

REVIEW
of
PHYSIOLOGICAL
CHEMISTRY

HAROLD A. HARPER

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PREFACE TO 5th EDITION

This book is intended to present the fundamentals of physiological chemistry with emphasis on the accepted facts and concepts of the subject. A concise presentation of the applied and established principles has been favored over that of the theoretical and controversial, leaving further discussion of such material to other texts and to the current reviews.

The Review is intended as a supplement to the standard texts in biochemistry and as a companion volume for the student in such courses. Much of the material in the book is drawn from the author's lectures in basic science given in conjunction with the graduate training of physicians. Thus it is hoped that the book will serve also as a review for the physician preparing for state and specialty boards. It should further aid him in keeping abreast of this rapidly expanding branch of medical science, which now contributes so much to the understanding and practice of all phases of medicine.

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Chapter 1

GENERAL AND PHYSICAL CHEMISTRY

Physiological chemistry is the science which is concerned with the chemical reactions associated with biological processes. It has developed as a natural outgrowth of the field of general chemistry, which deals with the reactions of all elements, radicals, and compounds, organic and inorganic, living and non-living. Physiological chemistry incorporates many of the working principles of physical chemistry, i.e., the theoretical and physical properties inherent in the chemical elements. Since physiological chemistry deals with biological processes, certain difficulties arise in our present state of knowledge in the application of physical laws. These difficulties stem from biological variability, species variations, and the complexity of the biological system and of the organic compounds of which it is composed. Certain physical chemical aspects, however, have been carried over logically into the field of physiological chemistry. These will be discussed in the following sections.

ELECTROLYTIC DISSOCIATION

Ions:

When an electric current is passed through an aqueous solution of a compound in an electrolytic cell, some elements collect at the anode, or positive pole; others at the cathode, or negative pole. This indicates that these elemental particles bear electric charges. Such charged particles are termed ions. In accordance with the physical principle that unlike charges attract each other and like charges repel, those ions which have migrated to the anode (anions) are electronegative, and those to the cathode (cations) are electropositive.

Electrolytes and Non-electrolytes:

Compounds composed of elements which may be electrolytically dissociated into ions are called electrolytes. Many other compounds, particularly organic compounds, cannot be so dissociated and are thus designated non-electrolytes.

ACIDS AND BASES

Definitions:

- A. Acids are compounds of electronegative elements or groups plus ionizable hydrogen [H^+].
- B. Bases are compounds of electropositive elements or radicals plus ionizable hydroxyl groups [OH^-].

Standard Solutions:

- A. A molar solution of an acid or base is one which contains in each liter an amount in grams of the acid or base equal to the molecular weight of that compound.

2 Acids and Bases

- B. A normal solution is exactly the same as a molar solution if only one hydrogen ion or hydroxide ion is available. If more than one H^+ or OH^- ion is available, a normal solution is one which contains in grams per liter an amount of acid or base equal to the molecular weight (the gram-molecular weight) divided by the number of such H^+ or OH^- ions present.

Examples:

1-molar acetic acid (CH_3COOH) contains 60.032 grams per liter. 1-normal acetic acid is the same since only one H^+ is available.

1-molar sulfuric acid (H_2SO_4) contains 98.016 grams per liter. But 1-normal sulfuric acid contains 98.016 grams divided by 2 (two H ions are available), or 49.08 grams per liter. It follows that a 1-molar solution of sulfuric acid is 2-normal.

Degree of Acidity:

This is expressed as:

- A. Actual Acidity: The concentration of hydrogen ions in a solution. (This is usually spoken of as the pH of the solution. See page 4). It must be remembered, however, that all of the hydrogen atoms available for ionization may not actually be in ionic form and so may not be chemically reactive.
- B. Titrateable Acidity: The concentration of hydrogen ions in a solution plus those available for ionization although not actually ionized at the time. This is determined by titration against a base, and the result is a measure of the total H^+ concentration, both actually and potentially ionized.
- Total acidity is a term sometimes used to express the amount of free acid in a solution in addition to that in combination with organic compounds (e.g., proteins) and that present in acid salts. Actually it is synonymous with titrateable acidity.
- C. Free Acidity: The amount of acid, not combined with other substances, which is present in a solution.

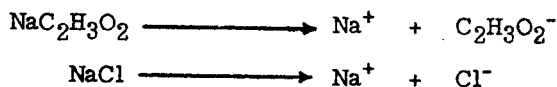
DISSOCIATION OF WATER

Dissociation:

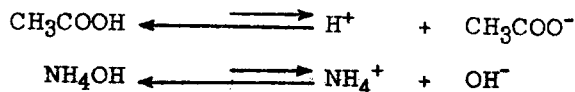
Acids, bases, and salts in solution dissociate into ions of the elements or radicals of which they are composed, although such dissociation is not to the same extent in each case.

Examples:

Sodium chloride and sodium acetate dissociate almost completely as follows:



Weak electrolytes such as acetic acid and ammonium hydroxide dissociate to a considerably lesser degree as follows:



Water itself dissociates, although to a very slight degree, in accordance with the following equilibrium:



Law of Mass Action: The relationship between the dissociated and undissociated molecules is a constant which may be expressed as:

$$(2a) \quad \frac{[\text{H}^+][\text{OH}^-]}{[\text{HOH}]} = K$$

or

$$(2b) \quad [\text{H}^+][\text{OH}^-] = K[\text{HOH}]$$

where the bracketed values refer to concentrations of the ions involved. This dissociation constant, K , for water is approximately 1×10^{-14} at room temperature. At the same time the amount of dissociated water is so small in comparison to the total amount present that the undissociated water (HOH) may be considered constant, with a value of unity. Equation (2b) may then be written as:

$$(3) \quad [\text{H}^+][\text{OH}^-] = K = 1 \times 10^{-14}$$

However, in pure water, $[\text{H}^+] = [\text{OH}^-]$

$$\text{Therefore,} \quad [\text{H}^+]^2 = 1 \times 10^{-14}$$

$$\text{and} \quad [\text{H}^+] = 1 \times 10^{-7} \text{ moles per liter.}$$

Effect of Acids and Bases on Dissociation of Water:

From the definition of acids and bases given above, it is evident that the addition of either acid or base to water will affect the ratio between the concentrations of H^+ and OH^- . Since the product of the two is a constant (see No. 3 above), the addition of one ion in excess requires a lowering of the concentration of the other. It should also be apparent from the above equation that in any aqueous solution of an acid there are still some hydroxide ions present, and vice versa.

Examples:

Give the OH^- concentration of a solution of acid with an H^+ concentration of 10^{-5} moles per liter.

$$10^{-5} [\text{OH}^-] = 10^{-14}$$

$$[\text{OH}^-] = 10^{-9} \text{ moles per liter.}$$

If base is added to water to make $[\text{OH}^-]$ of $1/10$ moles per liter, what is the $[\text{H}^+]$?

$$[\text{H}^+] \times 1 \times 10^{-1} = 10^{-14}$$

$$[\text{H}^+] = 10^{-13}$$

Methods of Expressing Concentration:

The concentration of a solution may be expressed by one of several methods (normality, moles per liter, etc.) as follows:

Examples:

What is the $[\text{H}^+]$ of a ten-thousandth-normal hydrochloric acid solution, assuming complete dissociation?

4 Concept of pH

Molar equivalent	0.0001 moles per liter
By powers of ten	1×10^{-4} moles per liter
By logarithms	$\bar{4}.0000$ ($\log 1 \times 10^{-4}$)

Express the $[H^+]$ of blood by various means, if it is known to be 0.0000000501 moles per liter.

By powers of ten	5.01×10^{-8}
By logarithms	$\log 5.01 \times 10^{-8} = \bar{8}.700$

THE CONCEPT OF pH

Hydrogen ion concentrations in the physiological range are usually considerably less than one. In order to express such concentrations in a convenient form, Sørensen introduced the concept of pH, in which whole numbers and decimal fractions thereof are used. All vital activities are affected by hydrogen ion concentration, and this convenient system of expressing it has become universally accepted in physiological chemistry.

pH may be defined either as (a) the negative of the logarithm (base ten) of the H ion concentration or, what is equivalent, (b) the logarithm of the reciprocal of the H ion concentration, i. e.:

$$\begin{aligned} \text{(a) } pH &= -\log [H^+] \\ \text{or (b) } pH &= \log \frac{1}{[H^+]} \end{aligned}$$

Note that the H ion concentration must be ascertained before the pH can be calculated. For strong electrolytes this may be substantially the same as the total concentration, if complete ionization is assumed; but for weak electrolytes the H ion concentration must be obtained by calculation from the ionization constant, as illustrated in subsequent paragraphs.

Examples:

What is the pH of a solution with a hydrogen ion concentration of 3.2×10^{-4} ?

$$\begin{aligned} [H^+] &= 3.2 \times 10^{-4} \\ pH &= \log \frac{1}{[H^+]} = \log \frac{1}{3.2 \times 10^{-4}} = \log \frac{1}{10.505 \times 10^{-4}} = \log \frac{1}{10^{-3.495}} = 3.495 \end{aligned}$$

Answer: pH = 3.495 (or 3.50)

In the above example one proceeds as follows:

1. Express H ion concentration by a power of 10.
2. Find the log of the coefficient, subtract this from the exponent, and then change the sign.

$$[H^+] = 3.2 \times 10^{-4}$$

$$pH = -4 \text{ less } \log 3.2^* = -3.50 = +3.50 \qquad * \log 3.2 = 0.50$$

To determine the H ion concentration from the pH, the order of calculation is reversed.

Example:

Give the H ion concentration of a solution at pH 4.72.

$$\text{pH } 4.72 = \log 10^{4.72} = \log \frac{1}{10^{-4.72}} = \log \frac{1}{10 \cdot 28 \times 10^{-5}} = \log \frac{1}{1.9 \times 10^{-5}}$$

Answer: $[\text{H}^+] = 1.9 \times 10^{-5}$

Variations in pH and Interpretation:

It will be noted that the greater the pH is, the lower will be the acidity, and that one unit of pH represents a difference of ten times in H ion concentration.

e.g., pH 5 = 0.00001 moles per liter $[\text{H}^+]$, pH 4 = 0.0001 moles per liter $[\text{H}^+]$.

From what has already been said concerning the ionization of water, it is apparent that when a solution is neutral (i.e., H and OH ions in equal proportions), it has a pH of 7.

$$[\text{H}^+][\text{OH}^-] = K_w = 1 \times 10^{-14} \quad (\text{pH} + \text{pOH} = 14)$$

$$[\text{H}^+]^2 = 1 \times 10^{-14}$$

$$[\text{H}^+] = 1 \times 10^{-7}$$

$$\text{pH} = 7$$

As the H ions increase, the pH will decrease (solution becomes more acid); and conversely, as the H ions decrease, the pH will increase (solution becomes more alkaline). Therefore, solutions whose pH values lie between 0 and 7 are acid solutions, and those whose pH values lie between 7 and 14 are alkaline solutions.

pOH:

This is the value obtained by subtracting the pH from 14. For example, a solution of pH 3 has a pOH of 11; a solution of pH 10 has a pOH of 4. This expression is based on OH ion concentration. It is useful in calculating the pH of an alkaline solution.

Example:

What is the pH of a 0.0004 normal solution of sodium hydroxide?

$$\text{OH ion concentration} = 4 \times 10^{-4}$$

$$\text{pOH} = 4.000 - 0.602 = 3.398$$

$$\text{pH} = 14 - \text{pOH} = 14 - 3.398 = 10.60$$

Answer: pH = 10.60

Determination of pH:

In order to arrive at the pH of a solution, one must first obtain the H ion concentration. In the case of a strong electrolyte this is obtained from the original concentration by assuming complete ionization. For weak electrolytes, with which physiological chemistry is more often concerned, this must be calculated by the use of the law of mass action, which is expressed as follows:

6 Concept of pH

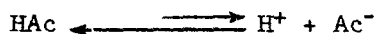
$$\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = K_a$$

Where H = cation
A = anion
HA = the undissociated acid

This states that the ratio of the product of the concentration of the ionized components to that of the un-ionized is equal to a constant (K_a), the dissociation (ionization) constant for that acid. This constant may be obtained from any handbook of chemistry. (See also Table II, page 9). The following examples illustrate the use of this expression in order to calculate the pH of a weak acid, such as acetic acid, or of a weak base, such as ammonium hydroxide.

Examples:

1. What is the pH of a 0.1 M solution of acetic acid (HAc)? (K_a , HAc = 1.86×10^{-5})



$$\frac{[\text{H}^+][\text{Ac}^-]}{[\text{HAc}]} = K_a = 1.86 \times 10^{-5}$$

Since $[\text{H}^+] = [\text{Ac}^-]$, we may represent the numerator as $[\text{H}^+] \times [\text{H}^+]$ or $[\text{H}^+]^2$

And therefore,

$$\frac{[\text{H}^+]^2}{0.1} = 1.86 \times 10^{-5}$$

Actually, the denominator should be $0.1 - \text{H}^+$, i.e., the original concentration (0.1 M) minus that in the ionized form, to give the un-ionized fraction. To avoid a quadratic expression, one may assume that the ionized fraction is so small that the original concentration of the solution, uncorrected for the ionized fraction, will suffice.

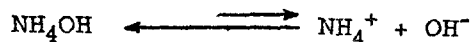
$$[\text{H}^+]^2 = 1.86 \times 10^{-6}$$

$$[\text{H}^+] = \sqrt{1.86 \times 10^{-6}}$$

$$[\text{H}^+] = 1.36 \times 10^{-3}$$

$$\text{and pH} = 3.00 - 0.13 = 2.87$$

2. What is the pH of a solution of NH_4OH , 0.1 N? (K_b , $\text{NH}_4\text{OH} = 2 \times 10^{-5}$)



$$\frac{[\text{NH}_4^+][\text{OH}^-]}{[\text{NH}_4\text{OH}]} = K_b = 2 \times 10^{-5}$$

$$\frac{[\text{OH}^-]^2}{0.1} = 2 \times 10^{-5}$$

$$[\text{OH}^-] = 1.42 \times 10^{-3}$$

$$\text{pOH} = 2.85$$

$$\text{pH} = 14.0 - 2.85 = 11.15$$

STANDARDIZATION OF ACIDS AND BASES

Solutions for Standardization:

To prepare solutions of known acidity, a primary standard of reference is used. This may be a chemically pure, dry substance (such as potassium acid phthalate), which can be weighed accurately and dissolved in a known volume of solution. The concentration of a solution of alkali is then obtained by titration against this primary standard. The solution of known alkaline strength may now be used to ascertain the strength of the acid solution. The following equation is useful in calculation of concentrations (normalities) from comparison with a solution of known strength:

$$N_1 V_1 = N_2 V_2$$

$$N_1 = \frac{N_2 V_2}{V_1}$$

where V_1 and N_1 refer to the volume and concentration (normality) of the unknown, and V_2 and N_2 refer to the corresponding known values.

Indicators:

An indicator is used to determine the end point of an acid-base reaction. The indicator is a very weak organic acid or base which undergoes a change of structure and a consequent change of color in the presence of certain concentrations of hydrogen and hydroxyl ions. The color change actually takes place over a range of concentration, as shown in Table I below.

Table I - Some Common Indicators

Indicator	pH Range	Color Change
Töpler's Reagent	2.9 - 4.0	Red - Yellow
Congo Red	3.0 - 5.0	Blue - Red
Methyl Orange	3.1 - 4.4	Orange Red - Yellow
Bromcresol Green	4.0 - 5.6	Yellow - Blue
Methyl Red	4.2 - 6.3	Red - Yellow
Litmus	4.5 - 8.3	Red - Blue
Alizarin Red	5.0 - 6.8	Yellow - Red
Bromcresol Purple	5.4 - 7.0	Yellow - Purple
Phenol Red	6.6 - 8.2	Yellow - Red
Phenolphthalein	8.3 - 10.0	Colorless - Red

Two factors must be considered in making titrations for the standardization of acids and bases: (1) the choice of the strength of the standard, which should usually be of approximately the same normality as that of the unknown; and (2) the end point of the reaction. If solutions of equivalent concentrations of a strong base and a strong acid are placed together, both will have approximately equal dissociation constants; the pH at the end point will be near 7. If, however, the acid is stronger than the base, the former will dissociate more completely than the latter, the $[H^+]$ will rise, and the pH will be less than 7; the solution will be acid in spite of the fact that equivalent concentrations of the acid and base have been used. Similarly, when a weak acid and strong base are together, the reaction at the end point will be basic. It is, therefore, necessary to choose an indicator which will undergo a change of color at the actual end point of the reaction for the particular system under study.

In gastric analyses, free acidity is determined by the use of an indicator which shows a color change at a low pH (2.8) (Töpler's reagent, dimethylaminoazobenzene). Phenolphthalein is then added and the titration continued to its end point to determine the total acid. The latter fraction measures the free acid plus the acid bound in acid-salt and protein combination.

8 Buffer Solutions

The determination of pH measures the effective or actual acidity of a solution, as it indicates the amount of H^+ present in the solution at the time, which is often the most important consideration from a physiological standpoint. Indicators may be used for pH measurement. (See Table I, above). Test samples are treated with various indicators which are sensitive to different pH values; they are then compared with standards held at a definite pH by means of a buffer (see below) in order to determine the exact pH. Electrometric methods of determination of pH are much better since they are very sensitive, more accurate, and can be applied to colored solutions. Hence, they find considerable application in biological work. The hydrogen electrode is the standard. In actual practice, the glass electrode together with a calomel electrode are used in most pH meters.

BUFFER SOLUTIONS

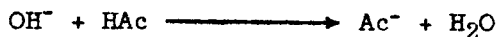
Buffers, which are usually mixtures of weak acids with their salts of strong bases, possess the ability (known as buffer action) to resist change in hydrogen-ion concentration. The buffer capacity is a measure of this ability to resist change in pH and is expressed as the rate of change in pH when acid or alkali is added. Buffer action is very important in physiological chemistry because it is part of the homeostatic mechanism whereby the neutrality of the body is regulated within the relatively narrow limits of pH compatible with the life of most cells.

Principles of Buffers:

To illustrate the action of buffers we may take the usual condition where a weak acid and its salt with a strong base are together in solution (e.g., acetic acid and sodium acetate). The conditions of the buffer mixture may be shown as:



If base (OH ions) is added to this system, it will react with the acid to form the salt:



which salt is less alkaline than the original base. The addition of acid (H ions) will cause another reaction:



to form a relatively undissociated acid and thus no free H ions. In either case the change in H ion concentration, and therefore the pH change, is relatively smaller than would be the case if the buffer were not present. The buffer acts almost as if it were "absorbing" the added free hydrogen or hydroxyl ions.

Buffers Are Used For:

- A. Making standard solutions in which it is desired to maintain a constant pH, as in colorimetric determination of the pH of unknown solutions. (See previous section).
- B. Maintenance of a given H ion concentration, which may be necessary for optimal activity of a reaction. This function of buffers is most important in the action of enzymes, both in vivo and in vitro, and buffer action is, therefore, of great importance in all physiological systems.

The pH of a Buffer Solution May Be Determined by the Henderson-Hasselbalch Equation:

$$\text{pH} = \text{pKa} + \log \frac{\text{salt}}{\text{acid}}$$

Where pH is $\log \frac{1}{\text{H}^+}$ and pKa is $\log \frac{1}{\text{Ka}}$ (Ka is dissociation constant of the acid in question)

The equation may also be used to determine the relative normal concentrations of the salt and acid to be used in reaching a desired pH. For practical purposes two systems of buffers are in common use, those of Clark and Lubs or those of Sørensen. Details are to be found in any hand-book of chemistry. The tables are arranged so that by mixing solutions as directed, buffers of any desired pH over the range from 1 to 14 may be prepared.

Example:

Calculate the pH of a solution prepared by mixing 25 cc. of acetic acid with 10 cc. of sodium hydroxide, both solutions 0.1-normal.

When these two solutions are mixed, the sodium hydroxide will neutralize 10 ml. of the acetic acid to form 10 ml. of the salt, sodium acetate. Fifteen ml. of unneutralized acid will remain. This produces a buffer mixture in which the ratio of salt to acid is 10:15. The Ka for acetic acid is 1.86×10^{-5} , and therefore the pKa ($\log 1/\text{Ka}$) is 4.73.

According to the Henderson-Hasselbalch equation,

$$\text{pH} = 4.73 + \log \frac{10}{15}$$

$$\text{pH} = 4.73 + \bar{1}.8195$$

$$\text{pH} = 4.73 + \log 0.66$$

Answer: pH = 4.55

It will be seen by examination of the equation that if the ratio of salt to acid is 1:1, i.e., at the exact halfway point in the neutralization of an acid by a base, the pH of the resultant mixture would equal the pKa ($\log 1:1$, $\log 1 = \text{zero}$). Under these circumstances the buffer capacity (see above) is at its maximum, for here it possesses maximal ability to react either as a base or an acid.

Table II - Dissociation Constants of Common Organic Acids

Acid	K	pKa
Acetic Acid	1.86×10^{-5}	4.73
Carbonic Acid	3.0×10^{-7}	6.52
second hydrogen	4.5×10^{-11}	10.35
Phosphoric Acid	8.0×10^{-9}	2.10
second hydrogen	7.5×10^{-8}	7.13
third hydrogen	5.0×10^{-13}	12.30
Lactic Acid	1.5×10^{-4}	3.82

THE SOLUBILITY OF GASES IN AQUEOUS SOLUTIONS

The normal environments of all life processes in both animals and plants are water and air. Water is the solvent in which the chemical reactions of the cell occur; and in order to gain access to the cell, the gases of the air must first be dissolved in this aqueous medium. Certain physico-chemical laws governing the solubility of gases in water or in other aqueous solutions such as the body fluids will, therefore, be discussed at this point.

The Amount of a Gas Dissolved in Water Varies With:

1. The nature of the gas.
2. The temperature.
3. The pressure.

Henry's Law:

For a given gas, Henry's Law applies: The temperature remaining constant, the amount (i. e., weight) of gas absorbed or dissolved by a given volume of liquid is proportional to the pressure of the gas.

Absorption Coefficient:

It is convenient to express the extent to which a gas is absorbed by a liquid as the absorption coefficient (α), that is, the volume of a gas [reduced to normal temperature and pressure (NTP, 273° absolute, 760 mm.)] which will be dissolved at a pressure of 1 atmosphere by 1 volume of the liquid.

Partial Pressure:

When dealing with a mixture of gases, such as air, the pressure under which each constituent is dissolved is called its partial pressure (p). The partial pressure of each gas can be calculated from its percentage (by volume) of the total.

Example:

Air is 79% nitrogen, 20.98% oxygen, and 0.04% CO₂. If the total pressure is 760 mm., after correction for the vapor pressure of water (17.5 mm. at 20° C.) it becomes 742.5 mm., and the partial pressure of each gas is then:

Nitrogen	0.79	x	742.5 mm.	=	586.6 mm.
Oxygen	0.2098	x	742.5 mm.	=	155.6 mm.
Carbon dioxide	0.0004	x	742.5 mm.	=	0.297 mm.

Calculation of Amount of Gas in Solution:

To calculate the amount, V (volume), at normal temperature and pressure of each gas dissolved:

$$V = \frac{\alpha \cdot v \cdot p}{760}$$

α = absorption coefficient for the gas
 v = the volume of the liquid
 p = the partial pressure of the gas

Example:

At an external pressure of 760 mm., how much (volume at NTP) oxygen, nitrogen, and carbon dioxide will be absorbed by 100 ml. water at 20° C. ?

After correction for vapor pressure of water, the external pressure becomes 742.5 mm.

<u>absorption coefficients</u>	Oxygen	0.0310
(in water at 20° C.)	Nitrogen	0.0164
	Carbon dioxide	0.878

Oxygen dissolved	$V = \frac{0.0310 \times 100 \times 155.6}{760} = 0.635 \text{ cc.}$
Nitrogen dissolved	$V = \frac{0.0164 \times 100 \times 586.6}{760} = 1.266 \text{ cc.}$
Carbon dioxide dissolved	$V = \frac{0.878 \times 100 \times 0.297}{760} = 0.034 \text{ cc.}$
Total gases dissolved	$= 1.935 \text{ cc.}$

Solubility of Gases in Solutions of Electrolytes and Non-electrolytes:

In the example above, the total, 1.935 cc., is the amount of air which would be dissolved in 100 ml. of water under those conditions. The solubility of a gas, however, is lower in solutions of salts or non-electrolytes than in water; the solubility decreases as the concentration of the solute increases.

These considerations are important because, with respect to their absorption of gases, blood and body fluids act like aqueous solutions of various salts. For instance, the α values in blood plasma at 38° C. are:

Oxygen	0.024
Nitrogen	0.012
Carbon dioxide	0.510

The amounts of each gas dissolved in 100 cc. of plasma at 760 mm. Hg may be calculated as follows: After correction for vapor pressure of water in the alveolar air (47 mm. Hg at 37° C.), the pressure of the gas mixture becomes 713 mm. Hg. The partial pressure of each gas is then (alveolar air-oxygen, 15%; nitrogen, 80%; carbon dioxide, 5%):

Oxygen	$0.15 \times 713 \text{ mm. Hg} = 107 \text{ mm. Hg}$
Nitrogen	$0.80 \times 713 \text{ mm. Hg} = 570 \text{ mm. Hg}$
Carbon dioxide	$0.05 \times 713 \text{ mm. Hg} = 36 \text{ mm. Hg}$

Oxygen dissolved	$V = \frac{0.024 \times 100 \times 107}{760} = 0.338 \text{ cc.}$
Nitrogen dissolved	$V = \frac{0.012 \times 100 \times 570}{760} = 0.900 \text{ cc.}$
Carbon dioxide dissolved	$V = \frac{0.510 \times 100 \times 36}{760} = 2.416 \text{ cc.}$

Oxygen and, to a certain extent, carbon dioxide are, however, absorbed by whole blood to a much greater degree than one would predict from Henry's law. This is due to the ability of hemoglobin to accept reversibly these gases (see page 162).

Varying Solubility of Gases With Varying External Pressure:

The relationship between external pressure and the solubility of gases in blood and tissue fluids becomes important when the external pressure varies significantly from normal.

- A. Low External Pressures: At high altitudes, although the composition of the air is substantially the same as at sea level, there is a relative lack of each gas because of the diminished barometric pressure. This lack particularly affects the supply of oxygen to the blood. At an elevation where the barometric pressure is half that at sea level, the partial pressure of oxygen and, consequently, the amount dissolved in the plasma would be reduced to a similar extent.

12 Osmotic Pressure

- B. **High External Pressures:** The various gases of the air are dissolved in the body fluids to an increased degree when the external pressure is higher than normal. With return to a normal environment, the gases leave the blood and tissue fluids. This may cause harmful effects in the remote areas of the body where gaseous equilibrium cannot be accomplished. Nitrogen is the most serious offender because of its high concentration in the air. Deep sea divers or other workers subject to high external pressures may suffer from the effects of this rapid evolution of gaseous nitrogen if returned to lower pressures too rapidly. These effects are described as "the bends."

OSMOTIC PRESSURE

Because of the nature of the cell membrane, osmotic pressure plays a very important role in the exchange of water and dissolved materials between the cell and its extracellular environment.

Diffusion:

When a soluble substance such as sodium chloride is added to water, it quickly dissolves and distributes itself equally throughout the liquid. This process of diffusion is produced by the constant movement of the ions of the salt when in solution.

Osmosis:

If, however, a semipermeable membrane is interposed between a solution and its pure solvent (e.g., water and a solution of sodium chloride), or between two solutions differing in concentrations of solute, the water molecules will diffuse through the membrane more rapidly than the salt molecules. If the salt solution is enclosed within the membrane, water will diffuse across the membrane into the salt solution. (See Fig. 1, next page).

Osmotic Pressure:

This movement of water (osmosis) results in the building up of a definite pressure (osmotic pressure) within the membrane-enclosed area. The amount of excess pressure which must be imposed on a solution in order to prevent the passage of a solvent into it through a semipermeable membrane is defined as osmotic pressure.

Determination of Osmotic Pressure:

- A. **Direct Measurement of Osmotic Pressure:** Osmotic pressure can be measured directly with an osmometer. The simplest form of an osmometer is a thistle tube, the opening of which is covered with a semipermeable membrane, containing a solution (of sucrose, for example). When the tube is inverted and immersed in water, the solvent (water) passes by osmosis into the thistle tube. The osmotic pressure which develops within the tube causes the liquid to rise in the stem of the tube. When the pressure is high enough to stop further inflow of water, a state of equilibrium is reached. The hydrostatic pressure of the column of solution is the measure of the osmotic pressure. (See Fig. 2, next page).
- B. **Indirect Measurement of Osmotic Pressure:** The direct determination of osmotic pressure by means of osmometers has been mentioned, but osmotic pressures are more conveniently determined by indirect methods. For physiological fluids, the depression of the freezing point, or the delta value (Δ), is used. According to the Law of Blagden, for a given solvent (e.g., water), the depression of the freezing point is proportional to the concentration of the solute. For aqueous solutions a 1.0 M. solution of a substance which does not ionize freezes at -1.86°C . Such a solution has an osmotic pressure of 22.4 atmospheres (1 atmosphere = a pressure of 760 mm. of mercury); therefore, a depression of 1.0°C . corresponds to an osmotic pressure of 12.04 atmospheres or 9150 mm. of mercury (12.04×760) at 0°C .
- The freezing point of mammalian blood is about -0.59°C ., corresponding to an osmotic pressure at body temperature of about 8 atmospheres. This pressure is maintained remarkably constant, largely by the regulatory action of the kidney. Solutions which have an osmotic pressure equal to that of the body fluids are said to be iso-osmotic or isotonic, while those which have lower or higher osmotic pressures are hypotonic or hypertonic, respectively.