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A Comparison of the determination of carbon dioxide content and carbon dioxide combining power as measures of the true bicarbonate concentration

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A COMPARISON OF THE DETERMINATIONS OF CARBON DIOXIDE CONTENT AND CARBON DIOXIDE COMBINING POWER AS MEASURES OF THE TRUE BICARBONATE CONCENTRATION

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INTRODUCTION

The following material has been gathered to show the measurement of carbon dioxide content is a more accurate and a more feasible method of determining the true bicarbonate concentration than is the measurement of carbonate dioxide combining power.

The reasoning for the work done in this paper was based on Peters and Van Slyke's (126b) work stating that

> "It (CO_2 combining power) does not give so exact an indication of the state of the acid base balance in the blood as does the direct determination of the CO_2 content made on plasma obtained under complete anaerobic precautions and without equilibrating with 5.5% CO_2. The equilibration with air of physiologically normal CO_2 tension at room temperature gives the plasma content of H_2CO_3 at $20^{o}$is 1.6 times as great as at 38^{o}. In consequence, the equilibrium H_2CO_3 + B-Protein = $BHCO_3$ + H-protein, is shifted to the right so that $BHCO_3$ is increased also. The bicarbonate content, or CO_2 capacity found in venous or arterial blood plasma by this procedure, in blood handled strictly without contact with air, was found in fact by Stadie and Van Slyke to be about 3 volumes percent higher than the total CO_2 content of the plasma analyzed immediately after it was centrifuged. The relationship is quite constant...In conditions where it is doubtful whether over or under ventilation of CO_2 rather than loss or retention of non volatile acids, is responsible for an acid-base disturbance it is necessary to determine both pH and the CO_2 content in plasma as nearly as possible unchanged from its condition in the circulation, and in these cases the Van Slyke-Cullen CO_2 capacity method can not be used in place of direct CO_2 analysis of the anaerobically separated plasma."

METHODS OF CO2 MEASUREMENT

Three methods which may be used to measure the bicarbonate concentration of blood are:

1. Titration method
2. Gasometric method
3. Calculation method, where total CO2 content and blood pH are measured and the bicarbonate concentration is calculated using the Henderson-Hasselbalch equation.

A chronological history of these methods will be reviewed but discussion will be limited to gasometric methods which will be used in this work.

Walter (166) in 1877 was the first to use CO2 measurements to find the bicarbonate concentration. Von Jaksch (165) and Magnus-Levy (102) in 1888 used titration of blood plasma but their results were only proportional to the bicarbonate concentration because of the acid binding properties of the phosphates and proteins. Hoppe-Seyler (81) in 1903 demonstrated that the proteins carried down considerable alkali during their precipitation. Cullen (29) and McClendon (114) were the first to combine the hydrogen electrode with titration methods. Van Slyke et al (162) transformed the plasma bicarbonate into the salt of the acid used and back titrated. Stillman (153) compared titration

methods with CO2 capacity and found an agreement of 2 mM per liter. Haskins and Osgood (62) used a modified titration procedure while Summer and Hubbard (154) recommended methods requiring the precipitation of proteins.

The major difficulties with titration methods are the buffer effect of proteins and the loss of CO2 during the titrations. Since 1949 Wooton and King (188) have suggested a capillary method using acid, phenol red and standards for comparison. Their accuracy is 4 volumes percent. Scribner (144) described a bedside determination method which gives an accuracy of one mEq when compared with Van Slykes gasometric method. Epstein (47) proposed a titration method using cationic exchangers and nonvolatile acids. He reports his results in alkaline groupings of albumin which supposedly bind chloride. Natelson and Barbour (116) did in vivo titrations with sodium bicarbonate of blood of patients in acidosis and correlated their results by using a pH meter. Kibrick et al (88) used a microdetermination method consisting of a glass electrode and back titration.

The analysis of blood gases has been studied by many men (75, 157,11,51,43) with Donald Van Slyke

contributing a major portion of the advancements.

In 1910 Barcroft and Roberts (10) made the first improvements in blood-gas analysis techniques. Henderson (75,76) suggested the effect of the chloride shift which was to become important in future work. Barcroft and Higgins (9) made the first attempt at establishing constants for use in blood-gas analysis. Christiansen, Douglas, and Haldane (171) were the first to saturate blood with air containing alveolar CO2 concentration but Van Slyke and Cullen (157) were the first to devise a practical method for measuring CO2 capacity of plasma. At this time McClendon et al (115) and Henderson and Morriss (74) discussed methods of determining CO2 in alveolar air and blood. Austin and Jonos (6) drew blood under oil and saturated it with air containing 6 percent CO2 and found that blood would hold more CO2 at room temperature than at 38 degrees centigrade. This also holds true for plasma. Stadie and Van Slyke (152) noted that venous CO2 content gave a closer approximation of the arterial CO2 concentration than did venous CO2 combining power. Scott (143) observed an increase in buffer in emphysema

and also an increase in CO2 content giving the first studies in respiratory acidosis. The result of Hasselbalch's work in 1916 and Van Slykes in 1922 made it possible to calculate the bicarbonate concentration by using the pH of blood (64,156).

The study of blood gas analysis continued in 1921 with the development of a mechanical shaker for the Van Slyke apparatus, further modifications of techniques and the discussion of CO2 absorption curves by Peters et al (150,122,123,124,125,61). Van Slyke (156) suggested the formula $(BHCO3) = \frac{1}{1 - 10^{pK' - pH}}$ (CO2) to be used in calculating the true bicarbonate concentration when the CO2 content and pH is known. This was derived from Hasselbalch's equation.

Austin and his co-workers in 1924 (2,4) found methods for the estimation of CO2 in the presence of ether which led to the finding of ether acidosis. They also established the CO2 pK' of 6.10 and Bohr solubility coefficient of .510. Hawkins (72) found that rabbits and guinea pigs were not satisfactory for acid base equilibrium experiments but that rats

were suitable.

At this point Austin et al (5) summed up the preliminary treatment of blood-gas analysis and found that experimental work on blood could be done with analytical accuracy if the following errors were considered:

1. Hemolysis
2. Formation of non-volatile acids in blood
3. Formation of CO2 and consumption of O2 by metabolism of whole blood.
   Harrop states that oxalated normal human blood loses 0.1 to 0.4 volumes percent CO2 in 6 hours at 38 degrees.
4. Uniform mixtures of cells and plasma
5. Change of equilibrium during separation of gasses and liquid phases
6. Collection and preparation of blood.

Van Slyke, Sendroy, Hastings and Neill (159) found that the difference in solubility of CO2 in serum and water was due to the following:

1. Salts ----- depress solubility by 3%
2. Proteins -- depress by replacing water
3. Lipoids --- raise solubility about 4% of normal serum.

The next major change in CO2 measurement techniques took place in 1924 when Van Slyke and Neill (158) combined the use of vacuum extraction and manometric measurement -- a method of greater accuracy than volumetric measurements. Harington and Van Slyke (61) continued investigation of techniques. Eisenman (45) in a report on the use of potasium oxalate as an anticoagulant stated that potasium oxalate has such a varied and inconsistant influence on the electrolyte distribution in blood that it is impossible to establish an average correction for its effects. If potasium oxalate is used in CO2 measurement the probably accuracy is around one volume percent.

Since 1928 Van Slyke et al (159,160) have added much information on gas and electrolyte equilibria. Schrock and Hastings (141) introduced a micro-technique which measured blood pH and CO2 with 2% accuracy. Exton, Schattner and Rose (49) in 1941 described a colorimetric measurement using only .1 cc plasma but taking the same time to do as a Folin Wu determination. Scholander and Roughton (133,134) described a micro-gasometric technique based on a syringe capillary method requiring 13

cubic millimeters of blood and they report an accuracy of one volume percent when compared with Van Slyke's method. Lilienthal and Riley (94) in 1946 modified the Scholander method and obtained their blood from heated ear lobes. They had a reproducibility of 0.7 volumes percent with a 0.6 volumes percent difference when compared with the Van Slyke method. Besides technical errors their method could be affected by the dilution of heparin, tissue fluids, ESR, and by blood O2 saturation. Fürst and Mrstad (53,54) again altered Scholander-Roughton technique but added little new knowledge. Gabardi and Davenport (55) introduced a new method of obtaining plasma and one which was used in obtaining the data for this paper. The method with minor changes makes the measurement of CO2 content much easier and more feasible than that of CO2 combining power.

Holmes (80), Vogt and Brench (164) and Kinoshita, Bunker and Scholander (89) continued work on the Scholander apparatus and they produced a workable method for determining CO2 content with small amounts of blood although the accuracy at the best is plus or minus 2 mM/liter.

The last minor alteration of the Van Slyke method was done by Lockhead and Purcell (98) in 1951 although most of their work was concerned with the measurement of O2, CO and hemoglobin contents. The new method has been suggested by Behrmann and Hartman (12) who propose to measure CO2 using a Beckmann O2 analyzer.

Singer and Hastings (147) found the normal CO2 content to average 28.2 mM/liter with a range of 24 - 33 mM/liter. They suggest that for adequate discussion of acid-base balance five readings are needed:

1. Hematocrit
2. Blood or alveolar pCO2
3. Plasma or blood pH
4. Whole blood buffer base concentration
5. Plasma or whole blood CO2 content.

METHODS OF pH MEASUREMENTS

Blood pH measurement has gone through various phases of development until the present time when the glass electrode is probably the easiest and most accurate instrument of measurement. In 1904 Friedenthal (52) and Salm (138) originated the principles of the colorimetric method for the determination of pH. Srensen (148) used their principles and devised a practical application for them. From this time on many different methods of colorimetry have been suggested.

Walpole (40) in 1910 used a comparator system. Bjerrum (17) introduced the "wedge" principle in 1914 but Levy, Rowntree and Marriott (93) in 1915 were the first to apply the colorimetric method to blodd. Dale and Evans (39) improved the above methods and Gillespie (57) suggested a bicolorimetric procedure. Michaelis (106) used mono-chromatic indicators and Cullen (31) in 1922 diluted the plasma 1 - 20 and determined the pH at room temperature by comparison with a standard buffer solution. Hastings and Sendroy (67) modified Cullen's method by introducing Gillespie's bicolorimetric procedure and reading both standard and unknown solutions at 38° centigrade. Myers and Muntwyler

(112) used this method and reported variations of 0.1 pH units. Myers, Schmitz and Booker (113) used Bjerrum's wedge principle and Cullens correction. Marriot also suggested a method of dialisis and colorimetry.

The colorimetric method is reasonably simple and rapid method but is unreliable with biological fluids because of variables such as salt effect, protein effect, loss of CO2, dilution effects, temperature changes, the affect of color indicators and difficulty in reading in the presence of hemolysis and high serum icterus index. The difference in pH measurements by electrometric methods and colorimetry and the effect of the above variables have been discussed by many authors (13,7,84,149,127,90,36).

Dialyzing blood and then doing colorimetric determinations was thought to be practicable by Dale and Evans (39) and Lindhard (96). Eisenman (46) in 1927 suggested a gasometric method for evaluating blood pH. Hawkins (71) suggested a micro-method using phenol red which gave an accuracy of plus or minus 0.03 pH units when compared with Cullens method. Holmes (78) and Holmes and Snyder (79) introduced photometric and spectrophotometric deter-

minatitions which increased the analytical accuracy but not the basic accuracy of the determinations. Linderstrom and Lang (95), Robinson, Price and Cullen and Robinson and Hogden and Cullen (129,130, 131) in 1941 discussed the effect of serum proteins on colorimetric tests. (19,145) Van Slyke et al (163) using photometric apparatus and comparing results with glass electrodes and hydrogen electrodes found an average deviation of 0.002 pH units with a maximal deviation of 0.04. Raabe (128) using micro methods obtained an accuracy of 0.1 pH units. Rutlledge (136) in 1948 using a spectrophotometric method found an average difference of 0.01 to 0.02 pH units.

The electrometric measurement of pH is probably the best method although there is as yet no method for exact calibration and comparison. The use of the hydrogen electrode is limited in blood because of both platinum poisons such as proteins and sulfur and of the difficulties in maintaining proper CO2 tension.

Nernst (117) in 1889 demonstrated the electrolytic solution tension of metals which was to become the basis for electrometric measurement.

Hober (77) in 1900 was the first to apply the hydrogen electrode for blood pH measurement. Hasselbalch and Lungsgaard (66) were the first to determine the "exact" pH of blood. Hasselbalch (63,64,65) made the study of pH practicable and Milroy (110) suggested that normal blood pH was 8.5. Parsons (121) showed that the electrometric determination of the pH of plasma is the same as that of whole blood if the loss of CO2 is prevented but Rosenthal (132) in 1948 showed that the plasma had to be obtained at 38° in order to be the same as whole blood at 38°. According to Donegan and Parsons (40), Warburg (167) and Cullen (30) and accuracy of 0.01 pH may be obtained with a Hydrogen electrode.

Helmholtz (73) in 1881 was the first to use a "glass electode." Haber and Klemenziewicz (60) were the first to formulate an equation for the electrode. Cremer (28), Clark (21), Horovitz (82), Brown (20), Kerridge (87), Michaelis (107), and Hughes (83) added further knowledge as did McClendon (114).

Kerridge applied the electrode to blood and found that it compared favorably with the hydrogen

electrode. MacInnes and Dole (99) descrived a "simple" technique for making glass membranes to be used as electrodes.

Early in the measurement of blood pH the quinhydrone electrode received much consideration. Einar Biilmann (14) demonstrated that the quinhydrone electrode could be used to measure blood pH as did various other authors (59,16,142,24,15). Lester (40) and Kolthoff (90) discussed temperature coeficients and salt and protein errors of this electrode. Varying results were reported for the quinhydrone electrode (27,34,135,97,111,124,104,105, 35,26,25,33) but Cornelius Daly (40) in his masters thesis summed up their efforts and experimentally showed that the quinhydrone electrode was of little use for biological fluids.

In order to correlate blood pH measurements with clinical conditions a CO2 pK was needed as was a correlation of blood bicarbonate and pH. (125) Cullen, Keeler and Robinson (37) recommended a pK_1 of 6.10 at 38° centigrade for blood. This was a modification of the work done by Hasselbalch (64), Parsons (121), Donnegan and Parsons (40) and Warburg (167). Hastings, Sendroy, and Van Slyke (68) in

1928 found the pK_1 value to be 6.10 using the CO_2 solubility coeficient of 0.510 as did Robinson, Price and Cullen (130) and Dill, Daly and Forbes (42).

In the early 1930's there was little change in techniques of pH measurement (11,3,22,137,151,101,108, 100) but with the advent of usable glass electrodes an attempt was made to test their validity. Stadie, O'Brien and Laug (151) found in testing serum at 38° with glass and hydrogen electrodes that the difference in readings was 0.01 pH units. Laug (91) in 1934 found that there is a slight decrease in the pH of whole blood at 38° immediately after removal from the body. Massive concentrations of NaF (1 - 2%) against the usual amounts of 0.05 - 0.1% are needed to stop this reaction which is thought to be caused by lactic acid. He reported that if the transfer of blood from the body to electrode took less than 2.5 minutes there was no acid change. Havard and Kerridge (70), Laug (91,92) Yoshimura (169,170) and Haugaard and Lundstien (69) also discussed this phenomena. Sendroy, Shedlovsky and Belcher (146) in 1936 reported as shown by Evans (48) that latic acid forms in freshly shed blood at 38° and with in one-half hour there is a fall of about

0.03 to 0.05 pH units.

The optimal technique for measuring blood pH was still not to be had. Nims (118) in 1938 suggested a method for direct recording of pH in vivo and used it with Marshal (119) to test blood reaction to respiration, acids, salts, dextrose and adrenalin. D'Elseaux, Frank, Blackwood, Palmer and Sloman (44) in 1942 reported that various types of heparin (products of various companies) produced different pH's but "...under basal conditions, the fluctuations in arterial pH of normal individuals are limited to changes in the third decimal place." Rosenthal (132) found that there was a linear relationship to blood pH and temperature. Kelsey and Leinbach (85) stated specifically that blood pH was more related to prognosis than was CO2 capacity. Graig, Lange, Oberman, and Carson (58) in 1952 have presented the newest techniques for the analysis of blood pH.

Cullen and Robinson (38) in 1923 were the first to give reasonably accurate blood pH ranges. They found in normal medical students that plasma pH varied from 7.28 - 7.41 and that 21 of 27 tests lay between 7.35 and 7.40. Kelsey and Leinbach (86) using a standard Beckman pH meter, open and

sealed glass electrodes, withdrawing the blood anaerobically and centrifuging under oil and considering but not allowing for the CO2 solubility in mineral oil, that the average variation of blood under oil from 15 minutes to 3 hours was a plus or minus 0.03 units. This was the same for blood centrifuged up to 10 minutes. The maximum error in split samples was considered to be 0.07 pH units. The greatest variations between open and closed electodes was 0.05. They found the average normal pH value to lie between pH of 7.3 and 7.56. They concluded that blood could be drawn at the bedside and then be brought back to a lab and pH measurement would be within clinical accuracy.

MATERIALS AND METHODS USED

Apparatus for withdrawing blood anaerobically.

1. Number 21 needles one and one-half inches long.
2. Five cc plain syringes to be described later.

Apparatus for CO_2 measurement.

1. Four Ostwald 1 ml pipettes with rubber tips.
2. Two Van Slyke volumetric apparatuses.
3. One centrifuge capable of holding 5 cc syringes with plungers extended.
4. Four 150 cc separatory funnels.
5. One apparatus for blowing air over glass beads.
6. One mercury barometer with brass scale.
7. One centigrade thermometer.
8. $NaHCO_3$ solution, 2.2568 grams per 1000 cc of solution.
9. 100 cc of 1 N lactic acid made by taking 10 cc of lactic acid of 1.21 specific gravity and diluting it with 90 cc of distilled water.
10. 100 cc of 0.1 N lactic acid made by taking 10 cc of 1 N lactic acid and diluting with 90 cc of distilled water and then bringing it to the boiling point to remove CO_2. Pour into stoppered bottle while still hot.
11. Dropper bottle full of CP caprilic alcohol.

12. One 2 cc delivery pipette.

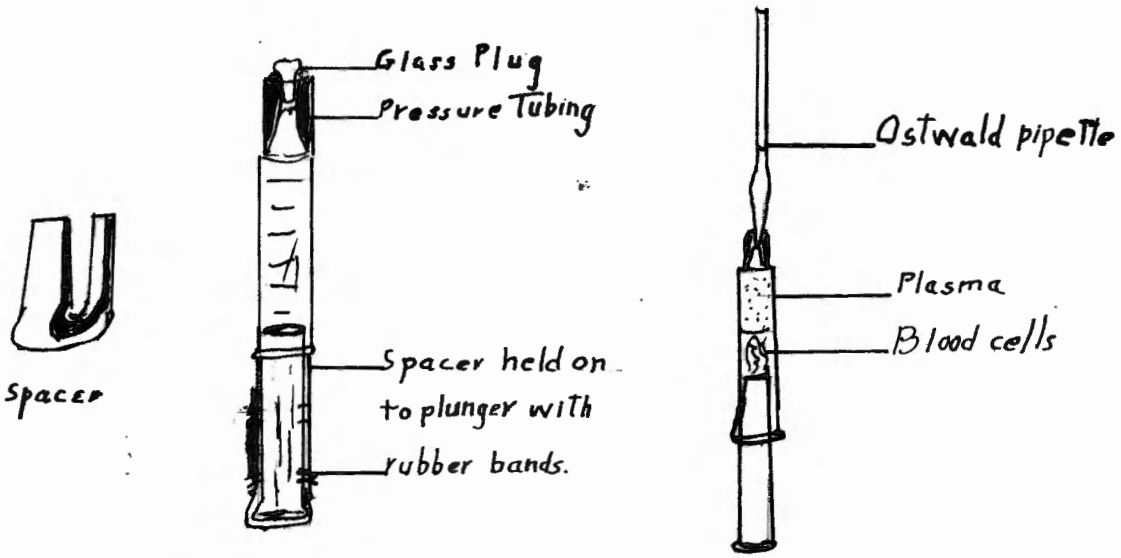
APPARATUS FOR pH MEASUREMENT

1. One Beckman 290-80 glass electrode assembly.
2. One Model G pH meter (Beckman).
3. One standard 270 RE glass electrode (calomel reference).
4. Potasium thalate solution of pH 4.
5. Beckman buffer solution which when mixed with distilled water 1-24 gives a pH of 7 at 20° and 6.97 at 40° centigrade.
6. One earthenware jar and large can with asbestos packing to hold water for a water bath.

METHODS FOR WITHDRAWING BLOOD

Blood is obtained from the patient by the Gabardi and Davenport (55) method with a few modifications. A 5 cc syringe with a plain tip is used. The spacers are made from a one centimeter strip of number 20 gauge aluminum and shaped as shown in figure 1. The entire plunger of the syringe is greased with a light bodied stop-cock lubricant. Dried heparin--one milligram per 5 cc of blood-- is placed on the top of the plunger and the barrel is placed over the plunger which is then rotated three times. (The Gabardi method calls for heparin solution to be used.) A number 21 needle is attached to the syringe and the blood is drawn. If a tourniquet is used, as soon as the vein is entered

FIGURE 1.



the tourniquet is released and a period of thirty seconds is allowed to lapse before the blood is drawn. Five and one-half to six cubic centemeters of blood are drawn, or by holding the needle in place three 5 cc syringes are filled. Approximately one cubic centimeter is injected into the Beckman glass electrode. A 1.5 centimeter length of pressure tubing is then placed over the tip of the syringe and with the syringe in a vertical position the plunger is forced to fill the lumen of the tube with blood.

A glass plug, fire polished on one end and flattened into a knob on the other is inserted into the lumen of the tubing. A spacer is placed around the plunger and tightly wrapped with rubber bands. The plug is removed and the plunger is pushed in until the spacer comes to rest against the barrel. While the spacer is held in position the plug is replaced. The assembly is then centrifuged for 15 - 20 minutes. After centrifugation the plug is withdrawn and the plasma forced into the blood gas pipet whose tip is inserted into the lumen of the tube. The contents of the Ostwald pipette are then transferred to the Van-Slyke apparatus and the CO2 content is measured. The remaining plasma is transferred to a separatory funnel and this plasma is used for the measurement of CO2 capacity.

VAN SLYKE METHOD OF MEASUREMENT OF PLASMA CO2

The measurement of the plasma CO2 content and capacity are the same except that the plasma is carried anaerobically from the syringe to the apparatus for measurement of CO2 content while the plasma is equilibrated with alveolar air for plasma

CO_2 combining power (capacity.) Two Van Slyke volumetric apparatus were used. They were cleaned with detergent and rinsed with copious amounts of distilled water before they were filled with filtered mercury. Since only plasma was used in the apparatus multiple determinations could be run without further cleaning.(126b) The stop-cocks of one apparatus were greased with a heavy stop-cock lubricant while those of the other were greased with a silicone lubricant. The apparatus greased with the heavy lubricant developed leaks three times during the three month period.

To test for leaks 3 cc of distilled water was admitted to the apparatus and its content of air was extracted in the usual manner and measured. The extraction process was then repeated and if the volume of air became greater a leak was indicated. The procedure used was exactly that of Peters and Van Slyke (126b) as follows:

> "Delivery from a rubber tipped pipette without a stop-cock.
>
> "This is the most economical precise method of delivering samples of blood into the gas apparatus.
>
> "...The pipette is filled above the mark on the upper stem with blood (plasma). It is then placed in a nearly horizontal position and the excess plasma is drawn from

the tip by absorption with a filter paper or towel, until the plasma surface in the upper stem has fallen to the level of the mark. The pipette is then tilted a little from the horizontal with the tip upwards, so that the surface of the plasma in the stem rises about 2mm above the mark. The upper stem of the pipette is then closed with a finger and the pipette tip is pressed into the bottom of the cup of the gas apparatus as shown in figure 2. When the pipette is changed from the horizontal to the vertical position the surface of the blood in the stem should move down the stem just far enough to return to the calibration mark."

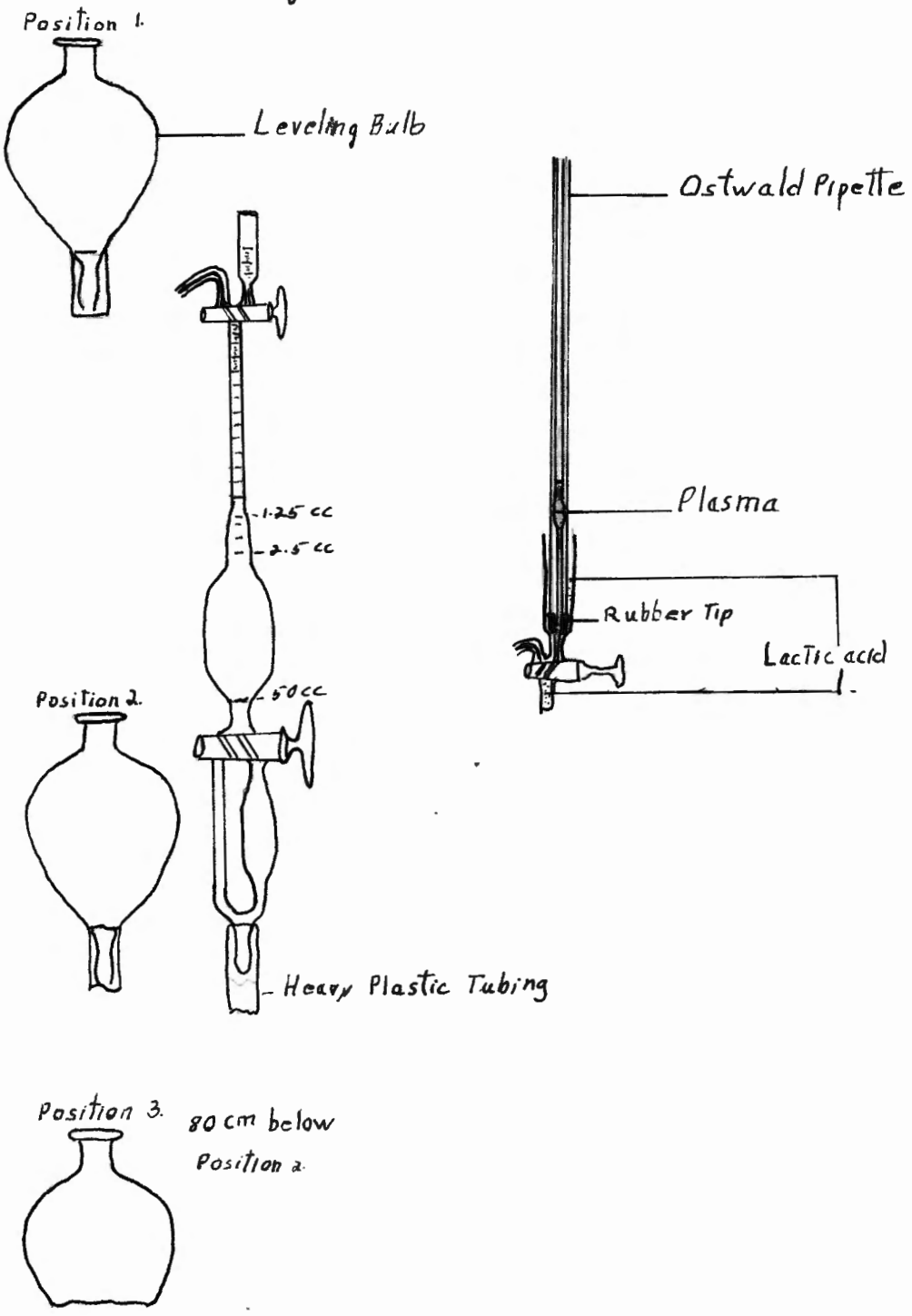
"Before the pipette tip is inserted into the cup of the gas apparatus the latter is arranged with the top cock open and bottom cock closed. The reagents with which the plasma is to be mixed in the chamber are to be partly in the chamber and partly in the cup.

"After the pipette tip has been pressed into position in the cup the finger is removed from the opening at the top of the pipette, and the flow of blood into the chamber is regulated by cautious opening of the cock which leads to the mercury leveling bulb. It is possible to regulate the flow of mercury through a cock more smoothly than the flow of blood....

"The delivery is continued until the plasma has entirely left the pipette, and a bubble of air has followed the column of plasma into the capillary beneath the cup of the gas apparatus. The pipette is then withdrawn from the cup. The bubble of air left in the capillary is dislodged by means of a fine wire that has been dipped in caprylic alcohol...."

Sufficient solution of 0.1 N lactic acid is admitted

Figure 2.


Position 1.
Leveling Bulb
Ostwald Pipette
Plasma
Rubber Tip
Lactic acid
1.25 cc
2.5 cc
5.0 cc
Position 2.
Heavy Plastic Tubing
Position 3.
80 cm below
Position 2.

until a total volume is 2.5 cc reading the bottom of the water meniscus. The top stop-cock is then sealed as follows:

> "....the cup is half filled with water, and about 0.2 cc of mercury is dropped in. The mercury is admitted into the chamber until just enough is left above the cock to fill the capillary leading to the cup."

After this the leveling bulb is then lowered so that the meniscus of the mercury reads at the 50 cc mark. This leaves a total volume of 47.5 cc of evacuated space. The apparatus is then shaken for 2-3 minutes and the bulb is lowered to station 3 to draw the solution into the receiving chamber. The leveling bulb is then placed at station 2 and the mercury is admitted through the lower stop-cock. It is admitted slowly so that no oscilation occurs. When the height of the mercury column has been reached, the leveling bulb is brought to the level of the column of mercury in the measuring tube and the lower stop cock is closed and the water meniscus is read. Since the height of the water column is less than 2 - 4 mm the correction for the weight of the water does not need to be considered.

The same technique is used for CO2 combining power determinations except that the plasma must be

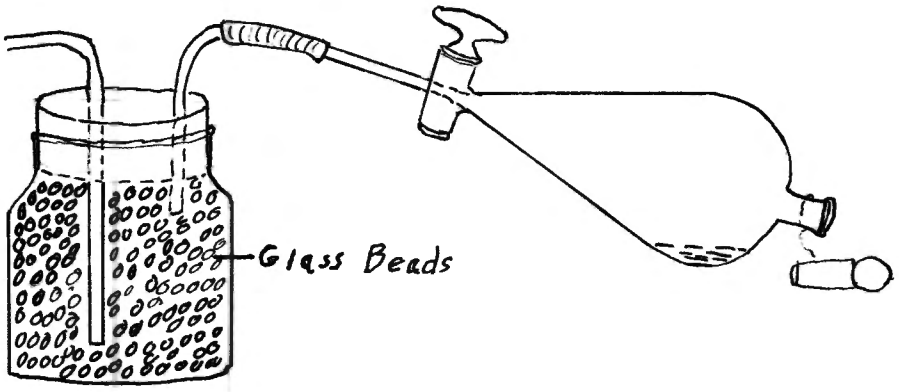
equilibrated. The technique was again taken from Peters and Van Slyke (126b) as follows:

> "In saturating plasma with alveolar CO2... the mixture must be passed over glass beads before it enters the funnel...
>
> "By passage over a large surface of either wet or dry glass beads at room temperature the expired air is colled, and the excess moisture in it is condensed, so that not enough is carried into the funnel to cause an appreciable error."

Thus alveolar air was passed through a 300 cc bottle containing dry glass beads for all tests. The very last air expelled was trapped in the funnel and the funnel was rotated from 2-3 minutes and then placed in a vertical position to allow the walls to drain. The plasma was then removed by use of an Ostwald pipette and placed in the Van Slyke apparatus as previously described.

To become acquainted with the Van Slyke procedure tests were run on a $NaHCO_3$ solution previously described. The technique was practiced until three successive runs on each apparatus could be done with accuracy.

FIGURE 3.



Apparatus to be used in equilibrating plasma with alveolar air.

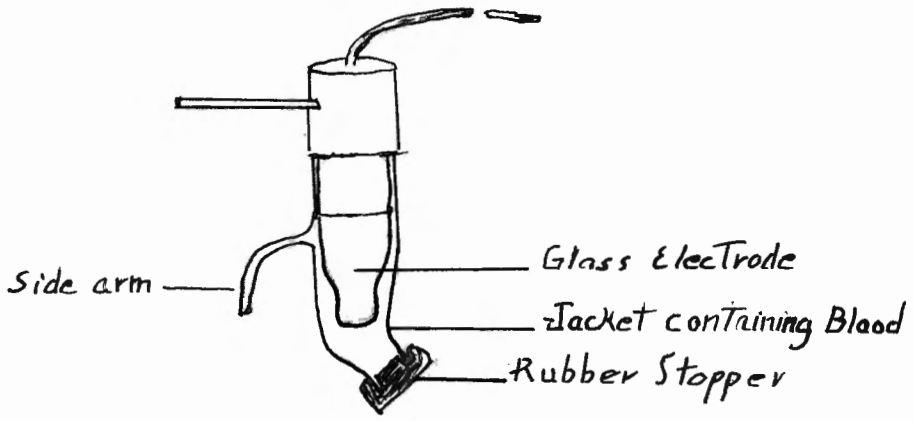
MEASUREMENTS OF BLOOD pH

For the measurement of blood pH a new Beckman 290 - 80 glass electrode assembly was used with a model B pH meter in conjunction with a standard 270 RE calomel electrode. Electrical contact was made through a beaker of saturated KCl solution.

PREPARATION OF ELECTRODE

The outer-body was removed and both parts were washed with distilled water. The electrode was reassembled and filled through the rubber cap with

FIGURE 4.



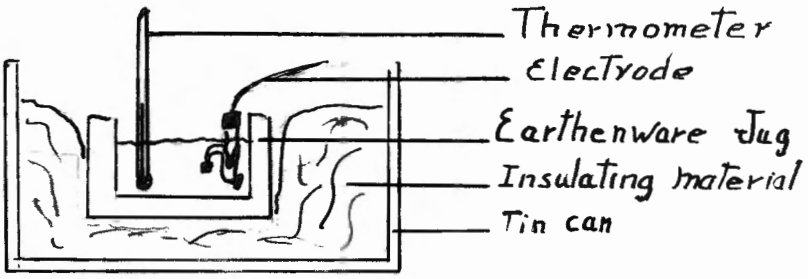
Glass electrode for anaerobic determinations

distilled water by inserting a number 22 needle attached to a water filled syringe. A new electrode or one that has not been used for some time should be allowed to soak for several hours in distilled water.

STANDARIZATION OF THE pH METER

Beckman buffer, previously described, is injected into the electrode and the entire assembly with the side arm covered by a rubber stopper is placed in a water bath at 40° centigrade for 15 minutes. The electrode assembly is then connected with the pH meter which has been set according to the directions of the machine. The pH dial is set for 6.97 and the

FIGURE 5.



pointer is brought to zero by the zero correction.

To check accuracy of the electrodes Beckman's buffer of pH 7 at 20° and a potasium thallate solution of pH 4 is used. The above procedures were done each day before any blood pH's were checked. After standardization of the machine the glass electrode was rinsed with distilled water and dried with tissue paper. It was then ready to be used for blood pH Measurement.

For the measurement of blood pH Comroe (23) recommends that the pH be standardized at a pH of 7 and then a solution of pH 7.4 tested. The results should be within 0,02 pH units. He states that with careful technique an accuracy of 0.01 to 0.002 pH units can be obtained at 38° centigrade. This is

the accuracy of the pH meter. He recommends that Rosenthal's (132) formula be used if blood pH is to be measured at room temperature. Table I shows the comparison of blood pH at room temperature, 38° and as calculated by Rosenthal's formula. It is apparent that Beckman's model G pH meter cannot be used to fulfill this formula.

To maintain the blood at 37 - 39° centigrade an earthen ware jar was surrounded by insulating material and placed in a deep can as shown in figure 5. Water was placed in the jar and kept at a temperature ranging from 36 - 39 degrees. The glass electrode and blood were kept in this water bath for periods up to one hour with no apparent change in pH. When possible the syringes and glass electrode were pre-heated to a temperature of 38° before the blood was drawn. This again did not seem to make any difference in the initial or final blood pH. (See Table II.)

If the pH of the blood was measured within a few minutes after it was taken with instruments at room temperature, it was found to be the same as the pH after fifteen minutes in the water bath at 37°.

TABLE 1.

Pt.	pH				Pt.	pH			
	37°	RT	cal	diff		37°	RT	cal	diff
GY	7.40	7.51	7.33	-0.06	JI	7.45	7.84	7.61	+0.16
LT	7.48	7.62	7.46	- .02	BW	7.43	7.80	7.59	+ .16
RH	7.30	7.58	7.37	+ .07	AY	7.45	7.71	7.58	+ .13
MMc	7.26	7.65	7.40	+ .14	LN	7.39	7.70	7.51	+ .11
HB	7.40	7.82	7.64	+ .24	HS	7.40	7.68	7.49	+ .09
VS	7.20	7.48	7.29	+ .09	VW	7.38	7.70	7.51	+ .13

The above table shows the blood pH at 37°, room temperature and as calculated by Rosenthal's formula

$$pH_{38} = pH_{rt} - 0.0147\ (38-t).$$

TABLE II.

Pt.	Initial Blood pH	pH at 15 minute intervals in a water bath ranging from 37 - 39°			
		15 min	30 min	45 min	60 min
GY	7.40	7.39	7.38	7.38	7.38
LT	7.48	7.47	7.47	7.47	---
RH	7.30	7.30	7.29	7.29	---
AY	7.34	7.34	7.34	7.32	7.32
In the following syringes and electrodes were at 37° before filling with blood.					
GY	7.41	7.41	7.40	---	---
AY	7.32	7.32	7.32	---	---

The above table demonstrates that the temperature of the blood was close to 37°, that it varied little and that blood could be kept in the anaerobic state at 37° for 45 minutes with little change inpH.

Combining the above methods, blood pH's, CO2 contents and combining powers were run on normal medical students and patients in acid-base imbalance. When possible duplicate determinations were carried out and the results are shown in Table III. The protocal concerning the patients will be found in the appendix. The diagnosis, treatment, condition of the patient and the condition under which the blood was drawn were noted.

TABLE III.

Pt.	pH	CO_2 content	CO_2 Combining Power	Calculated bicarbonate concentration	Diff. between cal. HCO_3 and CO_2 cont.	Diff. between cal. HCO_3 and CO_2 Combining Power
GY'	7.40	27.30	28.50	26.00	+1.30	+2.50
GY'	7.40	27.45	28.15	26.16	+1.29	+1.99
VW	7.36	29.13	---	27.61	+1.52	---
VW	7.38	22.17	23.15	21.07	+1.10	+2.07
VW	7.38	23.39	24.82	22.18	+1.21	+2.64
JI'	7.45	26.79	28.56	25.64	+1.15	+2.92
JI'	7.45	27.12	28.83	25.96	+1.16	+2.87
BW'	7.43	29.49	30.41	28.17	+1.32	+2.24
BW'	7.43	---	30.86	28.17	---	+2.69
DB	7.21	7.10	11.44	6.60	+0.50	+4.84
DB	7.28	17.59	19.00	16.50	+1.09	+2.50
DB	7.30	21.42	qns	20.15	+1.27	---
DB	7.27	20.99	22.30	19.66	+1.23	+2.64
RH	7.30	25.70	---	24.18	+1.52	---
MMc	7.26	17.94	17.97	16.78	+1.16	+1.19
HB'	7.40	16.10	16.76	15.33	+0.77	+1.43
HB'	7.40	16.10	17.66	15.33	+0.77	+2.33
HB'	---	---	17.24	15.33	+0.77	+1.91
VS	7.20	36.81	32.97	34.10	+2.71	-1.13
AY'	7.45	22.90	24.50	21.92	+0.98	+2.58
AY'	7.45	22.70	24.32	21.73	+0.97	+2.59

TABLE III continued.

Pt.	pH	CO_2 Content	CO_2 Combining power	Calculated bicarbonate concentration	Diff. between cal. HCO_3 and CO_2 Content	Diff. between cal. HCO_3 and CO_2 combining power
LN'	7.39	30.40	31.17	28.91	-1.49	-2.26
LN'	7.39	30.40	31.62	28.91	-1.49	-2.71
HS	7.40	27.89	28.15	26.56	-1.33	-1.59
LT	7.48	25.48	26.80	24.46	-1.02	-2.34

Determinations marked with (') are duplicate determinations.

DISCUSSION

All measurements in which there was any chance of personal error either in techniques of measurement or of taking the blood were eliminated from the final compilation. Thus errors caused by exposure of blood to air and those produced by hemolysis were eliminated. No attempt was made to obtain blood from the patient in a basal state. During the couse of measuring the CO2 combining power it was found that using wet beads decreased the ability to reproduce results and that most of the mistakes in technique occurred during this procedure. It was assumed that if there was any condesation of moisture on the flask the volume of serum would be increased thus lowering the CO2 combining power so dry glass beads were used.

The Van Slyke apparatuses and the Ostwald pipettes were not calibrated but since the same ones were used for all tests the error is constant and equal in all determinations. Assuming that any method of measurement is accurate to ½ the smallest division, the Van Slyke apparatus could be read to plus or minus 0.01 cc and the pH meter could be read

to plus or minus 0.05 pH units. The CO2 measurement error is 0.4 to 0.45 mEq per liter when measuring CO2 combining power.

Since the calculated bicarbonate concentration is always lower than the CO2 content and always lower except in respiratory acidosis than the CO2 combining power an error in pH measurement will not affect the desired result of this work. The pH meter can be estimated with reasonable accuracy to a plus or minus 0.02 pH units.

In this work the drop in pH due to the lactic acid effect, if present, was at the most no greater than 0.02 pH units, and since readings taken every 15 minutes on blood kept in a 37 - 39 degree water bath varied no more than 0.02 pH units, it can be considered that the blood pH was accurate with in a plus or minus 0.02 pH units.

The results of 25 acceptable measurements showed the plasma CO2 content to range from 0.77 to 1.52 mEq per liter higher than the true bicarbonate concentration while the CO2 combining power which is reported as mEq per liter of bicarbonate concentration varies from 1.19 to 2.69 mEq per liter higher than the true bicarbonate concentration. Thus the

difference between CO2 combining power and true bicarbonate concentration appears to be approximately twice as great as between the CO2 content and true bicarbonate concentration.

The technique of obtaining blood and measuring CO2 content that is presented here is much simpler and more accurate than that of measuring CO2 combining power. Thus the measurement of plasma CO2 content gives a more accurate estimation of the true bicarbonate concentration of plasma.

SUMMARY

1. A review of the literature pertaining to methods of blood CO2 measurement has been given.
2. A review of the literature pertaining to methods of blood pH measurement has been given.
3. A method for obtaining blood anaerobically using a five cc syringe has been given and its application to the measurement of CO2 content and combining power has been discussed.
4. An anaerobic method for the determination of blood pH using a glass electrode and pH meter has been discussed.
5. Combined measurements of plasma CO2 content, CO2 combining power and blood pH have been taken and have been correlated with the true bicarbonate concentration with the results showing that the CO2 content measurement is a simpler and more accurate method of estimating the true bicarbonate concentration of plasma.

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APPENDIX

TABLE I.

Measurements of $NaHCO_3$ solution to show accuracy of apparatus and technique.

Run	cc gas	ToC	Corr. BP	mEq CO_2
1.	0.686	23	730.2	26.41
2.	0.682	23	729.4	26.24
3.	0.690	24	730.6	26.41
4.	0.685	24	730.6	26.21
5.	0.690	24	730.6	26.41
6.	0.688	24	730.6	26.32

PROTOCOL

Patient: GY
Diagnosis: Normal Medical Student
Condition: Good
Treatment: None
Withdrawal of Blood: No tourniquet used, no air admitted to syringe.

pH at 37°--7.40 at RT 25°--7.51

CO_2	Content	Combining power
I.	0.716	0.79
II.	0.72	0.78

Corrected barometric pressure: 730.0

Patient: VW
Diagnosis: Normal medical student
Condition: Good
Treatment: None
Withdrawal of Blood: Tourniquet used, no difficulties, five cc drawn.

pH 37° -- 7.36 at RT 23°--

CO2	Content	Combining Power
I.	0.76	--

Corrected barometric pressure: 722.49

Patient: VW
Diagnosis: Normal Medical Student
Condition: Good
Treatment: None
Withdrawal of Blood:

pH 37° --7.38 at RT --25°--7.70

CO2	Content	Combining power
I.	0.59	0.660
II.	0.62	0.698

Corrected Barometric pressure: 730.89

Patient: JI
Diagnosis: Normal Medical Student
Condition: Good
Treatment: None
Withdrawal of Blood: Tourniquet used, 15 cc drawn with no difficulties.

pH 37°--7.45 at RT 22°--7.84

CO2	Content	Combining power
I.	0.692	0.781
II.	0.700	0.786

Corrected barometric pressure at 24° is 739.11

Patient: BW
Diagnosis: Normal Medical Student
Condition: Good
Treatment: None
Withdrawal of Blood: Tourniquet used, 15 cc drawn with no difficulty.

pH 37°--7.43 at RT 24 ° -- 7.80

CO2	Content	Combining Power
I.	0.775	0.840
II.		0.850

Corrected barometric pressure 725.85.

Patient: LN
Diagnosis: Normal medical student
Condition: Good
Treatment: None
Withdrawal of Blood: Tourniquet used, 15 cc drawn with no difficulty.

pH 37°--7.39 at RT 25°--

CO2	Content	Combining power
I.	0.780	0.880
II.	0.780	0.870

Corrected Barometric pressure 725.4

Patient: AY
Diagnosis: Normal female 6 months pregant
Condition: Good
Treatment: None
Withdrawal of Blood: Tourniquet used, 15 cc drawn with no difficulties

pH 37°--7.45 at RT 25°--7.77

CO2	content	Combining power
I.	0.62	0.705
II.	0.615	0.700
III.	---	0.700

Corrected Barometric pressure 714.39

Patient: HS
Diagnosis: Normal medical student
Condition: Good
Treatment: None
Withdrawal of Blood: Tourniquet used, 10 cc drawn with small amount of air admitted to one syringe.

pH 37°--7.40 at RT 25°--7.68

CO2	Content	Combining power
	I. ---	
	II. 0.730	0.780

Corrected barometric Pressure 729.13

Patient: DB at 1:30 pm
Diagnosis: Three year old with URI and nephritis
Condition: Poor--kusmal breathing
Treatment: 950 cc ½ strength lactated Ringer and glucose
Withdrawal of Blood: No difficulty
pH 37°--7.21 at RT 24o--

CO2	Content	Combining power
	0.22	0.375

Corrected Barometric Pressure 723.77

Patient: DB at 7:15 pm
Diagnosis: Same
Condition: Same
Treatment: Has received 150 cc blood, 300 cc 1/6 molar lactate and 250 cc NaCl
Withdrawal of Blood: Blood withdrawn from IV site.

pH 37°--7.29 at RT 22 o--

CO2	Content	Combining power
	0.48	0.57

Corrected Barometric Pressure 719.02

Patient: DB at 12 midnight
Diagnosis: Same
Condition: Improved, pt. resting
Treatment: 500 cc 5% glucose in ½ strength Ringers
Withdrawal of Blood: Was difficult and some air admitted to the syringe

pH 37°--7.3 at RT 24°

CO2 Content 0.58 Combining power qns

Corrected Barometric Pressure 719.42

Patient: DB at 2:45 pm
Diagnosis: Same
Condition: Good
Treatment: Parentaral fluids discontinued
Withdrawal of Blood: External jugular used with no difficulties

pH 37°--7.27 at RT 23°

CO2 Content 0.56 Combining power 0.64

Corrected Barometric Pressure 719.66

Patient: RH
Diagnosis: Hypertention and left heart failure
Condition: Poor--pulmonary edema
Treatment: Low Na diet--2 gm., morphine and digitalis
Withdrawal of Blood: Tourniquet used, no difficulties

pH 37°- 7.30 at RT 24°--

CO2 Content 0.67 Combining power error in technique

Corrected Barometric Pressure 737.11

Patient: MMcD
Diagnosis: 6½ months pregant, diabetes
Condition: Fair, epigastric pain, nausea, vomiting
Treatment: 500 cc 2½%dextrose in ½ strength Ringers lactated
500 cc 5% glucose
500 cc 5% glucose in saline
Withdrawal of Blood: Tourniquet used, no difficulty

pH 37°--7.26 at RT 21°--7.65

CO2	Content	Combining power
	0.48	0.54

Corrected Barometric Pressure 724.32

Patient: HB
Diagnosis: Diabetes, cirrhosis, hepatic insufficiency
Condition: Poor--
Treatment: Low sodium diet, cortisone
Withdrawal of Blood: Tourniquet used, no difficulty

pH 37°--7.40 at RT 26°--7.82

CO2	Content	Combining power
	I. 0.444	0.50
	II. 0.44	0.52
	III.	0.51

Corrected Barometric Pressure 734.3

Patient: VS--5 wk old baby
Diagnosis: Bilateral lobar and bronchial pneumonia
Condition: Very poor
Treatment: Sub-cue fluids and antibiotics, and O2
Withdrawal of Blood: Excellent femoral tap done, blood had a bluish black color.

pH 37°--7.20 at RT 25°--7.48

CO2	Content	Combining Power
	0.935	0.904

Corrected Barometric Pressure 725.55

Patient: LT
Diagnosis: Normal female labtech
Condition: Good
Treatment: None
Withdrawal of blood: No difficulties

pH 37°--7.48 at RT 24°--7.62

CO2 Content 0.670 Combining Power

Corrected Barometric Pressure 727.75 0.750
