

Biochemistry

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THIRD EDITION

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Preface to the Third Edition

Two changes which addicts may notice in this edition of the book are the absence of the calorie and of the Kenya blotched genet. The first has been replaced by the joule as a result of the very strong recommendation by the British Nutrition Society, although many of us will continue to be both figuratively and literally calorie conscious.

Genetta tigris erlangeri has disappeared because the complete chapter on inborn errors of metabolism has been omitted from this new edition. It is not that I feel that the subject is now unimportant; on the contrary, examples of congenital defects have accumulated at such a rate that it would have been impossible even to catalogue them adequately, let alone to have discussed such important topics as human polymorphism, or the still remote but exciting possibility of inserting suitable lengths of DNA into the chromosomes. Much of the material from this chapter has, however, been incorporated into the text elsewhere.

The space released has been used to extend the treatment of protein and nucleic acid synthesis, as I judge that these topics will become ever more important in medicine in the future. Elsewhere I have tried to bring the material up to date without greatly altering the basic structure. The book is indeed as complete as I can make it, and attempts that I have made to suggest excisions have brought cries of reproach from my colleagues. It is hoped, therefore, that the book in this new edition will once again have a warm welcome from students, doctors and teachers.

My thanks are due to my wife and Dr Ian Mason, for preparing the index. It is very thorough and should be of great assistance to all those who use this book.

From the Preface to The Second Edition

Fundamentally, this text is based on the courses of lectures given to medical students at University College, London and at Edinburgh University. Our experience of teaching students who are starting their training has, however, led us to expand our discussion of topics which are frequently puzzling to them, or which are in some way controversial.

The emphasis which is placed in the book on the biochemistry of blood, and particularly on acid-base status, probably makes it unsuitable for use as a text by students who are not in the medical sciences. Nevertheless, this comprehensive and, we hope, lucid account of this complex subject, whose clinical importance cannot be overemphasized, should help advance the spread of scientific medicine. We hope also that the book is still small enough to be of value to practising doctors whose days of full-time study are over.

**J. H. OTTAWAY
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Definitions

Before starting the book proper it will be useful to define some units which frequently occur in biochemistry.

Mole. One mole of a substance is the mass of that substance in grammes that is numerically equal to its molecular weight.

Molar solution. A molar solution (abbreviation: M) of a substance is one which contains one mole of that substance in one litre of solution.

The terms mole and molar solution are extended to ions, thus a one molar solution of disodium hydrogen phosphate (Na_2HPO_4) is one molar with respect to phosphate ions and two molar with respect to sodium ions.

Equivalent. One equivalent (abbreviation: equiv.) of an ion is the weight of one mole of that ion divided by the number of its electric charge. Thus one equivalent is equal to one mole for singly charged ions (Na^+ , Cl^-), to $\frac{1}{2}$ = 0.5 moles for doubly charged ions (Ca^{2+} , HPO_4^{2-}), to $\frac{1}{3}$ = 0.33 moles for triply charged ions (Fe^{3+} , citrate $^{3-}$), etc. Or, conversely, one mole of a singly charged ion contains one equivalent, one mole of a doubly charged ion contains two equivalents, one mole of a triply charged ion contains three equivalents, etc.

Multiples and submultiples of units are shown by prefixes as follows:

Multiplier	Prefix	Symbol	Example
1 000 000 = 10^6	mega	M	megaunit (Mu)
1 000 = 10^3	kilo	k	kilogramme (kg)
0.1 = 10^{-1}	deci	d	decimetre (dm)
0.01 = 10^{-2}	centi	c	centimetre (cm)
0.001 = 10^{-3}	milli	m	millilitre (ml)
μ -000 001 = 10^{-6}	micro	μ	microlitre (μ l)
10^{-9}	nano	n	nanometre (nm)
10^{-12}	pico	p	picrogramme (pg)

Only one multiplying prefix is used at one time to a given unit. Thus one thousandth of a millimole is not called one millimillimole (1 mmmol) but

is known as one micromole ($1\ \mu\text{mol}$) and instead of 1 millimicrometre ($0.001\ \mu\text{m}$) one writes 1 nanometre (nm).

Examples. The following examples will be found useful in the laboratory.

A 1 molar (M) solution contains 1 mole per litre ($1\ \text{mol/l}$), 1 millimole per millilitre ($1\ \text{mmol/ml}$), and 1 micromole per microlitre ($1\ \mu\text{mol}/\mu\text{l}$).

A 1 millimolar ($1\ \text{mM}$) solution contains 1 millimole per litre ($1\ \text{mmol/l}$), 1 micromole per millilitre ($1\ \mu\text{mol/ml}$), and one nanomole per microlitre ($1\ \text{nmol}/\mu\text{l}$).

Each millilitre of a solution which is 1 millimolar with respect to both KH_2PO_4 and Na_2HPO_4 contains $1\ \mu\text{mole}\ \text{K}^+$, $2\ \mu\text{moles}\ \text{Na}^+$ and $2\ \mu\text{moles}$ total phosphate ($\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$). *Each litre* of such a solution contains 1 meq. K^+ , 2 meq. Na^+ , and 3 meq. total phosphate, made up of 1 meq. H_2PO_4^- and 2 meq. HPO_4^{2-} since each mole of HPO_4^{2-} is equal to 2 equivalents.

Dalton, the atomic mass unit equal to $\frac{1}{12}$ of the mass of an atom of carbon-12.

Joule. The joule is a unit of energy and is numerically equivalent to 0.239 calorie. One calorie is equal to 4.184 joules (J) and one kilocalorie is equal to 4.184 kilojoules (kJ).

Acids and Bases

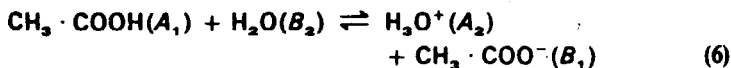
An acid is a molecular species tending to lose a hydrogen ion while a base is a species tending to add on a hydrogen ion. The dissociation of a hydrogen ion from an acid may be represented by the equilibrium



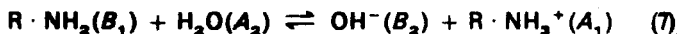
Since the dissociation is reversible the species B formed when A loses a hydrogen ion is in fact a base; when equilibrium (1) is displaced to the left B adds a hydrogen ion. Such a pair of species is known as a conjugate acid-base pair. Since an acid loses a hydrogen ion to form its conjugate base it follows that the acid must always have a charge which is one unit more positive than its conjugate base. These points are illustrated in the following equilibria:



In all biological systems the solvent is water which can itself act as an acid or as a base as is shown by the following equilibria:

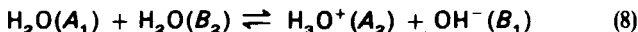


and



The Ion Product of Water

Because of the acidic and basic potentialities of water it follows that interactions between water molecules themselves will give rise to H_3O^+ and OH^- ions, thus



and the ion product of water, K_w , is given by

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-] \quad (9)$$

The constant, K_w , has a value of about 10^{-14} moles²/litre² at ordinary temperatures, and it follows from (9) that there is a reciprocal relation between $[\text{H}_3\text{O}^+]$ and $[\text{OH}^-]$. When $[\text{H}_3\text{O}^+] = [\text{OH}^-]$, the concentration of $\text{H}_3\text{O}^+ = \sqrt{K_w} = 10^{-7}$ moles/litre. This is the concentration of H_3O^+ ions at *neutrality*.

When acids dissociate in water, as in (6), they give rise to the hydronium ion H_3O^+ and not to the hydrogen ion H^+ though for simplicity we shall always refer to the hydrogen ion and write H^+ .

Strong Acids

When strong mineral acids are dissolved in water the dissociation of the hydrogen ion may be considered to be complete. Thus HCl , HClO_4 , HNO_3 and the first hydrogen of H_2SO_4 are completely ionized in dilute solution. In other words equilibrium (1) is completely over to the right.

Weak Acids

When weak acids such as $\text{CH}_3 \cdot \text{COOH}$, H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , HSO_4^- and $\text{CH}_3 \cdot \text{NH}_3^+$ are dissolved in water they are incompletely dissociated, that is to say both the acids and their conjugate bases are present in the solution in similar concentrations. All these dissociations may be represented by the general equilibrium



where the charge on the conjugate base, A^- , is one unit less positive than on the conjugate acid, HA .

Acid Dissociation Constants

The Law of Mass Action may be applied to these equilibria giving (from 10)

$$K_{\text{HA}} = \frac{[\text{A}^-][\text{H}^+]}{[\text{HA}]} \quad (11)$$

where K_{HA} is the equilibrium or acid dissociation constant of the acid HA . The constant K_{HA} has the dimensions of concentration and is a measure of the 'strength' of the acid, the larger the value of K_{HA} the 'stronger' the

acid. The following acids are arranged in order of their 'strengths' at 25°C. H_3PO_4 , $K = 8.91 \times 10^{-3}$; $\text{CH}_3 \cdot \text{COOH}$, $K = 2.24 \times 10^{-5}$; H_2PO_4^- , $K = 1.58 \times 10^{-7}$; $\text{CH}_3 \cdot \text{NH}_3^+$, $K = 2.40 \times 10^{-11}$, and $K_w = 10^{-14}$.

pH and pK

The numerical values of $[\text{H}^+]$ and K with which we have to deal are very small, such as the values of K listed above; $[\text{H}^+]$ at neutrality = 10^{-7} moles (100 nanomoles)/litre. To simplify calculations the pH and pK scales are used; these are defined as the negative logarithms to the base 10 of the hydrogen ion concentration and the acid dissociation constant respectively.

$$\text{pH} = -\log [\text{H}^+] = \log \frac{1}{[\text{H}^+]} \quad (12)$$

$$\text{pK} = -\log K = \log \frac{1}{K} \quad (13)$$

The hydrogen ion concentration of the blood, which is kept fairly constant, can also conveniently be expressed as nanomoles of hydrogen ion per litre. Normally blood has a pH = 7.4 or $[\text{H}^+] = 40 \text{ nmol/l}$ (see Chapter 14).

It follows from these definitions that the functions pH and pK have the following important properties:

(a) The higher the hydrogen ion concentration $[\text{H}^+]$, in moles/litre, the lower the pH and vice versa, e.g. if $[\text{H}^+] = 3 \times 10^{-7}$, pH = 6.523 and if $[\text{H}^+] = 2 \times 10^{-4}$, pH = 3.699. Similarly the lower the pK the greater K and the 'stronger' the acid; thus at 25°C for H_3PO_4 , pK = 2.05; $\text{CH}_3 \cdot \text{COOH}$, pK = 4.65; H_2PO_4^- , pK = 6.8; $\text{CH}_3 \cdot \text{NH}_3^+$, pK = 10.62, and $\text{pK}_w = 14$.

(b) A tenfold change in $[\text{H}^+]$ or K corresponds to a change of one unit in pH or pK; e.g.

$$[\text{H}^+] = 10^{-6} \text{ mol/l or } 1 \text{ } \mu\text{mol/l, pH} = 6$$

$$[\text{H}^+] = 10^{-7} \text{ mol/l or } 100 \text{ nmol/l, pH} = 7$$

$$[\text{H}^+] = 10^{-8} \text{ mol/l or } 10 \text{ nmol/l, pH} = 8$$

The Relation between pH and pK

We can now rewrite equation (11) in terms of pH and pK to give the very important equations:

$$\text{pK} = \text{pH} - \log \frac{[\text{A}^-]}{[\text{HA}]} \quad (14)$$

and

$$\text{pH} = \text{pK} + \log \frac{[\text{A}^-]}{[\text{HA}]} = \text{pK} + \log \frac{[\text{conjugate base}]}{[\text{conjugate acid}]} \quad (15)$$

From (15), the Henderson-Hasselbalch equation, it follows that $\text{pH} = \text{pK}$ when $[\text{A}^-] = [\text{HA}]$, i.e. when the acid is half neutralized.

The pH of a Solution of a Weak Acid

A weak acid dissociates in solution as shown in equations (10) and (11), and further it is necessary for the solution to remain electrically neutral, i.e. there must be the same *total* number of positive charges on the ions as there are negative charges. Then ignoring the small $[\text{OH}^-]$ in an acid solution and assuming HA is uncharged, the electroneutrality condition is:

$$[\text{A}^-] = [\text{H}^+] \quad (16)$$

Let the total concentration of acid be A_T , then from (10)

$$\text{A}_T = [\text{A}^-] + [\text{HA}] \quad (17)$$

and from (11)

$$[\text{H}^+][\text{A}^-] = K[\text{HA}] \quad (18)$$

Combining (16), (17) and (18) we have:

$$[\text{H}^+]^2 = K(\text{A}_T - [\text{H}^+]) \quad (19)$$

In a dilute solution of a weak acid $[\text{H}^+]$ may be assumed to be small compared with A_T , so (19) becomes

$$[\text{H}^+] \approx \sqrt{K\text{A}_T} \quad (20)$$

or

$$\text{pH} = \frac{1}{2}\text{pK} - \frac{1}{2} \log \text{A}_T \quad (21)$$

For example if we have solutions of acetic acid, $\text{pK} = 4.65$, at concentrations of 0.1, 0.01, and 0.001 molar their pH's will be given by:

$$\begin{aligned} \text{pH} &= \frac{1}{2}(4.65) - \frac{1}{2} \log (0.1, 0.01, \text{ and } 0.001) \\ &= 2.325 + 0.5, 1.0, \text{ and } 1.5 \\ &= 2.825, 3.325, \text{ and } 3.825 \text{ respectively.} \end{aligned}$$

Buffers

These are solutions of weak acids, HA, and their salts, MA; such systems resist changes in the pH when acid or alkali is added to the solution.

The acid HA is by definition a weak acid so we may assume that it is only very slightly dissociated and the concentration $[\text{HA}]$ is equal to the

total concentration of acid added. Further we may assume that the salt **MA**, if it is an alkali metal salt, is completely dissociated into M^+ and A^- and hence the concentration of the conjugate base, $[A^-]$, is equal to the *total concentration of salt added.* Equation (15) then becomes:

$$\text{pH} = \text{p}K + \log \frac{[\text{salt}]}{[\text{acid}]} \quad (22)$$

Both monobasic and polybasic acids form buffers. Typical examples of the first type are the acetic acid-acetate buffers and of the second type, the phosphate buffers. When the $\text{p}K$'s of the various groups of a polybasic acid are near each other (e.g. citric acid) the analysis of the buffer system is more complex and will not be considered.

Acetate buffers. The buffering action of mixtures of acetic acid ($\text{p}K$ 4.65) and Na acetate is illustrated in Fig. 1.1 which shows the changes in pH when 100 ml 0.2 molar $\text{CH}_3 \cdot \text{COOH}$ are titrated with 2M-NaOH.

On each addition of 1 ml of 2M-NaOH, the pH rises sharply from A to B and then less rapidly past C, the point of half neutralization, to D. Then the pH rises very sharply as the equivalence point is reached at E.

Fig. 1.1 shows that buffers most strongly resist changes in pH near the point of half neutralization (C), that is when $\text{pH} = \text{p}K$. Further it is seen that the range over which buffers are effective is about 1 pH unit on either side of the $\text{p}K$, i.e. from $[\text{salt}]/[\text{acid}] = 1/10$ to $10/1$ (equation (22)).

Putting this in another way, we can say that the rate of change of pH with titre is minimal when $\text{pH} = \text{p}K$, but it should be noted that the rate of change of hydrogen ion concentration, $[H^+]$, with titre is least at the

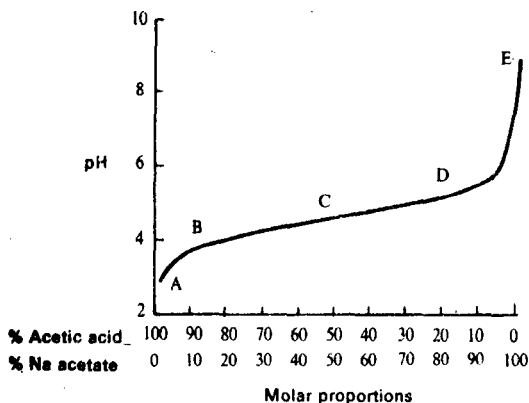


FIG. 1.1. pH titration curve of $\text{CH}_3 \cdot \text{COOH}$.

lowest values of $[H^+]$ (highest pHs). On the other hand, the rate of change of hydroxide ion concentration, $[OH^-]$, with titre is large at low $[H^+]$ (high pH) and low at high $[H^+]$ (low pH).

Phosphate buffers. In the titration curve of phosphoric acid shown in Fig. 1.2 three distinct regions of buffering can be distinguished; these correspond to the three dissociations of phosphoric acid. As phosphoric

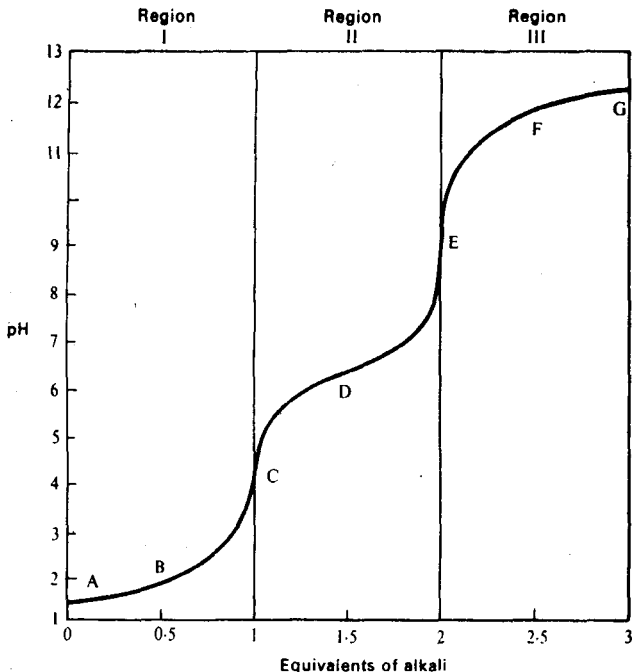


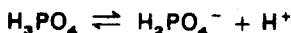
FIG. 1.2. pH titration curve of H_3PO_4 .

acid, H_3PO_4 , can lose 3 hydrogen ions per mole, 3 equivalents of alkali are required to neutralize it completely. There are, therefore, 3 sodium (or other metal) phosphates, NaH_2PO_4 , Na_2HPO_4 , and Na_3PO_4 . The main features of the phosphate system are indicated below and in Fig. 1.2.

The pH of any solution of phosphoric acid and/or its alkali metal salts can be determined from equations (23), (24) or (25) below. The addition of alkali to any phosphate solution will cause the pH to rise along the curve in Fig. 1.2, while the addition of a strong acid will cause the pH to fall along the curve.

Region I

Equilibrium



Curve

A to C

Mid-point of equilibrium, B

$$\text{p}K_1 = 2.0$$

$$\text{pH} = \text{p}K_1 + \log \frac{[\text{H}_2\text{PO}_4^-]}{[\text{H}_3\text{PO}_4]} \quad (23)$$

Region II

Equilibrium



Curve

C to E

Mid-point of equilibrium and point of maximum buffering, D

$$\text{p}K_2 = 6.8$$

$$\text{pH} = \text{p}K_2 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \quad (24)$$

Region III

Equilibrium



Curve

E to G

Mid-point of equilibrium, F

$$\text{p}K_3 = 11.7$$

$$\text{pH} = \text{p}K_3 + \log \frac{[\text{PO}_4^{3-}]}{[\text{HPO}_4^{2-}]} \quad (25)$$

The Measurement of pH

The fundamental instrument for the measurement of pH is the hydrogen electrode, though for routine use the glass electrode is more convenient.

The glass electrode. If a thin bulb of a special glass is placed in a solution it acquires a potential which depends on the pH in the same way as does that of a hydrogen electrode. In order to measure the potential of the glass membrane it is necessary to have a reference electrode (generally $\text{Ag} \cdot \text{AgCl} \cdot \text{HCl}$) inside the glass bulb as well as a reference electrode connected to the test solution by a salt bridge. Then the potential difference between the two reference electrodes is given by the equation:

$$E = E' + \frac{2.303RT}{F} \times \text{pH} \quad (26)$$

where R is the gas constant, F the Faraday, T the Kelvin temperature, and E' is a constant for the system.

In practice it is always first necessary to measure the potential of the glass electrode system in a standard buffer of known pH and then in the test solution. If E_s is the potential of the electrode system in a standard buffer pH_s, then the pH of the test solution, pH_x, is given by:

$$\text{pH}_x = \text{pH}_s + \frac{(E_x - E_s)F}{2.303RT} \quad (27)$$

The potential of the hydrogen-saturated calomel electrode system can be measured with an ordinary potentiometer. The glass electrode system, on the other hand, has so high a resistance that the potential has to be measured with a high input impedance voltmeter usually arranged as a pH meter, that is to say the potentiometer is divided to read directly in pH units. In spite of the scale calibrated in pH units it must be emphasized that a calibration measurement in a buffer of known pH must always be made before measuring the pH of the test solution.

Indicators. Indicators are weak organic acids which change colour on ionization. Thus the acid form of methyl red is red while the conjugate base is yellow. Similarly with phenolphthalein, the acid is colourless while the base is pink. The dissociation of the indicator, HI, may be represented thus



with a dissociation constant K_1 . Then equation (15) becomes:

$$\text{pH} = \text{p}K_1 + \log \frac{[\text{I}^-]}{[\text{HI}]} \quad (29)$$

Since the species HI and I^- are of different colours (red and yellow for methyl red, colourless and pink for phenolphthalein) the colour of the solution depends on the ratio $[\text{I}^-]/[\text{HI}]$, i.e. it depends on the second term on the right of equation (29) and therefore on the pH of the solution.

Because of the difficulty in discriminating between small changes in one colour in a large excess of another colour, the useful range of an indicator is only about 1 pH unit, i.e. over the range $\text{p}K_1 \pm 0.5$ pH (see Table 1.1). The measurement of pH then resolves itself into the choice of an indicator with a suitable $\text{p}K_1$, near the pH to be measured, and the determination of the concentration ratio of the two colours. This is most easily done by comparing the colour of the unknown solution containing a little indicator, with the colour of standard buffers of known pH containing the same concentration of indicator. Indicators are not so reliable as glass or hydrogen electrodes for the measurement of pH, as $\text{p}K_1$ is often affected by the presence of salts, proteins, etc. Indeed the 'protein error' of a paper strip soaked in

Table 1.1
Table of Indicators

Indicator	Useful range of pH and colour change
Thymol blue (acid range)	1.2 red-2.8 yellow
Tropaeolin—thymol blue Methyl orange	1.0 red-3.5 yellow 3.0 red-4.4 yellow
Bromophenol blue Methyl red	2.8 yellow-4.6 blue 4.2 red-6.3 yellow
Chlorophenol red Bromothymol blue	5.0 yellow-6.6 red 6.0 yellow-7.6 blue
Phenol red Phenolphthalein	6.8 yellow-8.4 red 8.3 colourless-10.0 violet-red
Thymol blue (alkali range) Thymol violet	8.0 yellow-9.6 blue 9.0 yellow-13.0 violet

a suitable indicator can be used as a test for the presence of protein, e.g., in urine.

Physiological Buffers

The buffers important in vivo are those which are effective around pH 7.4, the pH of blood. The pH of urine, however, can vary between 4 and 9. The chief systems are listed below.

Bicarbonate. The pK_1 of carbonic acid is 6.1. The ratio of base/acid at pH 7.4 is, therefore, 20/1, which means that the bicarbonate system is a good buffer when blood is being acidified, but very poor if it is being made alkaline. The concentration of HCO_3^- ions in plasma is about 0.03M. Bicarbonate is also useful in buffering urine.

Phosphate. The pK of the equilibrium $H_2PO_4^- \rightleftharpoons HPO_4^{2-}$ is 6.8, i.e. the ratio $[HPO_4^{2-}]/[H_2PO_4^-]$ in plasma is 4/1. This makes phosphate a more efficient buffer than bicarbonate at physiological pHs, but its concentration in plasma is only 0.002M. In cells, the various phosphate esters, which have very roughly the same pK as inorganic phosphate, come to about 0.08M, and are therefore important buffers. Inorganic phosphate is the chief buffer in urine.

Amino acids. Most of these compounds are dibasic, i.e. in going from pH 1-pH 10 they lose two protons. The pK s of the $COOH$ and NH_3^+ groups are, however, far removed from 7.4 and they are not important, except in buffering the HCl released in the gastric juice. The free amino acid concentration is also small.