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Investigation of Quantitative Polarographic Measurements of Oxygen Tension in Tissue

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INVESTIGATION OF QUANTITATIVE POLAROGRAPHIC MEASUREMENTS OF OXYGEN TENSION IN TISSUE

by

Albert Frederick Kelso

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 1959
BIOGRAPHY

Albert Frederick Kelso, born in Fort Wayne, Indiana, November 19, 1917, was the first of seven children in the family of Newton T. and Dorothy Leidolf Kelso. He received his elementary education in Fort Wayne at Miner and Hoagland grade schools, and graduated from South Side High School in 1936.

In the fall of 1937 he entered George Williams College, specializing in informal education in the fields of Health and Physical Education. In 1940 he was awarded the Edward D. Steinhaus Scholarship in Health and Physical Education. The scholarship provided the opportunity for training in physiological research. Working with Dr. Arthur N. Steinhaus, he completed a study of the effects of cold hip baths on sensori-motor performance. A degree of Bachelor of Science in Physical Education was granted in 1943, and the degree of Master of Science in Physical Education in 1946.

Margaret Esther Wade became his wife on October 21, 1943. The marriage followed the completion of his mid-shipmen training and his commissioning as Lieutenant, j.g., U.S.N.R. He was on active duty in the Pacific Area as communications officer of the U.S.S. Bellatrix and as Navigator and Assistant Executive Officer of the U.S.S. Lubbock.

Following his release to inactive duty, he instructed in
biology and in physiology as a member of the Faculties of George Williams College and Chicago College of Osteopathy. He also studied at Roosevelt University to complete the requirements prerequisite for entrance to a doctorate degree program. During this period three children, Elizabeth Ann, born December 5, 1946, Pamela Dorothy, born September 11, 1948, and Arthur Frederick, born September 18, 1950, and his wife assisted and encouraged him in the completion of his education.

In the spring of 1953 he began a program of study towards the degree of Doctor of Philosophy at Loyola University. While engaged in the doctorate training program he continued part-time teaching as a faculty member of the Chicago College of Osteopathy. He completed requirements for the degree of Doctor of Philosophy in the spring of 1959.
ACKNOWLEDGEMENTS

The author wishes to express appreciation to Dr. Mulder, deceased former chairman of the Department of Physiology, for affording him the opportunity to study for an advanced degree. He is most grateful to Dr. Clarence N. Peiss and Dr. Walter C. Randall for the stimulus and direction he has received while working with them, as well as the assistance they have rendered in the various phases of his training. The indirect assistance of his family, Dr. Shannon C. Allen, and Mrs. Betty Witte and Mrs. Joyce Kiley is acknowledged for the immeasurable contribution they have made.
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CHAPTER I
GENERAL INTRODUCTION, REVIEW OF THE BACKGROUND AND
IDENTIFICATION OF THE PROBLEM

This dissertation presents the information available on polarographic measurements of oxygen concentration in vivo and in vitro with a comparison of the relative accuracy in the two systems. The factors which might account for decreased accuracy in vivo are discussed, and experimental results are presented as an original critical test of quantitative polarographic measurement of oxygen tension in tissue. The introduction indicates the role of measurement of tissue oxygen tension in the more general problems of relating oxygen supply to vital processes. It also includes a discussion of diffusion and the principles of polarography pertinent to the investigations which are reported and to the material presented in the text.

Polarographic measurement of the tissue oxygen tension represents a recent approach to investigation of the relationship of oxygen to vital processes. Since the investigations of Boyle, Mayow, Priestley and Lavoisier indicated the fundamental role of oxygen in biological processes, biochemists, biophysicists and biologists have elucidated many aspects of this relationship. The present status of knowledge in this field is illustrated in Table I. Under vascular influences are listed the external respiratory, transport, and delivery factors related to oxygen
**TABLE I**

**DETERMINANTS OF TISSUE AVAILABILITY OF OXYGEN**

Compartments Involved and Processes Occurring Within Compartments

<table>
<thead>
<tr>
<th>VASCULAR</th>
<th>INTERSTITIAL</th>
<th>CELLULAR (Mitrochondria)</th>
</tr>
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<tr>
<td><strong>VASCULAR INFLUENCES</strong></td>
<td><strong>INTERSTITIAL FACTORS</strong></td>
<td><strong>CELLULAR INFLUENCES</strong></td>
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<tr>
<td>EXTERNAL RESPIRATION</td>
<td>DIFFUSION + 90-99? %</td>
<td>UTILIZATION</td>
</tr>
<tr>
<td>Oxygen saturation (Ventilation and Diffusing capacity)</td>
<td>CONVECTION (bulk transport)</td>
<td>(QO₂ : μl/mg/min)</td>
</tr>
<tr>
<td>TRANSPORT</td>
<td>Transcapillary + 0.3 %</td>
<td>For example:</td>
</tr>
<tr>
<td>Oxygen capacity</td>
<td>H₂O formation = 0.3</td>
<td>(Substrate = DPN(H)</td>
</tr>
<tr>
<td>(1.36 ml/gm Hb)</td>
<td>Temperature + ?</td>
<td>FAD(H) = (Cytochrome</td>
</tr>
<tr>
<td>(Dissolved O₂)</td>
<td>Pulsation + ?</td>
<td>system) = O₂)</td>
</tr>
<tr>
<td>PERFUSION OF TISSUE</td>
<td>Active 0</td>
<td>STORAGE</td>
</tr>
<tr>
<td>(ml/ml/min)</td>
<td></td>
<td>UTILIZATION</td>
</tr>
<tr>
<td>DELIVERY AT TISSUE</td>
<td>STORAGE</td>
<td>(eg. Myoglobin)</td>
</tr>
<tr>
<td>HbO₂ = HHb + O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH and T effects)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Diffusing capacity and rate at tissues)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
supply of the tissues. With the exception of the diffusing capacity and rate between capillaries and tissue these factors have been known for several decades. As indicated in the last column of the table many of the cellular influences have been intensively investigated. Respiratory quotients for tissues and cells and respiratory rates for small organisms have been established by various in vitro techniques. The specific chemical reactions for the metabolism of oxygen are known in general and in some respects in great detail. It has been possible to relate the chemical reactions by which oxidative processes are coupled with the formation of high energy bonds to portions of the mitochondria within the cell. In the center column of the table are the interstitial factors related to oxygen supply to the cells. August Krogh established diffusion as the fundamental process occurring at this level. Other factors and factors which modify diffusion have not been established. A quantitative method for measurement of oxygen concentrations at the cellular level would aid in the development of information on oxygen transport, circulation and metabolism at the cellular level in vivo.

DIFFUSION

An understanding of diffusion is basic to the development of a quantitative measurement of oxygen in tissues. In the following discussions of the diffusion current of polarographic measurement, the calculation of estimates of tissue oxygen tension and of
transfer of oxygen from the blood to the tissue involve various characteristics of diffusion. The following paragraphs present the information essential to the discussions, instrumentation and methods entailed in the investigations reported.

Studies of diffusion gained quantitative significance through Fick’s laws, in which he introduced a diffusion coefficient which makes it possible to calculate either the quantity of material crossing a surface in a diffusion system or the quantity of material accumulating within a portion of a diffusion system. Fick’s first law describes the flux of material in a system where area, concentration gradient and time have been defined,

\[ Q = -DA \frac{dc}{dx} dt \]  

(1)

- \( Q \) - Quantity
- \( \frac{dc}{dx} \) - gradient
- \( D \) - Diffusion coefficient
- \( A \) - Area
- \( dt \) - interval

Fick’s second law describes the rate of accumulation of a substance within a given volume of a diffusion system when the concentration gradient across that volume and the time have been defined.

\[ \frac{dc}{dt} = D \frac{d^2c}{dx^2} \]  

(2)

The diffusion coefficient, \( D \), has the same unit and dimensional values in both cases. In the first law \( D \) has been defined for a uniform concentration gradient and is identified as the differential \( D \). In the second law \( D \) has been obtained during a
variable concentration gradient and is identified as the integral \( D \) or \( \bar{D} \). The magnitude of \( D \) and \( \bar{D} \) is identical only for conditions in which \( D \) is linear with respect to concentration gradients of different magnitudes.

Fick's diffusion coefficients are widely used and it is frequently necessary to change the units of area, length, concentration, quantity or time for a particular use. Accurate changes can be made only on the basis of adequate knowledge of both the units and dimensional values involved. In the cgs* system the numerical value of \( D \) determined from Equation 1 is

\[
D = \frac{Q}{A} \times \frac{d^2}{dc} \times \frac{1}{t} = \frac{gm}{cm^2} \times \frac{cm}{(gm/cm^3)} \times \frac{1}{sec} 
\]  

(3)

Upon cancellation this reduces to \( cm^2/sec^{-1} \) or a dimensional value of \( L^2t^{-1} \).

Calculation of gas diffusion in liquid and biological systems is complicated by lack of information on one or more of the variables required to establish a diffusion coefficient for these systems. August Krogh* introduced the method of expressing the gas concentration in terms of the pressure of the gas in the gas phase, and quantitative treatment of diffusion in solution or biological systems became possible. In this method the unit and dimensional value for a diffusion coefficient** appear to be

---

*Centimeter gram second system of units.

**There is no advantage in using the term constant, since the confusion between Fick's \( D \) and Krogh's \( D \) arises from applicability and dimensions, rather than from absolute value.
nearly identical to those used by Fick. Krogh's units were
milliliter of gas for quantity, atmospheres of gas tension for
concentration gradient, microns for diffusion distance, and min-
utes for diffusion time. The unit value of Krogh's diffusion
coefficient as determined by Equation 1 is

\[ D_K = \frac{\text{ml}}{\text{cm}^2} \times \frac{1 \times 10^{-4} \text{cm}}{\text{atmos}} \times \frac{1}{60 \text{ sec}} \]  

(4)

Alpha, the absorption coefficient, can be used to convert tension
into concentration if the solubility of the gas is known.** Multiplying
the tension of the previous unit analysis by alpha results in

\[ D_K/\alpha = \frac{\text{ml}}{\text{cm}^2} \times \frac{1 \times 10^{-4} \text{cm}}{\text{cm}^2 \times \text{atmos}} \times \frac{1}{60 \text{ sec}} \]  

(5)

This reduces to the dimensions of \( L^2t^{-1} \), which is the same as the
dimensions of Fick's D. It is evident that if both D's are ex-
pressed in the cgs system, they differ only by the effect of
solubility of the gas or in the use of tension for calculation of
the concentration gradient. i.e.

\[ D_K/\alpha = D \]  

(6)

*Defined as the quantity of gas in solution at equilibrium
with the partial pressure of a gas phase at a given temperature.
ml/gas/ml solution.

**The major advantage of Krogh's diffusion coefficient is
that solubility, which in many cases could not be determined,"
need not be known.
Some biological systems may have an unknown diffusion distance and/or diffusion area, in addition to an unknown concentration gradient. Several methods allow quantitative treatment of the diffusion process in spite of the number of unknown variables. The methods are similar in that in each case all of the unknowns are incorporated into a proportionality constant of diffusion.

Diffusing capacity of the lungs is an example of a case in which area and distance are unknown, and permeability constants are examples of cases in which distance is unknown. Generally concentration gradient and area are known, but the derivation of the constant should be examined carefully to determine which unknowns are included with the diffusion coefficient.

There is considerable variation in the freedom which can be taken with constants and coefficients during their application. The diffusion coefficient represents properties of both the material which is diffusing and the system in which the diffusion occurs. Consequently the application of diffusion coefficients is limited to the system for which they have been defined. In the case of Fick's $D$ and to some extent of Krogh's $D_k$, useful application is extended to other systems because factors have been determined which allow corrections to be made. Useful corrections have been established for temperature, viscosity, and concentration differences. It is also possible to predict diffusivity for a given system on the basis of the inverse ratio of the molecular weights if the diffusion coefficient of one of the molecules is
known. Theoretically, proportionality constants of diffusion are limited to a system identical with the one for which they are determined. As they contain unknown variables, the extension of their application to other systems implies that the unknown factors are identical in the two systems.

Polarographic Principles

The principles of polarography and the fundamentals of polarographic instrumentation are presented in this section. A discussion of electrode reactions, modifications of electrodes and circuits applicable to the thesis problem is included. A review of the literature and a summary of polarographic measurements of oxygen concentration completes the section.

Polarography is an instrumental method of electrochemical analysis of the qualitative and quantitative nature of substances in solution. It differs from other forms of electrolysis used analytically. In conductometric and electrophoretic analysis the movement of charged particles in the electrolysis cell is analyzed. Potentiometric and electrolytic methods are similar to polarography in that they depend upon oxidation-reduction reactions. In the potentiometric method the analysis is based on the potentials established by the reaction, while in the electrolysis method the analysis is based on the quantity of material removed from the solution by the reaction. In both of these methods the body of the solution of the electrolysis cell is analyzed.
polarography both the reaction and quantity of substance are involved, and the body of the solution remains essentially unchanged by the reaction, which is limited to the small region in the immediate vicinity of the electrode.\textsuperscript{12}

Polarographic analysis is accomplished by establishing concentration polarization of the analysis electrode, relating the potential at which concentration polarization is established to the nature of the reactant, and the current flow following concentration polarization to concentration.

During electrolysis, conditions may cause one of the processes to become slower than other processes related to the electrode reactions.\textsuperscript{15} When this rate limiting process occurs, the electrode will assume an impressed voltage instead of the voltage characteristic of the electrolysis reaction, and it is said to be polarized. The causes of polarization include slowness of the formation and/or discharge of ions, the slowness of transfer of reactant or products in the region of the electrode, and the slowness of reaction rates during electrolysis.\textsuperscript{15}

In polarography conditions are controlled to cause concentration polarization (slowness of transfer of reactant to the electrode) by making the analysis electrode very small and keeping it either motionless or in constant motion relative to the solution.\textsuperscript{14} At the same time the other electrode is kept at constant potential, the reference potential of the cell, by prevention of polarization at this electrode.
In practice, a relatively positive potential is established at the analysis electrode and the current flow in the electrode circuit is recorded as the analysis electrode is made progressively more negative with respect to the reference electrode. A plot of the current observed relative to the analysis electrode potential is obtained, as illustrated in Figure 1. In the figure

![Diagram of polarograms](image)

**Figure 1.**

**Polarograms of Oxygen at Different Concentrations**

the portion of the c-v curve to the left of the negative two-tenths
volt is identical for all c-v curves of different concentrations. The electrode is polarized since no oxidation-reduction reactions occur at the applied voltages. Between a negative two-tenths and four-tenths applied voltage, the oxygen in solution is reduced at the electrode, and the electrode is depolarized by the presence of the reaction. The initial rise on the c-v curve represents the decomposition potential. The peak inflection represents the limitation of current flow due to the limitation of the reaction rate by slow rate of transfer of reactant from the body of the solution to the electrode surface. The voltage corresponding to the point halfway between the initial rise and the terminal inflection is the half-wave potential of the polarographic reaction. This is characteristic of the reaction and is independent of concentration. It is dependent upon the characteristics of the system since the nature of the supporting electrolyte solution, the material of the electrode and the condition of the electrode surface will modify the half-wave potential. Quantitative polarographic analysis is based upon the fact that for a given system the half-wave potential characterizes the oxidation-reduction reaction.

In Figure 1 the various curves are labeled with the volume per cent of the gas mixture which was used to equilibrate the

*It is only partial depolarization, since there should be no change in potential with increased current flow for complete depolarization.
solution. Each curve represents the total current flow, \( i_T \), for the particular concentration. Quantitative polarographic analysis depends upon resolving the total polarographic current, \( i_T \), into components and the identification of one of these components with the diffusion-limited concentration polarization of the analysis electrode. The components which are represented in Figure 1 can also be represented symbolically as indicated in Equation 7.

\[
i_T = i_r + i_d + [i_m + i_a + i_k]
\]  

(7)

- \( i_T \) - total current
- \( i_r \) - residual current
- \( i_d \) - diffusion current
- \( i_m \) - migration current
- \( i_a \) - adsorption current
- \( i_k \) - kinetic current

The values which appear in the brackets do not appear in Figure 1. The bracketed values represent factors which under certain conditions modify the polarographic current.

The residual current is defined as the current which would be present in the absence of the oxidation-reduction reaction related to the analysis.\(^{12-14}\) This current varies in different systems and varies with time following the application or change in potential during analysis. It is therefore necessary to determine the residual current of each system and to make allowances for the variation with time.

The residual current includes the current flow due to the resistance of the solvent, electrolysis of substances reduced at more positive applied potentials than the potential used during
analysis, and a charging current, $i_c$.\textsuperscript{12-14} The total current will be a function of the electrode surface area and will remain constant provided electrolysis current remains constant. The charging current or condenser current, as it is called in polarography, is a constant for a given electrode surface area and external resistance of the circuit.\textsuperscript{*}

On the basis of this analysis it is evident that the residual current, $i_R$, can be treated as a constant under the following conditions:

1. It is determined for the analysis system in use.
2. Modification of $i_R$ by extraneous oxidation-reduction reactions is ruled out.
3. The residual current is determined after the condenser current influence is essentially zero.

Since the residual current stabilizes within a few tenths of a second for most circuits,\textsuperscript{14, 16, 17} this component of the total polarographic current is easily resolved.

The diffusion current, $i_d$, is defined as that portion of the total current associated with electrolysis of the substance transferred to the electrode by diffusion.\textsuperscript{12-14} The gradient for diffusion is established by depletion of the concentration of substance at the electrode surface by electrolysis. If no other

\*A Helmholtz-Gouy-Stern layer (ion cloud) is formed at the electrode surfaces of electrolysis cells when a potential exists on the electrode. This layer acts like a condenser and the charging time is a function of the external circuit resistance. The capacity is a function of the effective surface area of the electrode and a value of 20 $\mu$F/cm$^2$ has been cited.\textsuperscript{13}
forces are responsible for the transport of substance to the electrode, the polarographic current becomes diffusion limited and will vary with the concentration of the substance in the body of the solution. When the diffusion component is resolved and the other factors discussed above are controlled, the diffusion current varies directly with concentration. The only requirement to be considered with respect to the diffusion current is that it must be a diffusion limitation of the electrolysis process.

A number of other forces supplement the polarographic current. Mechanical, gravitational, thermal and electrolytic forces tend to modify the diffusion of substance to the electrode. Transfer of substances to the electrode frequently occur as the result of convection associated with mechanical movement of the electrode or solution, as a result of thermal currents associated with electrolysis or temperature differences in the system, and as a result of convective currents associated with different densities of the solution during electrolysis. These factors are variable. Unless they are controlled, they decrease the accuracy of polarographic analysis. During electrolysis the charge on the electrode supplements the diffusion process and decreases the transport of ionized substances to the electrode. This effect, known as the migration current, $i_m$,\textsuperscript{12-14} is suppressed for ionized substances by addition of an indifferent electrolyte.

The effects of migration current and convection require the following additional considerations during analysis:
1. Minimization of density influences by positioning the electrode.

2. Using water baths and precise temperature control for the electrolysis cell.

3. Establishment of a constant diffusion gradient.

4. Minimizing the influence of the condenser current.

Several physico-chemical processes supplement the polarographic current. Substances adsorbed on the electrode increase the polarographic current when they are discharged during electrolysis.12-14 The discharge may be relatively rapid for surface adsorption, but may be quite protracted in some cases. In this discussion the chemical influences are incorporated into a single constant, the kinetic current, \( i_k \), of the equation. During electrolysis some products may decompose spontaneously causing increased availability of reactant and enhancement of the polarographic current.12 Enhancement also occurs in those reactions in which some part of the reaction which previously has been rate-limiting is modified by the electrolysis, resulting in increased availability of reactant.12 Occasionally electrolytic products or the products which they form with substances in solution will act as catalysts for the reaction being analyzed.14 The physico-chemical influences discussed above require the following considerations to be made in order to insure valid polarographic measurements:

1. Recognition and allowance for the presence of adsorption phenomenon.
2. Analysis of reactions associated with electrolysis for their influence.

3. Awareness of possible kinetic effects in the reactions, and analysis of data for this influence.

Polarographic analysis of oxygen tension in solution or in tissues depends upon the electrolytic reduction of oxygen in the presence of water. For neutral or alkaline solutions the reaction is

\[
\frac{1}{2} O_2 + 2 H_2 O + 2 e^- \rightarrow H_2 O_2 + 2 O^{-} \quad (8)
\]

\[
H_2 O_2 + 2 e^- \rightarrow 2 O^{-} \quad (9)
\]

\( E^{\frac{1}{2}} \) of reaction in Equation 8 is about -0.05 versus S.C.E.* for a dropping mercury electrode, d.e.,** and is approximately one-tenth volt more negative for bright platinum electrodes in physiologic saline solution. The \( E^{\frac{1}{2}} \) of the reaction in Equation 9 for the above systems is -0.9 volts and is not sharply resolved with either the mercury or platinum electrodes because of the extreme breadth of the wave.

Quantitative measurement of oxygen tension by the polarographic diffusion current depends upon the number of electrons involved in the reduction of oxygen. If the first reaction alone is

---

* S.C.E. will be used to indicate the Saturated KCL calomel reference electrode in the remainder of the text.

** In the remainder of the dissertation this electrode will be designated d.e.
involved it is a two electron transfer, while if both reactions are involved, it is a four electron transfer. There is still some question concerning the reduction, 13, 18-20 Davies and Brink 21 calculated the electrons involved in oxygen reduction and found evidence for a two electron transfer with one electrode and a four electron transfer with another. The two electron transfer would be expected at the more positive applied potentials except for the fact that the breadth of the hydrogen peroxide wave places the decomposition potential at applied potentials used for oxygen analysis. If it is assumed that the electrode surface determines either the decomposition potential of hydrogen peroxide or the transfer of the second pair of electrons the current-concentration relationship for a given electrode is constant.

It is generally accepted that below pH 9.0 the oxygen-water-hydrogen peroxide couple described by Equations 8 and 9 is not reversible. 18, 19, 22-24 Consequently, Faraday's laws predict only the equivalent current for the reduction of oxygen; the actual current will be somewhat more. The following equation is the result of incorporation of the diffusion limitation of polarographic currents into a mathematical expression for predicted current flow:

\[ \text{i}_d = nFCA \left[ \frac{D}{r} \right]^\frac{1}{2} \]  (10)
\[ i_d - \text{diffusion current} \quad \text{c} - \text{moles} \]
\[ n - \text{Faraday/mole} \quad A - \text{electrode area} \]
\[ F - \text{Faraday} \quad D - \text{diffusion coefficient} \]
\[ t - \text{time} \]

Krogh's coefficient can be used if \( n \) and \( C \) are converted to the system based on tension; however, it is easier to use Fick's \( D \), as solubility must be known in either case.

The magnitude of the current from the reactions in Equations 8 and 9 is independent of \( \text{pH} \) effects, but depends upon temperature, viscosity, and composition of the supporting electrolyte, since all of these affect the diffusion coefficient, and in some cases, the electrolysis reaction. A temperature correction of 1.3 - 1.6\% for each degree centigrade temperature change at physiologic temperatures is recommended by Lingane.\(^{13}\) In studies of the influence of viscosity, \( i_d \) has been shown to vary inversely with the square root of the viscosity of the supporting solution.\(^{20,25,26}\) The concentration influence on oxygen has not been studied; however, other small ions in solution have been studied with no recommendation made for correction of \( i_d \) on the basis of concentration of supporting solution.

Several of the physico-chemical factors mentioned earlier are known to influence polarographic measurement of oxygen concentration. Adsorption influences the current in two ways. Inactive substances adsorbed or precipitated on the electrode decrease the
effective surface area,²⁷,²⁸ causing decreased sensitivity of the electrode. This is a variable influence, at present it appears best to discount results obtained with such electrodes. Because oxygen is strongly adsorbed on platinum electrodes, it contributes considerably to the initial polarographic current. While studying this phenomenon for short periods of adsorption, Olson found that the major discharge is within the period of the condenser current. ¹⁶ Although quantitative studies of longer periods of adsorption have been made, it is possible that part of the influence of degassing electrodes and of the aging effect is the removal of adsorbed oxygen. Several studies in vitro have indicated that quantitative measurement of oxygen is modified by the presence of hydrogen peroxide.²⁹,¹³ In tissues this can probably be discounted. The hydrogen peroxide formed during the electrolysis of oxygen spontaneously decomposes, and in tissue the process is catalyzed by peroxidases and catalase.³⁰ It is doubtful that decomposition in tissues in presence of catalysts increases the oxygen availability to the electrode, since the oxygen is probably incorporated into metabolic reactions. This would cause a reactive error in the metabolism, but would not increase the polarographic current.

Metabolism can also be modified by other aspects of the oxygen analysis reactions. The electrolysis produces hydroxyl ion which by shifting the pH could markedly alter the state of the tissue. This occurs because the enzyme systems are sensitive to
pH and also because metals and alkaline earths tend to be precipitated at high pH's.

Based upon the preceding discussion polarographic measurements of oxygen in tissue are subject to the additional considerations listed below:

1. There is some uncertainty as to the number of electrons involved in the reduction of oxygen.
2. Temperature and viscosity variations will modify id.
3. Electrode sensitivity must be monitored to detect decreased effective surface area.
4. Allowance must be made for gas adsorbed during inactive periods.
5. The influence of hydrogen peroxide upon the id observed for oxygen may be different in tissues than in solution.
6. The pH change and the production of hydrogen peroxide during polarographic analysis may cause reactive errors in the tissues.

METHODS OF OBTAINING pO2 FROM POLAROGRAMS

Concentration or tension of oxygen in solution is obtained from the polarographic current in one of several ways. A discussion of the basic methods involved in obtaining reliable concentration analysis from polarographic measurements has been written by John Keenan Taylor of the National Bureau of Standards. This discussion has been used as the essential basis of the following presentation. Figure 2 represents typical c-v curves for oxygen at various concentrations and illustrates the
various methods.

In the absolute method, a c-v curve for each of several known and unknown concentrations is recorded. The plateaus before decomposition occurs and the plateau after concentration polarization has been established are extrapolated and interpreted in terms of the current ordinate of the polarogram. The concentration can be obtained from a graph of $i_d$ plotted as a function of concentration. It is also possible to use a constant ($k=i_d/C$) if it is established that there is a linear relationship between $i_d$ and concentration. In this method it is presumed that only diffusion is contributing to the current used for concentration analysis. The precision of the method is excellent since (a) the conditions have been controlled, (b) the system is the same for known and unknown analysis, and (c) the c-v curve is available for detection of extraneous influences. Taylor$^{31}$ states that an error of less than 1.5% occurs in the determination of concentration when there is (a) less than 2% error in the calibration, (b) less than 1% change in electrode surface area, (c) less than 0.5°C change in temperature, or (d) less than 0.5% alteration in parameters of the electrical circuit.

There are three comparative methods based on the principle that current at a constant voltage, rather than current over an applied voltage span under certain conditions, can be used to obtain concentration analysis. The method is illustrated in Figure 2, by the line drawn perpendicular to the abcissa at five-tenths.
**FIGURE 2.**

**THE POLAROGRAPHIC C-V CURVE**

Polarograms of oxygen reduction at solid electrodes. The dotted lines indicate the method of extrapolation. The vertical line at -0.55 volts indicates the voltage used for comparative analysis, and the arrows indicate for the comparative analysis. Equilibrated oxygen:

1. residual current, 0 mm Hg oxygen tension.
2. 133 mm Hg oxygen tension (21 vol% oxygen)
3. 380 mm Hg oxygen tension (50 vol% oxygen)
4. 760 mm Hg oxygen tension (100 vol% oxygen)

**FIGURE 3.**

**CURRENT-CONCENTRATION RELATIONSHIP**

1. Plot of $i_d$ against concentration (from Figure 2.)
2. Plot of $i_T$ against concentration. Note that the two plots differ by the slope of the residual current.
of a volt. At this voltage, slight variations in either the reference potential or in applied potential will not appreciably alter the current flow. Under these conditions polarographic current for known concentrations can be used to establish the basis for unknown concentration analysis. The simplest procedure is to plot \( i_T \) as a function of several concentrations in the range of the expected unknown concentration and to analyze the results by graphic interpolation. This method is presented as curve (2) in Figure 3. It is also possible to add a known amount of substance to the system and to use the constant obtained, \( k' = i_T/C_\text{added} \), for evaluating the currents obtained in analysis of unknown concentrations. Correction for residual current and non-linearity of the current-concentration relationship is necessary for satisfactory precision. The third method depends on the fact that \( i_d \) of two substances is determined by their electrochemical equivalence and diffusion rate. Consequently it has been possible to add a known amount of some substance to the system, which is reduced (or oxidized in anodic analysis) at the applied potential. A factor \( k'' \) similar to \( k' \) is obtained. \( k'' \) is converted to \( k' \) on the basis of the difference between electrical equivalence and diffusion of the two substances.

\[
k' = k'' \times \frac{n'}{n''} \times \frac{D'}{D''}
\]

(11)

In the pilot ion addition method just described above, the same limitations are implicit as in the use of \( k' \). In the three
comparative methods the precision could be as accurate as in the absolute method provided the conditions of calibration and analysis are similar. It is advisable to obtain occasional c-v curves with comparative methods to insure against instrumental and electrochemical artifacts.

Additional considerations are raised when concentration is obtained through the use of polarograms:

1. Precision of analysis is reflected from the precision of calibration, measurement of \( i_T \) or \( i_d \), accuracy of instrumentation, and in control of parameters of the analysis system.

2. Comparative methods are likely to be less precise than absolute methods unless additional consideration is given to the nature of the c-v curve and to the plateau region at the applied voltage.

3. Attention should be given to linearity between current and concentration.

POLAROGRAPHIC INSTRUMENTATION

There are a number of circuits in use in polarographic instruments. The three essential components are presented schematically in Figure 4. In this figure, B represents the source of applied e.m.f., P represents a polarographic cell consisting of an analysis electrode and a reference electrode, and G represents the means of measuring the current flow in the polarographic cell.
FIGURE 4.

SCHEMATIC DIAGRAM OF THE POLAROGRAPHIC CIRCUIT

In Figure 5 is a diagram of one of the simplest circuits. B in this figure consists of a battery, voltage divider and voltmeter, and possibly a polarity reversing switch. Flashlight batteries are adequate sources of e.m.f., and a suitable radio potentiometer will serve as a voltage divider. The voltmeter should be a vacuum tube type of high impedance to prevent loading the circuit and erroneous indication of the voltage.* The current recorder A can be a direct reading, optical or recording ammeter. Since the maximum current is only a few microamperes the meter should have a sensitivity of at least a tenth of a microvolt per scale division and should be readable to one hundredth of a microampere to insure maximum precision.

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*A precision potentiometer may be substituted for the voltage divider and voltmeter if circuit resistances are calculated and the applied voltage is calculated.
The reference electrode used in the polarographic cell must furnish a reliable reference potential and have a minimum tendency toward polarization. In biological investigations the calomel or silver-silver chlorine reference electrode is commonly used. The chloride content of the media or tissues makes these electrodes ideal selections as there is sufficient anion to prevent concentration polarization and to suppress the migration current. The characteristics of these electrodes are:

\[ 2\text{Hg} + 2\text{Cl}^- = \text{Hg}_2\text{Cl}_2 + 2\text{e}^- \]  \hspace{1cm} (12)

Hg, Hg_2Cl_2(s) sat KC1  
-0.2415 ± 0.00076 (t-25) volt

\[ \text{Ag} + \text{Cl}^- = \text{AgCl} + \text{e}^- \]  \hspace{1cm} (13)

Ag, AgCl(s) Cl^-(a=1)  
-0.2224 volt

Interface effects between the media of the reference electrode and the analysis portion of the cell, as well as variation in pH and
temperature, introduce stray potentials which affect the reference potential. For concentration analysis and biological application, a reference potential accurate to two significant figures is adequate; it is probable that the influences mentioned do not alter the first two significant figures cited. The reference electrode surface area should be ten times the area of the analysis electrodes to prevent concentration polarization. 14

Agar-agar bridges saturated with potassium chloride or solute of the analysis cell are used to connect the two portions of the polarographic cell and prevent contamination of the reference half cell. This increases the internal resistance of the circuit by several thousand ohms and introduces the possibility of additional interface potentials and diffusion potentials. 12 One investigator reported that a minute flow of solution out of the reference electrode through the bridge prevented the gradual decline in i_d which is sometimes observed and resulted in increased stability of the measurements. 32 With the exception of the last factor, the influence of the bridge on the reference potential is eliminated if the calibration and measurements are completed on similar systems. In other cases it must be evaluated.

Analysis electrodes may be either the dropping mercury or solid metal electrodes. 13 Although platinum is the metal used most frequently, gold electrodes have greater usefulness in some applications. 29 Other metals, including tungsten, silver, stainless steel and antimony, have been tried but the results have not
been promising. The electrode size varies from several square millimeters surface area to the ten-micra-diameter, gold-plated electrodes used by Misrahy. Concentration polarization is established more rapidly and appears to be more stable with the smaller surface areas.

The reactions which occur at the analysis electrode are influenced by a number of factors. For the reaction to be analyzed, no interfering substances may cause depolarization of the electrode. During analysis based on reduction, hydrogen evolution eventually causes depolarization. Theoretically this should occur at voltages that were more positive than those actually observed. The difference between the theoretical and observed voltage at which hydrogen evolves is called overvoltage. Acid media, nature of the electrode surface, current density, and duration of electrolysis are some of the factors which decrease overvoltage. Investigators have also reported influence of previous use of the electrode and film formation on the electrode surface as factors which modify the electrode reaction. The reaction for oxygen reduction is particularly susceptible to variation. Davies and Brink have reported a two-electron and a four-electron transfer for the same type electrode under identical conditions. These variations and the fact that to a great extent their origin is imperfectly understood indicate that a great deal of caution should be attached to new interpretations of variations in results.
Solid electrodes have additional disadvantages which are not present with the d.e. The short-term accuracy with most electrodes has been less than five per cent, and long-term accuracy is between fifteen and twenty five per cent for selected electrodes. Part of the variability is due to modified diffusion conditions in the region of the solid electrode which are minimal in the region of a d.e. Both thermal and mechanical effects have more influence on solid electrodes than on the d.e. In addition, the electrode surface is not constantly renewed and tends to become contaminated even though the reaction takes place in solution. Some of these factors will be emphasized in the review of the polarographic measurements of pO₂ in tissues and blood.

Instrumentation introduces the following factors for attention during quantitative concentration analysis:

1. The electrical components should be selected to allow an accuracy of at least 0.5% in the application or measurement of voltage or current.

2. Provision should be made to read the polarographic current to 10⁻⁸ ampere.

3. The reference potential and factors which modify it require monitoring, particularly with the comparative method of analysis.

4. The analysis electrode surface area should be minimized if at all possible.

5. The analysis electrode surface should be clean at the beginning of analysis and checked for contamination during and/or after experiments.

6. Factors disturbing the diffusion shell of the analysis electrode should be minimized, particularly with solid electrodes.
7. Maximum accuracy with solid electrodes will depend upon frequent calibration.

Several circuit modifications increase the utility of the polarograph. One of the most useful is the automatic step-wise increase in applied voltage over a selected voltage span with automatic recording of c-v curves. Provision can also be made to pass a bucking current through the recording circuit to reduce the magnitude of the recorded deflection.

Several advantages and increased types of analysis are introduced when alternating currents are used instead of direct current polarization or when the d.c. is modulated with an a.c. potential. This modulation allows an almost infinite number of types which can be classified as derivative, square wave, oscillographic and interrupted polarography. The last modification will be discussed in detail, as it is used in the research. The other methods will be presented to clarify the difference in these methods compared with interrupted polarography and to point up their potential use.

Derivative polarograms are obtained by recording the alternating current flow which results when a sinusoidal modulation of the applied potential is used. While the applied potential is swept through a selected voltage span, a small sinusoidal variation of a few hundredths of a volt at a frequency of sixty cycles per second is superimposed on the applied potential. Figure 6 presents the principles involved.
6 A. Horizontal arrows indicate 0.05 voltage variation for a given value of the applied voltage. The vertical arrows indicate the corresponding alternating current.

6 B. A continuous record of alternating current derived during a single sweep of the applied voltage span with superimposed a.c. modulation. The maximum current indicates the half-wave potential and the magnitude of the a.c. at any point indicates the d.c. slope.

The method quickly locates the half-wave potential which identifies the reacting substance. The slope of the residual current or plateau is readily obtained from the magnitude of the alternating current. The method would make it possible to check the residual current, identify the reacting substance, and to determine the extent to which the slope is changing in the region of comparative analysis in a matter of a few seconds and without serious interruption of analysis. The method has been reviewed by Lingane and Williams.33

Square wave polarography, illustrated in Figure 7, provides
A. Square wave modulation  
B. Charging current  
C. Diffusion current  
D. Polarogram

**FIGURE 7.**  
**PRINCIPLES OF SQUARE WAVE POLAROGRAPHY**

7 A. The periodic modulation of the applied potential by superimposed square wave potential.

7 B. The relative time course of the charging current during square wave potentials.

7 C. The relative time course of the diffusion current during square wave potentials.

7 D. Oscillographic record of the current flow in a period of modulated potential.

A method for separating the charging current from the diffusion current. Because the time courses of the charging and diffusion currents are different, it is possible to obtain the diffusion current after the charging current has decayed. In square wave
polarography the applied potential may remain constant or may be swept through the voltage spans as in differential polarography, and it may be used with either the d.e. or solid electrodes. In use with either type of electrode, its major advantage is that the operator can take immediate reading of the polarographic current instead of waiting for completion of the mercury drop or for stabilization of the current with the solid electrode. Solid electrodes may take several minutes for stabilization to be established. Even then, the errors are likely to be greater than fifteen per cent. Olson\textsuperscript{16} and Barker\textsuperscript{39} have shown that $i_d$ obtained with solid electrodes is proportional to concentration of reacting substance within a few tenths of a second following polarization of the solid electrode. In square wave polarography the choice has been to make the reading during the early phase of polarization. Under these conditions an accuracy of within two per cent of the probable value is claimed for concentrations of $10^{-6}$ molar.\textsuperscript{40} The method offers the advantages of increased sensitivity and accuracy and has no outstanding disadvantages.

Oscillographic polarography has developed from knowledge obtained with conventional methods of polarography for application to the study of reaction kinetics. The method utilizes a high resistance circuit and records potential-time curves with small electrodes which are polarized by the sweep voltage technique. It has the advantage of being rapid and offering an opportunity to alter rate limiting factors in the reaction with visualization of
the induced change in the oscillographic polarogram.\textsuperscript{12}

Although a number of investigators had observed that the solid electrode was more stable after a period of shorting to the reference electrode, the first thorough investigation of the factors and an evaluation of their influence was reported from the Laboratory of Physical Biology, National Institutes of Health.\textsuperscript{16} In the initial report a ten second cycle was used, and the analysis electrode in alternate periods of a cycle received first a positive, then a negative applied potential relative to the reference electrode, and was shorted to the reference electrode between applied potentials. The current recorded only during the terminal phase of negative applied potential, was found to be proportional to oxygen concentration. In a later study\textsuperscript{41} the circuit was modified to reduce the cycle to three seconds and to include a system for recording the rate of change in oxygen concentration based upon two consecutive measurements. The method has the advantage of increased stability and accuracy and probably tends to keep the electrode surface clean.

Early attempts to use solid electrodes for measurements in animal tissues and blood resulted in unreliable estimates of oxygen tension. These have been attributed to modification of the electrode surface and heterogeneous nature\textsuperscript{103} of oxygen distribution caused by the presence of red blood cells. As a result numerous electrode modifications have been introduced to circumvent these difficulties. In the original article on the use of
solid electrodes for measurement in tissues two methods were proposed for modification of the bare solid electrode. The recessed and covered electrodes which were described are presented in Figure 8 with other modifications introduced by Drenkhan and by Clark. The four types of electrode modifications and the Olson circuit represent the most effective methods of combating the difficulties encountered in tissue and blood measurements.

The recessed electrode stabilizes the diffusion conditions, thus increasing reliability and reproducibility of oxygen tension measurements. Keeping the recess bore small or filling the recess with a gel reduces the r.b.c. influence. The electrode will eventually be contaminated, but the process is delayed.

Drenkhan found that when a thick collodion stocking over the solid electrode was used with Olson's circuit, the measurements were insensitive to r.b.c.'s. He also observed increased
stability, reproducibility and precision over other solid electrodes. With the recommended care the electrodes will not show signs of contamination for several weeks.

Clark has reported electrodes partially isolated and completely separated from the tissue or solution by a membrane permeable to oxygen, but impermeable to the water soluble substances and water. These electrodes determine the oxygen concentration by the measurement of the oxygen which diffuses through the membrane. In the partially isolated electrode, contamination eventually occurs by diffusion of materials through the 'bridge' beneath the membrane which provides for electrolytic connection with the reference electrode. In the completely isolated electrode with an internal reference electrode, the only substances which reach the electrolysis measuring cell are those soluble in the membrane.

Any system which removes oxygen during the process of measurement reduces the oxygen concentration in the region of measurement and lowers the quantity present. Only the dropping mercury electrode and bare solid electrode with an unrestricted diffusion field are dependent upon the concentration representative of the body of the solution. If the ratio of the volume of solution to the volume of the diffusion field is large and the duration of measurement not too protracted, these two electrodes validly measure the oxygen tension of the system. In the use of Olson's circuit or with covered or isolated electrodes, the
measurement is a function of the concentration in the main body of solution, but is lower than the representative concentration. During measurements with Olson's circuit, adsorbed oxygen and oxygen within the diffusion field is consumed. This loss will be partially replaced during the period of alternate polarization and the two shorting periods, but the time is insufficient for equilibrium to be obtained between the diffusion region and the body of the solution in the absence of convective effects. In the case of covered electrodes or membranes, a diffusion gradient is established similar to one of those illustrated in Figure 9.

It is evident that the concentration or tension is less at the surface of a membrane or electrode covering than it is in the body of solution. Oxygen is less soluble in membranes and coverings in use at present than in solutions, and oxygen solubility in the membrane is the limiting factor in the quantity of oxygen electrolyzed in most systems.* Under these conditions it is the oxygen at the solution-membrane interface which is measured, and it is evident the oxygen at the interface is less than that in the body of the solution. With continuous measurement using the Olson circuit or the modified electrodes, equilibrium is established and a constant value for oxygen concentration is obtained, but it is

*It is impossible to determine the solubility for various membranes which have been used, but this statement is supported by the fact that there is decreased current obtained with modified electrodes in spite of shorter diffusion distances.
1. Oxygen more soluble in membrane than in solution.  
2. Oxygen less soluble in membrane than in solution.

A. SYSTEM ILLUSTRATED IN TERMS OF CONCENTRATION

B. SYSTEM ILLUSTRATED IN TERMS OF TENSION

FIGURE 9.
DIFFUSION IN A SYSTEM CONTAINING A MEMBRANE

evident from the above analysis that the concentration at the electrode will be less than that which exists in the body of the solution.

Modification of circuits or electrodes makes it possible to consider additional opportunities and introduces feasible changes in polarographic measurement of oxygen, such as:
1. Convenience features can be added to a circuit.

2. Additional features could be added to a circuit to permit monitoring of a reaction, \( i_r \), and the plateau region of comparative analysis.

3. Modified circuits make it possible to read \( i_d \) or its equivalent continuously and directly.

4. Modifications generally decrease current sensitivity, but in one case, at least, increase it.

5. Sensitivity, stability, speed of response, reproducibility, precision, reliability and validity are interrelated. An attempt to change one will modify one or more of the others.

6. Electrode modifications introduce problems in relating the measurement to the essential \( \text{PO}_2 \).

**REVIEW OF THE LITERATURE**

Analysis for oxygen was among the early developments of the polarographic method. Two years after he introduced the technique of polarographic analysis,\(^{12}\) Jaroslav Heyrovsky\(^45\) reported the reduction of oxygen at a dropping mercury cathode. These initial observations on oxygen analysis have been expanded by Heyrovsky\(^46\) and Vitek\(^47,48\) in Heyrovsky's laboratory at Charles University. Kolthoff and his associates\(^49-51\) at University of Minnesota in this country have verified and amplified many of the earlier findings concerning polarographic oxygen analysis. The polarographic literature on oxygen includes many reports by analytic chemists interested in the kinetics of oxygen reduction or the influence of dissolved oxygen upon other types of analysis. Only a few of these articles have been included in the dissertation. The
remainder of the literature represents application of the polaro-
graphic method to problems encountered in other fields. The re-
view indicates the problems and the contributions of polarographic
analysis to their solution. The bibliography contains the litera-
ture on tissue and blood oxygen tension measured polarogra phical-
ly through September 1958, and much of the related literature on
measurement of oxygen in solution.

The first suggestion for application to biological problems
was made by Prat. However, it was not until independent sug-
gestions for its application had been made by Bridcka and by
Baumberger and Muller, that investigation was begun using O2
measurement in biology. Bridcka's work discusses the broad
application including organic analysis with mention of the analy-
sis for dissolved oxygen. In the Stanford University Laboratory
Baumberger and Muller presented a report and demonstration of
application of the polarographic measurement of oxygen in a sus-
pension of yeast cells as a means of determining respiratory rate.

During the application of the polarographic method to their
problems, investigators in various fields of endeavor have com-
pared their results with the standard methods used in their field.
These comparisons indicate the potential value of the polarograph-
ic method, as well as its limitations, and at the same time reveal
the many contributions which have been developed through its
application.

In the field of engineering minute traces of oxygen in the
boiler feedwater of high pressure steam boilers and turbines leads to extensive corrosion. The engineers are interested in continuous monitoring and mechanical control of the quantity of contaminating oxygen. In a recent symposium the available methods were presented and an attempt was made to set up a standard as the basis for comparative studies. If the results are indicative, the old methods have very little precision in the range of ten to two hundred parts per billion. In contrast, instrumental methods which were reported indicated that the polarographic method was considerably better than older methods and as good as newer methods. The newer methods were continuous or semi-continuous with a precision of within two to ten per cent in the range of three to one hundred parts per billion.

Biologists and engineers have compared titration methods with polarographic methods in the investigation of the dissolved oxygen present in natural waters and industrial effluents and wastes which contaminate this natural resource. In routine use of the d.e. for oxygen analysis these men observed a precision of within two per cent when tonometric, Winkler titration, or colorimetric methods were used to establish the concentration of oxygen in solution. With solid electrodes or in the presence of contaminants, particularly those of organic origin, the accuracy was between two and five per cent of the value obtained by Winkler titration. In most cases the investigators were inclined to attribute the errors to the Winkler titration, which is extremely
sensitive to impurities present during titration. This contrasts with the five-tenths per cent error which the chemical analyst is able to obtain\(^\text{13}\) and emphasizes the importance of the control of the contents in solution in order to assure maximum accuracy.\(^\text{14}\)

The polarographic method has made available to the engineer and to the biologist an instrumental method for the determination of dissolved oxygen to replace a slower and less precise method. In addition it has made it possible to replace discontinuous sampling procedures with a continuous recording or monitoring system and made definite technological advances possible in these fields.

The area in which polarographic method has come closest to achieving its maximum potential value is in the application to the study of respiration in solutions. One of the limitations of the conventional respirometers is the time required for equilibrium to be established between the phases in the measuring system, interior of cell, cell membrane, solution, and gas phase. In Warburg and Barcroft type apparatus this is hastened by shaking the respirometer. In the respirometers used for single cells or small pieces of tissue the volumes must be made quite small to make it possible to measure rates of the order of \(10^{-6}\) liter per hour.\(^\text{68}\)

The polarographic method eliminates the necessity of shaking and has the same sensitivity as have the single cell respirometers, although it introduces the reactive errors of the possible toxicity of mercury\(^\text{69}\) and the consumption of oxygen by the
Both qualitative and quantitative measurements have been used in the application of polarographic measurements to the determination of respiration and photosynthesis. The maximum accuracy for the dropping electrode was claimed by Baumberger to be four thousandths per cent, favoring favorably with the three thousandths per cent obtained in the volumetric measurements. The greatest accuracy obtained with solid electrodes is reported by Olson. Using the modified circuit he was able to obtain an accuracy of within three per cent.

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Knowledge of respiratory processes and photosynthesis in unicellular organisms has been expanded in many areas by application of the polarographic method. A critical oxygen tension of a few millimeters of mercury at which respiration becomes oxygen dependent was verified for bacteria by Longmuir, and for yeast cells by Minzier. The absolute value of critical PO_2 was determined by Harvey.

Goodkind and Harfey were able to relate the dimming of photoluminescence to the release at the beginning of illumination, the influence of the length of dark intervals, and the quantum efficiency of photo-

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adaptation. Advantage has also been taken of the rapidity of the response of the polarographic measure of oxygen concentration to map the photosynthetic spectra and the intensity effects of light upon oxygen production. Baumberger has shown the usefulness of the d.e. as a rapid means of estimating the oxidation-reduction potential of a suspension and the relationship of oxygen consumption to cytochrome reduction.

Similar studies on animal tissues have not been as successful in quantitative determinations, although a great many advances in knowledge have accrued from the polarographic studies. Diracher was able to show that age had relatively little influence on mouse sperm respiration in vitro studies, and Warren tested Barcroft's theory of erythropoiesis showing a critical pO2 of six millimeters of mercury and a reciprocal relationship between respiration and glycolysis. D. K. Hill used frog muscle in vitro and sampled the solution from above the muscle during exposure of the lower surface to varying oxygen tensions. He was able to demonstrate a critical pO2 for frog muscle in the same range as that found for microorganisms. The Johnson Foundation investigators have utilized a flow respirometer for the study of respiration in isolated nerve and sympathetic ganglia. Their findings not only identify the respiratory rates of frog axons, the increases associated with conduction of impulses, and the kinetics of respiration, but also the probable mechanism by which oxygen respiration is increased.
Other studies on respiration in cells and suspensions have relied upon the qualitative polarographic determination of oxygen concentration. This method utilizes the rate of change or relative rates of change from steady state values as a means of determining the nature of respiratory processes. Connelly has described the principles of the method in detail. The essential aspects are presented in Figure 10. In Figure 10B the two constant levels of $i_T$ represent steady states of oxygen concentration. The irregularities at these levels and on the slope represent instability of the electrode, and it is apparent that these will not influence the findings. If the steady state levels are known, the rate can be given in absolute values. Otherwise, relative rate changes or the relationship between rate of change and simultaneous metabolic activity or other change is reported.

In Figure 10A the steady state system is diagramed. The sites of
oxygen consumption are indicated as \( X \) and \( X' \). For purposes of simplification, \( X \), the cellular consumption, is assured to be uniform throughout the cell. \( X' \), the electrode consumption, is dependent upon the oxygen in the region of the electrode. As indicated in the figure, this concentration for a steady state can be represented by regions of similar concentration (dotted lines). Alteration of the environmental oxygen concentration or in the rate of respiration results in transition to a new steady state. In the transition represented in Figure 10B the oxygen availability is decreased by a change in environmental oxygen from 156 mm Hg to ca. 0 mm Hg. This change is reflected by a fall in \( i_T \) to a new steady state.

This method was extensively applied to frog nerve by investigators in the Johnson Foundation Laboratories at the University of Pennsylvania. It was found that heat production and alteration of oxygen consumption associated with activity follow different time courses. Studies of the oxygen consumption relative to a resting membrane and a conducting membrane indicated that artificial polarization of the membrane did not increase oxygen consumption and that the change in oxygen consumption was not directly related to frequency of conduction. The addition of phosphate to preparations was one method of increasing the rate of oxygen uptake, and it was concluded that one possible regulation of the rate of oxygen uptake could be the availability of phosphate.
Britton Chance\textsuperscript{98} has used the rate of utilization of oxygen by a suspension of mitochondria in conjunction with spectrophotometric measurement of the oxidation-reduction states of various cellular constituents in elucidating the biochemical basis of respiration and formation of high energy phosphate. Through the use of a vibrating solid electrode in a spectrophotometric cell, the suspension and an added substrate could be mixed and then allowed to remain undisturbed for the time required to reduce the oxygen in solution. The vibrating electrode in one region of the cuvette followed the rate of oxygen removal while the other portion was used for spectrophotometric determination. Chance found\textsuperscript{98} by measuring fumarate production in a succinic oxidase system, that fumarate production began and ended with oxygen availability. Chance and Williams\textsuperscript{99-101} studied the respiratory chain in intact mitochondria and found that availability of ADP limited the amount of oxygen utilized. They used this knowledge to determine other rate limiting factors and the sequence of enzymes in the respiratory chain. Chance and others\textsuperscript{102} utilized these findings for the investigation of the sites involved in oxidative phosphorylation. The rapidity and sensitivity of the polarographic measurements contributed significantly to these studies.

In studies on blood and tissues, additional problems are encountered which increase the difficulty of obtaining quantitative results. The determination of oxygen in blood has been
evaluated by Berggren. He concludes, "---, the simultaneous presence of both chemically and physically combined oxygen gives rise to such complicated conditions in polarographic measurements that it seems impossible to arrive at a quantitative determination of either the oxygen content or tension directly in blood." Since the polarographic method measures tension, investigators have solved this difficulty by removing the influence of chemically combined oxygen or have converted it to dissolved oxygen by oxidizing hemoglobin to some ferric compound. As indicated in the following paragraphs, an accuracy of within two and five percent was obtained with the polarographic method based on the standard obtained by gas analysis of the equilibrating gas or the gas extracted under vacuum. Part of the variation in results may be attributed to failure of some investigators to correct for the variations in tension associated with the consumption of oxygen by blood or plasma and the influence of oxyhemoglobin dissociation, as well as variability in the polarographic measurement.

It is possible to make the polarographic measurement in whole blood dependent upon only the physically dissolved oxygen in several instances. Berggren and Clark point up the fact that if the oxygen tension remains above the level of oxyhemoglobin dissociation, there is no contribution of chemically bound oxygen to the \( T \). It is also possible to displace the chemically bound oxygen with carbon monoxide. Investigators, however, have shown that hydrogen peroxide decomposition is
catalyzed by the presence of hemoglobin, resulting in increased polarographic current. This can be prevented by the addition of cyanide ion\textsuperscript{107} or by the use of an applied potential which reduces oxygen directly to hydroxyl ions.\textsuperscript{103} Even then, the measurement of oxygen in whole blood is less than in plasma equilibrated with the same gas phase, a factor attributed by Berggren to the slower rate of diffusion of oxygen in red blood cells.\textsuperscript{103}

It is also possible to use a measured blood sample and to estimate its oxygen content through the conversion of the chemically dissolved oxygen into physically dissolved oxygen in a known volume of a reducing solution.\textsuperscript{108,109} Baumberger used acid to release the bound oxygen and convert the oxyhemoglobin to acid hematin. Tysarowski\textsuperscript{110} converted the oxyhemoglobin to cyanohemoglobin, using potassium ferricyanide in a manner reported earlier by Weissinger.\textsuperscript{111} Tysarowski reported no values for precision, but Baumberger claimed an accuracy similar to that obtained with van Slyke determinations, and Weissinger reported an accuracy of within two per cent.

A modification of the polarographic principle makes it possible to determine the oxygen in solution by potentiometric technique.\textsuperscript{112} Since very little oxygen is used by the electrode during measurement, it is equally effective in blood or homogeneous solution.

In other investigations the measurements of oxygen tension in blood have been directed toward measurement in plasma, based
either on physical separation or upon physical factors which markedly reduce the contribution of chemically bound oxygen to the polarographic measurement. Beecher and his colleagues\textsuperscript{113} were the first to use cell-free biological fluids as a means of estimating oxygen content or tension in blood and other body fluids. In their studies no special care was taken to make the separation at the temperature of the original sample. In later studies by Weissinger\textsuperscript{114} and by Wilson and coworkers\textsuperscript{115} special means for anaerobic separation such as used by the previous group was accomplished at body temperature. In addition, Wilson's work emphasized the consumption of oxygen by red blood cells and plasma during the interval between sampling, and separation and measurement. With adequate care and correction for oxygen consumption, these methods result in an estimation of the oxygen tension within five to ten millimeters of mercury of the oxygen partial pressure in the equilibrating gas phase.\textsuperscript{116}

As it is inconvenient and difficult to obtain accurate estimates of the blood oxygen tension by measurements made on plasma separated by centrifugation, other methods have been sought which do not require separation but have the advantage of being independent of chemically bound oxygen. Drenckhan\textsuperscript{117} introduced the use of a collodion stocking over the solid platinum electrode, and later included the use of Olson's circuit.\textsuperscript{42} Particularly with the second modification the contribution of chemically bound oxygen is minimal. The accuracy obtained is within one per cent of
the equilibrating gas partial pressure. Clark\textsuperscript{118} and his co-workers introduced a covered electrode and later the isolated electrolysis cell\textsuperscript{119} which were discussed earlier. These electrodes make it possible to measure tension, but unless some form of convection is present\textsuperscript{120,121} the tension is less than that of the equilibrating gas, because of the diffusion gradient to the surface of the electrode membrane. Dependent upon control of convective effects and temperature the accuracy of these modified electrodes is within two per cent of the partial pressure of oxygen in an equilibrating gas mixture\textsuperscript{122} or the pressure estimated from gas extraction analysis.\textsuperscript{120} When a bare platinum electrode is rapidly rotated in blood the diffusion layer around the electrode stabilizes to a cell free layer of plasma. Morgan and Nahas\textsuperscript{123} have applied the rotated electrode to whole blood and obtained a linear response for tensions of fifty to two hundred fifty millimeters mercury with less than one to five per cent deviation from the curve. These modified methods for blood increase the convenience of measuring tension of oxygen, but there are special problems with each method, as well as the constant problem of temperature and time influences on the sample characteristics.

Measurements of oxygen tension in blood indicate that similar factors may be expected in tissue measurements. The bare wire electrode must be used in tissue measurements and is subject to the same contaminations found in blood studies.\textsuperscript{27,28} One finding
in blood which indicates decreased sensitivity in tissue measurements is the decrease in its proportional to the red blood cell concentration.\textsuperscript{103,123} This would give experimental support to the mathematically derived inference of slower diffusion in cells\textsuperscript{124} and would verify Krogh's\textsuperscript{125} observations on tissue.

Technological advances and better understanding of physiologic functions as well as advances in polarography have resulted from investigations of blood oxygen tension. The improved polarographic electrode\textsuperscript{42-44} has provided the means for continuous monitoring of blood oxygenation and effectiveness of corporal perfusion during thoracic surgery,\textsuperscript{44,126-27} which requires utilization of the artificial heart-lung apparatus.

The simplified measurement of oxygen concentration with the polarographic method has made additional techniques available for blood analysis. In the Stanford laboratories it has been demonstrated that oxyhemoglobin dissociation curves\textsuperscript{128} and the nature of blood hemoglobin\textsuperscript{129} can be obtained in a few minutes without resorting to the use of slower and more cumbersome gasometric techniques.

Rapidity of the polarographic technique makes it feasible to study dynamic aspects of blood oxygenation. The fifty to one hundred blood gas analyses required in the course of a single experiment could hardly be handled with van Slyke or microgasometric methods of oxygen analysis. As a result of multiple blood oxygen analyses the shunting of blood from nonventilated alveoli
in normal \textsuperscript{103,130} and pathologic \textsuperscript{131} states of the respiratory system has become evident. Similarly, knowledge of pulmonary reserve capacity to maintain oxygen saturation\textsuperscript{132} the increased oxygen affinity of foetal blood\textsuperscript{133} and the role of counterflow between maternal and foetal circulations in oxygen transfer to the foetus has benefited from the reduced time required for oxygen analysis.

Polarographic measurement of tissue oxygen tension lacks a readily available method for estimating the accuracy of the measurements which are made.\textsuperscript{*} In the previous paragraphs application of polarographic measurements was made to systems in which a most probable value could be estimated or measured for actual oxygen tension. In tissue the parameters of oxygen tension, diffusivity, blood flow, blood oxygen capacity, oxyhemoglobin dissociation, tissue storage, metabolic rate, temperature and pressure, and tissue dimensions have varying degrees of influence in different states of the tissue and make it difficult to predict a reasonable value. Further complication arises in the heterogeneity of the oxygen tension pattern of the tissue. Under such conditions the term "oxygen availability" is most descriptive of the sum of the influences and the term indicates the uncertainty as to what is being measured polarographically in the tissue.

In spite of the difficulties mentioned above quantitative

\textsuperscript{*}The method of calculation and other available methods of measuring tissue oxygen tension will be discussed in the next section of the paper.
tissue oxygen tensions have been obtained in a few instances. Investigations using the recessed electrode\textsuperscript{134} report accurate measurements of the tension at the tip of the electrode, and polarographic measurements can be made on fluids which have been in contact with the tissue as a means of calculating the tissue oxygen tension.\textsuperscript{135,136}

Oxygen utilization in a tissue has been qualitatively estimated polarographically. Arrest of the circulation in the region of the electrode makes it possible for investigators to compare the rate of fall of $i_T$ under different conditions. This technique was first applied to the brain cortex by Bronk\textsuperscript{137} and later by Opitz and Kreuzer.\textsuperscript{138} Urbach used a similar procedure in measurement of relative rates of oxygen utilization of normal, inflamed, irritated and pathological states of the skin.\textsuperscript{139-141} As the ischemia was obtained by compression or tension of the tissues, the experimental conditions lack complete reproducibility. Less variation is encountered in investigations in which the circulation is arrested by occlusion of the vessels supplying the region under investigation or by cessation of perfusion until the oxygen tension falls to zero. This technique has also been applied to the brain cortex\textsuperscript{142-46} and in sympathetic ganglia.\textsuperscript{87-93,147-49} In a few investigations in which metabolism was made the independent variable, the interrelationship of oxygen availability and metabolism has been studied. Davies\textsuperscript{150} observed the rate of decline in $i_T$ during muscle contraction, and several investigators
have induced increased brain metabolism while making polarographic measurements. 151-53

During the course of investigations, changes in IT have been observed to be dependent upon variations in blood flow, arterial oxygen saturation and physical factors. These observations indicate the directional change to be expected in the polarographic measurement of oxygen tension, but offer little toward quantitative calibration of the observed diffusion currents. Decreases in the polarographic current are uniformly observed during partial and total obstruction of regional blood flow154-59 and during lowered arterial blood pressure. 160-63 When the circulation was altered by the use of drugs which affect flow, pressures, and vasmotor tone161-63 or by similar reflex changes,163-64 the polarographic oxygen tension in a few cases did not change, but usually was altered in the predicted direction. This variability in response may be due to interrelationships between blood flow through a tissue, which determines the mean capillary oxygen tension, and the ratio of the capillary radius to the radius of the tissue supplied by that capillary being modified and consequently changing the tissue oxygen tension. 165

Directional changes in polarographically measured oxygen availability are observed in studies in which arterial oxygen saturation has been varied. In two studies the arterial oxygen saturation was quantitated by oximeter measurement, 166-67 and in others the change was induced by alteration of the composition of
inspired gases. 134, 138, 168-72 In either case the relationship is bound to be non-linear because of the presence of hemoglobin.

Several other experimental designs have introduced factors which would modify the oxygen availability. The observed polarographic current in these experiments had the same qualitative characteristics as did those observed in other tissue measurements. Sonnenschein's experiment 153 placed the animal in an environment chamber and varied the pressure from one to six atmospheres. Quantitative results would be difficult to predict from the pressure changes because of associated alteration in the physically dissolved oxygen and the percentage of the metabolic requirements it supplies, and because of the circulatory and metabolic changes induced by the pressures in addition to the alteration of the other parameters of oxygen availability. Gravitational forces, 173-74 heat, 175 and cold 176-77 have been independent variables in investigations in which oxygen availability was measured polarographically. Again it would be difficult to quantitate the polarographic measurements because of alteration of the parameters of oxygen availability and the interaction of these with the physically imposed stimuli.

Even though polarographic tissue measurements of oxygen availability have been qualitative in nature the contributions of this method to knowledge of metabolism, circulation and clinical medicine have been extensive. Some of the contributions have a direct bearing on the investigations reported later in the thesis.
These contributions have been summarized below. Other aspects can be found in reviews by Montgomery\textsuperscript{178} and Pandazzi\textsuperscript{179} and a symposium on oxygen tension in tissue\textsuperscript{165}.

The fact has been known for many years that protozoa and tissues in vitro respire at a rate independent of oxygen tension above a very low minimal value,\textsuperscript{87,180-81} but this fact was not established for tissues in vivo prior to the application of polarographic measurement to the problem. A. V. Hill's\textsuperscript{124} speculation that there must be a marked difference between oxygen consumption of different regions of a cell or tissue have been substantiated by polarographic investigations of respiration in various regions of the nervous system\textsuperscript{137,182-84}.

The fact that oxyhemoglobin dissociation in tissues is the same as dissociation in vitro has been established\textsuperscript{163,166,186} to some degree. Other studies of oxygen availability in tissue emphasize the variability of the structural and functional characteristics of circulation in different tissues. Some tissues such as the brain\textsuperscript{152,182-83} and heart\textsuperscript{156-59} have little collateral circulation, while other tissues such as lung\textsuperscript{103,130} appear to have exceptional capacity to shunt blood. In studies made of kidney circulation\textsuperscript{185} it appears that the blood passing through the capillaries does not have the same red blood cell composition as blood in the veins and arteries.

Two facets of polarographic measurements of oxygen are revealed in an overall perspective of the literature on this
subject. The encouraging aspect of this new type of measurement for oxygen is the versatility and usefulness of application. The challenging aspect of polarographic measurement of oxygen is that while the measurements have an accuracy of within two per cent in vitro, it is difficult to obtain measurements in tissue that remain within fifteen to twenty-five per cent of the average steady state value.
CHAPTER II
EXPERIMENTAL PROCEDURES

The experimental procedures used in conjunction with the doctorate program included two experimental designs. The first design consisted of a method of measurement of diffusivity of oxygen in solution. This design is described (a) as a method of measurement and calculation of the coefficient of diffusion of oxygen in saline, (b) as the value obtained for the coefficient, and (c) as a discussion of the reliability of the coefficient and the implication of the reliability for the accuracy of the polarographic apparatus and technique being used in these experiments. The second experimental design was a method of testing the accuracy of polarographic measurements of oxygen tension in vivo. In this chapter the hypothesis and the experimental aspects of the second design are presented.

POLAROGRAPHIC INSTRUMENTATION

In these experiments the polarographic circuit, originally designed by Olson,16 was modified to allow the use of multiple electrodes. The modified circuit, in addition to having the features of the simple polarographic circuits described in the previous chapter, provides for:

1. Application of alternately positive and negative potentials to the analysis electrode with interspersed periods of shorting to the reference electrode.
2. Recording the current flow of a single analysis electrode only during the terminal portion of the period of negative applied potential.

3. Stabilizing all analysis electrodes by continuous cycling even when the electrode current is not being recorded.


This circuit differs from square wave and alternating current polarographic circuits in that the reference electrode potential is not modulated during polarization, while square wave and alternating current techniques modulate the polarized electrode and measure the modulated current flow.

Figure 11 illustrates the major features of the circuit. A block diagram at the top of the illustration indicates the circuit functions. The circuit in the center of the figure shows the location of various electrical components and the microswitches. Provisions one through four mentioned above are obtained by a set of programming switches which complete one cycle per minute and include six ten-second sub-cycles. The switching program is controlled by microswitches activated from a four-level cam driven by a synchronous one-rpm motor.* An illustration of programming sequence may be found in the lower left corner of the figure.

The one-minute cycle consists of six ten-second periods in which each electrode is placed in the recording circuit by its

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POLAROGRAPH

SCHEMATIC

POWER
&
VOLTAGE
REGULATOR

POLAROGRAPHIC CELL

AMMETER
& SHUNT

ELECTRODE
SELECTOR

INSTRUMENT

B = MERCURY DRY CELL
V = VACUUM TUBE

MS = MICROSWITCH
A = NANOAMMETER

R = REFERENCE
E = ELECTRODE
O₂ = ANALYSIS

SPST = SWITCH

R₁ = 1000 OHM
R₂ = 100 OHM
R₃ = R₂₀ 1000 OHM

R₃ = R₂₀ 1000 OHM

R₁ = 1000 OHM

R₂ = 100 OHM

R₃ = R₂₀ 1000 OHM

FIGURE 11.
respective microswitch (1-6). All ten-second cycles are identical. In the initial third of a ten-second cycle, microswitch 7 is activated. During the following one sixth of a cycle no voltage is applied, and the analysis electrodes are shorted to the reference electrode. Microswitch 8 is activated during the next one third of a cycle, which is again followed by a one-sixth cycle period in which the analysis electrodes are shorted to the reference electrode. While microswitches 7 and 8 are operating, the potential from the batteries* placed in the circuit with opposing polarity is applied to the polarographic cell. The recording circuit is activated for one electrode by microswitch 9 during the terminal portion of the applied voltage at the analysis electrodes. The electrodes are then negative with respect to the reference electrode potential. Two variable potentiometers, wired in parallel with the potential sources, regulate the applied voltage. A manually set range potentiometer** regulates the applied voltage span. A manually or solenoid operated stepping potentiometer*** allows the choice of a pre-set and constant applied voltage or of an automatic stepwise increase from zero to full span voltage in five per cent increments. Microswitch 9 actuates the

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*Mercury batteries, Mallory RM1R: 2 in series, 2.7 volts.

**2000 ohm radio potentiometer.

***1% precision 10 ohm resistors wired in series to the contacts of a multiswitch to form a 2000 ohm potentiometer.
solenoid* during the last five degrees of each one-minute 360-degree cycle. A vacuum-tube voltmeter** in parallel with the polarographic cell monitors the applied potentials.

The polarographic cell is composed of a saturated calomel reference electrode connected to the analysis portion of the cell via a saturated potassium chloride agar-agar bridge. Mercury and mercuric chloride purified for use in electrolytic cells are contained in the calomel half cell. The cross-sectional area is approximately twenty square centimeters for the calomel cell and approximately one square centimeter for the bridge. The bridges are four to six inches in length. The analysis electrodes (Figure 12) are constructed from platinum-iridium wire which varies from 10 to 65 microns in diameter and is approximately four millimeters long.

Electrode currents are recorded by means of a photokymograph*** from a reflecting galvanometer**** which is introduced

*24 volt power supply to solenoid is regulated by an off-on switch, SPDT. In off position of the SPDT switch the manually set position of the stepping potentiometer is not changed.

**Hico Model 221-K vacuum tube voltmeter. 25 megohm d.c. input impedance, 0-5 volt indicating range with provision for centering pointer to mid-scale.

***Waters-Conley 18-inch photokymograph.

****Kipp and Zonen, A-23, Micro, Moll-type galvanometer: 0.25 sec. undamped period, 2.25 to 3.3 x 10^-8 amp/mm deflection at 1 meter.
into the circuit with its critical damping resistance* by micro-switch 9. The critical damping resistance also serves as a variable sensitivity shunt.

DIFFUSION

METHOD OF MEASUREMENT OF DIFFUSIVITY

Both the apparatus and the method used to obtain an integral coefficient for diffusion of oxygen in saline depended upon the measurement of oxygen in a diffusion chamber with the polarographic apparatus which has been described. The diffusion chamber was composed of two cells separated by a cellophane membrane. One cell contained two polarographic analysis electrodes which were used to measure the concentration changes in oxygen as diffusion

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*G. W. Borg, Mod 205 micropot, a ten-turn precision potentiometer with 0.1% linearity.
occurred.

Figure 13 is a diagram of the diffusion chamber, which was constructed. The diffusion path, approximately one centimeter in cross sectional area, was drilled on the axis of the chamber after the two cell units had been fashioned and fastened together. A new 7/16-inch drill, which measured 0.437 inches in diameter, was used to drill the bore of the chamber. The diffusion cell was cut to measure two centimeters (0.788 inches by micrometer measurement). After the chamber had been drilled, the cells were sealed at the outer ends with plastic discs.

Both cells had openings for flushing and filling; the reference cell had an access opening to receive the agar-agar bridge from the calomel references electrode. An estimated cross sectional area of one per cent of the cross sectional area of the diffusion path was open to the atmosphere. Placing these openings at a maximum distance from the diffusion interface minimized their influence.

Electrodes in the diffusion cell were positioned accurately. The electrode nearest the membrane, $E_1$, was fastened in place with a set-screw while it was held firmly against a template that extended four millimeters (0.1570 inches by micrometer measurement) into the diffusion chamber. Electrode $E_2$ was flush with the end of the diffusion cell. After the electrodes had been positioned, they were cemented into place. During the experiments the cement seal was checked to insure that the electrodes had not shifted
DIFFUSION CHAMBER

A - REFERENCE CELL  (1 CM$^2$ DIAMETER, 2 CM DIFFUSION DISTANCE)
B - DIFFUSION CELL
E' & E - ANALYSIS ELECTRODES  (20 GAUGE PLATINUM WIRE)
C - CALOMEL CONNECTION
D - OVERFLOW TUBE
M - MEMBRANE
F & G - FILLING INLET

FIGURE 13.
During calibration and measurement of diffusion the chamber was supported in an oil bath from a plastic float which also supported the calomel reference electrode. The float was centered in the oil bath by weak helical springs to minimize transmission of vibrations to the system.

A dialysis membrane was stretched across the front of the diffusion cell to prepare the chamber for diffusion measurement. The two cells were then assembled and placed in the oil bath suspended from the plastic float.

The electrodes were calibrated prior to diffusion measurement against gas-equilibrated saline. The gases used included tank nitrogen, room air, and tank oxygen. A van Slyke apparatus was employed to measure the oxygen content of the saline of five milliliter samples. The calibration procedure included: (1) flushing the cells with the gas contained in the saline prior to filling the chamber, (2) filling the chamber with equilibrated saline and recording the polarographic current for at least one minute, (3) recording a c-v curve during the calibration with saline equilibrated against room air. All polarographic measurements, excluding the c-v curve, were comparative measurements made at an applied voltage of -0.59 volts relative to the calomel reference electrode.

To measure diffusion, a sharp concentration difference at the dialysis membrane was established at a known time, and the changes
in oxygen concentration at the electrodes in the diffusion cell were observed for the next hour. Both cells were drained and flushed with nitrogen gas. The gas flushing in the reference chamber was continued while the diffusion cell was filled with nitrogen-equilibrated saline, and all gas bubbles were carefully washed out of that side of the cell. The nitrogen gas was discontinued, and the reference cell immediately filled with saline equilibrated with tank oxygen. Within a second after the cell was filled, the polarographic circuit was turned on to indicate the start of the experiment, and the first pip on the trace registered the end of the first ten seconds. After the reference electrode had been inserted in the circuit by means of the agar-agar bridge, the system was undisturbed for the remainder of the hour.

Temperature was recorded with a precision mercury thermometer graduated in tenths of a degree centigrade. Gas analysis was made on five milliliter samples by the van Slyke gas extraction method described in a following section. The time was measured on the basis of the ten-second cycle controlled by a synchronous motor, with the initial time determined as described above.

MEASUREMENT OF THE DIFFUSION COEFFICIENT

In this experiment the critical dimensions and parameters of the measurement of the integral diffusion coefficient for oxygen
are time, distance and concentration dimensions and temperature and convective influences. Distance was controlled as reported in the preceding section; however, there were no available checks for accuracy of the distance between the two electrodes E₁ and E₂. The concentration measurements by gasometric analysis have an accuracy of within two per cent. Although the time was not checked, the synchronous motor stops if it is thrown out of phase by a resisting force; hence it should have an accuracy of better than 0.1%. The solutions and bath were at room temperature, which did not vary during the two-hour period of observation. No evidence of convective effects was observed in the polarographic record, a system which is extremely sensitive to convective effects.

The membrane in the system offers an unknown resistance to diffusion. It can be represented by an equivalent amount of resistance in the saline solution. In order to solve the diffusion equation an initial location of the boundary must be established. Since the time required for diffusion of a given concentration in a given system to reach points within that system is a linear function of the square of the distance from the initial boundary, the relationship between time and distance may be used to establish the initial boundary in terms of equivalent distance in a column of saline. The solution for the equivalent distance in this particular experiment is given in Table II.
TABLE II
ESTIMATION OF $x_0$ FROM DIFFUSION TIME

<table>
<thead>
<tr>
<th></th>
<th>$E_1$</th>
<th>$E_2$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$X$</td>
<td>$0.4$</td>
<td>$2.0$</td>
<td>cm from membrane</td>
</tr>
<tr>
<td>$t$</td>
<td>$28^*$</td>
<td>$450$</td>
<td>Seconds to appearance of $0.05$ $C_0$ at $E$</td>
</tr>
</tbody>
</table>

*Estimated from observation at 30 seconds.

$x_0 = \text{-}0.15 \text{ cm from } X_0$

Table III contains the tabulation of the results of the diffusion coefficient measurement. Calculations are based on methods discussed in Jost\textsuperscript{187} and H"{o}ber.\textsuperscript{188} The equation

$$\frac{2C}{C_0} = \left[ 1 - \frac{2}{\sqrt{\pi}} \int_0^q e^{-q^2} dq \right]$$

is applicable to systems which have an initial sharp boundary, and diffusion does not reach a boundary during the measurements. The argument for the error function, $q$, is related to diffusion coefficient, time and distance by the equation

$$q = \frac{x}{2\sqrt{DT}}$$

$C_0$ - concentration difference at time zero
$C$ - concentration difference at time 'n'
$q$ - the argument for the error function
$x$ - distance from the initial boundary
$t$ - time
$D$ - integral diffusion coefficient
since the membrane provides a means for establishing an initially sharp boundary and the measurements were concluded when the initial change in oxygen concentration occurred at the second electrode $E_2$, Equations 13 and 14 can be used to obtain the value of $D$.

Tables of the Error Function, AMS 41,189 were used to obtain the solution to equations $a$ and $b$. The integral is tabulated for arguments of $q$, and the tabulated values correspond to the value $(1 - 2C/Co)$. The value of $q$ thus obtained was used to solve equation $b$ for the value of $D$ for oxygen in saline.

In Table III the calculations of $D$ based on the above solution are tabulated. The values reported for concentration are for an initial galvanometer deflection of 56 millimeters, which corresponds to the measured oxygen concentration of 0.029 cc of oxygen per cc of saline. Measurements were made to the nearest 0.25 millimeters, and the ratio of each measurement to the galvanometer deflection for initial oxygen concentration is recorded. The mean variation in $D$ is calculated from the sum of squares of variation. The findings were $D_K = 7.70 \times 10^{-4} \text{ cm}^2\text{sec}^{-1}$ for a partial pressure gradient of 0.95 atmospheres per centimeter at 23° C in 0.9% saline.

**DISCUSSION OF DIFFUSION COEFFICIENT**

The diffusion coefficient, $D_K = 7.70 \times 10^{-4} \text{ cm}^2\text{sec}^{-1}$, corrected to standard conditions has a value of $D = 2.36 \times 10^{-5}$
## TABLE III
CALCULATIONS FOR DIFFUSION COEFFICIENT OF OXYGEN IN SALINE

### Determination from initial detection at E₁ and E₂

<table>
<thead>
<tr>
<th>t (sec)</th>
<th>C/C₀</th>
<th>1-2C/C₀</th>
<th>q</th>
<th>Dₓ cm² sec⁻¹ x 10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>0.05</td>
<td>0.99</td>
<td>1.822</td>
<td>6.82</td>
</tr>
<tr>
<td>450</td>
<td>0.05</td>
<td>0.99</td>
<td>1.822</td>
<td>8.40</td>
</tr>
</tbody>
</table>

### Determination from O₂ changes at E₁ at time 'n'

<table>
<thead>
<tr>
<th>t (sec)</th>
<th>C/C₀</th>
<th>1-2C/C₀</th>
<th>q</th>
<th>Dₓ cm² sec⁻¹ x 10⁻⁴</th>
<th>dDₓ</th>
<th>(dDₓ)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.01</td>
<td>0.99</td>
<td>1.645</td>
<td>7.41</td>
<td>29</td>
<td>841</td>
</tr>
<tr>
<td>40</td>
<td>0.015</td>
<td>0.97</td>
<td>1.518</td>
<td>8.00</td>
<td>30</td>
<td>900</td>
</tr>
<tr>
<td>50</td>
<td>0.025</td>
<td>0.95</td>
<td>1.386</td>
<td>7.87</td>
<td>17</td>
<td>289</td>
</tr>
<tr>
<td>60</td>
<td>0.035</td>
<td>0.93</td>
<td>1.282</td>
<td>7.67</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>70</td>
<td>0.045</td>
<td>0.91</td>
<td>1.199</td>
<td>7.53</td>
<td>17</td>
<td>289</td>
</tr>
<tr>
<td>80</td>
<td>0.06</td>
<td>0.88</td>
<td>1.100</td>
<td>7.82</td>
<td>12</td>
<td>144</td>
</tr>
<tr>
<td>90</td>
<td>0.065</td>
<td>0.87</td>
<td>0.994</td>
<td>7.35</td>
<td>35</td>
<td>1225</td>
</tr>
<tr>
<td>100</td>
<td>0.08</td>
<td>0.84</td>
<td>0.927</td>
<td>7.66</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>110</td>
<td>0.095</td>
<td>0.81</td>
<td>0.906</td>
<td>8.00</td>
<td>30</td>
<td>900</td>
</tr>
<tr>
<td>120</td>
<td>0.10</td>
<td>0.80</td>
<td>0.595</td>
<td>7.68</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>280</td>
<td>0.20</td>
<td>0.60</td>
<td>0.582</td>
<td>7.63</td>
<td>7</td>
<td>49</td>
</tr>
<tr>
<td>290</td>
<td>0.205</td>
<td>0.59</td>
<td>0.570</td>
<td>7.70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>0.210</td>
<td>0.58</td>
<td>0.559</td>
<td>7.76</td>
<td>6</td>
<td>36</td>
</tr>
</tbody>
</table>

\[
\frac{D_x}{n} = \frac{100.08}{13} = 7.70 \times 10^{-4} \text{ cm}^2\text{sec}^{-1}
\]

\[
s = (dD)/(n-1) = (4702/12)^{\frac{1}{2}} = 0.20
\]

Measured gas concentrations:
- \(N₂\) phase = 0 oxygen
- \(O₂\) phase = 0.029 ml/ml oxygen

\[
D = 0.029 \times 7.70 \times 10^{-4} = 2.23 \times 10^{-5} \text{ cm}^2\text{sec}^{-1}(23°C, 0.9\% \text{ NaCl})
\]
$\text{cm}^2\text{sec}^{-1}$ for a gradient of one milliliter of oxygen per centimeter distance at $25^\circ \text{C}$. The corrected value compares favorably with the values reported in the literature, summarized in Table IV.

**TABLE IV**

VALUES FOR THE DIFFUSION COEFFICIENT

OF OXYGEN IN WATER

<table>
<thead>
<tr>
<th>$D_{25^\circ\text{C}}$</th>
<th>Original measure</th>
<th>Author</th>
<th>Experiments and Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{cm}^2\text{sec}^{-1} \times 10^{-5}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.38</td>
<td>1.62 cm$^2$/day-$16^\circ\text{C}$</td>
<td>Hufner</td>
<td>(1) - $D$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stephan's Tables</td>
</tr>
<tr>
<td>2.03</td>
<td>1.72 cm$^2$/day-$18^\circ\text{C}$</td>
<td>Carlson</td>
<td>(3) - $D$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sum of trigonometric Series</td>
</tr>
<tr>
<td>2.42</td>
<td>same</td>
<td>Bridka and Wiesner</td>
<td>(1) - $i_d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polarographic equation</td>
</tr>
<tr>
<td>2.38</td>
<td>same</td>
<td>Kolthoff and Lingane</td>
<td>(4) - $i_d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polarographic equation</td>
</tr>
<tr>
<td>1.84-1.98</td>
<td>same</td>
<td>Muller and Pircher</td>
<td>(4) - $D$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LaPlace Transform</td>
</tr>
<tr>
<td>1.15-1.94</td>
<td>same</td>
<td>Kreuzer</td>
<td>(9) - $D$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LaPlace Transform</td>
</tr>
<tr>
<td>2.36</td>
<td>$2.23 \times 10^{-5}$</td>
<td>Kelso and Peiss</td>
<td>(1) - $D$</td>
</tr>
<tr>
<td>$\text{cm}^2$/sec-$23^\circ\text{C}$</td>
<td></td>
<td></td>
<td>Substitution in integral</td>
</tr>
</tbody>
</table>

Hufner$^{190}$ determined the value by the classical method of layer analysis using Stephan's tables to interpret the data obtained. Carlson$^{191}$ Müller,$^{192}$ Pircher$^{193}$ and Kreuzer$^{194}$ used a
Fourier-type analysis to obtain a constant for their systems. If the total oxygen diffusing is divided by the constant, the data can be interpreted in terms of diffusivity. In Carlson's experiments the system did not reach equilibrium and the system was several days in reaching the state upon which the analysis was performed. In the Fribourg Institute experiments (Muller, Pircher, Kreuzer) thin layers of hemoglobin-containing solution, 50 to 500 microns thick, were placed over a photocolorimetric detector, which followed the saturation of the hemoglobin. Bridka and Weisner\textsuperscript{195} and Kolthoff and Lingane\textsuperscript{196} have measured $i_d$ and applied the formula

$$i_d = nFAC \sqrt{D/\pi}$$  \hspace{1cm} (16)

$i_d$ - diffusion current  \hspace{2cm} $A$ - Area

$n$ - Faradays/mole  \hspace{2cm} $D$ - Integral $D$

$F$ - coulombs/Faraday  \hspace{2cm} $C$ - molar concentration

It is obvious that the definitive value for diffusivity of oxygen remains to be determined. Although the results of the Fribourg group of workers is indicative of linear nature of $D$, the indication was not a critical test of the assumption. Of all the methods applied to the problem up to the present time, the method presented in this dissertation offers the most promise as a diversified means of studying diffusivity of oxygen. Modification of the chamber not only will provide a method for a critical test of the linear nature of oxygen diffusivity, but will permit the
evaluation of the influence of viscosity, temperature, and salinity on diffusion of oxygen. Incorporation of a membrane into the system will allow other aspects of tissue permeability to be studied and permit the verification of Krogh's observations\textsuperscript{125} on oxygen diffusion in tissues.

The standard deviation of $D$ observed in these experiments indicates a precision of about five per cent for the measurements of oxygen concentration. In all probability, the agreement of the value of $D$ with other values reported in the literature is a better indication of the precision than is the variability indicated by the standard deviation. In either case, the use of the standard deviation or the use of values from the literature, the results indicate that reliable measurement of oxygen concentration is obtained through the use of the methods described in this chapter.

TISSUE MEASUREMENTS

The reliability which was observed when a diffusion coefficient was obtained for oxygen is similar to that observed by others for measurements made in solution. \textit{In vitro} polarographic measurements usually have an accuracy of within one per cent; rarely are they more than five per cent from the predicted concentration in solution. On the other hand, biologists, of necessity have accepted qualitative results, since it is difficult to obtain reproducible results with solid electrodes in biological tissues.
In human tissues it has not been unusual to observe fluctuations of fifteen to twenty five per cent from the average steady state value.21,156-59,178

It is proposed that polarographic measurements of tissue oxygen tension have the same inherent accuracy as the accuracy obtained in non-tissue measurements. If the variability of oxygen concentration in tissue were controlled, the tissue then would correspond to an in vitro system in which the solute composition varied and concentration and convection were not uniform during a period of measurement. Under the conditions just described in vitro, an accuracy of within five to ten per cent would be expected. If an accuracy of within five to ten per cent is observed under controlled conditions in tissue, such an accuracy would provide evidence to support the validity of the above hypothesis.

One difficulty in testing such a hypothesis is that no satisfactory method is available for measuring oxygen tension in the tissue. Therefore it is necessary to estimate the most probable value of the oxygen tension in the region of the electrode in order to test the hypothesis. The available methods for obtaining the estimate include spectrophotometric measurement of the concentration of oxygen compounds of tissue pigments, gas analysis of the gas phase in equilibrium with the tissue, and calculation of the oxygen tension from measured parameters of tissue oxygen
tension.* The method of calculation has been chosen from the three methods as the most suitable for providing an approximation of the oxygen tension in the region of the electrode.

Only myoglobin appears to have the proper characteristics for estimating local tissue oxygen tensions in the range of zero to one hundred millimeters of tension. Spectrophotometric measurements of the percentage of oxygen saturation of hemoglobin do not differentiate between the hemoglobin of the large blood vessels and the hemoglobin in blood in the tissue capillaries, making it impossible to determine the tissue oxygen concentration. Spectrophotometric measurement of respiratory pigment oxidation\textsuperscript{199} indicates that oxygen tension above a few millimeters of mercury does not affect the degree of oxidation of the pigments.

In previous investigation\textsuperscript{199} the measurements of myoglobin saturation have yielded only qualitative results. It would be necessary to develop techniques for the measurement of tissue myoglobin concentration and oxyhemoglobin dissociation curves in order to use this method quantitatively.

Gas analysis of oxygen in equilibrium with the gas phase of tissue solutions or injected air bubbles is limited by the

\*Two techniques that have been used to measure oxygen concentration in solution, bioassay and oxidation of dyes, have not been considered. Bioassay techniques\textsuperscript{197} do not yield quantitative results. The oxidation of dyes has been in disrepute for measurement of oxygen in biological systems for the past three decades.\textsuperscript{198}
quantities of solution obtainable and by the time required for gas bubbles to equilibrate with the tissues. In the proposed test of the hypothesis it is important that the tissue be relatively undisturbed and that determinations be completed within a minute.

The method of calculation of tissue oxygen tension from the measurement of blood flow, oxygen extraction during tissue perfusion and tissue dimensions was developed by the Danish mathematician Erlang for August Krogh. This equation has been extended by Kety for the calculation of the average tissue oxygen tension. The factors used in Erlang's equation are illustrated in Figure 14.

![Figure 14](image_url)

**FIGURE 14.**

**DIMENSIONS FOR CALCULATION OF DIFFUSION IN TISSUE**

The conditions and arguments for the use of Erlang's equation are:

1. Three concentric cylinders are assumed:
   a. The first with radius $r$ whose surface coincides with the capillary wall.
b. The second with radius $x$.

c. The third with radius $R$ has a surface which represents the limits of the tissue supplied by the capillary $r$.

2. During a steady state the oxygen diffusing across the surface of cylinder $x$ is just equal to the oxygen consumed by the tissue between the surfaces of cylinders $x$ and $R$.

3. The quantity of oxygen $Q$ at a given respiratory rate ($\text{ccO}_2/\text{cc tissue/min}$) for this volume of tissue is:

$$Q = m \left[ \pi R^2 L - \pi x^2 L \right]$$  \hspace{1cm} (17)

4. For a diffusion system defined by $D$ the rate of change in the oxygen tension, $p$, is described by:

$$\frac{dp}{dt} = \frac{2\pi D x L}{Q}$$  \hspace{1cm} (18)

5. An integral equation is obtained from Equations 11 and 12 when they are set equal to $Q$ and each other, and the boundary conditions of the diffusion system are defined:

$$-\int_{p_0}^{p_1} dp = -\frac{m}{2D} \left[ R^2 \int_{r}^{R} \frac{dx}{x} - \int_{r}^{x} dx \right]$$  \hspace{1cm} (19)

6. Integration of equation thirteen and solving defines the oxygen tension at $x$:

$$p_x = p_0 - \frac{m}{D} \left[ \frac{x^2}{2} \ln \frac{R}{r} - \frac{x^2 - r^2}{4} \right]$$  \hspace{1cm} (20)

The average oxygen concentration of the capillary is calculated from either the $a-v$ oxygen difference:

$$c_a = c_a - [c_a - c_v]L \%$$  \hspace{1cm} (21)
or from the oxygen extraction

\[ C_0 - C_o = \left[ \frac{m}{F} \right] [L \%] \] (22)

C - Concentration
F - Flow
L - Length
D - Diffusion coefficient, \( O_2 \)

a - arterial
v - venous
o - "x", assigned value
m - oxygen utilization

Using Erlang's equation (Equation 20) Kety has obtained an equation for the mean tissue oxygen tension by integrating the equation between the limits of \( r \) and \( R \):

\[ \bar{P} = P_0 + \frac{m}{D} \left[ \frac{3R^2 - r^2}{6} - \frac{R^4}{2(R^2 - r^2)} \ln \frac{R}{r} \right] \] (23)

Equations 22 and 23 provide the means for estimating the tissue oxygen tension in the region of the electrode. In the use of these equations, it is presumed that the electrode causes a minimal reactive error and that circulation and metabolism in the region of the electrode are similar to those of other regions of the tissue. The mean capillary oxygen concentration, obtained from Equation 22, is used to determine the related oxygen tension from Dill's oxyhemoglobin dissociation curves.\(^2\) Substitution of the oxygen tension for \( P_0 \) in Equation 23, in addition to substitution of the values obtained for \( m, r \) and \( R \) by measurement and with substitution of the published value of \( D \) provides the data for the calculation of the tissue oxygen tension.

The extent to which the calculated tissue oxygen tension
provides a critical test of the hypothesis depends in part on the correspondence between the tissue system and the mathematical analog provided by Kety's equation. Factors in tissue circulation and metabolism in certain cases (as will be shown in the following chapter) may make the mathematical model a poor choice for depicting the tissue oxygen tension. Furthermore, a steady state must be maintained while the required data is collected.

METHODS USED IN TISSUE MEASUREMENTS

A comparison of polarographic and calculated oxygen tension was made in the skin and in the muscle of the rabbit in order to test the hypothesis. Skin was selected for the original study because it was possible to make measurements with very little disturbance in the normal conditions of the animal. Eventually muscle was used, since the blood perfusion characteristics of muscle were more stable than those of skin; and the circulatory pattern would have a better correspondence to the assumptions made in the development of the Erlang - Krogh equation. Other aspects of oxygen availability in tissue, a) tissue temperature, b) constant perfusion characteristics and c) tissue metabolism were evaluated for the presence of a steady state in the tissue during measurements. In order to obtain several different tissue oxygen concentrations at which the polarographic and calculated tissue oxygen tension could be compared, the composition of the inspired gas was altered to vary the oxygen content of the
arterial blood.

White rabbits weighing 1.4 to 3.8 kilogram were used as experimental animals. Nembutal* was administered intravenously in sufficient quantity to depress the respiratory rate to 18-24/minute. This degree of anesthesia induced a mild hypoxia in the animal: An initial dosage of 30-50 mg/kilogram body weight was followed by a supplementary dose a few hours later. Heparin** was administered intravenously in doses of 1-2mg/kilogram body weight to prevent blood clotting.

In both skin and muscle experiments a tracheal cannula was inserted to insure a patent airway and in some cases to permit maintenance of respiration with an artificial respiator. In rabbit skin experiments the cervical sympathetic nerve was cut to denervate the blood vessels and prevent vasomotor changes induced by nervous activity. In the muscle experiments the nerve to the muscle was cut to prevent nervous stimulation of the muscle. Cutting the nerve to the muscle, in conjunction with stripping the arterial sheath or cannulating the artery, also served to remove vasomotor innervation of the muscle preparation.

Prior to tissue measurements the polarographic electrodes


**Heparin Sodium, 500 mg (50,000 U.S.P. units)/cc, diluted to 1 mg/cc. Furnished for research purposes by The Upjohn Company, Kalamazoo, Michigan.
were calibrated in saline freshly equilibrated with atmospheric air and a c-v curve was recorded. After the completion of tissue measurements the calibration and c-v record was repeated, and any difference in temperature of calibration was corrected on the basis of a change of 1.6% increase /degree centigrade.* Although this calibration indicated that the electrodes and circuit had been unaltered during an experiment, it gave no indication of the magnitude of oxygen tension in the tissues.

Polarographic electrode connections consisted of contact with the reference electrode and stabilized temporary implants of the analysis electrodes in either skin or muscle. To obtain the reference electrode contact, either the shaved and debrided rabbit ear, or the shaved and debrided front foot was placed in a beaker of saline, and the beaker was connected to the saturated calomel reference electrode by an agar-agar bridge.

Analysis electrodes were placed in the skin of the rabbit ear which had been shaved and depilated with a mild depilatory cream several days prior to experiment. The electrodes were located in the tip of the ear in a region in which no large vessels were visible. A small ball of cotton beneath the shaft of the electrode eased the tension on the skin, and the entire ear tip, including electrodes and cotton, was covered with several coats of collodion. This held the electrodes in position and prevented the

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*Kolthoff and Lingane\textsuperscript{13} indicate a correction of 1.4-1.6%/\textdegree C. Experimental determinations indicate that with Olson's circuit the value 1.6%/\textdegree C. is the proper value to use.
influence of atmospheric oxygen* on the preparation.

Analysis electrodes and the levator scapulae minor muscle in the neck of the rabbit were supported by a T-shaped bar. Each electrode was inserted perpendicular to the axis of the muscle fibers and it penetrated the width of the muscle. From one to four electrodes placed in the muscle were supported by clamps on the bar of the T which faced the muscle. The T-bar also contained two plastic jawed clamps which held each end of the muscle after it had been freed from adjacent tissues. The support bar and its associated clamps served to prevent the movement of the electrodes relative to the muscle and also minimized the mechanical disturbance of the preparation.

The simple method of timing the collection of one-tenth milliliter blood samples in a graduated 1 ml serological pipette was used to measure blood flow in the experiments. The marginal ear vein in the rabbit ear was catheterized with a polyethylene catheter,** the tip of which was located at the point representing the venous return from the region of the polarographic analysis electrodes. In the muscle experiments, the vein draining the region between the plastic-jawed clamps was catheterized with a

*Fitzgerald\textsuperscript{200} has estimated from experimental measurements that atmospheric oxygen can supply the oxygen needs of human skin to a depth of 100 microns.

similar polyethylene catheter. The blood flow from the catheter tip was collected in the pipette. After approximately one-tenth milliliter had collected in the tip of the pipette, then the catheter tip was pushed into the pipette to the depth of this sample. The time required for each additional one-tenth milliliter collection was recorded. A series of timed samples was made until the flow rate indicated a stable state of perfusion.

Blood gas analysis was made on one milliliter and one-half milliliter blood samples with the van Slyke-Neil apparatus\textsuperscript{203} for the first few experiments reported for rabbit ear skin. In all other experiments the gas analysis was made with a Kopp-Natelson 204,205 microgasometer.\textsuperscript{*} The apparatus is similar in principle to the van Slyke-Neil apparatus, but it requires only 0.03 milliliter blood samples for the gas analysis. All blood samples were analyzed within ten minutes of the time that the blood was sampled.\textsuperscript{**} Arterial samples from the carotid artery were used to determine arterial oxygen content, and were drawn immediately following the completion of venous flow measurements.

The oxygen was released for analysis from the blood sample by alkaline solution of senega root (a laking agent), potassium ferricyanid (a mild oxidizing agent), and evacuation. Oxygen in


\textsuperscript{**}The oxygen consumption by blood at room and body temperatures reduces the oxygen content of a blood sample very rapidly.104
the extracted sample was absorbed with a solution of sodium thiou­
sulfite containing anthroquinone. The oxygen content was deter­
mined from the partial pressure of the gas which was absorbed.
Either a stream of nitrogen or oxygen from tank sources was intro­
duced into the tracheal cannula or the intake of the respirator to
vary the arterial oxygen saturation.

To control the temperature of the tissue preparations the
rabbit ear was held over a water bath, or a water pack was applied
to the muscle. The water bath for the rabbit ear was covered
with a piece of brass shim which was in contact with the circulat­
ing water. The water pack consisted of a heavy piece of dialysis
tubing through which water circulated. A temperature regulated
water bath was maintained several tenths of a degree above the
desired temperature (31°C. for rabbit ear, 37°C. for rabbit
muscle). Altering the rate of flow of water from the water bath
regulated the final temperature at the tissue.

Temperatures of the tissue were measured by means of thermo­
couples, platinum wire resistance thermometers, and thermistor
probes.** The majority of the skin experiments were measured with
the platinum resistance thermometer, while the majority of the
muscle experiments were measured with the thermistor probe.

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*Copper-constantan wire fused by an arc-weld and recorded by
a reflecting galvanometer.

**Tele-thermometer, Model 43TA, Yellow Springs Instrument
Company, Yellow Springs, Ohio.
Blood samples were also analyzed for hemoglobin content and blood oxygen concentration. Hemoglobin was determined colorimetrically by the method recommended by a committee of the Medical Science Division of the National Academy of Sciences. The hemoglobin concentration determinations in skin experiments were made with Hycel* chemical reagents and in muscle experiments they were made with Acuglobulin** reagents. From a lambda micro-pipette 0.02 milliliter of blood was added to 5.00 milliliters of ferricyanide vehicle. The optical density of the solution was read on a Klett photocolorimeter using a green (540 mu) filter. Calibration charts prepared from measurements made with standard solutions furnished with these reagents were used for a graphic interpretation of the optical density of the blood sample to obtain the blood hemoglobin concentration.

Other procedures were instituted in a few experiments to provide additional information on the approximation of the tissue oxygen tension which were obtained by calculation. Temperature was varied in a few experiments. In some experiments potassium cyanide and dinitrophenol were used to alter metabolism. The substances were introduced in the arterial supply to the muscle

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*Hycel Chemical Reagents, Cyamhemoglobin standard and reagent. Hycel Hormone Chemistry Laboratory, Houston, Texas.

**Acuglobulin standard and diluent pellets for cyanmethemoglobin method. Ortho Pharmaceutical Corporation, Raritan, New Jersey.
or ear in microgram quantities over a period of several minutes. The cyanide caused an accompanying change in blood perfusion, and the results with dinitrophenol were variable. The directional changes observed in tissue oxygen tension with both drugs were indicative but did not quantitatively indicate the reliability of the calculated oxygen tension.
CHAPTER III

RESULTS, CALCULATIONS AND ANALYSIS OF THE
MEASUREMENTS OF TISSUE OXYGEN TENSION

The experimental results obtained for tissue oxygen tension in skin and muscle of the rabbit are presented in Tables V, VI, VII and VIII and Figures 15 and 16 at the end of this chapter. The id and calculated pO2 for electrodes in which reliable polarographic measurements were obtained during a steady state in the tissue are presented in Figure 15 for the rabbit muscle and in Figure 16 for the rabbit skin. In Tables VI and VIII and Figures 15 and 16 the material represents selected data, the selection being based on the criteria presented below. Table IX summarizes the analysis of the experimental data for factors which indicate a change in the system for which oxygen tension was calculated or a change in the polarographic electrodes.

Experimental results were evaluated for the existence of reliable polarographic measurements and for the presence of a reproducible steady state in the tissue before comparisons were made of the id of an electrode and the calculated pO2 of the tissue. Polarographic measurements were screened for conditions in which analysis for id would give valid measures of oxygen tension. The main considerations were that:

1. A c-v curve be present in tissue measurements and in calibrations preceding and following tissue measurements.
2. A constant electrode surface existed during experiments. It was presumed that if the electrode sensitivity was similar in calibration before and after tissue measurements, no change in the electrode surface occurred during experiments.

3. Conditions in the region of the electrode were stable during tissue measurements. Since it in vitro was observed to vary less than 2% over several hours, repeated fluctuations of over 5% in tissue measurements were regarded as indicating unstable conditions in the tissue.

The reproducibility and existence of a steady state were determined indirectly from the:

1. Presence of constant temperature during measurements.
2. Variation of less than ten per cent in three consecutive collection times for blood flow samples.
3. Variation of less than ten per cent between initial control and the initial control observation.

The rationale of the polarographic criteria was enumerated in the discussion of the polarographic method, instrumentation and literature in the first chapter. The reasons for the criteria selected for the tissue measurements will become apparent during the discussion of the results.

The calculated oxygen tensions have been obtained by two methods, both based on Equation 23. The data are reported for the second method using the following equation:
\( \bar{p} \) - mean tissue oxygen tension

\( P_0 \) - average capillary oxygen tension

\( m \) - oxygen extraction

\( A \) - factor based on ratio of capillary and tissue radii

\( R \) - tissue cylinder radius

\( D \) - Krogh's value for diffusion of oxygen in muscle

The second method has the advantage of emphasizing the contribution of capillary oxygen tension and of tissue dimensions and metabolism to the estimated average \( P_0 \) in the tissue.

In Tables V and VII the value for \( A \) was obtained from Kety's Table 1.\(^{165} \) The value for \( D \) represents milliliters of oxygen crossing a centimeter square surface per minute under a gradient of a millimeter of mercury pressure (tension) per centimeter path length. \( \bar{p} \) is obtained when the calculated factor, \( m(AR^2/4D) \), is subtracted from average capillary oxygen tension, \( P_0 \). This factor, \( P_0 \) corrected for deviations in temperature from 37°C., was obtained from Dill's oxyhemoglobin dissociation curve.\(^2 \) The blood gas determinations and measured hemoglobin concentration were used to obtain the hemoglobin saturation of the blood.

The data from Tables V and VII have been separated on the basis of analysis into two series. The first series consists of those measurements made in tissue during reproducible steady states with electrodes having constant characteristics. The second series represents measurements made when either the tissue steady-state or electrode characteristics were variable.

Tabulations for comparison of selected \( id \) and \( \bar{P}O_2 \) are given
for rabbit muscle in Table VI and for rabbit skin in Table VIII. This comparison represents simultaneous measurements of \( i_d \) with polarographic electrodes that had constant characteristics throughout an experiment, and \( \bar{F}O_2 \) obtained during reproducible steady states in the tissue. The tabulated material is presented graphically in Figure 15 for rabbit muscle and in Figure 16 for rabbit skin. In the graphs the \( \bar{F}O_2 \) has been made the abscissa and \( i_d \) the ordinate. In the measurements made on rabbit muscle seven series of measurements out of thirty-two series were made when tissue and electrode characteristics remained constant. Ten out of thirty-eight series of measurements in rabbit skin had similar constant conditions present in tissue and electrode. In these series of measurements when \( i_d \) and \( \bar{F}O_2 \) are comparable, there is less than 10 per cent variation between initial and final control measurements. If the variation of individual polarographic measurements is based on the electrode sensitivity and calculated \( pO_2 \) for an experiment there is less than 10 per cent variation for any individual measurement and the average variation in skin is 7.5 per cent, while a value of 5 per cent is observed for muscle.

Table IX summarizes the analysis of the experimental data for polarographic electrode characteristics and repeatable steady-states in the tissue. The column headings indicate the basis for exclusion of the electrode comparisons with calculated values. The number of each of the categories has not been summarized, since, in tissue, so many factors could contribute to the
variability. Any summary would tend to emphasize either electrode or tissue variability as the primary cause for variations which are usually observed in polarographic measurements of tissue oxygen tension, and it is likely that both factors are usually involved. The only clear-cut evidence for electrode unreliability is to be found in the in vitro calibrations.

In summary these experiments indicate that:

1. Under controlled conditions polarographic measurements are demonstrated to be within ten per cent of the average tissue oxygen tension.

2. Due to variability in tissue and electrodes polarographic measurements in a majority of in vivo situations will yield only qualitative estimates of tissue oxygen availability.

3. Only a few of the cases of variability in polarographically measured tissue oxygen tension can be attributed directly to faulty electrodes.

4. The id observed probably represents true tissue oxygen tension, but the relationship to tissue circulation, metabolism, and oxygen availability requires additional information to completely characterize local tissue conditions.
SYMBOLS USED IN TABLES V AND VII

Symbols Appearing Under Experiments

N = control
+ = increased arterial pO2
- = decreased arterial pO2
O = circulatory arrest
KCN = potassium cyanide
DNF = dinitrophenol

Symbols Appearing In Table

( ) = estimated value
* = not corrected for tissue weight
C = no calibration check
c-v = no c-v curve
IF = more than 10% difference in control values
M = unstable
TABLE V
MEASUREMENTS AND CALCULATIONS OF TISSUE OXYGEN TENSION IN RABBIT MUSCLE

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<th>R radius</th>
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**COMPARISON OF id and pO₂ IN THE RABBIT MUSCLE**

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OXYGEN TENSION IN MUSCLE.

COMPARISON OF \( i_d \) AND \( pO_2 \) IN RABBIT MUSCLE
## Table VII

Measurements and Calculations of Tissue Oxygen Tension in Rabbit Ear

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*Note: The table includes data for various experiments labeled REG 1 to REG 20, with columns for flow rate, tissue oxygen extract, Po pressure, and deflection levels. The data is presented in a tabular format with specific measurements and calculations for tissue oxygen tension in rabbit ear tissue.*
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### TABLE VIII

**COMPARISON OF $i_d$ and $P_{O_2}$ IN THE RABBIT SKIN**

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OXYGEN TENSION IN SKIN

FIGURE 16.
COMPARISON OF $i_d$ AND $pO_2$ IN RABBIT SKIN
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# refers to electrode
CHAPTER IV
DISCUSSION OF TISSUE OXYGEN TENSION MEASUREMENTS

The description of tissue oxygen distribution depends upon assigning values to factors related to delivery of oxygen to the tissues and to storage and utilization. Transient states are difficult to treat quantitatively and, with the exception of Roughton's treatment of the problem, have not been described in mathematical terms. Even in steady states only approximations of the distribution are available, and these approximations depend upon simplifying assumptions. The simplification is visualized thus: the many factors related to oxygen availability in tissue vary individually while all other factors remain constant. Mathematical treatment depends upon this simplified approach and upon separation of the factors. The initial separation is to consider delivery as one aspect and tissue extraction as the other aspect.

Delivery is dependent upon rate of blood flow and the oxygen saturation of the blood. An increase either in rate of flow or in saturation of the blood in the absence of any change in the blood pattern will result in an increased storage in the tissue because of the elevated diffusion potential and an increase in venous oxygen saturation in the new steady state, since metabolism has not changed. As the effects of either increased flow or increased saturation are similar, they can be represented by a single
factor. In the use of Bierang's equation or of equations derived
on a similar basis, the average capillary oxygen concentration
can be used to represent delivery. In Kety's equations and in
the experiments which were reported, delivery is represented by
Co, the average oxygen concentration of blood.

A further complication of the tissue description, and the
primary stumbling block in mathematical treatment, is the presence
of hemoglobin and the necessity of changing from the use of con-
centration to the use of diffusion gradient in order to complete
the description of the oxygen delivery to the tissues. The bulk
filtration will deliver less than one per cent of the oxygen re-
quirement of the tissue if it is calculated from Pappenheimer's data, which means that the only mode of distribution to the
tissues of any significance is diffusion. The diffusion gradient
from blood is dependent upon the dissociation of oxygen from
hemoglobin stores and the physically dissolved oxygen. At oxygen
tensions above hemoglobin saturation the storage in blood is dis-
regarded, but at oxygen tensions in the region of oxyhemoglobin
dissociation, the diffusion gradient becomes dependent upon the
hemoglobin saturation, which is a complex function of oxygen
tension. The great advantage in using Co is apparent, since a
single value may be assigned to the diffusion pressure head if Co
is converted to per cent saturation. This diffusion pressure is
represented as p0 in the experiments. In most experiments the
slight hypoxia and only moderate elevation or depression from this
slightly hypoxic state served to keep the values of $p_o$ in the relatively linear portion of the oxyhemoglobin dissociation curve. The low $p_o$ should also serve to make the pressure head for diffusion along the capillary more nearly linear and, to a certain extent, justify the simplifying assumptions made with regard to oxygen delivery.

The other aspect of description of oxygen in the tissue depends upon the details of oxygen extraction. Oxygen extraction depends on the diffusion gradient and two major factors which remove the oxygen along that gradient, storage and metabolism.

One factor in extraction is presumed to remain constant. In vitro tissue metabolism of oxygen is independent of oxygen tension above a critical level of a few millimeters of mercury. It is presumed that a similar situation exists in vivo, although no critical test of the assumption has been made.

The other major factor in extraction is storage, which varies directly with oxygen tension. Consequently, during fluctuations in tension there is a transient modification of the associated storage. In a steady state the only factor presumed to be in operation is oxygen metabolism; this factor is presumed to be responsible for one of the characteristics of the slope of the diffusion gradient. In Equations 17 and 17a, $a$ represents the metabolism of oxygen. However, the fact that storage may be included should be borne in mind.

The tissue dimensions and diffusion gradient, are the
remaining factors required to describe the system. \( \text{D, } r/R, \text{ and } r \) aid in determining the slope of the diffusion gradient. The only experimental value for the coefficient of diffusion of oxygen in tissues is Krogh's constant.\(^{125} \) The original constant, 0.13 cc of oxygen across a square centimeter surface per minute under a gradient of one atmosphere per micron at 20° C., corrected for temperature and changed to fit the conditions of the experiments, is 2.1 x 10\(^{-3} \) cc of oxygen crossing a centimeter square surface per minute under a gradient of one millimeter of mercury per centimeter at 37° C.

By using the capillary radius, \( r, \) and the tissue cylinder radius, \( R, \) to describe the system, Kety has simplified the calculation of the tissue dimensions for their influence on the characteristics of the diffusion gradient. This treatment simplifies Equation 23 to

\[
\bar{P} = P_0 - m \left[ \frac{AR^2}{4D} \right]
\]  

(24)

The constant \( A/4 \) summarizes a number of the factors in Equation 23 which are related to the ratio of \( r/R. \) The ration \( r/R \) is used to obtain the value of \( A \) from Kety's table.\(^{165} \) The validity of using \( r/R \) is presented in the original article, where it has been demonstrated that the point in the tissues which has an oxygen tension equal to the integrated mean oxygen tension of the tissue cylinder is independent of the magnitude of \( m, D \) and \( R, \) depending only upon the ratio of \( r/R. \) This fact has been stated in various ways by
Jacobs,\textsuperscript{208} Hill,\textsuperscript{124} and Rashevsky,\textsuperscript{209} who have not given explicit mathematical relationships for the concept.

The factors discussed above – \( C_0 \), \( P_0 \), \( m \), \( A/4 \), \( R^2 \), and \( D \) – are thus sufficient to give an approximation of the average tissue oxygen tension if the tissue system has a reasonable similarity to the simplified model represented by Equation 22 or the derived Equation 24. \( C_0 \) or \( P_0 \) are independent variables; \( m \) and \( D \) are constants. All that is required to fix the system for description is the ratio of \( r/R \) and the magnitude of \( R \). Since a change in flow rate, presuming that the blood pressure gradient has remained constant, implies that there has been a change in the ratio of \( r/R \), two of the restrictions in the application of Equation 23 or 24 are that \( r/R \) be constant during measurements, and that \( r/R \) be similar for comparison of two situations in a tissue or that it be accurately determined for each of the situations. The reason for including the requirement that flow remain relatively constant during measurements as criterion for evaluating the results was established from the considerations given in this paragraph.

The other criterion, that temperature be constant, is also a necessary requirement in the application of the equation to obtain an estimate of tissue oxygen tension. Temperature modifies the blood perfusion characteristics, metabolism, diffusivity of oxygen, and the ratio of \( r/R \). Consequently, each variation in temperature requires a new set of parameters to describe the tissue system; or alternatively, the requirement of a fixed
experimental temperature.

One other set of presumptions is implicit in the experiments. It is presumed that the electrode is in contact with tissue which has an average oxygen tension described by the mean oxygen tension of the tissue. It is also presumed that the perfusion characteristics in the region of the electrode are similar to the characteristics observed for the tissue sample. Obvious ischemia in the region of the electrode indicates that these presumptions are invalid. The best available evidence that the presumptions were valid is that there is a linear relationship between the observed and the calculated value of \( \bar{p} \).

The result of the application of Kety's equations to the estimation of tissue oxygen tension is that, for the first time, polarographic measurements of tissue oxygen tension in vivo have been evaluated on the basis of oxygen tension at the polarographic analysis electrodes. Other investigators have used the oxygen tension measured in blood, the rate of oxygen utilization, or respiratory changes in evaluating the measurement obtained with polarographic electrodes. Although these techniques served the purpose of validating the fact that oxygen tension was being measured, it is evident from the discussion presented in the first part of this chapter that they do not give quantitative estimates of the oxygen tension at the electrode.

Although there are no similar quantitative evaluations of the polarographic measurements in the literature, other investigations
of tissue oxygen tension obtained by the qualitative polarographic studies and by classical studies indicate the validity of the presumptions which have been made in the reported experiments. Furthermore, the data reported for flow and metabolism are similar in magnitude to the values reported in the literature.

Fitzgerald\(^{200}\) has summarized the evidence for oxygen diffusion through the skin. This permeability of the skin to oxygen has also been reported by Baumberger and Goodfriend,\(^{136}\) who used a polarographic method. In spite of the skin permeability to oxygen, studies such as Urbach's\(^{139-41}\) indicate that even in unstimulated skin metabolism depends primarily upon blood for its oxygen supply. This dependence upon blood flow would imply that access to oxygen from sources other than the blood perfusion will not modify the findings.

Studies at the Robinette Foundation of the University of Pennsylvania on the polarographic oxygen tension in skin indicate that the primary influence of temperature variation on skin oxygen tension is due to altered perfusion. Montgomery's\(^{164,176-78, 210-212}\) and Penneys's\(^{163,167}\) studies clearly reveal marked increases in blood flow with increasing temperature and an associated rise in skin oxygen tension dependent upon the initial state of the skin. The studies indirectly indicate that \(r/R\) is decreased by increased temperature, as it was noted that equilibrium with altered oxygenation of blood requires less than a minute in vasodilated skin and as long as an hour in vasoconstricted muscle
and skin.

Kaplan and Clark\textsuperscript{186} and Penneys\textsuperscript{163} have observed the \textit{in vivo} oxyhemoglobin dissociation curve, which has the same characteristics as those observed \textit{in vitro}. The polarographic studies on skin just mentioned, as well as those cited in the review of the literature, have varied by ten to twenty five per cent during successive measurements under similar conditions.

Fewer polarographic studies have been made of oxygen tension in muscle than have of oxygen tension in skin. Davies,\textsuperscript{150} who made one of the earliest studies, used relative changes in \( i_d \) during muscle activity to follow tissue oxygen characteristics. Montgomery's group,\textsuperscript{176-77,206} unable to estimate a sensitivity for their electrodes in muscle, relied entirely upon relative changes in observed \( i_T \) to interpret the influence of the applied experimental variables. Wolferth\textsuperscript{161 et. al.} also relied upon the rate of increase in muscle \( i_T \) to indicate the nature of \( pO_2 \) in muscle as the ventilated gas mixtures containing oxygen were varied. Kay\textsuperscript{171} and his collaborators, as well as Behrman \textit{et. al.},\textsuperscript{169} have had similar experiences in attempts to quantitate muscle oxygen tension.

Of the other classical studies on tissue oxygen tension the values obtained from gas equilibration techniques\textsuperscript{198,214} will probably have to be disregarded. Those of spectrophotometric nature\textsuperscript{215} or those dependent upon calculations\textsuperscript{215-18} similar to the ones reported in the last chapter indicate values for oxygen
tension in the same range as those reported in the thesis. In an analysis of the gas bubble technique, Rahn\textsuperscript{214} reported that the equilibrated gas tension probably reflects venous gas tension rather than tissue gas tensions. This fact could have been suspected from the studies of Bazett and Scribbyatta\textsuperscript{219} who clearly stated that they observed the spread of the gas bubbles along the major blood vessels during injection of the gas.

In studies on blood flow and oxygen consumption of muscle with the method described in this thesis, Verzar\textsuperscript{217} observed blood flows of 0.015 to 0.6 ml/gm/min and oxygen consumptions of 0.0024 to 0.0085 cc/gm/min in the hind limb of a resting dog. Millikan\textsuperscript{207} in his spectrophotometric determinations of oxygen consumption and blood flow, estimated blood flow at 0.036 to 0.600 ml/gm/min and oxygen consumption of 0.0078 to 0.0210 cc/gm/min for resting cat soleus muscle. Millikan also observed an increase in blood flow when the nerve to his muscle preparation had been severed. Mottram\textsuperscript{216} used a less drastic experimental procedure, retrograde catheterization of the muscle vein, and observed an oxygen consumption of 0.0024 ml/ml/min. This is one third larger than that reported for this method in earlier investigations, \textsuperscript{220-23} which Mottram attributes to a more representative venous sample in his experiments. These values for muscle blood flow and oxygen consumption as well as others published in the Handbook of Biological Data,\textsuperscript{5} indicate that the values obtained in the present study are within reason.
Studies on rabbit ear skin metabolism and blood flow have not been made by methods closely related to these experiments. Burton\textsuperscript{224} has observed pressure-flow curves in the entire perfused rabbit ear. One factor observed by Burton was also noted in the present experiments. While the preparation (rabbit ear) remains viable, there is a gradual change in the perfusion rate with time, indicating a different perfusion pattern. This may be associated with numerous a-v anastomoses observed in the rabbit ear.\textsuperscript{225-28} The values found for skin blood flow and oxygen consumption in the Handbook are of the same order of magnitude as those reported in the previous chapter.

One factor, the ratio of \( r/R \) for muscle, is substantiated in the literature, but the value used for skin could not be verified. In his initial studies on the capillarization of muscles Krogh\textsuperscript{229} indicated that there is about a one to one relationship between muscle fibers and open capillaries. This was also observed by Martin \textit{et al.}\textsuperscript{230} and is the summarized value reported in the Handbook\textsuperscript{5} for resting skeletal muscle. Clark's observations\textsuperscript{225-27} as well as those of others\textsuperscript{228} on the lucite chamber studies of blood flow in the rabbit ear indicate that the capillary size used for rabbit ear (3.5 \( \mu \) radius) is approximately right. Since no claim is made for the absolute accuracy of the blood vessel diameter, muscle fiber diameter, or thickness of the skin it is evident that there is a possibility that the ratio \( r/R \) is in error. This will have no effect on the linearity of the calculated tissue
oxygen tensions since it will only modify the final value by a fraction of the constant A which was used. Kety has shown that an error in R, which in these experiments should have been no more than twenty per cent from the true value, doubles the value of the estimated pO2 for a 50% underestimate of R and halves the value of the estimated pO2 for a 40% overestimate, with marked increases for larger errors. Hence, the absolute values of tissue pO2 have not been determined by the method of calculation. At the same time it is evident that if the ratio r/R is constant during an experiment, the calculated pO2 will bear a constant relationship to the true pO2. An approximation of the magnitude of error which might exist can be obtained from the limitation of altered blood flow to within ten per cent of the control value and application of Poiseuille's Law. Since flow will vary directly with R⁴, it is evident that a ten per cent increase in flow should limit changes in the capillarization of tissues to sufficiently small values which will not appreciably modify the system which was being studied. The ten per cent limit chosen for these experiments represents a change of about one capillary per hundred already active.

In summary, Kety's equations and the simplifications which are necessary in order to gain an approximation of the tissue oxygen tension indicate that, within the limitations used to evaluate the experimental data for comparison of polarographic and calculated tissue oxygen tensions, the calculated tissue oxygen tension
should provide a reasonable approximation of the oxygen tension existent at the electrode. The polarographic literature on tissue oxygen tension indicates that the presumptions used in these experiments are fundamentally sound, and that the quantitative evaluation of the polarographic measure of tissue oxygen tension is the first to have been reported. The calculated tissue oxygen tensions, metabolic rates and blood flows observed in these experiments are of the same order of magnitude as those reported in the literature.
CHAPTER V
CONCLUSIONS

The investigation of the accuracy of polarographic measurements of oxygen tension in tissue has made contributions to several areas of knowledge. These areas include 1) additional information concerning the behavior of oxygen in solution, 2) evaluation of two methods of measuring tissue oxygen tension, and 3) indications for the future developments needed to make absolute determinations of tissue oxygen tension.

A method has been developed and instrumented for the determination of the value for the diffusion coefficient of oxygen. Through the use of this method a value for the coefficient of $2.23 \times 10^{-5}$ cm$^2$ sec$^{-1}$ has been determined. It has been indicated that further application of the method will result in the determination of the influence of factors in the system upon diffusivity of oxygen. The method will also provide information on the dependence of the coefficient upon the nature of the diffusion gradient. In addition the method can be extended to the study of diffusion in membranes of oxygen and ions. Diffusion coefficients determined in a polarographic diffusion cell should have the same accuracy as diffusion coefficients studied in a refractometric or interferometric cell, because the accuracy and principles of the methods are similar.

Polarographic measurement of oxygen tension has been compared
with tension calculated from experimental measurements. This comparison supports the hypothesis that polarographic measurement of tissue oxygen tension has the same inherent accuracy as the accuracy obtained in non-tissue measurements. Since the comparison of polarographic and calculated oxygen tension during standardized conditions in the tissue indicates that there is a quantitative relationship between the two measurements, it is reasonable to assume that polarographic diffusion current indicates true oxygen tension of tissue even during variation in the parameters of tissue oxygen availability. The method of evaluation of polarographic measurements for variability and reliability has identified factors in both tissue and the polarographic method which will contribute to the non-quantitative nature of tissue measurements which have been reported in the literature. Analysis of the absolute values obtained for the two estimates of tissue oxygen tension indicate that neither method can give a definitive value for average tissue oxygen tension without further modification of the technique of measurement. The final contribution is to suggest that absolute values for tissue oxygen tension can be obtained by an electrode which can be calibrated and remains independent of its environment.
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APPROVAL SHEET

The dissertation submitted by Albert F. Kelso has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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Signature of Adviser