CHANGE IN BLOOD PO_2 AND PCO_2 WITH SHORT STORAGE TIME

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WITH

SHORT STORAGE TIME

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SUMMARY

The effects of short storage time on the partial pressures of oxygen and carbon dioxide in human blood were studied and the factors producing these effects were explored. Samples of freshly drawn heparinized blood were equilibrated with various oxygen pressures, stored in ice and analyzed at various periods of storage time. An identical experiment was performed using saline instead of blood. This was done to investigate the metabolism and material effects. During the above tests the blood samples were handled anaerobically. Another experiment was conducted to investigate the possibility of air contamination. Syringe material or the presence of small air bubbles seemed to have no effect on the blood samples and did not cause the oxygen partial pressure changes observed. Changes in PO_2 were found to be due to a shift in the oxyhemoglobin dissociation curve at constant oxygen saturations. The blood pH did not change during the period that the blood was stored and analyzed. Changes in PO_2 with time were measured and these changes strongly depended on the initial blood PO_2. There were no statistically significant changes in the partial pressure of carbon dioxide due to storage time.

CHAPTER I

INTRODUCTION

Blood gas measurements in vitro constitute an important factor in modern clinical diagnosis and medical research (2). Arterial and venous blood samples are usually drawn anaerobically into syringes containing heparine, iced and then sent to the laboratory for analysis. Results obtained from these analyses are used without taking into consideration the effects of the time elapsed between the moment blood is drawn from the subject and that when it is analyzed.

During an earlier investigation (1), it was discovered that the partial pressure of oxygen (PO_2) in blood changes with the storage time. This present investigation is a study of the changes in PO_2 with time of storage. Molecular oxygen exists in body fluids either free (as dissolved oxygen) or as loosely combined with respiratory pigments like hemoglobin. Hemoglobin is a chromoprotein and loosely combines with oxygen to form an unstable compound called oxyhemoglobin, O_2Hb. Oxygen being extremely insoluble in body fluids, free oxygen is in very low concentrations even in arterial blood and is best considered as an intensity factor which is expressed as the partial pressure of oxygen, PO_2 (Equation 1).

$$PO_2 = \frac{1}{.03} \times \text{Physically dissolved } O_2 \text{ (in cc/L)} \qquad (1)$$

As blood flows through a tissue, the partial pressure of oxygen can vary greatly along a capillary - from an arterial PO_2 of about 100 mm Hg to a venous PO_2 of about 40 mm Hg (2).

The other index of oxygenation of blood usually used in the blood gas analysis is the percentage oxygen saturation, % Sat (Equation 2).

$$\% \text{ Sat} = \qquad (2)$$

$$100 \times \frac{\text{Quantity of } O_2 \text{ bound to the Hb of blood sample}}{\text{Quantity of } O_2 \text{ that can be bound by this Hb}}$$

This index is thus the ratio of oxygen content of the blood (minus physically dissolved O_2) to the quantity of O_2 that can combine with the hemoglobin (minus phisically dissolved O_2). In the absence of carboxy-hemoglobin or other abnormal hemoglobin the percentage oxygen saturation can be expressed by Equation 3.

$$\% \text{ Sat} = 100 \times \frac{[O_2Hb]}{[O_2Hb] + [Hb]} \qquad (3)$$

where $[O_2Hb]$ is the concentration of oxyhemoglobin and $[Hb]$ is the concentration of reduced hemoglobin that is able to bind O_2.

The above two indices of oxygenation, PO_2 and % Sat, are related to each other by the oxyhemoglobin dissociation curve shown in Figure 1. This curve represents the relationship between the percentage saturation and the partial pressure of oxygen in the blood at some fixed carbon dioxide level. The pressure that oxygen exerts in blood depends on the concentration of free oxygen in plasma. The blood PO_2 does not depend simply on the concentration of O_2Hb nor does it vary linearly with the percent oxygen saturation. PO_2 may not even change when the total quantity of O_2 in the blood is changed. This is explained by the shift in the oxyhemoglobin dissociation curve, Bohr's effect (2).

Oxygen, in the body, exists almost exclusively in and is transported in blood as oxyhemoglobin, O_2Hb (2). Equations 4 and 5

Tissue		Lungs	
$CO_2 + H_2O \rightarrow H^+ + HCO_3^-$		$HCO_3^- + H^+ \rightarrow H_2O + CO_2$	(4)
$O_2 + HHb \leftarrow H^+ + O_2Hb$		$O_2Hb^- + H^+ \leftarrow O_2 + HHb$	(5)

represent the outlines of the major O_2 - Hb cycle for the oxygen and the carbon dioxide transport. From the tissue to the lungs, carbon dioxide is carried mostly as bicarbonate (HCO_3^-) where it diffuses into the lungs as free carbon

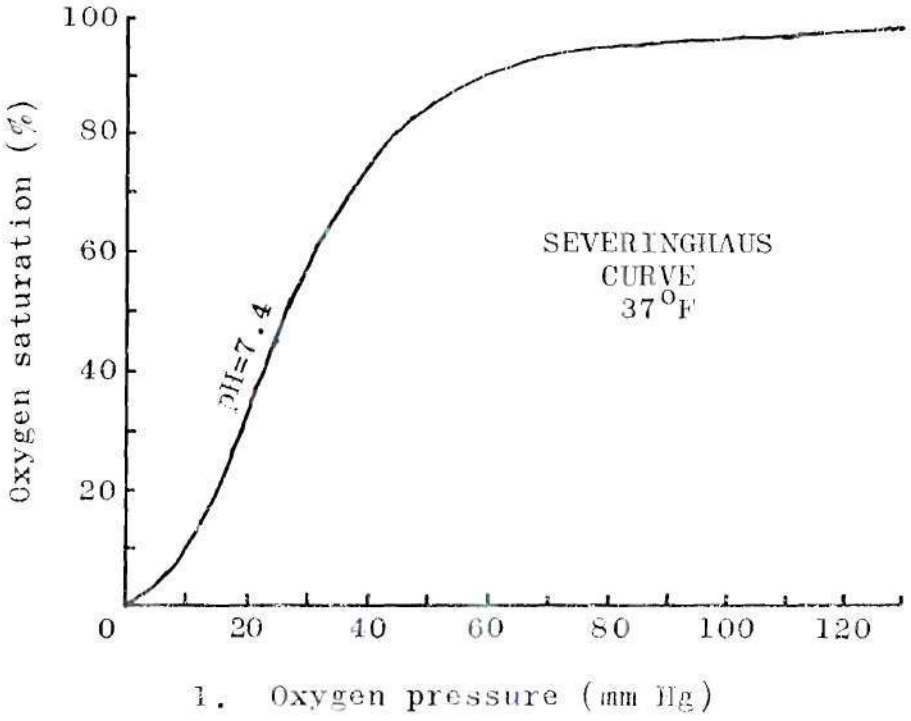


Figure 1. Oxyhemoglobin Dissociation Curve

dioxide. Oxygen from the lungs diffuses into the blood and combines loosely with the reduced hemoglobin (HHb) to form an unstable compound called oxyhemoglobin (O_2Hb^-) which is carried by the blood to the tissue. Here at the tissue, carbon dioxide released reacts with water in the blood to produce H^+ and HCO_3^- (Equation 4). HCO_3^- is carried by the blood with a slight increase in its PCO_2 (Haldane effect). H^+ is transferred to the O_2Hb^-. This fairly strong base becomes much stronger as it unloads its oxygen to the tissue (Equation 3). O_2Hb^- as well as the pH decreases as the arterial blood becomes venous. This drop in pH shifts the oxygen dissociation curve to the right so that the blood gives up oxygen with only a slight decrease in PO_2 (Bohr's effect). H^+ is known as the Bohr-Haldane proton and its transfer links the two (Bohr-Haldane) effects (2).

The partial pressures of oxygen and carbon dioxide in the blood are usually measured using electrodes. PO_2 is measured using polarographic system. This system uses a platinum electrode which is separated from the blood by a membrane permeable to oxygen but impermeable to water and ions. The availability of oxygen molecules at the platinum surface is proportionate to the current resulting from a given voltage applied to the platinum electrode.

The Severinghaus electrode is used to measure PCO_2 in the blood sample. This consists of a plastic membrane separating the blood from a carbonate solution containing a glass

electrode. Carbon dioxide from the sample (but not from HCO_3^-) diffuses through the membrane. This device responds to the pH, but the scale on the meter is calibrated for PCO_2. These two electrodes are discussed in more detail in appendix I.

CHAPTER II

LITERATURE SURVEY

Measurements of PCO_2, PO_2 and percent saturation can be made in a few minutes with modern instruments. The accuracy of these measurements depends on how well the instruments are cared for and how carefully they have been calibrated. The gas analysis technique and prehandling of blood samples are also determining factors in the accuracy of measured partial pressures and percent saturation.

Holmes,et al. (3) studied the methods, with particular interest in the accuracy, of calibration of O_2 and CO_2 electrodes. They calibrated the blood gas analyzer using a humidified gas and for various lengths of time. It was found that the oxygen and carbon dioxide partial pressures in samples of venous and arterial blood correlated well with those obtained by calibrating the electrodes with humidified gases. The different equilibration time did not result in any significant difference. It was concluded that the calibration of a blood gas apparatus with gas was satisfactory if the gas was properly humidified.

Rhodes and Mosers (4) studied several sources of error in the measurement of blood oxygen partial pressure by the polarographic system incorporating a microelectrode. Their (4)

study was mainly confined to the investigation of three sources of error in the determination of PO_2 in blood (a) the characteristics of the polarographic measuring system (particularly the membrane used to cover the electrode); (b) changes in the blood oxygen partial pressure prior to measurement; and (c) properties of blood which might change the measured oxygen partial pressure. They (4) compared the oxygen partial pressures of the tonometered blood and the tonometer gas. A range of 10 to 700 mm Hg was covered using both polyethylene and polypropylene membranes. Saline and buffers were substituted for blood to determine the correction factor and effect of varing pH on the response of the electrodes using polyethylene membrane only. They (4) also studied the effect of temperature with time of storage on PO_2 of blood. This study was extended to investigate the properties of blood that may change the blood PO_2. This was done by adjusting the hemocrits of blood. From their study, Rhodes and Mosers (4) found that the PO_2 of blood samples and gas was such that, over the range 0-65 mm Hg, using polyethylene membrane,

$$\text{Actual } PO_2 = 1.24 \times \text{measured } PO_2$$

and above approximately 65 mm Hg,

$$\text{Actual } PO_2 = 1.08 \times \text{measured } PO_2 + 10$$

When the polypropylene membrane was used, over the entire 10-700 mm Hg range, it was found that

$$\text{Acutal } PO_2 = 1.05 \times \text{measured } PO_2$$

This behavior was attributed to the difference in the characteristics of the membrane used. Comparison of the PO_2 in the blood samples maintained at three temperatures showed a significant difference. No change in the PO_2 was observed in blood samples maintained at $0^{o}C$ but the PO_2 declined with time in samples maintained at ambient temperature and fell more rapidly in samples kept at $37^{o}C$. The drop in PO_2 was considerable in samples of high PO_2. They (4) also found that hemocrit values ranging from 15-60% and heparine did not influence the PO_2 whether polyethylene or polypropylene membranes were used. It was concluded by them (4) that nature and extent of the error is dependent upon the specific characteristics of the polarographic system (electrode, cuvette design, membrane materials and amplifying-recording etc.) and the magnitude and the manner of correction should vary with details of instrumentation and technic. The rate of PO_2 decline was found to be a function of metabolic activity of cellular blood components which has been described by Rhodes and Mosers (4) to be dependent upon the temperature at which the blood is stored.

Evers, et al. (5) and Hilti, et al. (6) worked independently on the effects of syringe materials on the PO_2 in stored blood. Samples of equilibrated human blood were stored for various periods of time at room temperature or at 0^oC. In addition to this, Hilti, et al. (6) repeated the same experiments with ringer's solution as a control to evaluate the contribution of metabolic changes in the blood as well as material effects.

The results of the study conducted by Evers, et al. (5) showed that the pH of blood in both glass as well as plastic syringes dropped slightly with the drop somewhat more rapid in plastic syringes. The PCO_2 in both types of syringes showed a similar pattern with no significant difference. It dropped in the first thiry minutes followed by steady rise. The PO_2 in both types of syringes, within the first thiry minutes, dropped with no further marked change. The PO_2 loss in the plsatic syringes was less than that in the glass syringes, but the difference was insignificant. This brought Evers, et al. (5) to the conclusion that there was no difference in the use of plastic (polypropylene) or glass syringes and diffusion of gases through the syringe walls was not statistically or clinically significant.

The study conducted by Hilti and Karendal (6) showed that the use of glass syringes and immediate chilling of blood samples did not change the partial pressure of oxygen, whereas the plastic syringes under certain conditions had too high a

diffusion capacity for storing blood or solutions for gas analysis as they tend to come in equilibrium with the ambient oxygen pressure. Metabolic consumption was found to be dependent on the temperature but diffusion rather than metabolic consumption and other physiological changes was found to be dominant. As a general conclusion glass syringes (non-interchangeable) proved to be the best for storing blood for gas analysis, especially if immersed in iced water.

Earlier some work was done on gas diffusion between plastic syringes and room air by Laver and Seifen (7) in 1965 and by Fletcher and Jergen (8) in 1966. Comparing glass with plastic syringes Fletcher and Jergen (8) found a three fold greater oxygen loss from the plastic syringes. Laver and Seifen (7) on the other hand, stated that the plastic syringes can be used for blood gas analysis if no delay in reading the results is foreseen.

In short, from this literature survey, it is concluded that all authors agree with each other about the fact that storage of blood in iced water reduces the loss of oxygen from it, whereas the opinions differ in the case of comparing the use of different syringes (plastic or glass) for the storage of blood for gas analysis. Furthermore all authors have based and conducted their study on the assumption that diffusion of gases through the walls of the syringes is the major factor affecting the PO_2 in blood when stored in these syringes for gas analysis. Evers, et al. (5) came to the conclusion that

there was no difference whether the glass syringes were used or the plastic syringes. On the other hand Hilti and Karendal (6) suggested the use of glass syringes for storing blood and not that of plastic syringes. Other factors such as physiological changes in the blood have either been ignored or not been given much attention by them (5,6,7 & 8).

On this basis it was decided to conduct a study to investigate the effects of short storage time on the partial pressures of oxygen and carbon dioxide in human blood and to explore the reasons in order to attempt to explain any changes in oxygen and carbon dioxide partial pressures found during the short storage time. This study is described in detail in the following chapters.

CHAPTER III

EQUIPMENT

Various instruments used during the study are described as follows:

Equilibration Unit

Equilibration of the blood samples with a desired gas was obtained utilizing a tonometer (IL-237). This unit humidified the gas, brought it to a constant temperature (37^{o}C) and equilibrated the blood with the humidified gas that was fed to the tonometer. Blood samples from 0.5 to 8.0 milliliters in volume could effectively and precisely be equilibrated for a desired pre-set time in this equipment. These samples could be drawn from the tonometer directly into the syringes without the blood coming in contact with the air, thus making this process of blood transfer from the tonometer into the syringes anaerobic.

Measuring Units

Two measuring units were used, one Instrumentation Laboratories' model IL-182 co-oximeter and the other IL-313 ph Blood gas analyzer.

Co-oximeter (IL-182)

The co-oximeter (IL-182) was used for the measurements

of various concentrations (oxyhemoglobin, total hemoglobin and carboxy-hemoglobin) in the blood. It performs spectrophotometric analyses of blood oxygen, carbon monoxide and hemoglobin concentrations. It processes and analyzes samples automatically. A peristallic pump anaerobically draws up and moves blood samples into the measuring cuvette.

A 400 microliter blood sample is introduced and momentarily stored in a spiral reservoir at the front of the instrument. This is automatically drawn from the reservoir when the peristallic pump is activated. The mixed specimen is then passed through a mechanically hemolyzing assembly, brought to a constant temperature and passed to the cuvette for the measurement of the absorbance. After the measurements are completed, the pump is once again activated with zeroing solution, a trade product of the Instrumentation Laboratories, in the reservoir. After leaving the cuvette, sample is passed through the pump and out as waste. The oxygen concentration is maintained throughout the process. After each sample run, the co-oximeter was cleaned by running two or three samples of zeroing solution through the instrument.

The units of three blood concentrations (O_2Hb, Hb and COHb) displayed on the instrument (IL-182) are percent, gram-percent and percent respectively. Oxyhemoglobin and carboxy-hemoglobin are expressed as the ratio of oxyhemoglobin or carboxy-hemoglobin to the total hemoglobin whereas the total hemoglobin is expressed as grams of total hemoglobin per hun-

dred milliliters of blood. The instrument has a sensitivity of one tenth of a percent for O_2Hb and COHb, and one tenth of a gram for total hemoglobin.

pH Blood Gas Analyzer (IL-313)

The Blood gas analyzer (IL-313) of Instrumentation Laboratories was used to measure the pH and the partial pressures of oxygen and carbon dioxide in the tonometered blood samples.

This instrument (IL-313) has the capability for calibration with two different gases and separately adjusting the balance and the slope of calibration. Selection of any one of the three modes (Standby, Calibrate and Sample) of gas analysis operation calls into operation various individual functions such as flushing and washing of sample chamber and flow lines, introduction of gases and sample handling automatically and in precise timed sequence. The flush cycle washes the sample chamber and the sample lines with the cleaning solution equilibrated with the desired gas. This flush solution removes protein deposits and prevents occlusion of the lines. After the flush cycle, calibration gas starts flowing through the sample chamber. This gas is saturated with water as it bubbles through the bubble chamber. This calibration cycle automatically checks the calibration after each flush cycle that follows the sample cycle. The sensitivity of the instrument (IL-313) is 0.001 for the pH and one tenth of a millimeter of Hg for PCO_2. For PO_2, in the low range (0 to 200 mm

Hg) the sensitivity is one tenth of a millimeter Hg and the high range (200 to 2000 mm Hg) has a sensitivity of one mm Hg.

Some of the test runs in the study were repeated on a Blood gas analyzer (IL-113) in order to study the repeatability of the data from one instrument to another. The IL-113 is an older non-automated version of IL-313 but uses the same electrode configuration. This study was performed at the Pulmonary Laboratory, Grady Memorial Hospital in Atlanta, Georgia.

CHAPTER IV

EXPERIMENTATION

Procedure

This study was conducted using freshly drawn venous blood. Sodium heparine was used as the anticoagulant with the blood samples to be analyzed for PO_2 and PCO_2 using the Blood gas analyzer (IL-313) and for the blood samples to be analyzed using the co-oximeter, EDTA was used because its use with co-oximeter is recommended by the Instrumentation Laboratories. EDTA could not be used in the first case because it changes the acidic properties of the blood thereby changing its PO_2 and PCO_2 values. Sodium heparine does not have this effect on the blood.

Approximately eight milliliters of blood was equilibrated for each experiment, with a gas of desired composition (O_2, CO_2 and N_2) keeping each time the CO_2 content at about five percent of the total. This was done to avoid any side effects of CO_2 on the acidic properties of the blood and also because the main study was oriented towards the PO_2 measurements. While blood was being equilibrated in the tonometer, the IL-313 and the co-oximeter were set for calibration. The complete instrumentation set up is shown schematically in Figure 2.

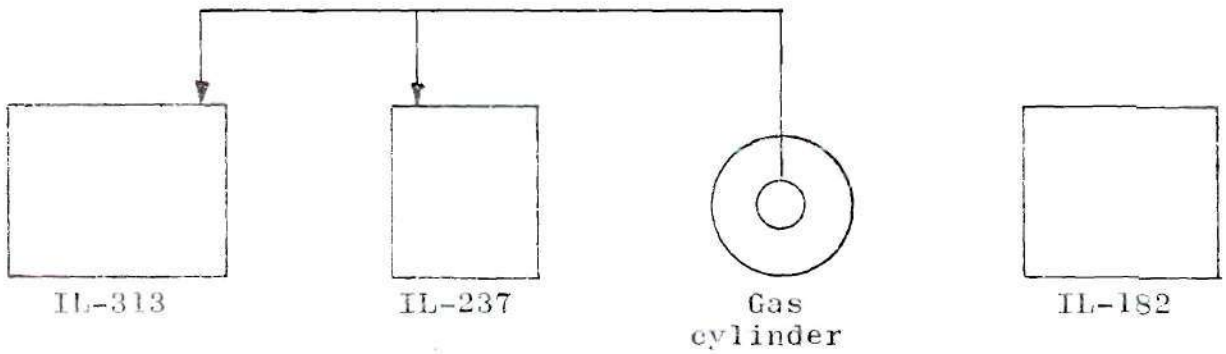


Figure 2. Instrumentation Arrangement

Series One Testing

The study was completed in a series of segments. Se- one was conducted to determine the reproducibility of the data. This was achieved using the blood and different gases in separate identical experiments. In each experiment the blood was equilibrated with a gas of known composition for thirty minutes. One milliliter of the equilibrated blood was drawn anaerobically into a three milliliter plastic disposable syringe and immediately analyzed on the Blood gas analyzer. The syringe was flushed ten times with the equilibration gas before the blood was drawn into it. Preflushing of the syringes was done during all experiments except one discussed in a later series. At intervals of ten to fifteen minutes, one milliliter blood samples were drawn from the tonometer and immediately analyzed for PO_2 and PCO_2. Similar experiments were performed using saline in place of the blood. The results of these tests are given in appendix II, Table 3. Number of data points, calculated oxygen partial pressure, standard deviation and the mean PO_2 are given in Table 1. Comparison of the mean PO_2 with the calculated PO_2 shows that the mean PO_2 in each experiment is lower than the calculated one. This difference is due to the solubility factor of the gas in blood and saline, and is also dependent on the diffusion characteristics of the electrode membrane (4).

The standard deviation of 1.3 mm Hg for saline, 1.4 for the blood in one experiment and 2.3 mm Hg in another indi-

Table 1. Results of Series One Tests

Specimen	No. of Data Points	Calculated PO_2 (mm Hg)	Standard Deviation (σ)	Mean PO_2 (mm Hg)
Saline	7	136.3	1.3	119.6
Blood	9	137.7	1.4	123.4
Blood	8	136.1	2.3	125.8

cates that the data obtained on the Blood gas analyzer (IL-313) was accurate and reproducible within the manufacturer's specifications.

Series Two Testing

Series two was conducted after it was made certain t at th data obtained on IL-313 could be reproduced. The experiments in this series investigate any change in the PO_2 and PCO_2 that may occur due to the storage of blood in ice. A part of this series was to determine if there was any differe ce in storing blood in a large syringe and analyzing the blood from the same syringe periodically or storing small samples in individual syringes. Equilibrated blood was collected in one ten milliliters plastic disposable syringe or separate three milliliters syringes and was stored in ice. The first analysis of each experiment was performed with no elapse of time after the blood was drawn from the tonometer. Other samples were analyzed at different intervals of time. During he measurements on IL-313 one of the gases used for the calibration of IL-313 was the same as that used for equilibrating blood. The flush solution was also equilibrated constantly with this gas. The purpose of this procedure was to expose the measuring electrodes in the IL-313 to the same gas as that used for the equilibration of the blood.

Data from the series two experiments is given in appendix II, Table 4. These results are shown in Figures 3-6. Fig-

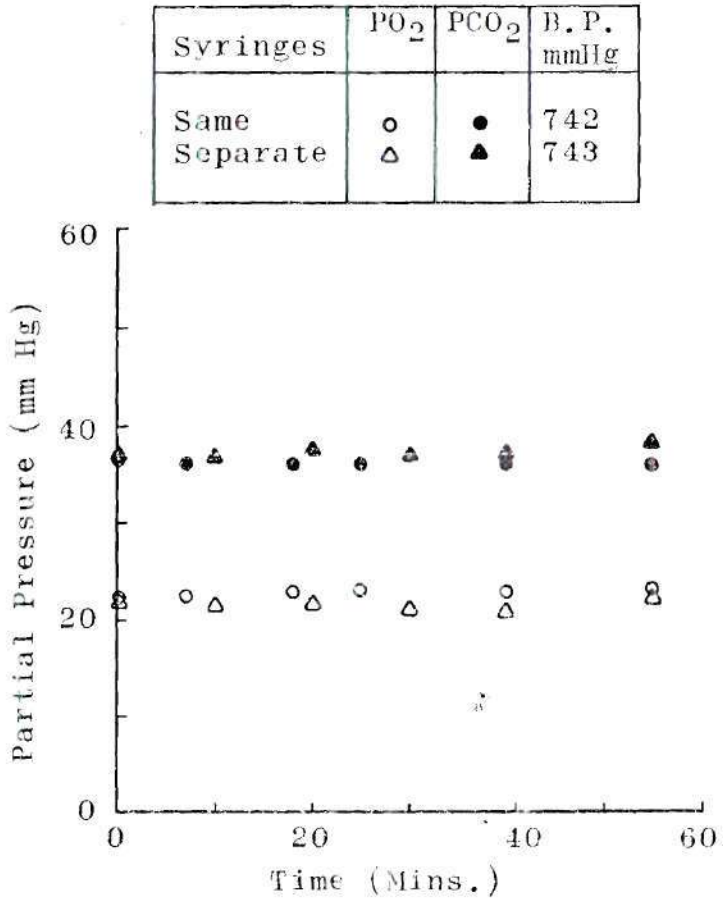


Figure 3. Oxygen and Carbon Dioxide Partial Pressures vs. Storage Time for Blood Equilibrated with 5.3% CO_2, 3.2% O_2 and Iced

ure 3 shows the variations in PO_2 and PCO_2 with storage time with the blood equilibrated with 5.3 % CO_2 and 3.2 % O_2. Plastic disposable syringes were used for storing blood in ice. No change in either PO_2 or PCO_2 is observed over a period of fifty five minutes irrespective of whether the same syringe was used or the separate syringes. The PO_2 varies within a range of 20-23 mm Hg whereas the PCO_2 varies within 36-39 mm Hg. Figure 4 shows the results for blood equilibrated with 4.8 % CO_2 and 7.2 % O_2. It is again seen that there is no appreciable change in either PO_2 or PCO_2. During fourty minutes the scatter of PO_2 data is found to be within 51-53 mm Hg while that of PCO_2 is within 32-34 mm Hg. Figure 5 shows the results for blood equilibrated with 5.1 % CO_2 and 19.7 % O_2. A marked increase in the PO_2 with storage time is observed whereas the PCO_2 does not show any variation. The PO_2 started out at about 136 mm Hg and after a storage period of sixty minutes it increased to a maximum value of about 147 mm Hg. Although there is a scatter among the data of various experiments, the PO_2 increased steadily with the storage time. Inspite of the scatter in the PCO_2 data, no significant change is observed.

The behavior of PO_2 and PCO_2 for the high pressure of oxygen with storage time is seen in Figure 6. The data shown in this figure is for the same syringe as well as separate syringes when the blood was equilibrated with 5.0 % CO_2 and 95.0 % O_2. In this range (600-650 mm Hg) the data is clearly

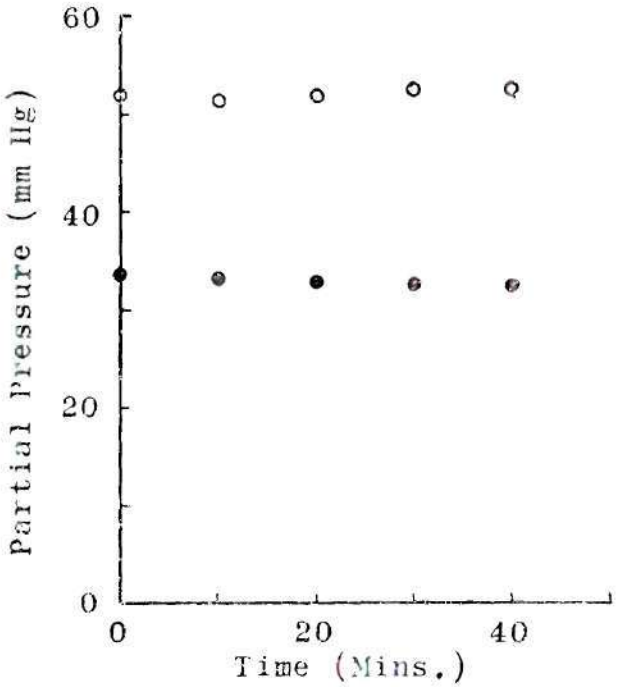


Figure 4. Partial Pressure of Oxygen and Carbon Dioxide vs. Storage Time for Blood Equilibrated with 4.8% CO_2, 7.2% O_2 and Iced in the Same Plastic Disposable Syringe. (B.P. 741 mm Hg; ●, PCO_2; ○, PO_2.)

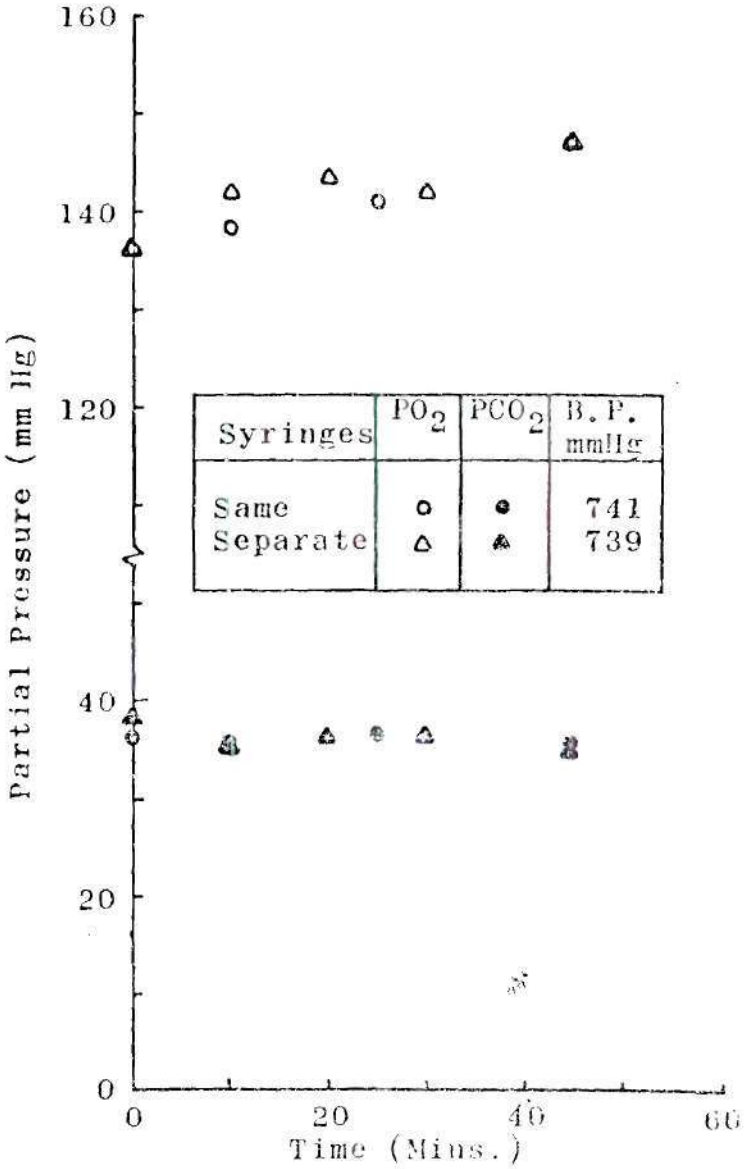


Figure 5. Partial Pressures of Oxygen and Carbon Dioxide vs. Storage Time for Blood Equilibrated with 5.1% CO_2, 19.7% O_2 and Iced

seen to be declining with the time of blood storage. Although there is some scatter in the data, the decline observed is distinct. Once again no change in the PCO_2 is seen. To summarize this, it is indicated from the results of these experiments that the equilibrated blood when stored in ice behaves in a manner depending upon the oxygen pressure with which the blood has been equilibrated.

As mentioned earlier in Chapter I, these changes in the partial pressure of oxygen in the blood can be caused by the following reasons:

1. Variations in the pH of the blood.

2. Air contamination or diffusion of gases through the syringe walls.

3. Shift in the oxyhemoglobin dissociation curve.

Series Three Testing

This series was performed to investigate the effects of air contamination and syringe material on the oxygen partial pressure of blood. The effect of syringe material was studied through two sets of experiments. The blood was equilibrated with 5.0 % CO_2 and 95.0 % O_2. It was then collected in a ten milliliters syringe preflushed with the equilibration gas. Each set consisted of two experiments, one using a plastic disposable syringe and the other using a glass reusable syringe. The first set was conducted similarly as those described in series two. The only difference in the procedure

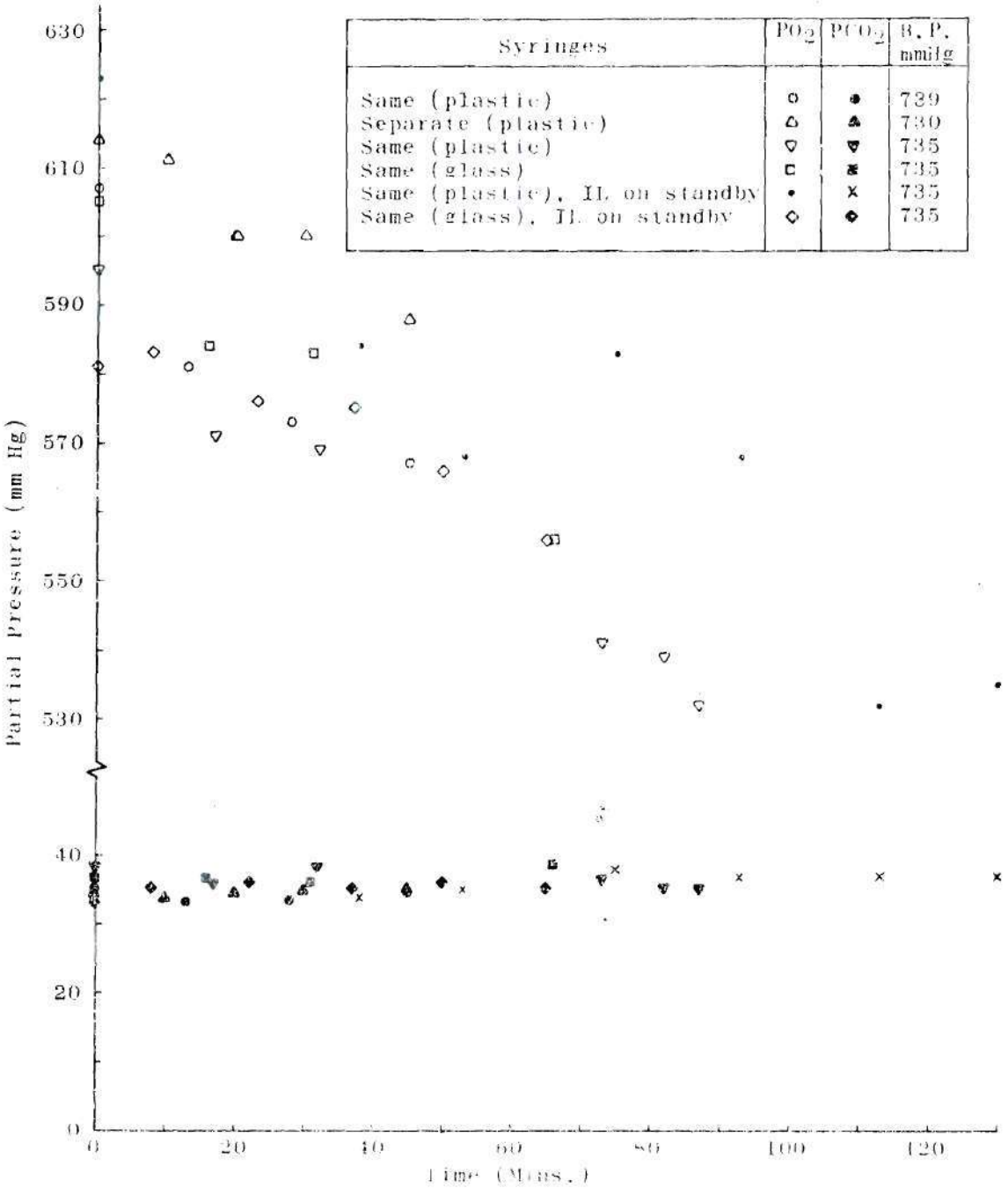


Figure 6. Partial Pressures of Oxygen and Carbon Dioxide versus Time for Blood Equilibrated with 5.0% CO_2, 95.0% O_2 and Stored in Ice.

of the second set was that the IL-313 was left in the standby mode between consecutive sample runs. This was done to prevent the electrode membrane from drying due to a constant flow of gas through the measuring chamber and also to determine if this produced any changes in the measured PO_2.

The results of these two sets are given in appendix II, Table 5. From Figure 6 it is indicated that the results of these two sets are not significantly different than those obtained in series two. Also the plastic as well as the glass syringes show the same pattern of PO_2 variations with the blood storage time. This is in agreement with the findings of Evers, et al. (5) and Laver, et al. (7) but does not agree with those of Hilti, et al. (6) and Fletcher (8).

The effect of air contamination was studied with particular attention given to the carbon dioxide partial pressure because the equilibration gas (5.1 % CO_2, 19.7 % O_2 and balance N_2) was used to equilibrate the blood. This gas is nothing but a mixture of carbon dioxide and air in the ratio of one to nineteen. Moreover equilibrated blood was collected and iced in syringes containing approximately one milliliter of room air. The first analysis of the experiment was performed with blood sample taken directly from the tonometer. Subsquent analyses were conducted at intervals of time using the blood stored in ice.

The results of this test are given in appendix II, Table 5. These results are shown in Figure 7. It is seen that

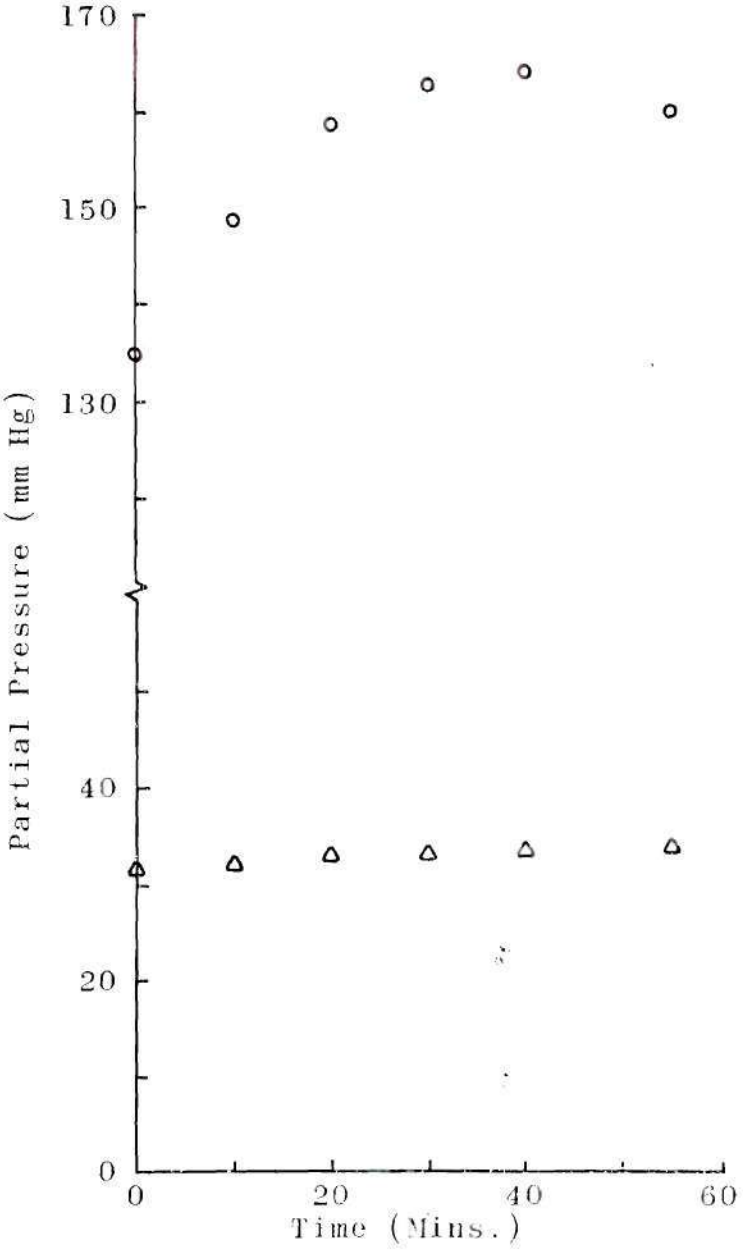


Figure 7. Partial Pressures of Oxygen and Carbon Dioxide vs. Storage Time for Blood Equilibrated with 5.1% CO_2, 19.7% O_2 and Iced in Plastic Disposable Syringes Containing Air. o, PO_2; Δ, PCO_2; B.P. 733 mm Hg

the PO_2 increases consistently with the blood storage time. It starts out at 134.5 mm Hg and is seen to reach a value of 164 mm Hg during a time interval of fifty-five minutes. This behavior of PO_2 is seen to be similar to that observed in previous experiments. Looking at the PCO_2 it is seen that there is no appreciable change, whereas it would be expected to drop had there been exchange of gases between air and the blood. This indicates that there is neither any effect of air contamination nor any diffusion of gases from the blood and through the syringe walls. This finding is in agreement with the conclusions of Hilti, et al. (6). It is also seen from the data (Table 5) that the pH of the blood does not change when the blood is stored for a short period of time. This indicates that changes in the partial pressure of oxygen in blood are not caused by the pH of the plasma.

Series Four Testing

Series four was conducted in order to determine if the percent saturation of oxyhemoglobin was also changing with the storage time. This information with the PO_2 data taken earlier will determine if there is a shift in the oxyhemoglobin dissociation curve with the storage time or not. This series was confined only to the plastic disposable syringes. Tests were conducted with blood stored in separate as well as same syringe. The procedure of blood equilibration and its storage was similar to that described in earlier series. EDTA was

used as the anticoagulant and the blood saturation measurements were made using the co-oximeter (IL-182).

Results of these experiments are given in appendix II, Table 6. Figure 8 shows the percent blood saturation at different storage times for blood equilibrated with 5.3 % CO_2 and 3.2 % O_2. It is seen that there is no significant change in the oxyhemoglobin saturation. Also there seems to be no difference in the use of same or separate syringes. Figures 9-11 show the data for blood equilibrated with 7.2 %, 19.7 % and 95 % oxygen respectively but none of them show any appreciable change in blood saturation. This indicates that O_2Hb, Hb and COHb saturations are independent of the equilibration gas, time of blood storage and use of same syringe or separate syringes for the blood gas analysis.

One experiment was conducted using sodium heparine in place of EDTA as the anticoagulant in the blood which was equilibrated with 5.1 % CO_2 and 19.7 % O_2. It was then collected anaerobically in a ten milliliter plastic syringe and analyzed in the same manner as described earlier in this series. The results from this experiment are shown in Figure 10 along with the results obtained using EDTA. From this figure it is indicated that the data from this experiment agrees well with that obtained using EDTA as anticoagulant in the blood. This indicated that the choice of anticoagulant does not affect the blood saturation with short storage time.

The last two experiments of the study were similar to

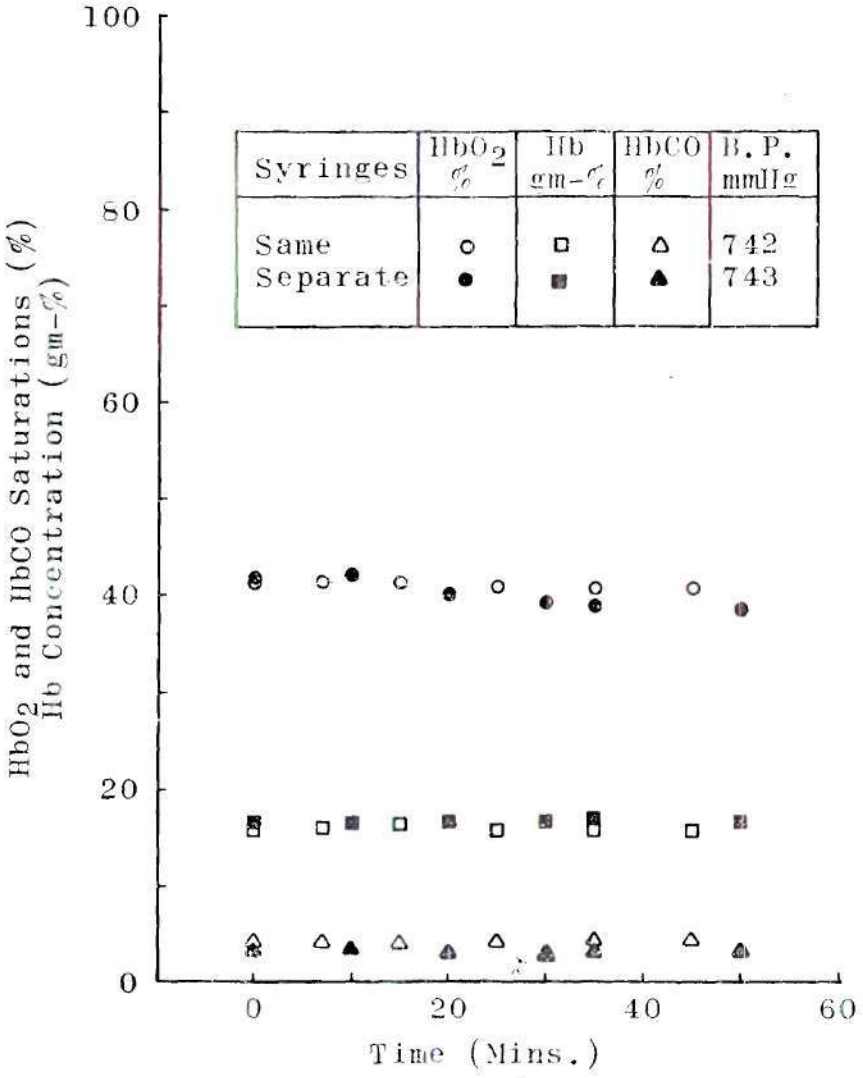


Figure 8. Concentrations vs. Storage Time for Blood Equilibrated with 5.3% CO_2, 3.2% O_2 and Iced in Plastic Disposable Syringes

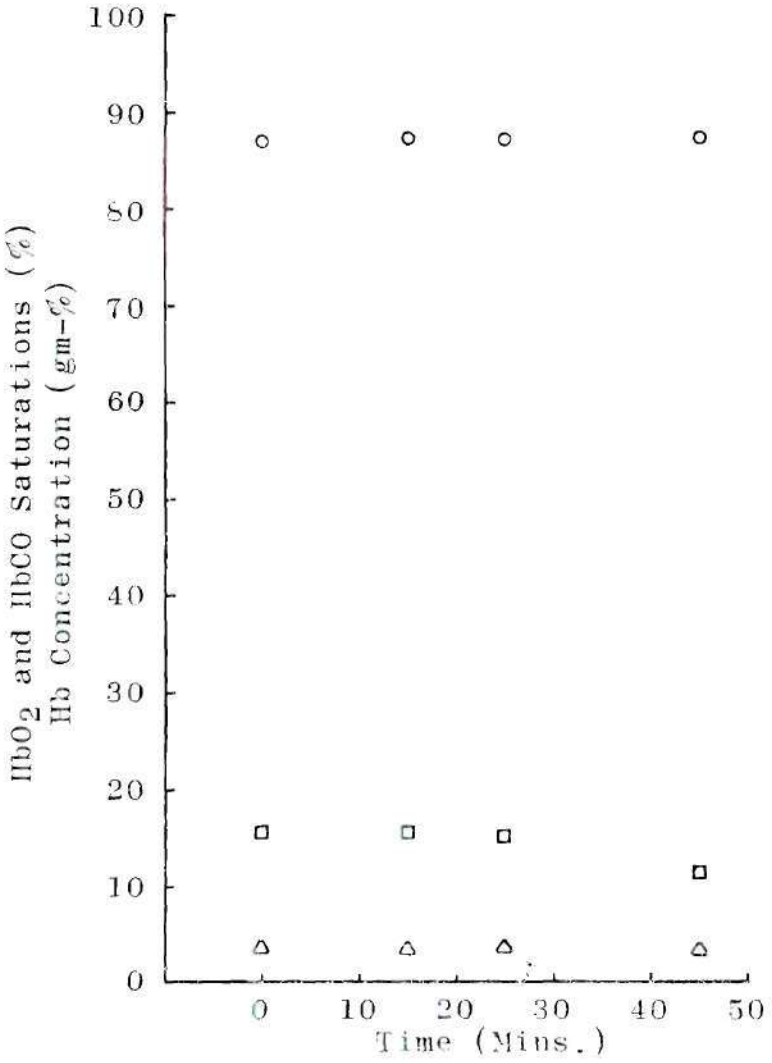


Figure 9. Concentrations vs. Storage Time for Equilibrated with 4.8% CO_2, 7.2% O_2 and Iced in Same Plastic Disposable Syringe. (○, HbO_2; □, Hb; △, HbCO and B.P. 741 mmHg.)

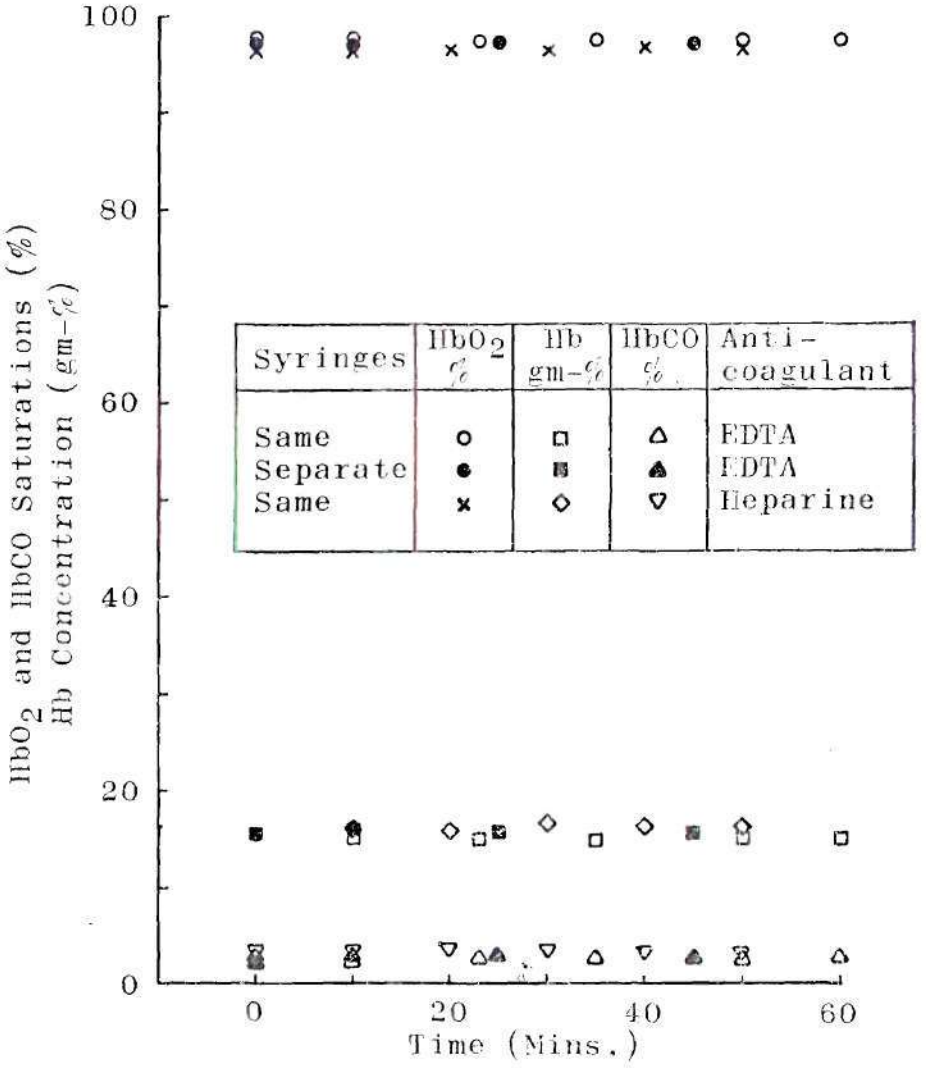


Figure 10. Saturations vs. Time of Storage for Blood Equilibrated with 5.1% CO_2, 19.7% O_2 and Iced in Plastic Disposable Syringes; (B.P. 739 mm Hg)

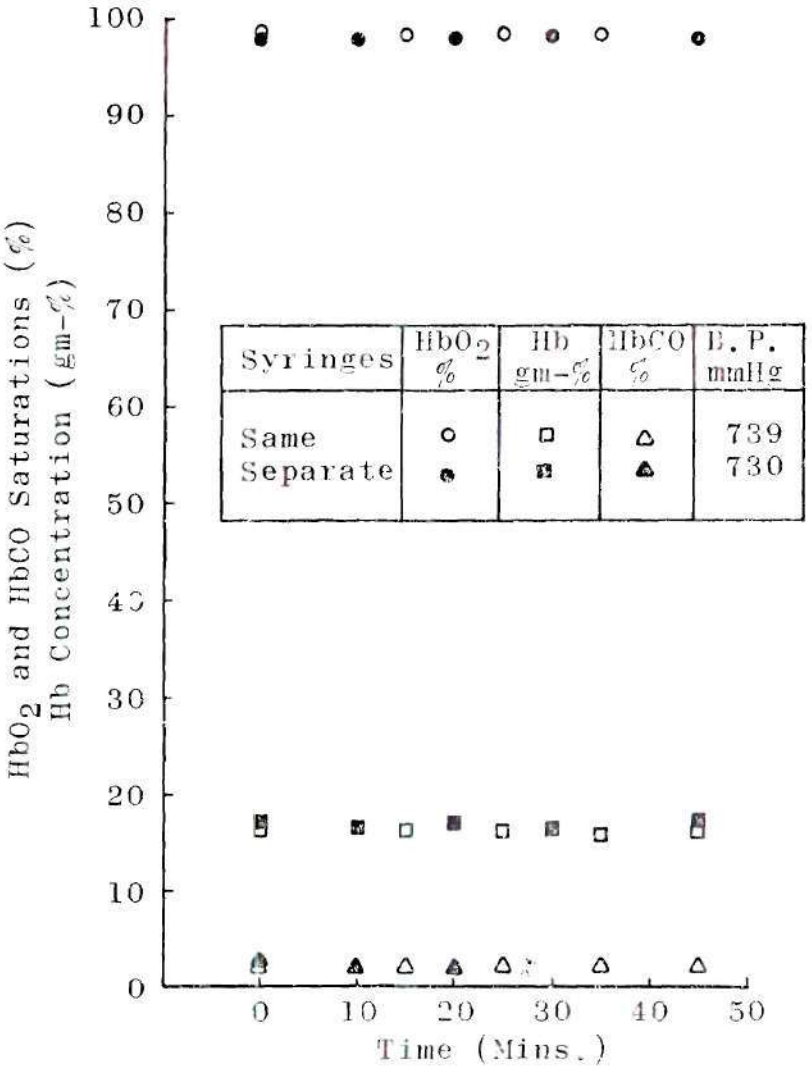


Figure 11. Concentrations vs. Storage Time for Blood Equilibrated with 5.0% CO_2, 95.0% O_2 and Iced in Plastic Disposable Syringes

the experiments described in series two except that IL-313 Blood gas analyzer was replaced by IL-113 Blood gas analyzer. Blood was equilibrated with 5.0 % CO_2 and 95.0 % O_2, and iced in separate plastic syringes. The IL-113 was checked for calibration after each sample run. This data is given in appendix II, Table 7 and shown in Figure 12. This figure shows the variation of PO_2 and PCO_2 with time of storage for equilibrated blood using the IL-113. It is seen that the partial pressure of oxygen in the blood immediately after it is equilibrated is in the range of six hundred mm Hg. After storing the blood in ice for a period of fifty minutes, the PO_2 dropped down to 578 mm Hg. Comparing Figures 6 and 12 it is seen that the drop in PO_2 in both cases is similar and does not make any difference whether the IL-313 or the IL-113 is used for the measurements of blood gases.

Data Analysis

A linear regression analysis was performed on each PO_2 group (very low, low, medium and high) separately. The correlation coefficient for the PO_2 group (very low) is 0.1519. Except for this particular group, the correlation coefficients for all other groups are significantly high (0.8928 for low; 0.6661 for medium and 0.8479 for high). This indicates that there is a strong dependence of blood oxygen partial pressure on the blood storage time and also on the initial PO_2 of the blood. If the blood is equilibrated with a gas, then the par-

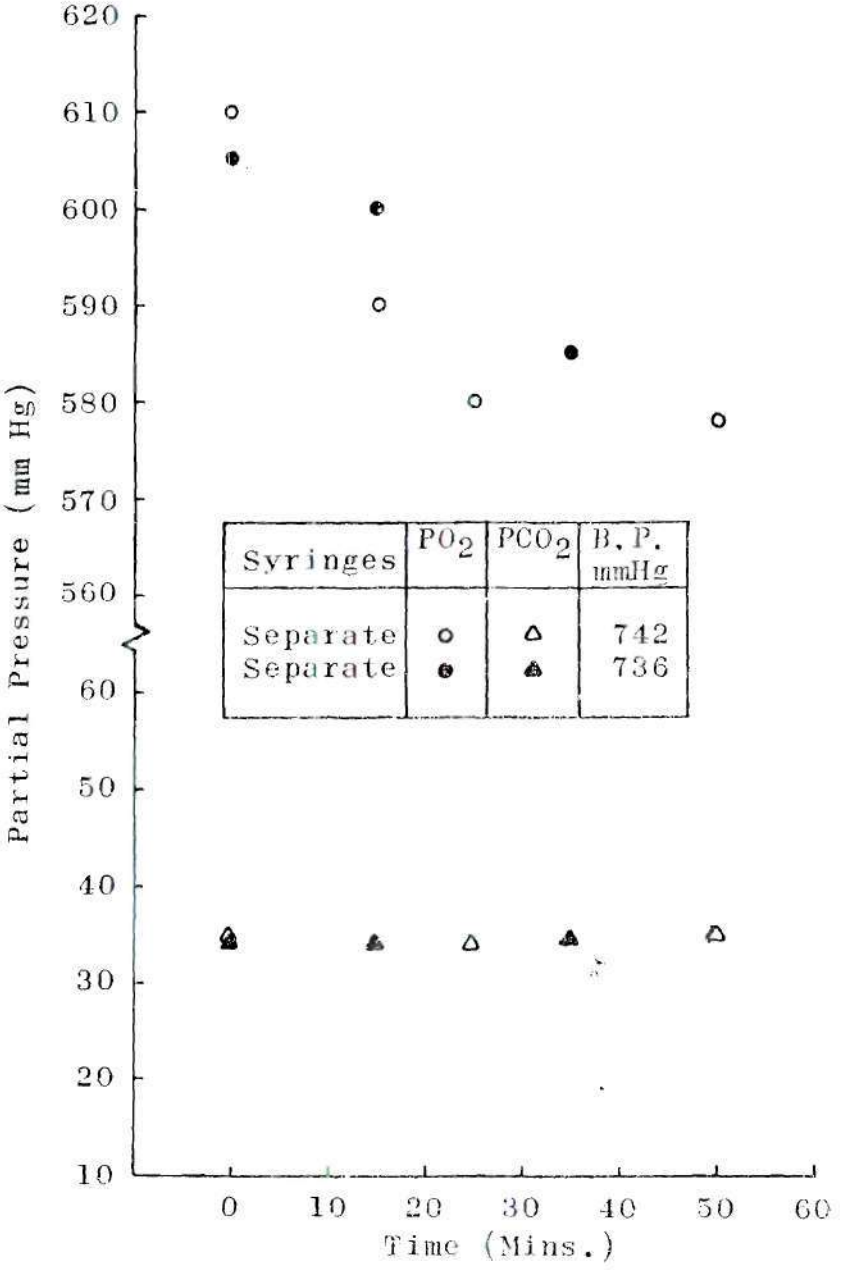


Figure 12. Oxygen and Carbon Dioxide Partial Pressures vs. Storage Time for Blood Equilibrated with 5.0% CO_2, 95.0% O_2 and Iced in Plastic Disposable Syringes; IL-113 Used for Gas Analyses

tial pressure of oxygen varies depending on the oxygen content of the equilibration gas. This is indicated by the slope of the regression line (Table 2). A negative slope indicates a drop while a positive slope indicates an increase in the PO_2. The partial pressure of oxygen in the high pressure range (approx. 600 mm Hg) is seen to drop with time (Figure 6) and is also indicated by a negative slope (Table 2). When PO_2 is around 150 mm Hg or below, it is found to increase (Figures 3-5 and Table 2). The rate of increase in PO_2 is proportional to the initial PO_2 of the blood (Table 2). Considering Figure 1 it is seen that at constant oxyhemoglobin saturation PO_2 increases with a shift in the curve to the left while it decreases with a rightward shift.

This shift in the oxyhemoglobin dissociation curve is caused either by a change in the pH value of the blood or by a change in the 2,3 DPG concentration. It is generally accepted (2) that 2,3 DPG is the most important regulator of the oxyhemoglobin dissociation at constant temperature and pH of the plasma. Lenfant, et al. (9) concluded that the PO_2 in the blood is directly dependent on the 2,3 DPG concentration. Mikeal Rorth (10) found that the 2,3 DPG concentration depended on the intracellular pH which increases with deoxygenation of the blood. The phenomenon of PO_2 variation with storage time might then be caused by a change in the 2,3 DPG concentration with storage time.

Table 2. Results of Linear Regression Analysis on PO_2 vs. Time

No. of data points	Equilibration gas (%)		Slope	Intercept	Correlation coefficient R*
	CO_2	O_2			
12	5.3	3.2	.006	22.02	.1519
5	4.8	7.2	.026	51.5	.8928
15	5.1	19.7	.369	138.3	.6661
39	5.0	95.0	-.585	600.7	-.8479

* R is the correlation coefficient which (as defined in Lectures on Biostatistics (12)) gives the degree of correlation between the variables - $\pm$1.0 indicating a perfect correlation, and 0 indicating no correlation.

CHAPTER V

CONCLUSTONS

From the results of the experiments performed during this study, it is concluded that

1. there is no statistical difference between the plastic disposable and glass reusable syringes when they are used for the storage of blood for short periods of time.

2. the change in blood PO_2 occur without any change in the pH value of the plasma.

3. the carbon dioxide partial pressure in the blood does not change with short storage time.

4. the blood saturation remains unaltered and is independent of short storage time.

5. with short storage time the blood oxygen partial pressure changes depending on the initial PO_2 to which the blood is saturated.

CHAPTER VI

RECOMMENDATIONS

The slopes of the regression lines (Table 2) present an interesting phenomenon. The magnitude and the direction of this slope show a dependence on the initial partial pressure of oxygen in the blood. If the blood is equilibrated with a gas then this slope bears a marked dependence on the oxygen content of the equilibration gas. This phenomenon can occur due to changes in the 2,3 DPG concentration and the slope of the regression line could be a measure of this change in the 2,3 DPG level.

If an experiment is designed for the measurements of the 2,3 DPG and carried out similarly as those for the measurements of PO_2, it would be possible to relate to it the findings of this study. In case the changes in 2,3 DPG are not found, some factors other than this must be sought to explain the drop or the rise in blood oxygen partial pressure with short storage time.

APPENDIX I

MEASURING ELECTRODES

MEASURING ELECTRODES

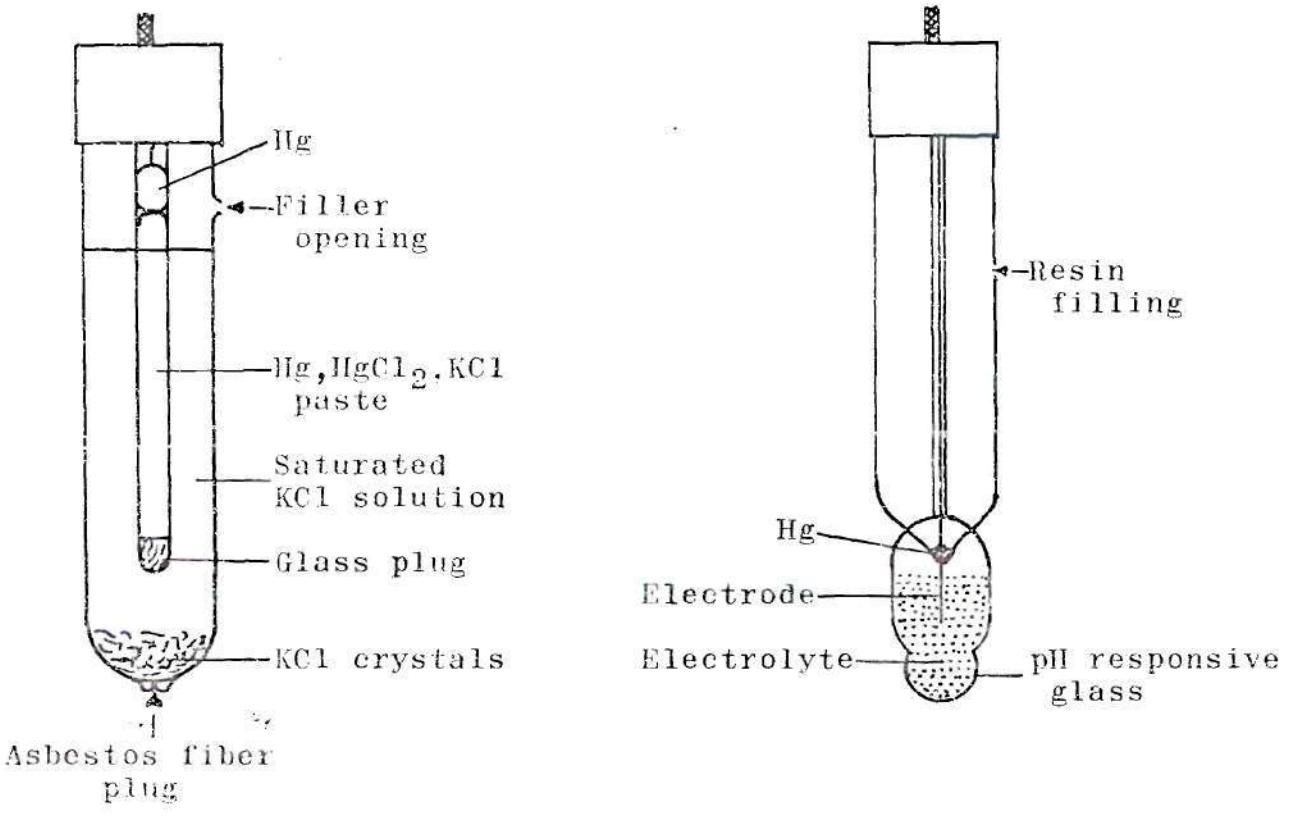
The partial pressures of oxygen and carbon dioxide in the blood are usually measured using electrodes. Principles involved behind these measurements are as follows.

pH Electrode

The blood pH is measured using a pH meter. It determines the potential difference between an indicator electrode and a reference electrode, amplifies this voltage difference and shows it on a meter either as pH or as millivolts. Figure 13 shows typical electrodes.

The most commonly used reference electrode is the Calomel electrode. This consists of metallic mercury in contact with a paste of calomel (mercuric chloride) and a solution of KCl saturated with calomel, as shown in Figure 13. As this electrode has the function to provide a steady reference voltage against which voltage changes at the glass pH membrane are referred, it is protected from contamination and dilution by the unknown solution, by housing it separately. This electrode makes contact with the unknown solution through a porous ceramic plug which allows current to flow without exchange of solution to the electrode.

The glass electrode is the most commonly used indicator electrode. The active element of a glass electrode is a special



CALOMEL ELECTRODE

GLASS ELECTRODE

Figure 13. Cross Sections of Calomel and Glass Electrodes

glass membrane. Contained on one side of this membrane is a solution of constant pH and on the other side is the solution of unknown pH. These two solutions differing in hydrogen ion concentration, produce across the glass membrane a potential difference (voltage) proportional to the difference in pH between the two solutions. The glass electrode has a small bulb of sensitive pH responsive glass filled with a solution of known pH. This solution is in contact with a wire of silver-silver chloride.

PCO_2 Electrode

The direct measurement of PCO_2 potentiometrically is an adaptation of pH measurement. A combination pH glass and reference electrode (Figure 14) is used. The pH glass electrode is covered by a Teflon membrane. This membrane is permeable to gas (CO_2) but not to ions. Nylon strands between the electrode glass and the membrane provide a space for a thin layer of KCl and a bicarbonate solution. This layer acts both for solution of CO_2 and to act as a liquid junction between glass and reference electrode. This reference electrode (silver - silver chloride) is located as shown in Figure 14. The combination electrode is inserted into the lucite jacket filled with KCl - Bicarbonate solution. Electric contact between the reference electrode and the measuring electrode is made via the port opening the reference chamber to the jack-

et electrolyte.

Carbon dioxide diffuses across the membrane in either direction in response to the partial pressure difference and in the electrolyte water it produces carbonic acid causing a change in the hydrogen ion concentration (See Equation 6).

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad (6)$$

The pH electrode picks up the change in CO_2 concentration as a change in pH of the electrolyte and develops a voltage exponentially related to the carbon dioxide partial pressure, PCO_2.

PO_2 Electrode

Determination of PO_2 is made amperometrically. The PO_2 electrode produces a current, at a constant polarizing voltage (0.6 V), which is directly proportional to the oxygen partial pressure (PO_2) diffusing to the reactive surface of this electrode. Figure 14 shows the general arrangement of the PO_2 electrode system. The PO_2 electrode consists of a platinum wire sealed in glass with its tip exposed. This platinum cathode is covered with an oxygen permeable membrane that is usually made of polypropylene, teflon or polyethylene. A thin film of KCl - phosphate buffer seperates the membrane from the platinum wire. Inside the membrane and in contact with the KCl buffer is the silver - silver chloride reference

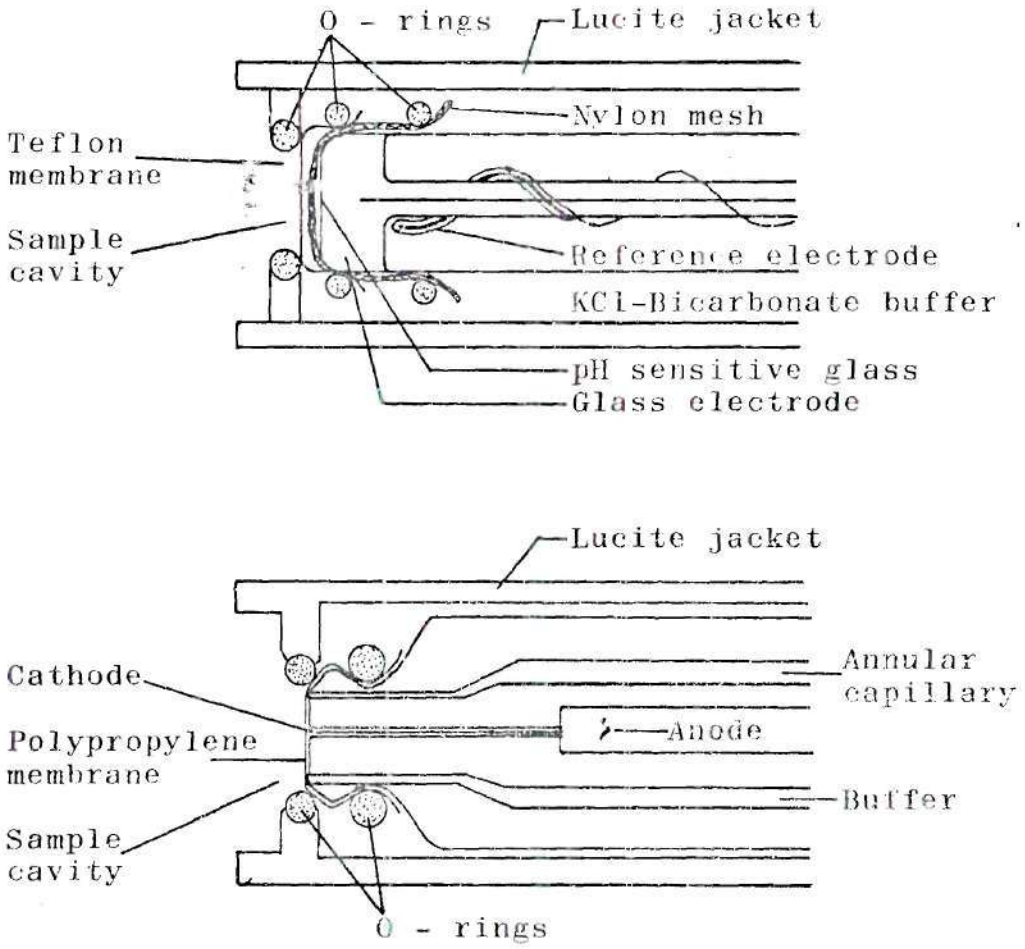


Figure 14. Cross Section of Carbon Dioxide and Oxygen Electrodes

electrode (anode). A voltage of (-)0.6 volts applied to the platinum cathode gives rise to a current directly proportional to the oxygen available at the membrane surface (Equation 7).

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \qquad (7)$$

This shows that each molecule of oxygen reacts with four electrons and to provide for these electrons at the platinum cathode, silver at silver chloride anode is oxidized providing these electrons (Equation 8).

$$Ag + Cl^- \rightarrow AgCl + e^- \qquad (8)$$

It is this flow of electrons that is measured.

APPENDIX II

TEST DATA

Table 3. Series One Test Data

Equilibration Gas: 5.2% CO_2, 19.7% O_2.

Specimen	B. P. (mm Hg)	Time (Mins.)	IL-313 (mm Hg)	
			PCO_2	PO_2
Saline	739.0	0	25.3	122.6
		20	28.8	112.3
		30	30.3	117.4
		40	31.7	119.0
		50	30.8	117.1
		60	30.9	120.2
		70	31.2	119.2
Blood	746.0	0	34.1	125.1
		5	32.4	123.0
		10	32.1	123.2
		15	31.9	120.7
		20	32.4	124.6
		30	32.2	123.5
		40	32.5	124.7
		50	32.9	122.2
		60	32.7	123.4
Blood	738.0	0	36.1	126.3
		5	33.8	122.4
		10	34.0	123.6
		20	34.2	127.8
		30	33.4	126.8
		40	32.8	124.0
		50	33.4	126.0
		60	33.8	129.4

Table 4. Series Two Test Data

Syringes	B. P. (mm Hg)	Time (Mins.)	IL-313 (mm Hg)	
			PCO_2	PO_2
Equilibration Gas: 5.3% CO_2, 3.2% O_2.				
Same	742.0	0	36.8	22.3
(plastic		7	36.1	22.6
disposable)		18	36.0	22.9
		25	36.0	23.0
		40	36.0	23.0
		55	35.7	23.1
Separate	743.0	0	37.0	21.7
(plastic		10	36.7	21.5
disposable)		20	37.5	21.8
		30	36.9	21.1
		40	37.1	20.8
		60	33.2	22.3
Equilibration Gas: 4.8% CO_2, 7.2% O_2.				
Same	740.5	0	33.6	51.7
(plastic		10	33.1	51.5
disposable)		20	32.9	51.9
		30	32.5	52.5
		40	32.5	52.5
Equilibration Gas: 5.1% CO_2, 19.7% O_2.				
Same	740.5	0	36.4	136.1
(plastic		10	35.9	138.3
disposable)		25	36.2	140.8
		45	35.4	146.8
Separate	739.0	0	38.2	136.3
(plastic		10	35.4	141.9
disposable)		20	35.5	143.2
		30	35.4	141.8
		45	34.9	146.8
Equilibration Gas: 5.0% CO_2, 95.0% O_2.				
Same	739.0	0	34.9	607
(plastic		13	33.1	581
disposable		28	33.3	573
		45	34.6	567

Syringes	B. P. (mm Hg)	Time (Mins.)	IL-313 (mm Hg)	
			PCO_2	PO_2
Equilibration Gas: 5.0% CO_2, 95.0% O_2.				
Separate (plastic disposable)	730.0	0	34.4	614
		10	33.9	611
		20	34.4	600
		30	34.6	600
		45	34.8	588

Table 5. Series Three Test Data

Syringes	B. P. (mm Hg)	Time (Mins.)	IL-313 (mm Hg) PCO_2	IL-313 (mm Hg) PO_2	pH
Equilibration Gas: 5.0% CO_2, 95.0% O_2.					
Same (plastic disposable)	735.0	0	38.0	595	7.42
		17	36.0	571	7.42
		32	38.0	569	7.42
		73	36.4	541	7.41
		82	35.0	539	7.42
		87	35.0	532	7.42
Same (glass reusable)		0	36.5	605	7.44
		16	36.5	584	7.46
		31	35.8	583	7.46
		66	38.7	556	7.37
Same (plastic disposable) with IL on standby.		0	36.0	623	7.42
		38	34.0	584	7.42
		53	35.0	568	7.42
		75	38.0	583	7.41
		93	37.0	568	7.42
		113	37.0	532	7.42
		130	37.0	535	7.42
Same (glass reusable) with IL on standby.		0	33.0	581	7.47
		8	35.0	583	7.43
		23	36.0	576	7.47
		37	35.0	575	7.47
		50	36.0	566	7.46
		65	35.0	556	7.46
Equilibration Gas: 5.1% CO_2, 19.7% O_2.					
Separate (disposable) with air.	733.0	0	31.5	134.7	
		10	31.7	148.9	
		20	33.0	158.3	
		30	33.4	162.3	
		40	33.5	163.9	
		55	33.7	159.9	

Table 6. Series Four Test Data

Syringes	B. P. (mm Hg)	Time (Mins.)	Co-oximeter HbO_2 (%)	Hb (gm %)	HbCO (%)
Equilibration Gas: 5.3% CO_2, 3.2% O_2.					
Same (plastic disposable)	742.0	0	41.3	15.9	4.1
		7	41.2	16.0	4.1
		15	41.3	15.9	4.1
		25	40.8	15.9	4.1
		35	40.7	16.0	4.2
		45	40.7	15.7	4.2
Separate (plastic disposable)	743.0	0	41.9	16.6	3.1
		10	42.0	16.6	3.2
		20	40.1	16.5	3.1
		30	39.3	16.5	3.0
		35	38.9	16.7	3.1
		50	38.5	16.7	3.1
Equilibration Gas: 4.8% CO_2, 7.2% O_2.					
Same (plastic disposable)	740.5	0	87.0	15.5	3.8
		15	87.1	15.4	3.5
		25	86.9	15.1	3.6
		35	87.2	15.2	3.5
		45	87.1	15.4	3.5
Equilibration Gas: 5.1% CO_2, 19.7% O_2.					
Same (plastic disposable)	740.5	0	97.3	15.4	2.6
		10	97.5	15.1	2.7
		23	97.3	15.0	2.7
		35	97.3	14.9	2.5
		50	97.4	15.2	2.6
		60	97.3	15.0	2.7
Separate (plastic disposable)	739.0	0	96.6	15.9	3.6
		10	96.5	16.0	3.6
		20	96.6	15.9	3.4
		30	96.6	16.3	3.4
		40	96.8	16.3	3.5
		50	96.6	16.1	3.2
Same (plastic disposable) with heparine	740.0	0	97.0	15.3	3.0
		10	97.0	15.7	2.9
		25	97.1	15.7	2.7
		45	97.0	15.7	2.9

Syringes	B. P. (mm Hg)	Time (Mins.)	Co-Oximeter HbO_2 (%)	Hb (gm %)	HbCO (%)
Equilibration Gas: 5.0% CO_2, 95.0% O_2.					
Same (plastic disposable)	739	0	98.4	15.9	2.1
		15	98.4	16.1	2.0
		25	98.4	16.0	2.0
		35	98.3	15.7	2.1
		45	98.3	16.0	2.1
		0	97.8	17.0	2.8
		10	97.8	16.9	2.7
		20	98.0	16.7	2.6
		30	98.0	16.6	2.3
		45	98.1	17.0	2.5

Table 7. Test Data Obtained Using IL-113

Equilibration Gas: 5.0% CO_2, 95.0% O_2.

Syringes	B. P. (mm Hg)	Time (Mins.)	IL-113 (mm Hg)	
			$\overline{PCO_2}$	PO_2
Separate (plastic disposable)	736.0	0	34.5	605
		15	34.0	600
		35	34.5	585
Separate (plastic disposable)	742.0	0	34.8	610
		15	34.0	590
		25	34.0	580
		50	34.8	578

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