1972

Sources of error in reflection oximetry

Michael Justin Brown
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Biomedical Engineering and Bioengineering Commons

Recommended Citation
Brown, Michael Justin, "Sources of error in reflection oximetry " (1972). Retrospective Theses and Dissertations. 5242.
https://lib.dr.iastate.edu/rtd/5242

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This dissertation was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in “sectioning” the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again – beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from “photographs” if essential to the understanding of the dissertation. Silver prints of “photographs” may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

University Microfilms
300 North Zeeb Road
Ann Arbor, Michigan 48106
A Xerox Education Company
BROWN, D.V.M., Michael Justin, 1944—
SOURCES OF ERROR IN REFLECTION OXIMETRY.

Iowa State University, Ph.D., 1979
Engineering, biomedical

University Microfilms, A XEROX Company, Ann Arbor, Michigan
Sources of error in reflection oximetry

by

Michael Justin Brown

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Physiology (Domestic Animals)

Approved:

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
For the Major Department

Signature was redacted for privacy.
For the Graduate College

Iowa State University
Ames, Iowa
1972
PLEASE NOTE:

Some pages may have
indistinct print.

Filmed as received.

University Microfilms, A Xerox Education Company
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>PART I. IMPROVED DESIGN OF A REFLECTION OXIMETER UTILIZING PHOTOCONDUCTIVE LIGHT DETECTORS</td>
<td>4</td>
</tr>
<tr>
<td>REVIEW OF THE LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>Theory of Reflection Oximetry</td>
<td>5</td>
</tr>
<tr>
<td>Light Sources</td>
<td>6</td>
</tr>
<tr>
<td>Photodetectors</td>
<td>9</td>
</tr>
<tr>
<td>Calibration</td>
<td>12</td>
</tr>
<tr>
<td>Computation and Display of Output</td>
<td>14</td>
</tr>
<tr>
<td>INSTRUMENTATION</td>
<td>16</td>
</tr>
<tr>
<td>Optical System</td>
<td>16</td>
</tr>
<tr>
<td>Electrical System</td>
<td>18</td>
</tr>
<tr>
<td>METHODS</td>
<td>20</td>
</tr>
<tr>
<td>Stabilization of the Light Source</td>
<td>20</td>
</tr>
<tr>
<td>Stabilization of Photocell Response Curves</td>
<td>20</td>
</tr>
<tr>
<td>Matching of Photocell Sensitivities</td>
<td>21</td>
</tr>
<tr>
<td>Calibration</td>
<td>25</td>
</tr>
<tr>
<td>Linearization of Oximeter Output</td>
<td>25</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>30</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>41</td>
</tr>
<tr>
<td>PART II. CYCLIC ERYTHROPOIESIS IN CANINE BLOOD DONORS</td>
<td>42</td>
</tr>
<tr>
<td>REVIEW OF THE LITERATURE</td>
<td>43</td>
</tr>
<tr>
<td>Recovery from Acute Hemorrhage</td>
<td>43</td>
</tr>
</tbody>
</table>
Nutrition of Blood Donors 44
Life Cycle of Erythrocytes 44
Prior Evidence for Cyclic Erythropoiesis 45

METHODS 47
Conditioning, Nutrition, and Care of Donors 47
Collection of Blood 47
Hematologic Monitoring 48

RESULTS 51
Plasma Protein Concentration, PCV, RBC, Total Hemoglobin, and Wintrobe Indices 51
Osmotic Resistance 58
Peripheral Blood Smears 59

DISCUSSION 64

SUMMARY 70

PART III. EFFECTS OF HEMATOLOGIC VARIABLES ON ACCURACY OF REFLECTION OXIMETRY 71

REVIEW OF THE LITERATURE 72
Optical Properties of Erythrocyte Suspensions 72
Hematologic Variables Affecting Reflection Properties 76

METHODS 82
Control of Hematologic Variables 82
Experimental Measurements 88

RESULTS 91
Differences Between Donors 91
Effects of Differences in Mean Erythrocyte Age 95
Effects of Changes in PCV 98
ABSTRACT

Stability, accuracy, and convenience of a fiberoptic reflection oximeter utilizing CdSe photocells were improved by simple alterations of the optical system and of the Wheatstone bridge readout circuit. Use of a halogen-cycle lamp and neutral-density optical wedges, together with a two-standard calibration technique, improved the stability and precision of the transducer. Continuous illumination of the photocells between periods of use and matching of the photocell sensitivities reduced losses of precision and accuracy previously encountered with these photodetectors. The output of the oximeter was made linear and direct-reading by simple adjustments of the Wheatstone bridge parameters.

Evidence of increased erythropoietic activity appeared every four months following acute massive blood loss in dogs. Each period of activity lasted approximately 30 days, and such crises were readily detected even a year after the hemorrhage. Each erythropoietic crisis was accompanied by decreases in RBC, PCV, and total hemoglobin; increases in MCV, MCH, MCHC, and resistance of erythrocytes to osmotic hemolysis; and increased anisocytosis and polychromatophilia on Wright's-stained smears of venous blood. Cyclic erythropoiesis results from synchronization of life cycles of erythrocytes produced in response to the hemorrhage; it is detectable by virtue of changes in physical characteristics of these cells as they age.

Effects of changes in PCV, plasma protein concentration, degree of hemolysis, osmotic pressure, pH, $P_{CO_2}$, temperature, and mean erythrocyte age on accuracy of reflection oximetry were studied using canine blood. Decreases in PCV below 30% or increased plasma osmotic pressure caused
systematic overestimation of relative oxygenation, whereas decreased plasma osmotic pressure or low mean erythrocyte age had the opposite effect. It is proposed that differences between calibration curves for samples of blood from different dogs are due to variations in mean erythrocyte age. Mechanisms which may be responsible for this are discussed. Possible significance of these findings in use of reflection oximeters in control systems for artificial hearts is also discussed.
INTRODUCTION

Blood consists of a suspension of cells in an aqueous solution called plasma. Of the several types of cells present, the most numerous are the erythrocytes, from five to eight million per cubic millimeter, constituting 40 to 50% of the volume of whole blood (17). They are discs with a mean diameter of 7 to 10 microns and a mean thickness of 1 to 2 microns (30), containing hemoglobin, a protein-porphyrin compound, in solution at a concentration of about 35 gm. per 100 ml. of cells.

Each molecule of hemoglobin contains a protein in combination with four heme groups. Each of these can bind reversibly with one molecule of oxygen, and binding by one facilitates binding by the other groups in the molecule, so that hemoglobin molecules tend to be either completely oxygenated (oxyhemoglobin) or completely reduced (reduced hemoglobin) (30). The percentage of oxygenated hemoglobin in blood is referred to as "relative oxygenation". Transport of oxygen is one of the primary functions of blood.

All tissues require a continuous supply of oxygen to maintain vital biochemical functions. This is derived from blood flowing through the tissues. Oxygen bound to hemoglobin is released in response to decreases in its partial pressure, \( P_0 \). A graph of relative oxygenation as a function of \( P_0 \) is known as an oxyhemoglobin dissociation curve. Both relative oxygenation and \( P_0 \) fall as the blood traverses capillary beds in the tissues until equilibrium is reached with the \( P_0 \) of the tissue. Since tissue \( P_0 \) is a dynamic value determined by the rates of inflow and utilization of oxygen, venous relative oxygenation values indicate adequacy
of blood flow. The normal value is about 70% oxyhemoglobin, at a $P_{O_2}$ of 40 mm. Hg.

Measurement of relative oxygenation is important in study of cardiac and pulmonary diseases (33) and in evaluation of effectiveness of artificial hearts and heart-lung machines (18,23). The standard method for determination of relative oxygenation is the gasometric method of Van Slyke and Neill (76) in which bound oxygen is chemically released and measured by volume. Various transmission spectrophotometric methods, in which the blood is hemolyzed, i.e., the erythrocytes are ruptured to form a hemoglobin solution, and the concentrations of total hemoglobin and of oxyhemoglobin are determined in accordance with Beer's law, are more commonly used. Both of these methods are time-consuming and result in destruction of a volume of blood withdrawn from the patient (41). Thus, dynamic studies are not possible, and transfusions may be required.

A third method has been studied which overcomes these limitations (5). Reflection spectrophotometry, or "reflection oximetry", can be performed either on a sample temporarily removed from the patient (64) or on blood within the cardiovascular system. The latter can be done either with a photometer attached to the skin (55) or with a fiberoptic waveguide conducting light through a catheter (58). The accuracy of the skin photometer or "ear oximeter" is limited by variations in skin thickness and pigmentation, distribution of vessels, and other factors (42). It is used primarily as a qualitative clinical tool (55). In contrast, claims of very good accuracy are made for the direct type of reflection oximetry (21).

In the direct method, the light source and photodetector are
separated from the blood only by optical elements of the instrument, thus eliminating many of the variables of percutaneous oximetry. Even so, the reflection properties of the blood are influenced by factors other than the relative oxygenation. These include the concentrations of cells and of hemoglobin, the color and refractive index of the plasma, the shape and orientation of the cells, the velocity of blood flow, and possibly other factors. The purpose of the research summarized in this dissertation was to systematically study the effects of changes in hematologic variables on the accuracy of direct reflection oximetry, both qualitatively and quantitatively.
PART I. IMPROVED DESIGN OF A REFLECTION OXIMETER UTILIZING PHOTOCONDUCTIVE LIGHT DETECTORS
REVIEW OF THE LITERATURE

Theory of Reflection Oximetry

Reflection oximeters are photoelectric instruments used for continuous estimation of relative oxygenation of blood. While their design is based on photometric principles, the need for simplicity of construction and operation in commercial instruments introduces constraints on accuracy and precision of practical measurements (73).

The basis of reflection oximetry is the functional relationship between relative oxygenation of blood and the ratio of its reflectances for light of different wavelengths (21). The intensity of reflected red light is proportional to the relative oxygenation (53) and parabolically related to the hemoglobin concentration (2,38). Thus, single-wavelength oximeters are sensitive to changes in erythrocyte concentration (18,67). For wavelengths in the near-infrared (e.g., 805 nm.), the intensity of reflected light is independent of relative oxygenation, yet related to hemoglobin concentration in the same manner as for red light. Hence, the ratio of intensities of reflected red and infrared light, the "reflection ratio", is linearly related to relative oxygenation but independent of hemoglobin concentration (21,26,79). The purpose of a reflection oximeter is, therefore, to measure the reflection ratio for two suitable wavelengths so that the corresponding value of relative oxygenation can be determined.

Clearly, this scheme works only if the reflection ratio is independent of variables other than relative oxygenation. The validity of this assumption is the subject of the studies reported in Part III of this dissertation.
Light Sources

Ideally, the optical system for a reflection oximeter should include a monochromatic light source of high output power and constant intensity for each of the two wavelengths. In practical instruments, these requirements are not completely fulfilled, so that problems of calibration, sensitivity and noise levels, and instability arise. The essential criterion for the light source is that the ratio of intensities integrated over the two bandwidths remains constant while the oximeter is in use. Changes in this ratio would result in drifting of oximeter readings or in higher frequency noise.

Light sources heretofore used in reflection oximetry fall somewhat short of ideal characteristics. The only report in which a truly monochromatic light source was used is that by Edgington and Cholvin (19). A helium-neon laser with an output of about 0.5 mw. at 632.8 nm. was used to study changes in reflectance due to pulsatile flow, velocity of flow, and relative oxygenation. Thus far, no reports of two-wavelength oximetry with lasers are available.

More recently, the light emitted at p-n junctions of certain semiconductors has been used in oximetry. These light-emitting diodes (LED's) produce light with a half-intensity bandwidth of 10 to 40 nm. However, present LED's yield only low intensities and are not available with peak emission at true isobestic wavelengths, e.g., 805 nm., of the hemoglobin-oxyhemoglobin system. Application so far has been limited to percutaneous and flow-through oximeters (11). In addition, both the intensity and peak wavelength of emission vary with temperature, so that thermal compensation must be included in the computation circuitry (10).
Others have modified commercial photometers (65) or spectrophotometers (2) for study of reflection properties of blood. These provide relatively narrow bandwidths, but are bulky, and of course two instruments are required for two-wavelength oximetry (65).

Thus far, the most popular light source is the tungsten-filament lamp in combination with a regulated power supply and optical filters to provide narrow bandwidths of light (21,22,25,26,33,50,51,55,58,59). For intra-vascular oximetry the optical system also includes fiberoptic waveguides to conduct light to and from the blood, but the principle is the same. Tungsten lamps supply electromagnetic radiation over a continuous range including parts of the ultraviolet and infrared as well as all of the visible spectrum. Either the incident light or the reflected light beam is split and passed through filters to produce bands centered around the desired wavelengths. The reflected light is then focused on the photodetector.

A number of problems occur with use of tungsten-filament lamps in oximetry. First, only a small percentage of the energy emitted is in the useful wavelengths; the rest must be absorbed by components of the oximeter, thus generating heat which must be effectively dissipated to protect heat-sensitive elements of the instrument. The emission spectrum of tungsten is similar to that of an ideal black body, except that it radiates only 43% as much power at any wavelength as the equivalent black body at the same temperature (37). Its emissivity is slightly higher in the blue than in the red, so that its spectral distribution curves resemble those of black bodies at temperatures somewhat higher than the actual filament temperatures. While the shape of its spectral distribution curve is constant,
its amplitude and position on the wavelength scale vary with filament
temperature, and hence with filament voltage or current. As temperature
is increased, the maximum emission occurs at shorter wavelengths and the
intensity of emission increases. Lamp output varies nearly as the square
of the applied voltage (28), so that a well-regulated power supply is
required to prevent fluctuations in both intensity and spectral distribu-
tion.

Drift in intensity ratio (spectral distribution) occurs as incandescent
lamps age, even with constant power. This results in a drop of as much as
100°K in color temperature over the lifetime of a lamp. An additional
filter or optical wedge was used in other oximeters to compensate for this
effect (25). In addition, deposition of tungsten evaporated from the
filament onto the inner surface of the bulb causes a loss of output power
and of efficiency as lamps age. Output is reduced by 10% at half-life and
up to 40% by the end of life (37). Compensation for this effect requires
adjustment of the power supply or light attenuators, or frequent replace-
ment of lamps. Operation of lamps in a base-up position slows the decrease
in output intensity.

Several of the problems associated with tungsten incandescent lamps
can be reduced by inclusion of a small amount of halogen vapor in the
filling gas. If such lamps are operated at sufficiently high filament and
bulb temperatures, the halogen will combine with tungsten vapor, preventing
its deposition on the bulb. Tungsten halide diffuses to the filament and
dissociates, increasing the vapor pressure of tungsten around the filament
and thus promoting its redeposition onto the filament. Practical halogen-
cycle lamps containing iodine are available in several wattages and bulb
configurations. Bulbs of fused silica are used to withstand the minimum operating temperature of 250°C. Advantages of quartz-iodine lamps also include up to double the operating lifetime, smaller size, and higher color temperatures, with virtually no change in output or color temperature with time. Use of quartz-iodine lamps in reflection oximetry was first reported in 1968.¹

Photodetectors

Three types of photodetection transducers are in common use for photometric and spectrophotometric measurements: 1) photoemissive tubes, including vacuum and gas photodiodes and photomultiplier tubes; 2) photovoltaic or barrier-layer cells; and 3) semiconductor photocells and photodiodes. The choice of a photodetector for a particular application should be based on the range of intensities of light to be measured, its spectral distribution, the necessary frequency response, and conditions under which the measurements are to be made (61).

In reflection oximetry the intensity to be measured depends on the output of the source and its proximity to the blood. Of particular importance is the use of fiberoptic waveguides which results in greater losses of intensity than occur with flow-through types of direct oximeters. This disadvantage may be acceptable, however, when fast dynamic response and the ability to sample remote compartments of the cardiovascular system are important. For obvious reasons, only detectors which are sensitive

to red and infrared light are useful in oximetry. The necessary dynamic response depends on whether one wishes to read average values of relative oxygenation or to follow fluctuations which accompany the respiration and heartbeat (41). A fast dynamic response is required in diagnostic cardiac catheterization (25), but a much slower response could be acceptable or even advantageous in a control system for an artificial heart (22).

Conditions which adversely affect the performance of certain photodetectors include variations in ambient temperature, prolonged or continuous operation, stray magnetic fields, and vibration or rough handling.

Of the photoemissive tubes, the photomultiplier is the most popular in reflection oximetry (18,21,33,38,41,51,59). In these devices, photons release electrons from a metallic cathode, and the resulting current is amplified within the tube. The spectral sensitivity depends on the composition of the cathode and the bulb. Most are highly sensitive in the ultraviolet and visible spectra, but only certain types are useful in the infrared. They require a well-regulated source of high voltage for the electrodes, and are rather sensitive to external magnetic fields. Their sensitivity changes with time, so that frequent recalibration may be required. Environmental conditions alter their dark current, sensitivity, and noise levels, and they are easily damaged by vibration. Exposure to intense light sources can cause them to overload and burn out. The chief advantages of photoemissive tubes are their very fast response times ($10^{-9}$ to $10^{-3}$ sec.) and the excellent linearity of the relation between intensity of incident light and magnitude of the output current (28).

Barrier-layer photovoltaic cells were used in the earliest reflection oximeters (5) and are still used in earlobe or percutaneous oximetry. In
these devices, photons generate a potential difference across a barrier separating two substances, and closure of the external circuit allows current to flow. The spectral sensitivity is high in the visible region and can be extended into the near-infrared. Since the current is generated by the cell, no external source is required and the signal is detected by a low-impedance galvanometer. The response time is quite long, usually 5 msec. or longer. Saturation occurs at high intensities and thermal effects are very important. Since power transfer efficiency, linearity, and temperature sensitivity of these cells all depend on the output impedance, optimization of performance is very difficult (28).

The use of photoconductive cells in oximetry was introduced in 1960 (58) but few reports of their use have appeared since then (22, 56). In these devices, photons cause release of electrons from the lattice of a semiconductor, resulting in increased conductivity. The spectral response peaks in the red or infrared, but large changes of resistance can be produced over a wide range of wavelengths. Their conductance varies linearly with intensity at high light levels, so that the resistance varies reciprocally with intensity. They are usually small, rugged, and inexpensive. The response time depends on the material and the level of illumination, but is seldom very fast (28). Both the sensitivity and response times of photoconductive cells are affected by their "light history". Long exposure to high intensity light causes a reduction in sensitivity and somewhat faster response. "A commonly used production-testing preconditioning schedule provides for exposure of the cells to a 500-footcandle fluorescent light for 16 to 24 hours before measurement of sensitivity." Their sensitivity at low light levels is also affected by changes in
temperature (61).

A potentially useful photodetector for oximetry is the semiconductor photodiode or junction photocell. With these devices, the position of the volt-amp characteristic curve depends on the level of illumination. They can be operated with either forward or reverse biasing and the output current is linearly related to light intensity. They have a wide spectral response and very high sensitivity, and require minimal regulation of the supply voltage. Response times are very fast, and the cells are compact, rugged, and fairly low in cost. They do not drift, are insensitive to temperature changes, and are quite uniform in quality from one unit to another of the same type. Their limitations are that they are directionally sensitive, requiring precise optical alignment, and have a very small output current, so that a high-gain, high-input-impedance amplifier is required (74).

Calibration

Because of the difficulties in making absolute measurements of light intensity and absolute reflecting power of sample surfaces, reflectance measurements are usually made relative to a white standard such as MgO, MgCO$_3$, or BaSO$_4$ (78). An ideal white reflectance standard would reflect all incident light regardless of wavelength. Real standards absorb at least 1% of any incident light, and the absorbance varies with the wavelength. Of the standards listed above, MgO exhibits the most uniform reflectance in the visible and infrared spectra (78).

While powdered MgO and MgCO$_3$ have been used to calibrate reflection oximeters in which the sample of blood is in a cuvette (58,59) the accepted
standard for fiberoptic reflection oximetry is Milk of Magnesia, U.S.P., a suspension containing 7 to 8 1/2% Mg(OH)$_2$ in water (21,25,26,33). While no critical data on the absolute reflectance of Mg(OH)$_2$ are available, it is assumed to reflect equally at the two wavelengths used in oximetry.

Calibration procedures used by the various investigators in reflection oximetry are somewhat similar in spite of the differences in instrumentation employed. In most cases, the white standard is placed in the light path where the blood would otherwise be and adjustments are made in the optical system to equalize the resulting signals at the two wavelengths. This can be done by adjusting an iris diaphragm (58) or an optical wedge (25) placed in either the red or the infrared light path. When only one photodetector is used for both wavelengths an optical wedge which absorbs at one of the wavelengths but is transparent at the other is used (26). Polanyi and Hehir (58) attenuated the light reflected from the white standard with a second iris diaphragm in order to calibrate their photocells at the intensity of 805 nm. light ordinarily reflected by blood. This was said to be necessary because the two photocells were matched over only a narrow range. Presumably, this precaution is not necessary when photomultiplier tubes are used as the detectors.

The white standard is used as a reference point for oximeter readings. The value of relative oxygenation of blood which gives the same reflectance ratio (i.e., unity) as the white standard apparently depends upon the geometry of the optical system, since various investigators give values from 97% oxyhemoglobin (33) to 70% (59) even though they used the same two wavelengths, 660 nm. and 805 nm.

As a refinement of the calibration procedure, several workers used a
second standard to verify the gain of the oximeter. Polanyi and Hehir (59) did not name the second standard; Gamble et al. (26) inserted a blue filter into the light path; and Frommer et al. (25) used three optical filters to get three points for calibration. None of these authors gave any data supporting the use of multiple standards as compared to using only the white standard.

Computation and Display of Output

The input to an oximeter consists of the relative reflectances of blood for each of the wavelengths used. The instrument may be designed merely to display the measured values of these reflectances or to compute and display the ratio between them or to compute and display an estimate of the relative oxygenation. With early oximeters (21,79) the ratioing procedure was done manually, but later instruments utilized mechanical (55,59), electromechanical (26), or electronic (25,51,59) ratioing devices. Those using photoconductive detectors used a Wheatstone bridge (22,58) or an operational amplifier (56) to perform the calculation automatically. Sutterer and Krovetz (71) described "an electronic analog system ... which performs the double scale calculation from the Wood oximeter and gives a single output as a log ratio and also gives a digital display of oxygen saturation .... This system ... consists of operational amplifiers to amplify the output of the red and infrared photocells, logarithmic devices to calculate the optical density as sensed by these two photocells, a division circuit to calculate the log ratio, a scaling circuit to facilitate digital display in percent oxygen saturation, and a digital voltmeter". Most other oximeters have a meter with a scale incorporating the ratio
function and reading directly in terms of relative oxygenation. When the reflection ratio is the only output, a graph or nomogram is used to convert it to relative oxygenation.
INSTRUMENTATION

Optical System

The optical system of the oximeter used in this study is similar to that used by Erickson (22) and by Peura (56) with the addition of two neutral-density optical wedges and substitution of a quartz-iodine lamp for the simple incandescent lamp. The light source is a modified high-intensity fiberoptic illuminator\(^1\) containing a 150 watt/120 volt quartz-iodine projector lamp\(^2\) which has an integral focusing reflector with a heat-transmitting mirrored surface. The modifications consist of rewiring to allow operation of the lamp from a regulated DC power supply\(^3\), and addition of an Inconel-coated circular neutral-density optical wedge\(^4\) between the lamp and the fiberoptic adapter. The optical wedge is rotated via a gear train connected to a knob on the front panel of the lamp housing.

The proximal end of the fiberoptic bundle\(^5\) is divided into three sets of fibers, the opposite ends of which are randomly distributed in the single bundle at the distal end. The waveguide is 46 cm. long with a nominal bundle diameter of 0.32 cm. One of the proximal bundles is connected to the lamp housing by an adapter and conducts light to the blood at the distal end. The other two conduct reflected light to the filter-photocell

---

\(^1\)Fiber-lite, Dolan-Jenner Industries, Inc., Melrose, Massachusetts.

\(^2\)Type DNE, Sylvania Electric Products Co., Salem, Massachusetts.

\(^3\)Model 6443B, Hewlett Packard, Berkeley Heights, New Jersey.


\(^5\)Type FF, Dolan-Jenner Industries, Inc., Melrose, Massachusetts.
assemblies. The distal or sensing end is fitted with a modified coaxial connector\(^1\) which joins it to a modified "T" connector\(^2\) through which blood may flow. The flat surface of the distal end of the fiberoptic bundle is set flush with the wall of the "T" connector to minimize turbulence. The depth of the layer of blood in front of the fiberoptic bundle is about 10 mm. This is sufficient to insure that all incident light is either reflected or absorbed by the blood and no external light reaches the fiberoptic bundle, except with very dilute erythrocyte suspensions.

The photodetectors are two matched CdSe photoconductive cells\(^3\) with a peak spectral response at 690 nm. The response at 660 nm. is approximately 17% of the peak value, while that at 805 nm. is about 25%, so that the cell used in the 805 nm. path is approximately one and one-half times as sensitive to changes in intensity as the one used in the 660 nm. path. The method used to equalize the effective sensitivities of the two cells is described in a later section.

The filters\(^4\) are a dielectric interference type with a maximum transmission of 35 to 45%. The bandwidth of the 660 nm. filter is approximately 10 nm., while that of the 805 nm. filter is approximately 13 nm. The filters and photocells are mounted in an insulated box to minimize

---

\(^1\)BNC type, Amphenol coaxial connector, Allied Electronics, Inc., Chicago, Illinois.

\(^2\)#T 5305, Scientific Products Co., Evanston, Illinois.

\(^3\)Type CL604L, Clairex Corp., New York, N. Y.

\(^4\)Baird Atomic, Inc., Cambridge, Massachusetts.
changes in the temperature of the photocells; the inside of the box is coated with a flat black paint to absorb stray light. The second optical wedge in the oximeter is located between the 660 nm. filter and the corresponding photocell, and is rotated by means of a gear train attached to a knob on the side of the box. The distance between the ends of the fiberoptic bundles and the front surfaces of the photocells is about 2 cm., including the filters and optical wedge. Electrical connections to the photocells are made through the back of the box. A small lamp¹ is mounted near each photocell, with leads emerging at the back of the box. Their function is described later.

**Electrical System**

The photocells are connected as two arms on one side of a DC Wheatstone bridge circuit as described by Erickson (22). The bridge is modified such that the arms opposite the photocells consist of 500 kilo-ohm (KΩ) and 50 KΩ potentiometers, respectively. A regulated DC power supply² has been substituted for the battery supplying the input voltage for the bridge. A switching circuit has been added to facilitate measurement of the photocell resistances, input and output voltages of the bridge, and the voltage across the lamp terminals. A pair of matched 20 KΩ resistors can be switched into the bridge in place of the photocells to facilitate

¹#1490, GE miniature lamp, Allied Electronics, Inc., Chicago, Illinois.
²Model 6200B, Hewlett Packard, Berkeley Heights, New Jersey.
testing of the system. Photocell resistances are measured by connecting them across a vacuum tube ohmmeter\textsuperscript{1}. All voltages are measured with a digital voltmeter\textsuperscript{2}.

\textsuperscript{1}Model IM-10, Heath Co., Benton Harbor, Michigan.

\textsuperscript{2}Model 405CR, Hewlett Packard, Berkeley Heights, New Jersey.
METHODS

Stabilization of the Light Source

Use of the quartz-iodine lamp with a regulated power supply was expected to provide a source of light sufficiently stable to preclude the need for recalibration of the oximeter during a 2 to 3 hour period of continuous operation. To test this hypothesis, the sensing end of the fiberoptic waveguide was immersed in milk of magnesia, a 32% (v/v) suspension of magnesium hydroxide in water, while the lamp was operated at 90.0 volts. The photocell resistances were measured periodically for 4 hours. The milk of magnesia was stirred thoroughly prior to each measurement. Operation of the lamp at only 75% of rated voltage extended its life from the rated 12 hours to approximately 400 hours. This provided adequate light for normal operation of the oximeter and was sufficient to maintain operation of the halogen cycle. The optical wedges were adjusted at the start of the experiment to equalize the resistances of the photocells at a value of 30 KΩ. Photocell resistances and their ratio \( q = \frac{R_{660}}{R_{805}} \) were then plotted as functions of time.

Stabilization of Photocell Response Curves

According to data supplied by the photocell manufacturer (57), the resistances of the photocells could be expected to vary as much as 50% depending upon their light history at the levels of illumination provided in use of this oximeter. The practical result of this phenomenon would be rapid drifting of photocell resistance values for several hours after the lamp was turned on. To circumvent this problem, small incandescent lamps located near the photocells were turned on whenever the oximeter lamp was
off and their filament currents were adjusted to maintain photocell resistances at 30 kΩ. Thus, the light histories of the photocells were already established whenever the oximeter was used and their response curves would not be expected to drift. To test the effectiveness of this method the experiment described in the preceding section was repeated after the photocells had been conditioned for many weeks ("infinite light history"). The results of this experiment were plotted for comparison with those of the preceding one, for which the photocells were at "infinite dark history".

Matching of Photocell Sensitivities

A technique used in equalizing thermistors (29) was used to equalize the sensitivities of the two photocells. At a given temperature two similar thermistors can always be made identical over a considerable range of temperatures by placing a resistor of the proper magnitude in parallel with the more sensitive one and another in series with one of them. The first resistor reduces the sensitivity of the thermistor and the second corrects for the difference in offset.

With the oximeter used in these experiments it was not possible to separate the difference in offset inherent in the photocells from that due to imbalance of the two optical paths between the blood and the photocells. Consequently, both were eliminated by adjustment of the optical wedge in the 660 nm. light path, and the series resistor was therefore unnecessary. Determining the proper value for the parallel resistor ($R_p$) proved more difficult.

An important consideration which has not been emphasized by others
who have used photoconductive detectors in oximeters is that the relationship between photocell resistance and the intensity of illumination of the photocells is highly nonlinear. It is, in fact, approximately hyperbolic or reciprocal. Data supplied by the photocell manufacturer show that the relationship can be approximated over a limited range by a linear logarithmic equation

\[ \log R = M - k \log I \]  

where \( R \) = photocell resistance in MΩ,
\( M \) = a constant,
\( I \) = photocell illuminance in footcandles, and
\( k \) = a constant.

This is easily transformed to the simple equation

\[ R = 10^{M/I^k}. \]  

Evaluation of the constants from the manufacturer's data yields approximate values of \( M = 3.6 \) and \( k = 0.7 \). Thus, the relationship is approximately reciprocal. If photocell sensitivity is defined as the rate of change in photocell resistance with change in illumination, it is apparent that the sensitivity is not constant. Indeed, the sensitivity is low at high levels of illumination, increases rapidly at intermediate levels, and approaches a steady high value at low levels. Hence, there exists a range of low levels of illumination at which the sensitivity is high and nearly constant, conditions which greatly simplify the design and operation of the oximeter.

Applying the "cell conversion factor" to the manufacturer's data for the photocells used in these studies shows that the region of constant
sensitivity begins at approximately 0.10 footcandles or 20 KΩ. Allowing for one order of magnitude variation in illumination of the photocells during use of the oximeter, the maximum photocell resistance would be about 100 KΩ at 0.01 footcandles. Thus, further study of photocell characteristics was limited to this range of resistances.

Since no accurate method for measuring absolute light intensities was available, the photocell sensitivities were compared rather than directly evaluated. For this purpose, the sensing end of the fiberoptic waveguide was immersed in the white reflectance standard and the resistances of the two photocells were equalized at an arbitrary value in the range of interest by adjustment of the optical wedges. Then the optical wedge in front of the lamp was rotated to increase its effective optical density by a small amount. The change in resistance for each photocell was recorded. Relative values of the "logarithmic sensitivities", defined by k in Equation 1, could then be estimated from the relationship

\[
k = \frac{\log R' - \log R}{\log I' - \log I} = \frac{\log (R'/R)}{\log (I'/I)} = \frac{\log (R'/R)}{\Delta \text{O.D.}}\]

where

- \( R \) = initial photocell resistance,
- \( R' \) = final photocell resistance,
- \( I \) = initial light intensity,
- \( I' \) = final light intensity, and
- \( \Delta \text{O.D.} \) = change in optical density of the wedge.

It follows directly that rotation of the wedge causes no change in the ratio of the resistances of the two photocells if their sensitivities are equal:
\[
k_\lambda = \frac{\log (R'_\lambda / R_\lambda)}{\Delta \text{O.D.}}
\]

\[k_{660} \frac{k_{805}}{k_{805}} = \frac{\log (R'_{660}/R_{660})}{\log (R'_{805}/R_{805})}.
\]

Thus, if \(k_{660} = k_{805}\), \((R'_{660}/R_{660})\) must equal \((R'_{805}/R_{805})\) and hence \((R_{660}/R_{805})\) must equal \((R'_{660}/R'_{805})\). Basically, then, the method for equalizing photocell sensitivities consists of finding a value of resistance which when placed in parallel with the 805 nm. photocell allows rotation of the optical wedge without altering the ratio of photocell resistances. In principle, this value could be calculated, but since absolute values of photocell sensitivities could not be determined with accuracy, a trial-and-error method was used.

The method described above would allow matching of the photocell sensitivities at an arbitrary point within the range of operation. Two further considerations are necessary, however. First, the effectiveness of the matching should be evaluated over the full range of operation, and, second, the hypothesis that the sensitivities are constant over the range of operation should be tested. Thus, the complete experiment for matching of photocell sensitivities consists of adjusting the wedge in the 660 nm. light path to provide various initial photocell resistance ratios and then rotating the wedge in front of the lamp and observing the changes in photocell resistances and resistance ratio. This is repeated for several values of the sensitivity-equalizing resistor \(R_p\) to determine a value which gives satisfactory matching.

Resistance ratios were plotted as functions of 805 nm. photocell resistance (i.e., \(R_{805}\)) for four values of \(R_p\).
Calibration

The technique for calibrating the oximeter was similar to that used by Polanyi and Hehir (58) and others. The sensing end of the waveguide was immersed in the white reflection standard and the wedge in front of the lamp was rotated to set the resistance of the 805 nm. photocell to 28 KΩ. The other wedge was then rotated to set the resistance of the 660 nm. photocell to the same value. The sensing tip was then cleaned and transferred to a gray standard prepared by adding approximately 0.25 ml. of a black drawing ink¹ to 100 ml. of the 32% milk of magnesia. The wedge in front of the lamp was then rotated to reset the 805 nm. photocell resistance to 28 KΩ. Because the reflectance of the gray standard was not exactly the same at the two wavelengths, the resistance of the 660 nm. photocell was then about 32 KΩ. The concentration of ink in the gray standard was such that the mixture gave almost exactly the same reflectance at 805 nm. as was given by the most highly reflective erythrocyte suspensions.

Linearization of Oximeter Output

The relationship between relative oxygenation of blood and the oximeter output can be considered in terms of two separate functions having a common variable, namely, the photocell resistance ratio \( q = \frac{R_{660}}{R_{805}} \). The two functions are: 1) the "calibration curve" relating \( q \) and relative oxygenation; and 2) the "output function" relating \( q \) and the oximeter output.

It is well-established that the relationship between relative oxygenation and the reflectance ratio is linear. If the response curves of the photocells were also linear, the calibration curve would necessarily be linear. However, because the range of operation of the photocells was selected to achieve a compromise between linearity and noise levels, there were slight increases in the slopes of the response curves (i.e., the sensitivities) at low levels of illumination. Since the reflectance of blood for 805 nm. light does not change while its reflectance at 660 nm. is directly proportional to relative oxygenation, a slight nonlinearity of the calibration curve results. That is, at low values of relative oxygenation the illumination of the 660 nm. photocell is much less than that of the 805 nm. photocell and its sensitivity is slightly higher. The result is that the slope of the calibration curve is slightly higher at low values of relative oxygenation than at high values.

Conveniently, the output function is also slightly nonlinear. To derive a simple mathematical expression for the output function requires the assumption that there is no loading of the output terminals of the bridge circuit. In actuality there is some loading whenever the terminals are connected to the voltmeter, but the simple model proves satisfactory for analyzing the oximeter circuit. The Wheatstone bridge circuit of the oximeter (Figure 3) is easily solved by applying Kirchoff's voltage law:

\[ v_0 = E \frac{R_2}{R_1 + R_2} - E \frac{R_{805}}{R_{660} + R_{805}} \]

where \( v_0 \) = output voltage,
\( E \) = input voltage,
\[ R_{805} = \text{resistance of the 805 nm. photocell, and} \]
\[ R_{660} = \text{resistance of the 660 nm. photocell.} \]

Then:
\[
V_0 = E \left[ \frac{1}{R_1/R_2 + 1} - \frac{1}{R_{660}/R_{805} + 1} \right]
\[
= E \left[ \frac{1}{p + 1} - \frac{1}{q + 1} \right] \tag{4}
\]

where \( p = R_1/R_2 \) and \( q = R_{660}/R_{805} \).

Equation 4 describes a hyperbolic relationship between \( q \) and \( V_0 \) when the values of the bridge parameters \( E \) and \( p \) are fixed. Since only positive values of \( p \) and \( q \) exist, only one-half of the hyperbola is to be considered as the output function.

Examination of Equation 4 shows that the parameter \( E \) establishes the range of values for \( V_0 \) while \( p \) controls the position of the output function relative to the coordinate axes. It is especially noteworthy that by proper selection of the values of \( E \) and \( p \) one can cause the output function to pass through any pair of points located above the abscissa and, to a degree, vary the curvature of the function between them. The practical result is that the relationship between relative oxygenation and oximeter output can be made linear and direct-reading by proper selection of the values of \( E \) and \( p \). The procedure is illustrated by the example below.

Suppose that a calibration curve gave values corresponding to those in Table 1. To linearize the relationship between relative oxygenation and \( V_0 \) over this range and simultaneously obtain values of \( V_0 \) corresponding exactly to those of relative oxygenation, select two points from the
Table 1. Resistance ratio versus relative oxygenation

<table>
<thead>
<tr>
<th>$R_{660}/R_{805}$</th>
<th>Relative oxygenation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>1.00</td>
</tr>
<tr>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td>1.15</td>
<td>0.80</td>
</tr>
<tr>
<td>1.40</td>
<td>0.70</td>
</tr>
<tr>
<td>1.71</td>
<td>0.60</td>
</tr>
</tbody>
</table>

calibration curve and insert their coordinates into two equations of the form of Equation 4. Then solve these equations simultaneously for the two unknown parameters. For example, using the points (0.96, 0.90) and (1.40, 0.70):

$$\frac{0.90}{E} = \frac{1}{p + 1} - \frac{1}{0.96 + 1}$$
$$\frac{0.70}{E} = \frac{1}{p + 1} - \frac{1}{1.40 + 1}.$$

Combining these two equations yields:

$$\frac{0.20}{E} = -\frac{1}{1.96} + \frac{1}{2.40},$$

$$E = \frac{0.20}{-0.093} = 2.15 \text{ volts}.$$

Substituting this value into either of the original equations yields:

$$\frac{0.90}{-2.15} = \frac{1}{p + 1} - \frac{1}{1.96}$$
$$p + 1 = \frac{1}{0.091} = 11.0,$$

$$p = 10.0 \text{ (no units)}.$$
The output function thus becomes

\[ v_0 = -2.15 \left[ \frac{1}{11.0} - \frac{1}{q+1} \right]. \] (5)

Comparison of Table 2 with Table 1 shows that values of \( v_0 \) calculated from Equation 5 correspond quite well with values of relative oxygenation which give the same resistance ratios.

<table>
<thead>
<tr>
<th>Oximeter output (volts)</th>
<th>( \frac{R_{660}}{R_{805}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>.999</td>
<td>0.80</td>
</tr>
<tr>
<td>.900</td>
<td>0.96</td>
</tr>
<tr>
<td>.801</td>
<td>1.15</td>
</tr>
<tr>
<td>.700</td>
<td>1.40</td>
</tr>
<tr>
<td>.599</td>
<td>1.71</td>
</tr>
</tbody>
</table>

To determine how well Equation 5 actually describes the output function of the oximeter, an experiment was conducted in which the calculated values of the bridge parameters \( E \) and \( p \) were used and \( q \) was varied by rotation of the wedge in the 660 nm light path. The photocell resistance ratio was calculated from values measured with the ohmmeter. They were plotted as functions of oximeter output. Values of \( q \) calculated from Equation 5 for the observed values of \( v_0 \) were plotted on the same scale for comparison.
RESULTS AND DISCUSSION

Data from the lamp stability test using unconditioned photocells (Figure 1) show an initially rapid drift for both of the photocell resistances and for their ratio. The rate of drift decreased rapidly during the first hour and more slowly thereafter. This behavior suggests that a significant portion of the drift was due to the light history effect on photocell response curves. This conclusion is supported by the results of the second experiment, in which the photocells were at "infinite" light history of 0.1 footcandles. This conditioning procedure reduced the drift in photocell resistances to almost unmeasurable levels. There was a slight shift in the ratio of photocell resistances with time, which amounted to about 1% in 4 hours. The change appeared to be linear rather than exponential with respect to time, suggesting that it was due to a gradual change in color temperature of the lamp rather than to the light history effect. With the usual setting of the bridge parameters this rate of drift would cause the oximeter output voltage to drop by 10 millivolts in the 4 hour period, causing underestimation of relative oxygenation by 1.0%. The rate of drift in individual photocell resistances was negligible in view of the limited accuracy of resistance measurements and the magnitudes of photocell resistance changes encountered in actual use of the oximeter.

Results of the sensitivity-matching experiments are plotted in Figure 2. With $R_p = 0$, the sensitivity of the 805 nm. photocell is clearly much higher than that of the 660 nm. photocell, and the difference is larger at high resistance ratios such as occur at low levels of relative oxygenation. As the value of $R_p$ was decreased, the slopes of the curves
Figure 1. Photocell resistances and resistance ratio versus time
Figure 2. Resistance ratio versus $R_{005}$ for four values of $R_w$, the sensitivity-reducing resistor, and five initial values of resistance ratio.
became less negative and eventually positive. With $R_p = 85 \text{ K}\Omega$, the sensitivity of the 805 nm. photocell had obviously been reduced below that of the 660 nm. photocell. Thus, the optimum value for $R_p$ was above 85 K\Omega. The data show, however, that neither of the other values tested gave ideal matching over the entire range of the test. The 150 K\Omega value gave the most consistent results at all values of the resistance ratio, but the 805 nm. cell was always slightly too sensitive. The 120 K\Omega value gave excellent matching at the two lower values of the resistance ratio, but appeared to overcompensate at the higher values. Inasmuch as the values of resistances and resistance ratio encountered in use of the oximeter were usually in these lower ranges, however, the 120 K\Omega value was selected for later use. On the basis of these tests it was estimated that the matching obtained was satisfactory in the range from 50 to 100% relative oxygenation, but that the 660 nm. photocell would be too sensitive at lower values unless the value of $R_p$ was increased to approximately 150 K\Omega.

The results of the final experiment were as in Figure 4. The agreement between Equation 5 and the behavior of the actual bridge circuit appears to be satisfactory. The apparent difference between the two at high values of the resistance ratio is probably due to relatively increased loading of the output terminals as the impedance of the bridge circuit increased.

The results of these experiments demonstrated that the light source and photocell response curves could be made sufficiently stable to permit continuous operation of the oximeter for periods of 4 hours without recalibration. The rate of drift of individual photocell resistances is not likely to invalidate comparisons of reflectance values at different levels of oxygenation, and the drift in the resistance ratio is significant.
Figure 3. Wheatstone bridge circuit of the oximeter
Figure 4. Measured and calculated values of resistance ratio versus oximeter output
Oxiometer Reading (Volts)

- Measured Values
- Calculated Values
only at longer times. Since the drift in \( q \) was linear with respect to time, correction for it could easily be made by recalibration at the end of an experiment. The range of accuracy of the present oximeter probably does not extend below 50% relative oxygenation, but a simple change in the value of the resistor regulating the sensitivity of the 805 nm. photocell would permit its use at lower values, if necessary.
SUMMARY

A fiberoptic reflection oximeter previously used in studies of control systems for an artificial heart (22) and of cardiovascular dynamics (56) was modified for use in systematic studies of reflection properties of blood. Use of a halogen-cycle lamp and continuous illumination of the photocells to stabilize their response curves produced acceptable stability for prolonged and continuous operation without recalibration. A technique was developed whereby the sensitivities of the two photodetectors could be equalized so that nonspecific variations in reflection properties of blood would not alter the oximeter output. The instrument was made more convenient to use by altering the values of circuit parameters to provide a linear, direct estimate of relative oxygenation with a digital voltmeter, eliminating the need for conversion tables or calibration graphs for interpreting the output.
PART II. CYCLIC ERYTHROPOIESIS IN CANINE BLOOD DONORS
REVIEW OF THE LITERATURE

Recovery from Acute Hemorrhage

Under normal circumstances, production of erythrocytes in the bone marrow proceeds at the same rate as erythrocyte destruction in the body so that the total mass of erythrocytes and of hemoglobin remains constant (unless, of course, the animal is growing). However, following acute massive loss or destruction of erythrocytes the rate of erythropoiesis can reach 50 to 100 times normal (30). The minimum lag time for appearance of new cells in the bloodstream following a stimulus is about four days (66). In extreme cases, the newly-formed erythrocytes may be released while quite immature so that even fully-nucleated forms may be observed in peripheral blood smears. The usual method for detection of increased erythropoiesis is by determining the percentage of RNA-containing (young) erythrocytes, which are called reticulocytes.

An acute loss of 8% of blood volume in normal men and women was followed by peaks in reticulocyte counts at nine days and again at 17 days. There was no significant change in MCHC and recovery was complete in three to four weeks (77). The usual limiting factor for erythropoiesis in both men and dogs is the preexisting store of iron in the tissues (24). While other nutrients are usually present in adequate amounts, protein requirements are increased during active erythropoiesis (66).

In iron deficiencies the regenerated erythrocytes are usually smaller than normal (microcytic) and have a lower hemoglobin concentration than normal (hypochromic). Otherwise, the young and immature erythrocytes are usually larger than mature cells (macrocytic) and have a greater affinity
for basic dyes (polychromatophilia); they may also contain nuclear remnants. The large cells disappear from the circulation within a few weeks. In deficiency states they are apparently replaced by new, normal erythrocytes, but in other cases they may be "remodeled" into normal cells while in the circulation (69).

**Nutrition of Blood Donors**

The administration of extra iron after each blood donation has been recommended for human donors (35) to prevent iron deficiency. Alternatively, a three-month interval between donations is suggested (31). While the preexisting store of iron is the critical factor regulating the rate of erythropoiesis following hemorrhage, adding iron to the diet during the recovery period appeared to increase the amount of available iron and the rate of erythropoiesis (24). Dogs maintained as blood donors on a commercial diet without iron supplementation showed tendencies toward iron-deficiency anemia as the frequency of bleeding was increased (60). Hemoglobin values for dogs bled every third or fourth week were within normal limits, but were as much as 12% below those of controls. Dogs bled more frequently had decreased PCV, RBC, hemoglobin, MCV, and MCHC, as well as increased reticulocyte counts. These results suggest that commercial feeds may not contain iron levels adequate for maintenance of normal erythrocyte populations in dogs used as blood donors.

**Life Cycle of Erythrocytes**

While it is generally accepted that erythrocytes are derived from large nucleated precursors, there is no agreement as to what morphological changes, if any, erythrocytes undergo during their lifetime in the
circulation. Similarly, there is no consensus as to how and where erythrocytes are destroyed although it is established that the spleen is normally involved but is not essential for this process (66). Studies in man (30) and dog (72) indicate that the average erythrocyte lifespan is about 120 days with a range of 90 to 140 days.

Physical and chemical changes associated with aging of erythrocytes in man and other mammals include progressively increasing specific gravity (6), increasing osmotic and mechanical fragility (13), decreasing activity of enzyme systems (1), increasing methemoglobin concentration (4), left shift in the oxyhemoglobin dissociation curve (20), and a decrease in hemoglobin content and cell volume (27). It is by no means certain that all of these changes accompany aging in canine erythrocytes. Indeed, contradictory results concerning the change in osmotic fragility have been reported for dogs. Stewart et al. (70) reported that young canine erythrocytes had higher osmotic fragility than older ones. The reverse of this relationship occurs in all other species studied (49). Since the three dogs were fed an unsupplemented diet of table scraps, it seems likely that they underwent an iron-deficiency anemia, with microcytic cells having pathologically elevated osmotic fragility.

Prior Evidence for Cyclic Erythropoiesis

Dogs subjected to acute destruction or loss of 50% of their erythrocyte mass recovered to their normal circulating hemoglobin concentration in about four weeks (34). Their rates of bile pigment formation decreased sharply during recovery, but became well above prebleeding levels about four months later as the replacement cells reached the end of their
lifespan and were destroyed. Their rates of erythropoiesis could be estimated to have averaged about twice normal during the first month of recovery since one-half of the erythrocyte mass was replaced in one-quarter of the normal erythrocyte lifespan. This would result in a large proportion of their erythrocytes being of nearly the same age so that they reached the end of their lifespan and were replaced nearly simultaneously, causing a secondary erythropoietic crisis. Blood samples obtained from such animals after recovery should exhibit a cycle of increasing and decreasing mean erythrocyte age (MEA). It should be possible to detect cyclic variations in physical and chemical characteristics of the erythrocytes and to correlate these with the changes in MEA.
METHODS

Conditioning, Nutrition, and Care of Donors

Sixteen random-source mongrel dogs weighing from 20 to 35 kg. (mean: 25 kg.) were used as blood donors. All were vaccinated\(^1\), wormed, and acclimatized to confinement prior to bleeding. Fecal examinations were performed periodically and treatment for parasites was repeated whenever necessary. A modified Knott's test for microfilaria was performed on each dog (52). The diet consisted of commercial dry dog food\(^2\) and water ad lib. A hematinic preparation\(^3\) containing 50.0 mg. of ferrous fumarate per tablet and various vitamins and minerals was fed at the rate of one tablet per day per dog. Two other dogs and four of the donor group were observed for several months as controls (prior to bleeding) and were treated in the same manner as donors.

Collection of Blood

Light surgical anesthesia was induced by intravenous injection of a short-acting barbiturate\(^4\) at a dosage of 20 mg./kg. body weight. Hair over the right fourth intercostal space at the level of the costochondral junctions was clipped, and the skin was scrubbed and disinfected. Blood

\(^1\)Canine Distemper Hepatitis Vaccine and Leptospira Canicola Icterohaemorrhagiae Bacterin, Bio-ceutic Laboratories, Inc., St. Joseph, Missouri.


\(^3\)Vi-sorbits, Norden Laboratories, Lincoln, Nebraska.

\(^4\)Sodium thiamylal, Surital, Parke-Davis and Co., Detroit, Michigan.
was drawn under light suction from either of the ventricles through a 13-ga., 2-inch (5.08 cm.) hypodermic needle and 20 cm. of 3.2 mm. I.D. polyethylene tubing into a dry, sterile 1-liter glass bottle containing 30 mg. of dry sodium heparin\(^1\). Following collection of 800 to 1000 ml. of blood, 500 ml. of isotonic saline solution was infused intravenously as rapidly as possible. Collection of the blood usually took 5 to 10 minutes. Blood obtained in this manner typically had 41.3 ±2.7% PCV and 5.8 ±0.5 gm.% plasma protein.

Hematologic Monitoring

Hematologic examinations were performed once every other week before and after bleeding. Five ml. of blood were drawn into an evacuated test tube\(^2\) containing 6.0 mg. of disodium EDTA, usually from the left jugular vein. Smears were prepared and stained with a modified Wright's stain\(^3\). The erythrocytes were examined at 970x (oil immersion) for anisocytosis and polychromatophilia. A rating was assigned on a scale from one to four for each of these variables. The lower ratings indicated only slight variation in size between the erythrocytes and nearly complete absence of basophilic erythrocytes; these were typical of dogs which had not been bled. The highest ratings were from slides showing the greatest variation in erythrocyte diameter and the largest percentages of basophilic erythrocytes;

\(^1\)Heparin sodium, Nutritional Biochemicals Corp., Cleveland, Ohio.

\(^2\)Vacutainer #4770, Becton, Dickinson and Co., Rutherford, New Jersey.

\(^3\)Camco Quik Stain, Scientific Products Co., Evanston, Illinois.
this appearance was typical of samples drawn the second week after bleeding.

Packed cell volumes were determined using microhematocrit tubes\(^1\), clay\(^2\), centrifuge\(^3\), and reader\(^4\). Plasma protein concentration (PPC) was estimated using plasma from the microhematocrit tubes and a clinical refractometer\(^5\). Total hemoglobin concentration (HGB) was measured using cyanmethemoglobin reagent\(^6\) and standard\(^7\), and a spectrophotometer\(^8\). Erythrocyte counts were made using pipettes\(^9\), shaker\(^10\), and counting chamber\(^11\) manually. For the last 140 days of the study osmotic fragility tests were conducted using the method of Stewart et al. (70). Whole blood

\(^1\)Capilets, Scientific Products Co., Evanston, Illinois.

\(^2\)Seal-Ease holder, Clay-Adams, Inc., New York, N.Y.

\(^3\)Adams micro-hematocrit centrifuge, Clay-Adams, Inc., New York, N.Y.


\(^6\)Cyanmethemoglobin stable reagent, Hycel, Inc., Houston, Texas.

\(^7\)Cyanmethemoglobin certified standard, Hycel, Inc., Houston, Texas.

\(^8\)Bausch and Lomb Spectronic 20 colorimeter, Scientific Products Co., Evanston, Illinois.


\(^10\)Pipette shaker, Burton Manufacturing Co., Van Nuys, Los Angeles, California.

was tested using a series of tubes with several concentrations of NaCl; the hemoglobin released after addition of blood was measured spectrophotometrically at 540 nm. The percentage of NaCl at which 10 and 100% hemolysis occurred was estimated from these measurements and the total hemoglobin concentration of the blood.
RESULTS

Plasma Protein Concentration, PCV, RBC, Total Hemoglobin and Wintrobe Indices

Hematologic data are plotted as functions of time (in days) after bleeding in Figure 5. The means shown include data from all donor dogs and for all recoveries. Since most of the dogs were bled several times, data for the early recovery period are much more plentiful than for the later period. One dog was bled five times, two four times, four three times, four twice, and five only once. One of the dogs died from cardiac tamponade and hypovolemic shock immediately after the second bleeding. Two others were euthanatized early due to chronic infections which were diagnosed after two or three bleedings.

Because the experimental design was not formulated until after some data had been collected, observations were not made continuously of all dogs during the initial months of this study. Thus, although 37 bleedings were performed, the maximum number of observations for any 14-day period for which means were calculated does not exceed 33. This figure decreases continuously as the length of time after bleeding increases, partially due to failure to perform hematologic tests at the indicated times and partially because many dogs were bled again in 50 to 90 days. Thus, there was a maximum of 21 observations per variable at 63 days, 12 at 133 days, five at 231 days, and two at 399 days. Prebleeding control data were obtained from only nine of the 16 donors. Long-term control observations were made on four of the donors prior to bleeding and on two other dogs. Standard deviations for the samples did not change significantly when six or more data were available for each mean. Thus, the means up to and including
Figure 5. Hematologic variables as functions of time after bleeding (days)

^SD denotes the mean value of the standard deviations of the means plotted, and SD is its standard deviation.
a. PPC (gm.%). \[ \text{SD} = 0.50 \pm 0.22 (\text{SD})^3 \]

b. PCV (%). \[ \text{SD} = 3.89 \pm 0.87 \]

c. HGB (gm.%). \[ \text{SD} = 1.56 \pm 0.51 \]

d. RBC \( (10^6/\text{mm}^3) \). \[ \text{SD} = 0.70 \pm 0.30 \]
Figure 5. (Continued)
e. MCV ($\mu^3$). $\bar{SD}=5.38 \pm 2.47$

f. MCHC (gm.%). $\bar{SD}=1.09 \pm 0.56$

g. MCH (μg/m.). $\bar{SD}=2.08 \pm 0.95$

h. Average number of observations per mean.

- Osmotic Resistances
- All Other Data
Figure 5. (Continued)

^Subjective ratings; see text for explanation.
i. Anisocytosis

j. Polychromatophilia

k. Minimum osmotic resistance (% NaCl). \( SD = 0.025 \pm 0.014 \)

l. Maximum osmotic resistance (%NaCl). \( SD = 0.020 \pm 0.010 \)
175 days are probably reliable.

Data for all of the hematologic variables exhibited cyclic variations which appear to have a period of approximately 110 days with a lag of about 20 days immediately after bleeding. There were apparently differences in phase between the variables, with decreases in PCV and hemoglobin preceding that in RBC. The rise in MCHC occurred several days before MCV and MCH reached their peaks. The peaks of MCV and MCH corresponded, of course, to the minima in RBC. Although only the means occurring at peak values of the Wintrobe indices were significantly different from prebleeding control values by Student's t test (P<0.05), the smoothness of the curves for the first 175 days indicated the clearly progressive course of the changes observed. Lack of sufficient data from long-term control studies precluded use of a more elaborate statistical evaluation.

Osmotic Resistance

Since osmotic fragility testing was not started until late in the study, these data were taken from only the last recovery for each of 13 surviving donors and one control; there was a maximum of eight observations per mean. The data plotted in Figures 5k and 5l represent the percentage concentration of NaCl in solutions in which 10% hemolysis (minimum osmotic resistance) and 100% hemolysis (maximum osmotic resistance) occurred, respectively. These data cover a period of 140 consecutive days for each donor, and include various phases of recoveries one through five. Five of the dogs were in the first recovery period, two in the second, three in the third, two in the fourth, and one in the fifth. Data from the control dog showed only minor random changes from one test to the next.
Peripheral Blood Smears

The number of data for each mean plotted in Figures 5i and 5j was always the largest of all the variables, since slides made were invariably kept even during the early part of the study. The means were calculated in the usual way. Since the observations represented by these data are of questionable objectivity, the data are presented only as a concise and accurate representation of the author's interpretation of the slides.

The photographs in Figures 6 through 9 illustrate the rating system used in evaluation of anisocytosis. Figure 6 is from a prebleeding sample which was assigned the lowest rating since the cells were quite uniform in size. Figure 7 is also from a prebleeding sample but was given a rating of two since a number of both large and small cells were present in most fields. Figure 8 is from the same dog as Figure 7, eight days after the first bleeding. It was assigned the highest ratings for both anisocytosis and polychromatophilia. Figure 9 is from another dog at 352 days after the first bleeding. This smear was given ratings of four for anisocytosis and of two for polychromatophilia. Polychromatophilia ratings above the minimum rarely occurred unless the anisocytosis rating was either three or four. Poikilocytosis is also evident in Figures 7 and 8.
Figure 6. Prebleeding erythrocytes; typical; x 1170; Wright's stain

Figure 7. Prebleeding erythrocytes; atypical; x 1170; Wright's stain
Figure 8. Erythrocytes eight days after bleeding; x 1170; Wright's stain

Figure 9. Erythrocytes 352 days after bleeding; x 1170; Wright's stain
DISCUSSION

The study reported herein was an outgrowth of an investigation of effects of changes in hematologic variables on the accuracy of a transducer used to measure relative oxygenation of blood. The original intent in gathering hematologic data was to monitor the progress of recovery in order to establish bleeding intervals which would yield normal erythrocyte populations for that study. Inasmuch as the need for the expanded investigation did not become apparent until late in the course of the original work, it was not possible to follow a satisfactory statistical design for its execution. Among the numerous shortcomings of the design are the paucity of both prebleeding and long-term control data. Of the 16 donors, complete prebleeding data are available for only nine. Only one control dog was observed for a period exceeding the length of the proposed cycle of erythropoiesis in the donor group. In addition, the number of donors for which data were taken decreases nearly linearly with the length of time after bleeding so that every opportunity for introduction of sampling bias is present. Certain phases of the recovery data are clearly biased toward either first recoveries or later recoveries. Dogs which were bled several times contributed data repeatedly to the early recovery period (up to 70 days); one dog went through this phase five times, two others four times. The middle third of the data was derived entirely from dogs bled two or three times, and the last third from two dogs bled only once; both of these were females, whereas 11 of the 16 donors were males. The long-term control dog was a female.

Examination of data from individual dogs revealed that many of them
consistently exhibited idiosyncrasies of one or more of the hematologic variables. Furthermore, nothing was known of the erythropoietic history of any of the dogs, except that at least four had apparently significant hookworm infections when acquired.

In view of the many deficiencies of the design of this study, it is clear that the greatest caution must be exercised in drawing conclusions from the data. One very useful criterion in this regard is the comparison of the data of Figure 5 with corresponding curves from individual dogs. In many cases the nature of the cyclic changes is apparent even in data from only one dog. Moreover, the timing of maximum and minimum values of the variables is often precisely as indicated by the mean values. The cyclic changes are clearly related to the time of bleeding in spite of the random distribution of bleeding dates in calendar time. Thus, while it is readily conceded that this study should be repeated under more rigidly controlled conditions and with complete collection of data from every donor and for uniform periods of time, it is difficult to completely dismiss the evidence that cyclic erythropoiesis can occur following acute massive hemorrhage.

It was rather difficult to estimate the proportion of erythrocytes removed from the dogs at each bleeding. The high control values of PCV and PPC indicated that the dogs were chronically dehydrated. No direct measurements of blood volume or of erythrocyte mass were attempted. Neither did the values of PCV and PPC in the donor blood give any indication of the percentage of erythrocytes removed. The PPC's averaged more than 1 gm.% lower than for small samples of venous plasma, indicating that a considerable amount of extracellular fluid was drawn into the vascular system even as the bleeding progressed. Hence, one could only
use the standard estimate of 40 ml. of blood per pound of body weight plus
some estimate of body PCV to calculate the erythrocyte mass. On such a
basis, the mean erythrocyte loss for all bleedings would be about 40% with
a range of perhaps ±10%.

That reticulocyte counts were not performed as a measure of
erthropoietic rates was due to limitations of time and technical assis­
tance, as well as the failure of the author to master a satisfactory
technique for the determination.

Several general features of the data deserve comment. The curves for
PPC, PCV, HGB, and RBC all initially overshoot the prebleeding control
values. Subsequently, all four curves appear to oscillate to both sides
of the control values. A degree of damping is evident in several of the
curves, indicating that the life cycles of the erythrocytes eventually
return to a state of desynchronization. Phase differences are most
apparent in the curves for the Wintrobe indices. The maximum values of
MCHC clearly occur before those of MCH and MCV. The maxima of MCH and
MCV appear to be associated with the low-point in mean erythrocyte age,
\( i.e. \), the young erythrocytes are large and hence contain more hemoglobin.
A consistent exception to this is the primary erythropoietic crisis. A
slight iron-deficiency anemia is indicated during this period resulting
from the physical removal of iron in the erythrocytes from the body during
bleeding. During subsequent crises the iron is largely retained so that no
deficiency occurs. Data from a few dogs bled before the iron therapy was
started indicated typical microcytic, hypochromic anemia during the
initial recovery period.

The variations in PPC require additional examination. Although
minimum values of PPC occur during each of the putative erythropoietic crises, the extreme values were not significantly different, on a statistical basis, from the prebleeding levels. Nevertheless, these variations should be expected since accelerated erythropoiesis increases the protein requirements of the body and no protein supplementation was provided for these dogs. Hence, it is reasonable to expect a mild protein deficiency to occur during periods of rapid destruction and regeneration of erythrocytes.

In general, therefore, the data appear to support the hypothesis that following acute massive hemorrhage cyclic changes occur in hematologic variables known to change as erythrocytes age. This implies that there is a cyclic variation in mean erythrocyte age caused by synchronization of the life cycles of the regenerated erythrocytes. The data further indicate that the length of the cycle is about 110 days, which is slightly shorter than the accepted mean value for erythrocyte lifespan in dogs (72). Possibly the mean erythrocyte lifespan for this group of dogs was shorter than for the general population. More likely, however, the lifespan of the erythrocytes produced during the erythropoietic crises was shortened by the conditions of stress under which they were produced.

The period between bleeding and the secondary erythropoietic crisis at 120 to 140 days is about 20 days longer than the period between subsequent crises. This is the lag time between the hemorrhage and the maximum rate of erythropoiesis. While it is not possible to determine from the data whether the regenerated erythrocytes were replaced or remodeled in this case, it is clear that if replacement occurred the replacement cells must have had a shorter lifespan than normal cells.
The values of MCHC observed during the erythropoietic crises were consistently above the normal range. While it is conceivable that the actual intracellular concentration of hemoglobin was elevated at these times, another explanation is possible. During erythropoietic crises the degree of anisocytosis was at its peak, so that cells of many different sizes and shapes were present in the blood in contrast to other periods when the cells were of more uniform morphology. This may have caused closer packing of cells in the microhematocrit tubes with a decrease in the proportion of trapped plasma, which would cause an apparent increase in MCHC. An inverse relationship between MCHC and trapped plasma has been reported (36).

Another artifact may have contributed to the rises in MCV and MCH following erythropoietic crises. It was repeatedly observed that the blood during these periods hemolyzed readily if shaken too long in the pipettes in preparation for erythrocyte counts. Although no systematic study of mechanical fragilities was done, it seems likely that some hemolysis occurred during the mixing process, resulting in lower erythrocyte counts and increased MCV's and MCH's.

As noted previously, MCV and MCHC were below prebleeding levels during the initial recovery period, indicating that mild iron-deficiency anemia was occurring. This was most evident after the first bleeding and appeared to decrease in severity with subsequent bleedings. Since the means for the first 70 days of recovery consist largely of data from the later recoveries, these curves are not an accurate representation of these indices following a single bleeding.

As would be expected, a double cycle was observed in several of the
dogs which had been bled more than once. That is, smaller decreases in PCV, HGB, and RBC were observed midway in cycles resulting from the most recent bleeding. Their amplitudes were less than in the primary cycle, since many of the cells originally involved were removed by bleeding. Another effect which appeared with repeated bleeding was an overall rise in the mean values of PCV, HGB, RBC, and Wintrobe indices, indicating that mean erythrocyte age was lowered progressively by repeated bleeding. However, mean osmotic fragility values were elevated in all three dogs which were bled four or more times, possibly indicating defective erythropoiesis.

Immediately prior to erythropoietic crises the mean erythrocyte age was at a maximum and many aged erythrocytes would be expected on the blood smears. In many cases, slides prepared at these times contained high percentages of microcytic spherocytes and disintegrating forms which appeared to be fragmenting erythrocytes whose fragments retained their hemoglobin. The most common form observed, however, could be described as a serrated poikilocyte, a cell having an irregular, angular border. None of these forms could definitely be identified as aged cells, of course.

Finally, one might speculate on the occurrence of cyclic erythropoiesis in human blood donors. Although the blood loss in such cases involves only about 10% of the erythrocyte mass, the available data on recovery rates suggest that synchronization of erythrocyte life cycles could occur. If the mean erythrocyte age was slightly elevated at 90 to 120 days after bleeding, repeated donation at that time might yield cells with a shortened posttransfusion survival time.
SUMMARY

Measurement of hematologic variables following acute massive blood loss in dogs revealed evidence of increased erythropoietic activity at 120 to 140 days after bleeding and about 110 and 220 days later. This cyclic pattern of erythropoietic activity resulted from synchronization of life cycles of erythrocytes produced in response to the hemorrhage. A cyclic variation in mean age of the erythrocyte population was evidenced by cyclic changes in physical properties of the erythrocytes.
PART III. EFFECTS OF HEMATOLOGIC VARIABLES ON ACCURACY OF REFLECTION OXIMETRY
REVIEW OF THE LITERATURE

Optical Properties of Erythrocyte Suspensions

Due to its complex nature reflection of light by blood has never been fully explained in theoretical terms. Because blood has a "mat" surface its reflection is considered as having two components: "specular" reflection and "diffuse" reflection. At any smooth dielectric interface the specular (mirror) component is completely governed by Fresnel's equations; its magnitude is dependent upon the refractive indices of the two media and the absorption index of the illuminated surface. No general theory exists to describe diffuse reflection completely, though most treatments of the problem are based upon a complex set of differential equations (45).

Assumptions necessary for application of the Kubelka-Munk equations are that the reflecting layer is composed of absorbing and light-scattering particles which are uniformly and randomly distributed and whose dimensions are much smaller than the thickness of the layer. The layer is assumed to be of infinite length and width and simplifications are possible when its thickness is large enough that further increases do not alter the reflectance. When these assumptions are satisfied, the diffuse component of reflection depends only on the values of absorption and scattering coefficients of the illuminated medium. Thus, total reflectance is determined by: 1) the absorption coefficient; 2) the scattering coefficient; and 3) the refractive indices of the second medium (blood) and of the first medium (e.g., fiberoptics).

Of the three optical properties of blood which control its reflectance the absorption coefficient is the one most obviously dependent on the
wavelength of the incident light. The absorption coefficient of oxyhemoglobin is much larger at 660 nm. than at 805 nm. while that of reduced hemoglobin is nearly the same at the two wavelengths and is equal to that of oxyhemoglobin at 805 nm. Obviously, the concentrations and absorption coefficients of other pigments in blood also affect its reflection properties.

Scattering coefficients of suspensions can also be wavelength-dependent. When the dimensions of the light-scattering particles are small relative to the wavelength of the incident light, the scattering becomes inversely proportional to the fourth power of the wavelength. This is known as Rayleigh scattering. When the particles are much larger than the wavelength, the scattering resembles specular reflection and is governed by the laws of geometric optics (Fresnel's equations). Since the dimensions of normal erythrocytes are large compared to the wavelengths used in oximetry their scattering coefficients are approximately the same for both wavelengths. The scattering coefficients for plasma vary strongly with wavelength, since the plasma protein molecules are quite small relative to these wavelengths.

Refractive index, the third optical property, is also wavelength-dependent. In general, the refractive index may be much higher or lower within an absorption band (i.e., 660 nm.) than at other wavelengths (i.e., 805 nm.). Hence, any variation in the composition of blood which changes the optical properties is potentially capable of affecting the reflectances at the two wavelengths unequally.

The theoretical basis of reflection oximetry is the functional relationship between the relative oxygenation of hemoglobin and the ratio
of the reflectances of blood for the two wavelengths of light (the "reflection ratio"). The literature describing this function has been reviewed by Edgington (18) and Erickson (22). The relationship is linear, with the values of its slope and intercept apparently being determined by the geometry of the optical system used to measure the reflectances. Thus, for quantitative applications reflection oximeters must be calibrated by comparison with the standard gasometric or spectrophotometric methods for determination of relative oxygenation.

The problem of calibration is complicated, however, by effects of variables other than relative oxygenation on the reflectances of blood. In principle, all three optical properties of blood are functions of the concentrations of plasma proteins and of erythrocytes, as well as the concentration of hemoglobin within the erythrocytes. In addition, the shape, dimensions, orientation, and degree of aggregation of the erythrocytes affect the magnitudes of the scattering coefficients.

Several theoretical approaches have been developed to predict quantitative effects of these and other hematologic variables on the optical properties and reflectances of blood. These include a multiple-scattering theory (47,48), a photon diffusion theory (12,40,80), and classical geometric optical theory (38). The classical approach, though admittedly oversimplified, has the advantage of utilizing variables which have obvious physical significance and which can be measured by standard techniques. In addition, certain consequences of the theory agree well with experimental observations.

Ishikawa's equations are derived as explained below. Reflection of light is considered to occur exclusively at the plasma-erythrocyte
Absorption occurs both within the plasma and within the erythrocyte. The erythrocytes are assumed to be cubes with edges of length $r$. For a columnar space extending perpendicular to the surface of the fiberoptics and composed of cubes having the dimensions of the erythrocytes the probability that incident light is reflected from cube no. $n+1$ is given by $(1-p)^n \cdot p \cdot r \cdot n$, where $p$ is the packed cell volume (expressed as a fraction). Light emerging from the fiberoptics ($I_0$) is attenuated exponentially before reaching the erythrocytes ($I_1$), and the reflected light ($I_2$) is similarly attenuated before reaching the fiberoptics ($I$):

$$I_1 = I_0 \exp[-kcd]$$

$$I = I_2 \exp[-kcd]$$

where

- $k = \text{extinction coefficient of plasma}$,
- $c = \text{relative concentration of plasma, regarded as unity in whole blood}$, and
- $d = \text{"mean reflection distance"}$.

The mean reflection distance ($d$) is calculated as a function of packed cell volume ($p$) and cell size ($r$):

$$d = \sum_{n=1}^{\infty} (1-p)^n \cdot p \cdot n \cdot r = \frac{1-p}{p} \cdot r .$$

The reflectance for a single wavelength is:

$$\frac{I_0}{I_0} = \frac{I_2}{I_1} \exp[-2kcd] = \frac{I_2}{I_1} \exp[-2kcr(\frac{1-p}{p})].$$
Ishikawa denotes the ratio $I_2/I_1$ as $\alpha$ and finds an empirical value for it of 3.4% at 800 nm.; values of $\alpha$ for oxyhemoglobin and reduced hemoglobin at 660 nm. are not reported, but can be determined by the same method. Derivation of values of $\alpha$ from first principles is impractical, since diffuse reflection theory is involved.

Ishikawa's equations describe the influence of plasma absorbance, plasma concentration, erythrocyte size, and packed cell volume on the reflectance for a single wavelength. His experiments with bovine blood verify the qualitative effects of packed cell volume (up to 60%) and of plasma protein concentration predicted by the equation. Further theoretical work is needed to determine the effects of variations in erythrocyte characteristics on the value of $\alpha$.

Quantitative usefulness of reflection oximetry is limited by the inaccuracy of the assumption that the effects of hematologic variables other than relative oxygenation on the reflectances are identical at the two wavelengths, so that the reflectance ratio depends only on relative oxygenation. It is recognized that this is not strictly true for all of the variables. For example, particle size has a marked effect on reflectance ratios when one of the wavelengths is weakly absorbed and the other is strongly absorbed. As the dimensions of the particles are reduced the mean depth of penetration of the medium by incident light is reduced, so that diffuse reflectance becomes relatively greater for the poorly absorbed wavelength than for the one strongly absorbed (78).

Hematologic Variables Affecting Reflection Properties

Few systematic studies of the effects of hematologic variables on reflectances of blood and accuracy of reflection oximetry have been reported. Such data are needed, however, if reflection oximeters are to
be useful for quantitative work in nonphysiologic circumstances such as in control systems for artificial hearts. Extreme values of hematologic variables can be encountered during extracorporeal circulation (63). These may result from dilution of the blood with plasma expanders, high levels of hemolysis due to mechanical or osmotic damage to the erythrocytes, abnormal values of pH and $P_{CO_2}$, nonisotonicity of plasma, and lowering of blood temperature, to say nothing of differences in physical characteristics among different species and among individuals of the same species.

One of the variables for which some data are available is the packed cell volume (PCV) or hemoglobin concentration (HGB). In normal blood PCV and HGB are directly proportional since the concentration of hemoglobin within the erythrocytes is constant. This proportionality can be altered, however, by changes in plasma osmotic pressure which change PCV without altering HGB, as well as in certain anemias and in hemolytic conditions.

Several workers report a hyperbolic increase in reflectances of blood with increasing PCV (2,21,38). With fiberoptic oximeters a decrease in reflectance occurs above 50 to 60% PCV (21,38). This is ascribed to an artifact, the "shading" of the erythrocytes by the afferent fibers of the catheter (38). Thus, with fiberoptic instruments the relationship between reflectances and PCV is nearly parabolic rather than hyperbolic.

The relationship between the reflectance ratio and PCV at 100% relative oxygenation appears to be hyperbolic. At 100% oxygenation the reflectance ratio is constant above 30% PCV (21); it decreases at lower PCV's causing underestimation of relative oxygenation. This effect is not explained but is important since the error is as high as 6% oxygenation at 10% PCV.
Plasma and erythrocyte cytoplasm can both be considered as solutions containing macromolecules which cannot cross the erythrocyte membrane which separates them. The osmotic pressure of such solutions is directly proportional to the concentration of dissolved molecules and ions. Any difference in solute concentration on the two sides of the membrane causes water to filter across it until the pressures are equalized. If the plasma is made hypotonic, the erythrocytes swell due to the influx of water. This can continue until the membrane is disrupted, releasing the cell contents into the plasma; this is termed "osmotic hemolysis". Conversely, if the plasma is made hypertonic, water filters from the cells and they shrink. They assume a "crenated" or spiked shape with the cell membrane remaining intact.

Drabkin and Singer (16) find that hypotonicity of the plasma has two effects on optical properties of blood. The increase in erythrocyte diameter causes increased reflection while the decrease in refractive index and absorption coefficient of the cells decreases reflection. The net result is a decrease in reflection. Others report increased reflectance with hypertonicity (18,62,64,67). Effects on oximeter calibration curves are not reported.

Studies of the effects of changes in composition of isotonic plasma or other suspending medium for erythrocytes are limited to the effect of dilution of whole plasma with isotonic solutions of NaCl, glucose, and mannitol (38,46). Reflectance increases as the plasma is diluted due to decreased absorption and increased change in refractive index at the plasma-erythrocyte interface. Again, effects on oximeter calibration curves are not reported.
Hemolysis of a portion of the erythrocytes decreases reflectance for monochromatic light (53,67). The optical changes are complex since hemolysis simultaneously lowers PCV, raises plasma refractive index, and increases the absorption coefficient of the plasma. Changes in oximeter calibration curves due to hemolysis are not reported.

Extraneous pigments such as carbon monoxide hemoglobin and methemoglobin reportedly interfere with oximetry (64). Ishikawa (38) discusses the effects of dyes used for dilution-curve estimation of cardiac output. No general solution for their effects on accuracy of reflection oximetry is available.

Changes in blood temperature alter the shape of erythrocytes (39). Sodium and potassium ions form salts with hemoglobin more readily as temperature increases so that bicarbonate ions diffuse from the cells, resulting in osmotic shrinkage. Reversal of this process causes the erythrocytes to swell at low temperatures. These changes could presumably affect reflectances in the same way that variations in osmotic pressure do. The increase in optical density of erythrocyte suspensions at elevated temperatures (54) is consistent with this concept.

Changes in flow characteristics of blood cause systematic errors with single-wavelength oximeters. Reflection of monochromatic light increases as the flow rate is increased to a critical velocity above which it becomes constant (18). Above an average linear flow rate of 20 cm. per second the shear rate is large enough to prevent "rouleaux formation", an inherent tendency of normal erythrocytes to attach face-to-face like a stack of coins when blood is stagnant. Formation of rouleaux decreases the effective surface area for reflection of light.
Other flow characteristics which may alter reflection include pulsatile flow and whether the flow is laminar or turbulent (18,22). Use of the two-wavelength principle appears to minimize oximeter errors arising from these conditions and changes in velocity (33,53). A tenfold increase in velocity produces very little change in oximeter readings at 100% oxygenation (33). Edgington (18) reports that changes in blood pressure have no effect on reflection at 632.8 nm.

In addition to hypotonicity and low temperature, high pH also causes swelling of erythrocytes provided there is an adequate concentration of bicarbonate (7); it acts by reversibly increasing the permeability of the cell membrane. High pH induced by lowering of $P_{CO_2}$ causes decreased optical density linearly related to the change in pH (65). The change in optical densities is nearly the same at 630 nm. as at 805 nm. resulting in negligible error with a transmission type of oximeter. The error due to neglecting $P_{CO_2}$ differences between arterial and venous blood with a single-wavelength oximeter is estimated to be between 3.2 and 6.1% oxygenation. Sinclair et al. (67) report that a bichromatic system compensates for effects of changes in $P_{CO_2}$ as well as tonicity, hemolysis, and hemoglobin concentration.

Changes in blood pH and $P_{CO_2}$ do not greatly alter the absorption spectra of hemoglobin and oxyhemoglobin since they do not alter the chemical composition of the heme group, which is responsible for the color of hemoglobin. They do alter the position of the oxyhemoglobin dissociation curve but this does not affect the relationship between relative oxygenation and the reflection and absorption coefficients of hemoglobin and oxyhemoglobin.
Reflectance of whole blood increases as sample depth is increased to about 3 mm. and does not change with further increases (2,44). Reflectance is also inversely related to the absorption coefficient of hemoglobin and the magnitude of this change increases with increasing sample depth. Thus, use of large sample depths in reflection oximetry has two advantages: 1) the absolute values of reflectance are greater; and 2) the changes in reflection ratio with changes in relative oxygenation are greater (2).

Interspecies differences in erythrocyte size and shape among mammals appear to have little effect on the accuracy of reflection oximetry. Comparisons between ovine and canine (22) and between human and canine (58) bloods are reported. Intraspecies variations in the in vivo relationship between reflection ratio and relative oxygenation are occasionally large enough to necessitate recalibration of the oximeter for each patient (41,64).

Many hematologic variables affect the absolute values of reflectances of blood at the two wavelengths used in reflection oximetry and at least some also alter the position or shape of calibration curves for oximeters. While systematic studies of this problem are few and limited in scope, continuing reference to it in reports on reflection oximetry indicate that further study is warranted. While it is said that in principle the reflection method is absolute in the same sense as the transmission method (58), the frustration of those attempting to use it for quantitative estimations is exemplified by a statement in Nilsson's review (53): "On the whole, it is evident that oximetry is best suited to the observation of changes rather than the establishment of absolute values".
METHODS

Control of Hematologic Variables

The objective of this study was to determine the effects of changes in composition of canine blood on its optical reflectances and on accuracy of reflection oximetry. The experiments consisted of a series of in vitro studies in which the complexity of a standardized erythrocyte suspension was gradually increased followed by in vivo studies for comparison with the more complex of the in vitro experiments. In the first studies only the identity of the blood donor was varied. Then the effects of changing PCV were studied. Subsequently the plasma protein concentration (PPC), the degree of hemolysis, the plasma osmotic pressure, the pH, and the $P_{CO_2}$ were varied. Finally, in vivo studies were made for comparison with in vitro results at 25°C. In addition, changes in optical properties of blood due to the cyclic changes in mean erythrocyte age (MEA) following recovery from hemorrhage were investigated.

Blood was obtained from 23 random-source mongrel dogs weighing from 20 to 35 kg. (mean: 25 kg.). All were vaccinated, wormed, and acclimatized to confinement prior to bleeding. Fecal examinations were performed periodically and treatment for parasites was repeated whenever necessary. A modified Knott’s test (52) for microfilaria was performed on each dog. The diet consisted of commercial dry dog food and water ad lib. A


hematinic preparation\(^1\) containing 50.0 mg. of ferrous fumarate per tablet and various vitamins and minerals was fed at the rate of one tablet per dog per day.

Prior to collection of blood the dogs were anesthetized by intravenous injection of a short-acting barbiturate\(^2\) at a dosage of 20 mg./kg. body weight. Blood was drawn by cardiac puncture through a 13-ga., 5 cm. hypodermic needle and 20 cm. of 3.2 mm. I.D. polyethylene tubing into a dry, sterile 1-liter glass bottle containing 30 mg. of dry sodium heparin\(^3\).

To facilitate adjustment of hematologic variables of donor blood to uniform values for the initial studies the cellular and plasma fractions of blood were partially separated by centrifugation\(^4\) at 2,000 rpm for 15 minutes at 25\(^\circ\)C. The plasma was siphoned into a separate bottle and the cell concentrate was filtered through eight layers of gauze\(^5\). The PCV of the concentrate was adjusted to 80% by addition of plasma prior to storage in a refrigerator at approximately 4\(^\circ\)C.

The cell concentrate and plasma were mixed in the desired proportions with isotonic saline or other solutions and were warmed to room temperature prior to use. For experiments in which the degree of hemolysis, osmotic

\(^{1}\)Vi-sorbits, Norden Laboratories, Lincoln, Nebraska.

\(^{2}\)Sodium thiamylal, Surital, Parke-Davis and Co., Detroit, Michigan.

\(^{3}\)Heparin sodium, Nutritional Biochemicals Corp., Cleveland, Ohio.


\(^{5}\)Curity gauze sponges, 3x3", 12-ply, Kendall Hospital Products Division, Chicago, Illinois.
pressure, pH, and $P_{CO_2}$ were to be held constant the suspending medium consisted of a mixture of isotonic saline and plasma; this was isotonic and contained either 1 gm.% or 6 gm.% plasma proteins. The lower value of PPC was used to simulate the effect of plasma expanders on the composition of blood. The PCV was adjusted in increments of 10% up to 50%, depending on the requirements of the experiment. Suspensions having 40% PCV were referred to either as standardized erythrocyte suspension (SES) or as standardized whole blood (SWB) depending on whether the PPC was 1 gm.% or 6 gm.%, respectively. These two suspensions were used in the majority of experiments in which changes were made in pH, $P_{CO_2}$, osmotic pressure, degree of hemolysis, and mean erythrocyte age.

All *in vitro* studies were done using the pumping system developed by Edgington (18). This consisted of two plexiglass cylinders 9 cm. in diameter containing pistons covered with a siliconized fabric. One of the pistons had an electromechanical drive system which permitted pumping of blood from one cylinder to the other without exposing it to air. The sensing end of the fiberoptics and the sampling port were located in the tubing through which the blood was pumped. The average linear flow velocity used in all of the studies was 30 cm./sec.

To vary the relative oxygenation of the erythrocyte suspensions and to control their $P_{CO_2}$ equal volumes of a suspension were equilibrated with different gas mixtures. Two methods of equilibration were used. For suspensions having 6 gm.% PPC two small disc oxygenators were used.

---

Polycarbonate disc oxygenator, 6-inch size, #2050016-52, Electro-dynamics Corp., Cardiovascular Division, Baltimore, Maryland.
Several experiments in which the PPC was 1 gm.% were also done with these oxygenators, but most of these were done using tonometers consisting of Kelly infusion jars with fritted glass dispersion tubes (9) to bubble gases directly through the blood.

The gases were prepared by mixing streams of compressed air, nitrogen, and carbon dioxide with proportioners \(^\text{1}\) and then bubbling the mixture through distilled water to saturate it with water vapor. It was then bubbled through the blood in the tonometers or passed through the oxygenators. One-half of each suspension was equilibrated with nitrogen and the other half with air. Carbon dioxide was added in equal proportions to the two gas streams so that the two tonometers contained suspensions having the same \(P_{\text{CO}_2}\) but different \(P_{\text{O}_2}\)'s. The flow system was initially filled with blood from one of the tonometers. When the measurements and samplings for that level of oxygenation were completed, a portion of the blood was removed and replaced with an equal volume from the other tonometer. By repetition of this procedure five different levels of relative oxygenation were produced for study of the optical properties of each suspension.

In the first set of experiments repeated studies were made of erythrocyte suspensions having identical values of PCV, PPC, pH, and \(P_{\text{CO}_2}\) at low levels of hemolysis and at room temperature, the only known difference among them being the identity of the blood donor. Most of these studies were done with blood from the first donation for a particular dog, but a few were made with blood from second, third, or fourth donations at

intervals of 56 to 84 days.

In the second set of experiments suspensions with five different values of PCV were compared at 1 gm.% PPC with balanced photocell sensitivities and again with the 805 nm. photocell having a 7% increase in sensitivity. Only 10, 20, and 40% PCV, and sometimes 30%, were compared with other degrees of photocell sensitivity balance. PCV's of 10, 20, and 40% were also compared at 6 gm.% PPC with balanced photocell sensitivities.

To determine the effects of changing PPC, comparisons were made between 1 gm.% PPC and 6 gm.% PPC at 10, 20, and 40% PCV with balanced photocell sensitivities and at 40% PCV with the 805 nm. photocell having a 7% increase in sensitivity.

Different levels of hemolysis were produced by treating a volume of SWB in an ultrasonic bath\(^1\) until it was completely hemolyzed. This was then mixed with untreated SWB to produce desired levels of hemolysis. Levels of 10 and 25% hemolysis were studied with matched photocell sensitivities and 30% hemolysis with the 805 nm. photocell having a 7% increase in sensitivity.

Osmotic pressure was varied by using hypotonic or hypertonic saline in preparing suspensions having 40% PCV and 1 gm.% PPC. Studies were made with osmotic pressures of 0.8x and 1.2x normal with matched photocell sensitivities.

Variations in pH were produced by adding 20 ml. of a 1% solution of HCl or NaHCO\(_3\) to 500 ml. of isotonic saline used to prepare suspensions having 40% PCV and 1 gm.% PPC. This yielded pH's of approximately 7.1 and

\(^1\)Ultrasonic Cleaner, Jensen-Salsbury Laboratories, Kansas City, Missouri.
7.5, respectively. Matched photocell sensitivities were used.

\( P_{CO_2} \) levels of 0, 20, 40, 60, and 80 mm. Hg were studied using SWB and matched photocell sensitivities. Also, \( P_{CO_2} \)'s of 0 and 40 mm. Hg were compared for PCV's of 10 and 30% with 1 gm.% PPC.

Effects of changes in mean erythrocyte age (MEA) were studied by comparing properties of SWB's prepared from blood drawn at 70, 85, 93, 139, and 152 days after a previous donation. In addition, comparisons were made of 119-day blood with 56-day blood at 10% PCV with 1 gm.% PPC and with first-donation blood at 20% PCV with 1 gm.% PPC.

Since erythrocytes of different ages could be partially separated by centrifugation (8), comparisons were made between suspensions prepared from the upper and lower halves of a column of centrifuged erythrocytes. The buffy coat was removed separately so that neither of the erythrocyte fractions had a significant leukocyte content. Blood from a first bleeding and from a 98-day bleeding were used. The comparisons were made at 20% PCV and 1 gm.% PPC.

Comparisons were made between in vitro data obtained at 25°C and in vivo data obtained at 35°C. The in vivo studies were made by diverting the flow of jugular venous blood in anesthetized dogs through the sampling port and oximeter cuvette and back into the vein in a closed loop. Anesthesia was induced by intravenous injection of pentobarbital at a dosage of 30 mg./kg. The right jugular vein was isolated and transected. Ligatures were used to connect the cut ends to two silicone rubber

\(^1\)Pentobarbital Sodium, Napental, S. E. Massengill Co., Bristol, Tennessee.
catheters. A thermistor was placed in series with the sampling port and oximeter cuvette between the open ends of the catheters. The left jugular vein was then isolated and temporarily occluded to maximize flow through the right jugular loop. Heparin was administered intravenously at 3.0 mg./kg. prior to insertion of the catheters. Variations in venous relative oxygenation were produced by varying the proportion of oxygen and nitrogen in the rebreathing circuit of an anesthetic machine to which the dog was attached via a cuffed endotracheal tube. Below about 40% relative oxygenation respiratory arrest occurred so that the dogs had to be resuscitated with a respirator and forced ventilation with oxygen. Attempts to raise relative oxygenation beyond 80% by forced ventilation were generally unsuccessful. Induction of hyperventilation by addition of CO₂ to the rebreathing circuit was similarly ineffective in sustaining venous relative oxygenation above 80%.

Experimental Measurements

The oximeter used in this study was similar to that used by Erickson (22) and by Peura (56). It consisted essentially of a tungsten-filament lamp powered by a regulated DC power supply, a trifurcated fiberoptic

1 Tele-thermometer, Yellow Springs Instrument Company, Yellow Springs, Ohio.

2 Heparin sodium, Nutritional Biochemicals Corp., Cleveland, Ohio.

3 Heidbrink Kinet-o-meter, Ohio Chemical and Surgical Equipment Co., Inc., Madison, Wisconsin.

4 Mark 4 anesthesia assistor/controller, Bird Corp., Palm Springs, California.
wageguide to conduct light to and from the blood, a pair of interference filters with peak transmittances at 660 and 805 nm., two photoconductive cells, and a Wheatstone bridge circuit. In this study the stability and precision of the transducer were improved by use of a halogen-cycle lamp and two neutral-density optical wedges, together with a two-standard calibration technique. Constant illumination of the photocells between periods of use and matching of photocell sensitivities minimized losses of precision and accuracy previously encountered with these photodetectors. The output of the oximeter was made linear and direct-reading by simple adjustments of the Wheatstone bridge parameters.

The method for equalizing photocell sensitivities involved placing a resistor in parallel with the more sensitive photocell, the one used for 805 nm. light. The magnitude of its resistance was designated by the symbol $R_p$. Best matching of the sensitivities was obtained with $R_p = 120 \, \text{K}\Omega$. Other degrees of sensitivity balance were used in a number of experiments to examine the possibility of automatically correcting for oximeter errors introduced by changes in several of the hematologic variables. The ratio of 805 nm. photocell sensitivity to 660 nm. photocell sensitivity was estimated to be 1.00 with $R_p = 120 \, \text{K}\Omega$, 1.06 with $R_p = 150 \, \text{K}\Omega$, 1.07 with $R_p = 175 \, \text{K}\Omega$, 1.09 with $R_p = 200 \, \text{K}\Omega$, 1.10 with $R_p = 220 \, \text{K}\Omega$, and 1.12 with $R_p = \infty \Omega$.

Measurements of instrumental parameters and variables and hematologic variables were made in identical fashion in the in vitro and in vivo experiments. At the start and at the end of each experiment the blood temperature, oximeter lamp supply voltage, and bridge voltage were recorded. Measurements and samples were taken only with the blood flowing from left
to right at 30 cm./sec. At each of five levels of relative oxygenation the oximeter output voltage \( v_o \) and the photocell resistances \( R_{660} \) and \( R_{805} \) were recorded, and the ratio \( R_{660}/R_{805} \) (q) was computed. A pair of 4.0 ml. blood samples was drawn for determination of relative oxygenation by the spectrophotometric method of Drabkin (15), as modified by Erickson (22). Another 4.0 ml. sample was drawn for measurement of total hemoglobin (HGB) and of plasma hemoglobin by the cyanmethemoglobin technique. Percent hemolysis was calculated as 100 times the ratio of plasma hemoglobin to total hemoglobin. The same sample was used for measurement of PCV by the microhematocrit method and for measurement of plasma protein concentration with a clinical refractometer\(^1\). A fourth 4.0 ml. sample was drawn for measurement of \( P_{O_2} \), \( P_{CO_2} \), and pH with an electronic gas analyzer\(^2\).

\(^1\)TS meter, American Optical Corp., Scientific Instruments Division, Buffalo, New York.

\(^2\)Physiologic Gas Analyzer, Model 160, Beckman Instruments, Inc., Spinco Division, Palo Alto, California.
RESULTS

Differences Between Donors

The results of all of the experiments could be comprehended best by comparison of the slopes and intercepts of two relationships contained in the data from each experiment. The first, a graph of photocell resistance ratio (q) as a function of relative oxygenation, was the oximeter calibration curve for the erythrocyte suspension in a given experiment. Its slope has the units \( \%^{-1} \); the intercept is dimensionless. The second, a graph of 805 nm. (IR) photocell resistance as a function of relative oxygenation, represented the reflectance of the suspension for infrared light; high intercepts indicated low reflectance and vice versa. Its slope has the units \( K\Omega/\% \) and its intercept the units \( K\Omega \).

Statistics for comparison of the slopes and intercepts were computed using a program based on the method described by Snedecor (68) for comparison of two linear regression lines. The computed "intercepts" were actually values extrapolated to 100% relative oxygenation, since these were nearer the actual range of the data than values at 0% relative oxygenation. A two-tailed F-test was used for evaluation of the probability that the slopes or intercepts of two lines being compared were identical. Levels of significance of differences between data given in the tables were indicated by asterisks: * for \( P < .050 \), ** for \( P < .025 \), *** for \( P < .010 \), and **** for \( P < .005 \).

Tables 3 and 4 contain data and statistics pertaining to comparisons

\(^1\)PDP 8/e computer, Digital Equipment Corp., Maynard, Massachusetts.
Table 3. Photocell resistance ratio vs. relative oxygenation; differences between donors

<table>
<thead>
<tr>
<th>Rp (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>10</td>
<td>1</td>
<td>.0204---</td>
<td>.865---</td>
<td>.0150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0212</td>
<td>.847</td>
<td>.0387</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0231*</td>
<td>.844*</td>
<td>.0350</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0228</td>
<td>.843</td>
<td>.0417</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0234****</td>
<td>.815</td>
<td>.0126</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0318****</td>
<td>.693</td>
<td>.0277</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0289****</td>
<td>.690</td>
<td>.0231</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0280**</td>
<td>.626</td>
<td>.1160</td>
</tr>
<tr>
<td>b. 120</td>
<td>40</td>
<td>6</td>
<td>.0251---</td>
<td>.610---</td>
<td>.0221</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0262</td>
<td>.500****</td>
<td>.0154</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0290</td>
<td>.455</td>
<td>.1003</td>
</tr>
<tr>
<td>c. 175</td>
<td>10</td>
<td>1</td>
<td>.0179---</td>
<td>.826---</td>
<td>.0301</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0189</td>
<td>.815</td>
<td>.0377</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0202*</td>
<td>.795</td>
<td>.0303</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0227</td>
<td>.647****</td>
<td>.0054</td>
</tr>
<tr>
<td>d. 175</td>
<td>40</td>
<td>1</td>
<td>.0238---</td>
<td>.726---</td>
<td>.0227</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0238</td>
<td>.697</td>
<td>.0342</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0242</td>
<td>.626****</td>
<td>.0327</td>
</tr>
<tr>
<td>e. 175</td>
<td>50</td>
<td>1</td>
<td>.0233---</td>
<td>.609---</td>
<td>.0064</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0272**</td>
<td>.444**</td>
<td>.0264</td>
</tr>
<tr>
<td>f. 200</td>
<td>30</td>
<td>1</td>
<td>.0184---</td>
<td>.707---</td>
<td>.0160</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0191</td>
<td>.706</td>
<td>.0375</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0216**</td>
<td>.698***</td>
<td>.0091</td>
</tr>
<tr>
<td>g. ∞</td>
<td>10</td>
<td>1</td>
<td>.0185---</td>
<td>.639---</td>
<td>.0085</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0238</td>
<td>.405****</td>
<td>.0232</td>
</tr>
<tr>
<td>h. ∞</td>
<td>40</td>
<td>1</td>
<td>.0198---</td>
<td>.728---</td>
<td>.0129</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0241</td>
<td>.625</td>
<td>.0275</td>
</tr>
</tbody>
</table>
Table 4. IR photocell resistance vs. relative oxygenation; differences between donors

<table>
<thead>
<tr>
<th>Rp (kΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept (kΩ)</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>10</td>
<td>1</td>
<td>0.0607---</td>
<td>47.3---</td>
<td>0.617</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1320</td>
<td>45.5</td>
<td>1.033</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0269</td>
<td>55.2****</td>
<td>0.601</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0882</td>
<td>52.1****</td>
<td>0.618</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0202*</td>
<td>51.9****</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.9330****</td>
<td>44.7****</td>
<td>0.445</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1270</td>
<td>48.8****</td>
<td>0.301</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0820</td>
<td>52.9****</td>
<td>0.644</td>
</tr>
<tr>
<td>b. 120</td>
<td>40</td>
<td>6</td>
<td>0.1080---</td>
<td>33.1---</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0730</td>
<td>35.2****</td>
<td>0.423</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0971</td>
<td>31.5****</td>
<td>0.369</td>
</tr>
<tr>
<td>c. 175</td>
<td>10</td>
<td>1</td>
<td>0.0446---</td>
<td>57.8---</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0876*</td>
<td>45.3****</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1760**</td>
<td>54.9</td>
<td>0.882</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1150*</td>
<td>55.7****</td>
<td>0.244</td>
</tr>
<tr>
<td>d. 175</td>
<td>40</td>
<td>1</td>
<td>0.0744---</td>
<td>28.0---</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0608</td>
<td>28.0</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0565*</td>
<td>27.6****</td>
<td>0.077</td>
</tr>
<tr>
<td>e. 175</td>
<td>50</td>
<td>1</td>
<td>0.0844---</td>
<td>28.5---</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0631*</td>
<td>28.7*</td>
<td>0.145</td>
</tr>
<tr>
<td>f. 200</td>
<td>30</td>
<td>1</td>
<td>0.0088---</td>
<td>28.5---</td>
<td>0.385</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0521*</td>
<td>26.6**</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0681**</td>
<td>27.6</td>
<td>0.130</td>
</tr>
<tr>
<td>g. ∞</td>
<td>10</td>
<td>1</td>
<td>1.2850---</td>
<td>50.1---</td>
<td>1.562</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.4060****</td>
<td>64.4</td>
<td>0.410</td>
</tr>
<tr>
<td>h. ∞</td>
<td>40</td>
<td>1</td>
<td>0.0438---</td>
<td>27.2---</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0683</td>
<td>28.2****</td>
<td>0.242</td>
</tr>
</tbody>
</table>
between blood from different donors under similar conditions. Eight
different combinations of photocell matching, PCV, and PPC were represented.
Each lettered division of these tables represents a set of replicated
experiments in which the only variable was the identity of the blood donor.
In each of these sets the data from the first experiment listed were
compared in turn with those from each replication. Significance levels of
the differences are indicated beside the data for the replicates.

In Table 3a the intercepts were nearly all identical, while the slopes
showed significant differences. Inasmuch as the comparisons were based on
a slope whose value was exceptionally low, and high intercepts appeared to
be associated with low slopes, the calibration lines could be considered
to be very close together. In subsequent sections of this table the
association of high intercepts and low slopes was also evident, but there
were significant differences between intercepts. More detailed study of
the data revealed that different samples of blood from the same donation
consistently exhibited either high intercepts and low slopes or low inter­
cepts and high slopes. Of 23 dogs used as donors, only six gave low
intercepts and high slopes on the first donation, so this combination was
designated as "atypical blood". Since the differences between intercepts
were relatively large compared to the differences in slopes, it appeared
that the atypical bloods gave calibration curves which were parallel to
those for typical blood but had lower intercepts.

In Table 4 the intercepts for 805 nm. photocell resistance were nearly
all different at a high level of statistical significance. This occurred
because the reflectance at 805 nm. was highly dependent on PCV or hemoglobin
concentration and these were never exactly the same even for sets of data
representing the same PCV. The atypical bloods gave 805 nm photocell resistance intercepts in the same range as typical blood did; the differences in calibration curves were due primarily to lower values of 660 nm. photocell resistance with atypical bloods. Thus, the atypical bloods had a higher reflectivity for red light at all levels of relative oxygenation than did typical bloods. Differences between slopes were generally at low levels of statistical significance and were most likely due to small differences in levels of hemolysis.

Effects of Differences in Mean Erythrocyte Age

Tables 5 and 6 contain the data and statistics pertaining to effects of variations in mean erythrocyte age on accuracy of reflection oximetry and on reflectance levels. Subdivisions a, b, and c of these tables represent sets of replicated experiments, data from the first member of the set having been used as the basis for comparisons. Subdivisions d and e show the results of comparisons of "top cells" and "bottom cells" from centrifuged samples of blood from a single donor. Subdivision f contains data from an experiment similar to those in d and e, but in which the cells were mixed after centrifugation and the buffy coat was not removed. The numbers in the "Days" column refer to the time elapsed since the last bleeding of the donor dog. The symbol ∞ in the first lines of the subsections c indicates that the blood used in that experiment was from a first donation.

When blood was collected at intervals longer than 85 days after a previous donation, there was a time-dependent change in the reflection properties. Tables 5a, 5b, and 5c show that progressive decreases in
Table 5. Photocell resistance ratio vs. relative oxygenation; effects of differences in mean erythrocyte age

<table>
<thead>
<tr>
<th>Rp (kΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>40</td>
<td>6</td>
<td>.0251</td>
<td>.610</td>
<td>.0221</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0269</td>
<td>.649****</td>
<td>.0379</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0250</td>
<td>.536****</td>
<td>.0154</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0232</td>
<td>.475****</td>
<td>.0240</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0217*</td>
<td>.434****</td>
<td>.0079</td>
<td>152</td>
</tr>
<tr>
<td>b. 175</td>
<td>10</td>
<td>1</td>
<td>.0189</td>
<td>.815------</td>
<td>.0377</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0239***</td>
<td>.617</td>
<td>.0174</td>
<td>119</td>
</tr>
<tr>
<td>c. 175</td>
<td>20</td>
<td>1</td>
<td>.0209</td>
<td>.763------</td>
<td>.0213</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0254</td>
<td>.606</td>
<td>.0603</td>
<td>119</td>
</tr>
<tr>
<td>d. 175</td>
<td>20</td>
<td>1</td>
<td>.0236</td>
<td>.742------</td>
<td>.0368</td>
<td>Bottom</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0249</td>
<td>.728</td>
<td>.0418</td>
<td>Top</td>
</tr>
<tr>
<td>e. 175</td>
<td>20</td>
<td>1</td>
<td>.0221</td>
<td>.755------</td>
<td>.0219</td>
<td>Bottom</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0240</td>
<td>.680</td>
<td>.0487</td>
<td>Top</td>
</tr>
<tr>
<td>f. 175</td>
<td>20</td>
<td>1</td>
<td>.0209</td>
<td>.763------</td>
<td>.0212</td>
<td>Mixed</td>
</tr>
</tbody>
</table>

calibration curve intercepts occurred between 85 and 152 days. Changes in 805 nm. photocell resistance for the same data (Tables 6a, 6b, and 6c) were also progressive but relatively small. Thus, the basic change was an increase in reflectivity of the blood for red light during this interval. This was the same period during which erythrocytes produced in response to the previous bleeding were being replaced by new, young erythrocytes.

Results of comparisons between erythrocytes from the top of centrifuged samples with those from the bottom of the same samples were shown in Tables 5d, 5e, 6d, and 6e. The differences between calibration
Table 6. IR photocell resistance vs. relative oxygenation; effects of differences in mean erythrocyte age

<table>
<thead>
<tr>
<th></th>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept (KΩ)</th>
<th>Std. Dev.</th>
<th>Days</th>
</tr>
</thead>
</table>
airel. |   120       |   40    |     6      | .1080--- | 33.1---         | .246      | 70   |
airel. |         |        |            | .0711** | 33.0****        | .127      | 85   |
airel. |         |        |            | .1500   | 34.2****        | .418      | 93   |
airel. |         |        |            | .1120   | 34.3****        | .517      | 139  |
airel. |         |        |            | .0576*  | 36.2****        | .271      | 152  |
b.  |   175       |   10   |     1      | .0876--- | 45.3---         | .330      | 56   |
airel. |         |        |            | .0653   | 50.7****        | .575      | 119  |
c.  |   175       |   20   |     1      | .0008--- | 31.9---         | .258      | ∞    |
airel. |         |        |            | .0268   | 34.5****        | .235      | 119  |
d.  |   175       |   20   |     1      | .0328--- | 34.8---         | .512      | Bottom |
airel. |         |        |            | .0762*  | 34.0****        | .107      | Top  |
e.  |   175       |   20   |     1      | .0250--- | 34.6---         | .279      | Bottom |
airel. |         |        |            | .0310   | 35.7****        | .223      | Top  |
f.  |   175       |   20   |     1      | .0008--- | 31.9---         | .258      | Mixed |

curves were not highly significant though in both comparisons the top
(young) cells yielded lower intercepts and higher slopes than the bottom
(old) cells. All four sets of data had higher 805 nm. photocell resistance
intercepts than the set of data for mixed cells (Table 6f) from another
donor under the same conditions. This may have been related to removal of
the buffy coat from the bloods which were centrifuged and separated into
top and bottom fractions.
Effects of Changes in PCV

Results of comparisons of different PCV's under identical conditions are presented in Tables 7 and 8. Six different degrees of photocell sensitivity balance and two levels of PPC were used. With equalized sensitivities ($R_p = 120 \text{ K}\Omega$) there was a progressive decrease in calibration curve intercepts (Table 7) as PCV increased from 10 to 40% at both 1 and 6 gm. % PPC. This trend was also evident at the other values of sensitivity balance but the magnitudes of the differences between intercepts were less; at $R_p = \infty$ the trend was reversed. With $R_p = 175 \text{ K}\Omega$ the calibration curves for 10, 20, 30, and 40% PCV were indistinguishable, while the curve for 50% PCV had a low intercept.

In Table 8 the relationships between 805 nm. photocell resistance intercepts and PCV appeared to be roughly parabolic with minimum resistance values (maximum reflectances) having occurred at 30 or 40% PCV.

Effects of Decreased Plasma Protein Concentration

Comparisons of 805 nm. photocell resistance intercepts at 1 and 6 gm.% PPC at 10, 20, and 40% PCV in Table 10 showed that the reflectances were always higher at 1 than at 6 gm.% PPC. This was expected, since the change in refractive index at the plasma-erythrocyte interface would be greater at the lower protein concentration.

In Table 9 the intercepts of the calibration curves for these experiments were not significantly different except at 10% PCV and at 40% PCV with unequal photocell sensitivities. Thus, the increase in reflectances with decreased PPC was very nearly the same at both wavelengths.
Table 7. Photocell resistance ratio vs. relative oxygenation; effects of changes in PCV

<table>
<thead>
<tr>
<th>( R_p ) (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (g.m.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>10</td>
<td>1</td>
<td>.0204</td>
<td>.865****</td>
<td>.0150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0225</td>
<td>.733***</td>
<td>.0400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0216</td>
<td>.728*</td>
<td>.0492</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0241---</td>
<td>.611---</td>
<td>.0450</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>.0229</td>
<td>.635</td>
<td>.0219</td>
</tr>
<tr>
<td>b. 150</td>
<td>10</td>
<td>1</td>
<td>.0280</td>
<td>.649</td>
<td>.0258</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0248</td>
<td>.729</td>
<td>.0405</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0291</td>
<td>.591</td>
<td>.0392</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>.0257---</td>
<td>.669---</td>
<td>.0306</td>
</tr>
<tr>
<td>c. 175</td>
<td>10</td>
<td>1</td>
<td>.0189</td>
<td>.815</td>
<td>.0377</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0209</td>
<td>.763</td>
<td>.0212</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0217</td>
<td>.752</td>
<td>.0137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0238---</td>
<td>.697---</td>
<td>.0342</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0233</td>
<td>.609****</td>
<td>.0064</td>
</tr>
<tr>
<td>d. 200</td>
<td>10</td>
<td>1</td>
<td>.0197**</td>
<td>.739</td>
<td>.0058</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0215</td>
<td>.716</td>
<td>.0109</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0184**</td>
<td>.707****</td>
<td>.0160</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>.0246---</td>
<td>.696---</td>
<td>.0267</td>
</tr>
<tr>
<td>e. 220</td>
<td>10</td>
<td>1</td>
<td>.0199****</td>
<td>.721**</td>
<td>.0126</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0206****</td>
<td>.744</td>
<td>.0035</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0232</td>
<td>.682**</td>
<td>.0229</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0261---</td>
<td>.678---</td>
<td>.0229</td>
</tr>
<tr>
<td>f. ∞</td>
<td>10</td>
<td>1</td>
<td>.0186</td>
<td>.639****</td>
<td>.0085</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0197</td>
<td>.732</td>
<td>.0184</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0198---</td>
<td>.728---</td>
<td>.0129</td>
</tr>
<tr>
<td>g. 120</td>
<td>10</td>
<td>6</td>
<td>.0246</td>
<td>.996****</td>
<td>.0830</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0236</td>
<td>.723****</td>
<td>.0321</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>.0251---</td>
<td>.610---</td>
<td>.0221</td>
</tr>
</tbody>
</table>
Table 8. IR photocell resistance vs. relative oxygenation; effects of changes in PCV

<table>
<thead>
<tr>
<th></th>
<th>Rp</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept (kΩ)</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>120</td>
<td>10</td>
<td>1</td>
<td>.0607</td>
<td>47.3***</td>
<td>.617</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
<td>.1920****</td>
<td>32.7****</td>
<td>.324</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1</td>
<td>.1030****</td>
<td>27.6</td>
<td>.082</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>---</td>
<td>.0492---</td>
<td>28.8---</td>
<td>.209</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>---</td>
<td>.0360</td>
<td>29.4*</td>
<td>.175</td>
</tr>
<tr>
<td>b.</td>
<td>150</td>
<td>10</td>
<td>1</td>
<td>.0379</td>
<td>56.0****</td>
<td>.317</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
<td>.0743***</td>
<td>38.7****</td>
<td>.058</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1</td>
<td>.1590****</td>
<td>36.9****</td>
<td>.395</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>---</td>
<td>.0136---</td>
<td>27.7---</td>
<td>.349</td>
</tr>
<tr>
<td>c.</td>
<td>175</td>
<td>10</td>
<td>1</td>
<td>.0876</td>
<td>45.3****</td>
<td>.330</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
<td>.0008***</td>
<td>31.9****</td>
<td>.258</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1</td>
<td>.0611</td>
<td>27.9</td>
<td>.298</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>---</td>
<td>.0608---</td>
<td>28.0---</td>
<td>.151</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>---</td>
<td>.0844*</td>
<td>28.5****</td>
<td>.127</td>
</tr>
<tr>
<td>d.</td>
<td>200</td>
<td>10</td>
<td>1</td>
<td>.0879</td>
<td>54.8****</td>
<td>.558</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
<td>.0755</td>
<td>34.6****</td>
<td>.099</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1</td>
<td>.0088***</td>
<td>28.5****</td>
<td>.358</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>---</td>
<td>.1220---</td>
<td>27.8---</td>
<td>.351</td>
</tr>
<tr>
<td>e.</td>
<td>220</td>
<td>10</td>
<td>1</td>
<td>.1700****</td>
<td>56.0****</td>
<td>.197</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
<td>.0226</td>
<td>31.8****</td>
<td>.163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1</td>
<td>.0433</td>
<td>29.2****</td>
<td>.239</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>---</td>
<td>.0389---</td>
<td>28.3---</td>
<td>.084</td>
</tr>
<tr>
<td>f.</td>
<td>∞</td>
<td>10</td>
<td>1</td>
<td>1.2850****</td>
<td>50.1****</td>
<td>1.562</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
<td>.0792</td>
<td>33.9****</td>
<td>.177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>---</td>
<td>.0438---</td>
<td>27.2---</td>
<td>.060</td>
</tr>
<tr>
<td>g.</td>
<td>120</td>
<td>10</td>
<td>6</td>
<td>.0859</td>
<td>76.1****</td>
<td>2.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>6</td>
<td>.0266</td>
<td>39.6****</td>
<td>.627</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>6</td>
<td>.1080---</td>
<td>33.1---</td>
<td>.246</td>
</tr>
</tbody>
</table>
Table 9. Photocell resistance ratio vs. relative oxygenation; effects of decreased plasma protein concentration

<table>
<thead>
<tr>
<th></th>
<th>Rp (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>120</td>
<td>40</td>
<td>1</td>
<td>.0240</td>
<td>.611</td>
<td>.0445</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0251</td>
<td>.610</td>
<td>.0221</td>
</tr>
<tr>
<td>b</td>
<td>120</td>
<td>20</td>
<td>1</td>
<td>.0225</td>
<td>.733</td>
<td>.0400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0236</td>
<td>.723</td>
<td>.0321</td>
</tr>
<tr>
<td>c</td>
<td>120</td>
<td>10</td>
<td>1</td>
<td>.0204</td>
<td>.865****</td>
<td>.0150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0246</td>
<td>.996</td>
<td>.0830</td>
</tr>
<tr>
<td>d</td>
<td>175</td>
<td>40</td>
<td>1</td>
<td>.0238</td>
<td>.726****</td>
<td>.0227</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0238</td>
<td>.617</td>
<td>.0609</td>
</tr>
</tbody>
</table>

Table 10. IR photocell resistance vs. relative oxygenation; effects of decreased plasma protein concentration

<table>
<thead>
<tr>
<th></th>
<th>Rp (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>120</td>
<td>40</td>
<td>1</td>
<td>.0492****</td>
<td>28.8****</td>
<td>.209</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.1080</td>
<td>33.1</td>
<td>.246</td>
</tr>
<tr>
<td>b</td>
<td>120</td>
<td>20</td>
<td>1</td>
<td>.1920****</td>
<td>32.7****</td>
<td>.324</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0266</td>
<td>39.6</td>
<td>.627</td>
</tr>
<tr>
<td>c</td>
<td>120</td>
<td>10</td>
<td>1</td>
<td>.0607</td>
<td>47.3****</td>
<td>.617</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0859</td>
<td>76.2</td>
<td>2.016</td>
</tr>
<tr>
<td>d</td>
<td>175</td>
<td>40</td>
<td>1</td>
<td>.0744****</td>
<td>28.0****</td>
<td>.119</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>.0259</td>
<td>33.3</td>
<td>.132</td>
</tr>
</tbody>
</table>
Effects of Increased Hemolysis

As the level of hemolysis increased the only consistent and significant change observed was decreased reflectance (i.e., increased 805 nm. photocell resistance intercepts, Table 12). Changes in the calibration curves (Table 11a) were significant but not consistent; massive hemolysis appeared to cause skewing of the calibration curves. The effect was similar with unequal photocell sensitivities (Table 11b). It is possible that the complex changes occurring during hemolysis resulted in nonlinear changes in the calibration curves; these could not be fully evaluated with the limited number of experiments performed. In any case, the high levels of hemolysis required to produce the observed errors in estimation of relative oxygenation were unlikely to occur in practical applications of the oximeter.

Effects of Alterations in Plasma Osmotic Pressure

Increases in plasma osmotic pressure (\(\pi\)) caused progressive increases in reflectance at 805 nm. (i.e., progressive decreases in 805 nm. photocell resistance intercepts, Table 14) and upward shifts of the calibration curves (i.e., increases in photocell resistance ratio intercepts, Table 13). There was virtually no change in reflectances at 660 nm.; the changes in calibration curves were entirely due to increased reflectances at 805 nm. These shifts in the calibration curves would result in a systematic overestimation of relative oxygenation of about 5% at 0.8x normal osmotic pressure, and underestimation of about 5% at 1.2x normal osmotic pressure.

Effects of Changes in \(pH\) and \(P_{\text{CO}_2}\)

The data for different values of \(pH\) and \(P_{\text{CO}_2}\) (Tables 15 and 17) showed only minor differences between the calibration curves. For different
Table 11. Photocell resistance ratio vs. relative oxygenation; effects of increased hemolysis

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>Hem.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>40</td>
<td>6</td>
<td>.0251</td>
<td>.610</td>
<td>.0221</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0298</td>
<td>.385</td>
<td>.0419</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0317</td>
<td>.507</td>
<td>.0256</td>
<td>25</td>
</tr>
<tr>
<td>b. 175</td>
<td>40</td>
<td>6</td>
<td>.0238</td>
<td>.617</td>
<td>.0609</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0325</td>
<td>.555</td>
<td>.0428</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 12. IR photocell resistance vs. relative oxygenation; effects of increased hemolysis

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>Hem.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>40</td>
<td>6</td>
<td>.1080</td>
<td>33.1</td>
<td>.246</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0519</td>
<td>38.5</td>
<td>.691</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0821</td>
<td>41.1</td>
<td>.082</td>
<td>25</td>
</tr>
<tr>
<td>b. 175</td>
<td>40</td>
<td>6</td>
<td>.0259</td>
<td>33.3</td>
<td>.132</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.1010</td>
<td>49.0</td>
<td>.185</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 13. Photocell resistance ratio vs. relative oxygenation; effects of alterations in plasma osmotic pressure

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>40</td>
<td>1</td>
<td>.0255</td>
<td>.678</td>
<td>.0169</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0241</td>
<td>.611</td>
<td>.0450</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0240</td>
<td>.443</td>
<td>.0396</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Table 14. IR photocell resistance vs. relative oxygenation; effects of alterations in plasma osmotic pressure

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept (KΩ)</th>
<th>Std. Dev.</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>40</td>
<td>1</td>
<td>.0386</td>
<td>26.1****</td>
<td>.160</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0492</td>
<td>28.8---</td>
<td>.209</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0590</td>
<td>32.2****</td>
<td>.176</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 15. Photocell resistance ratio vs. relative oxygenation; effects of changes in pH

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>40</td>
<td>1</td>
<td>.0299</td>
<td>.509</td>
<td>.0466</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0241</td>
<td>.611</td>
<td>.0450</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0262</td>
<td>.574</td>
<td>.0462</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Table 16. IR photocell resistance vs. relative oxygenation; effects of changes in pH

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept (KΩ)</th>
<th>Std. Dev.</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>40</td>
<td>1</td>
<td>.0531</td>
<td>31.8****</td>
<td>.1090</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0492</td>
<td>28.8---</td>
<td>.2090</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0486</td>
<td>29.8****</td>
<td>.0699</td>
<td>7.1</td>
</tr>
</tbody>
</table>
values of pH the 805 nm. photocell resistance intercepts (Table 16) were different, but were not related to the pH value. For high values of \( P_{CO_2} \), the 805 nm. photocell resistance intercepts (Table 18) were high, indicating decreased reflectance. It appeared that the reflectances were not affected by simple changes in pH, but were decreased equally at 660 and 802 nm. by increases in \( P_{CO_2} \).

Table 17. Photocell resistance ratio vs. relative oxygenation; effects of changes in \( P_{CO_2} \)

<table>
<thead>
<tr>
<th>( R_p ) (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>( P_{CO_2} ) (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>40</td>
<td>6</td>
<td>.0251*</td>
<td>.610</td>
<td>.0221</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0267*</td>
<td>.573</td>
<td>.0385</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0361--</td>
<td>.304--</td>
<td>.0991</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0349</td>
<td>.430</td>
<td>.0914</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0347</td>
<td>.424</td>
<td>.1310</td>
<td>80</td>
</tr>
<tr>
<td>b. 120</td>
<td>10</td>
<td>1</td>
<td>.0204*</td>
<td>.865</td>
<td>.0150</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0267--</td>
<td>.744--</td>
<td>.0920</td>
<td>40</td>
</tr>
<tr>
<td>c. 120</td>
<td>30</td>
<td>1</td>
<td>.0216*</td>
<td>.726</td>
<td>.0492</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0444--</td>
<td>.564--</td>
<td>.3080</td>
<td>40</td>
</tr>
</tbody>
</table>

Differences Between Measurements In Vitro at 25°C and In Vivo at 35°C

In the in vivo experiments it was not possible to control the values of PCV, PPC, pH, and \( P_{CO_2} \), but all of these variables remained within ranges which the previous experiments had shown to cause no systematic shifts in calibration curves. The comparisons of in vivo data with those from an in vitro experiment with \( P_{CO_2} = 40 \) mm. Hg (Tables 19 and 20)
Table 18. IR photocell resistance vs. relative oxygenation; effects of changes in $P_{CO_2}$

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept (KΩ)</th>
<th>Std. Dev.</th>
<th>$P_{CO_2}$ (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 120</td>
<td>40</td>
<td>6</td>
<td>.1080****</td>
<td>33.1****</td>
<td>.246</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0040**</td>
<td>41.9****</td>
<td>.144</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0336---</td>
<td>39.6---</td>
<td>.232</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0408</td>
<td>39.1****</td>
<td>.124</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0306</td>
<td>40.7****</td>
<td>.391</td>
<td>80</td>
</tr>
<tr>
<td>b. 120</td>
<td>10</td>
<td>1</td>
<td>.0607**</td>
<td>47.3****</td>
<td>.617</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0025---</td>
<td>59.7---</td>
<td>.478</td>
<td>40</td>
</tr>
<tr>
<td>c. 120</td>
<td>30</td>
<td>1</td>
<td>.1020**</td>
<td>27.6****</td>
<td>.082</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.0682---</td>
<td>32.4---</td>
<td>.245</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 19. Photocell resistance ratio vs. relative oxygenation; differences between measurements in vitro at 25°C and in vivo at 35°C

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Std. Dev.</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>40</td>
<td>6</td>
<td>.0361---</td>
<td>.304---</td>
<td>.0991</td>
<td>25</td>
</tr>
<tr>
<td>43</td>
<td>5.5</td>
<td></td>
<td>.0289</td>
<td>.373*</td>
<td>.1320</td>
<td>35</td>
</tr>
<tr>
<td>38</td>
<td>6.4</td>
<td></td>
<td>.0241</td>
<td>.664</td>
<td>.0279</td>
<td>35</td>
</tr>
<tr>
<td>44</td>
<td>5.5</td>
<td></td>
<td>.0282</td>
<td>.496</td>
<td>.0777</td>
<td>35</td>
</tr>
</tbody>
</table>

showed no major differences between the calibration curves. The 805 nm. photocell resistance intercepts were somewhat higher in vivo than in vitro. This discrepancy was probably due to the difference in blood temperatures, since the direction of the shift was not consistent with previous data relating PCV, PPC, and $P_{CO_2}$ to the level of 805 nm. reflectance.
Table 20. IR photocell resistance vs. relative oxygenation; differences between measurements *in vitro* at 25°C and *in vivo* at 35°C

<table>
<thead>
<tr>
<th>$R_p$ (KΩ)</th>
<th>PCV (%)</th>
<th>PPC (gm.%</th>
<th>Slope</th>
<th>Intercept (KΩ)</th>
<th>Std. Dev.</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>40</td>
<td>6</td>
<td>.0336---</td>
<td>39.6---</td>
<td>.232</td>
<td>25</td>
</tr>
<tr>
<td>43</td>
<td>5.5</td>
<td>.0269</td>
<td>46.5****</td>
<td>1.692</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>6.4</td>
<td>.0945</td>
<td>47.8****</td>
<td>.899</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>5.5</td>
<td>.1022*</td>
<td>40.5****</td>
<td>.731</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

In general, it appeared that the calibration curves for a given set of conditions were highly reproducible so that it was valid to ascribe deviations from them to changes which were made in the hematologic variables. The reflectances at the two wavelengths showed more variability, but this could probably be blamed on the failure to exactly replicate total hemoglobin concentration, plasma protein concentration, and degree of hemolysis in each series of experiments.

Certain of the variables studied appeared to exert truly nonspecific effects on the reflectances. Decreases in PCV from 50 to 40%, low $P_{CO_2}$, low PPC, and low temperature seemed to increase both reflectances equally so that the calibration curves were unchanged. Changes in pH had no effect on either of the reflectances. These results agreed with previously published data, but cover wider ranges of relative oxygenation and include information on accuracy of the oximeter which was not previously available.

While severe hemolysis consistently reduced reflectances at both wavelengths, there is no consistent pattern to the effect on the calibration curves. It seemed probable that the effect of hemolysis was more complex than could be analyzed with this experimental design. It was also possible that the manner in which hemolysis was induced significantly affected the resulting change in optical properties, since the size and shape of the erythrocyte fragments would be expected to depend on the manner in which the cells are disrupted.

The effect of decreasing PCV below 30% was to decrease the reflectance at 660 nm. more than that at 805 nm., resulting in a systematic
underestimation of relative oxygenation. The magnitude of the error was about 3% at 20% PCV and from 6 to 12% at 10% PCV. These results agreed both quantitatively and qualitatively with data of Enson et al. (21) for 100% relative oxygenation. The new data showed that the error was approximately constant in the range of 50 to 100% relative oxygenation.

Repeated experiments at different degrees of photocell sensitivity balance showed that it was possible to make the calibration curves coincide for 10, 20, 30, and 40% PCV by making the 805 nm. photocell 7% more sensitive than the 660 nm. photocell (i.e., $R_p = 175 \text{ K }\Omega$). This would provide a means of automatically correcting for the error caused by low PCV in applications where extreme dilution of blood was unavoidable. However, the imbalance in photocell sensitivities introduced significant shifts in the calibration curves when PCV exceeded 40% and when PPC changed; similar shifts could be anticipated with changes in $P_{CO_2}$ and temperature. Hence, unless these variables could be held constant nothing would be gained in trying to improve the accuracy of the oximeter by this method.

The increase in reflectivity for infrared light caused by increased plasma osmotic pressure was very striking. The curves relating 660 nm. photocell resistance to relative oxygenation in the three sets of data used in this study were virtually identical, while there were differences of 3 to 4 KΩ between the elevations of the curves relating 805 nm. photocell resistance to relative oxygenation. These results indicated that the accuracy of reflection oximeters was highly dependent on the osmotic pressure of the solution in which the erythrocytes were suspended. The mechanism responsible for this was not readily apparent; possibly the relative magnitudes of the diffuse and specular components of reflection at
the poorly-absorbed wavelength (805 nm.) were altered by changes in the size and shape of the erythrocytes. Changes in plasma osmotic pressure had not been previously reported as a source of error in reflection oximetry.

Probably the most important source of error identified in this study was the "atypical blood" phenomenon. Blood obtained from six of the 23 donors at the first donation had higher reflectivity for red light (660 nm.) than the blood of the other dogs. This caused a systematic overestimation of oxygenation by the oximeter varying in magnitude up to 10%. Errors such as these could obviously cause severe problems in artificial heart control systems in which relative oxygenation was estimated by reflection oximetry and the estimate used as a parameter for control of the output of the device, especially if there was no way to identify atypical blood except by running individual calibration curves.

In attempting to determine the reason for the abnormally high reflectance for red light given by the atypical bloods an important clue seemed to be that the phenomenon was not observed when the same dogs were bled the second time 56 to 85 days later. This indicated that the problem was not due to an abnormal type of hemoglobin continuously present in only a few dogs. However, it did not rule out the presence of some other abnormal pigment such as methemoglobin or carbon monoxide hemoglobin. Although no specific tests for detection of these pigments were done, there was no circumstantial evidence of their presence.

Bypassing the question of the presence of an abnormal pigment, it might have been supposed that some hematologic variable unrelated to the erythrocytes was abnormal in the atypical bloods. However, the only variables investigated in these studies which could have been expected to
produce errors of the magnitude observed were PCV's below 30%, hemolysis, and osmotic pressure. Low PCV and hemolysis were discounted since the values for atypical bloods were in the same ranges as for typical specimens. The effect of variations in osmotic pressure seemed to be unrelated to the problem since these affected only the reflectance at 805 nm., while the significant abnormality in the atypical bloods was the high reflectivity at 660 nm.

Interestingly enough, exactly the same aberration was observed with blood drawn from any of the donors 93 to 152 days after a previous bleeding. That is, there was a progressive decrease in the intercepts of the calibration curves due exclusively to increased reflectance for 660 nm. light. This period corresponded exactly to the time during which erythrocytes produced in response to the original hemorrhage were being destroyed and replaced. That the magnitude of the deviation from typical reflectances increased throughout this period indicated that the young replacement cells rather than the old erythrocytes were responsible for the atypical optical properties of the blood. Moreover, other physical properties of the erythrocytes exhibited progressive changes during this period: the mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular hemoglobin (MCH) all rose progressively during the same period of time, reaching maximum values at 133 to 147 days after bleeding. Whether the apparent increases in size and hemoglobin concentration of the erythrocytes were directly responsible for the atypically high reflectance for red light could not be determined.

Since the atypical bloods and blood obtained from repeat donors during the secondary erythropoietic crisis both exhibited unusually high
reflectances for red light, it was natural to investigate whether they had other similarities. Comparison of the mean values of Wintrobe indices for five of the atypical bloods with the means of values for 17 typical specimens showed no statistically significant differences. Inspection of stained smears of atypical bloods revealed a high degree of anisocytosis and poikilocytosis which was similar to what was seen during secondary erythropoietic crises. Since these observations could not be objectively quantified, however, no valid statistical comparisons could be made. It appeared, however, that the atypical bloods contained an abnormally high percentage of macrocytic spherocytes, microcytic spherocytes, and poikilocytes.

On the basis of these observations, it was concluded that a possible common characteristic of the two phenomena was the presence of an unusually high percentage of young erythrocytes and that for unexplained reasons these imparted to the blood an atypically high reflectance for red light.

A possible explanation for the presence of a high proportion of young erythrocytes in the dogs which yielded atypical bloods was that they had undergone some erythropoietic stress just prior to being obtained for experimental use and were in the process of recovery when first bled. While the histories of the donor dogs were unknown, two bits of circumstantial evidence suggested that this was not the proper explanation. First, the period for which the dogs which yielded atypical blood were held prior to the first bleeding varied from two to 15 weeks, so that it was unlikely that all of them could have been in recovery from an undetected erythropoietic stress. Second, the stained smears of atypical bloods contained a high proportion of microcytic spherocytes and poikilocytes.
which were interpreted to be aged erythrocytes, and their presence in large numbers would not be expected during the primary recovery from hemorrhage.

Other circumstances suggested another possible explanation for an abnormal age distribution of erythrocytes in the atypical bloods. The dogs which yielded atypical bloods at the first donation had all become very excited while being prepared for anesthesia prior to bleeding. Extreme excitement in dogs had been shown to cause contraction of the spleen and ejection of a concentrated mass of erythrocytes into the vascular system (43,66); circulating hemoglobin levels were raised 10 to 12% by this means. Moreover, the erythrocyte population of the spleen in dogs had been shown to contain up to 10% reticulocytes (i.e., young erythrocytes) (3) and an abundance of poikilocytes (66). There was evidence that the spleen could selectively sequester abnormal erythrocytes of several types (14,32,69,75). However, no published evidence was available to support the hypothesis that abnormal cells sequestered by the spleen could be ejected into the vascular system in times of excitement. Thus, there is still no proven explanation for the apparent presence of abnormal erythrocytes in the atypical bloods.
SUMMARY

Blood from 23 canine donors was used to study the effects of changes in hematologic variables on the accuracy of reflection oximetry. Changes in plasma protein concentration, $P_{CO_2}$, pH, and temperature had no net effect, but changes in reflectance levels were detected. Progressively greater underestimation of relative oxygenation occurred as PCV fell below 30%. High levels of hemolysis produced significant but unpredictable changes in the calibration curves. The oximeter was highly sensitive to changes in plasma osmotic pressure since reflectance at 805 nm. but not at 660 nm. was directly related to the osmotic pressure. Progressively increasing overestimation of relative oxygenation occurred during secondary erythropoietic crises due to increasing reflectance at 660 nm. A similar phenomenon was detected in several donors which had not previously been bled.

It was anticipated that the results of these studies would be the basis for development of computer-linked oximeters which would automatically correct for errors caused by changes in hematologic variables. This might be accomplished by measuring the critical variables simultaneously with the reflectances at the two wavelengths and integrating the resulting data with on-line computer processing to produce a corrected oximeter output.


61. RCA phototubes and photocells. Lancaster, Pa., Radio Corp. of America. c1963.


I would like to express my appreciation to my major professors, Drs. Neal R. Cholvin and Melvin J. Swenson, and to my entire committee for their assistance and encouragement during the course of this study. Special thanks are extended to Mrs. Joyce Feavel for her many months of technical assistance, and to Dr. Robert Carithers for his advice and assistance with the surgical procedures and procurement of dogs. Thanks are also extended to Mrs. Beverly Allfree for typing this manuscript.

This work was initiated with the financial support of a training grant (GM-1043) in physiology from the National Institute of General Medical Sciences, continued during the tenure of a Research Fellowship of the Iowa Heart Association (68-F-2), supported in part by the General Research Support Grant FR-05565, and completed with the support of a Postdoctoral Fellowship (F02-HE-42, 164) from the National Heart Institute.