STUDIES OF ACIDOSIS.

I. THE BICARBONATE CONCENTRATION OF THE BLOOD PLASMA; ITS SIGNIFICANCE, AND ITS DETERMINATION AS A MEASURE OF ACIDOSIS.*

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I. Blood Bicarbonate and Acidosis.

Free carbonic acid is present in the body fluids in such concentration that it automatically converts into bicarbonate all bases not bound by other acids. The bicarbonate therefore represents the excess of base which is left after all the non-volatile acids have been neutralized and is available for the immediate neutralization of further acids. In this sense it constitutes the alkaline reserve of the body. The bicarbonate concentration of the blood is representative of that of the body fluids in general, and is normally maintained at a definite level. Entrance of free acids reduces it to an extent proportional to the amount of the invading acid.

While in data to be published in this and subsequent papers we believe that we have broadened the foundation of facts on which the above statements stand, the latter are either contained in the propositions laid down by Henderson (1908, b; 1909, b), as the result of observations by himself and others, or are self-evident corollaries of those propositions. They establish the blood bicarbonate as a criterion of the acid-base balance of the body. Accordingly, for use in the present series of papers, we define acidosis as a condition in which the concentration of bicarbonate in the blood is reduced below the normal level. The definition appears a necessary preliminary because of present confusion in the literature, different authors regarding acidosis differently as "acid intoxication," as a condition in which acetone bodies are formed, or as an actual increase in the hydrogen ion concentration of the blood.

Acidosis in the sense defined may result, as in diabetes, from such an overwhelming rapidly production of acids that even an apparently undamaged eliminating mechanism working at several times the usual rate cannot dispose of them. Or it may result, as in nephritis, from inability to eliminate acids even at the moderate rate at which normal metabolism produces them. In either case the retained acid decomposes body bicarbonate, forming in its place the salt of the invading acid.

1 Henderson's monograph and the papers by Henderson and Palmer contain so complete an exposition of the mechanism by which phosphates and the kidneys assist in maintaining body neutrality that this portion of the subject is given minimum consideration in the present paper.
The bicarbonate not only represents the alkaline reserve of the body, but its normal concentration in the blood is so definite that it constitutes a physiological constant. The blood plasma of the normal adult contains 50 to 65 per cent of its volume of CO₂ gas bound as bicarbonate. The limits of variation are similar in magnitude to those of the pulse rate. By utilizing as a standard the normal bicarbonate concentration we can reduce the term "acidosis" to as definite a meaning as "fever" or "tachycardia." In each case a condition is indicated in which one of the physiological constants falls or rises to an abnormal level. The possible causes are numerous, but the result, in the case of acidosis a lowering of the blood bicarbonate, is an accurately definable and determinable phenomenon. Like accelerated pulse rate or increased temperature, it may occur temporarily even in health, e.g., as the result of muscular exertion and the consequent lactic acid formation (Christiansen, Douglas, and Haldane, 1914). It is not necessarily a pathological condition in itself, but is a symptom of disturbed function. Like fever or tachycardia, however, acidosis in itself becomes a danger when it has reached a sufficient degree of intensity.

The hydrogen ion concentration, Cₜ, of the blood is a physiological constant even less variable than the plasma bicarbonate (Lundsgaard, 1912), the normal value of approximately 10⁻⁷.₄₅ being maintained with the utmost tenacity by the normal organism. Nevertheless, as a standard for measuring changes in the acid-base balance, it appears less desirable than the bicarbonate, for the reason that, while the bicarbonate decreases progressively as soon as the normal excess of bases over acids begins to be depleted, rise of the blood Cₜ is usually one of the latest changes that follow.

Benedict (1906) and Michaelis (1914, p. 105), for example, have observed in diabetic acidosis an increased Cₜ only after terminal coma had set in, and Peabody (1914) has made similar observations in the acidosis of cardiorenal disease. In both types of cases coma occurs only after the blood bicarbonate has been reduced to a fraction of its normal value.

The reason for the lateness of the stage at which increase in blood Cₜ appears is the fact that, until a large part of the bicarbonate has been exhausted, the organism can, by accelerated
respiration, maintain the ratio\(^2\) \(\frac{H_2CO_3}{NaHCO_3}\) in the arterial blood at its normal value. And the \(C_H\), being directly proportional to this ratio, is thereby also kept normal.

The manner in which the body uses carbonic acid and bicarbonate in order to maintain its neutrality has been most clearly described by Henderson in his monograph (1909, b). The normal \(C_H\) is maintained by the mechanism for keeping the \(\frac{H_2CO_3}{NaHCO_3}\) constant. From the law of mass action

\[
C_H = K \frac{H_2CO_3}{CO_2} = K \frac{H_2CO_3}{\lambda NaHCO_3}
\]

\(\lambda\) being the degree of dissociation of NaHCO\(_3\) into Na\(^+\) and HCO\(_3^-\) in the blood, and \(K\) the ionization constant of H\(_2\)CO\(_3\). Since \(\lambda\) varies but slightly within the range of conditions encountered within the blood plasma, one may state that in the plasma the hydrogen ion concentration varies directly as the value of the molecular ratio \(\frac{H_2CO_3}{NaHCO_3}\). Hasselbalch (1916, b) has shown that this law holds so accurately that he regards the determination of the \(\frac{H_2CO_3}{NaHCO_3}\) ratio as an even more reliable means than the gas chain for determining blood hydrogen ion concentration. Whenever, either by increased rate of CO\(_2\) production or by decomposition of NaHCO\(_3\) by acid, the ratio \(\frac{H_2CO_3}{NaHCO_3}\) is increased, the \(C_H\) of the blood is proportionally increased, and stimulates respiration. More rapid ventilation follows until the H\(_2\)CO\(_3\) of the blood is so reduced that the normal \(\frac{H_2CO_3}{NaHCO_3}\) ratio, and consequently the normal \(C_H\), is restored.

The respiratory response is so sensitive to this stimulus that Campbell, Douglas, Haldane, and Hobson (1913) observed that an increase of only 1 mm. in the CO\(_2\) tension accelerated the rate of ventilation 60 per cent, and Boothby (1915) has observed that the heart output is similarly increased in the effort to rid the body of excess CO\(_2\).

We find that plasma, obtained by drawing blood from the arm vein and centrifuging at once, contains at 37\(^\circ\) and normal CO\(_2\)

\(^2\) A minor portion of the combined carbonic acid of the plasma is, of course, neutralized by bases other than sodium; but as the acid-neutralizing power of the strong bases does not differ greatly we follow Henderson's convenient practice of using "NaHCO\(_3\)" to indicate the total bicarbonate.
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tension approximately 60 volume per cent of CO₂ gas bound as bicarbonate. The concentration of CO₂ in the form of H₂CO₃ calculated from the average arterial CO₂ tension of 42 mm. is 3 volume per cent \( \left( \frac{42}{760} \times 100 \times 0.54 = 3.0 \right) \), 0.54 being the solubility coefficient of CO₂ in blood plasma at body temperature, as determined by Bohr). Consequently the normal ratio

\[
\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{3}{60} = \frac{1}{20}
\]

a value which agrees approximately with that calculated from the known values of the constants in the equation³

\[
\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{\lambda \text{C}_n}{K}
\]

The process of accelerating ventilation and circulation in proportion to the fall in plasma bicarbonate, so that the ratio \( \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} \) and the resulting \( \text{C}_n \) are kept constant, can apparently continue until acidosis is so intense that the respiratory and circulatory mechanisms are no longer able to eliminate carbonic acid so rapidly as to keep its concentration down to one-twentieth that of the depleted bicarbonate. The level to which the bicarbonate falls before this failure of compensation occurs must vary with the sensitiveness of the nervous control and the efficiency of the respiratory and circulatory mechanisms, and has never been definitely fixed, although in diabetes and nephritis it appears to be a small fraction of the normal (Michaelis, 1914, p. 105; Peabody, 1914).

To distinguish the stage of acidosis in which the respiratory mechanism no longer keeps the carbonic acid concentration of the arterial blood down to the normal fraction of approximately one-twentieth the bicarbonate, and in which consequently the \( \text{C}_n \) actually does increase, Hasselbalch and Gammeltoft

³The average \( \text{C}_n \) is approximately \( 0.35 \times 10^{-7} \). According to Michaelis and Rona (1912), \( K = 4.4 \times 10^{-7} \), \( \lambda \) for blood conditions = 0.605. From these constants,

\[
\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{0.605 \times 0.35 \times 10^{-7}}{4.4 \times 10^{-7}} = \frac{1}{21}
\]
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(1915) have already used the term "uncompensated acidosis," which seems well worth general adoption. So long, on the other hand, as the respiration, despite decreased bicarbonate, succeeds in keeping down to normal limits the $\frac{H_2CO_3}{NaHCO_3}$ ratio, and consequently the $C_n$, the condition is one of compensated acidosis. We shall in future use this nomenclature.

A maintenance of the $\frac{H_2CO_3}{NaHCO_3}$ ratio at a constant value can, of course, be expected only in arterial blood. The $H_2CO_3$ of venous blood is increased by absorption of $CO_2$ from the tissues. Consequently venous blood is less alkaline than arterial, and the difference must vary according to the activity with which the tissues perfused are producing carbon dioxide. As was shown by Zuntz (1868), the influx of $CO_2$ raises not only the $H_2CO_3$, but by reactions such as $Na_2HPO_4 + H_2CO_3 \rightleftharpoons NaH_2PO_4 + NaHCO_3$, also raises the $NaHCO_3$. Consequently the arterial blood bicarbonate must be accepted as the ideal measure of the alkaline reserve. In resting dogs, however, and therefore, it seems justifiable to conclude, in man, the differences between venous and arterial blood are small and fairly constant, the following being fair examples: arterial $pH = 7.44$, venous $7.41$; arterial $NaHCO_3 + H_2CO_3 = 50$ cc. of $CO_2$ per 100 cc. of blood, venous $= 55$ cc. The differences are such that analyses of normal venous blood drawn during rest and without stasis may be regarded as but slightly inferior in accuracy and significance to those of arterial blood.

The sense in which we have used the word "acidosis" is not that given it by its originator, Naunyn (1906), who used the term to denote the abnormal metabolic condition in which hydroxybutyric acid is formed. The departure from this use in the literature has been a matter of evolution. Apparently because the word "acidosis" is suggestive of acids in general, rather than hydroxybutyric in particular, when other types of acid intoxication were discovered they also were designated as acidoses. In this broader sense the term has in recent years been used in most of the important scientific papers in the field (for example, Henderson, 1909; Palmer and Henderson, a series of papers; Barcroft, 1914; Sellards, 1914; Peabody, 1914; Howland and Marriott,
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1916; Hasselbalch, 1916) "acidosis" being employed to indicate the effect of acids of any type in altering the acid-base balance of the organism. We have followed these authors, rather than those who maintain the original hydroxybutyric acid definition of Naunyn. Despite the value of Naunyn's great work, it appears probable that the confused ideas of acidosis and acid intoxication that have been general have been to a considerable degree due to his definition of the term, which does not differentiate ketone production, unaccompanied by significant effect on the acid-base balance of the body, from the condition in which the acids produced do lower or abolish the reserve of alkali.

The formation of acetone bodies has a significance of its own quite apart from the secondary effect which may or may not follow on the alkaline reserve of the body. It indicates that fatty acids, derived either from fats or from amino-acids, are being incompletely oxidized. The products, \(\beta\)-hydroxybutyric and acetoacetic acids, may or may not be so produced and eliminated that they lower the internal alkaline reserve. We have observed an excretion of 20 gm. of acetone bodies, calculated as hydroxybutyric, per liter of urine without an abnormally low plasma bicarbonate. It is desirable that the condition in which these substances are produced be designated by a name indicating the specific nature of the metabolic abnormality and not confusing it with the general question of the acid-base balance. Rowntree proposes that the excretion of acetone bodies be indicated simply as "ketonuria," while Allen (1917) suggests for the metabolic condition which gives rise to them the equally concise and specific name of "ketosis."

II. Other Methods for Detection of Acidosis Considered as Means for the Approximate Measurement of the Arterial Blood Bicarbonate.

Most methods which have in the past demonstrated some degree of quantitative accuracy in indicating the clinical severity of acidosis are seen when analyzed to constitute approximate determinations, either direct or indirect, of the blood bicarbonate. The following are important examples.
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1. Titration of Blood.—Titration of the blood plasma or of the filtrate obtained after precipitating the proteins with a neutral reagent is one of the oldest methods used in the study of acidosis. The source of error lies in the fact that at the high $C_H$ of the end-points usually employed the titrations measure, in addition to the bicarbonate, also an acid binding power of such buffers as the phosphates, and particularly the proteins, quite out of proportion to the amounts of acid which these substances bind within the $C_H$ limits that occur in the blood during life. Nevertheless, the bicarbonate seems to be the chief cause of variations in the titration figures, and results obtained by this method have consequently been of definite value in developing a knowledge of the changes that constitute acidosis (Jaksch, 1888; Magnus-Levy, 1899; Cullen, Paper III of this series).

2. Determination of the Carbon Dioxide Content of Venous Blood.—The use of the carbon dioxide content ($CO_2$ from $H_2CO_3$ and $NaHCO_3$) as a measure of the blood alkali dates back to Walter (1877), who, working in Schmiedeberg's laboratory, showed that the venous carbon dioxide of rabbits could be reduced to one-tenth its normal height by injection of acids. The significance of the determination does not differ essentially from that of the bicarbonate of the venous plasma, determined as described in this paper. In so far as the results indicate the bicarbonate of arterial blood, which must be considered as the true or compensated blood bicarbonate, the source of error lies in the fact that the blood in passing through the capillaries into the veins takes up an amount of carbonic acid which is variable with the rate of oxidation in the tissues and of blood flow through them. As a matter of experience, however, when the blood is drawn from a large vein without stasis, the difference between the venous blood and arterial appears to be sufficiently constant so that the figures for venous total carbon dioxide run approximately parallel to those for the arterial bicarbonate. The failure of Walter's method for detecting acidosis by determination of the venous $CO_2$ to attain general clinical use even in hospitals must be attributed chiefly to the lack of a sufficiently simple technique for the determination.
3. Determination of the Carbon Dioxide Capacity of Venous Blood.—In order to restore the venous blood to the condition of arterial and thus avoid the possibility of the error outlined in the above paragraph, Christiansen, Douglas, and Haldane (1914) saturated the venous blood with air containing carbon dioxide under the tension existing in normal arterial blood. As illustrated by Experiment IX, the fall caused by acidosis in the carbon dioxide capacity of venous blood is proportional to, and therefore a measure of, the fall in arterial bicarbonate.

In choosing the routine method described in this paper for measuring the alkaline reserve we have given preference to the CO₂ capacity of the plasma, rather than either the CO₂ content or the CO₂ capacity of venous whole blood, for practical reasons stated in the discussion of Experiment IX.

4. Determination of the Reduced Hydrogen Ion Concentration of the Blood.—Hasselbalch (1916, a) saturated blood with carbon dioxide at 37° under 40 mm. tension and determined under these conditions the Cₚ, which he calls the "reduced hydrogen ion concentration." The H₂CO₃ concentration being fixed by the constancy of the CO₂ tension and temperature, the hydrogen ion concentration determined must vary inversely as the NaHCO₃, since Cₚ = K \frac{H₂CO₃}{NaHCO₃}. Hasselbalch's "reduced hydrogen ion concentration" is therefore a measure of the blood bicarbonate.

5. Determination of the Oxygen Affinity of Hemoglobin under Standard CO₂ Tension.—Barcroft and Peters (Barcroft, 1914, p. 316) found that under changing CO₂ tension the proportion of oxygen bound by hemoglobin depended on the hydrogen ion concentration. The value of the oxygen affinity constant, K, in the equation \( \frac{y}{100} = \frac{Kx^n}{1 + Kx^n} \) (y = percentage saturation of hemoglobin with oxygen, x = oxygen pressure) varies inversely as Cₚ. The results of Barcroft and Peters have been confirmed by Hasselbalch (1916, b). Since under a given CO₂ tension the Cₚ varies inversely as the NaHCO₃, it is evident that the oxygen affinity under a given CO₂ tension is also an indirect measure of the bicarbonate; the NaHCO₃ fixes the Cₚ, and through it the oxygen affinity.
b. Determinations on the Alveolar Air.

1. Arterial Carbon Dioxide Tension (Haldane Method).—The alveolar air, as shown by A. and M. Krogh (1910) is in equilibrium in respect to its carbon dioxide content with the arterial blood. Consequently, in accordance with the law of gas solubility, the concentration of carbon dioxide in the alveolar air is directly proportional to that of free carbonic acid in the blood. And the latter has been shown (p. 293), with normal respiratory control, to be kept proportional to the bicarbonate concentration. Consequently the carbon dioxide concentration of the alveolar air is, through the intermediary parallelism of the blood $H_2CO_3$, kept proportional to arterial $NaHCO_3$. All three concentrations go up and down together, the blood bicarbonate fixing the level of the carbonic acid, and the latter that of the alveolar carbon dioxide. Consequently in normal individuals the Haldane determination of the carbon dioxide content of air expired without previous holding of breath (Haldane and Priestley, 1905) indicates approximately the bicarbonate concentration of the arterial blood. Under pathological conditions, or under the influence of drugs, of decreased atmospheric oxygen tension, or of anxiety or excitement, the sensitiveness of the respiratory control may vary (Hasselbalch, 1912; Michaelis, 1914, p. 97; Higgins, 1915; Straub, 1915; Peters, 1917; Stillman, Van Slyke, Cullen, and Fitz, 1917), so that the alveolar carbon dioxide is not under all conditions even an approximate measure of the bicarbonate reserve. Higgins (1914) found that even changing the position of the body from standing to lying could alter the alveolar CO$_2$ tension to the extent of 6 mm. Sonne (1915) has shown that a mechanical error may be added to those caused by changes in the nervous control. The air collected at the end of an expiration may fail to represent the average alveolar air, instances in a normal subject being observed in which its CO$_2$ content was as much as 1.4 volume per cent (corresponding to 10.6 mm. tension, or one-fourth the total normal value) lower than the CO$_2$ content of samples of air taken near the middle of the expiration. Apparently the completeness of the gas exchange varies in different parts of the lungs. All sources of error together, however, even in pathological conditions, are in most of the cases encountered within such limits that the clin-
ical utility of carbon dioxide determinations in the alveolar air as a measure of the alkaline reserve of the blood is thoroughly established (Beddard, Pembrey, and Spriggs, 1915; Straub, 1915); although the fact that so many factors besides the alkaline reserve of the blood can affect the alveolar carbon dioxide tension certainly makes the latter far from an ideal measure of the former.

2. Venous Carbon Dioxide (Plesch Method.)—The Plesch method (Plesch, 1909; Porges and Leimdörfer, 1915) differs from the Haldane in that the air analyzed, instead of being taken at the end of a single quick expiration, is breathed in and out of a rubber bag by the subject for 30 or 40 seconds. Consequently the carbon dioxide tension approaches more nearly that of the venous than of the arterial blood, the Plesch results being as a rule 4 to 6 mm. higher in carbon dioxide tension than the Haldane results. Since the venous carbon dioxide tension runs fairly parallel with the arterial, however, the Plesch results may be taken as indirect measures of the arterial bicarbonate, subject to the same errors as the Haldane results, and so to say, one degree less direct than the Haldane. An advantage of the Plesch technique is that it requires less cooperation on the part of the subject than the Haldane procedure, and has consequently been employed even with infants (Howland and Marriott, 1916).

c. Determinations in the Urine.

1. Determination of the Acid Excretion.—Since Magnus-Levy’s famous paper (1899) showed the significance of β-hydroxybutyric acid as the cause of the acid intoxication in diabetic coma, it has been a matter of common observation that symptoms of acid intoxication in diabetes are usually accompanied by the excretion of large amounts of β-hydroxybutyric acid, along with lesser amounts of acetoacetic, partly as ammonium salts, but also partly as free acids. That in diabetes the excretion of free acid plus ammonia by the kidneys bears a quantitative relationship to the blood bicarbonate concentration is demonstrated in the accompanying paper by Fitz and Van Slyke.

2. Alkali Retention.—The extremely practical alkali retention test devised independently by Palmer and Henderson (1913) and by Sellards (1914) appears also to be an indirect measure of the bi-
carbonate content of the body fluids, as represented by the plasma. Work by Palmer, which will shortly be published in this Journal, indicates that when the plasma bicarbonate (determined as described in this paper) has reached what may be called the critical level, near the upper extreme of the normal range, urine more alkaline than blood is excreted. The amount of bicarbonate which must be taken into the organism in order to turn the urine alkaline is approximately the amount necessary to raise the bicarbonate concentration of all the body fluids to this level, if the fluids are estimated at 0.7 of the body weight and assumed to equal the plasma in bicarbonate content. The amount of alkali necessary to administer in the retention test appears consequently to be proportional to the margin by which the plasma bicarbonate falls below the critical level at the time of administration, and therefore constitutes an indirect measure of the plasma bicarbonate.

III. The Influence of Free Carbonic Acid Concentration in the Blood on the Plasma Bicarbonate.

The plasma bicarbonate concentration is influenced by the free carbonic acid concentration, both of the whole blood at the time the plasma is separated from the cells, and of the plasma itself at the time the determination is made. The influence is exerted respectively through affecting the distribution of acids and bases between plasma and corpuscles, and through affecting reactions within the plasma itself. Both modes of influence must be considered in connection with any method for determining the concentration of plasma bicarbonate in venous blood, and we shall therefore discuss them from the standpoint of their effects on such determinations.

a. Influence through Effect on Equilibria within the Plasma.—To a minor extent the bicarbonate of the plasma can be affected by the equilibrium between normal carbonate, bicarbonate, and carbonic acid: $2\text{NaHCO}_3 \rightleftharpoons \text{Na}_2\text{CO}_3 + \text{H}_2\text{CO}_3$. As shown by Bohr, however, the conditions of this equilibrium are such that, with the concentrations of free carbonic acid existing in the plasma during life, the proportion of Na$_2$CO$_3$ is from a quantitative standpoint negligible, practically all the alkali not bound by other acid than carbonic being in the form of bicarbonate.
In a 0.155 per cent sodium carbonate solution (about the average carbonate concentration of plasma) at 38°, and with the physiologically normal carbon dioxide tension of 45 mm., Bohr calculated that 99.5 per cent of the sodium carbonate was in the form of bicarbonate, and confirmed the calculation experimentally within the limit of analytical error. Even at 12 mm. CO₂ tension, which is seldom if ever observed in life except in premonitory coma, 98 per cent was in the form of bicarbonate. Consequently one can, for quantitative purposes, regard the bicarbonate of the plasma as synonymous with its entire reserve of alkali in excess of that neutralized by acids other than carbonic.

There are other equilibria than that between carbonate and bicarbonate, however, which are more sensitive to changes in H₂CO₃ concentration. If carbon dioxide escapes from a sample of plasma, the latter loses not only free carbonic acid CO₂, but also part of the CO₂ normally combined as bicarbonate, which undergoes partial decomposition by such reversible reactions as NaHCO₃ + protein $\rightleftharpoons$ H₂CO₃ + Na proteinate. This reaction of the proteins appears in fact to be the one chiefly responsible for the variation in plasma bicarbonate caused by varying free carbonic acid (see Experiments II and III). (While in the cells the reaction NaHCO₃ + NaH₂PO₄ $\rightleftharpoons$ Na₂HPO₄ + H₂CO₃, studied by Henderson (1906), is important, in the plasma the phosphate concentration is too small to affect appreciably the bicarbonate (Greenwald, 1915).) If the H₂CO₃ falls greatly below normal even the reaction 2NaHCO₃ $\rightleftharpoons$ H₂CO₃ + Na₂CO₃ becomes appreciable. In each of these reactions a decrease in the free carbonic acid results in a shift of the equilibrium from left to right, and consequently in a decrease of the bicarbonate. Thus Jaquet found that at 42.7 mm. CO₂ tension the bicarbonate CO₂ of a normal plasma sample was 63.7 volume per cent, while at 17 mm. it was 58.5 per cent. The difference, though not great, is considerable, and becomes accentuated as the carbon dioxide tension is reduced still lower. Consequently in a solution such as the plasma the term bicarbonate content has a quantitatively definite meaning only for a definite concentration of free carbonic acid.

One has the choice of two alternatives. One may vary the free carbonic acid in proportion to the bicarbonate, maintaining the 1:20 ratio, and thus determining the genuine "compensated" bicarbonate which exists in the arterial blood. Or one may make all determinations at a fixed and definite carbonic acid concentra-
tion. The first plan has the theoretical advantage of duplicating natural conditions, but is impracticable for a routine method, as it would necessitate the use of a different carbon dioxide mixture in saturating every plasma. We have therefore, in the method described in this paper, adopted the plan of saturating all plasmas with carbon dioxide under normal alveolar tension. This has the theoretical disadvantage that in extreme acidosis the bicarbonate determined is not quite so low as that actually existing in the arterial blood. The fall below normal, however, is parallel to that of the arterial bicarbonate (see Experiment IX) and, as a matter of fact, the absolute difference between results by the two methods is not great. For example, the plasma of a diabetic patient with marked acidosis showed, when saturated with CO₂ at the reduced alveolar CO₂ tension of the patient, a bicarbonate yielding 23 cc. of CO₂ per 100 cc. of plasma, while the figure obtained after saturating with CO₂ under normal tension was 26 cc. We believe that under the conditions of constant CO₂ tension chosen the results are no less definite in their significance than they would be if we attempted to approximate the varying CO₂ tension existing in arterial blood.

b. Influence of Carbonic Acid on the Plasma Bicarbonate through Effect on the Transfer of Electrolytes between Plasma and Cells.—Gürber (1895) noticed that as the result of saturating the blood with carbon dioxide in vitro the titratable alkali of the plasma, which includes the bicarbonate, was increased. This phenomenon could be explained by assuming either that alkali diffuses from cells into plasma to meet the increased carbonic acid there, or that acids other than carbonic are, so to say, forced by the carbonic from the plasma into the cells, leaving in the form of bicarbonates the alkali with which they had been combined. Gürber claimed that no potassium or sodium at all passed from corpuscles into plasma when blood was saturated with carbon dioxide, that the entire change was due to passage of HCl from the plasma into the cells, the amount which passed being equivalent to the gain in titratable alkali in the plasma. As shown by Experiment X, however, the amount of HCl which disappears from plasma of blood saturated with pure CO₂ is equivalent only to about one-third the increase in bicarbonate. Hamburger (1916) has recently shown, furthermore, there is some transfer of K and Na
between plasma and cells. Consequently Gürber's belief that transfer of HCl alone was responsible for the alkali shift caused by saturating blood with CO₂ does not hold. That the alterations which occur within physiological limits of CO₂ tensions are chiefly due to transfer of HCl appears probable, however (see Experiment X). The reaction in the plasma, the consequent HCl transfer, and reaction of the transferred acid with phosphates inside the cell may be formulated as follows:

\[
\begin{align*}
\text{Plasma} & : \quad \text{H}_2\text{CO}_3 + \text{NaCl} \rightarrow \text{NaHCO}_3 + \text{HCl} \\
\text{HCl} & \rightarrow \text{Cell wall} \\
\text{Cell} & : \quad \text{HCl} + \text{Na}_2\text{HPO}_4 \rightarrow \text{NaH}_2\text{PO}_4 + \text{NaHCO}_3
\end{align*}
\]

\[i.e.,\quad \text{although the buffer salts may not readily pass out to neutralize acid in the plasma, the acid does pass in to meet the salts within the cells, so that the same effect is obtained in maintaining plasma neutrality. In fact Hamburger, to whose thorough researches we owe most of our knowledge in this field, has shown that it is highly probable that the corpuscles only typify the body cells in general, and that the transfer of acid to and from the latter is of such a nature that the plasma has practically all the buffer salts of the body at its disposal in maintaining its neutrality, despite the fact that it itself is not particularly rich in such salts. In confirmation of this view see Section 2 of the discussion of the results of Experiment IX.\]

The magnitude of the effect on plasma bicarbonate which can be caused by such loss of carbon dioxide as may occur when blood is drawn into an open receptacle may be seen by reference to Experiment VII, while the extreme effects obtainable, on the one hand by removing CO₂ as completely as possible, on the other by saturating the blood with pure CO₂ gas, are shown by Experiment X.

It is evident that in fixing conditions for the determination of the plasma bicarbonate as a measure of acidosis the concentration of free carbonic acid not only in the plasma at the time of the determination, but also in the whole blood at the time the corpuscles are separated from the plasma, must be considered.

The ideal determination would be made on arterial blood drawn without loss of CO₂, for only in the arterial blood is the constancy of the \[
\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}
\]
ratio exactly maintained. The use of venous
blood from human subjects being necessary, however, the nearest approximation to arterial conditions would be obtained by saturating the blood at body temperature with CO$_2$ at such a tension that the normal value of the $\frac{H_2CO_3}{NaHCO_3}$ ratio in the blood would be maintained. This can be approximated by saturating the blood at body temperature with the alveolar air of the subject. In clinical routine, however, such a procedure adds to the technique a complicating factor which our experience indicates is unnecessary. An alternative would be to determine the "carbon dioxide capacity" of the blood after saturating the whole blood with carbon dioxide at the average normal alveolar tension. This method and the practical drawback to its routine application are discussed on pp. 298 and 308. It proved in fact to be entirely practicable to centrifuge the blood as it was obtained directly from the arm vein.

Method for Determining the Plasma Bicarbonate under Constant Dioxide Tension.

I. Drawing Blood Sample—For at least an hour before the blood is drawn the subject should avoid vigorous muscular exertion, as this, presumably because of the lactic acid formed, lowers the bicarbonate of the blood (Christiansen, Douglas, and Haldane, 1914; Morawitz and Walker, 1914). The blood is drawn from the arm vein directly into a centrifuge tube containing enough powdered potassium oxalate to make about 0.5 per cent the weight of the blood drawn. In order to avoid accumulation of carbon dioxide and consequent effect on the electrolyte transfer between plasma and cells, it is desirable to avoid stasis, or when stasis is necessary, to release the ligature as soon as the vein is entered and allow a few seconds for the stagnant blood to pass. This is particularly important when the second procedure for collecting blood described below is used, for in this case no opportunity is given for excess of free CO$_2$ to escape.

For drawing the blood we have used two methods. For clinical purposes the McRae needle4 was chiefly employed

4 The McRae blood needle may be obtained from the Kny-Scheerer or the Tiemann Company of New York. The principle is similar to that of the Hallion and Bauer needle, described in Compt. rend. Soc. biol., 1912, lxxii, 232.
(first method). With this, the blood enters the collecting tube in a fine stream and falls through a height of several centimeters before it reaches the bottom. During this fall there is opportunity for the escape of carbon dioxide and for absorption of oxygen. The carbon dioxide loss, by its effect on the transfer of HCl between plasma and corpuscles, discussed in the preceding pages, measurably lowers the bicarbonate content of the plasma. As a matter of experience, however, the gas exchange which occurs in the interval of about 0.01 second during which the blood is falling is only enough to bring the carbonic acid content of the venous blood to approximately that of arterial (see Experiment VII). The tube is turned on its side and back to vertical position once or twice after the sample has been drawn, in order to mix the oxalate. The blood is subjected to no other agitation which might accelerate loss of carbon dioxide, and is centrifuged in the same tube within a few minutes after it has been drawn (for effect of standing see Table X). The results of Stillman, Van Slyke, Cullen, and Fitz (Paper VI of this series), who compared the CO₂ capacity of plasma drawn by this technique with the CO₂ capacity of the whole blood, indicate that the McRae tube can be used in routine clinical work, provided the above precautions are observed, without fear of error.

The other method is to avoid all loss of carbon dioxide and obtain strictly venous blood. For this purpose ordinary care in the use of a syringe is sufficient, the blood being drawn without suction, and free air space in the syringe being avoided. A satisfactory substitute for the syringe is shown in Fig. 1. The blood is collected and centrifuged under paraffin oil. The slight amount of agitation necessary in order to assure mixture of the oxalate is accomplished by stirring with the inlet tube, rather than by inverting or shaking. The paraffin oil, like most organic liquids, dissolves carbon dioxide in greater amounts than does water, and its action in preventing loss of carbon dioxide from the blood is due to prevention of free diffusion from the surface of the water rather than to the formation of a layer impermeable to gas. Consequently the tube is subjected to a minimum of agitation after the blood is in it. When this precaution is taken, the results are the same as those from blood drawn with a syringe (see Experiment VII, d).
FIG. 1. Centrifuge tube arranged for collecting blood under paraffin oil without loss of carbon dioxide.
When blood is drawn with momentary exposure to air (McRae needle) and analyzed in the manner described in this paper, the plasma being resaturated with air containing 5.5 per cent of CO₂, results of our own and the analyses of thirty normal bloods by Gettler and Baker (1916) show that the bicarbonate CO₂ yielded by 100 cc. of normal plasma varies from 53 to 75 cc., reduced to 0°, 760 mm. A great majority of plasmas show figures between 60 and 70 cc. Occasionally an alkaline diet may force the figure to the upper limit of 80 cc., but we have not yet seen it below 53 in any normal person.

With the second method (venous blood drawn under oil without loss of CO₂) the results of Austin and Jonas (1917) agree with the relatively small number of determinations on human subjects which we have made in placing the minimum normal figure at 60 instead of 53 cc. of CO₂ per 100 cc. of plasma. With either procedure, but especially with this one, where there is no opportunity for the escape of excess CO₂, the remarks in the first paragraph of this section concerning the avoidance of stagnation are pertinent.

If the difference in the level of the minimum normal values is kept in mind, it appears that the two methods of blood drawing may be used interchangeably (see, for example, Experiment VII, e). In this hospital we have used chiefly the McRae needle, but Austin and Jonas, to whom we have communicated both methods and who have tried both, prefer the oil tube.

II. Separation and Storage of Plasma for Analysis.—While results of similar significance are obtained by analysis of either

---

**Fig. 2.** Separatory funnel containing plasma and arranged for filling with alveolar air.
whole blood or plasma, we prefer the plasma for routine determinations for the following reasons. It can be measured and handled with greater convenience than whole blood. The oxygen bound by the hemoglobin does not complicate the determination when plasma is used. And, perhaps the most important reason, plasma can be kept for a long time without alteration in its carbon dioxide binding capacity, while whole or defibrinated blood begins to show a decrease in its alkaline reserve soon after it has been drawn. This apparent formation of acid in blood in which the corpuscles remain was discovered by Christiansen, Douglas, and Haldane, who found that at 37°, even within an hour after blood had been drawn and defibrinated, an appreciable fall in the carbon dioxide capacity occurs, so that they were able to obtain comparable results only when a constant interval, as short as possible, was allowed to elapse between the drawing of the samples and the determination of their carbon dioxide capacities. The change observed can be most readily explained as due to the formation of acids in the blood cells. The change certainly does not occur in their absence, for we find that sterile plasma, if kept cold and in tubes that have been paraffined in order to avoid solution of alkali from the glass, can be preserved for over a week without alteration in its carbon dioxide capacity. It may be well to state here, however, that plasma in ordinary glass can be kept for only a few hours, as sufficient alkali dissolves from the glass in longer intervals to increase measurably the carbon dioxide capacity.

In case it is necessary to separate the plasma by gravity, the sedimentation is allowed to occur in a closed tube which is completely filled with blood, so that no carbon dioxide can escape, and the plasma is drawn off in as short a time as possible.

Since during rest and normal circulation the carbon dioxide content of the venous blood is only a few per cent higher than that of the arterial, and the difference does not vary greatly, the plasma obtained without stasis might be analyzed at once and without further preparation. As a matter of routine, however, we have found it desirable to saturate the plasma with carbon dioxide at a definite tension, as described below, immediately before analysis, and thus avoid the possibility of error caused by loss of carbon dioxide while the sample is awaiting analysis.
III. Saturation of Plasma with Air Containing Carbon Dioxide under Normal Alveolar Tension.—For the saturation we have found the most convenient vessels to be ordinary separatory funnels capable of holding about 100 times the volume of the plasma that is to be saturated. The plasma is placed in the funnel, the latter is turned on its side, and the air within is displaced by either alveolar air from the lungs of the operator or with 5.5 per cent CO₂-air mixture from a tank. In either case the gas mixture must be passed over glass beads before it enters the funnel (see Fig. 1). Otherwise, when air from the lungs is used the plasma is appreciably diluted with the moisture which condenses from the breath on the inner walls of the funnel. By passage over a large surface of either wet or dry glass beads at room temperature the expired air is cooled, and the excess moisture in it is condensed, so that not enough is carried into the funnel to cause an appreciable error. When, on the contrary, a dry CO₂-air mixture from a tank is used, it causes an appreciable evaporation from the surface of the plasma, with consequent increase in its concentration and in the carbon dioxide capacity. This also is obviated if the gas mixture is passed over wet beads, so that it approaches saturation with water vapor.

For obtaining an artificial mixture of air containing 5.5 per cent of CO₂ we have used an ordinary metallic gas tank capable of standing 20 atmospheres pressure and provided with an accurate pressure gage. Carbon dioxide was run in from another tank until the desired pressure was indicated. Then air was run in until the total pressure of air plus CO₂ was 18.2 times that of the CO₂ (taking into account that the tank contains one atmosphere more than the gage registers). The tank was then laid on its side for a half hour to give the gases an opportunity to mix thoroughly, and samples were drawn for analysis before the mixture was used. The analysis had to be repeated every few days, as the CO₂ content of the gas sometimes changes unexplainably. In order to displace completely the air in the separatory funnel with the CO₂ mixture, five or more volumes were run through, the gas, after leaving the funnel, being collected in a gasometer or rubber bag so that the volume passed could be roughly estimated.

When alveolar air is used, the operator, without inspiring more deeply than normal, expires as quickly and as completely as possible through the bottle of glass beads and the separatory funnel connected as shown in Fig. 2. The stopper is inserted just before the expiration is finished, so that there is no opportunity for air to be drawn back into the funnel. With a little practice a normal
person can consistently fill a 300 cc. separatory funnel with air containing within a few tenths of a per cent of the desired 5.5 per cent of CO₂. The composition is not, of course, so constant as that obtained when an analyzed gas mixture is used to fill the funnel, but as a matter of experience we have never found that the deviations caused significant error in the results. A change of 0.5 per cent in the CO₂ concentration of the air with which the plasma is shaken causes a change of only about 1 volume per cent in the plasma in the amount of CO₂ gas taken up, of which the total is normally 60 to 80 volumes per cent.

The following figures exemplify the effect of CO₂ concentration in the air on the amount of carbon dioxide taken up by plasma. Samples of the same plasma were shaken in atmospheres of air containing respectively 3.2, 5.5, and 9.6 per cent of carbon dioxide. The results were:

<table>
<thead>
<tr>
<th>Volume per cent of CO₂ in air.</th>
<th>Gas obtained from 1 cc. of plasma.</th>
<th>Change in absorbed CO₂ due to 1 per cent change in CO₂ of air.</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>3.2</td>
<td>0.584</td>
<td>0.023</td>
</tr>
<tr>
<td>5.5</td>
<td>0.636</td>
<td></td>
</tr>
<tr>
<td>9.6</td>
<td>0.700</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Fig. 3. Rack for holding separatory funnels during saturation of plasma. The elastic cord which holds the stoppers, as well as the entire funnels, in place, is made of spiral wire.
In order to saturate the plasma the separatory funnel is turned end over end for 2 minutes, the plasma being distributed in a thin layer as completely over the surface of the funnel’s interior as is possible. We have found that 2 minutes’ shaking in this manner uniformly suffices for saturation, but that 1 minute is as a rule not enough. When there are several analyses to be done it is convenient to use a rotating rack such as is shown in Fig. 3. In this ten separatory funnels can be shaken at once, and the rack acts as a holder for them at other times.

As a rule, when plenty of plasma is available, we saturate 3 cc. of it in a 300 cc. separatory funnel. One then has sufficient for duplicate determinations on 1 cc. each. As it is possible even with the large apparatus to make a determination with 0.5 cc. of plasma, one can, when the supply is scanty, saturate a little more than 0.5 cc. in a 50 cc. funnel. In this case the volume of distilled water and acid used to wash the plasma into the apparatus is also halved, so that the total volume of water solution introduced is only 1.25 cc. The volume of gas observed is multiplied by 2 before it is used to calculate the volume per cent of CO₂ bound; i.e., a considerable error would be caused if the CO₂ capacity were first calculated from the observed reading, and the result multiplied by 2 after the calculation.

When the micro-apparatus for carbon dioxide determinations described in the next paper is used, one-fifth the above amounts of plasma suffices.

IV. Determination of Carbon Dioxide Content of the Saturated Plasma.—After saturation is completed the funnel is placed upright and allowed to stand a few minutes until the fluid has drained from the walls and gathered in the contracted space at the bottom of the funnel. A sample of 1 or 0.5 cc. for the large apparatus, or 0.2 cc. for the micro-apparatus, is drawn with a calibrated pipette and used for the determination of the carbon dioxide content, which is performed as described in the next paper.

When the plasma is being delivered from the pipette into the cup of the apparatus, the tip of the pipette is held below the surface of the liquid in the cup. If the plasma were allowed to run through the air in a fine stream loss of carbon dioxide would result.
It is convenient to use a small drop (0.02 cc.) of octyl alcohol to prevent foaming of the plasma and emulsification of the mercury.

The gas volume is measured after a single extraction, and the result is calculated, by means of the table on p. 316 into terms of volume per cent of carbon dioxide gas, measured at 760 mm., 0°, which is bound as bicarbonate by the plasma.

The carbon dioxide combining capacity of plasma varies appreciably with the temperature, so that human plasma at 20° binds as bicarbonate approximately 106 per cent as much carbonic acid as at 37°. The extent of the temperature effect is demonstrated and its nature discussed in connection with Experiment IV. After determining the temperature coefficients of a number of plasmas we have been able to introduce the average coefficient into the calculation, so that both saturation and analysis can be performed at room temperature without significantly affecting the constancy or reproducibility of the results.

V. Calculation of Results.—When from plasma, saturated as above described with alveolar air, gases are extracted for analysis one obtains not only the CO₂ bound as bicarbonate and set free by acidification, but also the CO₂ and air physically dissolved by the plasma and water. The gases thus dissolved are, of course, independent of the alkaline reserve, and are subtracted from the total in order that the carbon dioxide bound as bicarbonate may be estimated. The exact amount to be subtracted, which is about 0.10 cc. when 1 cc. of plasma is analyzed, but varies slightly with the room temperature, may be determined by blank analyses, or calculated from the known solubility coefficients of the gases.

a. Determination of Correction for Dissolved Gases by Blank Analysis.—A few cc. of acidulated water are saturated with alveolar air or 5.5 per cent CO₂, as described above, and 1 cc. is analyzed with the same technique used for plasma. The total amount of gas obtained is the “dissolved gas” correction. When subtracted from the volume of gas obtained in a plasma analysis made under similar conditions of temperature and pressure, the difference represents the CO₂ chemically bound as bicarbonate in the plasma. This value is multiplied by the factor given in column C, Table I of the next paper, which both reduces the gas to standard conditions (0°, 760 mm.) and corrects for the 4 or 5
Plasma Bicarbonate

per cent of the total CO₂ not removed from the water by the single extraction.

b. Formula Including Corrections for Temperature and Dissolved Gases. Table for Calculation of Results.—In order to calculate the carbon dioxide chemically bound (as bicarbonate) the basic equation of Paper II must be altered by introducing a term which deducts from the total carbon dioxide content of the plasma the amount dissolved as free carbonic acid. This amount in cc. of carbon dioxide gas per cc. of plasma is equal to \( p \alpha_{\text{CO}_2} \) when the plasma is in equilibrium with air containing \( p \) proportion of carbon dioxide by volume, \( \alpha_{\text{CO}_2} \) being the solubility coefficient of carbon dioxide in water. Introducing this subtraction into our basic equation, the latter becomes

\[
(1) \quad x = \frac{V - (2.5 - p) \alpha_{\text{air}}}{f} + 0.053 (V - [2.5 - p] \alpha_{\text{air}}) \alpha_{\text{CO}_2} - 0.975 p \alpha_{\text{CO}_2}
\]

The term \( (2.5 - p) \alpha_{\text{air}} \) is derived as follows. The volume of air dissolved in 1 cc. of plasma shaken with air containing \( p \) proportion of carbon dioxide is \( (1 - p) \alpha_{\text{air}} \). The volume held in solution under atmospheric pressure by the 1.5 cc. of water and dilute acid used in washing the plasma into the apparatus is 1.5 \( \alpha_{\text{air}} \). Hence the total correction for the air in the gas volume observed is \( (1 - p) \alpha_{\text{air}} + 1.5 \alpha_{\text{air}} \), or \( (2.5 - p) \alpha_{\text{air}} \). When \( p \) is only 0.055, as is the case when determining the carbon dioxide capacity of plasma in the manner described in this paper, its effect on this term is negligible; but if air containing proportions of carbon dioxide much higher than the physiological 5.5 per cent was utilized in saturating the plasma the effect of \( p \) would become measurable. This was the case in some experiments to be reported later, and the value of \( p \) is introduced into the equation so that it can be used in such cases.

The derivation of the other terms is self-evident. The last term has the coefficient 0.975 because Bohr has shown that the dissolved substances in plasma reduce the solubility of gases in it to 97.5 per cent of their solubilities in pure water. As in the case of \( p \), this factor, 0.975, exerts an
appreciable influence on the results calculated only when the plasma is saturated with gas containing a much higher percentage of carbon dioxide than the 5.5 per cent used in routine determinations of carbon dioxide capacities.

For routine determinations the equation is reduced to a working basis by substituting 0.055 for $p$, and by introducing the temperature coefficients for the various constants, in the manner employed in the derivation of Equation 4 of Paper II. We then have

\[
x = \frac{B}{760} (107.3 - 0.586 t) (V - 0.136 + 0.002 t)
\]

$t$ being the temperature centigrade.

As will be shortly shown, however, the carbon dioxide combining capacity of plasma decreases by an average of 0.36 per cent for each degree rise in the temperature at which the plasma is saturated with the CO$_2$-air mixture. In order to have results obtained at different room temperatures accurately comparable, therefore, we have introduced this additional temperature coefficient into the calculation in such a manner that the results calculated indicate the amount of carbon dioxide the plasma would bind if it were saturated at 20°. Introducing the temperature coefficient 0.0036 in this manner, we have

\[
x = \frac{B}{760} \frac{107.3 - 0.586 t}{1 + 0.0036 (t - 20)} (V - 0.136 + 0.002 t)
\]

The values of

\[
\frac{107.3 - 0.586 t}{1 + 0.0036 (t - 20)}
\]

may be accurately expressed between 15° and 30° by the term 100.8 - 0.27t. Hence the equation becomes

\[
x = \frac{B}{760} (100.8 - 0.27 t) (V - 0.136 + 0.002 t)
\]

$x$ expressing the cc. of CO$_2$ reduced to 0°, 760 mm., which 1 cc. of plasma will bind as bicarbonate when in equilibrium at 20° with air containing 5.5 per cent by volume of carbon dioxide.
| Observed vol. gas & Cc. of CO₂ reduced to 0°, 760 mm., bound as bicarbonate by 100 cc. of plasma. & Observed vol. gas & Cc. of CO₂ reduced to 0°, 760 mm., bound as bicarbonate by 100 cc. of plasma. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| R 760                       | 15°                        | 20°                        | 25°                        | 30°                        | R 760                        | 15°                        | 20°                        | 25°                        | 30°                        |
| 0.20                        | 9.1                        | 9.9                        | 10.7                       | 11.8                       | 0.60                        | 47.7                       | 48.1                       | 48.5                       | 48.6                       |
| 1                           | 10.1                       | 10.9                       | 11.7                       | 12.6                       | 1                           | 48.7                       | 49.0                       | 49.4                       | 49.5                       |
| 2                           | 11.0                       | 11.8                       | 12.6                       | 13.5                       | 2                           | 49.7                       | 50.0                       | 50.4                       | 50.5                       |
| 3                           | 12.0                       | 12.8                       | 13.6                       | 14.3                       | 3                           | 50.7                       | 51.0                       | 51.3                       | 51.4                       |
| 4                           | 13.0                       | 13.7                       | 14.5                       | 15.2                       | 4                           | 51.6                       | 51.9                       | 52.2                       | 52.3                       |
| 5                           | 13.9                       | 14.7                       | 15.5                       | 16.1                       | 5                           | 52.6                       | 52.8                       | 53.2                       | 53.3                       |
| 6                           | 14.9                       | 15.7                       | 16.4                       | 17.0                       | 6                           | 53.6                       | 53.8                       | 54.1                       | 54.1                       |
| 7                           | 15.9                       | 16.6                       | 17.4                       | 18.0                       | 7                           | 54.5                       | 54.8                       | 55.1                       | 55.1                       |
| 8                           | 16.8                       | 17.6                       | 18.3                       | 18.9                       | 8                           | 55.5                       | 55.7                       | 56.0                       | 56.0                       |
| 9                           | 17.8                       | 18.5                       | 19.2                       | 19.8                       | 9                           | 56.5                       | 56.7                       | 57.0                       | 56.9                       |
| 0.30                        | 18.8                       | 19.5                       | 20.2                       | 20.8                       | 0.70                        | 57.4                       | 57.6                       | 57.9                       | 57.9                       |
| 1                           | 19.7                       | 20.4                       | 21.1                       | 21.7                       | 1                           | 58.4                       | 58.6                       | 58.9                       | 58.8                       |
| 2                           | 20.7                       | 21.4                       | 22.1                       | 22.6                       | 2                           | 59.4                       | 59.5                       | 59.8                       | 59.7                       |
| 3                           | 21.7                       | 22.3                       | 23.0                       | 23.5                       | 3                           | 60.3                       | 60.5                       | 60.7                       | 60.6                       |
| 4                           | 22.6                       | 23.3                       | 24.0                       | 24.5                       | 4                           | 61.3                       | 61.4                       | 61.7                       | 61.6                       |
| 5                           | 23.6                       | 24.2                       | 24.9                       | 25.4                       | 5                           | 62.3                       | 62.4                       | 62.6                       | 62.5                       |
| 6                           | 24.6                       | 25.2                       | 25.8                       | 26.3                       | 6                           | 63.2                       | 63.3                       | 63.6                       | 63.4                       |
| 7                           | 25.5                       | 26.2                       | 26.8                       | 27.3                       | 7                           | 64.2                       | 64.3                       | 64.5                       | 64.3                       |
| 8                           | 26.5                       | 27.1                       | 27.7                       | 28.2                       | 8                           | 65.2                       | 65.3                       | 65.5                       | 65.3                       |
| 9                           | 27.5                       | 28.1                       | 28.7                       | 29.1                       | 9                           | 66.1                       | 66.2                       | 66.4                       | 66.2                       |
| 0.40                        | 28.4                       | 29.0                       | 29.6                       | 30.0                       | 0.80                        | 67.1                       | 67.2                       | 67.3                       | 67.1                       |
| 1                           | 29.4                       | 30.0                       | 30.5                       | 31.0                       | 1                           | 68.1                       | 68.1                       | 68.3                       | 68.0                       |
| 2                           | 30.3                       | 30.9                       | 31.5                       | 31.9                       | 2                           | 69.0                       | 69.1                       | 69.2                       | 69.0                       |
| 3                           | 31.3                       | 31.9                       | 32.4                       | 32.8                       | 3                           | 70.0                       | 70.0                       | 70.2                       | 69.9                       |
| 4                           | 32.3                       | 32.8                       | 33.4                       | 33.8                       | 4                           | 71.0                       | 71.0                       | 71.1                       | 70.8                       |
| 5                           | 33.2                       | 33.8                       | 34.3                       | 34.7                       | 5                           | 71.9                       | 72.0                       | 72.1                       | 71.8                       |
| 6                           | 34.2                       | 34.7                       | 35.3                       | 35.6                       | 6                           | 72.9                       | 72.9                       | 73.0                       | 72.7                       |
| 7                           | 35.2                       | 35.7                       | 36.2                       | 36.5                       | 7                           | 73.9                       | 73.9                       | 74.0                       | 73.6                       |
| 8                           | 36.1                       | 36.6                       | 37.2                       | 37.4                       | 8                           | 74.8                       | 74.8                       | 74.9                       | 74.5                       |
| 9                           | 37.1                       | 37.6                       | 38.1                       | 38.4                       | 9                           | 75.8                       | 75.8                       | 75.8                       | 75.4                       |
| 0.50                        | 38.1                       | 38.5                       | 39.0                       | 39.3                       | 0.90                        | 76.8                       | 76.7                       | 76.8                       | 76.4                       |
| 1                           | 39.1                       | 39.5                       | 40.0                       | 40.3                       | 1                           | 77.8                       | 77.7                       | 77.7                       | 77.3                       |
| 2                           | 40.0                       | 40.4                       | 40.9                       | 41.2                       | 2                           | 78.7                       | 78.6                       | 78.7                       | 78.2                       |
| 3                           | 41.0                       | 41.4                       | 41.9                       | 42.1                       | 3                           | 79.7                       | 79.6                       | 79.6                       | 79.2                       |
| 4                           | 42.0                       | 42.4                       | 42.8                       | 43.0                       | 4                           | 80.7                       | 80.5                       | 80.6                       | 80.1                       |
| 5                           | 42.9                       | 43.3                       | 43.8                       | 43.9                       | 5                           | 81.6                       | 81.5                       | 81.5                       | 81.0                       |
| 6                           | 43.9                       | 44.3                       | 44.7                       | 44.9                       | 6                           | 82.6                       | 82.5                       | 82.4                       | 82.0                       |
| 7                           | 44.9                       | 45.3                       | 45.7                       | 45.8                       | 7                           | 83.6                       | 83.4                       | 83.4                       | 82.9                       |
| 8                           | 45.8                       | 46.2                       | 46.6                       | 46.7                       | 8                           | 84.5                       | 84.4                       | 84.3                       | 83.8                       |
| 9                           | 46.8                       | 47.1                       | 47.5                       | 47.6                       | 9                           | 85.5                       | 85.3                       | 85.2                       | 84.8                       |
| 0.60                        | 47.7                       | 48.1                       | 48.5                       | 48.6                       | 1.00                        | 86.5                       | 86.2                       | 86.2                       | 85.7                       |
The temperature figures at the heads of columns represent the room temperatures at which the samples of plasma are saturated with alveolar CO₂ and analyzed. It is assumed that both operations are performed at the same temperature. The figures have been so calculated that, regardless of the room temperature at which saturation and analysis are performed, the table gives the volume (reduced to 0°, 760 mm.) of CO₂ that 100 cc. of plasma are capable of binding when saturated at 20° with CO₂ at approximately 41 mm. tension. If the figures in the table are multiplied by 0.94 they give, within 1 or 2 per cent, the CO₂ bound at 37°.

If the figures in the table are multiplied by 0.66 they give the mm. CO₂ tension of the alveolar air (Haldane method) if the relationship between alveolar carbon dioxide and plasma bicarbonate is the average normal. The physiological deviations from this average may be as great as 7 mm. (Paper V), the pathological much greater (Paper VI).

For convenience in the calculation the values for the ratio $\frac{\text{barometer}}{760}$ over the range usually encountered are given below.

<table>
<thead>
<tr>
<th>Barometer</th>
<th>Barometer $\frac{760}{760}$</th>
<th>Barometer</th>
<th>Barometer $\frac{760}{760}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>732</td>
<td>0.961</td>
<td>756</td>
<td>0.995</td>
</tr>
<tr>
<td>734</td>
<td>0.996</td>
<td>758</td>
<td>0.997</td>
</tr>
<tr>
<td>736</td>
<td>0.967</td>
<td>760</td>
<td>1.000</td>
</tr>
<tr>
<td>738</td>
<td>0.971</td>
<td>762</td>
<td>1.003</td>
</tr>
<tr>
<td>740</td>
<td>0.974</td>
<td>764</td>
<td>1.006</td>
</tr>
<tr>
<td>742</td>
<td>0.976</td>
<td>766</td>
<td>1.008</td>
</tr>
<tr>
<td>744</td>
<td>0.979</td>
<td>768</td>
<td>1.011</td>
</tr>
<tr>
<td>746</td>
<td>0.981</td>
<td>770</td>
<td>1.013</td>
</tr>
<tr>
<td>748</td>
<td>0.984</td>
<td>772</td>
<td>1.016</td>
</tr>
<tr>
<td>750</td>
<td>0.987</td>
<td>774</td>
<td>1.018</td>
</tr>
<tr>
<td>752</td>
<td>0.989</td>
<td>776</td>
<td>1.021</td>
</tr>
<tr>
<td>754</td>
<td>0.992</td>
<td>778</td>
<td>1.024</td>
</tr>
</tbody>
</table>

In order to express results in mg. of CO₂ bound by 1 cc. of plasma, the factor $\frac{\text{weight in mg. of 1 cc. CO₂}}{100} = 0.01964$ may be introduced, yielding

$$\text{Mg. CO₂ bound chemically by 1 cc. plasma} = \frac{B}{760} (1.982 - 0.0053 t) (V - 0.136 + 0.002 t)$$

In order to avoid the necessity of calculation in routine work, we have computed Table I by means of Equation 4. By means of
this table the readings on the apparatus can be directly transposed into cc. of CO₂ chemically bound by 100 cc. of plasma. It will be seen that when the gas volume reading is above 0.50 cc. the various temperature effects nearly neutralize each other, so that a reading of 0.70 cc., for example, indicates almost exactly the same carbon dioxide capacity, whether saturation and analysis are performed at 15° or at 25°.

EXPERIMENTAL.

I. The Non-Effect of Potassium Oxalate on the Carbon Dioxide Capacity of Plasma.

a. The Effect of Oxalate on the Carbon Dioxide Capacity of Water.
—Before oxalate could be used to prevent coagulation of blood it was necessary to demonstrate that its introduction causes no appreciable change in the carbon dioxide capacity. As shown by the following experiment, a solution of potassium oxalate dissolves under given conditions more carbon dioxide than does pure water.

Water and solutions containing respectively 1 and 10 per cent of potassium oxalate were shaken at 29° with air containing 23.0 per cent of carbon dioxide by volume. The carbon dioxide content of the solutions was determined as described in the preceding paper, with the exception that 2 cc. of each solution instead of 1 cc. were taken for analysis. The following results were obtained.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Vol. of CO₂ dissolved.</th>
<th>CO₂ observed</th>
<th>CO₂ observed by Bohr and Bock.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.345</td>
<td>0.75</td>
<td>0.753</td>
</tr>
<tr>
<td>1 per cent oxalate</td>
<td>0.435</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>10 per cent oxalate</td>
<td>0.480</td>
<td>1.04</td>
<td></td>
</tr>
</tbody>
</table>

It is evident that the oxalate imparts to pure water a slight alkalinity which can be measured by so delicate a means as the carbon dioxide capacity. Pure water is immensely more sensitive to the effects of solutes of slightly acid or alkaline nature, however, than are solutions containing buffers, like carbonates, phosphates, and proteins. Blood is preeminently such a solution,
and in order to make experiments on the effect of oxalate applicable to blood plasma, they must be performed upon solutions which imitate the buffer composition of the plasma.

b. Effect of Oxalate on the Carbon Dioxide Capacity of Phosphate Solutions.—Soerensen's solutions of pure phosphates in m/15 concentration were used. 10 cc. of KH₂PO₄ solution and 40 cc. of Na₂HPO₄ solution were mixed, the resulting solution having a pH of 7.38, approximately that of the blood. The carbon dioxide capacity of this solution was determined in exactly the manner described in the preceding pages for plasma, except that 5.0 per cent CO₂-air mixture instead of 5.5 per cent was used. The following results were obtained.

<table>
<thead>
<tr>
<th>Solution used</th>
<th>Vol. gas observed</th>
<th>CO₂ dissolved.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>mg.</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.260</td>
<td>0.396</td>
</tr>
<tr>
<td></td>
<td>0.265</td>
<td>0.405</td>
</tr>
<tr>
<td>Phosphate + 1 per cent oxalate</td>
<td>0.260</td>
<td>0.396</td>
</tr>
<tr>
<td></td>
<td>0.262</td>
<td>0.400</td>
</tr>
<tr>
<td>Phosphate + 10 per cent oxalate</td>
<td>0.260</td>
<td>0.396</td>
</tr>
<tr>
<td></td>
<td>0.262</td>
<td>0.400</td>
</tr>
</tbody>
</table>

The determinations were made at 24°, 760 mm., and the results in mg. calculated by the formula for 24° given in Table I of the succeeding article.

It is evident that oxalate even up to 10 per cent concentration, does not affect the carbon dioxide capacity of the phosphate solution.

c. Effect of Oxalate on the Carbon Dioxide Capacity of Sodium Carbonate Solution.—A 0.1 per cent solution of sodium carbonate was used for the experiment which in all details was similar to that preceding. The carbon dioxide capacity of this solution is slightly less than that of normal plasma.

<table>
<thead>
<tr>
<th>Solution used</th>
<th>Vol. gas observed at 24°, 760 mm.</th>
<th>Total CO₂ dissolved</th>
<th>CO₂ chemically bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>0.1 per cent Na₂CO₃</td>
<td>0.534</td>
<td>0.00</td>
<td>0.923</td>
</tr>
<tr>
<td>0.1 per cent Na₂CO₃ + 1 per cent oxalate</td>
<td>0.529</td>
<td>0.89</td>
<td>0.816</td>
</tr>
<tr>
<td>0.1 per cent Na₂CO₃ + 10 per cent oxalate</td>
<td>0.515</td>
<td>0.865</td>
<td>0.791</td>
</tr>
</tbody>
</table>
The presence of 1 per cent of oxalate had no effect on the carbon dioxide capacity of 0.1 per cent Na₂CO₃ solution outside the limit of error of the determination. 10 per cent of oxalate did not increase, but reduced the carbon dioxide capacity of the solution detectibly, an effect which may be attributed to the fact that the addition of so much solid oxalate to the carbonate solution appreciably increased its volume, so that it contained less than 1 mg. of Na₂CO₃ per cc. To a minor degree the effect is also due to the well known fact that the presence of salts reduces the solubility of gases in water, so that less CO₂ is dissolved as free H₂CO₃ than in pure water solution.

The results of both preceding experiments show that potassium oxalate in 1 per cent and even greater concentration does not affect the carbon dioxide capacities of solutions containing concentrations of phosphate or of sodium carbonate such as would bind the amounts of carbon dioxide held by the plasma. Consequently the oxalate appears to be excluded as a source of error in our determinations on plasma.

It may be noted that the amounts of CO₂ bound as carbonate, 0.826 mg. in absence of oxalate, 0.816 mg. in the presence of 1 per cent oxalate, are very near the amount, 0.830 mg., that must be bound to convert all the carbonate into NaHCO₃. The results harmonize with those of Bohr, who determined both by calculation and by analysis that nearly 100 per cent of the sodium carbonate in the presence of free carbonic acid at alveolar tension must be in the form of bicarbonate.

d. Comparison of Oxalate and Hirudin Plasmas.—Blood from an arm vein was drawn into a tube containing a few flakes of hirudin, and the carbon dioxide capacity of the plasma was determined. Duplicate readings were 0.74 and 0.74 cc. of gas at 23°, 760 mm., indicating a capacity of 61.6 volume per cent of carbon dioxide bound by 1 cc. of plasma. In 5 cc. of the plasma 0.050 gm. (1 per cent) of potassium oxalate was dissolved, and the determinations were repeated. The readings were again 0.74 and 0.74 cc., showing that the oxalate had no measurable effect on the results.

This experiment is final proof that even twice as much oxalate as is used in our routine is without significant effect on the carbon dioxide capacity of the plasma.
Experiment II. Effect of Concentration of Free Carbonic Acid on the Amount Bound as Bicarbonate by Plasma.—In order to obtain evidence concerning the magnitude of the effect which changes in the carbon dioxide content of the air used for saturating would have on results of our plasma analyses, we have determined the amounts of carbon dioxide absorbed by plasma in equilibrium with atmospheres containing from 3 per cent of carbon dioxide upwards. The results are given in Table V and Fig. 4.

The plasma was a mixture of several samples from diabetic patients, some of whom showed slight degrees of acidosis. The mixture of plasmas showed about the minimum carbon dioxide capacity which we have observed in plasma from normal individuals. The determinations of carbon dioxide were performed on 1 cc. samples in the usual manner. Because of the variation in the carbon dioxide content of the saturating air, however, the results could not be calculated by the table at the end of this paper, but had to be reckoned by direct application of Equation 1 (p. 314). The barometer was 760 mm. and the temperature 27°. Under these conditions the constants of Equation 1 have the following values: \( f = 0.827; \alpha_{\text{CO}_2} = 0.79; \alpha_{\text{air}} = 0.0165 \).

Experiment III. Effect of Free Carbonic Acid on the Amounts Bound by Sodium Carbonate, Sodium Phosphate, and Sodium Albuminate.—These data form but the preliminary steps of a study of the carbon dioxide carrying mechanism of the blood, but they are presented here because they are at least suggestive of the manner in which carbonic acid reacts with the blood constituents.

Sodium Carbonate.—A 0.1 per cent solution (0.0188 N) of Merck’s “reagent” \( \text{Na}_2\text{CO}_3 \) was saturated with carbon dioxide under varying tensions. The amounts of bound carbon dioxide were calculated, as in the case of the plasma in the preceding experiment, with the aid of Formula 1, which here, however, is altered by removal of the factor 0.975 from the last term, since such a dilute solution may be assumed to have practically 100 per cent of the dissolving power of water for gases, instead of the 97.5 per cent observed by Bohr in the case of plasma.

The experiment with sodium carbonate serves chiefly the purpose of a control of the methods as employed over a wide range of carbon dioxide tensions. As will be seen from the \( \text{Na}_2\text{CO}_3 \) curve of Fig. 4, the method gave practically theoretical results throughout the entire range of tensions, the \( \text{Na}_2\text{CO}_3 \) binding the amount of \( \text{H}_2\text{CO}_3 \) necessary to convert it into \( \text{NaHCO}_3 \).
Sodium Albuminate.—4 gm. of Merck's egg albumin were dissolved in 25 cc. of water in a 50 cc. flask, and 0.5 cc. of a 1 per cent solution of phenolphthalein was added. A solution of \(\text{N/7} \) sodium hydrate was then added from a burette until the albumin assumed the rose-color indicating a hydrogen ion concentration of approximately \(10^{-8.5}\), which is about that of plasma from which the free carbonic acid has been pumped out. The solution was then diluted up to the 50 cc. mark. The concentration of

<table>
<thead>
<tr>
<th>CO(_2) in air used for saturating plasma (per cent)</th>
<th>Vol. gas extracted from 1 cc. of saturated plasma (cc.)</th>
<th>Average V. (cc.)</th>
<th>CO(_2), reduced to 0°, 760 mm., in 100 cc. of plasma.</th>
<th>Molecular concentration of bicarbonate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>0.627</td>
<td>0.625</td>
<td>53.4</td>
<td>51.3</td>
</tr>
<tr>
<td>5.5</td>
<td>0.676</td>
<td>0.676</td>
<td>58.1</td>
<td>54.4</td>
</tr>
<tr>
<td>Alveolar air.</td>
<td>0.681</td>
<td>0.686</td>
<td>59.0</td>
<td>55.3</td>
</tr>
<tr>
<td>9.6</td>
<td>0.740</td>
<td>0.740</td>
<td>64.0</td>
<td>57.6</td>
</tr>
<tr>
<td>23.0</td>
<td>0.898</td>
<td>0.898</td>
<td>78.4</td>
<td>62.8</td>
</tr>
<tr>
<td>100</td>
<td>0.779</td>
<td>0.779</td>
<td>127.0</td>
<td>69.0</td>
</tr>
</tbody>
</table>

* Calculated on the assumption that the alveolar air from the analyst's lungs contained the average normal of 5.5 per cent of carbon dioxide. It may, of course, vary several tenths of a per cent from this.

The apparent agreement of most of the duplicates to within 0.001 cc. on the readings is somewhat misleading. The readings were made to the nearest 0.005 cc., and corrected according to the calibration of the burette. Absolute apparent agreement, therefore, indicates agreement not necessarily closer than 0.005 cc., which is about as close as one can read the instrument with a slightly milky solution like diluted and acidified plasma.
albumin, 8 per cent, was approximately that of the proteins of the plasma. The concentration of sodium in the solution was 0.0143, sufficient to bind as bicarbonate about one-half the amount of carbon dioxide held by normal human plasma.

![Graph showing the effect of free carbonic acid on the amounts bound as bicarbonate by plasma, and by sodium albuminate solution of alkali and protein concentrations similar to those of plasma.](http://www.jbc.org/)

**Fig. 4.** Effect of free carbonic acid on the amounts bound as bicarbonate by plasma, and by sodium albuminate solution of alkali and protein concentrations similar to those of plasma.

**Sodium Phosphate.**—A tenth molecular solution of Na₂HPO₄ prepared according to Soerensen was used. The phosphate solutions require for the attainment of equilibrium with atmospheric carbon dioxide considerably more time than the 2 minutes' shaking which suffices for plasma and carbonate solutions. The phosphate solutions were accordingly shaken
repeatedly with the gas mixtures, and analyzed after each shaking, until constant results showed that the maximum amounts of carbon dioxide had been absorbed by the reaction $\text{H}_2\text{CO}_3 + \text{Na}_2\text{HPO}_4 = \text{NaHCO}_3 + \text{NaH}_2\text{PO}_4$.

It will be seen that the curve obtained with the albuminate solution closely resembles that obtained with plasma. In equilibrium with 5.5 per cent carbon dioxide in the air the solution binds almost exactly the amount of carbon dioxide that the sodium hydrate alone would combine with if no albumin were present. When the carbonic acid content of the solution is greatly increased the albumin also binds an appreciable though small amount of carbon dioxide, as is evidenced by the fact that more is taken up than the sodium hydrate alone could account for. This does not prove that in the plasma all the carbon dioxide-binding power is due to alkaline carbonate and protein,—the plasma curve can also be almost exactly duplicated by a solution containing 0.01 M $\text{Na}_2\text{HPO}_4$ and 0.02 M $\text{NaHCO}_3$. It is known, however, that the plasma contains only about 3 mg. of inorganic phosphorus per 100 cc. (Greenwald, 1915)—which is too little to affect measurably the carbon dioxide capacity. Nor do ash analyses on the plasma indicate the presence of significant amounts of any other crystalloid buffers. The weight of evidence indicates that the only buffers of significance in the plasma are the proteins and the carbonates.

The whole blood, on the other hand, yields a constantly rising curve like the phosphate solution, indicating the participation of the relatively abundant phosphates of the corpuscles.

**Experiment IV. Effect of Temperature Saturation on the Amount of Carbon Dioxide Bound by Plasma.**

The determinations were made in the usual manner, except that in order to saturate at 10° and 40° the separatory funnels were shaken in baths at these temperatures. Short thermometers dipping into the plasma were placed inside the funnels, and the saturations were finished after the desired temperatures had been reached. Results were obtained with plasma from seven different individuals, and were calculated by means of Equation 1. The values for the solubilities of air and carbon dioxide used in the formula are, of course, partly the values for room temperature, partly for the temperature of saturation. The results are given in Table VI, and in the curves of Fig. 5.
The approximately linear form of the curves of Fig. 5 shows that the temperature effect between 10° and 40° is fairly constant. The maximum percentage decrease in carbon dioxide capacity caused by 1° rise in temperature was 0.47 per cent of the amount of carbon dioxide bound at 20°. The minimum was 0.25 per

**TABLE VI.**

*Effect of Temperature on the Carbon Dioxide Binding Power of Human Plasma.*

<table>
<thead>
<tr>
<th>Plasma No.</th>
<th>Temperature of saturation.</th>
<th>Room temperature.</th>
<th>Barometer.</th>
<th>Vol. of gas observed.</th>
<th>CO₂ per 100 cc. plasma.</th>
<th>Dissolved as free bicarbonate.</th>
<th>Bound as carbonic acid.</th>
<th>Decrease in bound CO₂ on increasing temperature °C.</th>
<th>per cent of CO₂ bound at 30°</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 24.5 758 0.700 60.6 6.5 54.1</td>
<td>24.5 759 0.650 56.3 4.1 52.2</td>
<td>0.13 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10 23.5 761 0.855 75.7 6.5 68.2</td>
<td>23.5 761 0.780 69.2 4.2 65.0</td>
<td>0.31 0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 24 757 0.721 62.5 6.5 56.0</td>
<td>24 757 0.671 58.1 4.2 54.2</td>
<td>0.14 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10 22 754 0.781 68.7 6.5 62.2</td>
<td>22 754 0.721 63.1 4.5 58.9</td>
<td>0.17 0.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10 24 757 0.791 69.0 6.5 62.5</td>
<td>24 757 0.711 62.5 4.2 58.3</td>
<td>0.24 0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10 24 757 0.721 63.6 6.5 57.1</td>
<td>24 757 0.671 58.6 4.2 54.4</td>
<td>0.14 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10 22 754 0.781 69.7 6.5 63.2</td>
<td>22 754 0.721 63.0 4.5 58.5</td>
<td>0.24 0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Plasma Bicarbonate

cent; the mean between the extremes is 0.36 per cent. The varia-
tion on both sides of the mean is large compared with the size of
the mean value itself. As the entire temperature effect is so
small, however, the mean temperature coefficient can be used
over the range of ordinary room temperature without introducing
significant errors.

The decrease in carbonic acid binding power caused by increase
in temperature appears to result chiefly from the lowering of the
solubility of carbon dioxide and the consequent decrease in the
concentration of free carbonic acid in the experiments at the higher
temperatures. Raising the temperature from 10° to 40° dimin-
ished the carbonic acid CO₂ from 6.5 volume per cent to 2.7.

The average effect on the bicarbonate CO₂ in the seven plasmas
was to lower it 6 volumes per cent. Interpolation on the curve of
Fig. 4 indicates that in the experiment there tabulated a lowering
of carbonic acid CO₂ from 6.5 to 2.7 volume per cent without any
temperature change decreased the plasma bicarbonate 5.1 vol-
ume per cent, or nearly as much as when the change in free car-
bonic acid was accompanied by an increase of 30° in temperature.
That temperature does influence the equilibria between carbon
dioxide and buffers independently of its effect on carbon dioxide
solubility is certain (Henderson, 1906), but the effect in the plasma
appears to be slight compared with that of changing concentra-
tions of free carbonic acid.
Experiment V. The Effect of Acids on the Carbon Capacity of Plasma.

β-Hydroxybutyric Acid.—Varying amounts of standardized solutions of Kahlbaum's hydroxybutyric acid were weighed into portions of a sample of human plasma, the concentrations of acid being such that the volume increase caused by its addition to the plasma was always less than 1 per cent. The plasmas were then saturated with 5.5 per cent carbon dioxide and analyzed.

Hydrochloric Acid.—Varying amounts of 0.1 N and 0.2 N HCl were added to 10 cc. portions of another plasma, with sufficient water in each case to bring the volume up to 12 cc. The mixtures were then saturated with 5.5 per cent CO₂ and analyzed.

The results with both acids are given in Table VII.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Concentration of acid, Mols. per liter</th>
<th>CO₂ bound as bicarbonate.</th>
<th>Decrease in bicarbonate caused by acid, Mols. per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mols. per liter.</td>
<td>Mols. per liter.</td>
</tr>
<tr>
<td>Hydrochloric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0000</td>
<td>58.0</td>
<td>0.0259</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.0042</td>
<td>48.5</td>
<td>0.0216</td>
<td>0.0043</td>
</tr>
<tr>
<td>0.0083</td>
<td>41.3</td>
<td>0.0184</td>
<td>0.0075</td>
</tr>
<tr>
<td>0.0167</td>
<td>25.3</td>
<td>0.0113</td>
<td>0.0146</td>
</tr>
<tr>
<td>0.0250</td>
<td>12.5</td>
<td>0.0056</td>
<td>0.0203</td>
</tr>
<tr>
<td>0.0333</td>
<td>2.8</td>
<td>0.0012</td>
<td>0.0247</td>
</tr>
<tr>
<td>0.0500</td>
<td>0.0</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>β-Hydroxybutyric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0000</td>
<td>67.6</td>
<td>0.0283</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.0096</td>
<td>43.3</td>
<td>0.0182</td>
<td>0.0101</td>
</tr>
<tr>
<td>0.0240</td>
<td>23.9</td>
<td>0.0100</td>
<td>0.0133</td>
</tr>
<tr>
<td>0.0481</td>
<td>3.7</td>
<td>0.0016</td>
<td>0.0267</td>
</tr>
<tr>
<td>0.0962</td>
<td>0.0</td>
<td>0.0000</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of the first and last columns of the table shows that until acid equivalent to about half the plasma bicarbonate has been added the fall in bicarbonate approximately equals in molecular equivalents the amount of acid added. As the amount of acid becomes greater, however, the drop in plasma bicarbonate begins to fall short of the added acid. This is due to the fact that the H₂CO₃ concentration is kept constant, instead of being reduced in proportion to the bicarbonate. The condition is similar to that of the blood in uncompensated acidosis. The $\frac{H_2CO_3}{NaHCO_3}$ ratio, and consequently the $C_M$, is increased. As a result the other
plasma buffers (chiefly proteins) bind a measurably greater amount of acid than they could at normal C\textsubscript{n}, and the acid so bound is prevented from decomposing bicarbonate. The effect on the routine plasma determination is that the bicarbonate determined by our technique denotes a fall in the more severe stages of acidosis which is not quite so great as the actual drop in bicarbonate \textit{in vivo}. The relationship between added acid and decrease in bicarbonate, however, is made so constant by saturating the plasma at a definite carbon dioxide tension that the lack of absolute numerical proportionality in the lower ranges is no practical detriment to the interpretation of results.

\textit{Experiment VI. Effect of Preservation on the Carbon Dioxide Capacity of Plasma.}

A sample of oxalated human plasma was placed in a paraffin-lined tube in a refrigerator at approximately +1°C. Samples were removed at intervals and analyzed with the following results.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Age of plasma sample kept at +1°C in paraffined tube. & Gas vol. read. & Temperature. & Barometer. & CO\textsubscript{2} capacity. (Cc. of CO\textsubscript{2} reduced to 0°C, 760 mm., bound chemically by 100 cc. of plasma.) \\
\hline
\hline
days & cc. & °C. & mm. & vol. per cent \\
\hline
0 & 0.70 & 21 & 750 & 56.8 \\
1 & 0.71 & 19 & 750 & 57.6 \\
2 & 0.71 & 21 & 753 & 57.8 \\
4 & 0.70 & 22 & 752 & 57.0 \\
6 & 0.70 & 22 & 752 & 57.1 \\
12 & 0.68 & 27 & 767 & 56.7 \\
20 & 0.61 & 20 & 764 & 49.4 \\
\hline
\end{tabular}
\end{table}

For 12 days the capacity of the plasma remained stationary at 57.3 ±0.5 volume per cent of carbon dioxide. The first change was noted in the 20 day sample, and was probably due to bacteria, as the samples for analysis were not drawn under sterile conditions. It may be well, however, to emphasize the fact that plasma kept in ordinary glass, instead of paraffin, may dissolve enough alkali over night to increase its carbon dioxide capacity by several per cent.
VII. Effects of Manner of Drawing Blood on the Carbon Dioxide Content of Whole Blood Samples, and the Carbon Dioxide Capacity of the Plasma.

Experiment VII, a. Blood from Different Veins Compared with Arterial. Effect of Momentary Exposure to Air.

An 18 kilo dog was etherized with a cone (no artificial respiration) and the femoral artery and vein of the right thigh, the brachial artery and vein of the left fore leg, and the left external jugular vein were exposed. Blood samples were drawn from each vessel both by means of a syringe, and of a needle connected with a 50 cc. Erlenmeyer flask. The latter was fitted with a two-hole rubber stopper through which passed two short glass tubes extending for 1 or 2 cm. both above and below the stopper. To one of the tubes was attached about 10 cm. of small bore rubber tubing, into the farther end of which was fitted the needle. To the other glass tube was attached a somewhat longer piece of rubber tubing, by means of which suction could be applied to accelerate the flow of blood from the vein. As in the case of the McRae needle, the blood was drawn with moderate suction into an open vessel, and fell through a height of several centimeters, so that for 0.01 second or less opportunity was given for escape of venous carbon dioxide.

Blood was allowed to flow into syringes simultaneously from the femoral and jugular veins. Then the syringes were immediately replaced by two collecting flasks of the kind described above, and samples of blood were drawn into them. Consequently these four samples may be considered as practically simultaneous.

The same procedure was then repeated with the femoral artery, brachial vein, and brachial artery. The entire operation, from beginning of anesthesia through the drawing of the second set of samples, consumed 30 minutes. Samples of the whole blood were analyzed for carbon dioxide content at once, the technique described on p. 361, of the succeeding article being employed. Other samples were meanwhile centrifuged, and the carbon dioxide capacity was determined, as described in this paper, after saturating at 20° with air containing 5.5 per cent of carbon dioxide. The results are given in Table IX.

The results may be summarized as follows:

1. Blood samples drawn without loss of carbon dioxide (syringe samples) within an interval of a few minutes from three different veins showed within 2.5 per cent the same carbon dioxide contents.

2. The carbon dioxide content of the venous blood from the resting dog (the animal was anesthetized without a struggle) was 2 to 5 per cent greater than that of the arterial blood.
drawn from the brachial vein showed 3 per cent more carbon dioxide than blood drawn simultaneously from the brachial artery.

3. The effect of drawing the blood into an open flask in which the blood stream was allowed to fall through the air for several centimeters was to cause a loss of carbon dioxide from the venous blood such as to bring its CO₂ content to approximately that of arterial. The carbon dioxide held by the venous blood in excess of that of the arterial appears to be given off readily, so that the instant’s exposure while the blood was falling into the receiving vessel approximately transformed venous into arterial blood, so far as the carbon dioxide was concerned.

4. The CO₂ capacity of the plasma rose and fell parallel with the CO₂ content of the whole blood from which the plasma was centrifuged. The carbon dioxide bound chemically by plasma saturated at 20° with air containing 5.5 per cent of CO₂ amounted to 15 ±1 volume per cent more than the total carbon dioxide content of the whole blood at the time it was centrifuged.
Within the limits of this experiment (compare Experiment VII, c), differences in the CO₂ content of the whole blood at the time of centrifugation resulted in approximately equal differences in the carbon dioxide capacity of the plasma, so that the latter remained at a level approximately 15 volume per cent greater than the former. The effect of conditions at the time of centrifugation on the bicarbonate content of the plasma separated is to be attributed to the transfer of electrolytes between plasma and corpuscles under the influence of changing free carbonic acid concentration, discussed on p. 303.

Experiment VII, b. Comparison of Blood Samples Drawn with Syringe and McRae Needle.

The experiment was performed like the preceding, except that for the samples drawn in open vessels a McRae needle was used, so that the conditions entirely simulate those obtained with the use of this apparatus for clinical purposes.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Vol. per cent of carbon dioxide in blood.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediately after being drawn.</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>37.1</td>
</tr>
<tr>
<td>Brachial vein</td>
<td>35.2</td>
</tr>
<tr>
<td>External jugular vein</td>
<td>37.1</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>34.0</td>
</tr>
</tbody>
</table>

The results confirm those of the preceding experiment. In addition they show that whole blood standing in a partly filled tube may lose in 6 hours up to 8 volume per cent of carbon dioxide.
Experiment VII, c. Effect of Different Methods of Drawing Samples on Results Obtained with Venous Blood Heavily Charged with Carbon Dioxide as the Result of Exertion.

The dog used in this experiment exerted himself strenuously against etherization, and consequently the carbon dioxide content of the venous blood was 10.2 volume per cent greater than that of the arterial, instead of the 3 to 5 per cent difference usually noted. The samples were all collected within an interval of a few minutes.

<table>
<thead>
<tr>
<th></th>
<th>Right jugular vein</th>
<th>Left jugular vein</th>
<th>Femoral artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ in whole blood as drawn.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drawn with syringe (no loss of CO₂)</td>
<td>42.5 vol. per cent</td>
<td>42.1 vol. per cent</td>
<td>32.0 vol. per cent</td>
</tr>
<tr>
<td>Drawn with McRae needle (blood stream falling through air)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂ bound as bicarbonate by plasma from above blood samples. Plasma is saturated with air containing 5.5 per cent CO₂.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma from blood drawn with syringe.</td>
<td>52.6 vol. per cent</td>
<td>51.6 vol. per cent</td>
<td>44.9 vol. per cent</td>
</tr>
<tr>
<td>Plasma from blood drawn with McRae needle.</td>
<td>47.9 vol. per cent</td>
<td>46.8 vol. per cent</td>
<td>44.0 vol. per cent</td>
</tr>
</tbody>
</table>

The results confirm those of the foregoing experiments in showing that allowing the blood to fall for a few centimeters through air when the sample is drawn (with a McRae needle) reduces it to approximately the carbon dioxide content of arterial blood. The fact has additional interest in this case, because the difference between arterial and venous bloods is, presumably on account of the animal's exertions, two or three times as great as that usually observed in resting dogs. Nevertheless the instant's exposure of the falling blood removed all the carbon dioxide in excess of that in the arterial blood, and even somewhat more. The result, however, was, as in the foregoing experiments, to bring the samples drawn with momentary exposure to air closer...
to arterial blood in carbon dioxide content than were samples of venous blood drawn without exposure.

Comparison of the carbon dioxide contents of the different whole blood samples and of the carbon dioxide binding powers of the corresponding plasmas affords more examples of the influence of carbonic acid on the acid-base transfer between corpuscles and plasma. Samples of whole blood from which some of the carbon dioxide escaped, during the momentary aeration connected with the use of the McRae needle, yielded plasmas which had also a reduced amount of base capable of binding carbonic acid.

**Experiment VII, d. Collection of Blood under Paraffin Oil without Loss of Carbon Dioxide.**—The following experiment shows that with centrifuge tube and needle arranged as shown in Fig. 1 one can collect blood samples without appreciable loss of carbon dioxide. The blood in entering the centrifuge tube mixes with the finely powdered oxalate, so that very little additional stirring is necessary in order to prevent clotting. After a sample was drawn the stopper was loosened, and the blood was stirred gently with the inlet tube. Previous experience had shown that if the mixing of the oxalate were attained by more vigorous agitation, such as shaking the tube or turning it upside down repeatedly, a measurable loss of carbon dioxide would occur, even if the layer of oil separating the blood from the air was not broken. Since carbon dioxide is more soluble in the oil than in the water, vigorous agitation of the two fluids results in a partial transfer of carbon dioxide from water to oil. The latter, however, prevents rapid diffusion of the gas away from the surface of the water layer, and if unnecessary agitation is avoided, this form of tube yields results identical with those obtained with syringe samples. The chief advantages over the syringe are in cost, and in the convenience of having the needle on a flexible connection.

The dog used, a female bull terrier of 16 kilos weight, was etherized with the Meltzer-Auer insufflation apparatus. The animal submitted to etherization very quietly; the resting condition is evidenced by the fact that there is only 3 per cent difference in carbon dioxide content between arterial and venous bloods.

It will be noted that the figures in the last column afford another illustration of the effect of CO₂ tension on the acid-base transfer between corpuscles and plasma.
TABLE XII.

Collection of Blood under Paraffin Oil without Loss of Carbon Dioxide.

<table>
<thead>
<tr>
<th>Blood vessel from which sample was taken</th>
<th>Instrument used in taking samples</th>
<th>Total CO₂ content of blood drawn</th>
<th>CO₂ bound as bicarbonate by plasma saturated at 20° with 5.5 per cent CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right jugular vein.</td>
<td>Syringe.</td>
<td>33.0 vol. per cent</td>
<td>41.3 vol. per cent</td>
</tr>
<tr>
<td></td>
<td>Oil tube.</td>
<td>33.0 vol. per cent</td>
<td>41.0 vol. per cent</td>
</tr>
<tr>
<td></td>
<td>McRae needle.</td>
<td>29.3 vol. per cent</td>
<td>32.9 vol. per cent</td>
</tr>
<tr>
<td>Left jugular vein.</td>
<td>Syringe.</td>
<td>33.9 vol. per cent</td>
<td>41.3 vol. per cent</td>
</tr>
<tr>
<td></td>
<td>Oil tube.</td>
<td>33.9 vol. per cent</td>
<td>41.3 vol. per cent</td>
</tr>
<tr>
<td></td>
<td>McRae needle.</td>
<td>33.0 vol. per cent</td>
<td>39.6 vol. per cent</td>
</tr>
<tr>
<td>Femoral artery.</td>
<td>Syringe.</td>
<td>30.3 vol. per cent</td>
<td>37.6 vol. per cent</td>
</tr>
<tr>
<td></td>
<td>Oil tube.</td>
<td>30.0 vol. per cent</td>
<td>37.1 vol. per cent</td>
</tr>
<tr>
<td></td>
<td>McRae needle.</td>
<td>25.6 vol. per cent</td>
<td>33.3 vol. per cent</td>
</tr>
</tbody>
</table>

Experiment VII, e. Effect of Manner of Drawing Blood on Results Obtained from Normal Human Subject.

A sample of blood was drawn from the right arm with a syringe, care being taken to avoid any chance for loss of CO₂. About 1 minute later a sample was taken from the other arm, conditions being the same except that the McRae needle was used in this case. In each case the arm was ligated with a rubber band for about 1 minute before the sample was drawn. The analyses yielded the results shown in Table XIII.

Comparison of analyses a and b shows, as in the foregoing dog experiments, that use of the McRae needle results in loss of some of the excess carbon dioxide of the venous blood, so that the venous blood is brought closer to arterial (analyses b) in its carbon dioxide content.

Comparison of c and d shows that the approximation of the results to the arterial standard, as a result of the momentary aeration connected with use of the McRae needle, is also noted when the carbon dioxide capacities of the plasmas are considered. The plasma from the venous blood drawn with the McRae needle approximated the plasma from arterialized blood in its carbon dioxide capacity.
TABLE XIII.
Comparison of Blood Samples Drawn from Normal Man with Syringe and McRae Needle.

<table>
<thead>
<tr>
<th></th>
<th>Syringe.</th>
<th>McRae needle.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. CO₂ content of whole blood as drawn.</td>
<td>67.7</td>
<td>62.1</td>
</tr>
<tr>
<td>b. CO₂ content of whole blood arterIALIZED by shaking at 37° with air containing 5.5 per cent of CO₂.</td>
<td>57.2</td>
<td>57.2</td>
</tr>
<tr>
<td>c. CO₂ bound as bicarbonate by plasma centrifuged from freshly drawn blood and saturated with 5.5 per cent CO₂ at 23°.</td>
<td>Plasma from syringe sample.</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>Plasma from McRae needle sample.</td>
<td>73.3</td>
</tr>
<tr>
<td>d. CO₂ bound as bicarbonate by plasma centrifuged from arterialized blood (analyses b above) and resaturated at 23° with 5.5 per cent CO₂.</td>
<td>Plasma from arterialized syringe sample.</td>
<td>71.0</td>
</tr>
<tr>
<td></td>
<td>Plasma from arterialized McRae needle sample.</td>
<td>71.5</td>
</tr>
</tbody>
</table>

Experiment VIII. Demonstration of Identical Bicarbonate Contents of Venous and Arterial Bloods under Identical Carbon Dioxide Tensions.

A dog of 24 kilos weight was placed under ether with the Meltzer-Auer apparatus and blood samples were drawn as indicated in the following table. Each blood sample was divided into three portions. In one the carbon dioxide content was determined directly (third column). In another the carbon dioxide was determined after saturation at 37.5° of the fresh blood with air containing 5.5 per cent of carbon dioxide. It will be noted that this treatment raised the carbon dioxide of the arterial blood by 10 volume per cent, indicating that the arterial carbon dioxide tension of this animal, presumably because of the artificial ventilation, was considerably less than that of the average man. A third sample of each blood was centrifuged immediately and the plasma bicarbonate CO₂ was determined in the routine way, after saturation of the plasma at room temperature with 5.5 per cent CO₂ in air.

Experiment VII, d, is confirmed in showing that blood can be drawn into the “oil tube” without measurable loss of carbon
dioxide. The results obtained are identical with those from samples drawn with the syringe.

The figures in the last column show that when arterial and venous bloods are brought to the same content of free carbonic acid, the bicarbonate contents are also equal. From this it follows that blood in passing from arteries to veins in the resting animal does not take up sufficient acid other than carbonic to affect the bicarbonate content appreciably. So far as the content in non-volatile acids is concerned, there is no appreciable difference in the resting animal between blood from the arteries and that from the jugular vein.

The figures in the middle column illustrate again the effect of carbonic acid concentration on the distribution of bases and acids between plasma and corpuscles. Plasma centrifuged from venous blood, with 3 per cent more total carbon dioxide than arterial, showed a bicarbonate CO₂ nearly 4 per cent higher than the arterial plasma.

**Experiment IX. Effect of Experimental Acidosis on the Carbon Dioxide Figures and the Hydrogen Ion Concentration of Venous and Arterial Blood.**

The animal used was a collie bitch of 14.5 kilos weight, in splendid condition.

At 2.00 p.m. the animal was etherized, and the femoral veins and arteries on both sides were exposed. Ether anesthesia was maintained throughout the experiment by the Meltzer-Auer insufflation method.

### TABLE XIV.

<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>Instrument used in drawing blood</th>
<th>Total CO₂ content of plasma after saturation at 20° with air containing 5.5 per cent CO₂</th>
<th>Bicarbonate CO₂ of plasma after saturation at 20° with air containing 5.5 per cent CO₂</th>
<th>Total CO₂ content of whole blood after saturation at 37° with air containing 5.5 per cent CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left jugular vein.</td>
<td>Syringe.</td>
<td>38.9</td>
<td>48.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39.3</td>
<td>48.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Oil tube.</td>
<td>39.2</td>
<td>48.2</td>
<td>46.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39.3</td>
<td>48.2</td>
<td>45.9</td>
</tr>
<tr>
<td>Left femoral artery</td>
<td>Oil tube.</td>
<td>36.4</td>
<td>44.5</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.4</td>
<td>44.5</td>
<td>45.9</td>
</tr>
</tbody>
</table>
At 2.15 blood samples of about 15 cc. each were taken from the right femoral artery and the left femoral vein, the samples being collected under paraffin oil with precautions to prevent loss of carbon dioxide (see p. 306).

At 2.20 the injection of $\text{N}_2\text{H}_2\text{SO}_4$ from a burette into the right femoral vein was begun.

At 3.00 50 cc. had been injected. The animal showed marked dyspnea and a pulse of 180. These symptoms disappeared after 10 minutes, and the injection was resumed.

At 3.30 75 cc. of $\text{N}_2\text{H}_2\text{SO}_4$ in all had been injected, and the injection was concluded.

At 4.10 a second pair of blood samples was taken, the vessels used being the right femoral artery and the left femoral vein.

At 5 two more samples were taken, in order to ascertain whether the animal's blood indicated that she was overcoming the acidosis.

The hydrogen ion concentrations were determined in a Clark electrode (Clark, 1915), successive portions of the same sample being shaken in the same hydrogen atmosphere until the latter had acquired the carbon dioxide tension of the blood, according to the principle of Hasselbalch's technique (Hasselbalch, 1911, 1913). The figures obtained consequently may be taken as representing the actual hydrogen ion concentration of the blood in the veins and arteries.

The gas analyses of whole blood and plasma were performed as described in this and the succeeding paper.

The results are given in Table XIV. Graphic comparison of the carbon dioxide figures is given in Fig. 6.

<table>
<thead>
<tr>
<th>TABLE XV.</th>
</tr>
</thead>
</table>

| Effect of Acid Injection on Blood. |

<table>
<thead>
<tr>
<th>Whole blood as drawn.</th>
<th>Plasma after saturation at 20° with air containing 5.5 per cent CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial.</strong></td>
<td><strong>Venous.</strong></td>
</tr>
<tr>
<td>Total CO₂</td>
<td>Re-</td>
</tr>
<tr>
<td>vol. per cent</td>
<td>action</td>
</tr>
<tr>
<td>Before injection</td>
<td>38.7</td>
</tr>
<tr>
<td>40 min. after injection of 75 cc. $\text{N}_2\text{H}_2\text{SO}_4$</td>
<td>10.1</td>
</tr>
<tr>
<td>90 min. after injection</td>
<td>10.1</td>
</tr>
</tbody>
</table>
Discussion of the Results of Experiment IX.

1. The curves of Fig. 6 show that in acidosis the arterial carbon dioxide is approximately paralleled in its fall by the other three values determined. For reasons discussed on p. 295 the arterial bicarbonate, or the nearly identical total arterial carbon dioxide, is the ideal figure to determine as a measure of the alkaline reserve of the body. The curves of Fig. 6 indicate, however, that any one of the other three values may also be used as an index of the alkaline reserve, provided the limits of the normal level, and the changes therefrom corresponding to different grades of acidosis, are determined for the value used. The necessity for employing in all clinical and much experimental work one of the values obtained on the venous blood rather than the theoretically preferable arterial figure is, of course, obvious. The value obtained by the technique for acidosis study outlined in this paper, *viz.*, the capacity of the venous plasma to combine with carbon dioxide, has been given the preference in our work chiefly for practical reasons, such as the ease and convenience of making determinations on the plasma as compared with whole blood, the facts that plasma can be preserved for days and even weeks and still show unchanged carbon dioxide binding power when resaturated with 5.5 per cent CO₂, and that unlike the carbon dioxide capacity of the whole blood the carbon dioxide capacity of the
venous plasma maintains its parallelism with the arterial carbon dioxide even in the severe grades of acidosis (Stillman, Van Slyke, Cullen, and Fitz).

2. The greater part of the injected acid did not remain in the blood, but was at once transferred elsewhere and presumably neutralized by the bicarbonate and phosphate reserves in other parts of the body. The amount of blood in a 14 kilo dog may roughly be estimated at 1 liter. The injection of 75 cc. of N acid into this volume of bicarbonate solution would decompose sufficient bicarbonate to reduce its molecular concentration by 0.075. The actual reductions of carbonate noted as the result of the acid injection were the following, the figures being transposed from terms of volume per cent carbon dioxide to molecular concentration:

<table>
<thead>
<tr>
<th></th>
<th>Arterial whole blood CO₂ content, reduced 0.0126 M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous</td>
<td>0.0115</td>
</tr>
<tr>
<td>Arterial plasma, CO₂ capacity,</td>
<td>0.0104</td>
</tr>
<tr>
<td>Venous</td>
<td>0.0119</td>
</tr>
</tbody>
</table>

As bicarbonate furnishes about nineteen-twentieths of the CO₂ of the whole blood, only one-twentieth being free carbonic acid, the whole blood figures as well as those of the plasma (where the CO₂ from H₂CO₃ is subtracted) may be taken as practically bicarbonate figures. The fall, on the average only 0.012 in molecular concentration of both whole blood and plasma, indicates that only about one-sixth of the injected acid remained in the blood, or was neutralized by the blood bicarbonate. The quick transfer between blood and tissues indicates that an acid-base equilibrium is continually maintained between them, even when there is such an enormously rapid influx of acid as occurred in this experiment. There is consequently direct experimental basis for assuming that the bicarbonate concentration of the blood is an index of the alkaline reserve of the entire body.

3. An actual increase in the hydrogen ion concentration of both arterial and venous blood occurred during the experiment. The acidosis was therefore partly uncompensated. Respiration did not lower the free carbonic acid enough to compensate entirely for the lowered bicarbonate, and the failure to do so is evidenced by an increase in the hydrogen ion concentration (fall in
Plasma Bicarbonate

pH) past the extreme normal limits. The failure may, however, be due to the effect of etherization on the respiratory control rather than to the reduction of arterial bicarbonate to one-fourth its previous level. Even severely sick patients appear able to compensate nearly if not quite so severe a grade of acidosis (Peabody, 1914). Michaelis and Davidoff (1912) have found, however, that narcosis of itself causes the respiratory centers to become less responsive to increase in hydrogen ion concentration, and consequently allow free carbonic acid to accumulate until the \( \frac{H_2CO_3}{NaHCO_3} \) ratio, and consequently the hydrogen ion concentration, is considerably above normal. Evidence that the etherization was responsible in this experiment is seen in the fact that a further decrease in pH (increase in hydrogen ion concentration) occurred between the second and third bleedings when no acid was injected.

4. The injection of acid apparently results in an increase in the gas exchange occurring with each circulation of the blood. Before the injection the difference in CO2 content between arterial and venous blood was 9 per cent; after the injection it had increased to 12 per cent. Similar increases in the gas exchange have been noted in other experiments after acid injection. In this case the increase in carbon dioxide occurring as the blood traverses the tissues cannot be attributed to CO2 set free in the latter by the injected acid, for it was still undiminished 1\(\frac{1}{2}\) hours after the injection had been finished. The increased gas exchange must be due either to increased rate of oxidation in the tissues, or to decreased rate of blood flow. The problem suggested by this observation, viz., whether acidosis, compensated or uncompensated, produced either experimentally or by disease, results in an increased rate of oxidation, we hope to attack later.

5. The effect of carbon dioxide on the acid-base transfer between corpuscles and plasma is again seen when the bicarbonate CO2 figures for venous and arterial plasmas are compared. The bicarbonate contents of the sets of plasmas from the two sources differ by nearly the same margins as the total CO2 contents of the respective whole bloods (see Fig. 6). The results obtained in the experiments of sections VII and VIII show the same relationship between venous and arterial blood and plasma. Con-
sequently the carbonic acid entering the blood from the tissues, during the passage from arteries to veins, must displace nearly an equivalent amount of other acid, which passes into the cells, leaving the plasma at its normal alkalinity. At least part of the acid thus displaced by combined mass action of the carbonic acid reaction, which is expressed as follows: 

\[ H_2CO_3 + NaCl = NaHCO_3 + HCl \]

The results shown in the next experiment indicate that this reaction is the one chiefly concerned when the carbonic acid concentration changes only within the limits likely to occur in the body. The physiological effect of the acid transfer into and out of the cells is to increase tremendously the power of the plasma to take up carbon dioxide without having its hydrogen ion concentration raised above the normal limits. The height to which it is possible to force the plasma bicarbonate by increasing the carbonic acid of the whole blood is indicated by the next experiment.

**Experiment X. Effect of Free Carbonic Acid on the Acid-Base Equilibrium between Plasma and Corpuscles.**

Blood was drawn with a McRae needle from the arm-vein of a healthy man, and the sample was divided into four portions. One was centrifuged at once, and the others were shaken each with 100 volumes of atmosphere containing the proportions of carbon dioxide indicated in Table XVI. Carbon dioxide in the blood plasma was determined in the usual manner, chlorides by the method of McLean and Van Slyke (1915). The experiment reported here is one of a number which yielded similar results. The relationship between chloride and carbonate concentrations is made readily evident by Fig. 7.

For furnishing the blood used in this experiment and performing the chloride determinations we are indebted to our colleague, Dr. Franklin McLean.

The figures for molecular concentration in the plasma of untreated blood (top line of Table XVI) show the relative importance of bicarbonate among the plasma electrolytes. It is, as pointed out by Henderson (1908, b), second only to the sodium chloride, the concentration of the bicarbonate being one-fourth to one-third that of the chloride. As shown by Hamburger, the average blood plasma is isotonic with a 0.9 per cent (0.155 molecular) solution of sodium chloride. The average chloride concentration may be taken, as in this blood, at about 0.105 molecular, the bicarbonate
Plasma Bicarbonate

at 0.030 M, the two together therefore at 0.135 M, leaving only about 0.020 M, or one-seventh the total, to be made up of all the other electrolytes in the plasma.

On examining the results from the three blood portions in which the carbonic acid was arbitrarily changed it becomes evident that a part of the resulting changes in plasma bicarbonate are

<table>
<thead>
<tr>
<th>Treatment of whole blood before centrifugation</th>
<th>Calculated free carbonic acid dissolved in blood as result of treatment</th>
<th>Bicarbonate of plasma centrifuged from treated blood, then saturated at 20° with 5.5 per cent CO₂</th>
<th>Plasma chloride</th>
<th>Changes in plasma concentration caused by treatment of whole blood, Mol. concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. per cent CO₂</td>
<td>Mol. concentration</td>
<td>Vol. per cent CO₂</td>
<td>Mol. concentration</td>
<td>Calculated as gm. NaCl per liter</td>
</tr>
<tr>
<td>No treatment, blood centrifuged as drawn</td>
<td>3.0</td>
<td>0.0013</td>
<td>69.8</td>
<td>0.0311</td>
</tr>
<tr>
<td>Shaken with 100 volumes of air.</td>
<td>0</td>
<td>0</td>
<td>42.2</td>
<td>0.0188</td>
</tr>
<tr>
<td>Shaken with 5.5 per cent CO₂ at 20°, 760 mm</td>
<td>5.3</td>
<td>0.0024</td>
<td>77.9</td>
<td>0.0357</td>
</tr>
<tr>
<td>Saturated with pure CO₂ gas at 20°, 760 mm</td>
<td>86.5</td>
<td>0.0386</td>
<td>135.8</td>
<td>0.0607</td>
</tr>
</tbody>
</table>

* Blood as drawn assumed to be saturated with 5.5 per cent CO₂ at 37°, 760 mm.

explainable by the migration of HCl from plasma into corpuscles. Changes within the limits of physiological possibility, such as that caused by changing the carbonic acid CO₂ from 3.0 to 5.3 volume per cent of the blood, may be chiefly accounted for by this shift in hydrochloric acid. When the carbonic acid is greatly altered other electrolytes also become involved in the transfer,
for the changes caused by saturating the whole blood with either pure carbon dioxide or with air practically free of carbon dioxide are, in molecular equivalents, only about one-third as great in the plasma chloride as in the bicarbonate (see last column of Table XV).

In connection with the technique for determining the plasma bicarbonate, these results, showing the extreme possible effects of the acid-base transfer, indicate the magnitude of the changes in plasma which it is possible to cause by varying the carbon dioxide tension of the whole blood at the time the plasma is separated from the cells. Although these extreme effects greatly exceed those which could be caused by accidental loss or gain of carbon dioxide in handling blood samples, they serve to emphasize the importance of this possible source of error, and the necessity of observing the precautions for handling blood samples given on pages 305 and 306.

Incidentally the data also indicate the effect which carbonic acid changes in whole blood may have on the chloride content.

**Fig. 7.** Effect of free carbonic acid concentration on the distribution of chloride and bicarbonate between corpuscles and plasma. Each square, ordinate or abscissa, represents a change of 0.01 in molecular concentration.
Plasma Bicarbonate

of the plasma. The effect in proportion to the total chloride is only one-third less than the relative effect on the bicarbonate, and if unrecognized could readily become a factor in the results of plasma chloride determinations.

SUMMARY.

Reasons are discussed for basing both the definition of acidosis and the methods for its detection on the blood bicarbonate.

Experiments are detailed showing both \textit{in vivo} and \textit{in vitro} the influence on the plasma bicarbonate of various factors, in particular of the shift of bases and acids between plasma and corpuscles under the influence of changing carbonic acid concentration.

A simple technique has been developed by means of which the capacity of the plasma to combine with carbonic acid under definite tension is determined as a measure of the alkali in excess of acids other than carbonic. The plasma, from oxalated blood, drawn and centrifuged under definite conditions, is shaken at room temperature in a separatory funnel filled with alveolar air from the lungs of the operator, or with an artificial air mixture containing 5.5 per cent of carbon dioxide. The carbon dioxide content of the plasma is then determined by the method described in the next paper. The results are calculated in terms of bicarbonate with the aid of the table on p. 316. The value determined appears to indicate not only the alkaline reserve of the blood, but also that of the entire body.

The results obtained with a given plasma are reproducible to within 1 volume per cent of CO$_2$, 65 volume per cent being the average normal value for man. In acidosis the carbon dioxide capacity of the plasma falls so far below normal that the method is a most sensitive indicator of this condition and its severity. The simplicity of the technique and the few minutes required for the determination make it available, not only for physiological experiments, but also for clinical routine. Results obtained with normal men and diabetic patients are given in Papers V and VI.
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STUDIES OF ACIDOSIS: I. THE BICARBONATE CONCENTRATION OF THE BLOOD PLASMA; ITS SIGNIFICANCE, AND ITS DETERMINATION AS A MEASURE OF ACIDOSIS
Donald D. Van Slyke and Glenn E. Cullen


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