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Oxygen Analysis:

Advances in Methodology

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THE THERAPEUTICALLY BENEFICIAL EFFECTS and the undesirable effects of oxygen therapy are well recognized. For the physician attempting to achieve "safe" therapy the complexities of pulmonary O_2 toxicity often create a dilemma best resolved by administering an inspired O_2 concentration (F_{IO_2}) sufficient to provide adequate tissue O_2 levels. To obviate the consequences of inadequate or excessive oxygenation, control of therapy requires measurement of F_{IO_2}, the corresponding partial pressure (P_{aO_2}) or content (O_2 content) of O_2 in arterial blood, and tissue O_2 concentrations; listed in order of increasing difficulty of measurement.

Several comprehensive reviews of principles and methodology of respiratory and/or blood–gas analysis are available.[1,2,9,17,56,64,70,73,87,99] They concern either single or multiple techniques for measuring O_2 and other gases. In this review we consider O_2 only, and describe available techniques, with particular attention to recent developments. To provide an orderly format applicable to clinical use, we discuss O_2 measurement in the gas phase and methods for blood analysis, followed by comment on the state of the art for measurement in tissues, and conclude with a discussion of methodology for evaluation of the hemoglobin–O_2 relationship.

Analysis of Oxygen in Gas

PARAMAGNETIC ANALYSIS

The paramagnetic O_2 analyzer was first described by Pauling, Wood, and Sturdivant.[7] Oxygen, being paramagnetic, is attracted to the strongest part of an inhomogeneous magnetic field, while other respiratory gases, being diamagnetic, will locate in a weaker portion of the field. The conventional analyzer (fig. 1) consists of a glass dumbbell containing a diamagnetic gas such as nitrogen (N_2), suspended by a quartz thread between the poles of a permanent magnet. Any O_2 contained in a mixture of gas which surrounds the dumbbell will displace it from the strong portion of the magnetic field. A beam of light reflected from a mirror affixed to the dumbbell onto a scale indicates the degree of rotation and thus, concentration of O_2 present in the mixture.

Accuracy is improved by use of a null-balance method. The torque of the dumbbell is opposed by means of a coil which forces return to the null position. Magnitude of the current necessary to return the dumbbell to the null position is controlled by a potentiometer which permits reading O_2 concentration directly from the dial. According to Nunn and associates,[75] one such system was found to provide great accuracy, improved linearity, and a means of providing temperature regulation. This system does not permit continuous analysis, since the potentiometer must be moved manually, but this difficulty may be overcome by utilizing two adjacent photocells on to which the beam from the mirror is deflected. The output from each photocell is fed into a differential amplifier, which in turn regulates the dumbbell-coil current to achieve null balance. Ellis and Nunn[29] have evaluated such a system extensively.

A major disadvantage of paramagnetic analysis is the prolonged response time owing to the low flow rates through the measuring cell. Cunningham *et al.*[19] have described a means of improving this by reducing pressure in the cell to increase volume flow without exceeding the mass flow.

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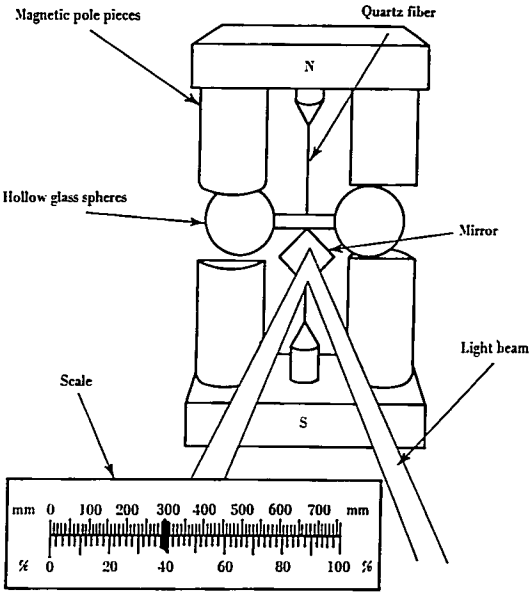

FIG. 1. Diagram of the paramagnetic O_2 analyzer. (Reproduced from Bellville, J. W.: Gas Analysis: A survey of measurements in anesthesiology, Techniques in Clinical Physiology. Edited by J. W. Bellville and C. S. Weaver, New York, Macmillan, 1969.)

The Rapox analyzer‡ utilizes the paramagnetic principle in combination with a pneumatic analogue of a Wheatstone bridge. It provides continuous O_2 analysis with a rapid (200 msec) response time and accuracy to within 2 per cent of full scale.

POLAROGRAPHIC ANALYSIS

The basic principle of the polarographic technique and its application to gas-phase analysis are considered at this point, although its major application lies in blood-gas analysis, where major advances have been made.

The basic electrode (fig. 2) contains four components, two terminals (platinum and silver), an electrolyte solution, and a gas-permeable membrane. The platinum terminal is the cross-sectional area (about 10–25 μm diameter) of a fine wire imbedded in a glass to which a negative voltage (about 0.5 to 0.8) is applied. A silver wire acts as the anode, or positive terminal. Electrical contact between terminals is achieved by immersion in an electrolyte solution (*e.g.*, KCl). A gas-permeable membrane (polypropylene) separates the electrolyte and terminals from the measured phase. O_2 molecules pass through the membrane and electrolysis occurs at the polarized platinum surface according to the following reactions:

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^- \quad (1)$$

$$H_2O_2 + 2e^- \rightarrow 2OH^- \quad (2)$$

The breakdown of O_2 alters the conductivity of the electrolyte solution, and as a result the current flow is proportional to the concentration of O_2 in the electrolyte solution.

Technical difficulties encountered in using the polarographic method for gas analysis have been described.[86,93] These include changes in sensitivity secondary to "cold stretch" of the membrane, alteration in electrolyte-layer thick-

‡ Godart/Statham, Belthoven, Holland.

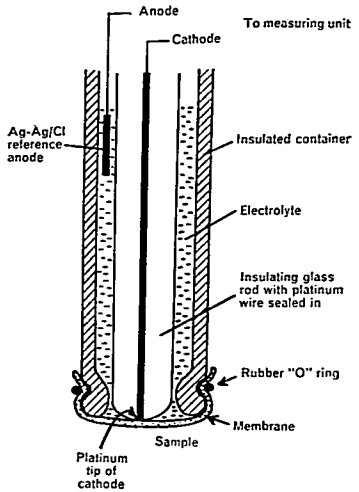

FIG. 2. Schematic diagram of Clark-type O_2 electrode. (Reproduced from Crampton Smith, A., and Hahn, C. E. W.: Electrodes for the measurement of oxygen and carbon dioxide tensions, Br. J. Anaesth. 41:732, 1969.)

ness, "dehydration" with changes in electrolyte concentration, deposition of silver on the platinum cathode, and artifacts due to gas bubbles in the electrolyte solution and pressure or temperature changes at the membrane.

In 1960, Kreuzer, Rogeness and Bornstein[54] reported a method for continuous analysis of O_2 by insertion of the electrode into the trachea. A response time of one second for 95 per cent deflection was reported. Disadvantages of the system included a response time too long for breath-to-breath analysis and the necessity for positioning the electrode within the trachea so as to overcome the changes in temperature and water vapor content which occur between inspired and expired gas when sampled outside of the airway (factors which must be considered with the polarographic method). Beneken Kolmer and Kreuzer[6] recently reported an additional modification, whereby a platinum/silver electrode covered with a Teflon membrane (6 μm thick) and maintained at constant temperature in a water bath allowed P_{O_2} outside of the airway to be measured with a rapid response time (0.2 to 0.3 sec for 95 per cent deflection). Similar modifications of platinum electrodes for respiratory O_2 analysis have been reported.[24,39,41,102,103] All include temperature control of the electrode environment and have rapid response times (less than 0.3 sec), making breath-by-breath O_2 analysis possible.

Mass Spectrometry Analysis

The mass spectrometer has seen limited application to respiratory-gas analysis since its introduction into medicine two decades ago.[49,68,90]

The principle of mass spectrometry is based on the ability to separate and identify substances according to molecular mass. A representative system, shown schematically in figure 3, consists of four basic components. Gas to be analyzed is drawn into an *inlet system* by a vacuum pump. A small fraction of this sample is admitted into the *source*, where ionization of the gas molecules is achieved by bombardment with an electron beam produced from a heated filament. The positively-charged ions are then accelerated and focused by means of an electrical field. This ion beam passes through a *magnetic field*, where it is deflected and split. Since the magnetic field is at right angles to direction of travel, the ions are deflected into a circular trajectory whose distance is determined by the mass, or more specifically, by ionic mass/charge ratios. The degree of deflection is greater for light than for heavy ions, assuming they have equal charges. By suitable positioning of *collector electrodes*, the quantity of ions at any particular mass number is detected, as the positively-charged ion releases its charge at the specific collector. The current is amplified and either recorded directly or displayed via an oscilloscope or digital voltmeter. This entire process of ionization through detection must be carried out in the presence of a high-vacuum system, with pressures in the range of 10^{-4} to 10^{-7} torr.

The basic design requirements for a respiratory-gas mass spectrometer and alternative methods for particle separation and detection have been extensively reviewed.[33,106,107] Application of respiratory gas analysis by mass spectroscopy with respect to pulmonary and

cardiovascular function has been considered in detail during a recent symposium.[95]

Numerous mass spectrometers for analysis of gases including O_2, N_2, CO_2, and also inert gases such as He, Ar, and Ne, are available commercially. These instruments offer several advantages over existing methods for gas-phase O_2 determinations. Continuous breath-by-breath analysis with a response time of approximately 100 msec for a 90 per cent step change in O_2 concentration is possible. Short-term accuracy (approximately 30 minutes) is to within ±0.1 per cent and to less than 2 per cent of full scale, with long-term drift of less than 2 per cent per day. The investigator can now have at his disposal an instrument that is compact, portable, and stable and requires little skill for calibration or operation.

Fuel-Cell Analysis

A solid electrolyte fuel cell § has recently been developed and is of potential value when a rapid response time and accuracy are needed in analyzing the gas phase. The cell (fig. 4) consists of calcium-stabilized zirconium oxide electrolyte molded into a tube. The internal and external surfaces of the tube are coated with a porous platinum film to form separate electrodes, the inner and outer tube surfaces. When the two surfaces are exposed to different O_2 concentrations, the following reactions occur:

$$\text{Cathode: } O_2 + 4e \rightarrow 2O^{=} \quad (1)$$

$$\text{Anode: } 2O^{=} \rightarrow O_2 + 4e \quad (2)$$

The outside of the tube is exposed to a known O_2 concentration (*e.g.*, ambient air) and the sample to be analyzed is drawn through the tube to come into contact with the inner surface. The electrolyte acts as a semipermeable membrane, conductive to O_2 ions but nonconductive to electrons. The conductivity of O_2 ions can be increased by heating the cell to 800–1,000 C. The electromotive force generated by the cell is expressed by the Nernst equation:

$$E = \frac{RT}{nF} \cdot \log \frac{P_1}{P_2}$$

where P_1 and P_2 are the O_2 partial pressures on the inner and outer surfaces, respectively.

Several modifications to improve stability, decrease response time, and improve configuration for physiologic analyses have been described.[28,91,105] The modified cell is capable of continuous on-line analysis, with response times in the range of 0.05–0.03 sec and accuracy comparable to that of Scholander analysis. In spite of these advantages, its limitations need comment. The high operating temperature (about 850 C) is hazardous in the presence of explosive gases. A combustible material, utilizing O_2 within the cell, will reduce the emf and create baseline instability. Halothane has been shown to produce sizable errors in measured O_2 concentration when present in the sample gas.[28]

A number of O_2 analyzers utilizing unheated fuel cells are available for routine clinical use, for monitoring infant environments, measurement during anesthesia and mechanical ventilation, etc. (An example is the Teledyne Percent Oxygen Detector.¶) Analysis is achieved with an electrochemical micro-fuel cell which consumes O_2 from the sample surrounding the detector. The cell generates an electric current proportional to the concentration of O_2 present; this current, in turn, drives a microammeter whose scale is calibrated in per cent O_2 (range 0–100 per cent). Accuracy and response time (±2 per cent full scale and 90 per cent response in 30 sec) are nowhere near the capabilities of the zirconium oxide cell, but are suitable for clinical and bedside use. Major advantages include ease of calibration, portability, ruggedness, and maintenance-free operation.

Analysis of Oxygen in Blood

Polarographic Analysis

Several recent reviews have considered the physical principles and design of the polarographic method for analysis of Pa_{O_2}.[1,2,17,30,44,56,84,87] Other articles have been concerned with specific difficulties associated with polarographic methods, *e.g.*, calibration, temperature, effects of storage, linearity, etc.[35,48,57,60,65,69,81,82,88] The reader is referred

§ Westinghouse Corp., Pittsburgh, Pennsylvania.

¶ Harris Calorific Company, Cleveland, Ohio.

FIG. 3. Schematic diagram of a magnetic respiratory-gas mass spectrometer designed for simultaneous recording of four gas concentrations. See text for detailed discussion of the major components. (Reproduced from Huygens, K: Experiences with the MAT Respiratory Air Mass Spectrometer. Paper presented at the "Mass Spectrometer Applied to Lung Physiology" meeting, London, May 1967.)

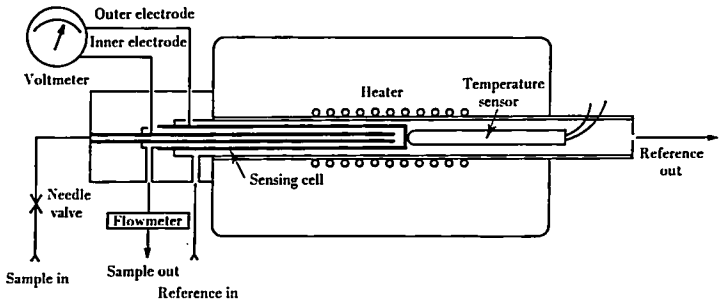

FIG. 4. Diagram of a solid electrolyte fuel cell. The reference gas (*e.g.*, ambient air) is passed along the external surface of the heated electrolyte tube, with the sample gas passing along the internal surface. See text for discussion. (Courtesy of Westinghouse Electric Corp., Pittsburgh, Pennsylvania.)

to the original literature for details. A basic description of the electrode is presented above; discussion here is restricted to recent advances in design and application for blood analysis.

The past several years have witnessed a concerted effort to realize continuous P_{O_2} analysis either by routing the arterial stream to an *in-vitro* system with continuous sampling or by direct insertion of an electrode-tipped needle or catheter into the artery. Numerous extracorporeal loop systems have been developed for the animal laboratory. Henningsen[45] has assembled a system for continuous measurement of P_{O_2}, P_{CO_2}, and *p*H, utilizing commercially available equipment. A continuous extracorporeal flow of 30–50 ml/min is passed through a constant-temperature (water-jacket) chamber containing electrodes. Feigl and D'Alecy[31] have recently described an O_2 electrode (Teflon membrane) cuvette within an extracorporeal loop to measure P_{O_2} of femoral and coronary-sinus blood continuously. The system offers the advantage of analysis at a low loop-flow rate (5–20 ml/min) with a rapid response time (90 per cent response in less than 3 sec). Gotoh[36] has successfully utilized an extracorporeal loop for continuous analysis and monitoring of arterial and venous gases and electrolytes in human subjects. Oxygen analysis is achieved by means of a modified Clark electrode housed in a multiple-electrode (*p*H, P_{O_2}, Na^+, K^+) thermostabilized acrylic cuvette. Distinct disadvantages of any extracorporeal loop include the necessity for multiple vessel cannulation, the need for pumping and thermal regulation within the loop, the possibility of sepsis, and the necessary heparinization of the subject. Veasy and associates[12,101] have described a computerized automated blood–gas analysis system designed to monitor critically ill infants. This system utilizes microelectrodes (*p*H, P_{CO_2}, and P_{O_2}) for analysis, with automated sampling, flushing, calibration, and data display governed by computer control. Measurements of P_{O_2} in the range of 10–150 torr were found to be as accurate as with the standard single-sample-*in-vitro* system. Marked, but reproducible, differences between results of the automated technique and values for individual samples measured *in vitro* were found at high P_{O_2}'s (>150 torr). These differences were attributed to mixing of the blood sample and the gas used for calibration in the automated system.

Continuous measurement of P_{O_2} with intravascular electrodes poses a number of technical problems.[80] Membrane-covered electrodes are flow- and pressure-sensitive.[53] Attempts to suppress flow sensitivity by increasing membrane thickness increase response time.[85] Thus, mean values rather than breath-by-breath changes are obtained. Oeseburg and associates[76] have recently described the clinical results of using a bare platinum electrode to measure changes in P_{O_2} during cardiac catheterization. Using a platinum-tipped cardiac

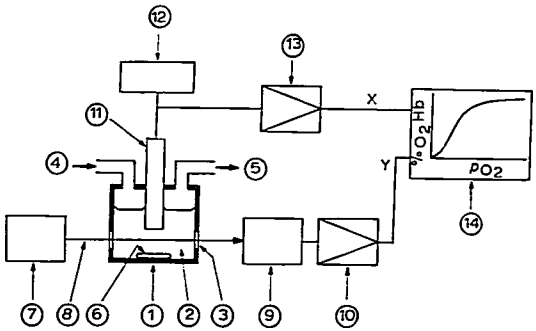

FIG. 5. Diagram showing the principle of automatic measurement of the oxygen equilibrium curve of hemoglobin. *1*, reaction cell; *2*, hemoglobin solution; *3*, window through which the light passes; *4*, inlet of nitrogen or air; *5*, outlet of gas; *6*, stirrer rod; *7*, light source and monochromator; *8*, monochromatic light; *9*, photomultiplier; *10*, amplifier for photomultiplier output; *11*, oxygen electrode; *12*, d.c. power supply; *13*, amplifier for oxygen electrode current; *14*, X–Y recorder.

catheter negatively polarized with reference to silver skin electrodes, spatial and temporal changes in P_{O_2} could be recorded. This electrode system has been successfully used to detect and localize intracardiac shunts by recording ΔP_{O_2} as the catheter is withdrawn through the heart. Although this system has a rapid response time and is flow-independent, it is inappropriate for long-term use because of changes in sensitivity that occur when protein is deposited on the platinum surface.

Galvanic electrodes (silver–lead) have been used to measure P_{O_2}.[46,51] A small, inexpensive catheter-tipped transducer for continuous monitoring has been described by Parker *et al.*[15,78] The transducer consists of a silver–lead galvanic cell located in the tip of a polytetrafluoroethylene (PTFE) catheter which is covered by a molded PTFE membrane, Galvanic electrodes, unlike that of Clark,[14] require no external applied voltage to produce reduction of O_2 at the cathode. The cell output is linear with P_{O_2} in the 0–150-torr range, is temperature- and flow-dependent, and has a response time of 2 seconds. Such cells have been reported to function *in vivo* for as long as 16 hours (72 hours *in vitro*, with actual membrane life being dependent upon deposition of fibrin and platelets).

Mass Spectrometer Analysis

The mass spectrometer has been adapted to measure blood gases *in vivo*. A flexible, gas-permeable, hollow, indwelling vascular cannula, which serves as an equilibrating tonometer, is connected to the mass spectrometer. The outer surface is in contact with blood and the inner surface is exposed to a low gas pressure (about 10^{-5} torr). Gas molecules diffuse through the membrane into the hollow cannula, then into the analyzer chamber, where the concentrations of specific gases are measured. P_{O_2} (mass 32) may be monitored continuously, obviating the need for blood sampling.

A technical review of membrane characteristics and their application to mass spectrometry has been published.[107] Woldring and associates[108] have described application of this technique to continuous analysis of blood–gas tensions in animals *in vivo*. Continuous intravascular measurement in humans has also been described.[42,104]

Factors limiting blood–gas analysis with such a system relate primarily to the mass range of the mass spectrometer and permeability characteristics of the membrane used. Mass range presents no problem for measuring O_2 (mass 32) and CO_2 (mass 44). Although attempts to prepare nonthrombogenic membranes have been made, long-term retention of membrane diffusivity is still a problem.[8] A heparinized silicone membrane covering a 22-gauge stainless steel cannula has been shown to have excellent diffusion characteristics for both O_2 and CO_2. Such membranes have considerable stability for as long as 24 hours of continuous use, with gas values comparing favorably with those determined by standard (electrode)

methods of PO_2 determination. Response time for a step change in O_2 concentration is generally 63 per cent in 15 seconds, with full equilibrium in less than a minute. Flow dependence (greater than 4–6 cm/sec necessary) and methodology for calibration *in vivo* are problem areas still to be resolved with commercially available devices.

Analysis of Oxygen Content

The O_2 content is that quantity of O_2 (O_2 in physical solution and O_2 in combination with hemoglobin) which can be extracted from a unit volume of blood. This is expressed as volume per cent, or volume of O_2 per 100 ml of blood at standard temperature (0 C), pressure (760 torr), dry, or STPD. The standard laboratory method by which all other determinations are judged is still the manometric method of Van Slyke and Neill.[100] However, despite its reliability, the method is time-consuming, and trustworthiness requires skill, neither feature being conducive to popularity.

Several attempts to develop simpler techniques have been made. A method extensively utilized is that first described by Baumberger in 1940,[3] and later confirmed by Neville.[71] The method involves release of O_2 combined with hemoglobin by potassium ferricyanide. The resulting change of P_{O_2} in the ferricyanide solution, measured with a polarographic O_2 electrode, can be used to calculate O_2 content. Several investigators have used a membrane-covered electrode to measure the released, dissolved P_{O_2}, whereby the calculations could

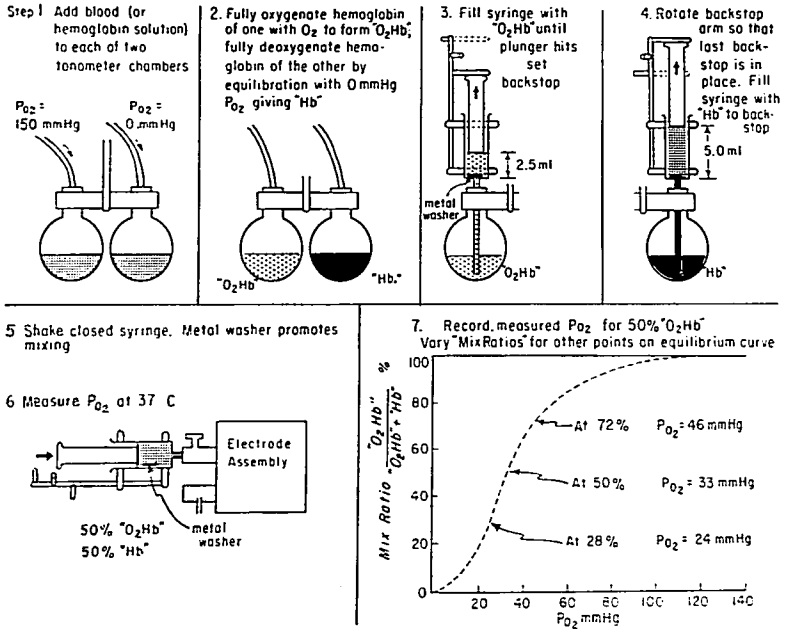

FIG. 6. Experimental procedure of mixing techniques.

be made.[22,42,53,59,67,83,92,96,97,98] Results appear uniformly reproducible, and are rapidly obtainable compared with the gasometric method (Van Slyke and Neill). Klingenmaier *et al.*[4,52] have described a similar method utilizing carbon monoxide (CO) to release O_2. It matters little whether O_2 is released from hemoglobin with ferricyanide or with CO (Klingenmaier *et al.*). All methods require small quantities of whole blood and careful temperature control (the electrodes are temperature-sensitive and the Bunsen solubility coefficient for O_2 necessary for the calculation is temperature-dependent). Accurate calibration of the membrane–electrode system is crucial. Each investigator has developed a special system which facilitates the necessary steps; some have chosen to mix blood with O_2-free solutions, other have taken advantage of the known contents of solutions in equilibrium with ambient air at known temperatures. There seems little to choose from when the data are analyzed. That numerous variations using the same principle have been developed bespeaks the problems inherent in the methodology. Widespread acceptance or routine use has not been part of the natural history of the technique. In the hands of the investigator or the technician aware of the need for scrupulous calibration of the PO_2 electrode, the method has worked well. This takes patience and skill. Unfortunately, neither can be expected to exist *a priori* in a laboratory dedicated to routine analyses.

Gas chromatography has had its moments in blood–gas analyses.[47] Extraction of the gases from blood is necessary (as with the Van Slyke–Neill manometric method). Individual components are separated by adsorption columns, while detection and quantification of individual gases (*e.g.*, O_2, CO_2, N_2) are provided by means of a katharometer utilizing thermal conductivity. The major technical difficulty with such analysis is the extraction of gas from blood and the use of carrier gases completely free of O_2. Davies[22,23] has described a system designed to simplify extraction and injection of gas obtained from an 80-μl sample of blood. Duplicate analyses may be accomplished in 8 minutes with excellent correlation with the results of Van Slyke–Neill analysis ($r = +0.999$). Unless a large number of samples is analyzed, this technique offers little over the manometric method when considered in terms of time and, above all, cost. It does, however, have advantages. First, the volume of blood used is small; second, it provides simultaneous readings for O_2, CO_2, and N_2 contents, which must be measured by additional manipulation of results obtained with the Van Slyke–Neill method.

An instrument** available commercially has been designed to provide automated measurement of blood O_2 content using a 20-μl sample of blood. Its accuracy and reliability in a clinical setting have yet to be established.

In the final analysis, we are still faced with the problem of measuring O_2 content rapidly and accurately. From the standpoint of pulmonary physiology, whole-blood CO_2 and N_2 contents can be of considerable interest. Clinical investigation is still hampered by the fact that routine determinations are not possible, and thus significant advances in this area are still desired.

Analysis of Oxygen in Tissues

"Tissue" P_{O_2} is a misnomer. In contrast to Pa_{O_2}, it exists not as a specific or average value, but rather as an O_2-tension field. The characteristics of this field, or O_2 gradient from the capillary wall to the mitochondria, depend upon a number of factors, such as the intracapillary tension, diffusivity across the extracellular and extravascular compartment, and finally, local O_2 consumption, which determines the gradients established for movement to take place. The implications of these factors with regard to measurement and the physiologic significance of tissue P_{O_2} have been reviewed by Lubbers,[62] Silver,[8], and Stuck and associates.[94] The present discussion is limited by necessity to description of two available techniques for measurement of tissue O_2, namely polarographic analysis and mass spectrometry utilizing a membrane-tipped capillary.

Polarographic Analysis

In the vast majority of attempts to measure tissue P_{O_2}, the platinum cathode has been used, without covering membrane, and negatively

** Lex-O_2-Con, Lexington Instruments Corp., Waltham, Mass.

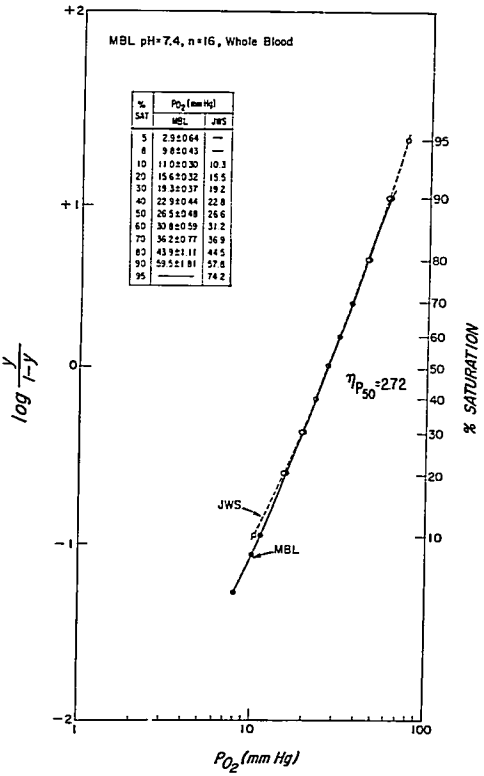

FIG. 7. Hill plot for mean O_2 dissociation curve from 16 samples of whole blood from a nonsmoker, obtained in a period of three weeks. Correction of individual curves to *p*H 7.40 was made according to Severinghaus. Mean carbon monoxide level (MBL): 0.14 ml/100 ml; methemoglobin level (MBL): 0.5 g/100 ml.

polarized to reduce O_2 at its tip.[10,24] A compromise must be achieved in the design: a large probe has a quick response time but consumes significant quantities of O_2 and, when inserted, can cause significant tissue distortion.[38] A small electrode surface (ultra-microelectrode), 1–5 μm^2, uses less O_2, has a response time, but is easily damaged.[11,18] Alteration of function secondary to protein deposition on the electrode surface is a major disadvantage of a non-membrane-covered electrode. Recent advances in design which employ platinum tips covered with gas-permeable membranes, thereby eliminating malfunction due to protein deposition, have been described.[62,89] The possibility of using this technique in man is still remote. Unless precise definition of the electrode tip position is possible, interpretation of results is impossible.

Mass Spectrometer Analysis

Woldring and associates have described analysis of tissue P_{O_2} *in vivo* by mass spectrometry.[20,21,77,103] Oxygen analysis is achieved via an indwelling tissue cannula connected to the mass analyzer. The cannula consists of a stainless-steel core covered with a Teflon membrane. A differential pressure between

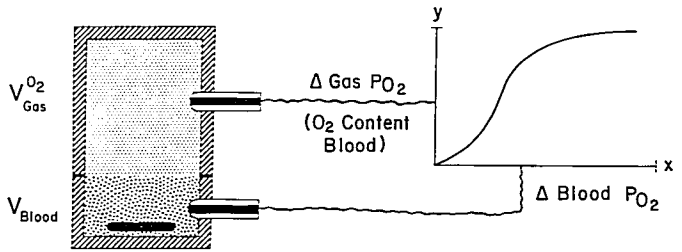

FIG. 8. Principle of the oxyhemoglobin-dissociation analyzer. Gas and blood chambers are shown in position for recording of curve. A volume of deoxygenated blood is exposed to a known volume of pure O_2 in a closed system. The change in P_{O_2} (O_2 molecules move from gas into blood), shown on the Y axis, is proportional to the change of blood O_2 content. The blood P_{O_2} is recorded on the X axis.

the inner and outer surfaces of the cannula results in continuous gas diffusion through the membrane, into the cannula, and subsequently to the analyzer chamber, where the concentration is determined according to the principles described above. A commercially-available instrument†† has a response time of several minutes (90 per cent step change in gas tension), with a typical sampling rate of 10^{-6} std ml/sec.

Again, it is difficult to interpret the values obtained by this measurement. We are dealing with a mean value for tissue P_{O_2} subject to error created by tissue damage secondary to placement of the catheter, while the long response time precludes real-time evaluation of these changes resulting from either O_2 delivery or O_2 utilization.

The Hemoglobin–Oxygen Relationship

In recent years substantial interest has been generated by the demonstration that products of erythrocyte glycolysis (*i.e.*, 2,3-diphosphoglyceric acid and ATP) can influence the affinity of hemoglobin for O_2. Since biochemical and physiologic aspects of this fascinating problem are discussed elsewhere in this symposium,[58] the comments which follow are confined to a discussion of methodology available for evaluation of this affinity.

The classic gasometric method relies on measurement of O_2 content of whole blood, hemolysates, or hemoglobin solutions, tonometered with known partial pressures of O_2–CO_2 under close control of *p*H. Once equilibration is achieved, oxyhemoglobin saturation is calculated from the ratio of O_2 actually bound to hemoglobin at the specified P_{O_2} to O_2 content present when hemoglobin is fully saturated (*i.e.*, O_2 capacity):

Per cent saturation

$$= \frac{O_2\ \text{content}^{TON} - 0.0031 P_{O_2}{}^{TON}}{O_2\ \text{content}^{SAT} - 0.0031 P_{O_2}{}^{SAT}} \times 000 \quad (1)$$

where:

$O_2\ \text{content}^{TON}$ = O_2 content at P_{O_2} of tonometry gas

$O_2\ \text{content}^{SAT}$ = O_2 content at P_{O_2} necessary for full saturation

Direct spectrophotometric measurement of oxyhemoglobin content in combination with P_{O_2} determinations using a membrane-covered electrode has simplified the procedure considerably. If changes in saturation are quantified after the addition of carefully measured volumes of O_2 (or ambient air) it is possible to eliminate the P_{O_2} measurement.[7,25] Unfortunately, spectrophotometric methods[50,72] are limited to evaluation of hemoglobin solutions or dilute erythrocyte suspensions that do not interfere with the path of the monochromatic beam of light (fig. 5). An alternative procedure for whole blood, the so-called "mixing technique," has been described by Edwards and Martin.[27] Following tonometry at the desired temperature, with a gas mixture of either

†† Scientific Research Instruments, Baltimore, Maryland.

N_2–CO_2 or O_2–CO_2, precise quantities of deoxygenated and fully oxygenated whole blood are mixed carefully in any desired ratio. The O_2 content of the mixture is known, since it is represented by the quantity of fully oxygenated blood added.

If we assume that the hemoglobin concentrations in the oxygenated and deoxygenated aliquots are equal, the calculations are made as follows:

O_2 content^{MIX} (ml O_2/ml blood)

$$= \frac{S}{100} \cdot O_2{}^{CAP} + P_{O_2}{}^{B} \cdot \alpha_{BLOOD}{}^{T} \cdot \frac{1}{760} \quad (1)$$

where

O_2 content^{MIX} = O_2 content after mixing (*i.e.*, O_2 added with known volume of oxygenated blood)

S = per cent saturation after mixing

$O_2{}^{CAP}$ = O_2 capacity or Hb (hemoglobin concentration in g/ml) times F (conversion ratio for ml O_2/g Hb)

$P_{O_2}{}^{B}$ = P_{O_2} of blood mixture

$\alpha_{BLOOD}{}^{T}$ = Bunsen solubility coefficient for O_2 in blood at temperature T

Solving equation (1) for S:

$$S(\%) = \frac{O_2 \text{ content}^{MIX} - P_{O_2}{}^{B} \cdot \alpha_{BLOOD}{}^{T}}{O_2{}^{CAP}} \times 100 \quad (2)$$

If we take into account the "mixing ratio," equation (2) is written as follows:

$$\text{Per cent SAT} = \frac{V_1[(Hb \cdot F) + \frac{\alpha}{760} P_{O_2}{}^{G}] - (V_1 + V_2)\frac{\alpha}{760} \cdot P_{O_2}{}^{B}}{(V_1 + V_2)Hb \cdot F} \times 100 \quad (3)$$

V_1 = volume (ml) of added oxygenated blood

V_2 = volume (ml) of added deoxygenated blood

$P_{O_2}{}^{G}$ = P_{O_2} of tonometer gas phase necessary for full saturation and in equilibrium with the blood.

At constant *p*H, the proper choice of mixing volumes (see fig. 6) will provide several points on the steep portion of the dissociation curve, and a graph of $\log \frac{\% \text{ SAT}}{1 - \% \text{ SAT}}$ versus $\log P_{O_2}$ (Hill plot; see fig. 7) will permit ready calculation of P_{50}, or P_{O_2} at 50 per cent saturation. Accuracy of the technique diminishes as saturation rises; small errors in mixing volumes can give rise to large differences in P_{O_2}. Further difficulties are encountered when working with abnormal hemoglobins having greater than normal affinity for O_2 (*i.e.*, high saturation at low P_{O_2}), since deoxygenation can be achieved only with difficulty. Unless the absence of O_2 is carefully demonstrated with the aid of the O_2 electrode, significant errors will be introduced on calculation of per cent saturation (*i.e.*, O_2 content of the mixture will be underestimated).

An additional modification in methodology, whereby dynamic registration of the O_2 content–P_{O_2} relationship is possible for whole blood at any desired hematocrit, as well as for hemoglobin solutions (fig. 8), has been achieved by Duvelleroy *et al.*[26] Disadvantages of the method include the cost and complexity of the equipment.

The problem of how to express changes in affinity found in whole blood has not been resolved. On first impression it appears that definition of $P_{50/7.40}$ (*i.e.*, P_{O_2} at 50 per cent oxyhemoglobin saturation and *p*H 7.40) is a convenient basis for comparison. Unfortunately, correction to a "standard" plasma *p*H fails to take into account the possible deviation from normal of the intraerythrocytic hydrogen-ion concentration. Most published data have assumed that the Bohr effect ($\Delta \log P_{O_2}/\Delta\, p$H) applies equally to normal and abnormal blood, but the validity of this assumption remains to be established. Second, definition of an abnormality at a standard *p*H (*i.e.*, 7.40) appears less than rational when the arterial value *in vivo* may deviate significantly from this number.

From this brief review, it should be evident that state of the art readily permits measurement *in vivo* of O_2 in all forms normally found in whole blood. Progress has been achieved with intravascular measurement to the point

where these values can be obtained with reasonable accuracy if care in calibration of the sensor is exercised. We still need a meaningful approach to the evaluation of oxygenation in individual organs and tissues. Its clinical use must await more extensive laboratory work.

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