Simultaneous Determination of pH, CO₂ Content, and Cell Volume in 0.1 ml. Aliquots of Cutaneous Blood

A Modification of the Shock and Hastings Technic

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In 1934 Shock and Hastings (1) described the construction and use of a micropipet, by means of which blood in 0.1 ml. aliquots could be used for the simultaneous determination of pH, CO₂ content, and red cell volume. In this method the blood in two pipets was diluted to 2.0 ml. with saline-phenol red solution, centrifuged, and used first for the determination of hematocrit value, second for the colorimetric determination of pH by visual comparison with previously prepared standards, and last, for the determination of CO₂ content in whole blood in the manometric Van Slyke apparatus. One or two additional pipets were filled with blood and 0.9% NaCl to serve as color blanks. A sample size of 0.5 ml. or less is thus sufficient for the determination of all three factors in duplicate, and cutaneous blood may therefore be utilized as an easily obtainable substitute for arterial blood. The method is particularly useful for the study of acid-base balance in infants.

Although the original method is suitable for most clinical purposes, the
following modifications were found, after a thorough trial, to offer certain advantages in operation and improvements in accuracy. The need for the preparation and checking of a large set of bicolor standards has been eliminated by adapting the micropipet in such a way that the optical density of the unknown can be compared with the optical density of a phenol red solution in phosphate buffer of known pH, in a suitable filter photometer. A similar method of photometric estimation of pH in blood or plasma in standard colorimeter tubes has been described by Van Slyke, Weisiger, and Van Slyke (2). To obviate the difficulty of filling the stem of the micropipet with blood under oil, without accidental admixture of the latter, we utilized the method of collection and handling of blood proposed by Lilienthal and Riley (3). In our hands this resulted in a more accurate measurement of the 0.1 ml. aliquot of blood, a crucial factor in any microtechnic. Since the technic thus modified has proved its value in five years of use at the Hospital of the University of Pennsylvania, it has seemed worthwhile to describe the modifications in detail to encourage a wider clinical application of the technic. Evidence will also be presented to support the accuracy of the determinations and the validity of handling blood samples in a way that appears to violate traditional anaerobic methods.

RELATIONSHIP BETWEEN pH AND PHOTOMETRIC MEASUREMENT

The basic principles involved in the adaptation of a pH method utilizing indicators from a visual colorimetric to a photometric measurement have been reviewed by Van Slyke et al. (2). Phenol red behaves like a monovalent weak acid, so that the dissociated salt and undissoicated acid components are related to the pH of the solution in the familiar Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}_{r^+} + \frac{(PR^-)}{(HPR)}$$

(1)

The total concentration of indicator, (PR), or the sum of (PR−) and (HPR), is kept very much less than the final buffer concentration of the mixture of the indicator solution with a test solution, such as blood or phosphate. It is therefore possible for the salt-acid ratio of the indicator to adjust to the pH of the test solution virtually without alteration of the latter.

Indicators like phenol red have the further property in both the dissociated and undissoicated forms of possessing distinctive colors in solution. The alkaline form, PR−, of phenol red is a deep purple color, and
the acid form, HPR, is yellow. Each form has a characteristic spectrophotometric absorption curve (4). In the pH range 6.7 to 8.6, mixture of the salt and acid forms gives a continuous series of intermediate colors, which can be analyzed visually by comparison with prepared standards or spectrophotometrically by making absorption curves or readings at two crucial wavelengths. The absorption curve for the alkaline phenol red has a maximum at \( \lambda = 560 \text{ m} \mu \), and that for the acid form, a maximum at \( \lambda = 430 \text{ m} \mu \), with virtually zero absorption at 560 m\( \mu \).

A reading of optical density or light extinction on a phenol red solution varies almost linearly with the concentration of alkaline phenol red, when a filter photometer with a green filter transmitting at about 540 to 560 m\( \mu \) is used. The diameter of the colorimeter tubes and other parameters must be constant to maintain this relationship. In solutions in which the total phenol red concentration is known and constant, the photometer reading can therefore be related to pH through the concentration, (PR−), and the factor pK′\( \text{pr} \) in Equation 1. A series of phosphate buffer solutions containing the proper amount of phenol red is read in the photometer, and a calibration curve is prepared for optical density or extinction against the known values of pH (2). It is convenient to use a photometer such as the Klett-Summerson, in which the scale reading is proportional to optical density rather than to transmission.

**APPARATUS**

*Micropipets* of the Shock and Hastings design, with certain dimensions specified so that the pipets can be easily centrifuged and also placed in a uniform and suitable position in the photometer. Critical dimensions of the micropipets used originally in our work are shown in Fig. 1. This design required the drilling of a hole at the bottom of the cuvette or tube holder of the photometer, and insertion of a cork spacer on the inside of the bottom cover for the purpose of aligning the pipet bulb vertically with the light aperture of the holder (see Fig. 1). The hole does not interfere with the use of the ordinary photometer tubes in the holder. However, to avoid the need for this modification, the micropipet has been redesigned by the supplier. The model currently available fits directly into a metal adapter which can easily be inserted into or removed from the standard tube holder of the Klett-Summerson filter photometer.\(^1\)

A filter photometer, capable of receiving the pipets and matching tubes of about 13 mm. outside diameter. A Klett-Summerson instrument was

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\(^1\) The adapter and micropipets made in accordance with such specifications are obtainable from Arthur H. Thomas Co., Philadelphia, Pa.
Aperture 0.5
not to exceed 6 mm.

Hole 6.5 mm. diameter
in floor of tube holder

Cylindrical part of bulb:
O.D. not to exceed 14 mm.
Length 12 mm.

Cork on bottom cover
to align bulb with
light aperture

Fig. 1. Sketch in cross-section, showing principal dimensions of the Shock and Hastings micropipet, and position of micropipet in the cuvette holder of the Klett-Summerson filter photometer. The walls of the cylindrical part of the bulb should be of clear glass, of uniform thickness, and free of defects. The actual outside diameter
used in all of our work. Although the micropipet was modified specifically for use in this photometer, it might be used in any filter photometer or spectrophotometer, provided that it could be positioned with the light path restricted to the cylindrical part of the bulb.

Test tubes for use as photometer tubes, matched as nearly as possible to the internal diameter of the micropipets (see specifications in Fig. 1). These can be selected from a quantity of round-bottom Pyrex test tubes of outside diameter 12.5 mm. and 100 mm. length.

A small water bath set at 38° and provided with a rack to hold micropipets and small photometer tubes.

A centrifuge with four brass shields (cups) of standard outside dimensions about 120 mm. by 19 mm. An angle centrifuge cannot be used because the stem of the micropipet is likely to break. A satisfactory small centrifuge is the Clay-Adams Standard Safeguard centrifuge, Model No. CT-1110, having a maximum speed of 2600 rpm.

Heavy rubber bands, approximately 75 mm. long and 6 mm. wide.

Syringes. A 1-ml. tuberculin syringe and 26-gage hypodermic needle for measuring the heparin solution; several 2-ml. syringes for collecting blood samples, with metal caps (Becton-Dickinson No. 425A); several 20-ml. syringes for storing solutions.

Collecting tips for the 2-ml. syringes, made from a piece of single bore #00 rubber stopper about 8 mm. in length, with a hollow cup ground in one end.

Adapters for use in filling the micropipet from the 2-ml. or 20-ml. syringes, made from pressure tubing of 1/8" bore and 1/8" wall. The pieces are 10 to 12 mm. in length and must be cut with a sharp knife so that the ends are flat.

Rubber adapters to center the stem of the micropipet in the centrifuge

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2 The instrument available for our use happened to be an early model that differs from current models in its optical system and in the galvanometer. For ease in reading, a box-type spot galvanometer had been substituted for the needle galvanometer that is a standard part of the Klett-Summerson instrument. However, the pipets have been successfully used in many other unmodified photometers of this manufacture. Any model can be used as long as the light aperture of the adapter, when in position, is within the light aperture of the instrument.

(O.D.) of the bulb averaged about 13 mm. A further improvement in design, made by Arthur H. Thomas Co., is incorporated in the current model of the micropipet. The neck has been shortened so that it is unnecessary to modify the Klett instrument by drilling a hole in the floor of the tube holder, as shown above. A removable metal sleeve has also been made available to slip into the tube holder. The purpose of this is to ensure accurate dimensions of the aperture and proper centering of the pipet.
shield. For use in the shields specified previously, these are made from pressure tubing of \( \frac{1}{4} \)" bore and \( \frac{3}{16} \)" wall, and are 15 mm. in length.

CALIBRATION

The volume contained in the stem of each micropipet to the nominal 0.1 ml. mark should be checked in order to insure accuracy of sample measurement. This calibration can be carried out to the nearest 0.0005 ml. by weighing the measured volume of mercury, by titrating with dilute alkali the measured volume of standard 1N acid and several rinsings of the stem, or by use of a calibrating microburet.

The micropipets and selected matching test tubes must also be calibrated with respect to their photometer reading, at both low and high readings. The micropipet is placed in the holder bulb down, and pipets and test tubes are always rotated so that the identifying mark faces the front of the instrument. With the green filter in place (Klett No. 54) readings are made (a) with water and (b) with an alkaline phenol red solution of 3.75 mg./l. or one of similar optical density. It is convenient in estimating the water corrections to make the original balance point with a scale setting of +10 divisions on the Klett instrument, since readings are not possible on the negative side of the zero setting. Failure to reproduce scale readings within one or two divisions on the Klett, or large correction values, may indicate that the aperture is not centered properly with respect to the cylindrical part of the bulb.

REAGENTS

1. For Collection of Blood

   *Mercury*, clean and free of acid or alkali.
   *Heparin sodium*, 1% in 0.9% NaCl solution (Abbott No. 3672).

2. For Hemoglobin Determination

   *0.1% Na\(_2\)CO\(_3\) solution* (1 Gm. per liter).
   *0.012M CuSO\(_4\)--4NH\(_2\)H\(_2\)O*. Dissolve 2.998 Gm. of CuSO\(_4\)--5H\(_2\)O and dilute to 1 liter with approximately 2M NH\(_4\)OH.

3. For Use in Micropipets

   *Phenol red, 0.1% stock solution*. Grind 1 Gm. of phenol red with 28.2 ml. of 0.1N NaOH until dissolved and dilute to 1 liter with distilled water. Filter if necessary. Allow to stand several days before using. Store in refrigerator.
Phenol red, 0.0075% working solution. Dilute 75 ml. of 0.1% phenol red to 1 liter with boiled distilled water. Store in refrigerator.

NaCl, 18% stock solution. Dissolve 180 Gm. of NaCl and dilute to 1 liter with distilled water. Store in refrigerator.

Saline-phenol red solution. Dilute 10.5 ml. of 0.0075% phenol red and 5.0 ml. of 18% NaCl to a total of 100 ml. with boiled distilled water. This solution should be freshly prepared at least once a week and, until used, is stored in a closed container or in a 100 ml. buret with a soda lime tube in the top.

4. For CO₂ Determination
(as described in Peters and Van Slyke (5))

Caprylic alcohol.

1N Lactic acid. CO₂-free stock solution, stored in refrigerator.

0.1N Lactic acid. Prepare fresh, free of CO₂ and air.

18N Sodium hydroxide (saturated). CO₂-free stock solution.

1N or 5N Sodium hydroxide. Prepare CO₂-free by dilution of 18N sodium hydroxide with boiled distilled water. The 1N solution must be fresh and air-free. The 5N solution is more stable because of lower solubility for gases (5, p. 284), but causes more rapid stopcock deterioration.

BUFFER STANDARDS AND CALIBRATION CURVE FOR pH MEASUREMENT

Stock solutions of M/3 Na₂HPO₄ (47.33 Gm./L.) and M/3 KH₂PO₄ (45.36 Gm. per liter). These solutions are prepared by dissolving the anhydrous reagent grade salt in freshly boiled redistilled water, and are stored in the refrigerator in glass-stoppered Pyrex bottles. This concentration has been reduced from that used by Van Slyke et al. (2) to lessen the amount of crystalline precipitation in the cold. Since the alkaline Na₂HPO₄ tends to pick up CO₂ if exposed to room air, the bottles are unstoppered only at the time of preparing desired solutions. The stock solutions appear to be stable for at least two years.

M/3 Mixed phosphate solutions. These are prepared in the proportions indicated in Table 1, so that the indicated pH will be obtained when each solution is diluted 1 to 5 to a final phosphate concentration of M/15. At 20° the pH will be 0.03 higher than the figures shown in Table 1 for 37°.

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4 The Merck Company provides anhydrous salts of high purity specially processed for buffer solutions.

4 The concentrations in Gm./L. given by Van Slyke et al. on page 746 of this reference (2) actually refer to molar concentrations, not 0.5 molar, as stated.
The column headed $R$ in Table 1 gives the molar ratio of Na$_2$HPO$_4$ to KH$_2$PO$_4$. If a mixed phosphate solution to make a pH 7.40 standard is desired, 10.00 ml. of $M/3$ KH$_2$PO$_4$ solution should be mixed with 10.00 $R$ or 43.5 ml. of $M/3$ Na$_2$HPO$_4$. If a final volume of 66.7 ml. is sufficient for each mixed phosphate solution, the figures for the ultimate concentrations of Na$_2$HPO$_4$ and KH$_2$PO$_4$ (in mM/L., Table 1, columns 4 and 5) can be used for the number of milliliters for each solution. These solutions are also stable if stored in Pyrex bottles in the refrigerator. The pH and pK$_r$ values corresponding to a given Na$_2$HPO$_4$/KH$_2$PO$_4$ ratio in Table 1 are taken from the experimental results of Hastings and Sendroy (6) and are based on $H_2$ electrode measurements at 38°. They are identical with the ones used by Van Slyke et al. (2). There is no significant difference in pH due to use of a temperature of 37° rather than 38°.

**Standard $M/15$ phosphate buffers.** Measure 20.00 ml. of mixed phosphate buffer, 10.00 ml. of the working solution of phenol red (75 mg./L.) into a 100 ml. volumetric flask and dilute to 100.0 ml. with freshly boiled redistilled water. This gives a standard $M/15$ phosphate of the designated pH at 37° containing 7.5 mg./L. of phenol red. Although such standard solutions are not so stable as the more concentrated phosphate mixtures, we have observed no significant change in photometer reading and pH over periods as long as two months, if the solutions are kept in Pyrex bottles in the refrigerator when not in use.
Calibration curve. At least two calibrated pipets or test tubes are filled for each standard buffer solution. If the pipets are used, the tips are dried and covered with adhesive tape as described in "Procedure." It is convenient to use one of the matched test tubes as a permanent water blank for the zero setting on the photometer in lieu of a calibrated pipet. Each buffer solution must be heated to 38° before reading in the photometer with a green filter, maximum transmission 540 μ. In the length of time necessary to place a pipet or test tube in the photometer and obtain a reading, the fall in temperature is generally no more than 1°. The zero setting is checked with the water blank after each reading; readings may be repeated, if desired, after rewarming. The appropriate pipet or tube correction is subtracted from each reading, and a graph is constructed of the mean photometer reading against pH.

It has been our practice to recheck the complete calibration curve only at infrequent intervals. However, the level of the curve is checked with each pH determination by reading at least one buffer, usually the one at pH 7.40, and a stable, colored reference solution, the CuSO₄·4NH₄·H₂O used in the hemoglobin determination. The level of the curve set by this buffer reading usually does not vary more than two or three scale divisions from day to day.

Because the pK' of phenol red in the saline-plasma mixture differs slightly from the pK' in phosphate buffer, it becomes necessary to apply a correction to the pH reading of the unknown solution to make it correspond to the buffer calibration curve. The pK' of phenol red in various salt solutions has been accurately determined by Sendroy and Hastings (7); values for the M/15 phosphate buffers and 0.9% NaCl solution (with

<table>
<thead>
<tr>
<th>pH at 38°C.</th>
<th>pK' PO₄ buffer</th>
<th>pK' 0.9% NaCl</th>
<th>Difference</th>
<th>pH Range at 37°C.</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.80</td>
<td>7.658</td>
<td>7.618</td>
<td>+0.040</td>
<td>under 6.94</td>
<td>-0.04</td>
</tr>
<tr>
<td>7.00</td>
<td>7.659</td>
<td>7.628</td>
<td>+0.031</td>
<td>6.94-7.18</td>
<td>-0.03</td>
</tr>
<tr>
<td>7.20</td>
<td>7.660</td>
<td>7.637</td>
<td>+0.023</td>
<td>7.19-7.42</td>
<td>-0.02</td>
</tr>
<tr>
<td>7.40</td>
<td>7.660</td>
<td>7.645</td>
<td>+0.015</td>
<td>7.43-7.67</td>
<td>-0.01</td>
</tr>
<tr>
<td>7.60</td>
<td>7.662</td>
<td>7.651</td>
<td>+0.011</td>
<td>7.66-7.91</td>
<td>0.00</td>
</tr>
<tr>
<td>7.80</td>
<td>7.669</td>
<td>7.657</td>
<td>+0.003</td>
<td>over 7.91</td>
<td>+0.01</td>
</tr>
<tr>
<td>8.00</td>
<td>7.663</td>
<td>7.662</td>
<td>-0.009</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Difference between pK' of phenol red in M/15 phosphate buffer and pK' in a solution containing 124 mM/L., NaCl and 30 mM/L. NaHCO₃, at the stated pH.

* Correction to be applied to the pH read with 0.9% NaCl solution as a diluent, in order to allow for the difference in pK' of phenol red in this solution as compared with the M/15 phosphate buffer standards.
H_2CO_3-NaHCO_3 buffer) are shown in Table 2, as taken from their work. The correction is given for various ranges of observed pH. Ordinarily this correction has been ignored as negligible in the past, but for the most accurate work it should be applied.

PROCEDURE

A syringe of 2 ml. capacity, graduated in 0.1 ml., is used for the blood collection. A constant amount, 0.05 ml., of heparin saline solution is measured in a tuberculin syringe and then injected through a 26-gage needle into the barrel of the 2 ml. syringe. After ejection of the air, about 0.3 ml. of mercury is drawn into the syringe and the rubber collecting tip is attached to it.

Two greased syringes of 20 ml. capacity are used for filling the micro-pipets with diluent. A satisfactory grease for the syringes is Arthur H. Thomas stopcock grease; silicone grease is too alkaline for this purpose. About 2 or 3 ml. of mercury are drawn into each syringe. Saline, 0.9%, to be used in the control pipets, is drawn into one syringe and the saline-phenol red solution into the other. A rubber adapter which will serve as a connecting piece between syringe and pipet is attached to each syringe. Before filling the pipets, the saline-phenol red solution is adjusted visually to pH 7.4 ± 0.2 by adding a fractional drop of dilute alkali or acid.

Collection of Blood Sample

To produce peripheral vasodilatation and fully "arterialize" cutaneous blood, the patient's hand (or the heel in infants) is first placed in warm water at 45° for 3–5 minutes. A sterilized spring lancet or blade is used to make the finger cut. Blood is collected in the rubber funnel tip as it falls drop by drop from the finger and is drawn into the syringe. The flow of blood should be free, but gentle pressure may be applied if necessary. The initial air bubble, if any, is ejected and the syringe is shaken gently to mix the blood with the heparin as the collection proceeds. About 1 ml. of blood should be collected in 1–3 minutes. If the first cut does not supply enough blood for the sample, a second cut may be made and the blood pooled with that of the first cut. The total amount of blood must be noted to the nearest 0.05 ml. so that the heparin dilution can be calculated. The rubber collecting tip on the syringe is replaced by a metal cap. The syringe is placed cap down in a beaker of ice. When the procedure is properly carried out, the exposure of blood to air during collection is without significant effect on the pH and CO_2 content.

Instead of the fingertip, the ear lobe can be used as a source of cutane-
ous blood, after warming by dry heat as described by Lilienthal and Riley (3).

**Determination of Hemoglobin Concentration**

The collecting syringe is shaken gently for thorough mixing. The metal cap is removed and the blood in the syringe tip is discarded. A rubber syringe adapter is attached to the syringe tip. A drop of blood is forced from the syringe and is drawn by suction into a 0.02 ml. (20 cu. mm.) pipet calibrated to contain that amount. This sample is delivered into a photometer tube containing 5.0 ml. of 0.1% Na₂CO₃ solution. The pipet is rinsed several times with the Na₂CO₃ solution and the tube is stoppered and inverted repeatedly. A tube containing 0.012M CuSO₄·4NH₃·H₂O is used as a standard. The solutions are read in a photometer against a distilled water blank. A 540 mμ (green) filter is used.

\[
\frac{\text{Reading Hb}}{\text{Reading CuSO₄·4NH₃·H₂O}} \times \frac{\text{volume blood} + \text{volume heparin}}{\text{volume blood}} \times 9.34
\]

\[
\frac{\text{Reading Hb}}{\text{Reading CuSO₄·4NH₃·H₂O}} \times \frac{\text{volume blood} + \text{vol. heparin}}{\text{volume blood}} \times 15.6
\]

The factors 9.34 and 15.6 in these equations are derived from the data of Drabkin (8), who proposed the use of CuSO₄·4NH₃·H₂O as a standard solution for photometric estimation of hemoglobin and other pigments. In the Klett-Summerson photometer with a 540 mμ filter, Drabkin found a ratio of 0.963 for the reading of a standard hemoglobin solution (8.98

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A detailed description is given for the hemoglobin determination because this is a slightly better index of blood nonbicarbonate buffers than is the packed cell volume, and because the reference standard can be used to check the photometer reading of the phenol red solutions. In our hands the copper sulfate photometric standard was stable and reproducible, and gave good results. However, more recent work by Drabkin and others on behalf of the National Research Council has indicated that slight variations may occur in the water content of the crystalline CuSO₄·5H₂O that make it undesirable as a primary photometric standard. Published recommendations have appeared in this journal, Vol. 1, page 151, to determine hemoglobin as cyanmethemoglobin, with the use of a stable, primary cyanmethemoglobin standard that is now available through Division of Medical Sciences, National Academy of Sciences-National Research Council. The use of this new standard is to be preferred.
mM./L. or 15.00 Gm./100 ml., diluted 1–251) and the reading of 0.012M copper sulfate standard. The reading of the standard, which varies somewhat from instrument to instrument, and serially in the same instrument, therefore corresponds to that of a solution containing 8.98/0.963 = 9.34 mM./L. of hemoglobin, or 15.00/0.963 = 15.6 Gm./100 ml. of hemoglobin, after dilution 1–251.

**Filling of Micropipets**

Before filling each micropipet with an aliquot of the blood sample collected, the collecting syringe is shaken gently and a small portion of blood is discarded by touching a clean cloth to the blood surface. The calibrated end of the micropipet is placed end to end against (not inside) the rubber adapter, and blood is forced carefully from the syringe until the pipet is filled slightly beyond the 0.1 ml. mark. Manipulation is facilitated by holding the barrel of the syringe and the pipet in the left hand while the right hand slowly pushes the syringe plunger (Fig. 2). When the pipet has been filled, it is grasped by the right hand with the forefinger covering the open stem above the bulb; contact with the syringe is then broken. The blood is adjusted to the 0.1 ml. mark by drawing off the excess with a clean cloth or by gently tapping the pipet end with a finger. The pipet is

![Fig. 2. Method of filling micropipet with blood from a 2-ml. syringe.](image)

The stem of the pipet is held between the thumb and forefinger of the left hand. The barrel of the syringe is held between the palm and fourth and fifth fingers of the same hand. The flat bottom of the pipet stem must be firmly apposed to the flat end of the rubber adapter on the tip of the syringe. Filling is controlled by gentle pressure on the plunger of the syringe with the right hand. The same procedure is used in filling the micropipet to the 2.0 ml. mark with diluent from a 20-ml. syringe.
then tilted slightly, bulb down, until there is a small air space between the blood and the calibrated end of the pipet. The outside of the calibrated end is wiped clean.

Saline-phenol red solution in the greased 20 ml. syringe is used as diluent for three pipets, and 0.9% NaCl for the fourth, which serves as a color blank for the pH determination. The dilution is accomplished by the filling technic just described; but to avoid trapping air in the pipet bulb, the syringe and pipet should be gradually raised to a vertical position while filling and then lowered to a horizontal position when the solution is slight above the 2.0 ml. mark. Care must be taken to hold the pipet firmly against the rubber adapter, maintaining the pressure fit. After adjusting the volume, a small air space is again left in the end of the pipet. The outside is dried carefully, and the tip of the calibrated end is sealed with a small piece of adhesive tape.

**Centrifuging and Determination of Packed Red Cell Volume (Vc)**

One of the heavy rubber bands is placed lengthwise about each pipet and flat over the adhesive tape seal to protect the pipet in centrifuging. The calibrated end of the pipet is placed in the cup adapter made from rubber pressure tubing. The pipets in this way are held firmly in the centrifuge cups. Centrifugation is carried out at 2600 rpm for 15-20 minutes. The rubber bands should be removed carefully after centrifugation since they sometimes stick to the adhesive tape seal.

The volume of packed red cells (Vc), noted to the nearest 0.5 per cent in the calibrated stem of the pipet, must be corrected for the heparin dilution.

\[
V_c \times \frac{\text{volume blood} + \text{volume heparin}}{\text{volume blood}} = \text{corrected } V_c
\]

**pH Determination**

To check against the standard curve, two pipets or test tubes are filled with pH 7.40 phosphate buffer containing phenol red. All the pipets and tubes except the saline blank are heated in a water bath to 38°. Readings are taken on each pipet after placing it bulb down in the holder of the photometer, checking stem orientation each time. A green filter with maximum transmission at 540 m\(\mu\) is used. All readings should be taken promptly or the red cells in the stem of the inverted pipet may flow into the bulb.

The reading of each micropipet must be corrected by applying the
appropriate pipet calibration. The final corrected reading, $R$, is calculated as $R = R_2 - R_1$, where $R_2$ is the corrected reading for the blood-phenol red sample and $R_1$ is the corrected reading for the blood-saline blank. The pH is estimated by the interpolation of $R$ on the standard curve of the pH of the phosphate buffers at 37° versus the photometer scale reading. This value, plus the appropriate pK’ correction (see Table 2), gives the final pH value.

**Determination of Whole Blood CO₂ Content (CO₂)ᵢ**

The CO₂ content of the blood diluted with saline-phenol red is determined in a Van Slyke manometric blood gas apparatus by the basic method of Van Slyke and Neill, as adapted to the characteristics of the sample (1, 5).

One drop of caprylic alcohol is drawn into the capillary and 3 ml. of 0.1N lactic acid are measured in the cup of the apparatus. After removing the adhesive tape seal from the pipet, a small piece of rubber tubing is placed on the tip of the calibrated end to provide a watertight seal between the pipet and the lactic acid. The contents of the pipet are delivered directly into the extraction chamber, delivery being halted with a small air bubble in the stopcock capillary. The air bubble is dislodged with a wire dipped in caprylic alcohol, and 2.5 ml. of the lactic acid are drawn into the machine. The stopcock is sealed with mercury and the mercury level in the chamber is lowered to the 50 ml. mark. After extraction the CO₂ is reabsorbed with 1 ml. of 1N NaOH. Pressure readings are taken at the 0.5 ml. volume.

A blank CO₂ determination is made in the same manner on a 2.0 ml. sample of the saline-phenol red solution used to dilute the blood.

The difference in pressure due to the blood, $\Delta P$, is calculated by subtracting the blank correction from the difference in the pressure readings for the blood diluted with saline-phenol red, $P_1 - P_2$ (see “Sample calculations”).

The CO₂ content of the blood in mM/L, $(CO₂)ᵢ$, is obtained by multiplying the pressure difference, $\Delta P$, by the appropriate factors for temperature, pipet calibration, and heparin dilution.

$$(CO₂)ᵢ = P \times \frac{\text{Temperature Factor}}{\text{Pipet Factor}} \times \frac{\text{volume blood + volume heparin}}{\text{volume blood}}$$

Temperature factors as calculated by Peters and Van Slyke (5) are reproduced in Table 3, page 304.
Centrifugation of Excess Blood for Determination of Plasma Constituents

Additional determinations, such as plasma Na, K, Cl, and CO₂ can be made if a larger sample of blood (1.5 to 2.0 ml.) is collected at the time of the finger or ear lobe puncture. When it is planned to collect a blood sample larger than 1.2 ml., it is advisable to grease the plunger of the collecting syringe before adding the heparin saline solution.

After the aliquots required for the pH, CO₂, and hemoglobin determinations have been taken from the syringe, the contents are mixed; the syringe is held vertically, tip down, while the mercury is carefully ejected. The blood is transferred to the bottom of a 1-ml. micro centrifuge tube through a 2-inch 21-gage needle. The centrifuge tube should be filled from the bottom to avoid trapping an air bubble. A small rubber stopper pierced by a short 24-gage hypodermic needle is quickly inserted in such a way that any trapped air and a small amount of excess blood are forced up through the needle. The needle is then removed and the tube is ready for anaerobic centrifugation. The brief exposure of the blood to room air while the tube is being filled does not result in a significant change in pH or CO₂ content, as is shown later. After centrifugation the stopper is removed and the plasma is carefully withdrawn through a 21-gage needle into a greased 2-ml. syringe. The empty needle is removed, the syringe held tip up, and the air bubble (from the needle) is ejected. The plasma can be stored anaerobically if the syringe tip is protected with a metal cap.

Sampling is accomplished by forcing a drop of plasma up into a collecting piece placed on the syringe tip and drawing up a 0.05 or 0.1 ml. aliquot into a suitable type of calibrated pipet such as the Linderstrom-Lang-Hølter semiautomatic constriction pipet. The pipet designed by Kirk is also suitable and in the sizes from 0.05 to 0.2 ml. can be utilized as an accurate delivery pipet despite the fact that it is designed “to contain.” In this pipet the volume to deliver is about 2 per cent less than the contained volume. Filling and delivery in these pipets are accomplished with the aid of mouth suction and pressure through a rubber tube and mouthpiece, or with the aid of a pipet controller (9).

If desired, the plasma CO₂ content can be measured, with 0.1 ml. aliquots delivered under water in the cup of the Van Slyke apparatus as described previously. The relative accuracy of this determination is greater than that of the whole blood CO₂ because the blank correction is

* The Linderstrom-Lang-Hølter and Kirk micropipets used by us were obtained from Arthur H. Thomas Co., Philadelphia, Pa.
more stable and the concentration of total CO₂ is about 20 per cent higher in plasma.

Unless plasma CO₂ is to be included, all plasma samples can be drawn directly from the centrifuge tube. If a potassium determination is desired, the blood must be transferred to the micro centrifuge tube before icing the collecting syringe to prevent the exchange of potassium across cell membranes.

Plasma sodium and potassium concentrations can be measured with the internal standard flame photometer (10). For the determination of chloride we have used Sendroy’s titrimetric iodate method, with modifications as described by Van Slyke and Hiller (11) on 0.05 ml. aliquots. Protein concentration could be estimated in one drop by means of the gradient tube (12) or falling-drop method (13). A variety of substances in plasma can be determined by suitable micro methods, up to the limit of plasma available, which would be about 0.5 ml. for a 1.5 ml. sample of blood.

Because the plasma is diluted with a heparin solution containing sodium and chloride, appropriate corrections must be applied to the measured concentrations of these ions, and to the concentrations of other substances estimated in heparinized diluted plasma. The corrections are calculated from the amount of heparin solution used in relation to the amount of plasma, and, in the case of sodium and chloride, from the concentration of these ions in the heparin solution.

SAMPLE CALCULATIONS

1. Dilution Factor

Suppose the total volume of blood and heparin diluent (measured in collecting syringe) to be 1.50 ml. Volume of heparin solution measured from tuberculin syringe—0.05 ml. Volume of blood = 1.50 – 0.05 = 1.45 ml. Consequently, the dilution factor for use in calculating hematocrit value and whole blood concentrations is 1.50/1.45 or 1.034.

2. Hemoglobin Concentration

Suppose that the corrected mean reading for the standard CuSO₄·4NH₃·H₂O solution in the Klett-Summerson photometer is 200, and the mean reading for the hemoglobin diluted 1/251 is 185. The hemoglobin pipets used have a calibrated volume of 20.2 cu.mm.

\[
Hb \text{ conc} = (9.34) \frac{(186/200) (20.0/20.2)}{(1.034)} = 8.9 \text{ mM/L.}
\]
3. Hematocrit Value

Suppose that the mean value in four micropipets is 0.440.

Corrected $V_c = (0.440)(1.034) = 0.455$

It is not useful to measure or calculate the hematocrit value more accurately than to the nearest 0.005.

4. Photometric pH

Suppose the mean reading of the plasma-indicator-saline mixture in three micropipets (with the individual pipet calibration applied to each reading) to be 156. The corrected blank reading is 10. The mean corrected reading for the pH 7.40 phosphate buffer is 152, and for the pH 7.20 buffer, 111.

Sample reading, corrected for blank = 156 - 10 = 146. The pH can then be read from a suitable graph on which photometer readings are plotted against pH, or estimated by the following equation in the pH range in which the relation is virtually linear:

$$pH = 7.40 - [(152-146)/(152-111)][0.20] = 7.40 - (6/41)(0.20) = 7.37$$

Finally, the correction from Table 2 must be applied (the difference of $pK'$ of phenol red in 0.9% NaCl solution as compared with M/15 phosphate buffer):

$$Corrected \ pH = 7.37 - 0.02 = 7.35$$

5. Whole Blood $\text{CO}_2$ Concentration

Assume the following data. Volume of micropipet = 0.099; calibration of chamber volume = 0.495 ml. Blank pressure difference for 2.0 ml of saline-phenol red = 14.0 mm. Calculated blank for 1.9 ml of saline-phenol red = (14.0)(1.9/2.0) or 13.3 mm. At 24° the two pressure readings obtained for the diluted blood sample are $P_1 = 182.6$ mm. and $P_2 = 114.0$ mm.

Corrected $\Delta P = (182.6 - 114.0) - 13.3 = 68.6 - 13.3 = 55.3$

The factor in Table 3 at 24°, for a chamber volume of 0.5 ml. and a sample of 0.100 ml. is 0.304.

$$(\text{CO}_2)_b = (55.3)(0.304)(0.100 \times 0.100/0.099)(0.495/0.500)(1.034) = (16.8)(1.034) = 17.4 \text{ mM/L.}$$

It is convenient to prepare a table of temperature factors that includes
the correction for the chamber volume. The sample and pipet corrections must be calculated in each individual determination; the dilution factor is usually applied only to the mean of the replicate CO₂ determinations.

6. Obtaining the Derived Acid-Base Factors

The calculated CO₂ pressure and total CO₂ or bicarbonate concentration of plasma can be obtained from the nomograms of Van Slyke and Sendroy (14) and Hastings and Shock (15). The whole blood buffer base concentration can be obtained from the nomogram of Singer and Hastings (16), which also gives values for CO₂ pressure and plasma total CO₂ or bicarbonate. In the application of all of these nomograms to whole blood CO₂ concentrations it is necessary to use the hemoglobin concentration or hematocrit value as one auxiliary parameter, and a known or assumed O₂ saturation as the other. Unless the patient is cyanotic or the sample shows obvious signs of unsaturation, it is assumed that cutaneous or arterial blood is fully oxygenated. If the oxygen saturation is less than 90 per cent, slight errors in CO₂ pressure and buffer base concentration will be introduced unless account is taken of this factor. In the example cited here, with Hb 8.9 mM/L. or Vc 0.455 and an assumed 100 per cent saturation, the whole blood CO₂, 17.4 mM/L., is found in Scale 1 of the nomogram of Singer and Hastings, and the pH, 7.35, on Scale 4. A straight line is drawn through these two points and the derived variables are read off.
Plasma \((\text{CO}_2)_p\) = 21.0 mM/L. (right-hand edge, Scale 1)
Plasma \((\text{HCO}_3)_p\) = 19.8 mM/L. (Scale 2)
Whole blood \((\text{BB}^+)_b\) = 43 mEq./L. (Scale 3)
\(P_{\text{CO}_2}\) = 37 mm. (Scale 5)

This represents for an adult a mild metabolic acidosis with a \(\text{CO}_2\) pressure at the lower edge of the normal range. However, in an infant, these acid-base conditions could all be within a normal range.

**RESULTS**

**Accuracy of Modified Microtechnic**

The respective errors of the various methods involved in the microtechnic have been estimated by an analysis of the deviations in a large number of sets of replicate determinations. The results in Fig. 3 show the distribution of the deviations from the mean. The standard error for the mean of duplicate hemoglobin determinations is \(\pm 0.029\) mM/L. or less than half of one per cent of the average concentration, a relative error equal to that of the calibration of the 20 cu.mm. pipets. The standard error of the mean of quadruplicate packed cell volumes was \(\pm 0.0023\). The standard error of the pH in triplicate was \(\pm 0.0051\), and that of the whole blood \(\text{CO}_2\) content in duplicate \(\pm 0.16\) mM/L. Confidence limits have been calculated on the basis of the difference between means (obtained with the usual number of replicates) that has only a 5 per cent probability of being due to chance variation; the ratio of this difference to the standard error of the difference, or \(\sqrt{2} \times \text{standard error}\), is 1.96 (17). This least significant difference is less than 0.1 mM/L. or 0.15 Gm./100 ml. for hemoglobin, 0.006 for cell volume, 0.014 for pH and 0.44 mM/L. for whole blood \(\text{CO}_2\) content. The accuracy of this whole blood \(\text{CO}_2\) determination can be compared with that of the determination of \(\text{CO}_2\) content in 0.2 ml. aliquots of plasma, measured in a stopcock pipet. The standard error of the duplicate plasma determination was \(\pm 0.11\) mM/L., and the least significant difference 0.3 mM/L.

**Effect of Collection or Transfer of Blood with Momentary Exposure to Air**

In Table 4 results are presented of a representative experiment in which heparinized blood was transferred dropwise from a 20-ml. syringe to four separate 2-ml. syringes in such a way as to simulate the collection of cutaneous blood. Two of the small syringes (A and C) were previously prepared with 0.05 ml. of heparin saline solution, and two (B and D) con-
Fig. 3. Frequency diagram showing percentage distribution of deviations from the mean in a large series of sets of replicate determinations utilizing the microtechnique. Below this distribution for each variable are given the standard deviation for a single reading, the standard error of the mean of the usual number of replicates in a single determination, and the least significant difference between two determinations (at the 95 per cent confidence level).
Table 4. Test of Collection: Dropwise Transfer of Blood

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Hb (mM/L)</th>
<th>Vc</th>
<th>pH at 37°</th>
<th>(CO2) b (mM/L)</th>
<th>Heparin in blood (ml./ml.)</th>
<th>Transfer time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>8.05</td>
<td>0.40</td>
<td>7.300</td>
<td>24.1</td>
<td>0.3/20</td>
<td>—</td>
</tr>
<tr>
<td>A</td>
<td>8.00</td>
<td>0.40</td>
<td>7.295</td>
<td>23.8</td>
<td>0.05/0.95</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>—</td>
<td>0.41</td>
<td>7.295</td>
<td>23.8</td>
<td>No heparin</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>8.30</td>
<td>0.41</td>
<td>7.285</td>
<td>24.0</td>
<td>0.05/0.95</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>8.10</td>
<td>0.405</td>
<td>7.290</td>
<td>23.8</td>
<td>No heparin</td>
<td>4</td>
</tr>
</tbody>
</table>

* Venous blood, drawn and stored in a 20-ml syringe containing 0.3 ml. heparin solution. By glass electrode the pH of this blood was 7.47 at 26°, or 7.315 at 37°, with temperature factor of Rosenthal, J. Biol. Chem., 173, 25 (1948).

Maintained no heparin; all syringes contained some mercury for mixing the blood in the sampling process. Two of the transfers were accomplished "rapidly" (about 1 ml. of blood in 1 minute) and two "slowly" (1 ml. in 4 minutes), to simulate easy and difficult fingertip collection. As is evident from Table 4, very slight decreases were observed in pH and CO2 content in the transferred blood as compared with the control blood. In syringe C the pH decrease of 0.015 was barely significant; all other differences in pH and CO2 content were smaller than the least significant differences determined above. The factor of dilution of blood with heparin saline introduces an additional uncertainty in the measurement of cell volume and hemoglobin and CO2 concentrations. This may explain the rather high hemoglobin concentration in Sample C (wetting of the syringe surfaces might reduce the effective amount of heparin saline dilution of the blood, in some instances). This experiment also shows that the heparin solution has had no significant effect on the pH of the blood to which it was added. Other experiments have confirmed this lack of effect, even when relatively much larger amounts of heparin solution were used.

Table 5. "Virtual" Anaerobic Technic: Transfer of Blood from Syringe to Centrifuge Tube

<table>
<thead>
<tr>
<th>Method of transfer</th>
<th>pH at 37°</th>
<th>(CO2) b (in blood) (mM/L.)</th>
<th>(CO2) b (in plasma) (mM/L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic control—syringe to syringe</td>
<td>7.34</td>
<td>24.30</td>
<td>28.60</td>
</tr>
<tr>
<td>&quot;Virtual&quot; anaerobic—Single transfer*</td>
<td>7.34</td>
<td>24.25</td>
<td>28.45</td>
</tr>
<tr>
<td>&quot;Virtual&quot; anaerobic—5 transfers*</td>
<td>7.32</td>
<td>24.00</td>
<td>28.10</td>
</tr>
<tr>
<td>Difference per transfer*</td>
<td>Single transfer</td>
<td>0.00</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td>Multiple transfers</td>
<td>-0.004</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

* Venous blood, exposed briefly to air during transfer from syringe to small centrifuge tube and back again, after stoppering tube so as to exclude air. Mixing of blood carried out with mercury. Blood heparinized in first syringe.
In another experiment we observed the effect of transferring about 8 ml. of blood from a syringe into an open centrifuge tube (a Pyrex round-bottom tube, 1.25 by 10 cm.). The blood was exposed to air during the filling process until a rubber stopper and needle were inserted as described previously, so as to eliminate any air bubble, and exposed again when the sample was taken up into a second syringe for storage. A portion of the blood in the syringe was then transferred aerobically to a 5-ml centrifuge tube, stoppered so as to exclude air, centrifuged, and the plasma drawn off and stored in a third syringe for analysis of plasma CO₂ content. The pH and the whole blood CO₂ content were determined on the blood in the second syringe, and control analyses performed on the original blood not subject to aerobic transfer. The results are shown in Table 5. In addition to the single tube transfer (as described above) the experiment was repeated with 5 successive transfers to separate tubes before the blood was drawn up in the storage syringe. The differences observed as a result of this multiple transfer were divided by 5, to give figures which should be a more accurate approximation of the differences due to a single transfer. Per single transfer the pH was found to decrease 0.004 (an amount too small to be detected in the single transfer), the whole blood CO₂ content decreased about 0.06 mM/L., and the plasma CO₂ content, 0.10 mM/L. These changes are well within the confidence limits found for the microtechnic and we can conclude that this method of aerobic transfer of blood for centrifugation introduced no appreciable error for clinical and most other purposes.

Comparison of Arterial and Cutaneous Blood Samples

Results are presented in Table 6 for the analysis of seven pairs of blood samples, one arterial and one cutaneous in each pair. The arterial sample was drawn in a 10-ml. syringe to which 0.3 ml. of heparin solution had previously been added. The syringe was attached to a manifold connected with a needle placed in the femoral artery. Because of risk of clotting in the long connecting tube of fine bore it was generally not possible to prolong collection of the arterial sample to correspond exactly with the time required for collection of the cutaneous sample. The average time for the latter was 2.0 minutes, and for the former about 0.5 minute. Slight mean differences were observed in packed cell volume and pH, and virtually none in CO₂ content. The only significant mean difference was that for hemoglobin, which was 0.19 mM/L. higher in cutaneous than in arterial blood. Although significant, this difference is small enough to ignore for most acid-base analyses, in which cutaneous blood can be considered almost identical with arterial blood.
Table 6. Simultaneous Arterial and Cutaneous Blood Samples
Comparison of Acid-Base Properties

<table>
<thead>
<tr>
<th></th>
<th>Arterial</th>
<th>Cutaneous</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (mM/L.)</td>
<td>8.50</td>
<td>8.60</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td>7.80</td>
<td>8.05</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td>8.45</td>
<td>8.60</td>
<td>-0.15</td>
</tr>
<tr>
<td></td>
<td>8.20</td>
<td>8.20</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6.65</td>
<td>7.00</td>
<td>-0.35</td>
</tr>
<tr>
<td></td>
<td>6.65</td>
<td>7.05</td>
<td>-0.40</td>
</tr>
<tr>
<td></td>
<td>7.85</td>
<td>7.85</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>-0.19</td>
</tr>
<tr>
<td>S.E.</td>
<td></td>
<td></td>
<td>±0.064</td>
</tr>
<tr>
<td>pH</td>
<td>7.41</td>
<td>7.41</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>7.53</td>
<td>7.55</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>7.35</td>
<td>7.35</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>7.45</td>
<td>7.46</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>7.39</td>
<td>7.37</td>
<td>+0.02</td>
</tr>
<tr>
<td></td>
<td>7.52</td>
<td>7.52</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>7.33</td>
<td>7.35</td>
<td>-0.02</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>-0.004</td>
</tr>
<tr>
<td>S.E.</td>
<td></td>
<td></td>
<td>±0.0083</td>
</tr>
<tr>
<td>Red cell volume (mM/L.)</td>
<td>0.425</td>
<td>0.435</td>
<td>-0.010</td>
</tr>
<tr>
<td></td>
<td>0.400</td>
<td>0.415</td>
<td>-0.015</td>
</tr>
<tr>
<td></td>
<td>0.420</td>
<td>0.420</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.395</td>
<td>0.395</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.345</td>
<td>0.365</td>
<td>-0.020</td>
</tr>
<tr>
<td></td>
<td>0.400</td>
<td>0.395</td>
<td>+0.005</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>-0.007</td>
</tr>
<tr>
<td>S.E.</td>
<td></td>
<td></td>
<td>±0.0058</td>
</tr>
<tr>
<td>Whole blood CO₂ (mM/L)</td>
<td>23.3</td>
<td>23.6</td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td>32.2</td>
<td>32.4</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>21.3</td>
<td>21.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>25.7</td>
<td>25.6</td>
<td>+0.1</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>21.6</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>26.4</td>
<td>26.0</td>
<td>+0.4</td>
</tr>
<tr>
<td></td>
<td>21.6</td>
<td>21.1</td>
<td>+0.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>-0.01</td>
</tr>
<tr>
<td>S.E.</td>
<td></td>
<td></td>
<td>±0.147</td>
</tr>
</tbody>
</table>

DISCUSSION

The suitability of this microtechnic for clinical studies of acid-base balance will be discussed from three aspects: the use of cutaneous blood; errors of collection and handling; and analytical accuracy.
Advantages of Cutaneous Blood

Our results confirm those of Lundsgaard and Møller (18), Saito (19) and Lilienthal and Riley (20) that cutaneous blood (drawn from hyperemic skin) is nearly identical with arterial blood in its acid-base properties. A comparable similarity has also been demonstrated for oxygen saturation (3, 18, 19). The only significant difference that we found was a slightly higher hemoglobin concentration, 0.2 mM/L. greater in cutaneous than in arterial blood. Part of the difference may have been due to dilution error but, regardless of the cause, the difference has a negligible effect on the use of this parameter in the calculation of CO₂ pressure and whole-blood buffer base concentration. Such a similarity of chemical properties is a fortunate circumstance for clinical studies, because the sampling of cutaneous blood is a much simpler process than an arterial puncture. The advantage of ease of sampling is thus combined with the further advantage of having arterial or cutaneous blood, rather than venous blood, for analysis of the state of acid-base balance of the patient. The respiratory factor in this state is most conveniently defined by the alveolar, or its equivalent, the arterial, CO₂ pressure, which can be readily calculated from the pH and CO₂ concentration of arterial blood or plasma. The usual arterio-venous differences with regard to O₂ saturation, CO₂ pressure, pH, and CO₂ content are greatly influenced by unpredictable variations in local venous flow. Consequently venous blood has the disadvantages of a wider range for "normal" values, a CO₂ pressure higher than in alveolar air, and a variable O₂ saturation, which introduces an additional factor in the calculation of Pco₂ and buffer base (14, 16). All these drawbacks are avoided by the simple expedient of using cutaneous blood for acid-base studies.

Collection, Handling, and Storage

The method described in this paper for the collection, handling, and storage of cutaneous blood samples is based on previous work (3, 21) and has here been shown to produce essentially no change in pH and CO₂ pressure, and only an insignificant decrease in bicarbonate and buffer base concentration amounting to less than 0.2 mEq./L. The anticipated effects of exposing blood to room air are a fall in Pco₂ and bicarbonate, a rise in pH, and no change in buffer base. However, a variety of effects may be produced by other sources of error, such as temperature change, the presence of an anticoagulant, glycolysis, hemolysis, and change in permeability of the red cell membrane. The combined error of a given method of handling blood therefore depends on a number of factors in addition
to exposure of the blood to air; in this method the results suggest a combination of slight accumulation of fixed acid, such as lactate, in the blood, and loss of displaced bicarbonate. The fact of importance in clinical chemistry is that the combined handling error is negligible if (1) heparin is used as anticoagulant, (2) the blood is chilled and stored in a closed syringe, and (3) exposure to air during collection or transfer is restricted to the conditions described herein.

Since the transient exposure to air does not introduce serious error, a technic of this sort might be called a "virtual anaerobic" technic. Undoubtedly the best method of avoiding this type of error is the "strict anaerobic" technic of Austin et al. (22), in which blood is handled in tonometers over mercury. However, these precautions are scarcely feasible or necessary in clinical studies of acid-base balance. On the other hand, such a "virtual anaerobic" technic appears to offer definite advantages over the common practice, in hospital laboratories, of relying on the sometimes uncertain protection (23–25) afforded by handling blood under oil, or of adhering to the outmoded "plasma CO₂ combining power" method (26), which was abandoned in Van Slyke's laboratory more than 30 years ago (27). Centrifuging blood under oil in the ordinary centrifuge tube is almost certain to produce appreciable loss of CO₂ (23), whereas use of a syringe (21) or a completely filled tube entails no appreciable loss (23; Table 6). Even with 1-ml. samples it is a convenience to avoid having to sample blood or plasma under oil; and we have demonstrated a definite decrease in accuracy of measuring 0.1 ml. aliquots when this is done from a sample under oil.

A certain amount of training is required for the analyst to attain proficiency in the collection of cutaneous blood samples and in the filling of the micropipets. The experience gained in acquiring such proficiency and in teaching others leads us to believe that the method is entirely suitable for any hospital laboratory where one or more individuals can be trained in its use, under the supervision of a clinical chemist.

**Accuracy**

The accuracy of the individual determinations is satisfactory for all clinical and many experimental purposes, as shown by the results in Fig. 3, and various papers describing each method (1, 2, 7, 28). The standard errors of measurement shown in Fig. 3 are estimates of reproducibility rather than of absolute error, which may be somewhat larger. For example, the hematocrit method is subject to errors due to trapping of solution between cells and to anisotonicity of the 0.9% NaCl diluent with respect
Table 7. COMPARISON OF COLORIMETRIC AND POTENTIOMETRIC pH IN HUMAN PLASMA AT 38°

Data from the Literature

<table>
<thead>
<tr>
<th>Source</th>
<th>No. comparisons</th>
<th>Difference, $pH_{\text{color}} - pH_{\text{pot}}$</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hastings and Sendroy*</td>
<td>5</td>
<td>$+0.007$ ± $0.011$</td>
<td>3 comparisons at 20°. In vitro equilibration.</td>
</tr>
<tr>
<td>Myers and Muntwyler 1928 (38)</td>
<td>41</td>
<td>$+0.020$ ± $0.037$</td>
<td>Venous plasma, as drawn from patients. Microcolorimeter for pH.</td>
</tr>
<tr>
<td>Foebinder and Schoono-\text{er} 1930 (39)</td>
<td>28</td>
<td>$+0.002$ ± $0.011$</td>
<td>Plasma diluted 1–20 with KCl-phenol red and equilibrated in vitro.</td>
</tr>
<tr>
<td>Austin, Stadie, and Robinson 1926 (36)</td>
<td>2</td>
<td>$+0.035$ ± $0.015$</td>
<td>One comparison with pH calculated from Henderson-Hasselbalch equation.</td>
</tr>
<tr>
<td>Van Slyke, Weisiger, and Van Slyke 1949 (2)</td>
<td>26</td>
<td>$-0.002$ ± $0.022$</td>
<td>Equilibrated in vitro, pH range 6.89 to 7.65. Photocolorimeter pH.</td>
</tr>
<tr>
<td>Weighted Mean</td>
<td>102</td>
<td>$+0.008$</td>
<td></td>
</tr>
</tbody>
</table>

* Hastings and Sendroy found a difference of $-0.015 ± 0.013$ in 13 comparisons in horse plasma, and $-0.010 ± 0.009$ in 4 comparisons in dog plasma.

to the actual red cell contents in a given blood. This uncertainty is not important in the calculation of acid-base factors, but of the two parameters, the hemoglobin concentration is a more accurate determination than the hematocrit value. The precision of the manometric blood-gas apparatus is well established for this as well as macro determinations (1, 28, 29). Unsuspected errors have been shown to be far greater in other methods commonly used in clinical laboratories (30), and best results cannot be obtained without careful attention to detail and accurate calibration of photometer tubes and pipets. The methods described in this paper have been successfully employed in a number of clinical studies (32–34).

The only part of the microtechnic to which objections have been raised in the past is the colorimetric pH determination. In comparison with the electrometric method (H, or glass electrode), the colorimetric method has been charged with being unreliable when applied to plasma or serum.

Stock volumetric pipets as purchased may deviate considerably from the stated volume and this is particularly true of the 30-cu.mm. hemoglobin pipets (31). In our experience the individually tested hemoglobin pipets supplied by Arthur H. Thomas Co. have been quite satisfactory; in one batch of 24 pipets calibration showed a maximum deviation from the 20-cu.mm. volume of only 0.3 cu.mm.
true

colorimetric-potentiometric

little

concentration

et

is

observed

between

pH

variability

human

colorimetric

doctrine

difference

sons

the

average

agreement

for

both

determined

and

comparisons

and

other

errors

the

final

result.

available

reports

comparisons

electric

potentiometric

pH

examined,

it

becomes

details

procedure

varied

widely.

comparisons

been

made

dog

human

blood,

serum

plasma,

colorimetric

pH

read

room

temperature

38°.

only

comparisons

relevance

basic

Hastings

Sendroy

technic,

Shock

Hastings

modifications

based.

summary

relevant

studies

human

plasma

given

Table

7.

Results

in

terms

the

colorimetric

potentiometric

pH,

both

determined

38°.

Most

papers

not

figures

mean

standard

deviation,

we

data

individual

differences.

evident

generally

good

agreement

several

series,

weighted

mean

difference

less

+0.01

pH

unit.

If

the

correction

Table

applied,

assuming

average

comparison

pH

7.40,

mean

difference

fall

between

0.00

and

-0.01.

reproducibility

the

adjudged

generally

small

standard

deviation,

is

good.

series

15

comparisons

electric

H

Van Slyke

found

mean

difference

-0.002

standard

deviation

±0.016

(2),

reproducibility

only

better

for

photometric

pH.

basis

result

appear

the

colorimetric

potentiometric

pH

determinations

38°

lies

-0.04

+0.04

in

90

per

cent

cases.

The

discrepancies

referred

previously

not

reported

human

plasma,

but

generally

on

dog

serum,

and

connection

variability

the

“C”

correction,

the

difference

the

colorimetric

pH

20°

photometric

pH

38°.

Species

differences

differences

between

serum

plasma

with

respect

“C”

correction

been

observed

Cullen

many

(35, 36, 40–42).

The

“C”
correction

+0.30

human

+0.23

human

plasma,

Robinson

et

demonstrated

the

correction

varied

the

serum

concentration

(43).

Some

results

dog

human

serum

characterized

large

standard

deviations,

ranging

±0.07

±0.11

(35–37).

On

the

other

hand,

Hastings

Sendroy

(6)

found

little

variation

the

plasma

one

another

the

colorimetric-potentiometric

difference

measured

38°.

Same

true

difference

human

compared

plasma,

38°.
(38, 41). From all this and other evidence (44) it seems clear that the final result in a colorimetric pH determination is influenced by a number of possible errors, which are generally larger when serum is used or when the reading is made at room temperature. For human plasma read at 37–38°, these errors either become very small or are self-compensating (44), as is demonstrated by the evidence in Table 7.

In their practical operation for the determination of pH in blood, both the H₂ electrode and glass electrode have serious disadvantages that must be weighed along with their advantages, in comparing them with the photocolorimetric method. For clinical work economy of sample size is often an important consideration, and in this the photometric method is superior to the usual glass electrode. The apparatus required for the photometric determination is cheaper and probably more reliable than the glass electrode and pH meter, but the technic of the determination is somewhat more complex. The standard error of measurement in the photometric pH determination appears to be smaller than that for any commercially available pH meter utilizing the glass electrode. After experience with both methods our preference in clinical work is definitely for the photometric.

Some of the features of the "virtual anaerobic" technic described here are applicable to the handling and centrifuging of larger amounts of blood, 2–20 ml., for the determination of pH and CO₂ concentration by other methods.

SUMMARY

1. A modification has been described for the Shock and Hastings technic of determining simultaneously, in 0.1 ml. aliquots of blood, the pH, CO₂ content, and packed cell volume. Advantages of the modification are collection and handling of the blood in a way that avoids the use of oil, and a modified design of the micropipets so that pH readings can be obtained in a filter photometer.

2. The standard error of measurement of a single reading was ±0.009 for the pH, and ±0.22 mM/L. for the whole blood CO₂ concentration. The reproducibility of the photocolorimetric pH measurement was slightly better than that obtainable with the most accurate commercially available glass electrode.

3. Results have been presented to show that blood can be handled in the presence of brief, limited, exposure to air with no detectable change in pH and only negligible change in CO₂ content (decrease of less than 0.2 mM/L). Errors attributable to collection and handling of cutaneous blood by this "virtual anaerobic" technic are not of significant magnitude.
4. Results have also been presented in confirmation of the fact that arterial and cutaneous blood are virtually identical in acid-base properties. A sample of as little as 0.5 ml. of cutaneous blood, analyzed by the microtechnic, provides the necessary and sufficient data for a description of the state of acid-base balance. This includes calculation of the physiologically important variables, arterial CO₂ pressure, and whole-blood buffer base concentration, thus furnishing a measure of the respiratory factor and the metabolic factor in any acid-base disturbance. With a sample size of 1.5 ml. it is also possible to determine other individual constituents of plasma, such as sodium, chloride, and total protein.

5. The technic is useful in the study of clinical disturbances of acid-base balance, especially in infants and children.

REFERENCES

7. Sendroy, J., Jr., and Hastings, A. B., J. Biol. Chem. 82, 197 (1929).
44. Hastings, A. B., Personal communication, 1944.