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Method for oxygen content and dissociation curves on microliter blood samples[1]

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TUCKER, VANCE A. *Method for oxygen content and dissociation curves on microliter blood samples.* J. Appl. Physiol. 23(3): 410–414. 1967.—Oxygen content of 7 μl of blood can be determined by injecting a blood sample from a calibrated pipette into a thermostatically controlled glass chamber containing a magnetic stirring bar and a solution of ferricyanide and saponin. The oxygen bound by the hemoglobin is released into physical solution and the resulting change in oxygen pressure is measured with an oxygen electrode. A determination can be completed every 7 min. Replicate determinations have a standard deviation of 0.14 ml O_2 per 100 ml blood. The difference between eight mean values of oxygen content determined by this method and that of Van Slyke and Neill had a mean value of 0.03 ml O_2 per 100 ml blood (standard error = 0.06). The method can be used with a microtonometer and gas-mixing system to construct oxygen dissociation curves from 200 μl of blood.

blood gases

OXYGEN CONTENT

The oxygen content of blood is an important measurement in clinical practice and in research on respiration and circulation. The method described here for making this measurement on 7 μl of blood is based on the tested principle of releasing hemoglobin-bound oxygen into physical solution and measuring the resulting change in partial pressure of oxygen (P_{O_2}) with an oxygen electrode (1, 8–10). Compared to other micromethods based on this and other principles, the present method requires one-third or less the sample volume without sacrificing accuracy and precision (Table 1).

Oxygen is released from hemoglobin in a water-jacketed glass chamber constructed to fit over the end of a vertical Beckman oxygen macroelectrode (no. 315780) (Fig. 1). The chamber contains a magnetic stainless steel stirring rod and opens to the outside by a capillary 1 mm in diameter. This capillary usually is closed with a glass stopper to prevent contamination of the chamber contents with atmospheric oxygen. The volume of the chamber (about .5 ml in this study) can be measured by weighing a .5-ml syringe before and after filling the chamber to the top of the capillary with water.

The chamber can be rinsed with distilled water from a siphon ending in PE-50 polyethylene tubing. Liquids are removed from the chamber by a vacuum line, also ending in PE-50 tubing. A magnetic stirrer actuates the stirring bar in the chamber.

The blood sample is transferred to the chamber with a calibrated overflow-type micropipette attached to a .25-ml syringe and 23-gauge needle with PE-50 tubing (Fig. 1). The micropipette used in this study contained 7.24 μl of blood.

Two solutions are used in the procedure. In one solution, 6 g of potassium ferricyanide [$K_3Fe(CN)_6$] and 3 g of saponin dissolved in 1 kg of water are used to release the oxygen bound by hemoglobin. Some of the ferricyanide solution is equilibrated with air and placed in a 5-ml syringe to serve as a standard. Another portion is placed in a well-greased 10-ml syringe and degassed by plugging the syringe needle with a piece of rubber, pulling back the plunger to create a vacuum, and shaking. The extracted gas is expelled and the procedure repeated four or five times until no more gas is extracted. A trace of caprylic alcohol in the syringe reduces foaming.

The second solution is oxygen free and consists of 1 mg sodium sulfite (Na_2SO_3) per 5 ml 0.01 M sodium borate ($Na_2B_4O_7$). It is prepared fresh daily by adding sodium sulfite to the borate solution in a 5-ml syringe, inserting the plunger, and expelling all air bubbles.

The current output of the oxygen electrode can be measured with a variety of devices. In this study, the electrode was connected to a Beckman dynograph preamplifier with a type 9871 input coupler. The preamplifier drove a 10-inch Honeywell potentiometric recorder. Since the electrode output is markedly temperature sensitive, water from a bath regulated at 32 ± 0.01 C circulated continuously through the water jacket of the electrode chamber. This temperature was chosen because it is sufficiently above laboratory temperature to allow regulation with a simple heater, yet solutions injected at laboratory temperature equilibrate rapidly.

Each analysis of blood oxygen content consists of two parts: measurement of the change in P_{O_2} when the oxygen bound by hemoglobin is released into physical solution in the degassed ferricyanide reagent, and calibration of the electrode by measuring a known P_{O_2} (aerated ferricyanide reagent). Periodically, the electrode reading at a P_{O_2} of 0 (sodium sulfite reagent) also is determined. This reading is fairly stable and need not be made for every analysis. The oxygen content of the blood sample can be calculated from the above data together with the volume of the chamber and the solubility coefficient for oxygen in the ferricyanide reagent.

The following is a detailed account of the operations involved in measuring the oxygen content of a blood sample. The chamber on the electrode is carefully filled with degassed ferricyanide solution from the 10-ml syringe. The chamber is stoppered and the stirring motor turned on. After equilibration (about 2 min) the reading of the electrode is noted, the stirring motor stopped,

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TABLE 1. *Comparison of sample volume, time, precision, and accuracy for various methods for blood oxygen content*

Method	Sample Vol, μl	Time per Analysis, min	Precision, (SD, ml O_2 per 100 ml blood)	Accuracy (Mean Diff Compared to Van Slyke Method, ml O_2 per 100 ml blood)
This study	7	7	0.14 (10 replicates)	0.03
Dixit and Lazarow (3)	20	15	0.19	not available
Natelson (7)	30	20	0.08–0.20	0.004
Roughton and Scholander (11)	40	12	0.13	0.05
Laver et al. (8)	50	3	0.21	0.42
Gas chromatography (5)	50	4	0.14	0.35
Mayers and Forster (10)	400	6–7	0.29	<0.37
Van Slyke and Neill (13)	2000	15	0.04	

the stopper removed, and the blood sample injected, care being taken to exclude any bubbles. During this procedure the ferricyanide solution overflows the chamber, and an insignificant part of this overflow re-enters the chamber when the micropipette is withdrawn. The stopper is replaced, the stirring motor is turned on, and the reading of the electrode is noted after equilibration (2–3 min). Stirring must be adequate to mix the blood and ferricyanide reagent thoroughly.

Calibration is accomplished by removing the blood and ferricyanide reagent with suction, filling the chamber with aerated ferricyanide reagent and stirring, and emptying the chamber and refilling again two-thirds full with aerated ferricyanide reagent. The chamber is stirred and left unstoppered, and the reading of the electrode is noted after equilibration (about 2 min). It is necessary to let the ferricyanide reagent equilibrate with a bubble of air in the chamber since the chamber will probably be at a different temperature than that at which the reagent was originally aerated. The electrode reading following this method of equilibration is identical to that when water equilibrated with air at 32.0 C in a water bath is placed in the electrode chamber. The reading of the electrode at 0 Po_2 is determined by injecting the sodium sulfite reagent into the chamber, stoppering, and stirring until equilibration. If more convenient, pure nitrogen may be bubbled through the chamber. Both methods give the same reading. The sodium sulfite must be thoroughly rinsed out with distilled water before the next blood analysis.

The following is an example of the measurements and calculations made during an analysis of blood oxygen content. Solubility coefficients (α) of oxygen in the ferricyanide reagent are shown in Table 2. Barometric pressure = 755 mm Hg, temperature of chamber = 32.0 C, vapor pressure of ferricyanide reagent = 35 mm Hg (Table 1), Po_2 in aerated ferricyanide reagent = (755 − 35) 0.2095 = 151 mm Hg, chamber volume = 458 μl, sample volume = 7.24 μl.

reading on degassed ferricyanide reagent = 13.7 chart divisions
reading after equilibration of ferricyanide reagent and blood = 64.5 chart divisions
reading on aerated ferricyanide reagent = 87.5 chart divisions
reading at 0 Po_2 = 8.8 chart divisions

Thus, 151 mm Hg oxygen corresponds to 87.5 − 8.8 = 78.7 chart divisions, or 1.92 mm oxygen per chart division. The change in Po_2 (ΔPo_2) after injecting the blood sample = (64.5 − 13.7) 1.92 = 97.6 mm.

oxygen content (ml O_2/100 ml blood)
= (ΔPo_2/760 mm) × α × vol of chamber × (100/sample size)
= (97.6/760) × 0.0252 × 458 × (100/7.24) = 20.5

The accuracy of the electrode method was determined by showing that the change in Po_2 in the chamber was proportional to the amount of oxygen introduced, and that the oxygen contents of blood samples as determined by the electrode were the same as those determined by the procedure of Van Slyke and Neill (13). The amount of oxygen introduced into the chamber was varied by injecting various volumes of a single blood sample equilibrated with air (Fig. 2). The relation between blood volume and change in Po_2 was proportional and was described by the least-squares equation μl blood = 0.0659 ΔPo_2 − 0.07 (standard error of estimate = 0.388, N = 16).

Comparative analyses of blood with the electrode and the method of Van Slyke and Neill showed that the means of these two methods agreed over a wide range of oxygen contents (Table 3). Blood was diluted with plasma to obtain samples with different oxygen contents. Each sample was equilibrated with air, and oxygen content determinations by the Van Slyke method were made on 1-ml aliquots by F. G. Hall. Determinations by the electrode method were made on 7.24-μl aliquots of the same sample.

The oxygen electrode method can be compared with other methods commonly used for blood oxygen content (Table 1). It is as accurate and precise as other micro methods, none of which are as precise as the macro method of Van Slyke and Neill. Its outstanding advantages are small sample requirements and simplicity. There is no obvious reason why it could not be used on samples of 1 μl or less by increasing amplification, using a smaller electrode, and reducing the electrode

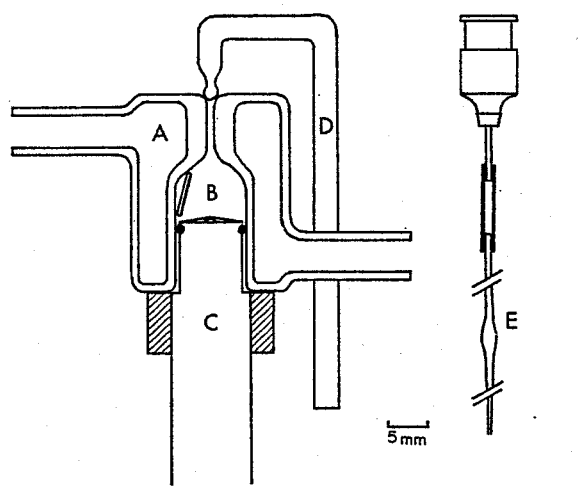

FIG. 1. Cross section of oxygen electrode in glass chamber. A, water jacket; B, chamber and stirring bar; C, oxygen electrode and rubber collar; D, glass stopper. The rubber collar is made from tubing and supports the chamber on the electrode. The stopper is made from 3-mm glass rod, drawn out and held over a flame until a ball forms at the tip. A micropipette attached by polyethylene tubing to a shortened hypodermic needle is shown at E.

TABLE 2. *Relation between temperature, oxygen solubility, and vapor pressure of the ferricyanide reagent used in this study*

Temperature, °C	α*	Vapor Pressure, mm Hg
25	0.0284	23
26	0.0279	24
27	0.0275	26
28	0.0270	27
29	0.0265	29
30	0.0261	31
31	0.0256	33
32	0.0252	35
33	0.0248	37
34	0.0243	39

* α is the volume of oxygen (at STP) absorbed by 1 volume of ferricyanide solution when the pressure of the oxygen itself, without the aqueous vapor pressure, is 760 mm Hg. These values and values at other temperatures may be calculated from refs. 6, 12.

chamber volume. With one electrode, an analysis including calibration can be completed every 7 min, and 85% of this time is spent waiting for equilibration. Two electrodes could be used simultaneously, allowing an operator to analyze a blood sample every 3 or 4 min.

A source of error in the oxygen electrode method is drift and noise in the electrode output. Drift errors are minimized by calibrating the electrode with aerated reagent following each determination. Noise can be reduced by adding Na_2SO_4 and a ground wire to the water circulating around the electrode chamber, and placing a 2-μf capacitor across the electrode leads.

A second possible source of error is the gain or loss of oxygen by the ferricyanide solution that overflows the chamber during injection of the blood sample and re-enters the chamber when the micropipette is withdrawn. The overflow is about 8 μl. It consists entirely of ferricyanide solution that has not contacted blood since the blood is injected near the middle of the chamber and falls rapidly. Only about 1 μl of the overflow re-enters the chamber. With a 458-μl chamber, addition of 1 μl of ferricyanide solution completely equilibrated with air would raise the P_{O_2} in the chamber by only 0.3 mm. This increase is insignificant; in the example given earlier, it would alter the calculated oxygen content of the blood by less than 0.4%. Furthermore, the oxygen electrode method yields values for the oxygen content of plasma that are within 0.1 ml O_2 per 100 ml plasma of the values determined by Van Slyke and Neill (Table 3), and the line shown in Fig. 2 has an equation that is not significantly different from a proportional relation. These observations demonstrate that no significant amount of oxygen is gained or lost during the overflow and reentry of the ferricyanide solution.

Another possible source of error is oxygen consumption by the electrode itself, but this is negligible with the Beckman macroelectrode. This electrode passes a current of approximately 400 $\times$ 10^{-11} amp in a solution with a P_{O_2} of 150 mm Hg. Four electrons flow through the electrode for every molecule of oxygen consumed (2), so that oxygen consumption by the electrode would change the P_{O_2} in the chamber by less than 0.0005%/min.

OXYGEN DISSOCIATION

Conventional methods of determining oxygen dissociation curves and pH on whole blood require several milliliters of blood. This is a major technical difficulty (14) since pooled blood samples are required from vertebrates weighing less than 100 g. There is almost no information on how dissociation curves of these animals vary among individuals, nor has it been feasible to follow temporal changes in individual dissociation curves after experimental treatment. The method described here has been in use in my laboratory for the past year and permits the construction of dissociation curves for individual vertebrates weighing as little as 10 g, or for larger animals without sacrificing the individual. In addition, the method is simple and requires only a modest investment in space and material.

The method uses a continuous gas-flow system similar to Hall's (4) with two special features: a method for mixing and analyzing gas flows to obtain a constant percentage of carbon dioxide and variable percentages of oxygen, and a microtonometer compatible with the previously described micromethod of analysis for blood oxygen content. Gas mixtures flow past an oxygen electrode, pass through a rotating tonometer containing the blood sample and exhaust to the atmosphere. The oxygen electrode and tonometer are mounted inside a glass jar that is submerged in a water bath for temperature control (Fig. 3).

Gas containing a constant percentage of carbon dioxide but variable percentages of oxygen is obtained by mixing gas streams in different proportions from two high-pressure cylinders, one containing the desired percentage of carbon dioxide in nitrogen and the other in air (or in oxygen for blood with low oxygen affinity). Each gas passes from its cylinder (I use small E cylinders and commercially mixed gases) through a two-stage regulator set at .2 atm gauge pressure, a fine needle valve (such as Whitey ORM2), a manometric flowmeter, and a humidifier to a three-way stopcock where it mixes with the other gas stream.

The humidifiers are made in two halves from 12-mm glass tubing drawn out at the ends to fit the rubber tubing carrying the gas. Each half contains approximately 3 cm of loosely packed glass wool, and the halves are joined together with rubber tubing. Once a day distilled water is sucked up into the humidifiers then blown out gently to remove any droplets. In

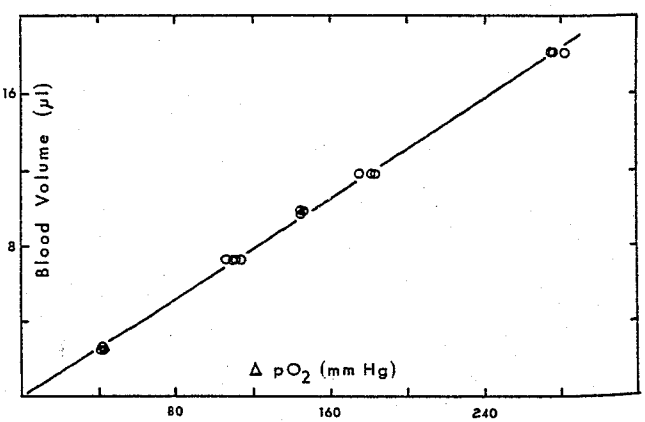

FIG. 2. Relation between volume of blood injected into a 505-μl chamber and change in P_{O_2} in the chamber.

TABLE 3. *Comparison of blood oxygen content by the method of Van Slyke and Neill and the oxygen electrode method, ml O_2 per 100 ml blood*

Sample	Van Slyke and Neill	Mean	Electrode	Mean	Diff
1	24.15, 24.25	24.20	24.00, 24.20, 24.60, 24.60, 23.80, 24.20	24.23	−0.03
2	18.36, 18.38	18.37	18.08, 17.66, 18.10	17.95	+0.42
3	10.45, 10.55	10.50	10.64, 10.23, 10.55	10.48	+0.02
4	9.65, 9.65	9.65	9.73, 9.77	9.75	−0.10
5	7.11, 7.13	7.12	7.25, 7.05, 6.95	7.08	+0.04
6	5.07, 5.00	5.04	5.15, 5.19	5.17	−0.13
7	2.92, 3.10	3.01	3.05, 3.03	3.04	−0.03
Plasma		0.5 (Ref. 12)	0.49, 0.44, 0.45, 0.37	0.44	+0.06
				Mean diff = 0.03 SE = 0.06	

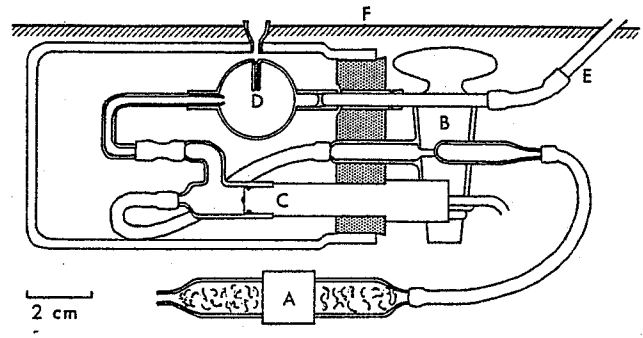

FIG. 3. Cross section of apparatus for equilibrating blood with analyzed gas mixtures. Except for the oxygen electrode, all parts are made of glass, Tygon, or rubber and are mounted on a rubber stopper (stippled). The apparatus is shown beneath the surface of a water bath. A, one of the humidifiers; B, three-way stopcock; C, oxygen electrode; D, tonometer: rubber tubing connects the tonometer to the shaft going through the rubber stopper; E, shaft to clock motor: rubber tubing forms a universal joint between the motor shaft and the shaft going through the rubber stopper—the motor is above the bath and is not shown; F, surface of water bath.

use, the humidifiers are immersed in the constant-temperature bath.

The three-way stopcock, oxygen electrode (Beckman macro-electrode no. 315780), and the tonometer are mounted on a rubber stopper. Gas flows from the stopcock through a chamber fitted over the end of the oxygen electrode and through a slip-joint into the tonometer. The stopcock is used in calibrating the electrode since it selects whether carbon dioxide in nitrogen, carbon dioxide in air, or a mixture of these flows over the electrode.

Gas flows continuously through the tonometer and exhausts to the atmosphere. The tonometer has a total volume of approximately 5 ml and can be rotated at 30 rpm by a synchronous clock motor. When the tonometer is stationary, blood can be sampled from it with a micropipette inserted through a tube attached to the inside wall of the tonometer. This tube prevents blood spillage when the tonometer rotates.

The rubber stopper and its attached components fit tightly into a wide-mouth jar that is immersed in a water bath. A hole drilled in the jar in line with the tonometer opening provides access to the blood. A tapered piece of Tygon tubing fits into the hole and forms a dam that prevents water from entering the jar. The bearing where the shaft from the motor passes through the stopper is made from close-fitting glass tubing and is watertight if lightly greased. Electrical connectors at the rear of some oxygen electrodes must be made waterproof before submersion.

In my laboratory, the oxygen electrode output is recorded with the same apparatus used with the electrode for blood gas measurement. pH is measured on 15 μl of blood with a Metrohm EA 521-3 capillary electrode and a Beckman no. 101900 research pH meter.

The procedure for constructing an oxygen dissociation curve is as follows: 0.2 ml of blood is placed in the tonometer which is rotated for 30 min and ventilated by carbon dioxide in air (or in oxygen for blood with low oxygen affinity) flowing at 15 ml/min. The oxygen electrode reading on this gas is noted. The tonometer is stopped and duplicate 7-μl blood samples are analyzed for oxygen content to establish the oxygen capacity of the blood. A blood sample for pH determination is obtained by inserting the inlet tubing of a capillary electrode directly into the tonometer. After blood analyses, the stopcock is turned and carbon dioxide in nitrogen flows past the oxygen electrode until a steady reading is obtained. The electrode readings on these two known partial pressures of oxygen constitute calibration, and any desired intermediate partial pressure may be obtained by setting the stopcock to the mix position and adjusting the needle valves while referring to the oxygen electrode reading. The total gas flow through the system should be approximately 15 ml/min.

The oxygen content and pH of the blood is determined after equilibration for 30 min with as many gas mixtures as desired. The oxygen capacity of the blood usually does not increase or decrease more than a few percent during an experiment, but it should be checked as often as necessary to provide an oxygen dissociation curve of the required accuracy. If necessary, fresh blood may be used for each point. A curve consisting of six points measured in duplicate and three pH determinations uses about 0.13 ml of blood and can be completed in 6 hr.

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CALIBRATION PROCEDURE. Tucker chamber.

1) The volume of the Tucker chamber. The chamber is weighed empty and dry, with the stirring magnet and the P_{O2} electrode mounted. The chamber is filled with water up to the capillary tube at the top of the chamber. The chamber is weighed again. The difference in weight in mg is the chamber volume in µl.

2) The chamber is thermostated to a specific temperature (e.g. ~~35~~ °C).

NB. A given chamber constant is only valid at specific conditions! (i.e. chamber volume, sample volume, temperature).

3) The stirring factor = the liquid-gas sensitivity ratio. The measured partial pressure of a gas is always a little higher than the gas tension measured in a liquid, equilibrated with the same gas mixture and at the same pressure, due to a diffusion barrier in the liquid. The stirring factor is estimated by measuring the oxygen tension, P_{O2} of water-saturated air, whereafter the P_{O2} of air-equilibrated distilled water is measured (3/4 filled chamber). The ratio between the two measured oxygen tensions is the stirring factor. (Approximately 1.02).

4) Calibration of the O_2 electrode. The chamber is filled with a "zero solution" made of a pinch (1 mg) of sodium sulfite Na_2SO_3 in 5 ml 0.01 M sodium borate $Na_2B_4O_7$. Stirring. At stable electrode output, the "Zero" knob on the meter is adjusted. NB. It is important to use freshly prepared zero solution and to ensure that no air bubbles are trapped in the chamber! This zero-adjustment is fairly stable and need only to be done once or twice a day.

The chamber is thoroughly rinsed with distilled water by filling and emptying the chamber 5-6 times. The stirring motor is started, and the air in the empty chamber equilibrates now with the waterphase in the small amount that is always left in the chamber.

The P_{O2} of the water-saturated air in the empty chamber is calculated: $(BP - P_w) \times 0.2095 \times SF$. BP means barometric pressure, P_w is the water vapour pressure at the given temperature (at ~~35~~ °C, 40 P_w = 55.32 mmHg), the figure 0.2095 is the fractional volume of oxygen in atmospheric air, SF means stirring factor.

The "Adjustment" knob is adjusted to this calculated P_{O2} value when the electrode output is stable. This adjustment should be done several times per day!

5) The chamber constant. The basis for the determination of the chamber constant is to apply a known amount of oxygen to the ferricyanide reagent in the Tucker chamber. Human blood is a well-studied liquid, which is able to accumulate a high amount of oxygen; the oxygen capacity is in vol.%: $HbO_2 = 0.460 \times Hct.$, where Hct. is hematocrit in %. This simple equation is empiric and holds for freshly drawn blood with pH ≈ 7.4. The donor should be a non-smoker.

...man blood is equilibrated with humid air (which saturates ...an blood more than 99%, in a tonometer, at for example 20 °C. The oxygen solubility coefficient of human blood at this temperature is).0037 vol.%/mmHg. The total amount of oxygen in the blood ctO_2 can :hen be calculated: $ctO_2 = HbO_2 + cdO_2$, where cdO_2 is the dissolved oxygen. (For blood with a Hct. of 44.5, at BP = 758 mmHg and at 20 °C, this gives a ctO_2 of 20.6 vol.%).

.) The Tucker chamber (with the newly adjusted O_2 electrode) is filled with degassed ferricyanide reagent. Stirring. Take care to exclude any air bubble! When the electrode output is stable (takes 2-4 min.), the meter is read ($P_{O2\ START}$).

A specific volume of saturated human blood is now injected into the chamber with a Hamilton syringe (Hamilton Gastight® #1705, Hamilton - Bonaduz. Schweiz). Stirring. At maximum P_{O2} electrode output, the meter is read ($P_{O2\ END}$).

The Tucker chamber is rinsed 3 times with distilled water and refilled with degassed ferricyanide reagent for a new determination. Stirring.

:) $\Delta P_{O2} = P_{O2\ END} - (P_{O2\ START} \times FD)$ is calculated for each determination. FD = factor of dilution = (chambervol. - samplevol.)/ chambervol.

The chamber constant, c_T is calculated from the mean of several determinations of ΔP_{O2}:

$$c_T = ctO_2 / \Delta P_{O2} \quad ; \text{vol.\%/mmHg}$$

$$= (0.460 \times Hct. + 0.0037 \times (BP - P_w)) / \Delta P_{O2} \quad ; \text{at } 20\ ^\circ C$$

:) This procedure is repeated for different sample volumes (e.g. 0, 20, and 30 µl). A plot of ΔP_{O2} versus sample volume gives a straight line. The chamber constant for a given sample volume can now be calculated using this plot.

It is convenient to calculate a series of c_T values and) values, corresponding to a series of sample volumes.

MEASUREMENT OF OXYGEN CONTENT.

1) The electrode is calibrated as described above.

2) The chamber is filled with degassed ferricyanide reagent. $P_{O2\ START}$ is read and multiplied with the factor of dilution, FD.

3) A sample of blood is injected into the chamber, immediately upon the $P_{O2\ START}$ reading. Stirring. The $P_{O2\ END}$ is read when the electrode output reaches a maximum.

4) ΔP_{O2} is calculated as $P_{O2\ END} - (P_{O2\ START} \times FD)$. ctO_2 is calculated as $\Delta P_{O2} \times c_T$. The hemoglobin bound oxygen is calculated by subtraction of dissolved oxygen, cdO_2.

$$HbO_2 = c_T \times (P_{O2\ END} - (P_{O2\ START} \times FD)) - cdO_2 \quad ; \text{vol.\%}$$

REAGENTS.

A) Ferricyanide reagent:

0.6 g potassium ferricyanide $K_3Fe(CN)_6$

0.3 g saponin

dissolved in 100 ml distilled water.

B) Zero solution:

1 mg sodium sulfite Na_2SO_3 dissolved in

5 ml 0.01 M sodium borate $Na_2B_4O_7$

Should be made freshly for each zero adjustment!

Solubility Coefficient (α): milliliters of gas dissolved per milliliter of fluid at 760 mm Hg pressure.

Temp °C	O_2 Solubility Coefficient							CO_2 Solubility Coefficient		
	NaCl[1]		Human	Whole Human Blood[2]				NaCl[1]		Ox
	0.119 N	0.155 N	Plasma	5 g Hb·100 ml^{-1}	10 g Hb·100 ml^{-1}	15 g Hb·100 ml^{-1}	20 g Hb·100 ml^{-1}	0.119 N	0.155 N	Plasma[2]
10	0.03715	0.03689	0.0338	0.0346	0.0348	0.0352	0.0361	1.181	1.177	.....
11	0.03631	0.03605	0.0330	0.0338	0.0340	0.0344	0.0353	1.141	1.137	.....
12	0.03550	0.03524	0.0322	0.0331	0.0333	0.0337	0.0345	1.104	1.100	.....
13	0.03472	0.03446	0.0315	0.0324	0.0326	0.0330	0.0337	1.070	1.066	.....
14	0.03399	0.03373	0.0308	0.0317	0.0319	0.0323	0.0330	1.037	1.033	.....
15	0.03328	0.03302	0.0302	0.0310	0.0312	0.0316	0.0323	1.006	1.002	0.916[4]
16	0.03216	0.03235	0.0296	0.0303	0.0305	0.0309	0.0316	0.972	0.968	0.889
17	0.03196	0.03170	0.0290	0.0297	0.0299	0.0303	0.0310	0.943	0.939	0.862
18	0.03133	0.03107	0.0285	0.0292	0.0294	0.0297	0.0304	0.915	0.911	0.835
19	0.03074	0.03048	0.0281	0.0287	0.0289	0.0292	0.0298	0.889	0.885	0.810
20	0.03015	0.02989	0.0277	0.0282	0.0284	0.0287	0.0293	0.865	0.861	0.787[4]
21	0.02957	0.02931	0.0273	0.0277	0.0279	0.0282	0.0288	0.841	0.837	0.764
22	0.02901	0.02875	0.0269	0.0273	0.0275	0.0277	0.0283	0.816	0.812	0.742
23	0.02847	0.02821	0.0265	0.0269	0.0271	0.0273	0.0279	0.791	0.787	0.721
24	0.02794	0.02768	0.0261	0.0265	0.0267	0.0269	0.0275	0.768	0.764	0.700
25	0.02744	0.02718	0.0257	0.0261	0.0263	0.0265	0.0271	0.746	0.742	0.681[4]
26	0.02696	0.02670	0.0253	0.0257	0.0259	0.0261	0.0267	0.725	0.721	0.664
27	0.02649	0.02623	0.0249	0.0253	0.0255	0.0257	0.0263	0.705	0.701	0.647
28	0.02604	0.02578	0.0246	0.0249	0.0251	0.0253	0.0259	0.685	0.682	0.631
29	0.02562	0.02536	0.0242	0.0245	0.0247	0.0249	0.0255	0.669	0.665	0.615
30	0.02521	0.02495	0.0238	0.0241	0.0243	0.0245	0.0251	0.652	0.648	0.601[4]
31	0.02487	0.02461	0.0234	0.0238	0.0239	0.0242	0.0247	0.637	0.633	0.587
32	0.02454	0.02428	0.0230	0.0235	0.0236	0.0238	0.0243	0.623	0.619	0.573
33	0.02420	0.02394	0.0226	0.0232	0.0233	0.0235	0.0240	0.608	0.604	0.560
34	0.02387	0.02361	0.0223	0.0229	0.0230	0.0232	0.0237	0.594	0.590	0.547
35	0.02353	0.02327	0.0220	0.0226	0.0227	0.0229	0.0234	0.579	0.575	0.535[4]
36	0.02326	0.02300	0.0217	0.0223	0.0224	0.0226	0.0231	0.567	0.563	0.525
37	0.02299	0.02273	0.0214	0.0220	0.0221	0.0223	0.0228	0.554	0.550	0.515[4]
38	0.02273	0.02247	0.0212	0.0217	0.0218	0.0220	0.0225	0.542	0.538	0.503[4]
39	0.02246	0.02220	0.0210	0.0214	0.0215	0.0217	0.0222	0.529	0.523	0.492
40	0.02219	0.02193	0.0208	0.0211	0.0212	0.0214	0.0219	0.517	0.513	0.482[4]
41	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.473
42	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.464
43	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.454[4]

in mM x $torr^{-1}$ = 1000/(22.4 x 760).

from Christoforides, C. & J. Hedley-Whyte. (1969). Effect of temperature and hemoglobin concentration on solubility of O_2 in blood. J. Appl. Physiol. 27: 592-596.