the following, the description is largely confined to an outline of the general procedure, details being given or

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[1] In practice, fresh material may not always be available. However, we have usually found it possible to preserve the same lot of blood with its capacity for CO absorption unimpaired for one week, by separating it into small portions, and storing them in stoppered flasks in the refrigerator. A previously unopened flask was used for each day's work.

The blood used in this work was supplied by The Armour Laboratories, through the courtesy of Dr. J. H. Glynn.

stressed only when they constitute modifications of, or departures from, the original technique (2), to which the reader desirous of using the method should refer for more complete information.

Admission and Measurement of Gas Sample — Approximately 35 cc. of gas are admitted into the cleaned and empty chamber of the Van Slyke-Neill apparatus and measured at 50 cc. volume, as described by Van Slyke and Sendroy (9, pp.512-18). The readings p_0 and p_1 correspond respectively, to the pressures in mm. Hg within the chamber at the observed temperatures, before and after the introduction of the gas sample.

Deoxygenation of Gas Sample — 5 cc. of the alkaline hyposulfite solution are placed in the cup, 3 cc. are allowed into the chamber, and the stopcock is sealed with mercury. The gas sample is slowly shaken with this solution at slight negative pressure (9, p.521) for 3 minutes. The residual gas, free of oxygen and carbon dioxide, is transferred from the apparatus, through a mercury seal, into a modified (stopcock) Hempel pipette (Van Slyke and Hiller, 6) containing glycerol-salt solution.[2] The technique used in transferring the gas is that described by Sendroy and Liu (3, p.136). The hyposulfite solution is allowed to ascend into the arm of the Hempel pipette until it just enters the bore of the stopcock, which is then turned so that the capillary may be flushed with mercury from the cup

[2]Transfer of the gas to this vessel eliminates the possible inconvenience of the attached gas sampling tube used in the original technique (2, p.600).

above. The hyposulfite solution is then ejected from the chamber, so that only the film adhering to the walls remains.

Reduction of Blood — A 5 cc. (± 0.1 cc.) portion of beef blood (at room temperature) is run into the unwashed chamber, followed by 5 cc. of water and 2 drops of caprylic alcohol. The blood is then reduced and deaerated by two extractions *in vacuo* of 3 and 2 minutes each, as previously described (2, pp.601-2), except that 2 drops instead of one, of hyposulfite solution are used for the second shaking and extraction. The liberated gas is ejected after each extraction as usual, without loss of fluid.

Absorption of Carbon Monoxide — The deoxygenated gas stored in the Hempel pipette is returned to the chamber over the reduced blood, and the CO is absorbed, with exclusion of light[3], by slow shaking (approximately 200 r.p.m.) for 30 minutes at slightly negative pressure. The upper portion of the chamber is then temporarily uncovered, while the unabsorbed gas is ejected, and 4 drops of caprylic alcohol are added (2, p.602). With the light shield replaced, the gases (including nitrogen and a mere trace of CO) dissolved in the diluted blood mixture are then extracted by rapid shaking *in vacuo*, for 5 minutes. The shield is removed, the extracted gas is ejected, and the stopcock of the chamber is sealed with mercury.

Liberation and Measurement of CO Absorbed by Blood — From a stopcock pipette, through the mercury seal, 1.0 cc. of the

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[3] Tin-foil or any opaque paper or cardboard may be used to cover the jacket of the apparatus.

neutral ferricyanide solution is admitted into the chamber, directly over the diluted blood. The evacuated chamber is shaken for 10 minutes. The CO_2 evolved is then absorbed with 1 cc. of air-free 1N NaOH, and the CO is measured at 0.5 cc. volume, in the manner previously described (6, p.812; 1, pp.545-546). The readings p_2 and p_3 correspond, respectively, to the pressures within the chamber, at the observed temperatures, before and after the ejection of the residual gas (CO) from the apparatus.[4]

Blank Analysis: Determination of the "c" Correction — This analysis ("whole" blank) is carried out exactly as described above, except that approximately 35 cc. of laboratory air (CO-free) are used in place of the unknown CO-containing sample. The readings p_2' and p_3', respectively, are taken before and after the final ejection of residual gas from the apparatus.

However, as will be shown, the above procedure may usually be shortened and the resultant "simple" blank used with a correction, to effect considerable economy of time and labor without loss of accuracy. Thus, after deoxygenation of an air sample,, the remainder of the air (nitrogen), instead of being transferred to the Hempel pipette, is discarded together with the 3 cc. of used hyposulfite absorbent. Five cc. of blood, 5 cc. of water, and 6 drops of caprylic alcohol are then made air-free by

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[4]For the readings at this point, the bottom of the meniscus may not be clearly observable through the blood-ferricyanide mixture. However, partial visibility through the layer of NaOH added, together with experience in reading a water meniscus, should enable the operator to estimate the position of the bottom of the meniscus accurately.

successive 3 and 2 minute extractions, with added hyposulfite, as above. After the second ejection of extracted air, the equilibration of the reduced blood with deoxygenated air (which has been described), is here omitted. Consequently, there immediately follows another extraction in vacuo, for 5 minutes, with the light shield in place. Any gas thus liberated is ejected, and the analysis is continued and finished as above ("Liberation and Measurement of Co....."). The final readings in this procedure are designated by the symbols p_2'' and p_3''.

Calculation

The pressure of the sample at 50 cc. volume is calculated as

(1) $$P_s = p_1 - p_o$$

The carbon monoxide pressure at 0.5 cc. volume is calculated as

(2) $$P_{CO} = p_2 - p_3 - c$$

The "whole" blank correction term, c, is obtained from readings in blank analyses carried out as described above. Thus, depending on whether the "whole" blank or the "simple" blank is determined analytically, the correction is calculated, respectively, as either

(3) $$c = p_2' - p_3',$$ or

(4) $$c = p_2'' - p_3'' + 1.0$$

Since composition of the gas with respect to CO is calculated as

(5) $$\text{Per cent CO} = \frac{100 \times \text{cc. CO in sample}}{\text{cc. volume of sample}}$$

pressures are converted to volumes in the equation

(6) $$\text{Per cent CO} = \frac{f_1 P_{CO}}{f_2 P_s}$$

in which f_1 is the factor by which P_{CO}, measured at the 0.5 cc. volume and the observed temperature, is multiplied to give 100 times the volume, in cc. at 0°, 760 mm., of carbon monoxide present in the gas sample analyzed; and f_2 is the factor by which P_s, measured at the 50 cc. volume and at the observed temperature, is multiplied to give the volume in cc. at 0°, 760 mm. of gas sample used.

The factor f_1 is derived as follows (2): Equation 4 of Van Slyke and Neill (1) gives the factor f for calculating the cc. of CO reduced to 0°, 760 mm., found in the blood sample analyzed, and representing CO absorbed from the gas sample. Thus,

(7) $$f = \frac{a}{760(1 + 0.00384\ t)} \left(1 + \frac{S}{A-S}\alpha'\right)$$

This factor f is multiplied by 1.020 to obtain the cc. of CO present in the gas sample, since under the conditions of analysis only 0.980 or $\frac{1}{1.020}$ of this is absorbed by the blood. Further multiplication by 100 gives results directly in terms of volumes per cent. The complete calculation of the factor f_1 is therefore expressed by the equation:

(8) $$f_1 = \frac{a}{760(1 + 0.00384\ t_1)} \left(1 + \frac{S}{A-S}\alpha'\right) \times 102.0$$

a is the volume (here 0.5 cc.), at which the pressure P_{CO} is measured, at t_1, the temperature in degrees centigrade; S is the volume of solution, 11 cc., present in the chamber when the carbon monoxide is extracted from the blood-ferricyanide mixture; A is the capacity of the chamber, 50 cc.; α' is the distribution (solubility) coefficient of carbon monoxide between gas and water phases, as shown in Table I of Van Slyke and Neill.

The factor f_2 is calculated as

$$(9) \qquad f_2 = \frac{a}{760(1 + 0.00384\ t_2)}$$

when a is the volume (here 50 cc.), at which the pressure P_S is measured, at the temperature t_2.

In practical use, the factors are not recalculated for each analysis, but are obtained from a table of values for 1° intervals of temperature, calculated as above from Equations 8 and 9.

Experimental

In principle, the types of experiments performed in establishing the accuracy of the method and the range of precision, were the same outlined previously by Sendroy (2) and consisted of the preparation and analysis of air samples containing added CO in amounts known, or measured by independent analyses. In certain details, however, the procedures were different from the above, as will be described in the following:

Analyses of Air Containing Known Amounts of Added Pure CO—In this group of experiments, the results of which are recorded in Table I, the gas samples were made by the accurate dilution

Table I.

Results of Analyses of Air Containing Known Amounts of Added Pure CO

Analysis group No.	Air Sample No.	Ox Blood lot No.	Percentage CO in air	Ratio of percentage CO found by analysis to that present	Average of ratio for group	Deviation from average ratio of 0.980*
I.	1	1	0.0571	0.969	0.980	-0.011
				0.987		+0.007
				0.962		-0.018
				1.020		+0.040
	1	2		0.957		-0.023
				1.006		+0.026
	2	3	0.0523	0.965		-0.015
				0.973		-0.007
II.	3	4	0.0905	0.992	0.990	+0.012
	3	5		1.003		+0.023
				1.002		+0.022
	3	6		1.012		+0.032
				0.987		+0.007
	4	6	0.0875	0.972		-0.008
				0.962		-0.018
				1.015		+0.035
	4	7		1.006		+0.026
				0.989		+0.009
				0.993		+0.013
	5	8	0.0955	0.960		-0.020
				0.994		+0.014
				0.995		+0.015
	6	9	0.1036	0.984		+0.004
				0.980		0.000
III.	7	10	0.217	1.002	0.979	+0.022
				1.000		+0.020
	8	11	0.207	0.965		-0.015
				0.959		-0.021
	9	12	0.187	0.978		-0.002
				0.970		-0.010
				0.988		+0.008
	9	13		0.967		-0.013
				0.975		-0.005
				0.981		+0.001
				0.959		-0.021
	10	14	0.238	0.985		+0.005
	11	15	0.277	0.991		+0.011
	12	16	0.218	0.972		-0.008
	13	16	0.238	1.000		+0.020
	14	17	0.269	0.982		+0.002
	15	17	0.169	0.975		-0.005
	16	18	0.198	0.961		-0.019
	17	19	0.214	0.985		+0.005
IV.	18	20	0.585	0.975	0.978	-0.005
	19	20	0.627	0.982		+0.002
	20	20	0.591	0.983		+0.003
	21	20	0.694	0.983		+0.003
	22	20	0.718	0.979		-0.001
	23	21	0.716	0.973		-0.007
	24	21	0.822	0.979		-0.001
	25	21	0.813	0.960		-0.020
	26	21	0.632	0.983		+0.003
	27	21	0.790	0.985		+0.005
Average				0.980		±0.013

* The value 0.980 represents the average of the ratio for CO found to that present, for all the results of Tables I and II.

of pure CO gas in CO-free air, to give mixtures containing from 0.05 to 0.8 per cent CO (2). The purity of the several lots of CO used, made from formic and sulfuric acids, was controlled in analyses by absorption with Winkler's cuprous chloride solution[5]. Five such analyses gave values of 99.1, 100.4, 99.5, 100.0, and 99.2 per cent. Since the indicated average impurity of 0.4 per cent was well within the limit of error of the analyses, no correction was made for it in the preparation of known CO gas mixtures. The air used for dilution was analyzed by a method developed in this laboratory, as yet unpublished, and sensitive to as little as 0.001 per cent CO in air. The results of repeated tests of the room air used in the preparation of known CO mixtures were consistently negative.

Mixtures of 0.05 to 0.2 per cent CO were made as follows:

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[5]The details of the independent technique used are as follows: Approximately 35 cc. of air were deoxygenated in the Van Slyke-Neill chamber with 3 cc. of alkaline hyposulfite + catalyst (11). The nitrogen was transferred to a Hempel pipette, and the apparatus cleaned. One cc. of glycerol-salt mixture (13) was extracted air-free for 3 minutes. Of this amount, all except that necessary to give a liquid meniscus above the mercury, was ejected. The CO gas to be analyzed was then admitted into the chamber in an amount to give approximately 100 mm. pressure, and was accurately measured at 2.0 cc. volume (p_0 and p_1). A portion of the deoxygenated air sufficient to give 300 mm. pressure at 2.0 cc. was admitted into the chamber, and the total pressure was measured (p_2). The CO was then absorbed with 5 cc. of air-free Winkler's solution, and a reading again taken after 2 minutes (p_3). The "c" correction was obtained by repeating the procedure without the admission of CO, but with an equal volume of nitrogen in its place. The percentage CO was given by the expression

$$\frac{(p_1 - p_0) \times 100}{p_2 - p_3 - c}.$$

A calibrated 300 cc. gas sampling (Barcroft) tube, with leveling bulb attached, was connected through a short piece of pressure tubing, to the capillary side arm of the Van Slyke apparatus. The inside walls of the tube were moistened with water, and the vessel filled with mercury. A suitable amount of CO was admitted into the Van Slyke apparatus, and the pressure measured at the 0.5 or 2.0 cc. volume (9, 12). Air was then admitted into the chamber almost to capacity, and the mixture passed over into the sampling tube. The washing with air and transfer to the gas sampling tube were repeated three or four times. The latter was then disconnected from the apparatus and the mercury withdrawn from it completely, under slight negative pressure. It was then placed in a water bath at a temperature 2 or 3 degrees below room temperature. After thermal equilibrium had been attained, the contents of the tube were equilibrated with the atmosphere. The temperature of the bath and the barometer reading were recorded.

The known CO percentage of the above mixtures was calculated according to Equation 5. Thus, the volume of CO, V_{CO}, at 0°, 760 mm., used to make the mixture, was calculated from the pressure measurements by the use of the factor f_2 (Equation 9), with a correction factor for the increase in volume of a (here 0.5 cc.) above a mercury meniscus, compared with that above a water meniscus, at the same mark (12). The final volume of the gas mixture, at 0°, 760 mm., was calculated as usual, with correction for vapor tension, from the volume of the gas-

sampling tube.

Mixtures in the higher range (0.8 per cent) of CO were made by dilution of the gas measured at 0.5 cc. volume, with approximately 35 cc. of air, directly in the Van Slyke chamber, to give individual samples which were analyzed without further ado. The known volume of CO in these mixtures was calculated as above.

Analyses of Air Containing Measured Amounts of CO Extracted from Blood — In this group of experiments, the results of which are recorded in Table II, CO was extracted from blood in the Van Slyke-Neill apparatus, measured, and then used directly in the preparation of 0.2 per cent mixtures in air (3, pp. 144-5; 2, pp. 608-9). The procedure, with slight modifications, was patterned after the blood CO capacity method of Van Slyke and Hiller (6): Samples of 3 cc. of blood, with 6.25 cc. of water added, were equilibrated in the Van Slyke-Neill chamber with 2.5 cc. of CO, by shaking for 5 minutes with the mercury level at the 50 cc. mark. The extracted gases and excess CO were ejected. The CO retained by the blood was then liberated by the addition of 0.75 cc. of acidified potassium ferricyanide (20 per cent $K_3Fe(CN)_6$ and 2 per cent saponin in 2 per cent lactic acid), and extraction in vacuo with shaking for 5 minutes.

For the measurement of CO liberated from the saturated blood, the procedure was continued as follows: after the addition of 1 cc. of air-free 1N NaOH, 1 cc. of air-free alkaline hyposulfite (in 1N KOH, with catalyst (11, p. 124)) was added and a reading p_1 taken at 2.0 cc. volume, in the usual way

Table II

Results of Analyses of Air Containing Measured Amounts of CO Extracted from Blood.

Air sample No.	Ox Blood lot No.	Percentage CO in air	Ratio of percentage CO found by analysis to that present	Deviation from average ratio of 0.980*
1	22	0.232	0.981	+0.001
			0.981	+0.001
			0.982	+0.002
			0.982	+0.002
			0.975	-0.005
			0.974	-0.006
			0.973	-0.007
			0.989	+0.009
2	23	0.216	0.955	-0.025
			0.972	-0.008
			0.955	-0.025
			0.968	-0.012
			0.976	-0.004
			0.964	-0.016
			0.984	+0.004
Average			0.974	±0.008

*The value 0.980 represents the average of the ratio for CO found to that present, for all the results of Tables I and II.

(1, 11)[6]. The gas was ejected, and the reading p_2 was recorded. The "c" correction for the small amount of nitrogen liberated from the ferricyanide reagent, and therefore included in the measurement of p_1, was found by a repetition of the above procedure with an equal volume of water in place of blood, and omission of CO (equilibration or saturation) for the first 5 minute shaking and extraction. Thus, this method provided data for the calculation:

$$P_{CO} = p_1 - p_2 - c$$

From this there was obtained, by the use of the factor f_2 (Equation 9), the value of V_{CO}, the volume of free CO at 0°, 760 mm., extracted from the 3 cc. blood sample, and available for dilution with air after its measurement at the 2.0 cc. volume.

For the preparation of gas samples containing the same amounts of CO thus extracted and measured, the above procedure

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[6]The addition of alkaline hyposulfite, after introduction of the 1N NaOH, at this point was a departure from the technique of Van Slyke and Hiller (6), and had its origin in the observation that in these determinations, readings after the usual addition of air-free 1N NaOH were markedly lowered by the subsequent further addition of oxygen absorbent. This lowering of mercury was greater than that caused by the mere addition of absorbent solution (1, p.537) and could be attributed either to an absorption by the hyposulfite of oxygen not displaced by CO, or to the reabsorption by reduced hemoglobin of CO liberated from it (1, p.563). Since this point was not investigated further, the use of hyposulfite seemed necessary as a precautionary measure. If oxygen were present, hyposulfite would prevent the error of including it in the measurement of CO extracted from the blood. The CO reabsorption effect of the hyposulfite, on the other hand, could be ignored, inasmuch as the end in view was not the exact total amount of CO in the blood, or extracted from it, but the accurate measurement of the amount (after reabsorption) diluted and used in the subsequent mixture with air.

was repeated with the following changes: At the end of the second 5 minute extraction period (with ferricyanide), with the mercury level still at the 50 cc. mark, a calibrated gas sampling tube was attached to the capillary side arm as described in the preceding section. Hyposulfite was added to the contents of the extraction chamber, as above, and the reading p_1 taken at 2.0 cc. volume. The mercury level was then lowered, and air was admitted to the chamber for the dilution of the CO and transfer of the mixture to the gas sampling tube. The reading p_1 observed in this procedure, provided a check of the value obtained in the analysis described above. Although no p_2 reading was here available, it could be assumed to be the same observed by such previous measurement, if no significant temperature change had taken place in the interim. The final volume of the gas mixture was calculated from the tonometer volume, as above.

Results: The Evaluation of the Correction Factor 1.020 — Tables I and II indicate that the average of the results obtained for CO in the analysis of air mixtures containing 0.05 to 0.8 per cent of that gas, by the technique described, is 98.0 ± 1.2 per cent of the true concentrations (known, or obtained by independent measurement). There was no significant variation from this value, either with variation in CO concentration (97.8 to 99.0 per cent for the group averages in Column 6, Table I), or with difference in the type of experiment employed (total averages, 98.2 and 97.4 per cent, respectively, for Tables I and II). The corresponding empirical correction factor 1.020 was

therefore incorporated in the calculation of CO concentrations or content, of gas mixtures analyzed by this method (Equation 8).

Relationship of "Simple" Blank to "Whole Blank" Corrections — Since P_{CO} represents the CO initially in the gas sample (or 98.0 per cent of it) the readings actually obtained must be corrected for the presence of other gases extracted in the final 10 minute shaking with ferricyanide, namely, (1) the slight amount of CO present in normal blood, (2) dissolved air in the added ferricyanide reagent, and (3) that portion of the nitrogen absorbed during the 30 minute equilibration with deoxygenated air, which is not liberated in the following 5 minute extraction. The "whole" blank provides a complete correction for all of these factors of error (Equations 2 and 3).

Since the CO content of normal blood is variable and its determination requires the use of ferricyanide, the corrections for factors (1) and (2) must be determined for samples of the same blood and ferricyanide used for analysis. By the elimination, from the "whole" blank determination, of the 30 minute equilibration of blood with deoxygenated air, there is provided a control of factors (1) and (2) in what has been described above as the "simple" blank. Factor (3), as might be expected, is so constant (and small) that, once determined, the same correction for it may usually be used for all analyses. The average value for this correction, in a series of 14 comparisons of "whole" and "simple" blank analyses, was found to be 1.0 mm., measured at 0.5 cc. volume (Table III). Thus, this value, when

Table III

Comparison of "Whole" and "Simple" Blank Analyses.

Blood Sample No.	Pressure in mm., at 0.5 cc. volume, for "Whole" blank W	"Simple" blank S	Difference between blanks W - S	Deviation from average difference of -1.0
1	22.1	20.7	-1.4	-0.4
	23.2	21.1	-1.1	-0.1
	22.8	21.3	-1.5	-0.5
	23.1	22.2	-0.9	+0.1
	22.0	21.5	-0.5	+0.5
2	15.0	13.4	-1.6	-0.6
	16.8	17.2	-1.6	-0.6
3	20.7	20.0	-0.7	+0.3
	21.1	20.1	-1.0	0.0
4	18.1	17.4	-0.7	+0.3
	18.3	17.1	-1.2	-0.2
5	18.7	18.7	0.0	+1.0
6	21.1	20.1	-1.0	0.0
7	20.8	19.8	-1.0	0.0
Average			-1.0	±0.3

added to the determined "simple" blank result, gives the "whole" blank correction (Equation 4). This simplified procedure is recommended for the analysis of all samples except those in the region of 0.05 per cent CO. Although the average deviation from the above value of 1.0 mm. is only ± 0.3 mm., a possible maximum deviation of ± 1.0 mm. would involve an error of 4 per cent at this concentration of CO. Hence for such samples, the "whole" blank correction should be determined directly.[7]

Factors Affecting the Results — For the method described in this paper, the extent to which the CO found by analysis is a true measure of that present in the air sample, is determined by several factors of error which are not controlled by the blank analyses. These factors represent the extent to which there is conformity with the following theoretical requirements, (a) the complete elimination from the blood mixture of any oxygen, either bound (as oxyhemoglobin) or dissolved, which might be evolved upon the addition of ferricyanide and subsequently be measured and accounted for as carbon monoxide, (b) the complete absorption by the blood of CO in the air sample, and its subsequent quantitative liberation and measurement.

(a) The hyposulfite mixed with the blood reduces the oxyhemoglobin and removes the oxygen as such, by chemical reaction.

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[7]An obvious further simplification of the "simple" blank analysis would be the omission of the initial step, namely, the deoxygenation of the air sample which is immediately discarded. For some unknown reason, however, we did not find it possible to establish a sufficiently constant relationship between the results of such blanks, and those of "whole" blanks.

Preliminary deoxygenation of the air sample avoids the presence of a large amount of extra oxygen possibly interfering with the complete reduction of the hemoglobin. Because the alkaline hyposulfite used in this method was of different composition than that previously used[8], its efficiency in this respect was tested by the analysis for oxygen, of samples of air thus "deoxygenated". The results showed a content of 0.6 per cent O_2 in the gas sample.

Apparently, however, when the blood is equilibrated with "deoxygenated" gas containing this amount of unabsorbed O_2, the hyposulfite previously added to the blood removes the oxygen and prevents the formation of oxyhemoglobin. That this is so, is proven by the fact that the difference between the analysis of the "whole" blank, in which the blood is equilibrated with a residual air sample, and the "simple" blank, in which it is not, amounts to only 1 mm., a difference wholly attributable to dissolved nitrogen. It is reasonable to conclude, therefore, that no oxygen is included in the readings for $\underline{P_{CO}}$ in the present method.

(b) The ratio, 0.980, of CO found by analysis to CO present in the air sample is 4.3 per cent higher than that of the method as previously developed by Sendroy (2), who suggested that dif-

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[8]In order to retard the rapid deterioriation of grease in the stopcock below the cup, and the consequent leakage of air into the extraction chamber, sodium hydroxide was used in place of potassium hydroxide, and the concentration of hyposulfite reduced to one-half that used by Van Slyke and Sendroy (9) for oxygen analysis.

ferences in quantitative technique or control might to some extent be responsible for the variation in results obtained by different workers using this principle in quantitative work. The method of CO measurement employed in the present work is more convenient, and easier to carry out, but it is slightly less accurate (4), than that used in (3) in the determination of the factor 0.937 (2). Since method of measurement is precluded as a factor accounting for the improved recovery of from 93.7 to 98.0 per cent, the change must represent a real increase in that proportion of total CO in the gas sample, absorbed by the blood. That this is indeed the case, is shown by preliminary experiments in which either value could be obtained, depending on whether the blood used for equilibration with the gas sample was undiluted, as in Sendroy's procedure, or diluted as in the present modification. Apparently, dilution increases either the affinity of hemoglobin for CO under these conditions, or its rate of absorption of the gas.

Summary

A modification of Sendroy's method is described, whereby small amounts of carbon monoxide in air, in concentrations of from 0.05 to 0.8 per cent, are analyzed in the Van Slyke apparatus. By a change in reagents, procedure, and technique of measurement, there has been effected an economy of time and labor, and an increase in accuracy and precision of the results.

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