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The In Vitro Determination of Human Blood Oxygen Saturation in the Presence of High Concentrations of Carbon Dioxide

Elizabeth D. Armstrong

THE IN VITRO DETERMINATION OF HUMAN BLOOD OXYGEN SATURATION IN THE PRESENCE OF HIGH CONCENTRATIONS OF CARBON DIOXIDE

By

Elizabeth D. Armstrong

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Thesis committee

Douglas G. Hamm

CHAIRMAN

Wm. D. Slack

C. Clayton Hoff

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CHAPTER I. HISTORICAL REVIEW

Mayow's work in 1673 is cited by Landois (1905) as the first demonstration of the liberation of gases from blood in a vacuum. In 1863 Meyer, Ludwig, and Pfleuger established that the oxygen leaving the lung was transported in a combined form with hemoglobin and that the oxygen was released into the tissues accompanied by a change in color of the blood.

Paul Bert (1878) found that the physiological action of oxygen was a function of its partial pressure and stated that the formation of oxyhemoglobin was related to the partial pressure of oxygen. Haldane in 1897 (cited by Haldane 1932) while studying oxyhemoglobin noticed that the same reagents which produced methemoglobin also liberated bubbles of gas from blood. Haldane identified the gas as oxygen. This discovery was quickly applied to a method use of the mercurial pump and simplified further studies in the field.

One of the first graphs of an oxygen dissociation curve was drawn by Hüfner (cited by Stewart 1900) from calculated data assuming the following equation for the reaction involved.

$$Hb + O_2 \rightleftharpoons HbO_2$$

The curve obtained was an hyperbola and a reproduction is shown in figure 1. Investigation of the oxygen dissociation curve by later workers apparently confirmed Hüfner's

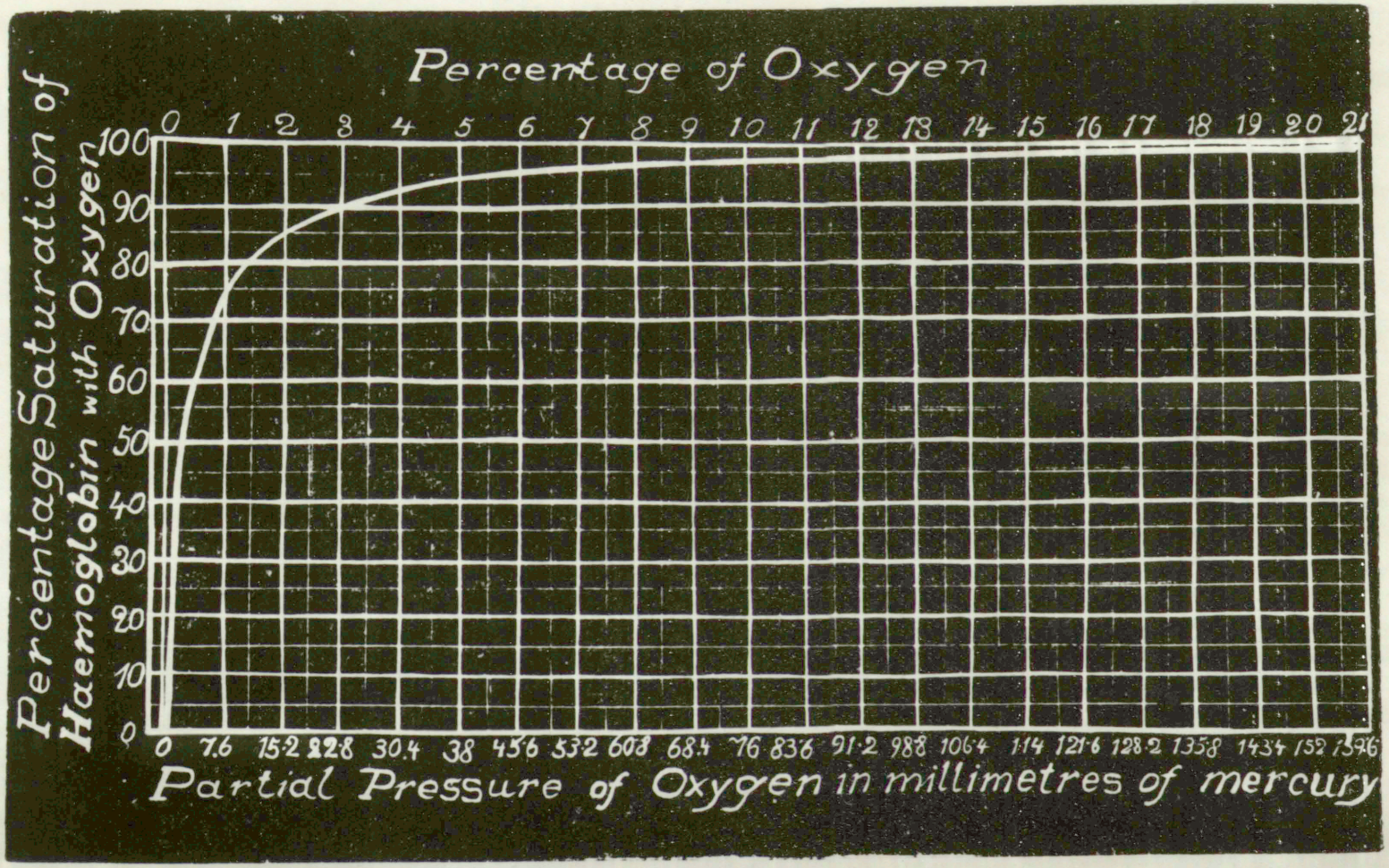


FIG. 84.—CURVE OF DISSOCIATION OF OXYHÆMOGLOBIN AT 35° C. (AFTER HÜFNER'S RESULTS.)

calculations. However, modern methods have shown the curve to be S-shaped for whole non-hemolyzed blood and hyperbolic for hemoglobin in solution.

Paul Bert (1878) and Hüfner (cited by Stewart 1900) first showed that the release of oxygen from the blood occurred at a higher rate when the temperature was raised. This effect was confirmed by Barcroft and King (1909) who stated further that the rate of dissociation at 41° centigrade is 1.7 times the rate of oxygen release at 36°. The results of Barcroft and Hill (1910; cited by Haldane 1935) suggested a loss of heat when the oxygen united with hemoglobin. They determined experimentally that to keep a solution of hemoglobin 64% saturated, the oxygen pressure must be increased from 12.5 mm. to 31 mm. Hg for a rise in temperature from 26° to 38°C. This represents a change in partial pressure oxygen at 64% saturation of 1.5 mm. Hg of oxygen tension per degree centigrade. Brown and Hill (1922; cited by Haldane 1935) showed in a series of experiments that a fall in temperature did not alter the shape of the curve, but instead shifted the entire curve to the left along the abscissa as shown by figure 2. It is significant to note that Haldane (1935) shows a change of 1.2 mm. Hg partial pressure of oxygen per degree centigrade at a blood oxygen saturation of 50% with a change in temperature from 38° to 37°C.

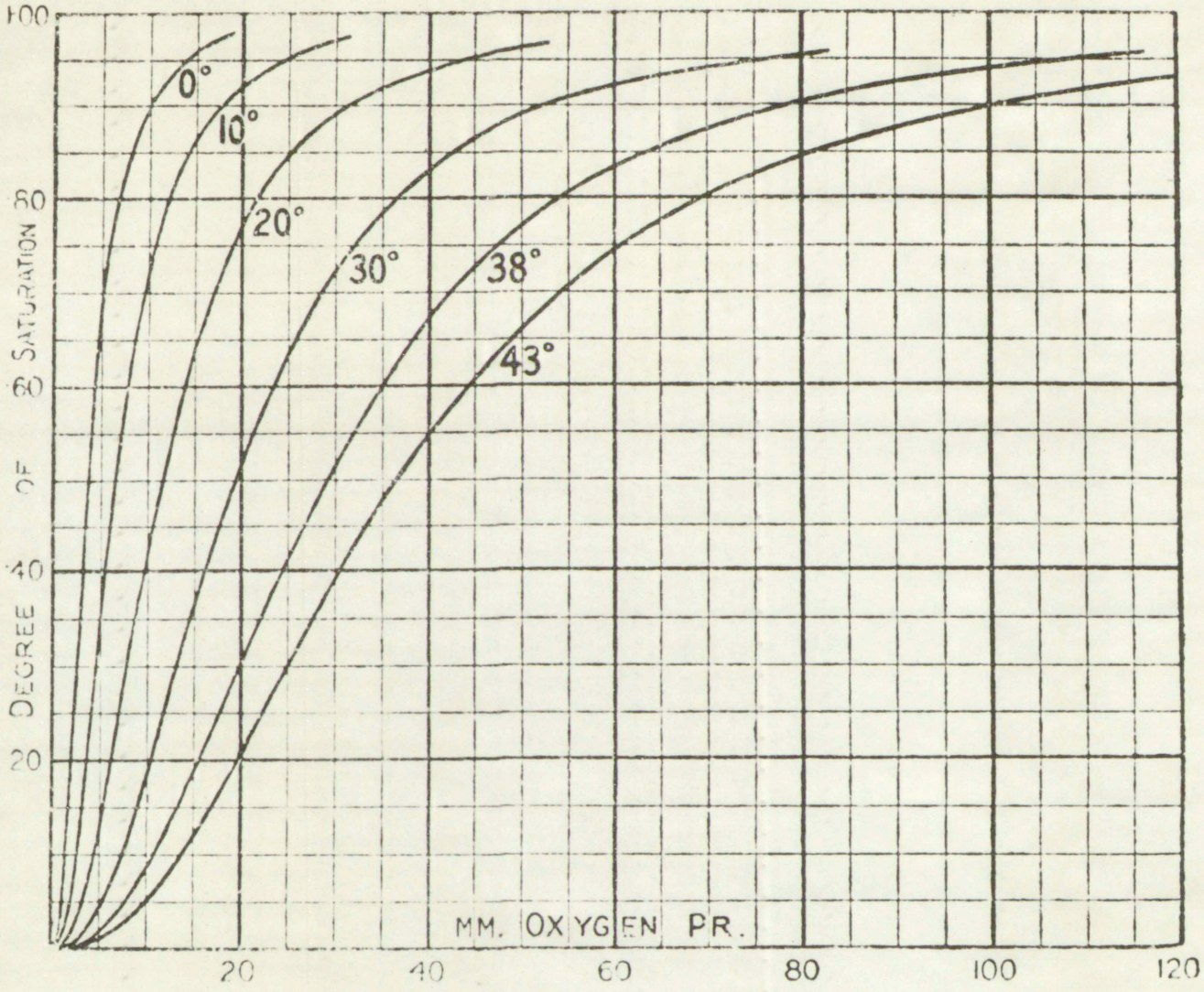

100
80
60
40
20
% SATURATION OF
DEGREE
0°
10°
20°
30°
38°
43°
MM. OXYGEN PR.
20
40
60
80
100
120

Fig. 2

In 1904 Bohn, Hasselbalch and Krogh (cited by Henderson 1928) noted the effect of carbon dioxide as an important factor in determining an oxyhemoglobing dissociation curve and showed that the partial pressure of carbon dioxide must also be considered. In normal body metabolism the carbon dioxide gathered by the blood flowing through tissues aids in the dissociation of oxyhemoglobin and shifts the entire curve to the right. This is known as the Bohr effect and will be considered in more detail later.

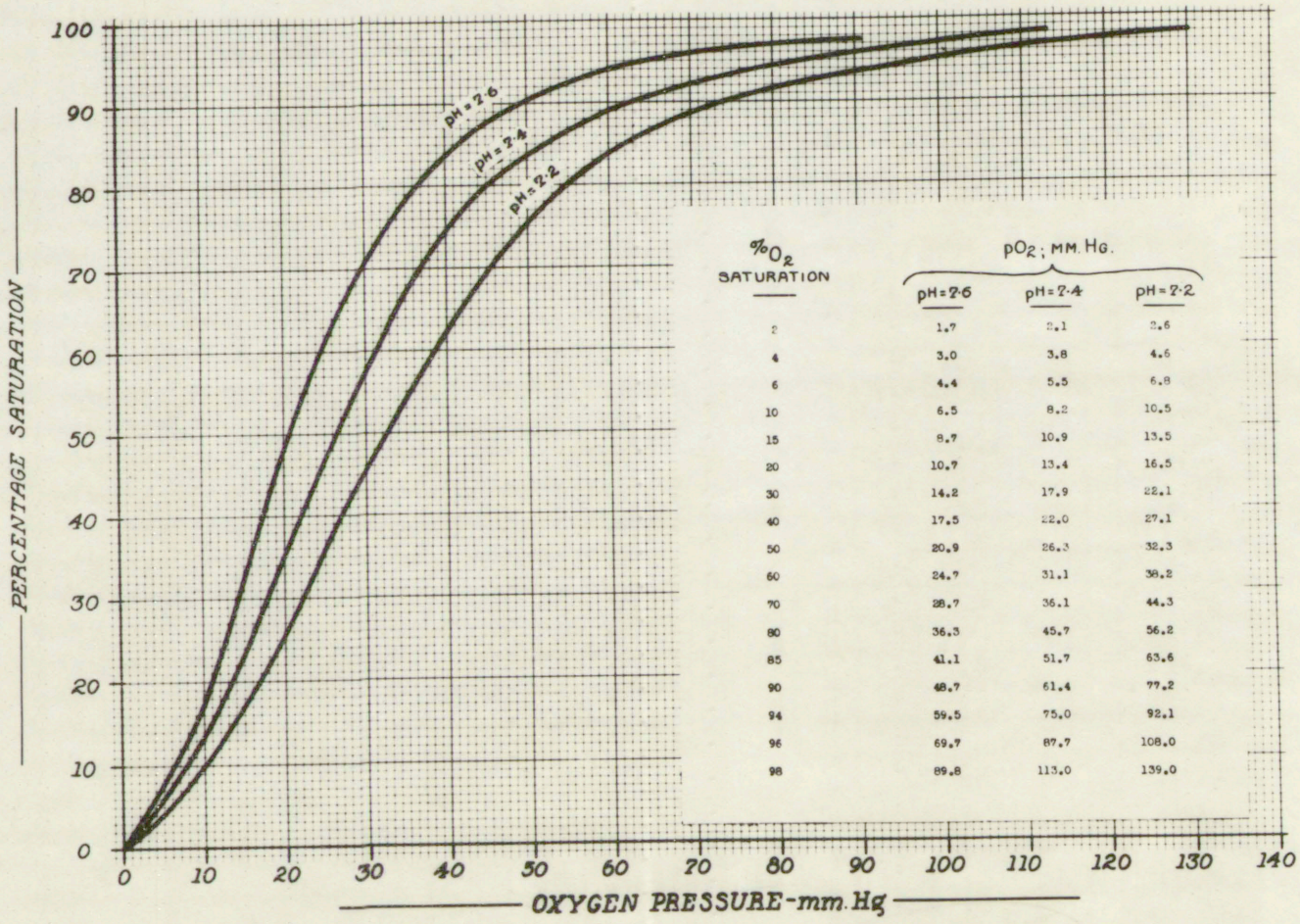
Work by Barcroft and Camis (1909) showed that the oxyhemoglobin dissociation curve is also influenced by the concentration of salts present. In their experiments however, the pH effect was neglected. In an acid medium the affinity of hemoglobin for oxygen is reduced and the results obtained by Barcroft and Camis must be considered in the light of a change in pH as well as a variation in concentration of salts.

These factors, therefore, must also be considered in constructing an oxyhemoglobin dissociation curve in addition to the partial pressure of oxygen. The lack of appreciation of the influence of all these factors explains the lack of agreement found in the form of oxyhemoglobin dissociation curves as published by early writers.

The oxygen dissociation curves shown in figure 3 were compiled by Dill (1944) from the data of Bock and Adair (1924),

OXYGEN DISSOCIATION CURVES FOR HUMAN BLOOD

Dill (1944) Handbook of Respiratory Data in Aviation



$\%O_2$ SATURATION	pO_2; MM. Hg.		
	pH=7.6	pH=7.4	pH=7.2
2	1.7	2.1	2.6
4	3.0	3.8	4.6
6	4.4	5.5	6.8
10	6.5	8.2	10.5
15	8.7	10.9	13.5
20	10.7	13.4	16.5
30	14.2	17.9	22.1
40	17.5	22.0	27.1
50	20.9	26.3	32.3
60	24.7	31.1	38.2
70	28.7	36.1	44.3
80	36.3	45.7	56.2
85	41.1	51.7	63.6
90	48.7	61.4	77.2
94	59.5	75.0	92.1
96	69.7	87.7	108.0
98	89.8	113.0	139.0

Fig. 3

Henderson (1928) and Dill (1932). These curves consider the factors mentioned above and are drawn at three different pH values. These curves have been accepted as the most accurate expression of the effect of pH on the oxyhemoglobin dissociation curve. Recent work by Lilienthal (1944) serves to show that any dissociation curve for human blood now available must be regarded as accurate only within limits defined by several technical factors to be discussed later.

Bohr (1904; cited by Henderson 1928) as stated previously first noted that carbon dioxide might cause a deviation from the standard oxygen dissociation curve and explained the variation in the percentage saturation of hemoglobin in two bloods exposed to atmospheres of the same oxygen tension on the basis of different carbon dioxide tension. Barcroft and Camis (1909) verified Bohr's work and concluded that carbonic acid tended to liberate the oxygen from the hemoglobin. They suggested further that work on blood should be done at a carbon dioxide tension equal to that found in the body.

Christiansen, Douglas and Haldane (1914) suggested that the greater capacity of reduced blood to combine with carbon dioxide might be due to the fact that reduced hemoglobin is less acid than oxyhemoglobin.

Investigation in this field continued and according to Roughton (1935) by 1928 it was generally agreed that

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dissolved carbon dioxide, carbonic acid (H_2CO_3) and bicarbonate ions (HCO_3^-) constituted the three forms in which carbon dioxide was carried in the blood. The evidence for this and the description of the changes between these substances during respiration were presented by Van Slyke (1922) and Henderson (1928) and summarized by Roughton (1935).

When carbon dioxide is dissolved in water the following reactions occur:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad (2)$$

In blood the buffers remove the hydrogen ions and the resultant displacement of the equilibrium in equation 2 to the right was believed to result in 95% of the carbon dioxide being taken up in the form of the bicarbonate ion. The main buffer in the red blood cells is the alkaline salt of hemoglobin, potassium hemoglobinate, often expressed as KHb. The ionization of this salt releases hemoglobin ions which combine with the hydrogen ions.

The ability of the alkaline hemoglobinate to take up the hydrogen ions is increased by the loss of oxygen from the red cells at the same time as the carbon dioxide enters. Reduced hemoglobin is known to be a weaker acid than oxyhemoglobin and readily takes up the extra hydrogen ions from the solution. Van Slyke (1922) explained this by the concept of a particular hydrogen ion which he called the "oxy-labile hydrogen ion" that dissociates from, or combines with a

"spot" near the heme nucleus in the hemoglobin molecule. The attachment of oxygen to the heme nucleus thus increases the degree of dissociation of this ion and the removal of oxygen increases the ease of binding hydrogen ions. Therefore, reduction of the hemoglobin is a significant factor in aiding the blood to bind more carbon dioxide as bicarbonate.

During the uptake of carbon dioxide by the blood, there is an increase in the concentration of the bicarbonate in the corpuscle, relative to the concentration in the plasma. The Donnan equilibrium between red cells and plasma is disturbed and some of the bicarbonate ions from inside the red cells exchange for chloride ions from the plasma until the equilibrium is once more restored. This chloride shift is sometimes called "secondary buffering". During the release of carbon dioxide from the blood in the lungs the processes are reversed. Roughton (1935) suggested two considerations which had not been referred to in earlier schemes. These were the speed at which gaseous carbon dioxide is formed from the bicarbonate ions at the pH and temperature existing in the capillaries of the lung and the possibility of a form of bound carbon dioxide other than carbonic or free bicarbonate ions.

These points were not original with Roughton, but were a culmination of several other investigations, in

particular Henriques (1928) who suggested that the blood in the lung capillaries comes into equilibrium with the carbon dioxide of the alveolar air, and if the average time spent by the blood in the lung capillaries is one second, then the blood must contain a catalyst which accelerates the reaction,

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

under physiological conditions at least 150 times. Hawkins and Van Slyke (1930) confirmed Henriques' observations that the rate of carbon dioxide evolution was greatly accelerated in blood as well as in hemoglobin solutions and these workers attributed the catalytic power to hemoglobin itself.

Brinkman and his coworkers (1932) next showed that, if the hemoglobin was split, no activity was found in the heme fraction while the globin showed almost as much activity as the original hemoglobin.

Meldrum and Roughton (1932) isolated the catalyst from blood and gave it the name of carbonic anhydrase. No trace of the enzyme could be found in oxalated plasma; all seemed to be in the red cells. The importance of this enzyme is indicated by the calculations showing that in its absence only one one-hundredth of the carbon dioxide given off from bicarbonate could escape during the period in which the blood is in the lung capillaries.

Since 1928 several studies have been made to determine the state of carbon dioxide in the blood. Investigations

by Henriques (1928) on the rate of evolution of carbon dioxide from hemoglobin solution were confirmed by Meldrum and Roughton (1933). These workers also observed the rate of uptake of carbon dioxide by blood initially free of carbon dioxide. The uptake was found to occur in two phases, the first rapid and the second at a slow rate, comparable to the uptake of carbon dioxide by buffer solutions containing no carbonic anhydrase.

The carbon dioxide which was taken up in the rapid phase was more than could be accounted for by physical solution; therefore, the excess must have been due to the rapid formation of some carbon dioxide compound and not carbonic acid or bicarbonate. Further experiments suggested that this non-bicarbonate form was bound to hemoglobin since oxygenation affected the rate of combination of the carbon dioxide. This fraction was called carbamino compound.

The summary, the carbon dioxide of the blood is carried in physical solution and as bound carbon dioxide. The bound carbon dioxide exists as unstable carbonic acid which forms bicarbonates in the blood and dissociates to form the bicarbonate ion. The remainder was first designated by Roughton (1935) as the x-bound carbon dioxide, which includes a carbamino compound formed with hemoglobin, and the hypothetically-bound carbon dioxide believed to be the bicarbonate salts of proteins.

Many different views have been suggested as to the inter-relationships between oxygen and carbon dioxide bound by hemoglobin. The hemoglobin molecule contains many hydrogen ions which dissociate at various pH levels. Henderson (1920) and Van Slyke (1922) first suggested the "oxy-labile hydrogen ion" mentioned previously. It was formerly thought that the extra carbon dioxide taken up by reduced hemoglobin solution when in equilibrium with a given pressure of carbon dioxide was entirely due to differences in acidic strength of oxy-labile ionising groups. Ferguson and Routhton (1934), however, suggested that carbon dioxide and hydrogen ions compete for an oxy-labile amine group located near each heme nucleus. These workers also suggested that the affinity of this amino group for both hydrogen ions and carbon dioxide was decreased by oxygenation of the hemoglobin molecule. On the other hand, the attachment of either carbon dioxide or hydrogen ions to the oxy-labile amine groups decreases the affinity of hemoglobin for oxygen.

A new idea as to the type of link between hemoglobin and oxygen was given by Haurowitz (1948) at the Barcroft symposium; namely, that hemoglobin is an aquo-compound with the aqueous moiety being active in binding oxygen. The following equation would illustrate the relationship:

$$Hb\,(H_2O) + O_2 \rightleftharpoons HbO_2 + H_2O$$

This type of structure gives another possible explanation for the effects of carbon dioxide, salts, and hydrogen-ion concentration on the oxyhemoglobin dissociation curve. According to this idea, these factors modify the affinity of hemoglobin for oxygen through changes in the aqueous portion of the molecule.

In most of the studies mentioned above, the blood was exposed to a vacuum and the gases were extracted and measured. But in recent years the use of spectophotometry in measuring oxygen content of blood has been utilized. This began with the work of Drabkin and Austin (1935) who suggested the use of whole, rather than diluted blood. The results of these experiments, done with dog's blood, showed Beer's law[1] applied for reduced and saturated hemoglobin and made possible the use of whole bl od in spectrophotometric methods.

The first description of an ear oximeter, according to Wood (1950) was given by Matthes (1935) who measured blood oxygen solution by photoelectric means. Necolai (1932)

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[1] Beer's law states that light of intensity I traversing a medium d layers thick suffers a logarithmic reduction in intensity so that the intensity I of the transmitted bean is given by the equation

$I' = I \frac{1}{n} d$ when N = a constant for a given molecule.

Kramer (1934) cited by Wood (1950) and Millikan (1942) developed similar instruments that depend on the spectral transmission characteristic of oxyhemoglobin and reduced hemoglobin. Millikan (1942) described a compact and convenient type of oximeter with an ear piece containing photocells which received filtered light transmitted through the pinna of the heat flushed ear. Goldie (1942) compensated for the change in reading due to variations in ear thickness by setting the galvanometer at 100 while light was being transmitted through the bloodless ear. None of these instruments were entirely satisfactory since the results were relative and the absolute values for blood oxygen saturation were unobtainable.

Wood and Geraci (1950) modified the ear oximeter. Their instrument was designed to measure the absolute value of arterial oxygen saturation and this eliminated one of the serious difficiencies of the earlier instruments. These workers also successfully developed the cuvette or oximeter for whole blood. This device allows in vivo or in vitro estimations to be carried out and is the photoelectric instrument used in the present work.

The cuvette, compared with the Van Slyke apparatus is a relatively simple and rapid means of determining blood oxygen saturations. However, data are not available to show that results produced by the cuvette are valid for blood with a carbon dioxide content much outside the physiological

range. The Van Slyke apparatus on the other hand, has been the classical method for determining blood oxygen saturations and has been used on blood which was equilibrated with carbon dioxide tensions up to and including 90 mm. Hg. Validation of the results obtained by the cuvette when used on blood with comparatively high carbon dioxide tensions will significantly extend the utility of the photoelectric method and is one of the objectives of this study.

A second objective of this research is to develop by in vitro technique a simplified method for studying the effects of carbon dioxide on the human oxyhemoglobin dissociation curve. The method should be applicable to in vivo use and valid under conditions in which blood will be in equilibrium with carbon dioxide partial pressures of near 200 mm. Hg. Such a method is needed currently in the fields of toxicology, pathophysiology, clinical medicine and psychiatry.

It is not usual in clinical medicine, for instance, to see a case of bulbar poliomyelitis in which atelectasis has occurred (Bennett 1950). In such a case, the cardiopulmonary function is embarrassed by low oxygen and high carbon dioxide in the blood. This occurs because the arterial blood is in reality a mixture of blood from the functioning lung and from the abnormal lung which is circulated but not aerated. In emphysema, blood carbon dioxide in

excess of 100 volumes percent have been found (Hickam 1951). Meduna (1950), a Chicago psychologist, reports the use of 35% carbon dioxide in oxygen in the treatment of over 200 cases of minor neuroses and carbon dioxide in near this concentration is being used currently as shock therapy in major psychotics. Also, carbon dioxide intoxication was found by the Civil Aeronautics Board to be the probable cause of a major airline accident in 1947. (O'Connell, 1949)

Problems of carbon dioxide tensions are therefore of great current significance in many fields of study. There is need for practical methods offering research talent a valid and efficient time saving approach to allow study of the effects of high carbon dioxide tensions on the cardio-respiratory systems.

CHAPTER II. METHODS

Human blood was used in this investigation and was obtained by venipuncture. The blood was then placed in a "saturator" and exposed to gas mixtures of known tensions of carbon dioxide, oxygen, and nitrogen. After exposure to the gas mixture the blood was run through a whole blood oximeter (cuvette) and the blood oxygen saturation quickly determined. Blood from the cuvette was collected under oil and used for pH and Van Slyke estimations. In the latter, the Van Slyke manometric apparatus was used to determine the oxygen capacity, oxygen content, the percentage oxygen saturation and the carbon dioxide content.

A 50 milliliter syringe coated with liquid heparin was used for drawing the blood. The blood run through the saturator was obtained from three apparently normal subjects, one male and two females. Each blood was exposed to the gas mixtures within one hour after venipuncture. If more than 5 minutes elapsed between drawing the blood and its exposure to the gas mixtures, the syringe containing blood was placed in an icebox at 10° to 15° C.

The gas mixtures were made by "cascading" gas from larger cylinders of pure oxygen, carbon dioxide, and nitrogen into smaller cylinders. The amounts of each gas were judged by noting pressure changes, the temperature being held

approximately constant. Pressures were metered with stock gauges. Since the total volume was a constant, the percentage of each gas was proportional to the pressure rise produced by the respective gas. The mixtures were then analyzed with the Scholander micro-gas analyzer. (Scholander 1947). The percentage composition of the four gas mixtures utilized are shown in Table I.

A Beckman pH meter was used to determine the pH of each sample. The glass electrode was an hypodermic type first filled with gas of the same concentration as that saturating the blood. The blood was then drawn into the electrode without exposure to air. The pH obtained at room temperature could be corrected to 38° C. according to the formula given by Rosenthal (1947).

$$\text{Blood pH } 38^{\circ} = pH_t - 0.0147\ (38-t)$$

Previous investigations have reported several technical factors which cause inconvenience and which are potential sources of error. One inconvenience is the length of time necessary to saturate blood with gas mixtures, especially with mixtures of low oxygen tension. Several methods of equilibrating blood with gas mixtures were tried. Blood was placed in a tonometer filled with nitrogen and then evacuated. Also, a stream of nitrogen was passed through the tonometer during this procedure. With this technique and with the temperature held at 37.5°C. equilibration

TABLE I

COMPOSITION OF THE EXPERIMENTAL GAS MIXTURES

Mixture Number	Percent Oxygen	Percent Carbon Dioxide	Percent Nitrogen
1	11.94	6.80	81.26
2	11.04	15.09	73.87
3	11.24	27.90	60.86
4	12.00	33.30	54.70

required as long as 2 hours.

Tonometer equilibration offers sources of error as noted by Roughton (1944). He reported a concentration of erythrocytes due to unequal drainage of red cells and plasma from the sides of the vessel. This difficulty is serious if whole blood oximetry is to be used, because blood flow must be constant and a concentration of cellular constituents produces an error due to difraction of light. In addition, transferring blood without exposure to air from a tonometer to a cuvette is difficult.

Equilibration of blood in a tonometer is valid only if there is a negligible change in the concentration of gases contained in the tonometer. With whole blood and relatively high carbon dioxide percentages, there will be a significant change because the whole blood has a comparatively high carbon dioxide combining power. This fact was pointed out by Peters in 1932.

With these considerations in mind, a more satisfactory means of equilibrating blood with gas mixtures was desirable. Accordingly, several models of a circulating saturator were designed and tried. These all embodied the principle of obtaining a large surface of exposure between the liquid and gas phases by using a large number of small bubbles obtained by forcing gas through bacteriological filters. A fritted glass filter gave rapid equilibration, but produced

considerable hemolysis probably because the gas bubbles emerging from the filter, under a pressure of 9 to 12 pounds per square inch, traumatized the membrane of the erythrocyte. The model which proved most satisfactory utilized a Mandler filter. This operated well at gas pressures from 0.5 to 2 pounds per square inch.

This apparatus was used in accord with many of the principles described by Clark (1950a,b) who published a description of similar devices used as oxygenators for the blood of living dogs breathing pure nitrogen.

The circulating saturator used in this investigation consisted of a blood and gas circulating system as shown in figure 4. The blood system contained two units made of Pyrex glass.

The first unit consisted of a saturation chamber, a degassing column, and a reservoir. The saturation chamber was cylindrical, 2.5 cm. in diameter. One end contained a Mandler filter (E. H. Sargent Company Catalog No. S-32 395, size E) mounted in a recessed, perforated rubber stopper which filled the bottom of the cylinder. Above the saturation chamber was a cylindrical degassing column 1 1/2 cm. in diameter. This chamber contained glass beads coated with resin (D. C. Anti-foam A; Dow Corning Company). The resin was necessary to control the release of excess gas from the blood without a great deal of foaming. The second unit of the saturator consisted of a short cylindrical degassing

column contained inside a larger tube. A reservoir was located between the inner and outer walls. The inlet tube was continuous with the center column. An outlet tube led from the reservoir.

The gas system of the saturator consisted of a gas containing cylinder, a pressure reducing valve, a humidifier surrounded by a heating jacket, rubber tubing, a Y-tube and a 3-way stop cock. The gas passed through the humidifier to the Y-tube. One stream of gas entered the Mandler filter while the other passed to the 3-way stop cock. One limb of the stop cock was open to the atmosphere; the other to a perforated rubber stopper pushed into the top of the second unit of the saturator. Proper use of this stop cock allowed washing of the pH electrode or the cuvette with the experimental gas mixture and made possible a pressure in the second unit to force blood back into the saturation chamber. After passing through the Mandler filter and blood, the gas escaped from the open end of the first unit of the saturator. Such an arrangement served to protect the blood from atmospheric air.

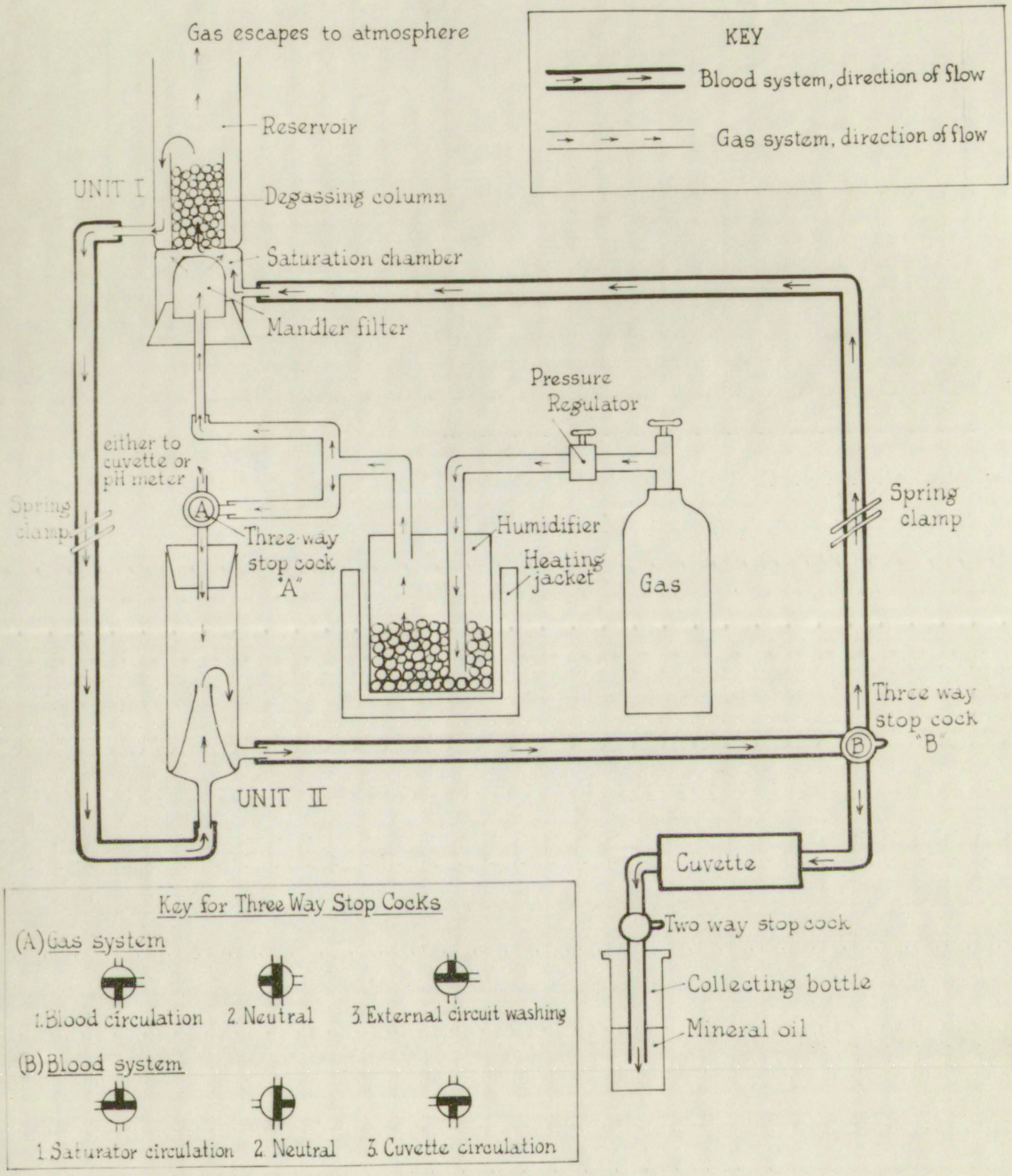
The first and second glass units of the saturator and a 3-way stop cock were connected with Polythene tubing in such a way that blood could be circulated. The route of circulation was from the saturation chamber through the outer portions of the first unit by gravity to the second unit,

through it and out to the 3-way stop cock. This stop cock allowed blood to be returned to the saturation chamber or to pass on to the cuvette. Two spring clamps were intermittently used on the tubing between the two units to avoid back flow of blood during the gravity and pressure phases of the circulation. The entire blood containing portion of the circulating saturator and the cuvette were coated with a silicone resin (Dri-Film No. 9987; General Electric Company). This layer made the glass parts easier to clean and helped to prevent clotting.

The whole blood oximeter used was described by Wood and Geraci (1947) and Wood (1950). The instrument noted in figure 4, consisted of a cuvette, two galvanometers, and a control panel. The cuvette consisted of two metal plates which slightly compressed a Polythene tube through which the blood flowed. Above the tube were 10 battery-operated 3V incandescent light bulbs. Below the tubes were located two filters and three selenium barrier-layer photo-electric cells. One photo-electric cell recorded light passed through the contents of the Polythene tube and a Wratten 29F filter (red range beginning at 600mμ. The other two photo-electric cells were covered with a double thickness, Wratten 88A filter and passed light in the near infra-red region (beginning at about 570mμ. The blood-filter combinations transmit light in two different spectral regions; namely, near

Diagram of a Circulating Blood Saturator

Fig. 4



infra-red of 750-900 mμ. and red light of 600-750 mμ. Transmitted light was registered simultaneously metering the output of the photo-electric cells using two galvanometers, one for each spectral region mentioned above.

Oxyhemoglobin and reduced hemoglobin both transmit approximately equal amounts of light in the near infra-red regions. Oxyhemoglobin, on the other hand, transmitted light in the red region quite readily but reduced hemoglobin did not. Thus light transmitted in the region from 750-900 mμ was a function of the total hemoglobin in the blood, while transmission in the range of 600-750 mμ was a function of the total amount of oxyhemoglobin. Therefore, the amount of oxygen saturation was a function of the ratio of the red to the near infra-red light transmission. To determine the relative blood oxygen saturation from the galvanometer readings, it was necessary to calculate the ratio of the relative transmissions of red and near infra-red light by the blood. The relative absorption of infra-red light by the blood was equal to the logarithm of the ratio of the respective galvanometer deflections obtained with saline and blood in the cuvette respectively:

$$(1) \qquad IR_B = \text{Log } IR_o/IR_n$$

The relative absorption by the blood of red light was:

$$(2) \qquad R_B = \text{Log } R_o/R_n$$

The ratio of R_B to IR_B was equal to the relative oxygen

saturation

$$(3) \qquad R_B/IR_B = \frac{\text{Log } \frac{Ro}{Rn}}{\text{Log } \frac{IRo}{IRn}}$$

The absolute oxygen saturation is determined empirically by calibration of the cuvette. This is done using the Van Slyke manometric apparatus and plotting the percentage oxygen saturation against the ratio, noted in equation (3) above, obtained by running the blood through the cuvette. Such a calibration curve is given in figure 5 which is based on the data given in Table II. The Van Slyke figures are averages of duplicate determinations. The ratio R_B/IR_B was determined using a double scale technique and employing a blood flow through the cuvette of 10 cc. per minute.

The blood samples showing low blood oxygen saturations were obtained using the human subject as a tonometer. An arm was immersed in ice water of approximately 10° C. for 5 to 10 minutes after which a tourniquet was applied around the mid-upper arm. The subject then rhythmically flexed and extended the fingers. Venipuncture was performed with the lower arm and hand still in the ice water. The exercise was continued while the blood was being drawn into a heparinized syringe.

Use of the cuvette eliminates many errors inherent in gasometric methods. Since whole blood is used, no dilution

factor need be considered. Possible errors which could accompany the introduction of gas absorbing reagents can be ignored and no correction need be made for physically dissolved oxygen.

Spectrophotometric methods must include consideration of certain principles of spectrophotometry such as monochromatic light, parallel cuvette walls, and a homogeneous absorbing medium. The oximeters now in use are constantly being modified in these and other respects such as the possibility of obtaining a higher sensitivity separating the red and infra-red spectral bands and eliminating the overlap or crossover band now being used. Elam and his co-workers (1949) suggest that the mounting of the photoelectric cells with red in the center and infra-red on either side could also induce an error. The use of a cuvette such as the Wood oximeter for whole blood described here has a further disadvantage in that each cell must be calibrated separately against analyses on the Van Slyke apparatus.

However, all these factors are mechanical and are being eliminated gradually by the many workers interested in the field. Results obtained using the cuvette are easily reproduced. This, plus ease of operation and the short time necessary to obtain the percentage saturation of oxygen in a sample of blood far outweigh the various disadvantages and suggest the use of the cuvette in clinical analyses where the

above considerations are so important.

CHAPTER III. RESULTS

1. Calibration of Cuvette.--The cuvette was calibrated by determinations of the log ratio $R/_{IR}$ on sixteen samples of blood. Duplicate analyses were run on the Van Slyke apparatus. The results of the calibration curve for the cuvette used are shown in figure 5 and table II. The shape of this curve is similar but more nearly linear than that reported by Wood (1950) on 235 blood samples. The part of the curve below 20% oxygen saturation is fixed by only four determinations in this study and by five in Wood's data.

2. Comparison of whole blood oximeter and Van Slyke manometric apparatus.--Data were available on 29 blood samples analyzed by both methods. Sixteen of these involved the determinations shown in figure 5. The oximeter results were read from the calibration curve. The results are shown in figure 6 and table III in which is given the percentage blood oxygen saturation as determined by the Van Slyke apparatus against the results obtained using the cuvette. Thirteen of the samples were exposed in a circulating saturator to carbon dioxide concentrations of 33, 27, 15 and 6.8%. The blood samples run through the saturator show a Van Slyke-oximeter relation similar to the results obtained with venous blood run directly through the oximeter or equilibrated in a tonometer except for the two points marked with an arrow in

CUVETTE CALIBRATION DATA

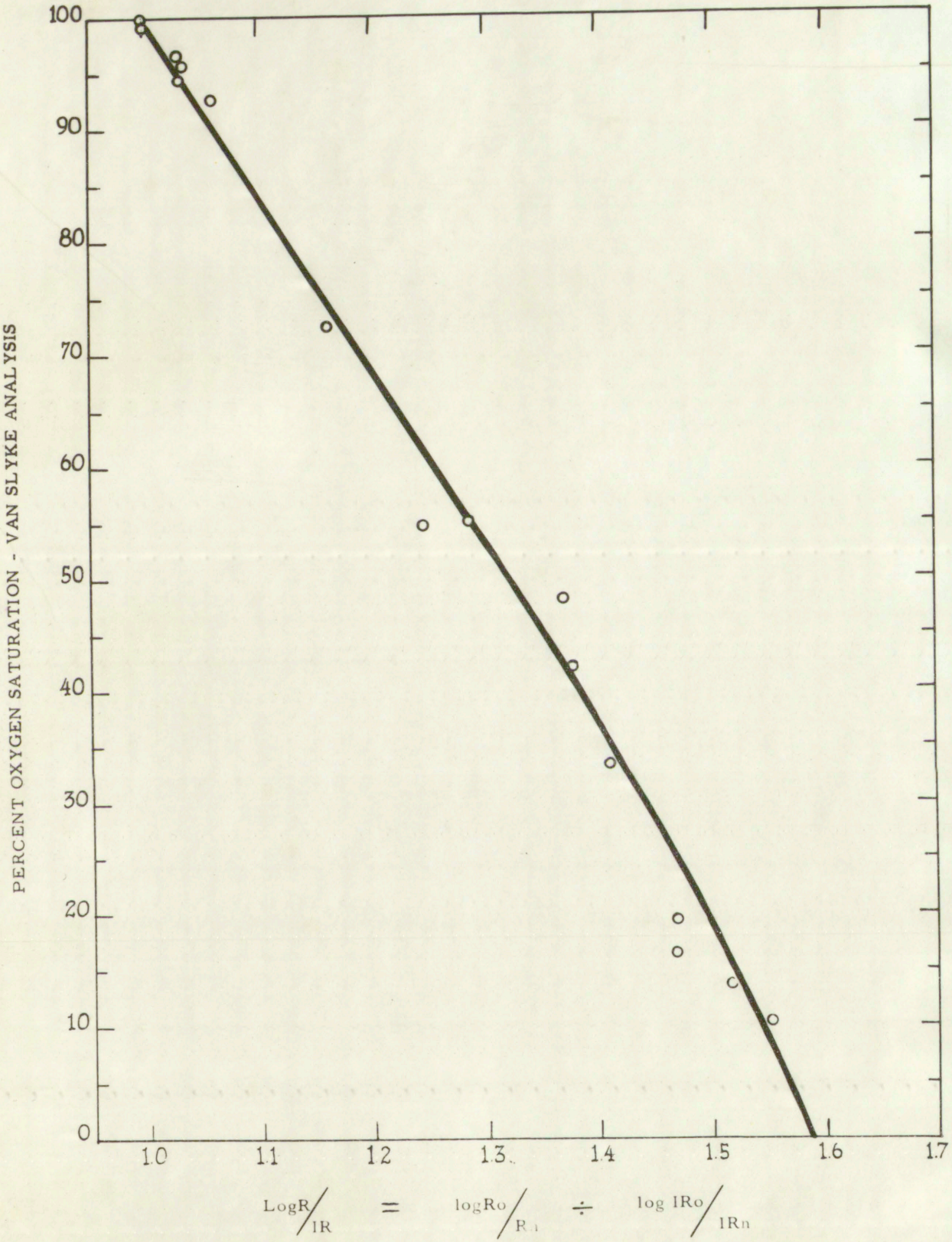


TABLE II

DATA FOR THE CUVETTE CALIBRATION CURVE

Sample Number	Blood Oxygen Saturation in Volumes Percent -- Duplicate Van Slyke Analyses	Ratio: R_B/IR_B obtained on the Cuvette
1	100.60	1.000
2	99.21	1.000
3	96.65	1.033
4	95.60	1.035
5	94.60	1.032
6	92.70	1.060
7	72.52	1.163
8	55.10	1.285
9	54.76	1.245
10	48.29	1.378
11	42.06	1.378
12.	33.28	1.413
13	19.50	1.468
14	16.57	1.468
15	13.80	1.518
16	10.50	1.555

TABLE II

DATA FOR THE COMPLETE CALIBRATION CURVE

Sample Number	Duplicate Analyses	Blood Oxygen Saturation in Volumes Percent	Ratio: R/IR_0 obtained on the Curette
1		120.30	1.000
2		99.41	1.303
3		94.41	1.071
4		95.00	1.038
5		94.70	1.042
6		98.70	1.000
7		72.52	1.159
8		75.10	1.295
9		74.75	1.275
10		84.89	1.178
11		84.06	1.218
12		34.25	1.413
13		19.50	1.802
14		16.37	1.805
15		13.90	1.810
16		10.70	1.852

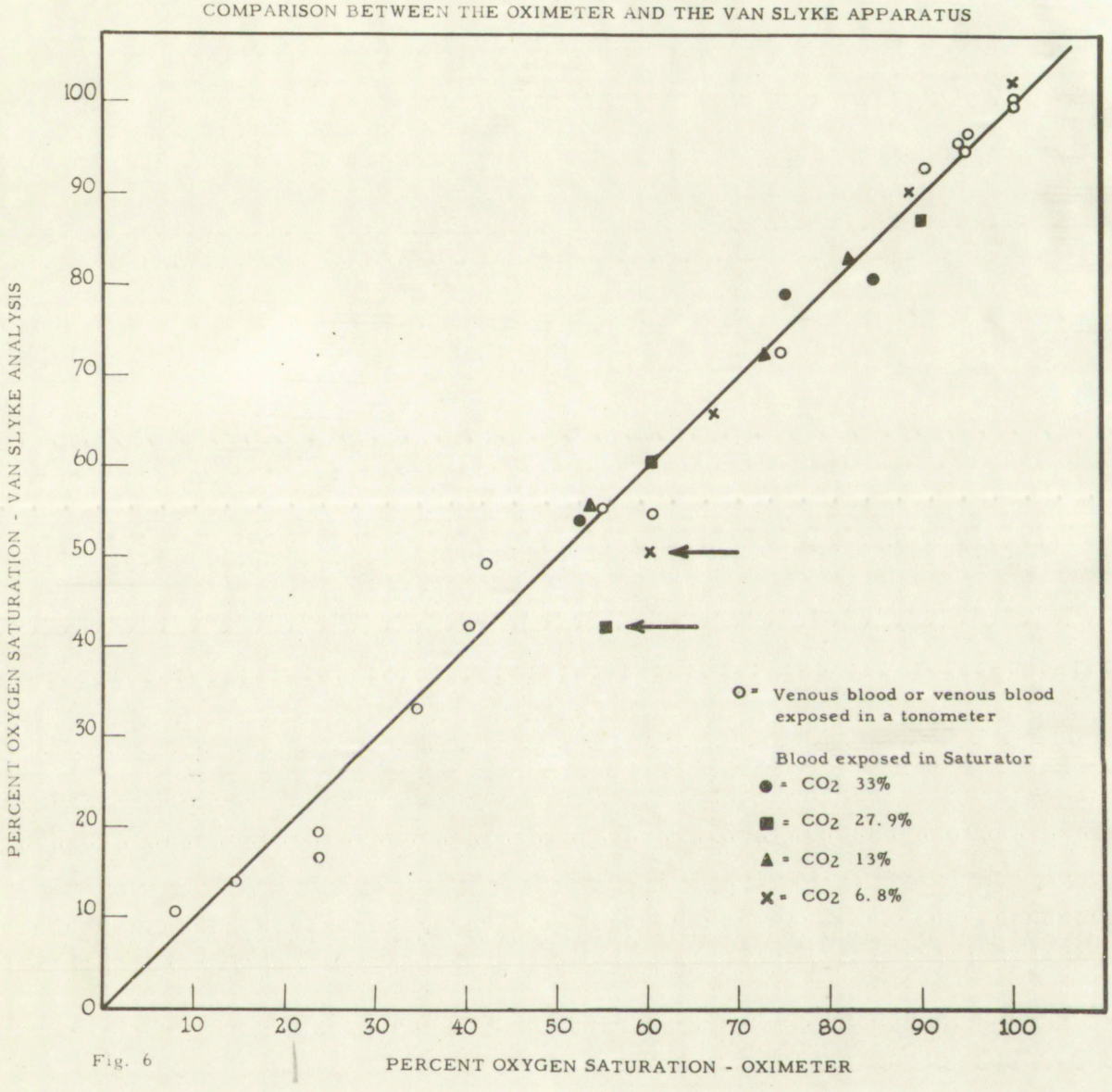

COMPARISON BETWEEN THE OXIMETER AND THE VAN SLYKE APPARATUS
PERCENT OXYGEN SATURATION - VAN SLYKE ANALYSIS
PERCENT OXYGEN SATURATION - OXIMETER
0
10
20
30
40
50
60
70
80
90
100
O = Venous blood or venous blood exposed in a tonometer
Blood exposed in Saturator
● = CO2 33%
■ = CO2 27.9%
▲ = CO2 13%
× = CO2 6.8%

Fig. 6

TABLE III
Data for Figure 6

COMPARISON BETWEEN BLOOD OXYGEN SATURATION OBTAINED USING THE VAN SLYKE MANOMETRIC APPARATUS AND THE OXIMETER FOR WHOLE BLOOD

Sample Number	Ratio log R/IR	Oxygen Percent Saturation -Oximeter	Oxygen Percent Saturation Van Slyke	Partial Pressure of Carbon Dioxide
1	1.208	67.00	66.24	41.30
2	1.253	60.20	49.95	41.20
3	1.000	100.00	102.10	40.38
4	1.070	88.80	90.16	41.31
5	1.293	53.80	55.07	91.80
6	1.134	82.20	83.33	89.67
7	1.117	73.10	72.27	91.67
8	1.253	60.20	61.07	180.17
9	1.284	55.30	42.52	180.17
10	1.065	89.50	86.98	168.00
11	1.156	75.40	78.33	197.70
12	1.096	84.80	81.17	201.07
13	1.301	52.50	53.90	201.92
14	1.033	95.00	96.65	
15	1.000	100.00	100.60	
16	1.035	94.20	95.60	
17	1.060	90.50	92.70	
18	1.285	55.00	55.10	
19	1.163	74.40	75.52	
20	1.468	23.80	16.57	
21	1.555	8.00	10.50	
22	1.378	40.50	42.06	
23	1.413	34.50	33.28	
24	1.245	61.40	54.76	
25	1.518	14.50	13.80	
26	1.000	100.00	99.21	
27	1.378	42.00	48.29	
28	1.032	94.50	94.60	
29	1.468	23.80	19.50	

TABLE III

Data for Figure 6

COMPARISON BETWEEN MICRO OXYGEN SATURATION GRADIENT USING THE VAN SLYKE MANOMETRIC APPARATUS AND THE OXIMETER FOR MUSCLE TISSUE

Sample Number	Ratio $\log \frac{r_2}{r_1}$	Saturation - Oximeter	Oxygen Percent Saturation Van Slyke	Partial Pressure of Carbon Dioxide
1	1.202	67.00	64.24	41.70
2	1.253	60.20	44.52	40.20
3	1.000	100.00	102.10	40.39
4	1.070	86.80	90.16	40.34
5	1.202	54.30	45.07	41.00
6	1.153	82.20	84.12	40.07
7	1.117	73.10	72.27	40.07
8	1.252	60.25	61.37	40.17
9	1.230	57.50	42.52	40.17
10	1.065	87.50	86.90	40.09
11	1.136	75.40	76.33	40.20
12	1.053	84.60	84.12	40.07
13	1.301	52.50	42.90	40.09
14	1.033	95.00	96.05	
15	1.000	100.00	100.00	
16	1.035	94.20	95.60	
17	1.059	90.50	92.70	
18	1.284	52.00	55.10	
19	1.163	74.40	75.32	
20	1.169	73.80	76.27	
21	1.558	8.00	10.96	
22	1.379	40.50	42.06	
23	1.143	74.50	73.40	
24	1.215	61.40	64.76	
25	1.318	44.50	42.80	
26	1.000	100.00	97.21	
27	1.378	42.00	42.29	
28	1.092	94.50	94.80	
29	1.468	23.80	19.50	

figure 6. Both these points were obtained with blood from the same subject and the determinations were done on the same day. No explanation for the variation shown by these two points is known. A technical error or a change in the physiological condition of the subject are both possible explanations.

3. The carbon dioxide dissociation curve obtained using the saturator.--The circulating blood saturator was used at room temperatures of 24° to 27° C. in order to equilibrate the blood with the gas mixtures shown in table I. Blood carbon dioxide content was measured on the Van Slyke apparatus and the results of these analyses are shown in figure 7, table IV. Data from observations made at body temperature by Henderson (1928) and Haldane (1932) are also shown. Results obtained in this study follow those of other workers fairly well, although in some instances the amount of determined carbon dioxide was low. Some variability is to be expected, however, since the blood in this study was equilibrated at room temperature.

The carbon dioxide dissociation curves were extended to tensions of 180 and 200 mm. Hg. which are tensions higher than those previously reported in the literature.

4. Relationship between carbon dioxide content and the pH of whole blood.--Figure 8 shows graphically the carbon

CARBON DIOXIDE DISSOCIATION CURVES ON THE BLOOD OF THREE SUBJECTS

Data from Haldane (1932) and Henderson (1928) Included

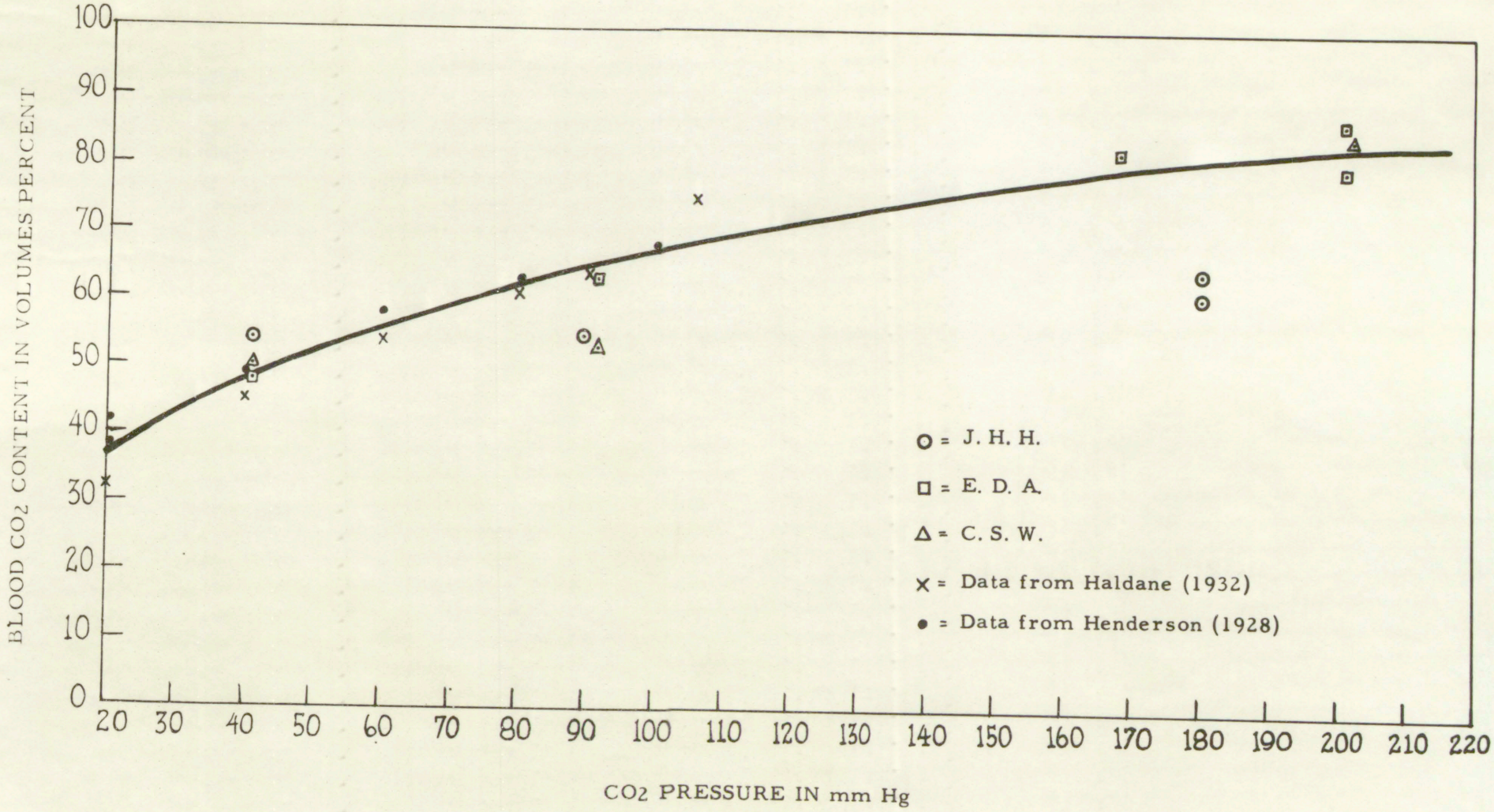


Fig. 7

TABLE IV
Data for Figure 7

DATA FOR CARBON DIOXIDE DISSOCIATION CURVES ON THE BLOOD OF THREE SUBJECTS.
DATA FROM HENDERSON (1928) AND HALDANE (1935) INCLUDED

	Data from this Investigation		Henderson (1928 figure 28, page 126)		Haldane (1935 figure 12, page 47 and 52)	
Sample Number	Blood CO_2 in volumes present	Partial pressure of CO_2	Blood CO_2 in volumes present	Partial pressure of CO_2	Blood CO_2 in volumes present	Partial pressure of CO_2
1	50.22	41.30	38.00	20.00	32.00	20.00
2	53.90	41.20	42.00	20.00	45.00	40.00
3	48.00	41.30	49.00	40.00	54.00	60.00
4	53.03	91.80	58.00	60.00	61.00	80.00
5	54.92	89.67	63.00	80.00	64.00	90.00
6	63.20	91.67	68.00	100.00	75.00	106.00
7	61.01	180.17				
8	64.70	180.17				
9	81.90	168.00				
10	51.50	197.70				
11	87.30	201.07				
12	84.78	201.92				

TABLE IV

Data for Figure 7

DATA FOR DARROW PROTEIN DISSOCIATION CURVES ON THE BLOOD OF NORMAL SUBJECTS.
DATA FROM HENDERSON (1928) AND HASSELBALCH (1915) INCLUSIVE

Sample Number	Data from this Investigation (Figure 25, page 126)			Henderson (1928 Figure 17, page 47 and 52)	
	Dilute CO_2 in volume per cent of Pressure	Partial pressure of CO_2	Blood CO_2 in volume per cent of Pressure	Partial pressure of CO_2	Blood CO_2 in volume per cent of Pressure
1	50.22	42.30	26.00	20.00	70.00
2	80.30	44.20	28.00	25.00	80.00
3	100.00	47.30	30.00	30.00	90.00
4	82.03	48.00	32.00	35.00	100.00
5	84.72	48.07	34.00	40.00	110.00
6	87.20	51.07	36.00	45.00	120.00
7	89.01	51.07	38.00	50.00	130.00
8	91.70	120.17	40.00	55.00	140.00
9	93.86	120.17	42.00	60.00	150.00
10	95.50	123.00	44.00	65.00	160.00
11	97.20	127.79	46.00	70.00	170.00
12	98.78	201.07	48.00	75.00	180.00
		201.92	50.00	80.00	190.00
			52.00	85.00	200.00
			54.00	90.00	210.00
			56.00	95.00	220.00

RELATIONSHIP BETWEEN CO_2 BLOOD CONTENT AND HYDROGEN ION CONCENTRATION

Results Corrected to 38°C. after Rosenthal (1947)

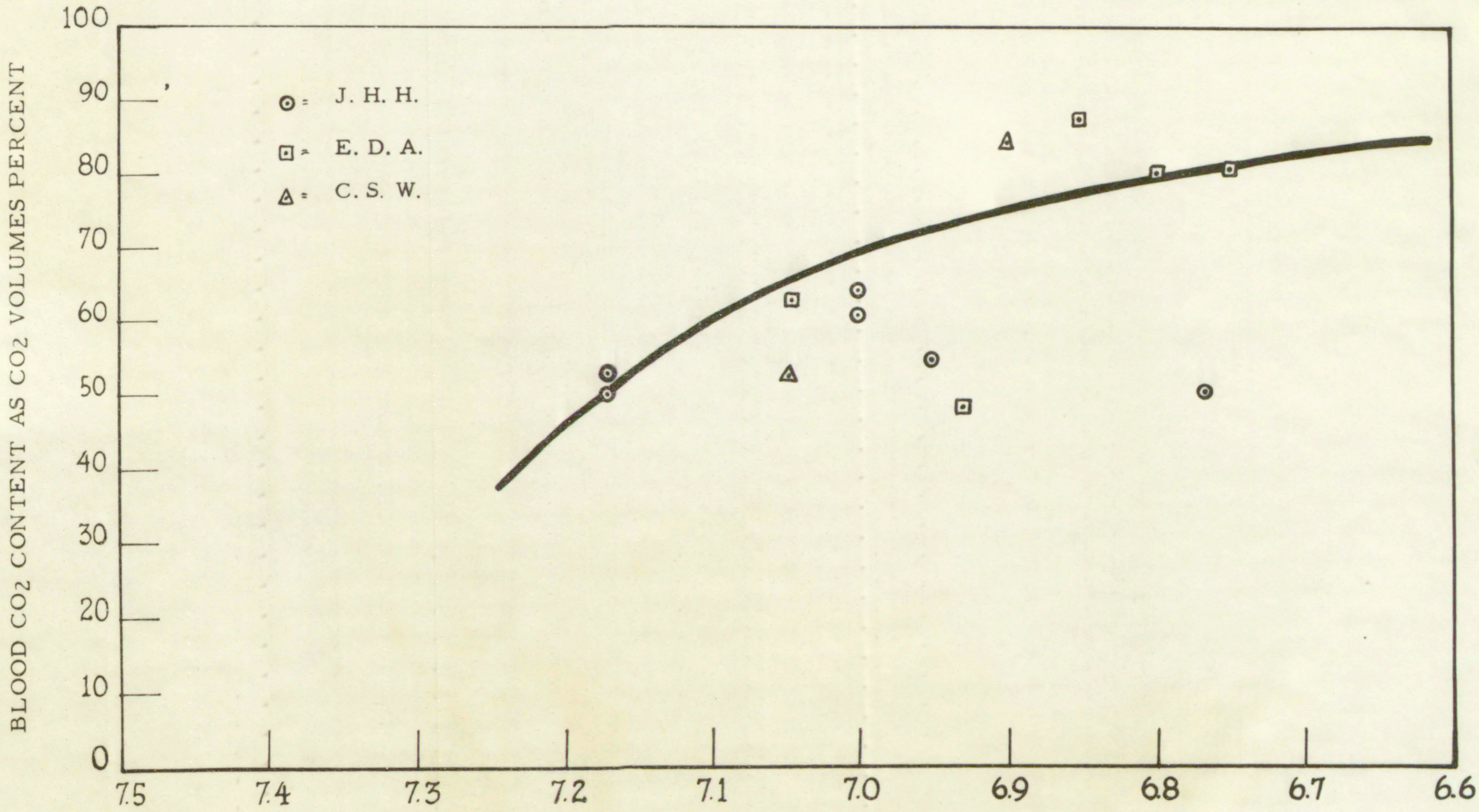


Fig. 8

TABLE V
Data for Figure 8

RELATIONSHIP BETWEEN pH
AND CARBON DIOXIDE BLOOD CONTENT

Sample Number	Blood Carbon Dioxide Content in volumes percent	pH Corrected to 38°C. after Rosenthal (1947)
1	50.22	7.17
2	53.90	7.17
3	48.00	6.94
4	53.03	7.02
5	54.92	6.95
6	63.20	7.04
7	61.01	7.00
8	64.70	7.00
9	81.90	6.70
10	51.50	6.74
11	87.30	6.85
12	84.78	6.90

dioxide content and the pH of blood samples exposed to the experimental gas mixtures in the blood saturator. The results were corrected to 38° C. Data for this graph are in Table V.

5. Relation of blood oxygen saturation and pH.--Data relating blood oxygen saturation to pH at room temperature are plotted in figure 9, and listed in table VI. The results are variable but indicate a 5.5% change in the oxygen saturation per 0.1 increment of pH at an oxygen tension of 66 to 70 mm. Hg. The data of Dill (1944) show a variation of 2.5% per 0.1 unit change in pH. This discrepancy and the wide range indicate one or more uncontrolled variables as for example, a distribution of partial pressure of oxygen from 65 to 72 mm. Hg will vary the blood oxygen saturation from 2.0 to 2.5%. Variations in the acid-base balance would also influence the pH. It is known that these results of pH determinations are questionable, but they are included here for comparative purposes.

Tables VII, VIII, IX and X show the complete data obtained from 13 samples of blood exposed to the 4 experimental gas mixtures.

Relationship Between Blood Oxygen Saturation - Oximeter-and pH at Room Temperature - Oxygen Approximately 70 mm Hg

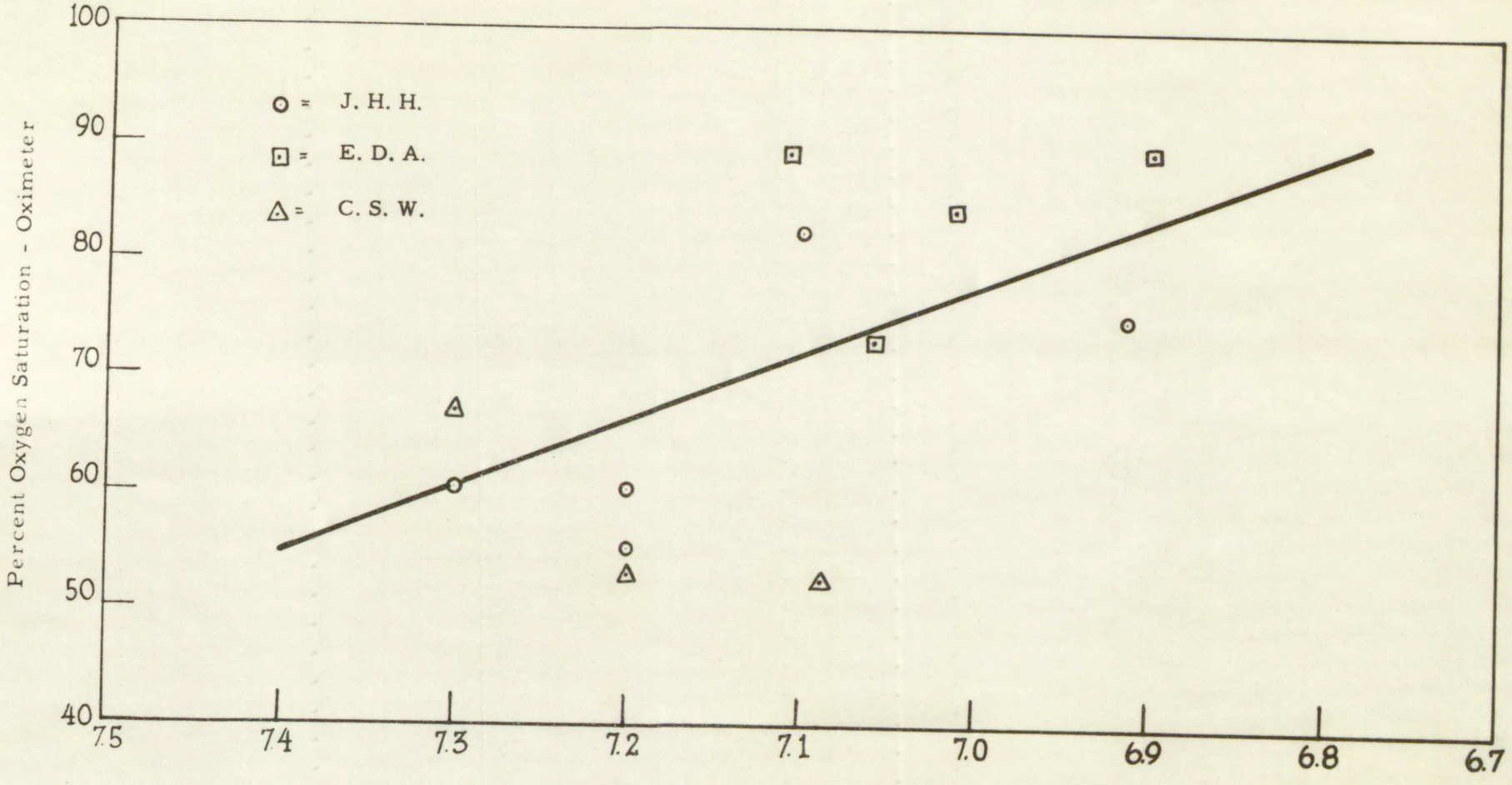


Fig. 9

TABLE VI
Data for Figure 9

RELATIONSHIP BETWEEN pH AND BLOOD OXYGEN PERCENTAGE SATURATION AT ROOM TEMPERATURE

Sample Number	Oximeter Oxygen percent Saturation at Room temperature	pH at Room temperature
1	67.00	7.30
2	60.20	7.30
3	88.80	7.11
4	53.80	7.20
5	82.20	7.10
6	73.10	7.06
7	55.30	7.20
8	60.20	7.20
9	89.50	6.90
10	75.40	6.91
11	84.80	7.01
12	52.50	7.09

TABLE VII

Gas Mixture No. 1 11.94% oxygen 6.8% Carbon Dioxide					Van Slyke Manometric Apparatus				
	Oxygen	Carbon Dioxide	Subject	Oximeter Oxygen percent Saturation	Oxygen Content	Oxygen Capacity	Oxygen percent Saturation	Carbon Dioxide Content	pH
			C. S. W.						
Tension in mm. Hg	72.60	41.30	at room Temperature	67.00	12.75	19.20	66.24	50.22	7.30
			at 38°C.	59.93			57.17		7.17
	72.20	41.20	J. H. H. at room Temperature	60.20	10.43	20.90	49.95	53.90	7.30
			at 38°C.	50.94			40.69		7.17
	70.90	40.38	J. H. H.						
			at room Temperature	100.00*	24.51*	24.00*	102.12*	81.05*	7.02*
			at 38°C.	91.89			94.01		6.85
	72.53	41.31	E. D. A.						
			at room Temperature	88.80	24.21	26.85	90.16	48.00	7.11
			at 38°C.	79.80			81.09		6.94

TABLE VIII

Gas Mixture No. 2
11.04% oxygen
15.09% Carbon Dioxide

	Oxygen	Carbon Dioxide	Subject	Oximeter Oxygen percent Saturation	Van Slyke Manometric Apparatus: Oxygen Content	Oxygen Capacity	Oxygen percent Saturation	Carbon Dioxide Content	pH
			C. S. W.						
Tension in mm. Hg	67.10	91.80	at room Temperature	53.80	11.70	21.17	55.07	53.03	7.20
			at 38°C.	43.46			44.73		7.02
			J. H. H.						
	65.56	89.67	at room Temperature	82.20	19.90	24.00	83.33	54.92	7.10
			at 38°C.	72.96			74.06		6.95
			E. D. A.						
	67.07	91.67	at room Temperature	73.10	14.20	19.66	72.27	63.20	7.06
			at 38°C.	64.25			63.42		7.04

TABLE IX

Gas Mixture No. 3 11.24% oxygen 27.90% Carbon Dioxide					Van Slyke Manometric Apparatus				
	Oxygen	Carbon Dioxide	Subject	Oximeter Oxygen percent Saturation	Oxygen Content	Oxygen Capacity	Oxygen percent Saturation	Carbon Dioxide Content	pH
			J. H. H.						
Tension in mm. Hg	68.10	180.17	at room Temperature	55.30	8.87	20.86	42.50	61.01	7.20
			at 38°C.	45.20			32.40		7.00
			J. H. H.						
	68.10	180.17	at room Temperature	60.20	13.18	21.58	61.07	64.70	7.20
			at 38°C.	50.10			50.97		7.00
			E. D. A.						
	67.60	168.00	at room Temperature	89.50	15.02	17.30	86.98	81.90	6.90
			at 38°C.	81.56			79.04		6.70
			J. H. H.						
	66.75	165.68	at room Temperature	85.70*	12.20*	24.00*	51.60*	81.50*	6.90*
			at 38°C.	79.12			41.72		6.73

TABLE IX

TABLE X

Gas Mixture No. 4 12.00% oxygen 33.30% Carbon Dioxide				Oximeter	Van Slyke Manometric Apparatus				
	Oxygen	Carbon Dioxide	Subject	Oxygen percent Saturation	Oxygen Content	Oxygen Capacity	Oxygen percent Saturation	Carbon Dioxide Content	pH
			J. H. H.						
Tension in mm. Hg	71.20	197.7	Room Temp.	75.40	18.80	24.00	78.30	51.50	6.91
			38°C.	66.49			70.19		6.74
			E. D. A.						
	72.45	201.07	Room Temp.	84.80	21.00	25.80	81.17	87.30	7.01
			38°C.	77.67			74.04		6.90
			C. S. W.						
	72.76	201.92	Room Temp.	52.50	10.60	19.70	53.90	84.78	7.09
			38°C.	44.08			45.48		6.90
			C. S. W.						
	72.74	201.86	Room Temp.	81.00*	46.00*	19.70*		46.00*	6.92*
			38°C.	77.77					6.75
			E. D. A.						
	72.45	201.07	Room Temp.	77.70*	11.51*	25.87*	44.49*	80.10*	6.98*
			38°C.	71.87			37.36		6.80

TABLE X

CHAPTER IV. DISCUSSION

The calibration and use of a whole blood oximeter with bloods exposed to high concentrations of carbon dioxide and the development of a rapid method for saturation of blood with gas mixtures at room temperature form the basis for this investigation. The results obtained must be interpreted in respect to many factors.

To the author's knowledge, there are no data on human carbon dioxide dissociation curves for blood equilibrated with carbon dioxide partial pressures above approximately 100 mm. Hg. In this investigation blood was exposed to four gas mixtures, two at carbon dioxide tensions below 100 mm. Hg, the other two at tensions of 180 and 200 mm. Hg. Results obtained on the first two mixtures confirm the results of previous workers. The blood oxygen percentage saturations as obtained on the oximeter and Van Slyke apparatus did not vary more with one gas mixture than with another. This would seem to indicate that the oximeter is an efficient instrument for measuring blood oxygen saturation on blood containing low as well as high carbon dioxide tensions.

When the Van Slyke manometric apparatus was used to determine the carbon dioxide content of the bloods exposed to the higher tensions, definite drifting of the miniscus occurred and a constant reading was difficult to obtain.

Two normal sodium hydroxide was substituted for the usual normal concentration and the stop cocks of the apparatus were greased to eliminate the possibility of leaks. The use of two normal sodium hydroxide at lower carbon dioxide tensions gave results which checked with those obtained using a reagent of normal concentration on duplicate samples.

Two normal sodium hydroxide was used to absorb the carbon dioxide in the Van Slyke determinations on blood exposed to tensions above 100 mm. Hg. Calculations would indicate 1 cc. of normal sodium hydroxide enough to absorb the carbon dioxide. In an apparatus like the Van Slyke, however, the possibility of leaks must be considered.

The results obtained on the carbon dioxide content of blood exposed in the circulating saturator seem to indicate that the saturator is an efficient method of equilibrating human blood with varying tensions of carbon dioxide. However, use of a smaller saturator is suggested before the efficiency of the saturator with other gases is tested.

Hemolysis was apparent in the blood samples exposed to gas mixtures in the saturator. It occurred, however, in varying degrees and some correlations with technique can be drawn. In particular, hemolysis was noticed on blood samples exposed to gas passing through the fritted glass disk used early in the investigation. The gas passed through the blood at 5 to 6 pounds pressure. When the glass disc was replaced

by a Mandler filter which required only one to two pounds of pressure, the amount of hemolysis decreased.

Only one determination could be done on a blood sample and after a cuvette reading was taken, the saturator was emptied and refilled with fresh blood. Several samples were run twice but the results obtained were not consistent and because of the hemolysis were not considered as giving a true picture of whole blood relationships. For this reason these results are not plotted on the graphs but are marked with an asterisk and included in tables VII, VIII, IX and X. The relation between the time the blood was in the saturator, the pressure of the gas passing through the blood, and the amount of hemolysis would seem to indicate a physical basis for the destruction of the erythrocytes. It is suggested that the gas bubbles hitting the blood cell could rupture the cell membrane. A relationship between the amount of hemolysis and the concentration of carbon dioxide was also noted. A red blood cell in the venous circulation is larger than in arterial blood due to osmotic changes and it is suggested that the rapid exchange of substances through the cell membrane could result in rupture of the membrane due to osmotic changes. Further work on this point is suggested.

The pH data obtained in this investigation are lower than those reported in the literature and show a wide variation. Singer (1948) has pointed out that at least five and

possibly six factors are involved in the acid-base balance of blood. They are: the carbon dioxide content of blood and plasma, the hematocrit, the plasma pH, the buffer system of the whole blood, the carbon dioxide pressure, and possibly the oxygen saturation. Some variation is to be expected but there is no one explanation for the wide variation obtained in this investigation.

In this investigation no attempt was made to control the conditions under which the blood sample was drawn. Environmental factors as well as the physiological condition of the subject will affect results obtained in an investigation of this type. Perhaps the biggest factor in causing the wide range found in the data is the change of method at various times during the investigation. The method used was constantly modified to eliminate the technical errors previously mentioned, hemolysis in particular, which would also explain some of the wide variation in results obtained.

CHAPTER V. SUMMARY

The cuvette, an oximeter for whole blood is described. A method for rapid saturation of blood with gas mixtures at room temperature is given as modified from recent oxygenators.

The cuvette was calibrated using freshly drawn human blood from 16 subjects. The determinations obtained from the oximeter were checked by duplicate analyses on the Van Slyke manometric apparatus. A calibration curve was drawn from these data.

Blood from three subjects was exposed in a circulating saturator to experimental gas mixtures of varying carbon dioxide tensions. The carbon dioxide content of the 13 blood samples exposed to gases of carbon dioxide tensions of below 100 mm. Hg agrees with previous investigations, which indicated that for carbon dioxide equilibration the saturator was efficient. Carbon dioxide dissociation curves are extended beyond those previously reported by exposing the blood to carbon dioxide partial pressures of 180 and 200 mm. Hg.

Agreement between the Van Slyke apparatus and the oximeter on the blood oxygen saturation of 13 bloods of both low and high carbon dioxide content suggest that the oximeter is not influenced by amounts of carbon dioxide beyond the physiological range.

LITERATURE CITED

Barcroft, J., and M. Camis, 1909. The Dissociation Curve of Blood. J. of Physiol., 39:118-131.

_____, and W. O. R. King, 1909. The Effect of Temperature on the Dissociation Curve of Blood. J. of Physiol., 39: 113-160.

Bennett, Ray V., 1950. Personal Communication.

Bert, Paul, 1878. Barometric Pressure; Researches in Experimental Physiology translated by Hitchcock. Columbus: College Book Company, 1943. 1055 pp.

Bock, A. V., G. S. Adair, and H. Field, Jr., 1924. The Oxygen and Carbon Dioxide Dissociation Curves of Human Blood. J. of Biol. Chem., 59:353-378.

Brinkman, R., R. Margaria, N. O. Meldrum, and F. J. W. Roughton, 1932. The CO_2 Catalyst Present in Blood, J. of Physiol., 75:3-4.

Christiansen, Johanne, C. G. Douglas, and J. S. Haldane, 1914. The Absorption and Dissociation of Carbon Dioxide by Human Blood, J. of Physiol., 48:244-262.

Clark, Leleand C., Vishwa Gupta, and Frank Gollan, 1950a. Dispersion Oxygenation for Effecting Survival of Dogs Breathing pure Nitrogen for Prolonged Periods, Proc. Soc. Exp. Biol. and Med., 74:268-271.

_____, 1950b. Oxygenation of Blood by Gas Dispersion, Science 111:85-87.

Dill, D. B., H. T. Edwards, Marcel Florkin, and R. W. Campbell, 1932. Properties of Dog Blood, J. of Biol. Chem., 95:143-152.

_____, 1944. Mayo Aero-Medical Unit.

Drabkin, David, and J. Harold Austin, 1935. Technique for the Analysis of Undiluted Blood and Concentrated Hemoglobin Solutions, J. of Biol. Chem., 112:105-115.

Elam, James O., John F. Neville, Jr., William Sleator, William N. Elam Jr., 1949. Sources of Error in Oximetry Annals of Surgery, 130:755-773.

Ferguson, J. K. W., and F. J. W. Roughton, 1934. The Chemical Relationships and Physiological Importance of Carbamino Compounds of CO_2 with Hemoglobin, J. of Physiol., 83:87-102.

Goldie, E. A., 1942. A Device for the Continuous Indication of Oxygen Saturation of Circulating Blood in Man, J. of Scien. Instru., 19:23-25.

Haldane, J. S., and J. G. Priestley, 1935. Respiration. New Edition; New Haven: Yale University Press, 1935. 493 pp.

Haurowitz, Felix, 1949. Section II, the Bond between Haem and Globin (In Haemoglobin. Barcroft Symposium.) New York: Interscience Publishers, Incorporated. pp. 53-56.

Henderson, L. J., 1920. Equilibrium between Oxygen and Carbonic Acid in Blood, J. of Biol. Chem., 41:401-410.

_____, 1928. Blood: a study in general physiology. New Haven: Yale University Press, 1928. 397 pp.

Henriques, O. M., 1928. Die Bindungsweise des Kohlen dioxyds in Blut. III. Der Experimentelle Nachweis eines CO_2--Hämoglobin Komplexes in Lösun en von CO_2 und Hämoglobin, Biochem. Z., 200:10-17.

Horecker, B. L., 1943. Absorption Spectra of Hemoglobin and Its Derivatives in the Visible and near Infra-red Regions, J. of Biol. Chem., 148:173-183.

Hickam, John, 1951. Department of Medicine,
Duke University School of Medicine,
Personal Communication.

Landois, L., 1905. Textbook of Human Physiology. Philadelphia: Blakiston, Son and Company, 1027 pp.

Lilienthal, J. L., and R. L. Riley, 1945. The Relationships of Oxygen, Carbon Dioxide and Hemoglobin in Blood of Man, Research Project X-490 (Av-260-f). Naval School of Aviation Medicine, U. S. Naval Air Training Bases, Pensacola, Florida.

Meduna, L. J., 1950. Carbon Dioxide Therapy. Springfield, Illinois, Thomas. 236 pp.

Meldrum, N. U. and F. J. W. Roughton, 1932. Some Properties of Carbonic Anhydrase the CO_2 Enzyme Present in Blood, J. of Physiol., 75:15-16.

Meldrum, N. U. and F. J. W. Roughton, 1933. The State of Carbon Dioxide in the Blood, J. of Physiol., 30:143-170.

Milliken, G. A., 1942. The Oximeter, an Instrument for Measuring Continuously the Oxygen Saturation of Arterial Blood in Man, Rev. Scien. Instru., 13:434-44.

Peters, J. P. and D. D. Van Slyke, 1932. Quantitative Clinical Chemistry, Volume II, Methods. Baltimore: Williams and Wilkins Company, 1932. 957 pp.

Rosenthal, T. B., 1948, Effect of Temperature on the pH of Blood and Plasma in Vitro, J. of Biol. Chem., 173:25-30.

Roughton, F. J. W., 1935. Recent Work on Carbon Dioxide Transport by the Blood, Physiol. Rev., 15:241-296.

_____, R. C. Darling and W. S. Root, 1944. Factors Affecting Determination of Oxygen Capacity, Content and Pressure in Human Arterial Blood, Am. J. of Physiol., 142:708-720.

Scholander, P. F., 1947. Analyzer for Accurate Estimation of Respiratory Gases in One-half Cubic Centimeter Samples, J. of Biol. Chem., 167:235-250.

Singer, R. B., and A. B. Hastings, 1948. An Improved Method for the Estimation of Disturbances of Acid Base Balance of Human Blood, Medicine, 27:223-247.

Stewart, G. N., 1900. Manual of Physiology with Practical Exercises. Philadelphia: W. B. Saunders. 894 pp.

Van Slyke, D. D., Baird Hastings, Michael Heidelberger, and James M. Neill, 1922. Studies of Gas and Electrolyte Equilibria in Blood. III The Alkali Binding and Buffer Values of Oxyhaemoglobin, J. of Biol. Chem., 54:

_____, and James A. Hawkins, 1930. The Evolution of Carbon Dioxide from Blood and Buffer Solutions, J. of Biol. Chem., 87:265-269.

Wood, E. H., and J. C. Geraci, 1949. Photo Electric Determination of Arterial Oxygen Saturation in Man, J. of Lab. and Clin. Med., 34:387-401.

Wood, E. H., 1950. Oximetry (In Medical Physics. Chicago: Chicago Year Book Publishers. Vol. 2, pp. 664-679).

O'Connell, Joseph J. et al., 1949. Accident Investigation Report, United Air Lines Incorporated, near Mt. Carmel Pennsylvania, Civil Aeronautics Board (mimeographed), file No. 1-0075-48:18 + iii, June 17, 1948.