Medical Acid–Base Balance
THE BASIC PRINCIPLES
I dedicate this book with gratitude and affection to my parents; also to the many individuals of the surgical, medical and nursing staff of The Royal Infirmary of Edinburgh to whose professional excellence, teamwork and tenacity I owe so much.

M. L. G. G.
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Preface

Acid–base balance is a large subject in its own right and its understanding requires an integrated and interdisciplinary approach to the several systems involved in acid–base homeostasis. Before a working knowledge of clinical and practical aspects can be acquired it is essential that the basic principles of biochemistry, physical chemistry and physiology, and the interactions between the various organs and systems, be mastered. This is the objective of Medical Acid–Base Balance.

In spite of the existing literature and although the subject is acknowledged to be of enormous and obvious relevance to patient care in many areas of contemporary medicine, it is one which the majority of medical students and many clinicians find both difficult and unattractive. Some existing texts treat the subject as a pure science and present it in almost mathematical form with little or no reference to human patients and their ills. At the other extreme, some clinically oriented books oversimplify, or even neglect totally, the fundamental physicochemical and physiological background. This book aims to come between these two extreme attitudes and to spell out in detail the basic principles of acid–base balance and, where appropriate, to illustrate their relevance with reference to clinical examples. It does not purport to teach clinical medicine or therapeutics, and it does not give advanced accounts of the physiology and pathology of pulmonary and renal function. Few current texts discuss adequately the principles underlying modern methods of the measurement of "blood gases" and the assessment of acid–base status, and so these too are covered here in some detail.

Both S.I. and metric units have been adopted throughout the text, since the transition to S.I. units has not been uniformly completed and since much of the existing literature uses metric units. Any change of units’ systems poses major communication problems and at a time of transition it is particularly important that users be familiar with both systems.

Areas of controversy such as the relative merits of actual bicarbonate, standard bicarbonate and base excess values, etc., have been aired with, it is hoped, as little prejudice as possible. Again, severe problems of communication arise if users are not familiar with the several different conventions favoured in various centres and used in the existing literature.

Medical Acid–Base Balance is intended primarily as an introductory text for preclinical and clinical students. However, it is also hoped that it will be useful to those clinicians who require to learn or re-learn the fundamental principles of acid–base balance. The content is based largely on a course which has been taught to preclinical students over a number of years, and I have been greatly helped by the
constructive comments made by many students who have also highlighted the areas which they have found to be of particular difficulty.

I am most grateful to many friends and colleagues who have given me valuable encouragement, advice and criticisms, both general and specific. In particular I wish to thank Dr I. B. R. Bowman, Professor G. S. Boyd, Professor R. B. Fisher, Sister Jeannie Fisher, Dr M. George, Dr R. Hume, Dr Anne T. Lambie, Dr G. J. R. McHardy, Dr Anne G. Morton and Dr I. A. Nimmo. However, responsibility for any errors and omissions remains my own.

In addition, I wish to thank the following for permission to reproduce their published material: Churchill Livingstone; Professor H. W. Davenport; Dr D. C. Flenley; Instrumentation Laboratory (U.K.) Ltd.; The Lancet; Dr R. W. Logan; The New England Journal of Medicine; Radiometer A/S; Professor W. B. Schwartz; University of Chicago Press; Yale University Press.

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Introduction

Acid–base balance is a fascinating and important aspect of modern medicine. Few subjects can claim to be more interdisciplinary or to combine more closely the pre-clinical sciences with clinical medicine. The basic sciences of physiology and biochemistry merge to explain the normal functioning of the organs and systems concerned in acid–base homeostasis: in some clinical situations this normal functioning either has failed or is at risk and therefore medicine, surgery and therapeutics are all concerned in its maintenance and restoration. Although the subject has a long history, there are still many problems on which our current knowledge is incomplete and on which the way is open for further research.

An understanding of both the theoretical and practical aspects of acid–base balance is vital to patient care in many branches of contemporary medicine. While the physico-chemical theory of buffers and equilibria may appear to some clinicians and medical students to be an uninteresting and even irrelevant realm of pure science, there is no doubt that a firm grasp of basic principles really does provide essential background for diagnosis, interpretation and treatment of many respiratory and metabolic disturbances encountered in both medical and surgical practice.

For example, the diagnosis and particularly the assessment of severity of many respiratory conditions, even in the out-patient clinic, relies on measurements of the ‘blood gases’, that is determination of $P_O_2$ and $P_CO_2$ values. The same is true for the management of patients on artificial ventilators (where the $P_CO_2$ values achieved in the arterial blood are regulated solely by the medical and nursing staff) and for the control of ventilation during anaesthesia especially during major procedures such as those involving cardiopulmonary surgery. Since the kidney is fundamentally involved in the regulation of the electrolyte concentrations in the body fluids and the reabsorption and excretion of acids and bases it is obvious that an understanding of these processes is required by all who undertake the treatment of renal disease. Many other metabolic disorders involve disturbances of acid–base balance: simple examples include the excess metabolic production of non-volatile acids (e.g. keto-acids in diabetic keto-acidosis) and the direct loss of the base bicarbonate in some forms of renal failure. Other important but less obvious examples include the lactic acidosis sometimes associated with shock or with side-effects of certain drugs or caused by cardiac arrest and the excess $H_+^+$ ion excretion caused by hyperaldosteronism.

Investigations of acid–base status also often play an important part in the diagnosis of paediatric conditions and in some cases here speed and accuracy of measurement and interpretation are particularly vital. The advent in recent years of intensive care (critical care) units has also demanded more attention to the monitoring and management of acid–base and electrolyte status. Particularly in these units and in operating
theatres correct and rapid assessment of acid–balance considerations saves patients’ lives. In some centres physiotherapists refer to blood gas measurements on ventilated patients, especially infants, as a guide to the degree of chest treatment which is necessary or which can be tolerated and also as an index of the efficacy of treatment.

Hence no clinician, especially in hospital practice, should be without an intimate understanding of the principles and practice of acid–base balance: indeed in many intensive care areas the nursing staff also are expected to be familiar with the basic principles and practice of the subject.

In some units the various physical and chemical measurements are made by technical and laboratory staff; however in others the clinician personally makes the measurement on the spot. In any case it is the clinician’s responsibility to provide a suitable and valid sample (generally blood, sometimes CSF), then to interpret the numerical results as correctly as possible, and to initiate the appropriate therapeutic action and any further monitoring that may be subsequently required. Thus both practical and theoretical knowledge are absolutely essential.

It will be clear both from this book and from personal clinical experience that many topics in acid–base and electrolyte balance are incompletely understood. There is therefore a continuing need for first-class clinical and experimental research, and for this an interdisciplinary attitude embodying the principles of physiology, biochemistry and medicine is demanded.
2

The Need for Acid–Base Regulation

pH dependence of metabolism

The normal functioning of metabolism requires that the compositions of the environments and contents of cells be kept constant, or at least within certain limits. Thus the pH of extra- and intracellular fluids must be kept fairly constant. It is not possible to point to any single reason for this; the activities of virtually all the thousands of enzymes within cells are to some extent pH-dependent. However, the pH-dependence of the activity of most enzymes is much less dramatic than the pH-dependence of the overall normal functioning of the body and other factors must be involved. Also the ionic states of all substrates which have acidic or basic chemical groups are determined by the pH. Membrane transport processes are markedly pH-dependent: therefore the composition of intracellular fluids and of the contents of subcellular organelles, and hence the metabolic activities of cells, can be affected to an important extent by pH change.

While any one metabolic process may be affected only to a small extent by a change in pH, the whole overall pattern of metabolism can be drastically changed by pH variation. For example, lactate production from glucose by isolated rat diaphragm muscle falls at pH 7.1 to 63% of the rate at pH 7.4. Also the contractile force of heart muscle varies considerably as the extracellular pH is changed. Furthermore, since the acid or base form of every buffer is ionized (by definition), and the relative amount of the ionized species is intimately related to pH, it follows that the amount of counter-ion required is pH-dependent. Hence there is a close association between electrolyte balance and acid–base balance.

The need for regulation of acid–base balance can best be judged by the practical consequences of a disturbance from normal blood pH (7.40). The consequences of a blood pH as low as 6.8 are so severe as to be almost certainly lethal. In practice, the blood pH in a healthy individual is normally maintained between 7.35 and 7.45 by the physiological mechanisms involving lungs and kidneys and by the physicochemical action of buffers which are discussed in the following chapters. While this range of 'normal' pH values may seem very narrow it is worth bearing in mind that the pH scale is a logarithmic scale and that these values correspond to a range of H⁺ ion concentration of 45–35 nmole/litre*; i.e. a 25% change in [H⁺] is quite acceptable, and in this sense the body regulates hydrogen-ion concentrations far less precisely than, for example, plasma sodium-ion concentration. A logarithmic scale which relates pH and [H⁺] values is given in Appendix II (p. 94).

* A nmole, or nanomole, abbreviated to nmol is 10^{-9} moles. One mole is one gram molecule. See also Appendix II.
**Extremes of pH**

It is not possible to state a definite extreme range of pH values encountered in disease, although pH values of as low as 6.8 ([H+] = 158 nmol/litre) and as high as 7.8 ([H+] = 16 nmol/litre) have been observed for brief periods in patients who have survived. However it is emphasized that these are particularly extreme abnormal values and that far smaller deviations from pH 7.40 should cause concern.

**Metabolic production of acids**

Under normal physiological circumstances the body produces a substantial quantity of acid since many end-products of metabolism are acids. Thus a normal sedentary individual produces carbon dioxide (which becomes hydrated to carbonic acid especially rapidly since the enzyme carbonic anhydrase is present in erythrocytes) equivalent to about 13 000–15 000 nmol (mEq) of hydrogen ions per day, i.e. the same amount of H+ ions as are present in 13–15 litres of 1 m HCl. Clearly, if this were allowed to accumulate then the effect on blood, tissue fluid and intracellular fluid pH would be dramatic and lethal. However since carbon dioxide is volatile it can be excreted via the lungs and it is normally possible to excrete it as fast as it is produced; hence no accumulation should occur. In addition a much smaller quantity of non-volatile acids, the so-called ‘fixed acids’, is produced metabolically.

On a normal diet the main non-volatile acid which has to be excreted is sulphuric acid formed during the oxidation of sulphur-containing amino acids (i.e. methionine, cystine and cysteine) from proteins. A smaller amount of phosphoric acids is also excreted; these arise mainly from oxidation of nucleic acids, phospholipids and phosphoproteins such as casein and egg albumin. A small contribution is made by non-metabolizable organic acids of dietary origin (or at least whose rate of metabolism is slower than their rate of production or intake).

The amount of non-volatile acid produced and excreted by a normal individual thus depends largely on the dietary intake, especially of protein; it is generally of the order of 50–100 mmol (mEq) H+ per day. Clearly this is a trivial amount compared with the amount of the volatile component (CO₂) which has to be excreted by the lungs each day. However the addition of non-volatile acids to the blood poses an additional problem which has to be considered since they cause depletion of the plasma base, bicarbonate, as follows:

Consider the equilibrium:

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

By the law of mass action the addition of H+ will displace the equilibrium towards the left. Therefore the HCO₃⁻ concentration decreases and more CO₂ is formed. This can also be seen in another way by taking a specific example such as the addition to blood of sulphuric acid which is a very strong acid (i.e. virtually fully ionized):

\[
\text{H}_2\text{SO}_4 \rightarrow 2\text{H}^+ + \text{SO}_4^{2-}
\]

Then we have:

\[
\text{H}_2\text{SO}_4 + 2\text{HCO}_3^- \rightarrow 2\text{CO}_2 \uparrow + \text{SO}_4^{2-} + 2\text{H}_2\text{O}
\]

In practice the CO₂ concentration normally hardly increases since the respiratory centre controls respiration and causes hyperventilation, thus venting off CO₂ (see below) to keep the Pco₂ in arterial blood fairly constant, close to 40 mmHg (5.3 kPa). However the loss of HCO₃⁻ (converted into CO₂ which is then exhaled) is a potentially
The buffering capacity of each buffer obviously depends on, among other factors, the concentration or total amount of that buffer present. Hence loss of buffer is undesirable.

**Conservation of bicarbonate**

Therefore the kidney in a healthy individual on a normal diet has two important tasks in the conservation of bicarbonate: (1) all the filtered HCO$_3^-$ must be reabsorbed (normal urine does not contain any HCO$_3^-$: this would be a waste of an important buffer) and (2) any HCO$_3^-$ lost through addition of non-volatile acids to the blood as explained above must be resynthesized or regenerated within the renal tubule cells. The processes of H$^+$ excretion into the urine and of reabsorption of filtered HCO$_3^-$ and also of regeneration of lost plasma HCO$_3^-$ by the kidneys are all closely related and are discussed in detail in Chapter 6.

**Accumulation of excess acid**

The problem of pH regulation is clearly even more crucial in severe exercise and in pathological conditions where, for example, more acid end-products of metabolism may be produced faster than they can be oxidized to the volatile acid CO$_2$. Normally lactic acid is oxidized to CO$_2$ and H$_2$O at approximately the same rate as that at which it is formed. If, however, its oxidation is impaired and/or its production is increased then lactic acid may accumulate in blood, especially during tissue hypoxia, e.g. owing to circulatory or respiratory failure. Lactic acid is quite a strong acid with a pK value of about 3-7 and so it will deplete the plasma bicarbonate. In starvation or in diabetic keto-acidosis the relatively strong acids acetoacetic acid (pK 3-6) and $\beta$-hydroxybutyric acid (pK 4-4) are formed faster than they can be oxidized and so deplete the HCO$_3^-$, the so-called 'alkali-reserve'. Furthermore when these acids are excreted they, being strong acids and therefore mainly ionized, must be accompanied by cations. Excess loss of Na$^+$ and K$^+$ ions into the urine would obviously be undesirable, and therefore a mechanism is required to minimize or 'spare' the loss of these cations during excretion of strong acids.

**Accumulation of bases**

While the normal end-products of metabolism tend to be acids, bases can accumulate in the body under certain circumstances, such as the ingestion of large quantities of anti-acid preparations or of fruits containing citrate as salts. Citrate is metabolized to form a volatile acid (CO$_2$) which is easily excreted plus a non-volatile or 'fixed' base (HCO$_3^-$) which has to be disposed of together with a counter-cation via the kidneys. Normally the kidneys can cope, but if renal function is impaired this can create a disturbance of acid-base balance.

The excessive excretion of H$^+$ ions such as in hyperaldosteronism or hypokalaemia (see Chapter 6) is equivalent, in its effect on acid-base balance, to retention of excess base. This is also discussed further in Chapter 8.

**Defences against variation in acid-base status**

Thus in the face of a continual production of acids the body has to minimize the pH changes in the body fluids. Furthermore, even in a healthy individual, the amount of acid produced varies from time to time and so the mechanisms have to be capable of
recognizing' the requirements for correct regulation and of adapting the regulatory systems accordingly.

In general, the defence mechanisms can be divided into two distinct categories. (1) The chemical buffers which by their physicochemical nature minimize the effect which addition of acids or bases would have on the H+ ion concentration of the body fluids. These are dealt with in detail in Chapter 3. (2) The physiological mechanisms which, largely through the functioning of the respiratory system and the kidneys, regulate the composition of the body fluids. Thus the lungs can excrete CO2, an acid, and the kidneys can excrete H+ ions and can reabsorb and regenerate bicarbonate, the base, as required. These mechanisms are discussed in detail in Chapters 5, 6 and 7.

**Requirement for regulation of carbon dioxide concentration**

The concentration of carbon dioxide, expressed as the partial pressure (Pco₂), in the blood and other body fluids must be regulated also in its own right and not only as a means of regulating the pH. Quite apart from effects which could be due to change in pH, changes in Pco₂ can have serious effects on the body. In particular the cardiovascular and central nervous systems are at risk. For example, accumulation of carbon dioxide (respiratory acidosis, hypercapnia) causes important changes in blood flow such as an increase in cerebral circulation and peripheral vasodilation, but there is vasoconstriction in the pulmonary vascular system. In addition there is depression of the central nervous system. The respiratory system therefore is well adapted to minimize severe changes in Pco₂ under normal circumstances and the mechanisms are discussed in Chapter 5.
Physiological Buffers

Definitions of buffers, acids and bases

A buffer solution is one which resists or minimizes change of pH when acid or base is added. Thus the buffers in the body fluids act as a physicochemical defence against pH change; other mechanisms such as the physiological control mechanisms of the kidneys and lungs also operate to minimize changes in pH and also to restore the composition of the body fluids to normal following a disturbance. These physiological mechanisms will be considered in subsequent chapters.

A buffer always consists of a mixture of a weak acid (or base) and its conjugate base (or acid) respectively. Such a mixture is said to constitute a conjugate pair. These terms can be defined according to Brönsted and Lowry as follows.

An acid is a proton donor, that is anything which dissociates into one or more protons, i.e. a hydrogen ion, plus a conjugate base.

e.g. \[ \text{HA} \rightleftharpoons \text{H}^+ + \text{A}^- \]

acid proton conjugate base

example: carbonic acid \( \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \)

Conversely a base is a proton acceptor, i.e. anything which can combine with a proton to form a conjugate acid.

e.g. \[ \text{B} + \text{H}^+ \rightleftharpoons \text{BH}^+ \]

base conjugate acid

or \[ \text{BH}^+ + \text{H}^+ \rightleftharpoons \text{BH}_2^+ \]

base conjugate acid

example: ammonia \( \text{NH}_3 + \text{H}^+ \rightleftharpoons \text{NH}_4^+ \)

bicarbonate \( \text{HCO}_3^- + \text{H}^+ \rightleftharpoons \text{H}_2\text{CO}_3 \)

Other definitions of acids and bases have been proposed but those of Brönsted and Lowry are adequate for all medical purposes.

Note that some biological substances (e.g. phosphates and amino acids, see below) can behave as both bases and acids, i.e. they can both accept and donate protons respectively. Such substances are described as being amphiprotic or amphoteric.

e.g. \[ \text{H}_3\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-} \]

acid

\[ \text{H}_3\text{PO}_4^- + \text{H}^+ \rightleftharpoons \text{H}_2\text{PO}_4^- \]

base
In aqueous solution hydrogen ions, i.e., protons, can themselves be hydrated to form hydroxonium (or hydronium) ions, $\text{H}_3\text{O}^+$:

$$\text{H}^+ + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+$$

Therefore the ionization of an acid in aqueous solution is more correctly written as:

$$\text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{A}^- + \text{H}_3\text{O}^+$$

Nevertheless it is convenient to consider the more simple dissociation equilibria and to ignore the hydroxonium ion for practical purposes.

It can thus be seen that water behaves as an amphoteric molecule since it can both donate and accept protons:

$$\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$$

and

$$\text{H}_2\text{O} + \text{H}^+ \rightleftharpoons \text{H}_3\text{O}^+$$

### Equilibrium and dissociation constants and pK values

The equilibrium position of any reaction is described quantitatively by an equilibrium constant, $K$. This is defined as follows:

For the equilibrium $\text{A} + \text{B} \rightleftharpoons \text{C} + \text{D}$

$$K = \frac{[\text{C}][\text{D}]}{[\text{A}][\text{B}]}$$

and for the equilibrium $\text{A} + \text{B} \rightleftharpoons \text{C}$

$$K = \frac{[\text{C}]}{[\text{A}][\text{B}]}$$

Therefore for the dissociation of dihydrogen phosphate:

$$\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-}$$

the equilibrium constant is

$$K = \frac{[\text{H}^+][\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

which has a value of $1.6 \times 10^{-7}$ mole $\cdot$ litre$^{-1}$.

Since typical values of $K$ are very small it is often convenient to use pK values. The pK for any equilibrium is defined as

$$\text{pK} = \frac{1}{\log K} = -\log K$$

in an analogous way to Sørensen's definition of

$$\text{pH} = \frac{1}{\log [\text{H}^+]} = -\log [\text{H}^+]$$
where \( K \) is the equilibrium constant for the equilibrium in question. Thus the pK value for the dissociation of dihydrogen phosphate mentioned above is 6.8.

The lower the pK value, the stronger is an acid. This reflects that the stronger an acid is, the further to the right lies the equilibrium

\[
\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-
\]
i.e. the greater is the degree of dissociation. Hence, the equilibrium constant (dissociation constant), \( K \), will be larger and pK smaller. Thus acetoacetic acid (pK 3.6), which accumulates in diabetic keto-acidosis, is a stronger acid than acetic acid (pK 4.7).

The equilibrium constant (\( K \)), and hence the pK value, is constant under stated conditions of temperature, etc. Note that for substances which can dissociate or associate in several ways (i.e. the so-called polybasic, polyprotic, amphiprotic or amphoteric acids and bases) there are several pK values, one for each possible association or dissociation equilibrium.

Thus the amino acid histidine has three separate pK values, 1.8, 6.0 and 9.2. These values are associated with the \( \alpha \)-carboxyl, imidazole and \( \alpha \)-amino groups respectively.

1. Dissociation of the \( \alpha \)-carboxyl group (pK\(_1\) = 1.8):

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} \\
\text{NH}_2 & \quad \text{COOH} \\
\end{align*}
\quad \rightleftharpoons \quad
\begin{align*}
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} \\
\text{NH}_2 & \quad \text{COO}^- \\
+ & \quad \text{H}^+ \\
\end{align*}
\]

2. Association of the imidazolium side-chain group (pK\(_2\) = 6.0):

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} \\
\text{NH}_2 & \quad \text{COO}^- \\
+ & \quad \text{H}^+ \\
\end{align*}
\quad \rightleftharpoons \quad
\begin{align*}
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} \\
\text{NH}_2 & \quad \text{COO}^- \\
+ & \quad \text{H}^+ \\
+ & \quad \text{HN}<^\text{N} \\
\end{align*}
\]

3. Association of the \( \alpha \)-amino group (pK\(_3\) = 9.2):

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} \\
\text{NH}_2 & \quad \text{COO}^- \\
+ & \quad \text{H}^+ \\
\end{align*}
\quad \rightleftharpoons \quad
\begin{align*}
\text{N} & \quad \text{N} \\
\text{CH}_2 & \quad \text{CH} \\
\text{NH}_2 & \quad \text{COO}^- \\
+ & \quad \text{H}^+ \\
+ & \quad \text{HN}<^\text{N} + \text{H}^+ \\
\end{align*}
\]
The Henderson–Hasselbalch equation

From the definitions

\[ \text{Acid} \Leftrightarrow H^+ + \text{base}^- \]
\[ \text{pH} = -\log [H^+] \]
\[ \text{pK} = -\log K \]
\[ K = \frac{[H^+] \cdot [\text{base}^-]}{[\text{acid}]} \]

one can simply derive the Henderson equation:

\[ [H^+] = \frac{K \cdot [\text{acid}]}{[\text{base}^-]} \]

or the more useful logarithmic version, the Henderson–Hasselbalch equation (see Appendix I for detailed derivation), which is:

\[ \text{pH} = \text{pK} + \log \left( \frac{[\text{base}^-]}{[\text{acid}]} \right) \]

This equation is fundamental to the consideration of all acid–base equilibria and should preferably be memorized.

Obviously, according to the law of mass action, addition of \( H^+ \) ions to a mixture containing acid and base

\[ \text{acid} \Leftrightarrow H^+ + \text{base}^- \]

will displace the equilibrium towards the left as written above; thus the concentration of the base component will fall, while the concentration of the acid component must rise by an exactly corresponding amount. The Henderson–Hasselbalch equation enables one to relate quantitatively the change in \([\text{base}], [\text{acid}]\) and pH. One therefore can see that the pH of a solution depends on the logarithm of the ratio of base to acid concentration* and not on the ratio itself or on one or other concentration by itself.

Consider the equilibrium:

\[ \text{acid} \Leftrightarrow H^+ + \text{base}^- \]

Addition of \( x \) mmole of \( H^+ \) ions per litre will reduce \([\text{base}]\) by an amount \( x \), and will increase \([\text{acid}]\) by an equal amount \( x \), so that the pH becomes

\[ \text{pH} = \text{pK} + \log \left( \frac{[\text{base}^-] - x}{[\text{acid}] + x} \right) \]

It can be shown that the smallest change in the ratio will be produced (for a given value of \( x \)) if initially \([\text{acid}] = [\text{base}]\). This means that a buffer is best, i.e. it minimizes

* In turn, the hydrogen ion concentration itself depends on the ratio of base to acid concentration (not logarithm) according to the Henderson equation:

\[ [H^+] = K \cdot \frac{[\text{base}^-]}{[\text{acid}]} \]
pH change most effectively, when it consists of an equimolar mixture of its acid and base forms. Therefore a buffer mixture is best when:

$$\text{pH} = \text{pK} + \log \frac{[\text{base}^-]}{[\text{acid}]}$$

$$= \text{pK} + \log (1)$$

$$= \text{pK}$$

Hence a buffer functions best when the pH is close to the buffer's pK value; roughly speaking it is only useful as a buffer if the pH is within 1 pH unit of the pK value. Hence acids with pK values in the vicinity of 2 to 3, such as $\alpha$-COOH groups in amino acids and proteins, are of no value as buffers at around physiological pH (7-4).

**Titration curves for buffers**

Titration curves can be drawn for buffers, either from theoretical calculations based on the Henderson-Hasselbalch equation or from practical experiments in which the pH is determined following addition of successive known aliquots of acid or alkali. A typical curve shows an inflexion (Fig. 3.1). The region where the slope (pH change per equivalent or mole of acid added) is least is therefore the region of best buffering and is centred on the pK value. So A in Fig. 3.1 corresponds to the pK of the buffer, and B (Fig. 3.1) to 0.5 equivalents of alkali added per equivalent of buffer present, i.e. half the buffer has been neutralized. Histidine therefore gives a titration curve (Fig. 3.2) with three inflexions on it, each corresponding to one pK value (see above). At very low pH (i.e. high $H^+$ ion concentration) all the dissociating and associating groups will be fully protonated:

![Diagram](attachment:image.png)

Complete protonation at high $[H^+]$ or low pH should be self-evident from consideration of the law of mass action. As the pH is raised the molecule will become progressively less protonated. At pH 1.8 (the pK of the $\alpha$-carboxyl group) half the molecule will be in each of the following forms:

![Diagram](attachment:image.png)

50% at pH = 1.8

At above pH 3.5 the $\alpha$-carboxyl group will be virtually wholly dissociated.
As the pH is further increased the imidazole side-chain group (pK 6·0) loses its proton, so that at pH = pK₂ = 6·0 half the molecules are protonated on this group:

\[
\begin{align*}
\text{pK}_2 &= 9·2 \\
pK_2 &= 6·0 \\
pK &= 1·8 \\
\end{align*}
\]
Further increase in pH results in deprotonation of the α-amino group so that at pH = pK₃ = 9.2 the histidine exists as an equilibrium mixture of the two forms:

\[
\begin{align*}
\text{Histidine} & \rightleftharpoons \text{Histidine}^+ \\
& \text{at pH = 9.2}
\end{align*}
\]

Finally, at very high pH values this equilibrium will lie to the right, with virtually all the histidine molecules (or ions) in the fully deprotonated form.

The ionic form(s) of a buffer predominating at any pH can be easily worked out from fundamental principles in the manner shown above if the pK values are known. When pH = pK the protonated and deprotonated forms exist in 1:1 ratio: the effect on the equilibrium of raising or lowering the pH can then be seen by application of the law of mass action; i.e. at pH ≤ pK (i.e. at relatively high H⁺ ion concentration) the equilibrium A ⇌ B + H⁺ will lie to the left and vice versa.

**Buffers of the body fluids**

We will now consider the various buffer systems in blood and other extracellular fluids. The two principal buffers in blood are the bicarbonate/CO₂ system and the haemoglobin system. Plasma proteins and phosphate make minor, but sometimes significant, contributions. Except for haemoglobin, the same substances are important buffers in the other extracellular tissue fluids, including cerebrospinal fluid (CSF). Proteins and phosphates (organic and inorganic) are probably the principal intracellular buffers, but since one cannot measure readily the composition of intracellular fluids.

**Table 3.1**

*Principal buffer systems in body fluids*

<table>
<thead>
<tr>
<th>Blood</th>
<th>CO₂/HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HHb/Hb⁻ and HHbO₂⁻/HbO₂⁻ i.e. haemoglobin</td>
</tr>
<tr>
<td></td>
<td>H⁺ Protein/Protein⁻</td>
</tr>
<tr>
<td></td>
<td>(H₂PO₄⁻/HPO₄²⁻)</td>
</tr>
<tr>
<td><strong>CSF, ECF</strong></td>
<td>CO₂/HCO₃⁻</td>
</tr>
<tr>
<td></td>
<td>(H⁺ Protein/Protein⁻)</td>
</tr>
<tr>
<td></td>
<td>(H₂PO₄⁻/HPO₄²⁻)</td>
</tr>
<tr>
<td><strong>Intracellular fluids</strong></td>
<td>H⁺ Protein/Protein⁻</td>
</tr>
<tr>
<td></td>
<td>H₂PO₂⁻/HPO₂⁻</td>
</tr>
<tr>
<td></td>
<td>Porganic/P⁻organic</td>
</tr>
<tr>
<td></td>
<td>CO₂/HCO₃⁻</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td>H₂PO₄⁻/HPO₄²⁻</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺/(NH₃)</td>
</tr>
</tbody>
</table>
fluids in practice they will not be considered further in this book. These buffers are summarized in Table 3.1.

It is important to bear in mind that measurements on specific extracellular fluids such as blood and CSF do not necessarily give a true picture of the acid–base status of the intracellular fluids or of the body fluids as a whole. This is discussed further below in the section on whole-body titration curves.

**Bicarbonate/carbon dioxide system**

This system is of major importance, especially because the acid component (or, strictly, the acid anhydride) carbon dioxide is volatile and its concentration can be regulated physiologically by the respiratory system, that is by pulmonary ventilation together with control from the chemoreceptors and respiratory centre (see below). Additionally the conjugate base, bicarbonate, can be independently regulated to a certain extent by the kidneys although this is not very rapid. There is a fairly large quantity of this buffer present owing to the large carbon dioxide production during even basal oxidative metabolism since carbon dioxide is the principal end-product of normal carbohydrate and fat oxidation. A sedentary adult produces about 13 000 mmol CO₂ per day and an active person may produce over twice this amount.

Consider the equilibrium:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

In the presence of the enzyme carbonic anhydrase which is found in red blood cells, the formation of carbonic acid by the hydration of carbon dioxide is rapid:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \]

Since carbonic acid cannot easily be measured, and since it is carbon dioxide (the acid anhydride) rather than the H₂CO₃ (the acid itself) which is regulated by ventilation, it is convenient to ignore the ‘true’ acid–base equilibrium

\[ \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

which gives

\[ pK = -\log \frac{[\text{H}^+] \cdot [\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \]

and to consider the overall equilibrium:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

and to define an ‘overall dissociation constant’, K’, so that

\[ pK' = -\log \frac{[\text{H}^+] \cdot [\text{HCO}_3^-]}{[\text{CO}_2] + [\text{H}_2\text{CO}_3]} \]

This pK’ has a value of 6·1 at 37°C and is frequently used in acid–base and blood gas calculations.

Carbon dioxide obeys Henry’s law of gas solubility and so its concentration in solution is directly proportional to its partial pressure (Pco₂) in the gas phase in equilibrium with the solution. Thus

\[ S \cdot P_{\text{CO}_2} = [\text{CO}_2] \text{ dissolved} + [\text{H}_2\text{CO}_3] \]

where S is the solubility coefficient, which has a value of approximately* 0·03 mmole . litre⁻¹ . mmHg⁻¹ at 37°C, or in S.I. units 0·23 mmole . litre⁻¹ . kPa⁻¹. Hence the

* Values for pK' and for S are generally assumed to be constant, at a given temperature, and independent of the pH and chemical composition of the plasma. Although this is not necessarily strictly true, the approximation is normally acceptable in clinical usage. It must also be recognized that correction factors are necessary if the temperature differs appreciably from 37°C.
Henderson–Hasselbalch equation for carbonic acid is often written (with $PCO_2$ in mmHg)

$$\text{pH} = 6.1 + \log \frac{[HCO_3^-]}{0.03 \times PCO_2} \quad \text{at } 37^\circ C$$

or (with $PCO_2$ in kPa)

$$\text{pH} = 6.1 + \log \frac{[HCO_3^-]}{0.23 \times PCO_2}$$

At pH 7.4 the normal value of the ratio

$$\frac{[HCO_3^-]}{[CO_2]+[H_2CO_3]}$$

is 20/1.

The $HCO_3^-/CO_2$ system is a better buffer against acidification (which is the normal condition physiologically since the end-products of normal metabolism tend to be acids) than against alkalinization, since addition of acid takes the pH nearer to the $pK$ value and the ratio $[\text{base}]/[\text{acid}]$ becomes nearer unity.

It was pointed out above that a buffer is only effective approximately over a pH range from $(pK - 1)$ to $(pK + 1)$. The one exception to this general rule of thumb can be considered to be the bicarbonate/CO$_2$ system. At a physiological pH of 7.4 one might conclude that a buffer with a $pK$ of 6.1 would be extremely ineffective since a small addition of, say, acid would cause a relatively large variation in the ratio $[\text{base}]/[\text{acid}]$ and hence a large change in the pH. However, in this exceptional case of the bicarbonate/CO$_2$ system, the acid component, the carbon dioxide, is volatile and so its concentration can be kept constant through regulation of ventilation: hence a much smaller change occurs in the $[\text{base}]/[\text{acid}]$ ratio than would otherwise be expected. The ratio only changes to $[\text{base} - x]/[\text{acid}]$ instead of to $[\text{base} - x]/[\text{acid} + x]$ on addition of $x$ mmol/litre of acid.

**DEMONSTRATION OF SUPERIOR BUFFERING CAPACITY OF BICARBONATE/CO$_2$ SYSTEM**

A simple experiment can be set up in the laboratory which shows quite convincingly the superiority which the volatile CO$_2$ component confers on this buffer system over any other. Set up two beakers, one containing sodium bicarbonate and the other containing an equivalent amount of some other buffer such as phosphate. Maintain a constant $PCO_2$ (CO$_2$ tension, i.e. CO$_2$ concentration) in each by bubbling in 5% CO$_2$ (e.g. 5% CO$_2$: 95% O$_2$) from a gas cylinder: this mimics the physiological action of the lungs in keeping the CO$_2$ constant. While monitoring the pH of each buffer, add an aliquot of a strong acid such as lactic acid to mimic the addition from muscle or red blood cells of lactic acid to blood. Observe the pH falling in each case as

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$

$$\text{acid} \quad \leftarrow \quad \text{base}$$

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

$$\text{acid} \quad \leftarrow \quad \text{base}$$

each equilibrium moves to the left and the ratio of $[\text{base}]/[\text{acid}]$ falls. However the elevated CO$_2$ concentration in the bicarbonate buffer will not remain high: the continued bubbling of the gas mixture from the cylinder ensures that the CO$_2$ concentration returns gradually to ‘normal’ (5%) and the pH rises. Of course the $[HCO_3^-]$ will still be low: some of the bicarbonate was converted into CO$_2$ and has been ‘blown off’ on the addition of the acid. No such mechanism operates in the phosphate buffer.
to return the pH towards normal, hence demonstrating the superiority of the bicarbonate/CO₂ system when it is used in conjunction with a means (e.g. ventilation) of keeping the concentration of volatile CO₂ fairly constant. The physiological control of ventilation by the respiratory system is discussed in Chapter 5.

REGULATION OF BICARBONATE CONCENTRATION

In addition to the lungs regulating the PₐCO₂ in blood, the kidneys can regulate the bicarbonate concentration to some extent, although this regulatory mechanism acts more slowly than does the respiratory one (see Chapters 6 and 9). The kidneys can regulate the plasma bicarbonate concentration by controlling the rate of tubular reabsorption of bicarbonate and the rate at which bicarbonate is regenerated in the renal tubule cells via the rate of H⁺ secretion into urine. These mechanisms are discussed in detail in Chapter 6.

The HCO₃⁻/CO₂ system can be regarded as the main buffer system in extracellular fluids.

Haemoglobin

PROTEINS AND AMINO ACIDS AS BUFFERS

All proteins are, to some extent, buffers by virtue of the acid–base dissociations or associations of their component amino acids. However the equilibria of the α-amino and α-carboxyl groups, viz.

\[
\begin{align*}
\text{R} & \quad \text{R} \\
\text{CH-NH₂} + H^+ & \rightleftharpoons \text{CH-NH₃⁺} \\
\text{COOH} & \quad \text{COOH} \\
\text{base} & \quad \text{acid}
\end{align*}
\]

and

\[
\begin{align*}
\text{R} & \quad \text{R} \\
\text{CH-NH₂} & \rightleftharpoons \text{CH-NH₂} \\
\text{COOH} & \quad \text{COO⁻ + H⁺} \\
\text{acid} & \quad \text{base}
\end{align*}
\]

are not important since these two groups are involved in the formation of the peptide linkage between amino acids and so any protein has these particular associating or dissociating groups only at the N- and C- termini respectively. Furthermore their pK values are of the order of 9 (α-amino group) and 2 (α-carboxyl group) respectively, and so they make a negligible contribution to buffering around the physiological pH of 7.4 (recall that buffer capacity is greatest when pH = pK and is only useful in the approximate range from pH = pK - 1 to pH = pK + 1). For this reason too the acid–base dissociations or associations of most side chain groups are unimportant as buffers. Side chain carboxyl groups as in aspartic and glutamic acids have pK values of around 3–4 (the exact pK value of a group in a protein is slightly different from that in the free amino acid, and depends to some extent on its neighbours in the primary and tertiary structure) which is too far from 7.4 to be useful. Side-chain amino groups such as lysine have pK values of around 9. Some can thus be significant buffers at pH 7.4,
although their importance must not be overestimated. However the imidazole side-chain of histidine

![Chemical Structure](image_url)

has a pK of around 6.0–7.0 (again the exact value in a protein depends on its immediate environment) and this is well suited to buffering around pH 7.4. It is chiefly to this side-chain group that most proteins owe their physiological buffering properties. Haemoglobin is no exception: it has an unusually high histidine content (36 residues/molecule).

In addition haemoglobin is present in blood at a remarkably high concentration of around 15 g/100 ml and since the buffering capacity of any buffer depends on its concentration this is a very powerful buffer system.

**The Isohydric Shift**

A third, and rather special, feature of haemoglobin which accounts for its importance as a physiological buffer arises from the important fact that oxyhaemoglobin is a stronger acid (i.e. has a lower pK) than is deoxyhaemoglobin.

Thus the dissociation of the oxy-form (relatively stronger acid, i.e. lower pK)

$$H HbO_2 \rightleftharpoons H^+ + Hb^-O_2$$

lies relatively further to the right than does the dissociation of the deoxy-form (relatively weaker acid, i.e. higher pK)

$$H Hb \rightleftharpoons H^+ + Hb^-$$

Therefore on removing oxygen from oxyhaemoglobin one will have fewer anions of $Hb^-$ (and fewer $H^+$ ions) than were present as $Hb^-O_2$ anions.

The changes on deoxygenation can be summed up either by the dotted line in the equations

$$H HbO_2 \rightleftharpoons \overset{\text{---}}{H^+ + Hb^-O_2}$$

$$H Hb \rightleftharpoons H^+ + Hb^-$$

or by the equation

$$Hb^-O_2 + H^+ \rightleftharpoons H Hb + O_2$$

Thus there are fewer hydrogen ions (and base anions too) present after deoxygenation than before. Therefore the pH must rise. Obviously if a weak acid (oxyhaemoglobin) is removed and replaced by the same amount of an *even weaker* acid (deoxyhaemoglobin) the pH must rise. However at the same time as oxyhaemoglobin is being

---

*It is convenient to refer to the pK of haemoglobin in this context, although it must be appreciated that this is not strictly valid: a pK value refers to a single dissociation or association and there are of course many such associating and dissociating groups in a protein. However, an overall 'average' value of pK is useful as an operational definition of the region of maximum buffering capacity.*
deoxgenated in the body carbon dioxide is being added by the tissues to the blood stream. As this CO₂ is being hydrated carbonic acid is formed, and clearly the H⁺ ion concentration must rise, i.e. the pH must fall. These two effects tend to have opposite effects on blood pH: removal of O₂ tends to raise the pH while addition of CO₂ tends to lower the pH. Clearly there exists a situation, at least in theory, where the two effects exactly cancel each other out. Knowledge of the pK values for oxyhaemoglobin (about 6.6), the stronger acid, and for deoxyhaemoglobin (8.2), the weaker acid, enables one to calculate that the two phenomena would have exactly equal and opposite effects on pH when the respiratory coefficient (RQ) is about 0.7, i.e. when 0.7 moles of CO₂ are produced per mole of O₂ consumed. Under these circumstances there would be no pH change in blood, that is no arteriovenous pH difference. Obviously realistic RQ values are greater than this with values at around 0.8 at rest, 1.0 on exercise or greater than 1 in hyperventilation or a lactic acidosis. Therefore more CO₂ is produced per mole of O₂ consumed. Hence the pH-lowering effect of the CO₂ (or, strictly, of the carbonic acid) predominates. This is one reason why the pH of venous blood is less than that of arterial blood. If it were not, however, for the difference in acidity between the oxy- and deoxy-forms of haemoglobin the pH would have fallen very much more dramatically. Since the 'break-even' point is an RQ of 0.7 (see above) it follows that, at an RQ of 1.0, 70% of the CO₂ added to blood has no pH-lowering effect. In the light of the very large amount of CO₂ produced (at least 13 000 mEq or mmol per day) it can be appreciated that this phenomenon (although it is not buffering in the strict physicochemical sense of the word) is of major quantitative physiological importance. This phenomenon is known as the 'isohydric shift'. Some texts refer to it as the Haldane effect: however, although Haldane was probably the first to observe the pH shift on oxygenation of haemoglobin, this term is usually reserved for the greater capacity of deoxyhaemoglobin than of oxyhaemoglobin to bind carbon dioxide (see below).

Since deoxgenation is accompanied by a decrease in the total number of osmotically active particles within the erythrocytes (i.e. two ions are replaced by one undissociated molecule) the osmotic pressure within the cell changes. This results in flux of water across the red cell membrane.

The pK of oxyhaemoglobin (the stronger acid) is about 6.6 while that of deoxyhaemoglobin is about 8.2. Thus at pH 7.4, the pH of normal blood, both are equidistant from their pK values and so both are theoretically equally 'good' buffers. However oxyhaemoglobin is more able to resist a lowering in pH while deoxyhaemoglobin is a better buffer when the pH tends to rise. These are relatively unimportant theoretical considerations since the relative proportions of the two are continually changing as blood circulates.

CARBAMINO COMPOUNDS

A small portion (about 10%) of the CO₂ carried by the blood stream is carried in combination with proteins including haemoglobin which is of course the most abundant protein in blood. Carbon dioxide can form a covalent bond with α-amino groups to form carbamino compounds:

\[
\text{Protein } \ldots \text{NH}_2 + \text{CO}_2 \Leftrightarrow \text{Protein } \ldots \text{NH}–\text{COO}^- + \text{H}^+ 
\]

The reaction is very rapid and no enzyme is required. Carbamino compounds have pK values of around 5. Therefore they are virtually wholly dissociated into carbamate ions and H⁺ ions in the physiological pH range. Although this reaction lowers the CO₂ concentration (and hence the H₂CO₃ concentration) in blood it does not cause any net change in pH since for every mole (or equivalent) of CO₂ bound in this way
Physiological Buffers

1 mole of H\(^+\) is liberated which in turn is buffered, probably by the imidazole groups of haemoglobin. So while carboxyramino compounds do have a role in the carriage of CO\(_2\) they have no buffering significance. While only about 10\% of the total CO\(_2\) in blood is carried in this form, measurement of arteriovenous differences of carboxyramino compounds, CO\(_2\), HCO\(_3^-\), etc., shows that carboxyramino compounds are of major importance in the transport of CO\(_2\) for excretion (see Table 5.1).

Carbon dioxide, however, does not react with protonated amino groups —NH\(_3^+\). The affinity of deoxyhaemoglobin for CO\(_2\) is about three to four times greater than that of oxyhaemoglobin. This is known as the *Haldane effect* and is of considerable physiological significance for the release of CO\(_2\) for excretion in the lungs as blood is being oxygenated. Indeed, it and the *isohydric shift* are probably of more physiological importance than the *Bohr effect*, which is the decrease in oxygen-binding capacity of haemoglobin as H\(^+\) ions are added to blood, i.e. the pH-induced shift of the oxygen dissociation curve. Note that these three phenomena are good examples of the structure of a protein being adapted to its function.

**Plasma proteins**

All proteins contain dissociating and associating side-chain groups in their constituent amino acids and hence they are capable of acting as buffers. As discussed above, the imidazole groups of histidine (and a few side-chain amino groups in certain environments) are the only groups with pK values near enough to pH 7-4 to be of physiological significance as buffers. While proteins are likely to be of major importance for intracellular buffering they have limited capability of buffering in blood because most of the plasma proteins are present only in quite small concentrations. Albumin (3-4.5 g/100 ml) is the most abundant plasma protein and does contribute significantly to blood buffering.

**Phosphate**

Phosphoric acid is a tribasic acid which can dissociate thus:

\[
\begin{align*}
    H_3PO_4 & \rightleftharpoons H^+ + H_2PO_4^- & pK_1 & = 2.0 \\
    H_2PO_4^- & \rightleftharpoons H^+ + HPO_4^{2-} & pK_2 & = 6.8 \\
    HPO_4^{2-} & \rightleftharpoons H^+ + PO_4^{3-} & pK_3 & = 11.7
\end{align*}
\]

At physiological pH values virtually all the phosphate is in the H\(_2\)PO\(_4^-\) and the HPO\(_4^{2-}\) forms, and therefore only the second equation above need be considered.

Since pK = 6.8 is so close to pH 7-4 one can see that this is potentially a good buffer at blood pH on physicochemical grounds. However the phosphate concentration in plasma is so low (0.8–1.4 mmol/litre in fasting) that this system is normally of trivial importance.

However, as discussed below in Chapters 6 and 7, phosphate is the principal buffer in normal urine. The concentration in urine obviously can vary over a wide range, depending mainly on phosphate and fluid intake, but a figure of 50 mmol/litre may be typical.

**'Bone buffering'**

In prolonged acidosis phosphate buffering can become important, although it is often loosely stated that under these conditions bone acts as a buffer. Calcium phosphate, which is present in hydroxyapatite form in the inorganic part of bone, is relatively insoluble; however the solubility is greater at lower pH. Hence if the pH falls below normal some calcium phosphate in bone goes into solution and the plasma levels of...
both calcium and phosphate rise. The increased phosphate is thus able to buffer $H^+$ ions.

\[
(CaPO_4^2) \rightleftharpoons Ca^{2+} + PO_4^{3-}
\]

insoluble

\[
PO_4^{3-} + H^+ \rightleftharpoons HPO_4^{2-} \quad \text{(equilibrium to right)}
\]

\[
HPO_4^{2-} + H^+ \rightleftharpoons H_2PO_4^- \quad \text{at physiological pH}
\]

Thus calcium phosphate in bone can be regarded as an 'alkali reserve', which is mobilized in response to reduced pH.

Not only can an abnormally low pH be buffered in this way, but if there is bone dissolution due to some other pathological condition the plasma calcium and phosphate levels will be elevated. The extra phosphate will combine with hydrogen ions as shown above and the pH will rise: this is one rare form of metabolic alkalosis (see Chapter 8).

**Ammonia**

Ammonia is excreted by the renal tubule cells into the urine where it acts as a buffer (or, strictly, as a proton 'sink') by combining with $H^+$ ions to form ammonium ions:

\[
NH_3 + H^+ \rightleftharpoons NH_4^+
\]

With a $pK$ of about 9.8 this equilibrium lies almost entirely to the right at physiological pH values. Ammonium buffering in urine becomes increasingly important as the amount of acid to be excreted increases such as in metabolic acidosis. This is discussed in detail in Chapter 7. Ammonia, being a toxic molecule, is not found in appreciable concentration in the blood of a normal individual except perhaps in portal venous blood.

**Relative importance of the various blood buffer systems**

Table 3.2 summarizes approximately the relative contribution made by the various buffer systems to the buffer capacity of whole blood. The predominant importance of the bicarbonate system, and also the important contribution made by haemoglobin, are obvious.

**Table 3.2**

<table>
<thead>
<tr>
<th>Contribution to buffering of whole blood (% approx)</th>
<th>Buffer system</th>
<th>Buffer acid (HA) $\rightleftharpoons H^+$+Conjugate base (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>$H_2CO_3$</td>
<td>$H^+ + HCO_3^-$</td>
</tr>
<tr>
<td>1</td>
<td>$H_2PO_4^-$</td>
<td>$H^+ + HPO_4^{2-}$</td>
</tr>
<tr>
<td>6</td>
<td>$HPr$</td>
<td>$H^+ + Pr^-$</td>
</tr>
<tr>
<td>29</td>
<td>$HHb$</td>
<td>$H^+ + Hb^-$</td>
</tr>
</tbody>
</table>


**Whole-body titration curves**

So far we have discussed only the properties of the individual buffer systems *in vitro*, that is outside the body. It must be recognized that these properties *in vitro* do not
fully reflect the buffering capability of the intact organism *in vivo*. However they are a close approximation and are accepted by many authorities to be reasonably accurate. Indeed the acid-base status of a patient is often assessed by studying the *in vitro* titration curve of blood (see Chapter 10). It is assumed that this gives enough information about the whole-body acid-base status. However the validity of this assumption is often questioned, especially on the grounds that about half the body's buffers are intracellular. Furthermore it is now known that the *in vivo* CO₂ titration curve (whole-body titration curve) does differ significantly from the *in vitro* titration curve.

The *whole-body titration curve* has been defined as the relation *in vivo* between the PCO₂ and the plasma actual bicarbonate concentration (or the H⁺ ion concentration) when the PCO₂ is systematically varied and the intact organism is allowed to achieve a new acute steady state.

The *in vitro titration curve* is the relation between the PCO₂ and the actual HCO₃⁻ concentration (or H⁺ ion concentration) in a sample of blood which is equilibrated *in vitro* in a tonometer with gas in which the PCO₂ is systematically varied. The properties of *in vitro* titration curves are described in detail in Chapter 10 in connection with the Astrup equilibration technique.

---

**Fig. 3.3** Comparison of the *in vitro* and *in vivo* carbon dioxide titration curves of human blood. The lower curve was drawn through the average plasma bicarbonate concentrations observed in seven subjects during exposure to 7% and 10% CO₂. The dotted extension of this curve is a calculated extrapolation. The upper curve was drawn through the average bicarbonate concentration observed during *in vitro* titration of blood from nine normal subjects. (Reproduced from Brackett, N. C., Cohen, J. J. & Schwartz, W. B., (1965) New Engl. J. Med., 272, 6-12, by kind permission of the authors and Editor).

The classical studies of Schwartz and his associates have shown the differences between these two titration curves for PCO₂ values ranging from 40 to 106 mmHg (5.33 to 14.1 kPa) in man. These workers exposed normal unanaesthetized subjects to elevated PCO₂ levels in a large environmental chamber while maintaining normal oxygen tensions in the inspired gas. Arterial blood samples were taken at intervals and it was possible to show that the subjects had reached a new acute steady state. In the short time of exposure no renal compensation occurred as judged from the net acid excretion in urine. Blood samples were also titrated *in vitro* in the Astrup apparatus (which is described in Chapter 10).
Their results are summarized in Fig. 3.3 which clearly shows that the buffering properties of blood \textit{in vitro} do not accurately represent (but in fact overestimate) the buffering properties of the intact human. This discrepancy is particularly marked at very high $P_{CO_2}$ values.

The facts that this discrepancy might invalidate some acid–base studies in blood \textit{in vitro}, and also that it is desirable to know what extent of physiological compensation can be expected in chronic (long-term) disorders have prompted much controversy. Some clinicians use as a diagnostic aid whole-body titration curves and nomograms constructed from data from actual patients whose acid–base disturbances were well characterized. One such example, the Flenley diagram, will be described later (Appendix IV).
Acidosis and Alkalosis

Definitions: acidosis and alkalosis
All acid–base disturbances can be classified using the terms acidosis and alkalosis, although these are not easy to define.

It is not adequate to define an acidosis as a cause of a fall in plasma pH, since a mild acidosis can exist accompanied by a normal pH owing to intervention of compensatory mechanisms. Recall that

\[ \text{pH} = \text{pK} + \log \left( \frac{[\text{base}]}{[\text{acid}]} \right) \]

and so pH depends on the logarithm of the ratio of [base] to [acid] rather than on base concentration or acid concentration alone. Some authors favour the definition: 'an acidosis is an acid–base disturbance which would cause pH to fall if no compensatory or other factors were operating'.

An alternative definition, which is more satisfactory, is to regard an acidosis as either a deficit in buffer conjugate base or a gain in buffer acid with respect to normal levels. The converse definition is used for an alkalosis.

In some of the earlier Scandinavian literature the term 'basosis' was used with the same meaning as alkalosis, but this terminology has not been widely accepted.

Acidaemia and alkalaemia
The terms acidaemia and alkalaemia are sometimes used to indicate that the plasma pH is low or high respectively: in correct usage they are not synonymous with acidosis and alkalosis. An acidaemia (low pH) can only be caused by an acidosis, but an acidosis is not necessarily accompanied by an acidaemia (since there may be compensation or a secondary or mixed disturbance present which raises the pH to normal or above).

The analogy with a mechanical balance shown in Fig. 4.1 emphasizes that an acidaemia can be associated with either an increased acid (respiratory) component or a decreased base (metabolic) component or with both.

Metabolic and respiratory disturbances
Since an acidosis (or alkalosis) can result from an abnormality of either buffer base or acid concentration it is convenient to classify all disturbances as either metabolic or respiratory. The base of the CO₂/HCO₃ buffer system is the bicarbonate; it is affected by metabolic production of acids and bases and by the activities of the kidney. Hence
it is often referred to as the *metabolic factor* or metabolic component or sometimes as the *non-respiratory component*. An acid–base disturbance caused by loss or destruction of bicarbonate is therefore classified as a *metabolic acidosis*. Any disturbance associated with a direct increase in bicarbonate concentration is a *metabolic alkalosis*.

![Fig. 4.1. This analogy with a mechanical balance gives a visual representation of the roles of base and acid components in acid–base balance. Note that the pH can be lowered (i.e. acidaemia) by either an increase in the acid (respiratory) component, PCO₂, or by a decrease in the base (metabolic) component, [HCO₃⁻]. The analogy with the mechanical beam balance is, of course, only partly valid; the equilibrium position of the mechanical balance depends on the difference between the weights on the two pans, while the pH depends on the ratio of the [HCO₃⁻] to the Pco₂ according to the Henderson–Hasselbalch equation. It must not be presumed from this simple diagram that the CO₂ and HCO₃ are completely independent, or that the actual bicarbonate concentration by itself is a useful index of the metabolic component.

The acid component of the CO₂/HCO₃⁻ system is of course the CO₂ (or the H₂CO₃) whose concentration is controlled by respiration: hence an excess of CO₂ is classified as a *respiratory acidosis*. The PCO₂, unlike the [HCO₃⁻], is unaffected by the excess production of metabolic acids. Carbon dioxide excess may also be referred to as *hypercapnia* or *hypercarbia*.

Comroe has pointed out that the terminology, respiratory and metabolic, may be misleading because a respiratory acidosis is due to metabolically produced CO₂. Furthermore one cause of a so-called metabolic acidosis may be an ingested (but not metabolically produced) acid. Similarly metabolic alkalosis may result from ingestion of excess base or by loss of gastric acid although neither of these is necessarily caused by abnormal metabolic processes. Therefore some authorities prefer to use the term ‘non-respiratory’ instead of ‘metabolic’ in classification of an acid–base disturbance.

**Mixed disturbances**

The term mixed disturbance is used when there is evidence, either from clinical signs or from interpretation of the acid–base data (see, for example, use of the Flenley nomogram, Appendix IV), that two or more aetiological factors are primarily responsible for production of the acid–base disturbance. Thus cardiac arrest causes both
Acidosis and Alkalosis

respiratory acidosis due to failure to excrete carbon dioxide and metabolic acidosis due to tissue hypoxia causing excess production of lactic acid. A diabetic patient who suffers renal failure might have a metabolic acidosis caused both by accumulation of ketone body acids and by failure to reabsorb bicarbonate completely in the renal tubules.

Interdependence of metabolic and respiratory components

While separate physiological mechanisms in the lungs and kidneys respectively regulate the respiratory factor, \( P_{\text{CO}_2} \), and the metabolic factor, \([\text{HCO}_3^-]\), the two factors must not be regarded as strictly independent. The law of mass action applies at all times to the equilibrium:

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- 
\]

Hence a high \( \text{CO}_2 \) concentration, i.e. in respiratory acidosis, will inevitably result in an elevated \( \text{HCO}_3^- \) concentration (and an elevated \( \text{H}^+ \) concentration) simply because of this equilibrium. However in this instance the increase in \( \text{HCO}_3^- \) cannot be regarded as a metabolic alkalosis because it has not arisen through any metabolic abnormality. Thus a simple measure of the actual bicarbonate concentration in arterial blood cannot define unequivocally the metabolic acid-base status of the individual: the actual bicarbonate concentration is influenced by the respiratory factor as well as by the metabolic factor. Therefore other indices must be used to quantitate the metabolic factor free from influence by the respiratory factor such as standard bicarbonate and base excess. These are discussed in Chapters 8 and 10.

It might be asked why the pH of blood falls when, for example, there is a high \( P_{\text{CO}_2} \) since the \([\text{HCO}_3^-]\) will also be elevated according to the equilibrium

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- 
\]

Therefore the ratio \([\text{HCO}_3^-]/[\text{CO}_2]\) might be expected at first sight to remain constant, and the pH to remain constant, since

\[
\text{pH} = pK + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]} 
\]

where, strictly, \([\text{CO}_2]\) refers to \([\text{H}_2\text{CO}_3] + [\text{CO}_2]\).

In fact when the \( \text{CO}_2 \) concentration is increased the \( \text{HCO}_3^- \) concentration does rise, but the increase in \( \text{HCO}_3^- \) concentration is proportionally very much smaller than the increase in \( \text{CO}_2 \) and so the ratio \([\text{HCO}_3^-]/[\text{CO}_2]\) does in fact fall. This can be easily seen if one considers a solution containing only \( \text{CO}_2 \) and \( \text{HCO}_3^- \),

\[
K = \frac{[\text{H}^+].[\text{HCO}_3^-]}{[\text{CO}_2]} 
\]

Since \([\text{H}^+] = [\text{HCO}_3^-]\) (anions must balance cations), then

\[
K = \frac{[\text{HCO}_3^-]^2}{[\text{CO}_2]} \quad \text{or} \quad [\text{HCO}_3^-] = \sqrt{K}.[\text{CO}_2] 
\]

Hence a doubling in \( \text{CO}_2 \) concentration causes a rise in \( \text{HCO}_3^- \) concentration by a factor of only \( \sqrt{2} = 1.414 \) times. Hence the \([\text{HCO}_3^-]/[\text{CO}_2]\) ratio must fall by a factor of \( 1/414 \) times: the pH therefore falls.

When there are other buffers present, as in the body fluids, some of the \( \text{H}^+ \) ions
formed during the hydration of CO₂ are buffered, e.g. by haemoglobin. Thus, in effect, these H⁺ ions are removed and so the carbonic acid dissociation proceeds further, i.e.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

\[
\text{(H}^+ \text{ ions buffered)}
\]

\[
\text{H}^+ + \text{Hb}^- \rightleftharpoons \text{HHb}
\]

Dissociation moves to the right (law of mass action) to restore equilibrium

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

Therefore the amount of extra bicarbonate formed from excess CO₂ in practice depends on the concentration of other buffers especially haemoglobin.

**Compensation and repair**

The corrective processes of the physiological defence mechanisms against acidosis and alkalosis are known as compensation. 'Compensation' must be distinguished from 'repair'; the latter term is used to describe the return to normality of all acid–base parameters (see Chapter 9). In contrast, compensation, generally only a temporary expedient (exceptions are to be found in certain chronic conditions, e.g. bronchitis, which have become permanent) only brings the pH back to, or towards, normal. This can be achieved by restoring the ratio [HCO₃⁻]/[CO₂] towards the normal value of 20 : 1 by regulatory mechanisms so that both [HCO₃⁻] and [CO₂] are abnormal in the same direction. For example, in a metabolic acidosis without compensation (i.e. acute metabolic acidosis) the [HCO₃⁻] will be low. As compensation proceeds the CO₂ concentration in the blood will be lowered, so that if compensation were complete the ratio [HCO₃⁻]/[CO₂] would be normal—hence the pH would be normal (given by pH = pK + log [HCO₃⁻]/[CO₂]). However both the [HCO₃⁻] and the [CO₂] would be low; this is potentially still a serious situation, since if the cause of the metabolic acidosis (e.g. metabolic production of relatively strong acid or loss of HCO₃⁻) is not removed the HCO₃⁻ concentration will fall further (i.e. the so-called alkali reserve* will become progressively depleted) and potentially could become zero. In this case one would have lost all of one's most important buffer system and the pH would fall precipitously. Thus compensation does not remove or 'cure' the metabolic acidosis; it merely alleviates the abnormality in pH by, in effect, causing a temporary secondary respiratory alkalosis.

On the other hand repair entails returning the [HCO₃⁻] to normal so that all the acid–base parameters are normal. Compensation and repair mechanisms are discussed in detail in Chapter 9.

*The use of the obsolete term 'alkali reserve' is not encouraged. It is not often used in clinical practice, and can be misleading.
Regulation of $P_{CO_2}$: The Respiratory Factor

The organ of gas exchange between blood and the atmosphere is, of course, the lung. Excretion of carbon dioxide and the maintenance of normal $P_{CO_2}$ is just as vital as is oxygenation of the blood. In complete apnoea hypercapnia develops, that is the $P_{CO_2}$ increases so that there is a respiratory acidosis, at a rate of around 4 mmHg (0.5 kPa) per minute. Of course tissue hypoxia, i.e. a fall in $P_{O_2}$, also develops very rapidly. As already stated in Chapter 2, a sedentary individual produces about 15 000 mmol or mEq CO$_2$ per day. If this were not excreted but were all retained in the body fluids the pH of blood would potentially fall from 7.40 to about 4.5 in a single day, i.e. the hydrogen ion concentration would increase from 40 to 32 000 nmol/litre.

Regulation of ventilation—chemoreceptors

Ventilation is regulated to a large extent by the respiratory centre, located in the medulla oblongata; the respiratory centre in turn is controlled by information about the chemical composition (pH and $P_{CO_2}$) of the blood from the peripheral and central chemoreceptors.

The respiratory centre of course does not only regulate ventilation so as to maintain appropriate CO$_2$ excretion, but so as to maintain also the correct $P_{O_2}$ in arterial blood. Although a considerable fall in $P_{O_2}$ can occur before the hypoxic (hypoxaemic) stimulus to ventilation becomes significant, the ‘hypoxic drive’ can be an important stimulus to respiration. The $P_{O_2}$ is largely recognized by the peripheral chemoreceptors. Indeed in some patients with long-term respiratory disease such as chronic bronchitis the provision of pure oxygen to breathe can remove the hypoxic drive and therefore respiration may be depressed or even cease. The administration of oxygen to such a patient must be a compromise, sufficient to avoid tissue hypoxia with subsequent ischaemia and necrosis and yet insufficient to remove the ‘hypoxic drive’ stimulus to ventilation.

That regulation of carbon dioxide concentration in blood was more important than oxygen concentration under normal circumstances was proved by the classical experiments of Haldane. In one series of experiments Haldane and Priestley measured the resting $P_{CO_2}$ and $P_{O_2}$ in their own alveolar air (which was taken to be a fair estimate of the values in arterial blood) at the summit of Ben Nevis which is the highest mountain in the United Kingdom, at the bottom of a mine in Cornwall, at Oxford and in a hyperbaric pressure chamber. Although their $P_{O_2}$ values varied considerably at these different barometric pressures, their $P_{CO_2}$ values hardly varied at all. They concluded that only when the concentration of inspired oxygen fell very drastically did the respiratory centre respond to oxygen deficiency (‘hypoxic drive’) rather than to CO$_2$ excess.
The peripheral chemoreceptors are located in the carotid and aortic bodies. The carotid bodies are near the division of the common carotid into the internal and external carotids, while the aortic bodies (which may be of less importance in adults) are located in the aortic arch. The carotid bodies have an enormous blood flow of about forty times that to muscle and brain when expressed in terms of unit tissue weight. These peripheral chemoreceptors are particularly responsive to hypoxia; they are also thought to be sensitive both to the pH and to the \( P_{\text{CO}_2} \) of the fluids bathing them, although the intracellular mechanisms involved are not at all understood. It is debated whether both \( P_{\text{CO}_2} \) and pH act as independent controlling factors since the two are obviously related. Current evidence favours that these are independent stimuli, and this has been expressed by Gray's 'multiple factor theory' which attempts to predict the ventilatory response mathematically from empirical equations containing terms in \( P_{\text{CO}_2} \), pH and \( P_{\text{O}_2} \). However the carotid and aortic bodies are much less sensitive to changes in \( P_{\text{CO}_2} \) and pH than the central chemoreceptors.

The central chemoreceptors are located in the medulla and are highly sensitive to changes in pH and \( P_{\text{CO}_2} \). The sensitivity of these central chemoreceptors to \( P_{\text{CO}_2} \) (and pH) may however be depressed or even abolished in hypoxia or by drugs, including many general anaesthetics, or by a very high \( P_{\text{CO}_2} \). They are probably not very sensitive, or even are insensitive, to \( P_{\text{O}_2} \).

Relative importance of peripheral and central chemoreceptors

Under normal circumstances the medullary central chemoreceptors probably have the dominating role, but when hypoxia is present or when the central receptors are depressed or damaged the carotid and aortic bodies become more important. The hypoxic (i.e. hypoxaemic) drive to respiration may be crucial in some diseases such as chronic bronchitis (see above), but it is regarded as of only minor importance in a normal individual.

However the relative importance of the central and peripheral chemoreceptors is open to some debate. Probably the central receptors regulate respiration in such a way as to control the composition of cerebrospinal fluid (CSF) while the peripheral chemoreceptors are acting so as to control the blood composition. Changes in the acid–base status of blood are reflected by changes in acid–base status of CSF but there may be a considerable time lag, since the blood–brain barrier is not freely permeable to ions such as \( H^+ \) and bicarbonate although it is freely permeable to carbon dioxide. Hence a change in blood \( P_{\text{CO}_2} \) causes an immediate change in the \( P_{\text{CO}_2} \) (and therefore pH) in blood. Also the pH change in CSF will be relatively large since CSF, lacking haemoglobin, is a relatively much poorer buffer than blood. Consequently both the central and the peripheral chemoreceptors will operate in the same direction towards restoring a normal \( P_{\text{CO}_2} \) in both blood and CSF. On the other hand, a change in \( \left[ \text{HCO}_3^- \right] \), say for example a fall, in blood will cause the peripheral chemoreceptors to sense a fall in blood pH and to stimulate respiration. Owing to the relative impermeability of the blood–brain barrier the fall in blood \( \left[ \text{HCO}_3^- \right] \) will not immediately affect the CSF \( \left[ \text{HCO}_3^- \right] \) and so the central chemoreceptors would not be expected to stimulate respiration. On the other hand the stimulation of respiration by the peripheral chemoreceptors will cause an immediate fall in \( P_{\text{CO}_2} \) in both blood and CSF. This fall in \( P_{\text{CO}_2} \) in CSF will be detected by the central receptors which may then inhibit respiration. Hence the peripheral and central chemoreceptors can be acting in opposite directions on respiration. It is because the acid–base status of blood and of CSF are not necessarily the same that CSF samples are occasionally taken for 'blood gas' (or 'acid–base') analysis although this is not a routine practice. However it must be remembered that the CSF is not a uniform fluid in a well mixed compartment as
Regulation of \( P_{CO_2} \): The Respiratory Factor

blood is, and so the composition may depend on the site of sampling. In some diseases such as some types of meningitis the blood–brain barrier appears to be damaged since ions can then equilibrate across it relatively rapidly.

Information about the chemoreceptors is briefly summarized in Table 5.1.

### Table 5.1

**Summary of information on chemoreceptors**

<table>
<thead>
<tr>
<th></th>
<th>'Central'</th>
<th>'Peripheral'</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td>Medulla oblongata</td>
<td>Carotid and aortic bodies</td>
</tr>
<tr>
<td><strong>Sensitivity to ( P_{CO_2} )</strong></td>
<td>Highly sensitive to CSF ( P_{CO_2} )</td>
<td>Less sensitive to ( P_{CO_2} )</td>
</tr>
<tr>
<td><strong>Sensitivity to ( pH )</strong></td>
<td>Highly sensitive to CSF ( pH ) (sensitivity depressed in hypoxia, etc.).</td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity to ( PO_2 )</strong></td>
<td>Insensitive to ( PO_2 )</td>
<td>Sensitive to ( PO_2 )</td>
</tr>
<tr>
<td><strong>Importance</strong></td>
<td>Normally dominant (except in hypoxia or when central receptors are otherwise depressed or damaged)</td>
<td>Important in hypoxia and other cases of central receptor depression (central respiratory depression)</td>
</tr>
</tbody>
</table>

**Respiratory compensation**

An increase in \( P_{CO_2} \) from 40 to 50 mmHg (5-3 to 6-7 kPa) will stimulate respiration so that the ventilation rate in litres/min increases by about four-fold. Since the chemoreceptors are sensitive to \( pH \) as well as to \( P_{CO_2} \) it follows that they can regulate respiratory compensation for acidaemia or alkalaemia in a non-respiratory, i.e. metabolic, acidosis or alkalosis. It is estimated that the ventilation rate changes about two-fold for a change of 0-1 \( pH \) unit. Thus when \([HCO_3^-]\) falls in a metabolic acidosis the \( pH \) falls; ventilation is stimulated and the \( P_{CO_2} \) falls to some value below normal (respiratory compensation) so that the ratio \([HCO_3^-]/[CO_2]\), and therefore the \( pH \), rises and becomes normal or almost normal once again.

**Reflex regulation of respiration**

In addition to the chemical regulation of respiration an important role is played by nervous reflexes. There are many different reflexes: many of them originate in stretch receptors in the lungs and are transmitted via the vagus nerves. Separate reflexes for inspiration and expiration are recognized. These vagal stretch reflexes are often called the Hering–Breuer reflexes, and can be demonstrated by bilateral vagotomy. The tidal volume suddenly increases and the frequency of respiration slows. There are also a number of respiratory (and cardiovascular) reflexes which originate in the heart and great vessels.

Physical changes in the lungs associated with disease, especially the 'stiff lung' diseases, modify the reflexes. For example, pulmonary vascular congestion, atelectasis and pulmonary oedema cause the lungs to become stiffer, that is less compliant. The stretch receptors then become sensitized, so that they are activated at a lower than usual increase in lung volume. Volatile anaesthetics and some bronchoconstrictor
drugs also affect these reflexes and this may explain the rapid breathing which is observed during administration of some anaesthetics.

The chemoreceptors probably control the primary drive to respiration, with a less important part played by the reflexes.

**Carbon dioxide transport**

Carbon dioxide, the major acid end-product of metabolism, is carried in the blood stream as:

1. **Dissolved CO₂** in both plasma and erythrocytes.
2. **Carbonic acid**, and hence since this dissociates \( \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \) as bicarbonate, again both in plasma and erythrocytes. The hydration of CO₂

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3
\]

is, in vitro, a very slow reaction. However in vivo it is catalysed by the enzyme carbonic anhydrase which is present in high activity in the red blood cells. It is estimated that the presence of this enzyme allows the hydration to proceed about 3000 times more rapidly in erythrocytes than in plasma. Carbonic anhydrase is also found in renal tubule cells and gastric and intestinal mucosa; although it is catalysing the same reaction in these tissues the physiological functions are different from those in the red blood cells.

3. **Carbamino compounds.** Only about 5–10% of CO₂ is carried in this form. It forms covalent bonds with amino groups at the termini of proteins (plasma proteins

### Table 5.2

**Distribution of carbon dioxide in 1 litre of normal arterial and venous blood**

<table>
<thead>
<tr>
<th></th>
<th>Arterial blood</th>
<th>Venous blood</th>
<th>A–V difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol (%*)</td>
<td>mmol (%*)</td>
<td>mmol (%†)</td>
</tr>
<tr>
<td><strong>PLASMA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dissolved CO₂</td>
<td>0.71 (3.3)</td>
<td>0.80 (3.4)</td>
<td>0.09 (5.4)</td>
</tr>
<tr>
<td>bicarbonate</td>
<td>15.23 (70.7)</td>
<td>16.19 (69.8)</td>
<td>0.96 (57.1)</td>
</tr>
<tr>
<td>Total CO₂ in plasma (600 ml)</td>
<td>15.94 (74.0)</td>
<td>16.99 (73.2)</td>
<td>1.05 (62.5)</td>
</tr>
<tr>
<td><strong>ERYTHROCYTES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dissolved CO₂</td>
<td>0.34 (1.6)</td>
<td>0.39 (1.7)</td>
<td>0.05 (3.0)</td>
</tr>
<tr>
<td>bicarbonate</td>
<td>4.28 (19.9)</td>
<td>4.41 (19.0)</td>
<td>0.13 (7.7)</td>
</tr>
<tr>
<td>carbamino compounds</td>
<td>0.97 (4.5)</td>
<td>1.42 (6.1)</td>
<td>0.45 (26.8)</td>
</tr>
<tr>
<td>Total CO₂ in red cells (400 ml)</td>
<td>5.59 (26.0)</td>
<td>6.22 (26.8)</td>
<td>0.63 (37.5)</td>
</tr>
<tr>
<td><strong>WHOLE BLOOD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total in whole blood (1 litre)</td>
<td>21.53 (100)</td>
<td>23.21 (100)</td>
<td>1.68 (100)</td>
</tr>
</tbody>
</table>

The arteriovenous difference is also shown to demonstrate the importance of the various forms in carriage of CO₂ for excretion. Note that although the actual quantity of CO₂ bound as carbamino compounds is small, the arteriovenous difference is much higher. This is due to the Haldane effect and illustrates the physiological importance of carbamino compounds.


* % of total CO₂ in whole blood.
† % of total A–V difference in whole blood.
and haemoglobin): each CO₂ molecule bound in this way gives rise to one H⁺ ion, which in turn has to be buffered elsewhere on the carbamino compound molecule (see Chapter 3). Deoxygenated blood has a greater capacity for carbon dioxide than oxygenated blood has (Haldane effect). This is because deoxyhaemoglobin forms carbamino compounds some three to four times more readily than oxyhaemoglobin does. Hence as blood is reoxygenated in the lungs there is a tendency to give up carbamino-bound CO₂. This is clearly an advantageous adaptation for the transport and excretion of CO₂. While only about 5–10% of the total CO₂ carried in whole blood is carried as carbamino compounds, the arteriovenous difference in carbamino concentration is relatively large. This means that CO₂ in carbamino compounds is freely exchangeable and that it can account for over one-quarter of the CO₂ lost to the lungs (Table 5.2).

The amount of carbon dioxide in blood which is carried in these various forms can be seen from Table 5.2.

**Body stores of carbon dioxide**

Under ideal circumstances in the resting normal subject the rates of metabolic production of CO₂ and of its excretion are equal and so the Pco₂ in arterial blood remains fairly constant, and at a value of around 40 mmHg or 5·3 kPa (range 35–45 mmHg or 4·6–6·0 kPa). Typically the Pco₂ in mixed venous blood is some 6–8 mmHg (0·8–1·1 kPa) greater.

The whole body contains about 120 litres of CO₂ in the various forms and in the various body compartments. Of this, only about 100 ml is in the gas phase. This gives rise to the concept of a ‘body CO₂ store’. Since this is so large it is obviously of homeostatic value: in a sense this large ‘pool’ of CO₂ can be regarded as ‘buffering’ changes in free gaseous CO₂ and the other forms when changes in the rate of production or excretion of CO₂ occur, although this is not buffering in the strict physico-chemical sense. If the ‘body CO₂ store’ were smaller, then a small change in the rate of production or of excretion of CO₂ would produce a much larger change in the pH and the Pco₂ of the body fluids.

A similar concept is applied to the ‘body O₂ store’ which is much smaller. A total amount of about 1 litre of oxygen is contained in the whole body (including the body cavities), of which only 300 ml is present as free gaseous oxygen.

One other implication inherent in a body gas store is that metabolism can proceed for a while at least while gas exchange with the atmosphere is disturbed or impaired.

**The normal CO₂ excretory process**

The normal process of carbon dioxide excretion depends on three factors:

1. **Ventilation.** This in itself depends on both the nervous system—chemoreceptors and respiratory centre—and the mechanical aspects of respiration, such as the correct functioning of chest muscles and rib cage. Also, the airways must be unobstructed.

2. **Diffusion.** The CO₂ has to cross the semipermeable alveolar epithelium by diffusion before it can be exchanged with alveolar air and be passed out of the body.

3. **Perfusion.** The blood supply within the lungs must deliver the CO₂ to the alveolar epithelium.

**Abnormal CO₂ excretion**

Abnormalities in any of these processes will be reflected in an abnormal carbon dioxide excretion, and can therefore result in low or high Pco₂ values in arterial blood.
Since the CO₂ composition of venous blood depends largely on the metabolic activity of the region drained it cannot normally give any useful information about ventilation and respiratory function: for this arterial, or capillary, blood is obligatory (see Chapter 11).

In a normal adult the alveoli are ventilated by about 4 litres of air per minute, while the lungs are perfused with some 5 litres of blood per minute. The ventilation–perfusion ratio (\(V_A/Q\)) is then said to be 0.8. Even in a healthy individual neither ventilation nor perfusion is perfectly uniform, and so values for different regions of lungs vary from one extreme where a well ventilated alveolus has negligible blood perfusion (\(V_A/Q \to \infty\)) to the other extreme with \(V_A/Q \to 0\). However, gross abnormalities in the ventilation–perfusion relation are common in diseased lungs and are frequently reflected by abnormal \(PCO_2\) and \(PO_2\) values in arterial blood or by abnormal \(PO_2\) but normal \(PCO_2\) values. The detailed investigation of ventilation–perfusion defects relies on the use of specialized techniques using radioactive tracers (e.g., \(^{133}Xe\)) to outline the areas of adequate ventilation and perfusion and to measure the kinetics of gas exchange between the lungs and the atmosphere.

Excessive or deficient production of CO₂ can be regarded as metabolic defects and so need not be considered further here.

Examples of respiratory acid–base disturbances

Some examples of causes of respiratory acidosis and alkalosis are summarized below.

**Respiratory acidosis**

Respiratory acidosis, hypercapnia or hypercarbia is defined by a \(PCO_2\) in arterial blood greater than about 45 mmHg (60 kPa). While the \(PCO_2\) is rising carbon dioxide is being excreted more slowly than it is being produced and so the body's 'CO₂ stores' increase to a new steady state.

**Defective ventilation**

This may be caused by depression of the respiratory centre due to cerebral injury such as tumours, 'mechanical' head injury (increased intracranial pressure) or viral infections (e.g., bulbar poliomyelitis) and lesions of the spinal cord. Hypoxia, very high \(PCO_2\) and many drugs (notably general anaesthetics and all sedatives and tranquillizers such as morphine and barbiturates) depress the respiratory centre. It is claimed that some antibiotics can abruptly depress respiration in anaesthetized patients, e.g., neomycin, streptomycin and kanamycin administered intraperitoneally or intrapleurally, but this must be very rare. In a severe chronic metabolic acidosis with pH below 7.2 the respiratory centre may be inhibited so that \(PCO_2\) rises, thus superimposing a primary respiratory acidosis on the metabolic disturbance.

Mechanical injury, e.g., rib-cage injuries (crushed chest), neuromuscular disease of the respiratory muscles, and in some severe, almost lethal, cases of loss of compliance (elasticity) in lungs, pneumothorax or haemothorax. (Chronic cases of unilateral pneumothorax and 'stiff lung' diseases are often, however, accompanied by a decreased \(PCO_2\).) Any obstruction of the airways such as by a foreign body, tumour, or a constriction in bronchial asthma will also hinder the ability of alveolar gas to exchange properly with the atmosphere.

**Defective diffusion**

Transport of carbon dioxide from the blood in the alveolar capillaries to the gas in the alveolus is a purely passive process depending on diffusion. It is, however, con-
Regulation of $P_{CO_2}$: The Respiratory Factor

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troversial whether diffusion defects are relevant as causes of respiratory acidosis in patients: the current view is that they are not likely to be major causative factors of clinical relevance. Owing to its greater water solubility, $CO_2$ diffuses across the alveolar barrier about twenty times faster than oxygen. Thickening of the diffusion barrier, or changes which reduce its permeability, between the alveolar capillary blood and alveolar gas may occur in inflammation (pneumonia), pulmonary fibrosis as in diffuse fibrosing alveolitis, or pulmonary oedema and some uncommon conditions.

DEFECTIVE PERFUSION

Inadequate or non-uniform perfusion of the pulmonary capillaries with blood will restrict the effective surface area of alveolar membrane at which gas exchange may take place. This is expressed as a low ventilation–perfusion ratio, $V_A/Q$ (see above). Pulmonary infarction or embolism, general destruction of lung tissue and compression of vessels by extravascular lesions (e.g. tumours) can all lead to impaired distribution of blood flow. However, lung area is sufficiently large that the overall $P_{CO_2}$ often remains normal in a subject at rest.

Abnormalities in the ventilation–perfusion ratio are more often reflected by hypoxia than by hypocapnia. The hypoxia, if severe enough, will stimulate ventilation and so may cause hypocapnia rather than the reverse condition.

CONSEQUENCES OF RESPIRATORY ACIDOSIS (HYPERCAPNIA)

Hypocapnia can affect severely the central nervous and cardiovascular systems. There is an increase in total cerebral blood flow caused by cerebral vasodilation. This may be accompanied by raised CSF pressure. There is also peripheral vasodilation, but this may be opposed by the vasoconstrictive effects of sympathetic stimulation. Vasoconstriction of the pulmonary vascular system is also caused, and this may precipitate right heart failure. The heart rate tends to increase.

The depression of the central nervous system can be extremely dangerous to the extent of loss of consciousness. Some stimulation may also occur, resulting in twitching and convulsions.

Respiratory alkalosis

Respiratory alkalosis, hypocapnia, or hypocarbia is defined by a $P_{CO_2}$ in arterial blood less than about 35 mmHg (4.6 kPa). While the $P_{CO_2}$ is falling carbon dioxide is being excreted faster than it is being produced and the body's $CO_2$ stores become depleted. Although hypocapnia raises the blood pH until renal compensation lowers the blood bicarbonate concentration (see Chapter 9), this condition does not usually create a direct or immediate threat to life. However, there is a serious indirect threat since hypocapnia causes cerebral vasoconstriction and hence a danger of cerebral hypoxia.

In addition to the effects on the central nervous and cardiovascular systems, respiratory alkalosis produces a number of non-specific effects. These include fatigue and headache and features such as numbness and tingling of the extremities and twitching or even convulsions which are characteristic of increased neuromuscular excitability.

A number of drugs stimulate the respiratory centre directly (e.g. salicylates), as also does progesterone which can cause mild overventilation in pregnancy. Thus in pregnancy the $P_{CO_2}$ may fall to about 28 mmHg (3.72 kPa) and, in compensation, the base excess to $-5$ mmol/litre. Excessive ventilation can also be caused by fever, cerebral disease or damage affecting the pons and occasionally by severe infections by Gram-negative organisms. Obviously passive overventilation by an incorrectly adjusted artificial ventilator is a potentially important cause. It cannot be over-stressed
that the management of patients on ventilators requires regular monitoring of the blood gases, $P_{\text{CO}_2}$ and $P_{\text{O}_2}$.

A decreased $P_{\text{CO}_2}$ is easily produced by voluntary overbreathing or by a hysterical patient. Hence care must be taken during blood sampling that the patient is relaxed and that the respiration is not affected by the procedure or surroundings.

People living at very high altitudes have a pronounced hypoxic drive (due to the rarefied atmosphere) which can produce a significant chronic respiratory alkalosis.
The Kidney as a Regulatory Organ

Conservation of useful solutes

The kidney must be regarded not merely as an excretory organ but chiefly as a regulatory organ. Obviously the stage at which solutes are excreted from the body represents an ideal point for the control of the composition of the body fluids. Note that the body's carbon dioxide content also is regulated by its organ of excretion, the lung.

While the waste solutes such as urea have to be disposed of into urine, useful solutes must be conserved by partial or total reabsorption from the glomerular filtrate. Examples of solutes which are normally totally reabsorbed, and therefore conserved, by the kidney are glucose and bicarbonate; hence urine does not normally contain these.

It has already been stressed that bicarbonate is a particularly important buffer constituent of all the extracellular fluids. Thus any loss of this would normally represent a reduction in the buffering capacity of the body fluids and it is therefore important that all HCO$_3^-$ ions in the glomerular filtrate under normal circumstances be conserved by reabsorption. Furthermore losses of bicarbonate utilized in the buffering of H$^+$ ions produced metabolically must be made good by the regeneration of HCO$_3^-$ ions, and this is another important role of the kidney. As already mentioned in Chapter 2, some 50–100 mmol (or mEq) of H$^+$ ions in the form of fixed (non-volatile) acids are normally produced each day, and this amount may be greatly increased in pathological conditions. An equivalent amount of HCO$_3^-$ ions is destroyed and so in a healthy individual 50–100 mmol (or mEq) of H$^+$ must be excreted and 50–100 mmol (or mEq) of HCO$_3^-$ must be regenerated by the kidney per day.

Additionally the body content of Na$^+$, K$^+$ and other ions depends on their renal excretion as well as on their intake and the kidneys are the site of physiological control of these electrolytes. In fact, the renal regulation is so powerful that the body content of most cations is virtually independent of intake provided kidney function is normal. Maintenance of the extracellular volume and osmolarity are vital functions of the kidneys. Hence normal electrolyte balance requires adequate renal function and it will be seen that the body’s electrolyte and water balance is inevitably intimately associated with acid–base balance.

Formation of urine involves the glomerular filtration of plasma, which is a physical process of ultrafiltration, followed by the reabsorption of water and ‘valuable’ solutes in the proximal and distal tubules and the collecting ducts (see Fig. 6.1). In addition, some solutes are actively secreted by the tubule cells into the urine.
Reabsorption of bicarbonate

**General features**

The 'normal' plasma bicarbonate concentration of a healthy individual is about 24 mmol/litre (mEq/litre), that is in a healthy individual on a normal European-type diet. Therefore the glomerular filtrate contains 24 mmol HCO$_3^-$/litre. Urine normally contains no HCO$_3^-$, so (assuming a glomerular filtration rate of 125 ml/min) 3 mmol of HCO$_3^-$ are reabsorbed per minute. The nature of this process is not straightforward, as it is generally accepted that most or all of the bicarbonate ions are not reabsorbed as such. They are in effect reabsorbed indirectly by a process which involves the active secretion of H$^+$ ions by the proximal and sometimes distal tubule cells into the tubular lumen. In studies on rats in normal acid–base balance it appears that about 85% of the filtered bicarbonate is absorbed in the proximal tubules. In the normal human individual passing acid or neutral urine this process is largely completed in the proximal tubules. However in the formation of an alkaline urine, which contains bicarbonate, reabsorption in the distal nephron becomes significant. Electroneutrality is maintained by reabsorption of Na$^+$ ions from the tubular fluid in exchange for the excreted H$^+$ ions.

The addition of H$^+$, produced from dissociation of cellular carbonic acid, to the bicarbonate in the tubular luminal fluid (i.e. the fluid that was the glomerular filtrate and will become the urine) displaces the equilibrium

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$$

\[\text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}\]

This reaction is catalysed by intracellular carbonic anhydrase, a key enzyme in the renal reabsorption of HCO$_3^-$ and excretion of H$^+$ ions. This enzyme is present in high activity in kidney. The carbonic acid formed inside the cells dissociates thus:

$$\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$$

and the H$^+$ ions produced are excreted into the tubular luminal fluid (to convert HCO$_3^-$ to CO$_2$) and the HCO$_3^-$ ions pass into the peritubular fluid, i.e. the tissue fluid which surrounds the tubules and ultimately exchanges with the renal venous plasma. It has been claimed that hypercalcaemia may promote urinary H$^+$ ion excretion by stimulation of carbonic anhydrase activity. However it is not certain that this step is rate-limiting.

Electroneutrality is maintained by the simultaneous passage of Na$^+$ across the renal epithelium. Thus Na$^+$ and HCO$_3^-$ ions pass together out of the cells into the peritubular fluid. Therefore the overall net effect of this process is the loss (reabsorption) of HCO$_3^-$ and Na$^+$ ions from the tubular luminal fluid and the gain of these ions by the peritubular fluid as though NaHCO$_3$ had been simply reabsorbed with the concomitant loss (excretion) of H$^+$ ions into the urine.
The process of effective bicarbonate reabsorption by means of acid secretion is summarized diagrammatically in Fig. 6.1. For clarity this figure shows each step occurring separately but it must be stressed that these are not time lapse sequence diagrams. All the steps are occurring simultaneously.

**Hydrogen ion secretion**

The process of H⁺ ion secretion from the tubule cell into the tubular luminal fluid is one of active transport. The pH of normal urine is on the acid side of neutrality (say about 6 to 6.5) and it may fall to a value as low as pH 4.5. The normal pH of the intracellular fluid is not known owing to the extreme difficulty of making a reliable measurement. If it is assumed to be 7.4, that is the same as the normal pH of plasma and the peritubular fluid, then it follows that H⁺ ions can be extruded from the tubule cell against a very substantial (about 800-fold) concentration, or strictly electrochemical, gradient (Table 6.1). This process obviously requires energy, and so the process of H⁺ ion excretion must involve active transport and the utilization of metabolic energy, probably in the form of ATP (Fig. 6.1b). The transport process, or pump, is linked to the reabsorption of Na⁺ ions from the tubular luminal fluid into the cell (hydrogen-sodium exchange) and it can be stimulated by cortisol and by aldosterone, which is the principal mineralocorticoid hormone secreted by the adrenal cortex. The action of aldosterone is mediated by protein synthesis. Thus it is characterized by a time-lag, by an increase in RNA synthesis (demonstrable by incorporation of radioactively labelled nucleotide, ³H-uridine-P) and susceptibility to blockage by inhibitors of protein synthesis such as actinomycin D. However, the action of aldosterone is thought to be largely or solely confined to the distal nephron, i.e. the distal tubule and collecting ducts. As stated above, the majority of Na⁺/H⁺ exchange in the production of a normal acid urine occurs in the proximal tubule.

**Table 6.1**

| Calculation of the average H⁺ gradient across luminal membrane in renal tubule cells |
|----------------------------------------|---------------------------------|-------------------|
| pH<sub>in</sub> = 7.4 | [H⁺]<sub>in</sub> = 10⁻⁴.⁷ mmol/litre |
| pH<sub>out</sub> = 4.5 | [H⁺]<sub>out</sub> = 10⁻⁴.⁵ mmol/litre |
| [H⁺]<sub>out</sub>/[H⁺]<sub>in</sub> = 10²⁻⁹ | ≈ 800 |

The nature of the linkage between Na⁺ and H⁺ transport is not clear. While it is convenient to regard these processes as closely linked as in Fig. 6.1b, this view is not necessarily strictly accurate, and certainly should not be interpreted as involving a single molecular ‘carrier’ species.

**TESTS OF ACID SECRETION**

Investigations are sometimes carried out on the ability of the renal tubule cells to secrete H⁺ ions in the assessment of renal disease. A simple test (see Chapter 7) entails creating a metabolic acidosis by the administration of ammonium chloride. If tubular secretion of H⁺ ions is normal then the urinary pH should fall below 5.3, and there should be a small decrease in the plasma bicarbonate concentration.

**Potassium secretion**

The specificity of the transport mechanism for H⁺/Na⁺ exchange is not absolute, since K⁺ ions can also be transported from the cell in exchange for Na⁺ ions (potassium-
Fig. 6.1. Diagrammatic explanation of bicarbonate reabsorption by tubular acid secretion.

A, Intracellular production of $H^+$ ions.
B, Active secretion of $H^+$ ions in exchange for $Na^+$ ions.
C, Conversion of bicarbonate in tubular lumen to $CO_2$ which then diffuses into cells.

$sodium$ exchange). Thus $H^+$ ions and $K^+$ ions can be regarded as competing for exchange with $Na^+$ ions although this competition may not be valid in a strict biochemical sense. While the entire nephron can absorb $K^+$ ions (of which absorption is normally complete), it is in the distal tubule that potassium secretion occurs. The
D, As A. Intracellular hydration of CO₂ catalysed by carbonic anhydrase and dissociation of carbonic acid.

E. Passage of sodium bicarbonate (active transport?) from cell to peritubular fluid.

F. Overall effect in apparent reabsorption of sodium bicarbonate.

Relative amounts of H⁺ ions and K⁺ ions excreted by this pump depend on their relative intracellular concentrations which in turn depend to some extent on their concentrations in the peritubular fluid (ECF). Hence a rise in H⁺ ion concentration (fall in pH) is automatically reflected by an increase in H⁺ pumping (excretion) and a decrease in K⁺ pumping, and vice versa. If the enzyme carbonic anhydrase is inhibited by, for example, a sulphonamide diuretic drug, then the number of H⁺ ions available for excretion falls and the amount of K⁺ ions excreted will increase. This explains the potassium-losing side-effects of many diuretics. However, the extra K⁺ ions lost will
be less than the reduction in H\(^+\) ions excreted, so the pump will in fact function more slowly and thus less Na\(^+\) (and in consequence less H\(_2\)O) will be reabsorbed.

**Direct reabsorption of bicarbonate**

The possibility that at least some bicarbonate may be reabsorbed directly as such either actively or passively is suggested currently by some authorities, and a dogmatic statement is not possible at the present time.

**Electrolyte interrelationships**

It can be seen that the excretion of H\(^+\) and K\(^+\) ions and the reabsorption of Na\(^+\) and HCO\(_3^-\) ions and water are very closely interrelated. Therefore any physiological, pathological or therapeutic disturbance affecting the balance of one will inevitably affect the balance of the others. This emphasizes the close physiological relationships between acid–base balance and electrolyte and water balance.

**Excretion of acid urine and regeneration of bicarbonate**

So far we have discussed only the excretion of H\(^+\) ions in so far as this is required for the reabsorption of filtered bicarbonate. However, in addition to this any H\(^+\) ions produced metabolically must be excreted otherwise they will accumulate and themselves cause an acid–base disturbance. As was discussed in Chapter 2, many of the end-products of normal metabolism are acids, and the non-volatile ones, the so-called ‘fixed acids’, are excreted by the kidneys. Hence the pH of normal urine is somewhat acidic, the actual value depending greatly on the diet but generally in the range of 5–6.5. The principal non-volatile acid requiring to be excreted by a normal individual is sulphuric acid produced by the oxidation of the sulphur-containing amino acids. Some phosphoric acid is also excreted and in certain circumstances lactic, acetoacetic and \(\beta\)-hydroxybutyric acids. The last three acids are normally completely oxidized to carbon dioxide and water but in certain circumstances may be produced faster than they can be oxidized. As was seen in Chapter 2, the addition of an acid which is stronger than carbonic acid displaces the equilibrium

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

to the left according to the law of mass action. For example, the addition of sulphuric acid to plasma reduces the bicarbonate concentration thus:

\[
\text{H}_2\text{SO}_4 + 2\text{HCO}_3^- \rightarrow 2\text{CO}_3^- + 2\text{H}_2\text{O} + \text{SO}_4^{2-}
\]

bicarbonate volatile

lost

The strong acid has been neutralized and is now available for excretion as a neutral salt. The CO\(_2\) is volatile and so can be blown off by the lungs. But the bicarbonate has been lost: thus the buffering capacity of the blood is reduced. The blood pH falls slightly according to the Henderson–Hasselbalch equation:

\[
\text{pH} = \text{pK} + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}
\]

The pH drop is of course far less than if no bicarbonate buffer were present. If further acid is added to the blood more bicarbonate is lost and ultimately all the bicarbonate, the so-called alkali reserve, could be lost. Further addition of acid would then cause a precipitous fall in pH.
The kidney counteracts this continual depletion of the bicarbonate buffer by regenerating $\text{HCO}_3^-$ and simultaneously excreting $\text{H}^+$ into the tubular luminal fluid. Hence the non-volatile acid is eliminated from the body and excreted into the urine.

The process of bicarbonate regeneration is therefore exactly the same as the mechanism for bicarbonate reabsorption. The sources of $\text{CO}_2$ which became hydrated for the purposes of reabsorption and regeneration within the tubule cell are indistinguishable. They are both acidified bicarbonate in the glomerular filtrate (luminal fluid) and $\text{CO}_2$ from the peritubular fluid and also from the tubule cells’ own aerobic metabolism. Note that the work in actively transporting $\text{H}^+$ ions out of the cells requires considerable expenditure of metabolic energy, and that the kidneys have a remarkably high $\text{QO}_2$ and hence an associated high $\text{CO}_2$ production.

The process of bicarbonate regeneration is summarized schematically in Fig. 6.2.

---

The overall effect is as though intracellular acid is being ‘split’ to produce acid ($\text{H}^+$) which is passed into the urine and base ($\text{HCO}_3^-$) which is passed into the blood (Fig. 6.3). Thus for every one mole of acid which is excreted into the urine one mole of buffer base is regenerated and passes into the blood.
In a normal individual the amount of acid thus excreted and the corresponding amount of bicarbonate regenerated is quantitatively relatively small (about 50 to 100 mmol/day or mEq/day) compared with the amount of volatile acid, CO₂, excreted (about 13 000–25 000 mmol/day or mEq/day) and the amount of filtered bicarbonate which is reabsorbed in the proximal tubule (about 4300 mmol/day or mEq/day). Nevertheless it is an extremely important aspect of renal function. In disease the amount of ‘fixed acid’ to be excreted, and thus of bicarbonate to be regenerated, may be very much greater, say up to 750 mmol or mEq/day in diabetic ketoacidosis. In renal disease the ability of the kidney to do this and to reabsorb filtered bicarbonate may be greatly impaired.

Sodium and water reabsorption

Although the details of the regulation of sodium and water reabsorption are outside the scope of this book and may be found in any textbook of renal physiology, it is important to appreciate the close interrelationships between acid–base balance, electrolyte balance and water balance.

The cation exchange (Na⁺/H⁺ and Na⁺/K⁺) mechanisms discussed above, however, account for only a quite trivial fraction of sodium reabsorption. Hydrogen ion excretion amounts to only about 4400 mmol (mEq) per day (i.e. 4300 mmol for HCO₃⁻ reabsorption and 100 mmol appearing in urine as titratable acid, that is H⁺ buffered by phosphate and ammonium ions), and K⁺ ion excretion to about 50 mmol/day. In contrast the total sodium ion reabsorption is of the order of 25 000 mmol (or mEq) per day. Thus the major route of reabsorption of Na⁺ is independent of this exchange mechanism, but is absorbed in association with chloride anions by other active transport system(s).

About 60–70% of salt and water reabsorption occurs in the proximal tubules: the distal nephron appears to be the site of fine control of reabsorption. Sodium reabsorption appears to be the primary active process, occurring against both electrical and chemical gradients, and inhibited by metabolic inhibitors: chloride follows passively to maintain electroneutrality and water follows as a secondary passive process to maintain osmotic balance. However measurements of electrical potential suggest that it may be chloride transport which is the active process in the thick ascending limb of the loop of Henle (diluting segment): it is in this region that several major diuretics are thought to act. Inhibition of sodium reabsorption by ouabain, a cardiac glycoside, suggests that a Na⁺–K⁺-dependent ATPase enzyme may be involved in the transport process, as in many other types of cell.

Control of sodium excretion

Renal control of net sodium excretion is via the rate of sodium reabsorption. It is normally so effective that the amount excreted in the urine can be regulated from about 1 to 600 mmol (mEq) per day in order to keep the total body Na⁺ content constant and such that excretion can be kept constant in spite of large changes in the glomerular filtration rate.

Control of sodium reabsorption

Control of sodium reabsorption is effected by a number of factors, physical, chemical and hormonal. The efferent fibres of the sympathetic nervous system also appear to affect Na reabsorption. The physical factors involved are mainly haemodynamic and osmotic: the peritubular colloid osmotic pressure and the hydrostatic pressure in the tubular capillaries are likely to be particularly important. The relevant chemical factors
include the capillary bicarbonate and potassium concentrations. Hormones which are implicated include aldosterone, angiotensin and the proposed but elusive natriuretic hormone.

Aldosterone stimulates sodium reabsorption in the distal nephron: it (and cortisol) increases both Na\(^+\) and Cl\(^-\) reabsorption and Na\(^+\)/H\(^+\) exchange as already discussed. However since its action involves protein synthesis there is a time lag and so this hormone cannot be implicated in the minute-to-minute regulation of sodium reabsorption. Aldosterone secretion is relatively unaffected by ACTH but appears to be largely controlled by the extracellular volume (probably via baro- and osmo-receptors) and the ECF sodium content.

Angiotensin may also be involved in the regulation of Na\(^+\) excretion although many facts are uncertain. For example one author states that angiotensin infusion increases sodium retention in normal individuals while the opposite effect was recorded in hypertensive patients. Studies in dogs have suggested that angiotensin II acts as an inhibitor of distal tubular sodium reabsorption, and hence this factor has been implicated as a likely regulator of distal salt transport. Secretion of the hormone appears to be dependent on the arterial perfusion pressure and it has been suggested that this is sensed by the juxtaglomerular apparatus. In the dogs the sodium reabsorption increased when the arterial perfusion pressure fell. The active angiotensin appears in the blood stream following the release of an enzyme, renin, from granular cells in the juxtaglomerular apparatus in the kidney in response to stimuli similar to those responsible for aldosterone secretion. This enzyme catalyses the conversion of ‘renin substrate’ in the plasma into angiotensin I which is then transformed into angiotensin II, the active form. Angiotensin may also have a trophic effect on the secretion of aldosterone from the adrenal cortex.

A natriuretic hormone (the so-called ‘third factor’) is also thought to exist which finely controls sodium reabsorption over short time intervals. It appears to reduce Na\(^+\) reabsorption in the proximal tubules. There is experimental evidence from cross-circulation experiments in animals that it may originate in brain, possibly the hypothalamus. A satisfactory assay has not yet been devised, however. This hormone may be one of the important mechanisms by which expansion of extracellular volume, e.g. by infusion of isotonic NaCl, leads to increased sodium excretion.

**Control of water reabsorption**

While some 180 litres of glomerular filtrate are formed each day, the urinary output is only 1–1.5 litres in normal circumstances. Thus about 99.5% of the filtered fluid is reabsorbed. Water reabsorption is a purely passive process, the driving force coming from an osmotic gradient created mainly by Na\(^+\) and Cl\(^-\) ions. Sodium is the major extracellular cation, being present in normal plasma at about 145 mmol/litre. Together with accompanying anions (mainly Cl\(^-\), some HCO\(_3\), and traces of others) which must be present in equivalent amounts this accounts for 290 mmol/litre or mEq/litre. An ideal solute would thus exert an osmotic pressure of 290 mOsm/litre: deviation from ideality however results in an osmotic coefficient of around 0.93 so the true osmotic pressure associated with extracellular Na\(^+\) ions and their accompanying anions is about 269 mOsm/litre. The mean normal osmotic pressure of plasma is 291 mOsm/litre and so it is clear that the Na\(^+\) ions plus their accompanying anions account for 92% of the total osmotic pressure of plasma and are therefore crucially important in maintaining water balance and in the regulation of extracellular volume.

The active reabsorption of Na\(^+\) ions into the peritubular fluid therefore generates an osmotic gradient which draws water across the renal epithelium. Nevertheless the layer of cells does present a barrier to diffusion and so water reabsorption is retarded.
However the permeability of the renal epithelium in the distal tubules and particularly in the collecting ducts to water is increased by vasopressin (antidiuretic hormone, ADH), one of the polypeptide hormones released from the posterior pituitary in response to stimulation of osmoreceptors. Hence ADH increases water reabsorption and reduces diuresis. Defective synthesis or secretion of this hormone is seen in diabetes insipidus. The mechanism of its action is not well understood: it is thought to activate adenyl cyclase and thus to elevate the intracellular concentration of cyclic AMP. Its effect in increasing permeability may be regarded as analogous to creating pores in the cell membrane which are permeable to water molecules. Some workers believe that ADH may regulate the permeability of the intercellular cement by causing the secretion of hyaluronidase-like activity. However, the significance of water transport between cells as opposed to via the transcellular route has not been fully resolved.

The plasma levels of ADH and of aldosterone appear to be regulated by both the Na⁺ ion concentration and the osmotic pressure in plasma, the latter being sensed by osmoreceptors in the hypothalamus and carotid sinus. It is important to remember that regulation of the extracellular volume, which is critical to the maintenance of tissue perfusion, is one of the very important functions of the kidney. Indeed the need to maintain the volume and osmolarity of the extracellular fluid probably takes precedence in practice over the maintenance of acid–base homeostasis. Secretion of ADH may also be controlled by volume or pressure (baroreceptors) receptors, neurogenic stimuli and angiotensin. Its production, and also possibly its effect on the kidney, can be depressed by hypercapnia (high $P_{CO_2}$) with resultant diuresis.

Hence water reabsorption is regulated by secretion of ADH and by the reabsorption of Na⁺ ions. The latter in turn is controlled by many factors, some of which have been discussed briefly above, and part is related to the energy-dependent excretion of $H^+$ and $K^+$ ions and the reabsorption of $HCO_3^-$ ions. Thus it will be appreciated that acid–base, electrolyte and water balance are intimately related and that a disturbance in one is frequently reflected in imbalance in other factors.

**Excretion of lithium**

The renal excretion of lithium is of some interest since Li$^{2+}$ salts are used in psychiatry. Many features are similar to the renal handling of Na⁺ and it may be that lithium is reabsorbed by the Na⁺ pump in exchange for $H^+$ or $K^+$. Thus lithium is retained on a high Na⁺ intake and rapidly excreted (though not as rapidly as Na⁺) on a low sodium intake. However its excretion, unlike that of Na⁺, is unaffected by diuretics such as frusemide (furosemide) and ethacrynic acid; also Li$^{2+}$ excretion can somehow be hastened by infusion of urea, a fact which is sometimes exploited in the treatment of lithium poisoning in overdosage.
The Buffering of Urine

The need for urinary buffers

We have already seen how the renal tubule cells excrete H+ ions into the tubular luminal fluid which becomes urine (Chapter 6). If there were no buffers in urine the pH would fall drastically and the H+ ion concentration gradient between the tubule cells and the luminal fluid would steepen dramatically. A point would soon be reached where the work required to eject H+ ions from the cells against their concentration, or electrochemical, gradient would be so great that sufficient energy could not be supplied for the active transport process and so H+ excretion would cease. In practice it appears that the minimum pH of urine is about 4.4-4.5. This corresponds to a urinary H+ ion concentration which is probably 800-1000 times greater than that within the tubule cells (see Table 6.1).

Sources of urinary buffers

Glomerular filtrate contains the non-protein buffers of blood. The two principal buffer systems in normal urine are the phosphate system (wholly of filtered origin) and NH₃/NH₄⁺ (generated mainly in the tubule cells, see below). Normal urine is somewhat acidic (pH 5-6.5 or so, depending largely on the diet) and contains virtually no bicarbonate. It has already been stressed that bicarbonate is an important extracellular buffer which must be conserved in a normal individual and indeed any which is lost (e.g. in the buffering of strong acids) must be regenerated. However if the plasma [HCO₃⁻] is slightly higher than normal, as in a metabolic alkalosis, then reabsorption of HCO₃⁻ may not be complete since the renal threshold for bicarbonate excretion is normally around 26 mmol/litre. This value is however not constant. Then bicarbonate ‘spills over’ into the urine which becomes alkaline and the loss of HCO₃⁻ tends to ‘repair’ the alkalosis by bringing the abnormal plasma [HCO₃⁻] towards normal (see Chapter 9).

Production of an alkaline urine

While the kidney can produce an alkaline urine when there is an excess of base in the body such as arising from a pathological metabolic alkalosis or from the ingestion of certain diets such as citrate fruits, it must be noted that there is no actual mechanism for the tubular secretion of alkali. The production of an alkaline urine is achieved simply by the reabsorption of water, together with Na⁺ and Cl⁻ ions from the glomerular filtrate while there is failure to secrete H+ ions into the filtrate. Reabsorption of water from the filtrate raises its bicarbonate concentration, and if the CO₂ in the filtrate is in equilibrium with intracellular and peritubular CO₂, the [HCO₃⁻]/[CO₂] ratio rises. (In practice the Pco₂ in the filtrate is slightly higher than in the
plasma; however the ratio \([\text{HCO}_3^-]/[\text{CO}_2]\) is still raised in urine because proportionately the rise in \([\text{HCO}_3^-]\) is greater.) Therefore the pH of urine rises.

**Phosphate buffering**

The phosphate in the urine is derived from the diet and from the turnover of endogenous phosphorus compounds. As was seen in Chapter 3, the physiologically important dissociation of phosphoric acid is:

\[
\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-} \\
\text{acid} \quad \text{base}
\]

with a pK of around 6·8.

The other two dissociations of this tribasic acid:

\[
\text{H}_3\text{PO}_4 \rightleftharpoons \text{H}^+ + \text{H}_2\text{PO}_4^- \\
\text{HPO}_4^{2-} \rightleftharpoons \text{H}^+ + \text{PO}_4^{3-}
\]

have pK values of around 2·0 and 11·7 respectively. These pKs are sufficiently far from the pH values encountered in urine or body fluids, even in abnormal circumstances, that they are not at all physiologically relevant. Therefore \(\text{H}_3\text{PO}_4\) and \(\text{PO}_4^{3-}\) can be ignored since they can exist at physiological pHs only in negligible concentrations.

The phosphate concentration in plasma is too low for it to be important as a blood buffer. However as reabsorption of filtered phosphate is not complete, the concentration in the filtrate rises as water is reabsorbed. Hence the phosphate concentration in urine is high enough for this to contribute substantially to buffering. The actual urinary concentration varies greatly with the diet.

At the normal pH of 7·40 plasma and therefore glomerular filtrate (as it is filtered) contains \(\text{HPO}_4^{2-}\) (base) and \(\text{H}_2\text{PO}_4^-\) (acid) in the ratio 4 : 1.

Since

\[
\text{pH} = \text{pK} + \log \frac{[\text{base}]}{[\text{acid}]}
\]

\[
7·4 = 6·8 + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}
\]

so

\[
\frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = 3·98
\]

Secretion of \(\text{H}^+\) ions into the tubular luminal fluid (i.e. the process of acidification of urine) displaces the equilibrium:

\[
\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-} \\
\text{H}^+ \quad \text{H}_2\text{PO}_4^- \\
\]

to the left. If \(x\) mmol (mEq) of \(\text{H}^+\) ions are secreted then the amount of the base \(\text{HPO}_4^{2-}\) will fall by \(x\) mmol (mEq) and the amount of the acid \(\text{H}_2\text{PO}_4^-\) will increase by
the urinary buffer of major importance is the ammonium ion. Since ammonia is a relatively weak acid (or strong base) with a pK_a of about 8.2 the equilibrium:

\[ \text{NH}_3 + H^+ \rightleftharpoons \text{NH}_4^+ \]

must lie, for practical purposes, entirely to the right at any pH below 8, i.e. \( \text{NH}_3 + H^+ \rightarrow \text{NH}_4^+ \). Hence urine may contain much ammonium ion but it contains negligible ammonia. This should be obvious because one can detect low concentrations of \( \text{NH}_3 \) by smell, and uncontaminated fresh urine has no such smell. Strictly speaking, ammonia does not behave as a buffer at the pHs of glomerular filtrates and urine if a buffer is defined as a mixture of a weak acid and its conjugate base. In fact \( \text{NH}_4^+ \) behaves as an effective ‘sink’ for \( H^+ \) ions, since ammonia combines irreversibly with \( H^+ \) ions at the pH of urine.

The high pK of this buffer is very valuable, because it means that the kidney can continue excreting \( H^+ \) ions into a tubular luminal fluid which has already reached the minimum pH of 4.4-4.5 provided that one equivalent (or mole) of \( \text{NH}_3 \) is excreted for
each equivalent (or mole) of H\(^+\) ion excreted. The NH\(_3\) thus can combine with the H\(^+\), so that the H\(^+\) ion concentration does not rise in spite of the addition of H\(^+\) ions to the urine. Hence continued excretion of acid is possible, even when the minimum urinary pH is reached. However this should not be taken to imply that NH\(_4^+\) buffering becomes significant only when the minimum urinary pH is reached or approached. All urine contains some NH\(_4^+\) but the amount excreted increases as the amount of H\(^+\) ions to be excreted increases so that under some circumstances the amount of NH\(_4^+\) is inversely proportional to the urinary pH. However the control mechanisms governing ammonia excretion are not fully understood (see below). Thus in a severe metabolic acidosis where renal function is normal, e.g. a diabetic ketosis, the amount of NH\(_4^+\) in the urine can be ten times the amount in a normal urine. The latter is of the order of 30–50 mmol or mEq/day.

Regulation of ammonia excretion

Some measure of control over ammonia excretion is given by physicochemical considerations. The more acid the tubular luminal fluid is the steeper is the NH\(_3\) concentration gradient between cell and tubule lumen. Hence the faster NH\(_3\) diffuses ‘downhill’ out of the cells. However, more sensitive adaptive mechanisms are almost certainly operative as well. Many studies have shown that glutaminase appears to be induced in chronic (long-term) metabolic acidosis; however this is apparently not essential for increased NH\(_3\) excretion since actinomycin D, an inhibitor of protein synthesis, blocks this increased synthesis of enzyme but apparently has no effect on ammonia production. Also no induction of glutaminase occurs in respiratory acidosis in spite of an increase of NH\(_3\) excretion. The actinomycin D experiments also appear to rule out the possibility that adaptive induction of other enzymes is involved.

Glutaminase is inhibited by ammonia and glutamic acid, an example of product inhibition. In turn the intracellular NH\(_3\) concentration depends on the concentration gradient between the cell and the tubular luminal fluid. Thus when a highly acid urine is being produced ammonia diffuses out of the cells faster, the intracellular NH\(_3\) concentration falls, and so the synthesis of further NH\(_3\) by deamidation of glutamine is promoted. This is a form of feedback control.

Direct or indirect effects of pH on enzymes concerned in ammonia production have also been postulated. For instance the citrate cleavage enzyme which catalyses AcCoA + oxaloacetate → citrate is pH-sensitive.

Also glutamate, whose intracellular concentration is pH-dependent and which falls in acidosis, is known to inhibit glutaminase (i.e. product inhibition). Some authorities believe that the regulation of mitochondrial membrane permeability, which governs the access of glutamine to the intramitochondrial enzymes glutaminase and glutamate dehydrogenase, may be the key to the control of ammonia production. Although this theory is attractive it cannot be said to be proved.

Sources of ammonia

The source of the urinary ammonium ion is ultimately protein, both dietary and endogenous, which is of course always turning over. The normal end-product of protein metabolism is urea which is neutral: in a prolonged severe acidosis urea excretion is diminished while NH\(_4^+\) excretion increases. This is not because urea is a precursor of urinary ammonia but because amino acids are a common precursor of both. The principal source of urinary ammonia is glutamine, an amino acid which is the amide of glutamic acid:
Glutamate acid

Glutamine is the main form in which ammonia is carried in the blood and is present in higher concentrations than any other amino acid; unlike ammonia, it is non-toxic and is formed in several tissues especially liver. Its formation requires energy in the form of ATP and is catalysed by glutamine synthetase. Hydrolysis of the amide group is, however, catalysed by glutaminase and the free energy of hydrolysis is not trapped by ATP. Kidney contains a high activity of glutaminase. The α-amino groups of glutamine and of other amino acids also make a smaller contribution to the excreted ammonia following transamination and oxidative deamination catalysed by glutamate dehydrogenase. Free ammonia in blood also makes a small but significant contribution to the total urinary ammonia.

The unionized ammonia thus formed within the tubule cells passes, supposedly by passive diffusion, down its concentration gradient into the tubular luminal fluid where it combines with H+ ions. The tubule cell membrane is relatively impermeable to ammonium ions according to the theory of non-ionic diffusion, and so the ammonium ions formed in the tubular lumen are excreted in the urine instead of diffusing back into the cells.

Cation ‘sparing’ effect of ammonium ion excretion

A further major advantage in the urinary excretion of NH4+ ions is that this ‘spares’ other cations. Thus for example when acetoacetic acid (pK = 3.6) is being excreted a

\[ \text{NH}_3 \rightarrow \text{NH}_4^+ \text{ in urine} \]

...
large fraction is present as the ionized form, i.e. base or salt, acetoacetate. This must be accompanied by an equivalent amount of cations. If Na⁺ or K⁺ ions are lost this can represent a major undesirable loss of these ions and indeed loss of Na⁺ or K⁺ ions can be a serious consequence of, for example, diabetic ketoacidosis. When however NH₄⁺ ions are excreted they can balance the anions so that, in effect, ammonium acetoacetate is excreted, and Na⁺ and K⁺ ions are ‘spared’. There is a delay during the initial phase of a metabolic acidosis in the ammonia excretion mechanism reaching its maximum capacity and ‘catching up with’ H⁺ ion production: during this lag period loss of other cations can be particularly important.

It might be thought that the formation of NH₄⁺ is of no net benefit in the excretion of H⁺ ions since the hydrolysis of glutamine results in the production of glutamic acid which is itself acidic (see foot of page 49). With a pK around 4 the γ-carboxyl group of glutamic acid is thus wholly ionized at physiological pHs. Thus, considering the kidney alone there has been no net loss of H⁺. But if one considers the whole body situation, and considers also the synthesis of glutamine in the liver, there is a net loss of H⁺.

**Summary of processes in ammonia excretion**

Fig. 7.1 summarizes the process of renal excretion of ammonia.

**Creatinine and other buffers**

Creatinine, which is derived from creatine, is excreted in urine. It is a weak acid with a pK of about 4.9 and so behaves as a buffer.
Other weak acids, especially those excreted in abnormal urine such as \( \beta \)-hydroxybutyric acid (\( \text{pK} 4.5 \)) can contribute significantly to the titratable acidity, e.g. in diabetic ketoacidosis.

Tests of urinary acidification power

In some investigations of renal function a metabolic acidosis is created by the ingestion of ammonium chloride. As noted in Chapter 8, this is equivalent to the ingestion of acid. Essentially, one version, the 'short test', consists of making serial collections of urine in a fasted patient at hourly intervals after administration of 0.1 g NH₄Cl/kg body weight. Blood samples may also be collected. If renal tubular function is normal, then the urinary pH should fall to below 5.3 in at least one of the samples. The total \( \text{H}^+ \) ion excretion rate should rise to above 60 \( \mu \text{mol/min} \). This should include titratable acidity excretion of over 25 \( \mu \text{mol/min} \) plus ammonium ion excretion of over 35 \( \mu \text{mol/min} \).
Definition of metabolic disturbances
All acid-base disturbances of non-respiratory origin are conveniently classed together as metabolic disturbances. A metabolic acidosis is associated with a decreased plasma bicarbonate concentration; conversely a metabolic alkalosis is associated with an elevated bicarbonate concentration. Obviously, if there is no respiratory compensation and no other primary acid-base disturbance the plasma pH will be low in a metabolic acidosis and high in a metabolic alkalosis.

Indicators of the metabolic factor
As explained in Chapter 4 the elevated \( P_{CO_2} \) associated with a respiratory acidosis inevitably causes a rise in the plasma bicarbonate concentration since the equation:

\[ CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \]

must be slightly displaced to the right according to the law of mass action. Furthermore, when a buffer such as haemoglobin is present to combine with the \( H^+ \) ions formed the equilibrium can move further to the right than would otherwise be possible. Hence more bicarbonate is formed. Such an elevation of plasma bicarbonate is a consequence solely of a respiratory disturbance and is not indicative of a metabolic acidosis. Therefore measurement of the actual bicarbonate concentration in plasma cannot by itself show with infallible reliability whether the metabolic component is normal. Assessment as to whether there is metabolic acidosis or alkalosis can be made from the actual bicarbonate concentration if the \( P_{CO_2} \) or pH is known, or from derived parameters, the standard bicarbonate or base excess values if these values are known. These terms are defined below. Abnormal values will be obtained either if there is a primary metabolic disturbance or if there is a secondary compensatory disturbance, i.e. secondary to a respiratory disturbance. However, such secondary compensatory events are not normally described as acidosis or alkalosis since the terms acidosis and alkalosis should be reserved to describe primary disturbances.

Standard bicarbonate
Standard bicarbonate is a theoretical concept which estimates numerically what the actual bicarbonate concentration would be if the respiratory component (\( P_{CO_2} \)) were normal at 40 mmHg (5.33 kPa). Thus the influence of the respiratory component on the bicarbonate concentration can be eliminated, and one can assess directly whether the metabolic component is normal (standard bicarbonate = 22–26 mmol/litre or mEq/litre) or whether there is metabolic acidosis (standard bicarbonate < 22 mmol/litre or mEq/litre) or metabolic alkalosis (standard bicarbonate > 26 mmol/litre). The
premise that the standard bicarbonate is unaffected by the respiratory factor is, however, not strictly valid if a respiratory disturbance alters the distribution of \( \text{HCO}_3^- \) between blood and the tissue fluids.

Some workers criticize the use of standard bicarbonate on the grounds that it is an artificially derived value with, it is claimed, little physiological value. A more severe drawback is that the calculation of standard bicarbonate involves certain assumptions about the behaviour of bicarbonate in blood in vivo. In particular, it is assumed that the 'in vivo titration curve' is accurately reflected by the 'in vitro titration curve'. Although this may be true over a limited range, there is evidence that this assumption is not universally valid (see Chapters 3 and 10).

**Total buffer base**

The sum of the concentrations of all buffer bases is given by:

\[
[\text{Total buffer base}] = \sum [\text{HCO}_3^-] + [\text{Hb}^-] + [\text{HbO}_2^-] + [\text{traces of other anions}]
\]

This has a normal value for each individual which depends on the total haemoglobin concentration, and any deviation from the normal value reflects a metabolic abnormality. Respiratory disturbances, although they affect the actual bicarbonate concentration, do not alter the total buffer base concentration since they also affect the haemoglobin anion concentration. Thus:

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \\
\text{H}^+ + \text{Hb}^- \rightleftharpoons \text{HHb}
\]

So an elevated \( \text{PCO}_2 \) causes an increase in the actual bicarbonate concentration but a corresponding decrease in the (oxy)haemoglobin anion, i.e. base, concentration. Therefore the total buffer base concentration remains constant.

**Base excess**

An alternative parameter or variable which some clinicians prefer as the index of the metabolic factor is termed the *base excess*. The base excess is the difference between the observed total buffer base and the 'normal' buffer base value taking into account the particular patient's (total) haemoglobin concentration. **Definition:** the base excess is equal to the amount of base which has been added to the blood and so is equal to the amount of strong acid which would have to be added to the blood in order to titrate it back to pH 7-4 at a \( \text{PCO}_2 \) of 40 mmHg (5·33 kPa) at 37°C. A negative value of base excess signifies a base deficit and in this case is equal to the amount of base which would have to be added to the blood in order to titrate it back to pH 7·4 at a \( \text{PCO}_2 \) of 40 mmHg at 37°C. Obviously there should not be any base excess or deficit, so the normal value is 0±2 mmol/litre or mEq/litre; negative values of base excess signify a metabolic acidosis and positive values a metabolic alkalosis.

Advocates of base excess use this value to calculate the quantity of acid or base required to 'titrate' the patient back to normality. However, formulae for this are, at best, approximations and rigid adherence to such formulae is highly inadvisable or downright foolish. Typical formulae and their inherent assumptions are discussed in Chapter 9.

An additional difficulty arises in the interpretation of negative values of base excess. Although the physiological meaning of such values is straightforward in that there is a deficit of base in the blood—a metabolic acidosis exists—which can be corrected by the administration of the appropriate amount of base, some clinicians find negative values confusing. It is important that a clinician should be sufficiently familiar with the meaning of the numerical data that he generates or receives from a laboratory to be able to interpret them without doubt or apprehension.
Respiratory acidosis and alkalosis do not produce changes in the standard bicarbonate, total buffer base or base excess unless secondary renal compensation occurs.

**Difficulties in evaluating the metabolic component**

It will be apparent from comments above and from the fact that special terms have had to be coined to describe the metabolic acid–base balance of patients, that evaluation of the metabolic factor is much less straightforward than that of the respiratory factor. There are two main reasons for this: (1) the fact that the actual bicarbonate concentration in the plasma depends also on the respiratory component as discussed above, and (2) the plasma bicarbonate concentration itself cannot be directly measured (unlike the $P_{CO_2}$) but is inferred indirectly from several other measurements. These are discussed in Chapter 10. These difficulties are reflected in the controversies which surround the use of the various parameters and their associated nomenclature. This is a typical area where heated differences of opinion as to the superior methods exist between different centres. Regardless of one’s personal preference it is essential that the clinical and physiological implications of the data are recognized, whether they be expressed as, for example, standard bicarbonate or base excess.

**Table 8.1**

*Typical values characteristic of metabolic acidosis*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard bicarbonate</td>
<td>$&lt; 22$ mmol/litre (or mEq/l)</td>
</tr>
<tr>
<td>Base excess</td>
<td>$&lt; -2$ mmol/litre</td>
</tr>
<tr>
<td>Actual bicarbonate</td>
<td>$&lt; 22$ mmol/litre</td>
</tr>
<tr>
<td>$P_{CO_2}$</td>
<td>$&lt; 35$ mmHg (4.66 kPa)</td>
</tr>
<tr>
<td></td>
<td>or 35–45 mmHg (4.66–6.00 kPa)</td>
</tr>
<tr>
<td></td>
<td>$&gt; 44$ mmHg (6.00 kPa)</td>
</tr>
<tr>
<td>pH</td>
<td>$&lt; 7.35$</td>
</tr>
<tr>
<td></td>
<td>or 7.35–7.45</td>
</tr>
<tr>
<td></td>
<td>$&gt; 7.45$</td>
</tr>
</tbody>
</table>

i.e. base deficit $> 2$ mmol/litre unless there is also a complicating primary respiratory acidosis (i.e. $P_{CO_2} > 44$ mmHg, 5.86 kPa) which may be of unrelated aetiology if there is respiratory compensation and/or a complicating respiratory alkalosis. Respiratory compensation would be expected in a chronic metabolic acidosis only if there is also a complicating respiratory acidosis which may be of unrelated aetiology if there is no (or incomplete) respiratory compensation. There may be complicating (primary) respiratory acidosis only if there is a complicating (primary) alkalosis which may be of unconnected aetiology
Metabolic acidosis

The clinical picture very much depends on the underlying cause of the acidosis. However, any severe metabolic acidosis is likely to be accompanied by weakness, possibly nausea and vomiting, and characteristic deep respirations. The compensatory ventilation stimulated by the reduced plasma pH is often very deep and has been described as ‘air hunger’ or Kussmaul respiration. Some values characteristic of metabolic acidosis are shown in Table 8.1.

Causes of metabolic acidosis

The causes of metabolic acidosis can be divided into three broad categories:

1. Excessive production of metabolic H+ ions (i.e. rate of production of acids exceeds their rate of catabolism) or ingestion of H+ ions.
2. Failure to excrete H+ ions as fast as they are produced (i.e. impaired renal function).
3. Direct loss of bicarbonate.

Excess metabolic production or ingestion of acid

Hydrogen ions accumulate in the body when the production or ingestion of acids exceeds the rate at which the kidney is able to dispose of H+ ions. Typical examples of excess production of H+ ions include accumulation of acetoacetic and β-hydroxybutyric acids in diabetic ketoacidosis and, to a lesser extent, in starvation where carbohydrate sources have been exhausted and lipid stores are being mobilized and of lactic acid in tissue hypoxia. Tissue breakdown in shock, severe infections, major trauma or fever and thyrotoxicosis (where the metabolic rate greatly increases) also can cause excess production of ketoacids from partial oxidation of fat stores and of sulphuric acid from oxidation of the sulphur-containing amino acids of protein origin. Phosphoric acid may also accumulate due to the increased breakdown (catabolism) of endogenous nucleic acids and phospholipids.

Lactic acidosis

Lactic acid can be formed faster than it is oxidized in a variety of circumstances ranging from severe exercise (when muscular tissues develop an oxygen debt and utilize carbohydrate by anaerobic glycolysis) to any pathological cause of tissue hypoxia. Thus lactic acidosis may complicate shock (where the peripheral circulation may be so reduced as to cause hypoxia), acute respiratory hypoxia (but lactic acidosis is not often seen in chronic respiratory insufficiency), use of cardiopulmonary bypass (where oxygen supply is inadequate), acute myocardial infarction (again, local hypoxia both in the myocardium and in other consequently poorly perfused tissues) and pancreatitis. Indeed any local cause of tissue hypoxia is a potential cause of lactic acidosis.

Cardiac arrest or circulatory failure causes accumulation of lactic acid and the resuscitation procedure should include the urgent injection of bicarbonate (NaHCO₃) because myocardial contraction is impaired by the acidosis; 200–500 mmol (mEq) in a sustained arrest is recommended by some authorities although such a large Na load can itself pose subsequent cardiac problems. Normal rhythm is therefore more likely to be reinstated if the acidosis has been alleviated. Careful monitoring of acid–base and potassium status is often required in a post-arrest patient, in addition to the obvious ECG surveillance.

Aerobic oxidation of glucose can be inhibited, especially in susceptible individuals, by the oral antidiabetic biguanides such as phenformin and metformin since these
drugs can inhibit electron transport in the respiratory chain as well as uncoupling oxidative phosphorylation. Therefore the cellular [NADH]/[NAD] ratio rises and so the ratio of [lactate] to [pyruvate] rises correspondingly:

\[
\text{pyruvate} \rightleftharpoons \text{lactate} \\
\text{NADH} \quad \text{NAD}
\]

Accumulation of lactate can also accompany acute alcohol intoxication since the oxidation of ethanol causes the reduction of NAD to NADH so that the [NADH] to [NAD] ratio again increases. (Ingestion of methanol can cause metabolic acidosis directly due to the metabolic production of formic acid.)

Lactic acidosis is also seen in type I glycogen storage disease (von Gierke's disease) where there is a hereditary deficiency of glucose-6-phosphatase. Glucose-6-phosphate accumulates since glucose cannot be formed; hence levels of all glycolytic intermediates including lactic acid accumulate. Administration of adrenaline which promotes the mobilization of glycogen reserves also elevates blood lactate levels.

Occasionally a severe lactic acidosis (‘spontaneous lactic acidosis’) is encountered which is of unknown origin and does not appear to be caused by hypoxia.

**BLOOD TRANSFUSION**

On storage the pH of blood falls due to the addition of ACD (acid–citrate–dextrose anticoagulant and preservative) and the production of lactic acid by the cells (N.B. red cells do not have mitochondria and therefore cannot oxidize lactate; only anaerobic glycolysis is possible). Transfusion of large quantities of banked blood, as in major surgery, can therefore cause a metabolic acidosis. Indeed the pH of stored blood can be as low as 6.5 after 21 days. This complication can be aggravated by the fact that on storage some of the intracellular K⁺ is lost into the plasma since the maintenance of the high cellular K⁺ requires expenditure of metabolic energy. The increase in extracellular K⁺ in the transfused blood (up to 15–25 mmol/litre instead of 4 mmol/litre) means that the recipient’s kidneys will excrete more K⁺ and less H⁺ than usual (since both ions in effect compete for exchange with Na⁺). Hence H⁺ is retained. Additionally the hyperkalaemia caused by the transfusion may cause serious cardiac arrhythmias. These considerations make fresh blood desirable in certain circumstances, especially where transfusion of large volumes is contemplated. However, massive transfusions can also lead to a metabolic alkalosis, since the citrate in the ACD can be metabolized to bicarbonate (see below).

**INGESTION OR INFUSION OF ACIDS**

Ingestion or infusion of acids such as HCl or drugs which are strong carboxylic acids such as aspirin causes metabolic acidosis. If ammonium chloride is ingested the ammonium moiety is excreted as urea, a neutral molecule. The Cl⁻ then is equivalent to HCl and so causes a metabolic acidosis: hence NH₄Cl can be used therapeutically to correct a metabolic alkalosis.

Diagrammatically the sequence of reactions can be written:

\[
\begin{align*}
\text{NH}_4\text{Cl} & \rightarrow \text{NH}_4^+ + \text{Cl}^- \\
\text{NH}_3 + \text{H}^+ & \rightarrow \text{urea (neutral)} \\
& \text{excreted}
\end{align*}
\]

\[
\text{net gain of HCl}
\]
or

\[
\text{NH}_4\text{Cl} \rightleftharpoons \text{NH}_4^+ + \text{Cl}^- \rightleftharpoons \text{NH}_3 + \text{H}^+ + \text{Cl}^-
\]

\[
\downarrow \text{urea (neutral)}
\]

\[
\text{excreted}
\]

The same fate is shared by lysine hydrochloride and arginine hydrochloride.

Infusion of large quantities of saline can cause an acidosis because the ratio of Na\(^+\) : Cl\(^-\) in sodium chloride saline is 1 : 1 while the ratio in normal extracellular fluid is about 145 : 100, the remaining Na\(^+\) being counterbalanced by HCO\(_3^\)\(^-\). Hence, relative to the sodium concentration, the Cl\(^-\) concentration rises and the HCO\(_3^\)\(^-\) concentration must fall. In effect, sodium is then excreted as sodium bicarbonate, while Cl\(^-\) is retained as HCl. This is referred to as a dilution acidosis, since the extracellular fluid (ECF) bicarbonate is in effect being diluted by the infusion.

In practice chlorides are often given in the correction of a metabolic alkalosis (see below) if the alkalosis is associated with potassium deficiency then KCl is indicated. If the alkalosis is accompanied by extracellular volume depletion and NaCl deficiency then NaCl should be administered.

**Inadequate acid excretion**

In Chapter 6 it was seen how the formation of an acid urine required a renal mechanism for the active transport (secretion) of H\(^+\) ions against a concentration gradient into the urine in exchange for Na\(^+\) ions. In Chapter 7 it was seen how the continued functioning of this process was facilitated by the urinary buffers which minimize the H\(^+\) ion concentration gradient against which the ions have to be pumped. Impairment of glomerular filtration or of the active transport mechanism (which requires metabolic energy and in the distal tubule is stimulated by aldosterone) or of ammonia production will result in inadequate H\(^+\) excretion and necessarily in an exactly equivalent reduction in bicarbonate reabsorption and regeneration by the kidney.

**Impaired renal function**

Many kinds of renal disease, chronic and acute, thus cause metabolic acidosis. A decrease in glomerular filtration accompanied by a decrease in the total number of functional nephrons causes the partial retention of urea (hence uraemia or azotaemia, i.e. retention of nitrogen), creatinine and acids such as sulphuric and phosphoric acids. As already discussed (p. 4) these relatively strong acids can reduce the plasma bicarbonate concentration, the lost bicarbonate being converted to volatile CO\(_2\) which is blown off by the lungs. This is sometimes referred to as depletion of the alkali reserve.

If glomerular filtration is nearly normal but there is severely impaired renal tubular function (e.g. in chronic pyelonephritis) then there is renal tubular acidosis but no uraemia. Potassium loss due to excess secretion (e.g. due to excess aldosterone) and possibly occasionally due to incomplete reabsorption leads to hypopotassaemia (hypokalaemia). The urine in this case may be of relatively higher pH than normal, generally above pH 6.5.

In disease the renal production of ammonia may be impaired. Hence the buffer capacity of urine may be reduced and this may aggravate a metabolic acidosis since the kidney's ability to excrete H\(^+\) ions into the urine is reduced as the H\(^+\) ion concentration gradient is increased (see Chapter 7).
CARBONIC ANHYDRASE INHIBITORS

Excretion of H⁺ ions is also impaired when carbonic anhydrase activity is inhibited. As already seen in Fig. 6.1, this enzyme in renal tubule cells plays a key role in both the reabsorption of bicarbonate and the excretion of H⁺ ions. Carbonic anhydrase inhibitors such as acetazolamide (Diamox) are used therapeutically as diuretics. Acetazolamide is also prescribed in epilepsy and glaucoma. Some thiazides, and modified thiazides, which are used as diuretics are also carbonic anhydrase inhibitors. However in most cases this effect is so mild as not to cause a disturbance of acid–base balance: they tend to cause more chloride than bicarbonate to be lost into the urine. Inhibition of the hydration of CO₂ results in less H⁺ ions being available for exchange with Na⁺ ions. Thus Na⁺ ion reabsorption is reduced and there is therefore less osmotic force for the reabsorption of water—hence the desired diuretic action. Since smaller amounts of H⁺ ion are available for excretion, greater amounts of K⁺ ion are lost (recall that H⁺ and K⁺ ions in effect compete with each other for exchange with sodium). Thus large doses of carbonic anhydrase inhibitors are associated with increased loss of water, K⁺, Na⁺ and HCO₃⁻ and with increased retention of H⁺ and hence metabolic acidosis.

HYPOALDOSTERONISM

As already noted, the H⁺/Na⁺ exchange active transport mechanism is stimulated by aldosterone. Hence if there is a lack of aldosterone due to adrenocortical insufficiency, as in Addison’s disease, or in the presence of an aldosterone antagonist such as spironolactone, which is a synthetic diuretic, H⁺ and K⁺ excretion will be impaired. Hence a metabolic acidosis is produced. Sodium (and Cl⁻), and therefore water, loss will be increased. In untreated Addison’s disease there may also be failure to form renal ammonia, although the reasons for this are not understood.

Table 8.2

Summary of general causes of metabolic acidosis

<table>
<thead>
<tr>
<th>Cause</th>
<th>Example (see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input of excess H⁺ ions</td>
<td>Ingestion or infusion of acids or potential acids</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl, HCl, lysine and arginine hydrochlorides, lactic acid (in banked blood), aspirin (salicylates), methanol</td>
</tr>
<tr>
<td>Metabolic production of strong acids</td>
<td>Lactic acid (hypoxia, cardiac arrest, biguanides, ethanol, shock, glycogen storage disease, exercise, etc.)</td>
</tr>
<tr>
<td></td>
<td>Keto-acids (diabetes, starvation, trauma, thyrotoxicosis, etc.)</td>
</tr>
<tr>
<td>Diminished renal output of H⁺ ions</td>
<td>Renal failure</td>
</tr>
<tr>
<td></td>
<td>Inadequate NH₃ secretion, lack or antagonism of aldosterone, inability to pump H⁺ ions out of tubule cells, inhibition of carbonic anhydrase</td>
</tr>
<tr>
<td>Loss of base</td>
<td>From gastrointestinal tract</td>
</tr>
<tr>
<td></td>
<td>Diarrhoea, fistulae or uretero-enterostomy</td>
</tr>
<tr>
<td></td>
<td>From kidneys into urine</td>
</tr>
<tr>
<td></td>
<td>Renal failure, inhibition of carbonic anhydrase</td>
</tr>
</tbody>
</table>
Direct loss of base

In a healthy individual on a normal European-type diet, the urine is, to all intents, free of bicarbonate. If, however, renal function is impaired the reabsorption of bicarbonate may be incomplete in just the same way as the excretion of $H^+$ may be incomplete as discussed above. Hence bicarbonate is lost.

Pancreatic, biliary and jejunal secretions are distinctly alkaline, having a much greater bicarbonate concentration (about 120 mmol/litre) than that in plasma (about 24 mmol/litre) and therefore a lower [Cl$^-$] than in plasma. Therefore loss of these secretions in diarrhoea or through fistulae causes a direct loss of buffer base (HCO$_3^-$) and therefore a metabolic acidosis.

Severe metabolic acidosis sometimes develops in patients with ureteroenterostomy (ureterointerostomy, i.e. transplantation of the ureters to drain into the colon carried out in severe disease of the bladder). If the urine is permitted to remain in the colon for several hours significant exchange of urinary chloride with plasma bicarbonate can occur across the colonic mucosa. Then the urine voided depletes the bicarbonate directly. This is normally prevented by the frequent emptying of the colonic bladder if such a patient becomes bed-bound an indwelling rectal catheter becomes necessary. A similar exchange between urinary Na$^+$ and plasma K$^+$ ions can also occur and results in hypokalaemia.

These examples of typical causes are summarized in Table 8.2.

Metabolic alkalosis

Again the signs and symptoms are non-specific and are mainly those of the underlying cause. There may be reduced cerebral blood flow, with resultant confusion. The plasma ionized calcium concentration may fall and cause tetany. A metabolic alkalosis is caused by the loss of acid stronger than carbonic acid from the body or by the net gain of bicarbonate. Thus when gastric fluids are lost by vomiting or by aspiration there is a net loss of hydrochloric acid. Even if the fluid lost is not strongly acid it will contain a negligible amount of bicarbonate or, at any rate, a much lower bicarbonate concentration than that in plasma, i.e. more water than HCO$_3^-$ is lost, relative to plasma. Hence there is a net gain in plasma bicarbonate concentration and this constitutes a metabolic alkalosis.

Some values characteristic of metabolic alkalosis are shown in Table 8.3.

Causes of metabolic alkalosis

GENERAL FEATURES

Healthy kidneys are so well adapted to the excretion of excess bicarbonate, i.e. when the renal threshold is exceeded, that it is remarkably difficult to create an alkalosis simply by the administration of bicarbonate to a normal subject. Only when the renal mechanisms are impaired and there is an elevated bicarbonate threshold can a metabolic alkalosis be sustained. Thus the existence of an alkalosis implies lesions leading to both the generation and maintenance of the disturbance.

EXTRACELLULAR EXPANSION AND CONTRACTION

Expansion of the extracellular volume suppresses the reabsorption of HCO$_3^-$ as well as of Na$^+$, Cl$^-$ and $H_2$O although the mechanisms are multiple and not well understood. Extensive observations on this have been made in animals and there is now good evidence that the same is true in man. Conversely, contraction of extracellular volume causes augmented bicarbonate reabsorption and can therefore cause the maintenance of a metabolic alkalosis.
### Table 8.3

**Typical values characteristic of metabolic alkalosis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard bicarbonate</td>
<td>$&gt;26$ mmol/litre (or mEq/litre)</td>
<td>unless there is also a complicating primary respiratory alkalosis (i.e. $P_{CO_2} &lt; 35$ mmHg, 4.66 kPa) which may be of unrelated aetiology</td>
</tr>
<tr>
<td>Base excess</td>
<td>$&gt; +2$ mmol/litre</td>
<td></td>
</tr>
<tr>
<td>Actual bicarbonate</td>
<td>$&gt;26$ mmol/litre</td>
<td></td>
</tr>
<tr>
<td>$P_{CO_2}$</td>
<td>$&gt;45$ mmHg (5.86 kPa)</td>
<td>if there is respiratory compensation, and/or a complicating primary respiratory acidosis</td>
</tr>
<tr>
<td></td>
<td>$or$ 35–45 mmHg (4.66–5.86 kPa) if there is no respiratory compensation or complicating respiratory acidosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$or &lt;35$ mmHg (4.66 kPa)</td>
<td>only if there is also a complicating primary respiratory alkalosis (i.e. mixed respiratory and metabolic alkalosis)</td>
</tr>
<tr>
<td>pH</td>
<td>$&gt;7.45$</td>
<td>if there is no (or incomplete) respiratory compensation. There may be a complicating respiratory alkalosis</td>
</tr>
<tr>
<td></td>
<td>$or$ 7.35–7.45</td>
<td>if there is complete respiratory compensation, and no complicating primary respiratory disturbance</td>
</tr>
<tr>
<td></td>
<td>$or &lt;7.35$</td>
<td>only if there is also a complicating primary respiratory acidosis (i.e. of independent cause)</td>
</tr>
</tbody>
</table>

When much extracellular fluid including NaCl is suddenly lost, as in the action of potent diuretics which tend to cause selective excretion of NaCl, then the bicarbonate concentration rises. Obviously the $P_{CO_2}$ would also tend to rise due to the removal of water if it were not for the respiratory control of the carbon dioxide. The loss of NaCl leads to extracellular contraction which in turn leads to an elevated renal threshold for bicarbonate and to increased Na$^+$, Cl$^-$ and HCO$_3^-$ reabsorption. Thus a metabolic alkalosis can be caused and the plasma pH rises. This is sometimes called a *contraction alkalosis*. Other complex changes accompany the change in extracellular volume.

Other important causes of metabolic alkalosis include excesses of adrenal steroids and hypokalaemia.

**ALDOSTERONISM**

Excessive H$^+$ ion (and K$^+$) excretion, together with increased Na$^+$, Cl$^-$ and water reabsorption, are associated with aldosterone hypersecretion in aldosteronism since the aldosterone appears to stimulate directly the active ion pump exchange mechanism. Therefore primary or secondary aldosteronism (e.g. from an adrenal tumour, cirrhosis or cardiac failure) is liable to cause metabolic alkalosis and hypokalaemia. It is thought that increased adrenocortical activity may be responsible for the metabolic alkalosis which sometimes follows severe burns. Pharmacological doses of administered steroids can produce similar effects.
INTAKE OF BASES OR 'POTENTIAL BASES'
Ingestion or infusion of excessive amounts of bases such as NaHCO₃ or of substances which are metabolized to bicarbonate such as acetate, citrate or lactate can cause metabolic alkalosis. A typical source of excess NaHCO₃ is in anti-acid preparations, e.g. as in the milk-alkali syndrome. While such preparations may not often be prescribed, it should be remembered that several are available 'over the counter' without prescription. As stated above, an alkalosis caused simply by ingestion of excess base is uncommon and supposes a degree of renal damage also. Healthy kidneys would rapidly excrete the excess bicarbonate as soon as the threshold is reached.

If an acidosis is due to an accumulation of a metabolizable acid and bicarbonate is administered to normalize the pH, then if the acid is subsequently metabolized a base excess may remain. Hence correction of a metabolic acidosis, such as a lactic acidosis, may in due course create an 'overshoot' metabolic alkalosis (see p. 68).

Transfusion of large quantities of banked blood containing citrate as preservative (ACD) may cause a metabolic alkalosis. The citrate is metabolized via the citric acid cycle (Krebs or tricarboxylic acid cycle) to CO₂ which becomes hydrated to bicarbonate. However, massive transfusion of blood which has been stored can also cause metabolic acidosis (see p. 56).

DISEASES INVOLVING DESTRUCTION OF BONE
Where disease has caused severe chronic mobilization of bone, the levels in plasma and therefore glomerular filtrate of Ca²⁺ and phosphate (HPO₄²⁻ + H₂PO₄⁻) ions are elevated. There is therefore more phosphate buffering capacity in the urine, and additional H⁺ ion excretion may occur. Also the process of bone mobilization involves exchange between free H⁺ ions and the cations in bone. Thus hydrogen ions become bound in the bone and are taken out of solution: in this sense one may regard the bone as 'buffering' H⁺ ions.

Hypercalcaemia is often associated with a metabolic alkalosis. In addition to the above mechanism which is operative if the excess Ca²⁺ originates from skeletal dissolution, it has been suggested that hypercalcaemia stimulates carbonic anhydrase activity in the renal tubule cells. Thus H⁺ ion excretion is promoted. However, it is not clear that the step catalysed by this enzyme, the hydration of CO₂ to carbonic acid, is actually rate-limiting for H⁺ ion excretion.

POTASSIUM DEPLETION
Potassium depletion frequently causes a metabolic alkalosis. The reasons for renal tubule malfunction in this case are not completely understood. However, hypokalaemia results in a diminished excretion of K⁺ and thus a reciprocal excessive secretion of H⁺ ions. This in turn permits greater reabsorption of bicarbonate than normal. In mild potassium deficiency the alkalosis appears to be almost wholly due to contraction of the extracellular volume (ECV) and so can be rectified by administration of saline to re-expand the ECV. In severe hypokalaemia there appears to be an additional effect related directly to the potassium deficiency itself so that there is less competition with H⁺ ions for excretion. In the latter case infusion of NaCl could only repair part of the alkalosis and administration of KCl is indicated.

Since severe potassium depletion often accompanies a metabolic alkalosis this must not be forgotten when treating the alkalosis. Ingestion of ammonium chloride may rectify the alkalosis, but clearly ignores the K⁺ loss. Infusion of KCl and ingestion of quite large supplements, of the order of 10–20 g per day, of KCl for several days or even weeks may be necessary. Metabolizable salts of potassium should not be used. Note that the blood concentration of K⁺ is not necessarily a suitable index of the total
body content of potassium since the bulk of the body's potassium is intracellular. The ECG is often a useful indicator of intracellular potassium concentrations.

**Relationship between potassium and $H^+$ ions.** There is a reciprocal relationship between $H^+$ ions and $K^+$ ions inside and outside cells so that:

\[
\frac{\text{extracellular } [K^+]}{\text{intracellular } [K^+]} \text{ is directly proportional to } \frac{\text{extracellular } [H^+]}{\text{intracellular } [H^+]}
\]

Thus movement of potassium into cells is often accompanied by movement of $H^+$ ions out of cells into ECF. Conversely if there is potassium depletion, $K^+$ ions move out of cells (the major source of body potassium) and $H^+$ ions move into the cells, thus creating an extracellular alkalosis and an apparent intracellular acidosis. Hence it will be appreciated that the acid–base status of blood does not always reflect perfectly that of intracellular water.

**OPEN-HEART SURGERY**

The use of cardiopulmonary by-pass poses considerable problems in acid–base management. As well as regulating $O_2$ and $CO_2$ exchange between the blood and the environment the anaesthetist has to contend with abnormal production and disposal of acids and potential bases. For instance, poorly perfused tissues produce lactic acid, stored blood for transfusion may have a low bicarbonate concentration (p. 56), but citrate in the transfused blood may be metabolized to bicarbonate (p. 61) and poor perfusion may reduce renal function. In addition, hypothermia complicates the situation further: the basal metabolic rate falls and there are important changes in regional blood flow and distribution. Thus there are many interacting factors which may influence acid–base balance: the net result may be either acidosis or alkalosis. Blood gases should be checked regularly especially once by-pass has been established. Once the patient is in a steady state they should still be monitored, say, every half hour.

In the postoperative period monitoring is still necessary. Artificial ventilation requires careful attention to the blood gases, and the 'post-perfusion' lung syndrome may cause ventilatory problems. Renal function may not be fully restored to normal and any circulatory insufficiency will be reflected in tissue hypoxia and consequent lactic acidosis.
Compensation and Repair Mechanisms

We have already seen how the physicochemical properties of buffers minimize the effect on pH of addition (or loss) of acids and bases to the body fluids. In addition, physiological compensatory mechanisms often come into operation which tend to alter the pH towards normality. Compensation is to be distinguished from repair: physiological compensation involves a secondary acid–base disturbance which simply tends to annul the pH change caused by the primary disturbance. On the other hand, repair mechanisms tend to restore the pH towards normal by correction of the primary acid–base disturbance.

Recall that the pH is determined by the ratio of the concentration of buffer base and acid components, i.e.

\[ \text{pH} = \text{pK} + \log \frac{\text{[base]}}{\text{[acid]}} \]

or

\[ \text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03 \times P_{\text{CO}_2}} \text{ at } 37^\circ C \]

Compensation

Compensation for a metabolic acidosis

Consider a case of, for example, diabetic ketoacidosis. Increased production of acetoacetic and \( \beta \)-hydroxybutyric acids causes the plasma bicarbonate concentration to fall:

\[ \text{H}^+ + \text{AcAc}^- + \text{HCO}_3^- \rightarrow \text{AcAc}^- + \text{CO}_2 + \text{H}_2\text{O} \]

acetoacetate

The \( P_{\text{CO}_2} \) would tend to rise if it were not regulated by blowing off carbon dioxide at the lungs.

Thus the pH falls:

\[ \text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03 \times P_{\text{CO}_2}} \]

(1) plasma bicarbonate concentration falls

owing to production of strong acid

(2) pH falls correspondingly

The fall in pH is detected by the peripheral chemoreceptors in the carotid body, aortic arch and especially the central chemoreceptors in the medulla oblongata. The respiratory centre accordingly stimulates ventilation; characteristic deep respiration
('air hunger' or 'Kussmaul breathing') is often seen in severe metabolic acidosis. Carbon dioxide excretion is then increased and so the $P_{CO_2}$ falls:

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03 \times P_{CO_2}}$$

Partial compensation is often seen, i.e. the pH is lower than normal, but not as low as it would be on account of the loss of bicarbonate alone. Complete respiratory compensation (i.e. with pH fully restored to normal) is not common because as the $P_{CO_2}$ is lowered so the carbon dioxide drive to respiration is reduced.

Thus if respiratory compensation were complete one would have a normal pH. However both the $[\text{HCO}_3^-]$ (primary metabolic acidosis) and the $P_{CO_2}$ (secondary compensatory respiratory disturbance, although such a secondary disturbance strictly should not be classified as an alkalosis) are lower than normal in the same ratio. On the other hand, repair entails the restoration of both $[\text{HCO}_3^-]$ and $P_{CO_2}$ (and hence also pH) to their normal values. During recovery from a metabolic acidosis the $[\text{HCO}_3^-]$ increase either by the repair action of the kidneys (see below) or by the therapeutic administration of base. However, the hyperventilation often persists and does not fall as fast as the bicarbonate recovers; hence recovery from a compensated metabolic acidosis is often followed by a transient respiratory alkalosis. The reason is probably that there is a time lag before the CSF attains a normal $[\text{HCO}_3^-]$ and so the central chemoreceptors continue to cause ventilatory stimulation even though the plasma $[\text{HCO}_3^-]$ may have reached its normal value.

Compensation for a metabolic alkalosis

Respiratory compensation for a metabolic alkalosis entails hypoventilation so that the $P_{CO_2}$ rises in response to the increased pH associated with the high $[\text{HCO}_3^-]$. However this compensation may be greatly limited by the hypoxic drive which will tend to prevent ventilation from being markedly reduced. Complete compensation is rarely seen.

Renal compensation in respiratory disturbances

Primary respiratory disturbances are compensated by renal regulation of the plasma bicarbonate concentration. A high $P_{CO_2}$ will tend to increase the rate of bicarbonate reabsorption and regeneration, and hence the rate of $\text{H}_+^+$ ion excretion into the urine. Conversely a low $P_{CO_2}$ will automatically reduce bicarbonate regeneration and reabsorption (law of mass action) and unreabsorbed bicarbonate may be excreted in the urine. Hence the plasma bicarbonate concentration will fall.

Speed of compensation

Respiratory compensation is characteristically a much more rapid event than metabolic compensation. Respiratory compensation for a primary metabolic disturbance often commences rapidly and may reach its maximum extent within a few hours even in a severe disturbance. On the other hand metabolic compensation by the kidneys for a respiratory disturbance often takes up to a week to reach its full extent.

Maximum extent of physiological compensation

In theory, compensation may be complete if the secondary compensatory processes restore the ratio [base]/[acid] and hence the pH to normal. Although such complete
compensation is sometimes seen, partial compensation is much more common. The drive to respiration is regulated by pH, $P_{CO_2}$ and $P_{O_2}$ all acting in concert as discussed in Chapter 5. Thus, while a low pH elicited by loss of bicarbonate in a metabolic acidosis will stimulate ventilation so that the $P_{CO_2}$ decreases, the consequent fall in $P_{CO_2}$ will tend to reduce the ventilatory drive. The two effects act in opposition, so that in practice the $P_{CO_2}$ normally falls to some extent but insufficiently to return the pH to normal.

Similarly in a metabolic alkalosis ventilation is depressed so that the $P_{CO_2}$ rises in compensation. However, the $P_{O_2}$ also falls and so the extent of respiratory compensation for a metabolic alkalosis can be restricted by the opposing hypoxic drive which acts to stimulate ventilation.

In assessing whether a simple acid–base disturbance exists or whether there is a mixed disturbance (i.e. more than one primary disturbance) it is useful to know the normal extent of physiological compensation which can be expected to accompany a simple disturbance. For this purpose some clinicians use nomograms or diagrams constructed from in vivo whole-body titration curves (see Chapter 3) or from data obtained in patients whose acid–base status is well defined. One useful example is the Flenley diagram: this is discussed later (Chapter 10 and Appendix IV).

**Repair**

**Physiological repair**

Repair of an acid–base disturbance entails the removal of the underlying cause. Then the affected component must be restored to normal. The chemoreceptors sense $P_{CO_2}$ as well as pH so that the respiratory centre tends to correct the $P_{CO_2}$ to within normal limits. A plasma bicarbonate concentration in excess of the renal threshold of about 26 mmol/litre (mEq/litre) automatically means that renal reabsorption is incomplete. This bicarbonate ‘spills over’ into the urine, which then becomes alkaline and hence is lost from the body. However, if the renal threshold for bicarbonate is elevated, then this mechanism is not operative and so the alkalosis can be sustained. As noted in Chapter 8, it is not easy to generate a metabolic alkalosis in a healthy subject simply by the administration of base. The maintenance of an elevated plasma bicarbonate concentration generally implies a degree of renal malfunction.

Conversely, when the plasma bicarbonate concentration is low in a metabolic acidosis reabsorption of bicarbonate is complete provided renal function is unimpaired, and gradually the $H^+$ ion excretion and plasma $[HCO_3^-]$ are increased. The mechanism of this is not known, but may involve induction of glutaminase or changes in mitochondrial permeability since ammonia excretion gradually increases in the first few days of a prolonged acidosis (see p. 50).

Calcium is sometimes regarded as a long-term buffer. Calcium phosphate (e.g. in bones) is not entirely insoluble and its solubility is increased at low pHs. Thus a prolonged acidosis is associated with mobilization of bone calcium and phosphate. Phosphate buffering in extracellular fluids may become significant. The phosphate is excreted, and the phosphate buffering capacity of urine is increased. Thus the urinary pH (or strictly, the pH of tubular luminal fluid) falls less for each $H^+$ ion excreted than it would otherwise have fallen. The $H^+$ ion gradient between tubule cell and tubular luminal fluid is therefore less steep and $H^+$ ion excretion may proceed faster, or at least more $H^+$ ions can be excreted.

**Therapeutic repair**

Therapeutic repair of acid–base disturbances should always involve in the first place the removal or alleviation of the underlying cause wherever possible. In some, but by
no means all, cases it may be desirable to restore the \(PCO_2\) (e.g. by assisted ventilation or by the use or disuse of drugs which act on the respiratory centre) or to restore the bicarbonate concentration (e.g. by the administration of potential acids or bases as appropriate), especially if the patient's clinical condition demands rapid restoration of normal acid-base status.

Potential acids which may be used to correct a positive base excess (i.e. in metabolic alkalosis) include ammonium chloride, lysine or arginine hydrochlorides; sodium, potassium or calcium chlorides may also be used even though these are not potential acids. However, it must be remembered that such an alkalosis is often accompanied by severe \(K^+\) loss which may require gross potassium supplementation and in this case KCl infusion would be indicated. Note that the more palatable potassium citrate should not be used: the metabolizable anion will give rise to further bicarbonate.

Often attention to the electrolyte imbalances attending acid-base disturbance is of as much, or even more, practical clinical importance as the restoration of acid-base equilibrium.

Bases, or potential bases (i.e. compounds which are metabolized to bases), which are often employed to correct a base deficit include sodium bicarbonate (orally or by intravenous infusion), sodium acetate lactate or citrate and ‘Tham’ (i.e. ‘Tris’, tris-hydroxymethyl aminomethane).

‘Tham’ is a weak base with a \(pK\) of 7.8:

\[
(CH_2OH)_3-C-NH_2 + H^+ \rightleftharpoons (CH_2OH)_3-C-NH_3^+
\]

Sodium lactate and ‘Tham’ (‘Tris’) have the advantage that they cross cell membranes and therefore equilibrate with intracellular fluid more rapidly than bicarbonate does. Rapid administration of ‘Tham’ is, however, said to cause a risk of cardiac arrest and opponents of its use argue that it is a toxic foreign compound.

<table>
<thead>
<tr>
<th>Alkalizing agents</th>
<th>Acidifying agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>Ammonium chloride</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>Tham (Tris or tris-hydroxymethyl aminomethane)</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td></td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td></td>
<td>Calcium chloride</td>
</tr>
</tbody>
</table>

Lactate is contraindicated in hepatic failure or in lactic acidosis where metabolism of lactate is impaired. The metabolizable salts should not be used where an immediate effect is required. Thus bicarbonate affects the extracellular acid-base status immediately, but only slowly enters cells. On the other hand, lactate enters cells relatively faster, but it of course has no effect on extracellular acid-base status until it has been converted into bicarbonate inside the cells.

Likewise, ammonium chloride should not be administered to patients in hepatic failure since their ability to convert the toxic ammonium moiety into urea will be impaired.

The decision as to whether therapeutic correction is required depends on one's clinical judgement. However there are some instances in which corrective measures
are invariably indicated. For example, the resuscitative measures following cardiac arrest should always include the immediate injection of sodium bicarbonate since the acidosis caused by accumulation of lactic acid during the tissue hypoxia associated with the circulatory failure impairs myocardial contractility and so would lessen the chance of successful resuscitation. Both acute and chronic renal failure often require correction of the base deficit by administration of adequate doses of base since the normal metabolic repair mechanisms of the kidney will be impaired.

In acute renal failure dialysis may be used to dispose of $\text{H}^+$ ions and to replace the plasma $\text{HCO}_3^-$. The various alkalinizing and acidifying agents which are commonly encountered are summarized in Table 9.1.

**DOSE OF BASE OR ACID REQUIRED**

The actual amount of acid or base to be administered is also best judged from clinical experience combined with serial measurements of the pH and standard bicarbonate or base excess. Some clinicians use approximate formulae such as:

\[
\text{amount of base (or acid) required in mmol (or mEq)} = 0.3 \times \text{base deficit (or excess) in mmol/litre (or mEq/litre)} \times \text{body weight in kg}
\]

However, faith in such over-simplified approximations is often unwise since it is assumed that the only change in acid–base status will be the theoretical physicochemical one due to the administration of the base (or acid). The pH change associated with part of the dose may cause physiological or metabolic changes which themselves act so as to produce or destroy base.

Also, it is assumed in the application of these formulae that the 0.3 correction factor is appropriate to account for the distribution of the administered base (or acid) between the body fluids and this may not be valid, especially in sick patients; in such patients the distribution of electrolytes between, for example, extracellular and intracellular fluids is thought to be frequently abnormal. Thus the *in vitro* titration curve of the blood may be a poor approximation to the *in vivo* titration curve of the body fluids. That is to say, the buffer properties of the blood sample which is convenient to analyse are not necessarily a valid or accurate reflection of the state of the whole body buffers especially in sickness. Simple measurements on blood (and occasionally on CSF) are convenient and more often than not do give a fairly accurate picture of the acid–base status, but they obviously cannot take into account intracellular buffering or $\text{H}^+$ ions buffered in bone.

It is therefore often more appropriate to administer several quite small doses of the base, or acid, and to monitor serially every 2 hours or so the changes in acid–base status than to rely implicitly on simple theoretical equations. Plasma $[\text{K}^+]$ should also be frequently monitored since considerable ion shifts may occur.

Furthermore while administration of NaHCO$_3$ causes an immediate change in the plasma bicarbonate concentration, the [$\text{HCO}_3^-$] in the CSF does not change rapidly, probably because the formation of CSF bicarbonate is an active process involving hydration of CO$_2$ in the choroid plexus and is not a simple filtration of plasma. In contrast, changes in blood $P_{\text{CO}_2}$ are accompanied by equally rapid changes in CSF $P_{\text{CO}_2}$ since the blood–brain barrier is freely permeable to carbon dioxide. Hence administration of a large amount of bicarbonate causes an increase in the $P_{\text{CO}_2}$ since:

\[
\begin{align*}
\text{HCO}_3^- + \text{H}^+ &\rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O} \\
\text{HCO}_3^- &\rightarrow \\
\end{align*}
\]
Thus the plasma bicarbonate and $P_{CO_2}$ both rise abruptly, but the plasma bicarbonate concentration rises proportionately far more than the $P_{CO_2}$ so that the blood pH rises. Simultaneously the CSF $P_{CO_2}$ rises slightly, but the CSF bicarbonate concentration hardly rises at all at once; thus the CSF pH may actually fall while the blood pH rises.

OVERSHOOT IN CORRECTION OF METABOLIC ACIDOSIS

If a metabolic acidosis is caused by accumulation of an oxidizable acid such as lactic acid then this can be removed by normal oxidation once cellular metabolism returns to normal. Thus, as noted on p. 61, if base has already been administered, this metabolic removal of the lactic acid would cause a metabolic alkalosis, i.e. there is now a base excess. In practice this is sometimes seen: often, however, metabolic removal of the lactic acid cannot be abruptly and fully returned to normal. Therefore, for example, sustained metabolic acidosis is more frequently observed than an ‘overshoot’ alkalosis following successful cardiac resuscitation with administration of bicarbonate.

CORRECTION OF RESPIRATORY DISTURBANCES

The administration of acids or bases is theoretically only indicated in metabolic disturbances, i.e. when plasma bicarbonate is abnormal. However some clinicians have advocated the use of bases such as ‘Tham’ or bicarbonate in respiratory acidosis as an interim measure of therapeutic compensation, on the grounds that the advantages of restoration of normal pH outweigh the disadvantages attending the imposed metabolic alkalosis. However others disagree with this therapy, and hold that a respiratory acidosis should be treated by removal of the cause and thus reduction of the $P_{CO_2}$. There is also some evidence that Tham in adequate doses acts as a respiratory depressant: this is clearly undesirable in a respiratory acidosis. Opponents to the use of Tham argue that ‘Tham cures the pH but not the patient’.

Severe respiratory acidosis may require artificial ventilation to obtain adequate excretion of the accumulating CO$_2$. Thus pH correction by administration of base in these cases is only a temporary measure.
Measurement of Acid–Base Status

The need for complete characterization

As already explained, the presence of an abnormal blood pH (i.e. acidaemia or alkalaemia) does not tell much about the underlying acid–base disturbance. While an acidaemia can be caused only by an acidosis, this may be of respiratory or of metabolic (non-respiratory) origin or of both (i.e. mixed acidosis): also there may or may not be compensation and/or a secondary disturbance. Additionally the presence of a normal blood pH tells absolutely nothing about the normality or otherwise of the respiratory and metabolic components. This is because physiological compensation can adjust the pH to normal, or near normal, while the concentrations of both the buffer acid and base components may be abnormal. For example, a metabolic acidosis can cause a reduction in the plasma bicarbonate concentration, while respiratory compensation can lower the $P_{CO_2}$ to such an extent that the blood pH is close to normal. However, the loss of bicarbonate results in a reduction in the plasma buffering capacity and, in theory at least, a point could be reached where further addition of metabolic acid had destroyed all the buffer base (the so-called 'alkali reserve'), but the pH could be almost normal. Further addition of $H^+$ ions to the now almost unbuffered plasma would then cause a precipitous fall in pH.

Therefore quantitation of both the plasma $P_{CO_2}$ and the bicarbonate concentration (some clinicians regard the standard bicarbonate or the base excess as more useful than the actual bicarbonate concentration) is essential in order to assess both the respiratory and metabolic components. The information required, and the use made of it, obviously depends on the individual clinical circumstances. Thus determination of $P_{O_2}$ and $P_{CO_2}$ is of paramount importance in the management of many respiratory patients, while particular attention is attached to plasma bicarbonate and electrolyte concentrations in a renal unit. The decision as to whether to make acid–base measurements, and what measurements are likely to be useful, can only be made in the light of clinical experience together with an understanding of the physiological and physicochemical implications.

Thus while complete characterization demands knowledge of both respiratory factor (plasma $P_{CO_2}$) and metabolic factor (e.g. either standard bicarbonate, base excess or total buffer base) determination of one factor alone perhaps approximately may suffice in certain clinical circumstances where the patient's diagnosis is known. Therefore in a patient in known chronic renal failure the bicarbonate concentration may provide all the information required for correct management. The plasma bicarbonate concentration is technically difficult, or impossible, to determine directly. Hence it is invariably determined by an approximation (see below) or indirectly by the
determination of other variables. Since the Henderson–Hasselbalch equation relates simply the three variables, pH, PCO₂ and \([\text{HCO}_3^-]\) thus:

\[
\text{pH} = \text{pK} + \log \frac{[\text{HCO}_3^-]}{0.03 \times \text{PCO}_2}
\]

it follows that if two out of the three variables are known then the third can be calculated. The knowledge of PCO₂ and pH enable the [HCO₃⁻] (i.e. the actual bicarbonate concentration) to be calculated. Calculation of other parameters such as total buffer base, base excess and standard bicarbonate is also possible, but in these cases the haemoglobin concentration must also be known. While numerical solution of the Henderson–Hasselbalch equation in its simplest form is easy, a number of diagrams (nomograms) and also special slide rules have been devised: these are discussed separately in Appendix IV.

**Modern methods of measurement**

Of the four general methods discussed below the first two are probably obsolescent and so are described very briefly. As the necessary equipment becomes available in hospitals and laboratories these methods are being superseded by the Astrup equilibration technique and direct determination by micro-electrodes. These modern methods owe their inception to the invention of glass micro-electrodes capable of determining blood pH to a high degree of accuracy on minute blood samples, to the introduction of the equilibration technique by Astrup and his colleagues in Copenhagen around 1960, and to the refinement of PCO₂ electrodes for the direct electro-metric determination of PCO₂ on small blood samples, especially by Severinghaus. The theoretical basis of these methods will be described in detail below.

Modern sophisticated equipment for blood gas analysis is currently available from a number of manufacturers. Much of the equipment has been designed so that it can be used at the bedside or in a side-room or operating theatre rather than at a distant clinical chemistry laboratory. There is a trend towards increasing automation: many instruments are self-calibrating, and report results via a mini-computer which performs all the necessary calculations.

**Intracellular pH measurement**

Although intracellular pH measurements are currently a specialized procedure used only in research laboratories, it is worth stating that some authorities predict that they may become useful in clinical practice.

**Determination of total CO₂ content**

*Theoretical basis*

Although [HCO₃⁻] and [H₂CO₃] are difficult to determine, measurement of ‘total CO₂ content’ is relatively easy. If an excess of strong acid is added to blood all the bicarbonate, carbonic acid and also carbonates and carbamino compounds are converted into carbon dioxide. Gaseous CO₂ can then be measured manometrically by the Van Slyke method or the Natelson method or colorimetrically on an AutoAnalyzer (see below).

In a normal individual the mean value for [HCO₃⁻] is 24 mmol/litre (mEq/litre) and that for \([\text{CO}_2]+[\text{H}_2\text{CO}_3]\) is 1.2 mmol/litre (mEq/litre). Thus:

\[
\text{Total CO}_2 \text{ content} = \text{[CO}_2]+[\text{H}_2\text{CO}_3]+[\text{HCO}_3^-] = 1.2+24 = 25.2 \text{ mmol/litre (mEq/litre)}
\]
Of this the majority (24/25.2 = 95%) is contributed by the bicarbonate concentration. Even in a severe respiratory disturbance the total CO₂ content therefore approximates to the bicarbonate concentration. The total CO₂ content can therefore be used as a fairly accurate index of the metabolic component.

If the blood pH is also known, then use of the Siggaard-Andersen Alignment Nomogram (see p. 105) allows calculation of the P_co₂ and the true actual bicarbonate concentration. If the haemoglobin concentration is also known then base excess and standard bicarbonate values can also be read off from this nomogram.

**Practical procedures: Van Slyke and Natelson manometry**

Briefly, the procedure with the Van Slyke or Natelson manometric method entails introducing a known volume of plasma into a gas burette while the sample is continuously kept anaerobic, i.e. out of contact with air. Lactic acid or acid ferricyanide reagent is then added which converts all the bicarbonate plus carbonic acid, etc., into carbon dioxide. Evacuation, together with the acidic conditions, which together reduce the solubility of gaseous CO₂, removes the ‘total CO₂’ from the plasma into the gas phase, and the total volume of this gas is measured manometrically in terms of a pressure, i.e. the sum of partial pressures, at constant volume. Sodium or potassium hydroxide solution is then introduced anaerobically into the apparatus and this absorbs all the CO₂ in the gas phase: the gaseous CO₂ is thus converted into CO₃⁻ in the liquid phase:

\[
2\text{OH}^- + \text{CO}_2 \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O}
\]

The volume of the remaining gas (i.e. all previously measured minus the ‘total CO₂ content’) is determined again in terms of a pressure at constant volume. By difference the ‘total CO₂ content’ of the plasma sample is determined.

The Van Slyke manometric gasometer is a classical method capable of high accuracy. However its successful use requires considerable experience, and a certain amount of patience. At least 1–2 ml of blood is required and the extensive handling of the sample must be with great care to avoid CO₂ loss, etc. Nevertheless, in experienced hands it can be used as a standard method for the calibration of other procedures.

**AutoAnalyzer method**

In the AutoAnalyzer colorimetric method, the ‘total CO₂’ liberated from the plasma following the addition of strong acid is allowed to equilibrate with a gas phase which is originally free of CO₂. This gas phase, now containing carbon dioxide, is then allowed to equilibrate with a weakly buffered bicarbonate solution containing phenolphthalein indicator. This equilibration is achieved by the segmented flow technique familiar to AutoAnalyzer users. A liquid stream of the bicarbonate buffer containing the indicator is segmented with gas bubbles containing the CO₂ and as this stream is continuously pumped through the apparatus turbulence, or stirring, is created in the slugs of liquid due to a surface tension effect, so that the buffer-plus-indicator slugs rapidly come to equilibrium with the gas bubbles. The addition of CO₂ to the buffer lowers the pH and so partially decolorizes the phenolphthalein indicator. The colour of the liquid stream is continuously monitored by passing it through a spectrophotometer or colorimeter equipped with a flow-through cuvette.

The instrument is calibrated by including standard solutions of bicarbonate. A calibration graph is then constructed by plotting the concentration of CO₂ in the standards against percentage decolorization (i.e. percentage transmittance), and the
values for unknowns can be read off directly. A diagrammatic representation of such an AutoAnalyzer system is shown in Fig. 10.1. Large numbers of samples can be processed rapidly in this way in a laboratory which is equipped for this type of automated analysis, but care is needed so that \( \text{CO}_2 \) is not lost from the plasma samples, e.g. by equilibration with air prior to analysis.

![Diagram](image)

**Fig. 10.1.** An AutoAnalyzer system for automated determination of total \( \text{CO}_2 \) content.

### Determination of \( \text{CO}_2 \) combining power

This obsolescent measurement is another attempt to get around the problems in determining plasma bicarbonate concentration. The \( \text{CO}_2 \) combining power is defined as the total \( \text{CO}_2 \) content of plasma which has been equilibrated at a \( P\text{CO}_2 \) of 40 mmHg (5.3 kPa). It is thus equal to

\[
[\text{CO}_2] + [\text{H}_2\text{CO}_3] + [\text{HCO}_3^-] \text{ standard} \\
= (0.03 \times 40) + [\text{HCO}_3^-] \text{ standard} \\
= (1.2 + \text{standard bicarbonate}) \text{ mmol/litre (or mEq/litre)}
\]

Therefore the \( \text{CO}_2 \) combining power is approximately equal to the standard bicarbonate, i.e. the [\( \text{HCO}_3^- \)] of plasma equilibrated at a \( P\text{CO}_2 = 40 \) mmHg.

Measurement of the \( \text{CO}_2 \) combining power is made using the same techniques as those used for total \( \text{CO}_2 \) content (see above) but following equilibration of the plasma sample with gas at a \( P\text{CO}_2 \) of 40 mmHg (5.3 kPa). Obviously this extra equilibration step allows more scope for error; also a correction factor must be applied if the \( P\text{CO}_2 \) of the equilibrating gas is different from 40 mmHg (5.3 kPa) and of course the partial pressure of any gas mixture varies from day to day as the barometric pressure changes.
Astrup equilibration technique

Theoretical basis of in vitro titration curve

On theoretical grounds one can predict that it should be possible to determine both the respiratory factor (P\textsubscript{CO2}) and the metabolic factor (base excess, standard bicarbonate, etc.) from the in vitro CO\textsubscript{2} titration curve of the blood sample. That is to say, one measures the pH of the blood sample following its equilibration in vitro with CO\textsubscript{2} mixtures at several P\textsubscript{CO2} values, and constructs a titration curve.

Consider the Henderson–Hasselbalch equation:

$$\text{pH} = \text{pK} + \log \frac{[\text{base}]}{[\text{acid}]}$$

For the CO\textsubscript{2}/HCO\textsubscript{3} system:

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03 \times P_{\text{CO}_2}}$$

at 37°C

Expanding the logarithmic term we obtain:

$$\text{pH} = 6.1 + \log [\text{HCO}_3^-] - \log 0.03 - \log P_{\text{CO}_2}$$

Rearranging terms gives:

$$\log P_{\text{CO}_2} = 6.1 + \log [\text{HCO}_3^-] - \log 0.03 - \text{pH}$$

This equation shows how the pH of a bicarbonate buffer varies as the P\textsubscript{CO2} is changed.

In any one blood sample [HCO\textsubscript{3}] is constant. If it is assumed that this remains constant while the P\textsubscript{CO2} is changed (see below), then we see that the first three terms on the right hand side of the equation are all constants at 37°C.

$$\log P_{\text{CO}_2} = 6.1 + \log [\text{HCO}_3^-] - \log 0.03 - \text{pH}$$

constant

Therefore this equation becomes of identical form to the general equation for a straight line:

$$y = mx + c \quad (\text{or } y = bx + a)$$

$$\log P_{\text{CO}_2} = \text{constants} - 1 \cdot \text{pH}$$

$$y = c + m \cdot x$$

The equation \(y = mx + c\) gives a straight line with slope = m and intercept = c, as shown in Fig. 10.2. Correspondingly we see that the relationship between pH (on the x axis) and log P\textsubscript{CO2} (on the y axis) is a straight line (Fig. 10.3). Its slope is -1 (the coefficient of the pH term), and the intercept on the y axis is equal to:

$$6.1 + \log [\text{HCO}_3^-] - \log 0.03$$

However it was assumed above that log [HCO\textsubscript{3}] remains constant while the P\textsubscript{CO2} (and pH) are changed. Clearly this is not necessarily valid: we have already seen that the actual bicarbonate concentration in plasma depends on the P\textsubscript{CO2} since the law of mass action demands that the equilibrium

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$$

moves to the right when P\textsubscript{CO2} is elevated, and vice versa. Therefore the straight line
in Fig. 10.3 should be drawn as a curve as shown in Fig. 10.4. But if one considers a relatively small portion of this curve, e.g. the region between pH 6 and 8, it can be seen to approximate very closely to a straight line. If one thus restricts oneself to modest changes in log \( P_{\text{CO}_2} \) the corresponding percentage change in \([\text{HCO}_3^-]\) (compared to its existing value) is small and the corresponding percentage change in log \([\text{HCO}_3^-]\) is even smaller. Thus for operational purposes a section of the curve over a small pH change can be accepted as being a straight line, as shown in Fig. 10.5.

\[ y = m \cdot x + c \]

\[ y = b \cdot x + a \]

Fig. 10.2. The general equation for a straight line, \( y = m \cdot x + c \) or \( y = b \cdot x + a \).

\[ \text{intercept} = (6.1 + \log [\text{HCO}_3^-] - \log 0.03) \]

\[ \text{slope} = -1 \]

Fig. 10.3. The theoretical linear relationship between log \( P_{\text{CO}_2} \) and pH, assuming that \([\text{HCO}_3^-]\) remains constant.
When this theory is put to test in practice by determining the *in vitro* CO₂ titration curve of a blood sample it is found to behave closely as predicted. Over the range pH 6 to 8 deviation from linearity cannot be detected.

However the slope of the curve obtained in practice is not exactly −1 for whole blood, although it is for plasma. The above theoretical derivation assumed that bicarbonate was the sole buffer system. The presence of another buffer, haemoglobin, will tend further to minimize the pH change of blood in response to a change in $P_{CO_2}$.

![Figure 10.4](image1.png)

*Fig. 10.4.* The relationship between log $P_{CO_2}$ and pH is slightly curvilinear since [HCO₃⁻] changes slightly as log $P_{CO_2}$ is changed.

![Figure 10.5](image2.png)

*Fig. 10.5.* The relationship between log $P_{CO_2}$ and pH is approximately linear over the limited pH range of 6 to 8.
Therefore the true buffer line or titration curve obtained for whole blood is a straight line, but with slope slightly steeper than $-1$: the actual slope depends on the haemoglobin concentration (Fig. 10.6). This additional factor, the haemoglobin concentration, does not in fact complicate the issue from the practical viewpoint.

**Fig. 10.6.** The presence of non-bicarbonate buffers, notably haemoglobin, makes the CO$_2$-titration curve steeper. Whole blood is thus a better buffer than plasma.

**Fig. 10.7.** The position of the CO$_2$-titration curve (buffer line) depends on the bicarbonate concentration, while the slope depends mainly on the haemoglobin concentration.
The position of the line is defined by the intercept, which is equal to
\[ 6.1 + \log [\text{HCO}_3^-] - \log 0.03 \]
so that it is in fact determined by the bicarbonate concentration. Thus two blood samples with identical haemoglobin concentrations but with different bicarbonate concentrations will give parallel *in vitro* CO₂ titration curves or buffer lines, but with the curve for the sample with higher [HCO₃⁻] displaced to the right as shown in Fig. 10.7.

![Standard bicarbonate scale on the log Pco₂ vs pH diagram](image)

*Fig. 10.8. Standard bicarbonate scale on the log Pco₂ vs pH diagram. The value of standard bicarbonate is read off where the buffer line (CO₂-titration curve) intersects the standard bicarbonate scale.*

A scale can be drawn on the diagram so that it intersects the log Pco₂ axis at Pco₂ = 40 mmHg (5.3 kPa) and is calibrated so that the standard bicarbonate (i.e. bicarbonate concentration of blood equilibrated at Pco₂ = 40 mmHg at 37°C) concentration can be read off directly (Fig. 10.8). The standard bicarbonate value is read off where the drawn buffer line cuts the standard bicarbonate scale.

As an aid to drawing the titration curve, the data are normally entered on a calibrated chart, the Siggaard-Andersen Curve Nomogram, supplies of which are commercially available. These nomograms also have calibrated scales for base excess and total buffer base; the values for these parameters are read off where the drawn buffer line (or titration curve) intersects each scale (see below).

**Practical procedure for two-point equilibration method**

The Astrup equilibration procedure is carried out as follows. Three portions of the blood sample (whole blood) are required, but these need not exceed about 0.08 ml each with currently available equipment. As with any technique utilizing whole blood, the samples must be thoroughly mixed immediately before analysis. Two portions are equilibrated with gas mixtures whose Pco₂ values are accurately known. The gases, supplied from cylinders, generally contain about 4% v/v CO₂ (low CO₂) and 8% v/v CO₂ (high CO₂) and are saturated with water vapour at 37°C. Equilibration is
achieved in a tonometer, or microtonometer, which is a device designed to expose a large surface area of the blood to the humid gas. The tonometer is immersed in a water bath at 37°C and is agitated by an electrical shaker so that the blood sample is thrown up in a thin film around the walls of the tube, and is in intimate contact with the equilibrating gas (Fig. 10.9).

![Diagram of equilibration process](image)

**Fig. 10.9.** A micro-tonometer with two equilibration chambers. Only one is shown in use.

Three to four minutes in a microtonometer is adequate for complete equilibration: by this time the $P_{\text{CO}_2}$ of the blood sample has been changed completely to that of the gas mixture. Agitation is then stopped, and the pH of each of the two equilibrated (or tonometered) samples is determined in turn using a glass pH micro-electrode (see below). The pH values found after equilibration with each gas mixture are entered as points (A and B, Fig. 10.10) having coordinates equal to the $P_{\text{CO}_2}$ in the gas mixture on the $y$-axis and to the pH on the $x$-axis on a Siggaard-Andersen Curve Nomogram as shown in Fig. 10.10.

![Nomogram illustration](image)

**Fig. 10.10.** Points A and B are entered on a Siggaard-Andersen Curve Nomogram following equilibration of the blood at two known $P_{\text{CO}_2}$ values.
The buffer line \((in \text{ vitro} \text{ titration curve})\) is then constructed by joining points \(A\) and \(B\) with a straight line. Values for total buffer base, standard bicarbonate, and base excess can be read off where the buffer line cuts the respective scales at \(C\), \(D\) and \(E\) respectively in Fig. 10.11.

![Graph showing buffer line and scales](image)

**Fig. 10.11.** The buffer line is drawn through points \(A\) and \(B\). Values for the total buffer base, standard bicarbonate and base excess are read at \(C\), \(D\) and \(E\) where the buffer line intersects the respective scales.

The third portion of the original blood sample is then taken for direct pH measurement. It is most important that it is kept anaerobic; its \(PCO_2\) must remain the same as it was in the patient. The pH value is then noted on the nomogram's pH axis \((F)\) and a corresponding point marked at \(G\) on the buffer line already drawn (Fig. 10.12). The corresponding plasma \(PCO_2\) is then read directly off the \(PCO_2\) axis at \(H\) (Fig. 10.12).

The Astrup method is capable of high accuracy in experienced hands, and with practice full measurements on a blood sample can be made in about 5–6 minutes. For maximum accuracy the 'actual pH point' \((G)\) should lie between the two points corresponding to the artificially equilibrated portions of the blood sample \((A\) and \(B)\). If \(G\) lies outside these limits, then the buffer line has to be extrapolated, as opposed to interpolated, and accuracy is impaired.

It can be noted that it is not necessary to know the haemoglobin concentration to perform a complete acid–base assessment by this method. The haemoglobin concentration alters the slope of the buffer line and the slope is determined by the measurements on the two equilibrated blood samples. If the haemoglobin concentration is known then it can be used as a rough internal check on the validity of measurements made as follows.

**CHECKING THE VALIDITY OF THE BUFFER LINE**

One reads the base excess or deficit value from the base excess scale. Note that the base excess value, unlike the slope of the buffer line, is quite independent of the
Fig. 10.12. Point G, corresponding to the actual pH of the blood sample (without equilibration), is inserted on the buffer line. The $PCO_2$ is then read off at H.

Fig. 10.13. This buffer line shows that the total buffer base and base excess values are 38 and $-10$ mmol/litre respectively. Therefore the 'normal buffer base' value must be $(38 + 10) = 48$ mmol/litre.
haemoglobin concentration. If there is a base deficit, then addition of this value to the total buffer base value observed gives a value for the normal buffer base at the prevailing haemoglobin concentration. A subscale (the ‘Hb NBB’ scale) on the buffer base scale shows the haemoglobin concentration, [Hb], corresponding to that normal buffer base value. This value for [Hb] should agree within about ±3 g/100 ml with the value obtained independently. Note that the haemoglobin concentration alters the slope of the buffer line so trivially that this method would never be used for the accurate measurement of [Hb]. An example may make this check method clearer.

A blood sample gives a buffer line (Fig. 10.13) which shows that the base excess is −10 mmol/litre (i.e. base deficit of 10 mmol/litre) and the total buffer base concentration is 38 mmol/litre. Therefore the normal buffer base value must be:

\[
\text{observed buffer base} + \text{base deficit} = 38 + 10 = 48 \text{ mmol/litre}
\]

![Graph showing normal buffer base subscale](image)

**Fig. 10.14.** The haemoglobin at normal buffer base subscale ('Hb NBB') gives a value of [Hb] = 15 g% corresponding to a normal total buffer base concentration of 48 mmol/litre.

Reference to the ‘Hb NBB’ (haemoglobin at normal buffer base) subscale on the total buffer base scale shows that this value corresponds to a haemoglobin concentration of 15 g/100 ml (Fig. 10.14). Therefore the buffer line which has been drawn is probably valid if the actual haemoglobin concentration in the blood is within the range 12–18 g/100 ml.

**One-point equilibration method**

A similar method can be used if only a single portion of blood is equilibrated with a standard gas mixture in the tonometer and the haemoglobin concentration is known
so that the slope of the buffer line can be determined. This method has no advantages and is much less accurate than the two-point equilibration technique of Astrup which is more commonly used.

Use of nomograms
As an alternative to the Siggaard-Andersen curve nomogram the alignment nomogram may be used. Detailed examples of the use of both nomograms are given in Appendix IV.

Direct determination of $P_{CO_2}$ and pH by micro-electrodes

Clearly if the $P_{CO_2}$ and pH are both known then it is possible to calculate the actual bicarbonate concentration and total CO$_2$ content simply by solution of the Henderson-Hasselbalch equation either manually or by the aid of a nomogram or special slide rule. If the haemoglobin concentration is also known (an approximate value generally suffices) then the base excess and standard bicarbonate values can likewise be calculated.

The $P_{CO_2}$ electrode

Electrodes for direct measurement of $P_{CO_2}$ have been available for about 15-20 years, but it is only recently that technical developments have improved the reliability of these instruments to the point that they are in great demand. At the present time a number of instrument manufacturers are offering acid-base (or blood-gas) analysers equipped with micro-electrodes for direct determination of pH, $P_{CO_2}$ and $P_{O_2}$ on about 0.1 ml of blood.

The principle of the CO$_2$ electrode is very simple and is explained diagrammatically in Figs 10.15 and 10.16. Consider a pH glass electrode A immersed in a closed system containing water at B. One wall of the system is made of a very thin plastic membrane, C (e.g. of Teflon) which is permeable to CO$_2$ but not to H$^+$ ions (Fig. 10.15). If a drop of blood containing CO$_2$ is placed at D in contact with the membrane C, CO$_2$ will diffuse from the blood across the membrane into the water at B until an equilibrium is set up. The CO$_2$ in B will become hydrated and the pH of B will change; the steady state pH value recorded by the pH meter at E therefore depends solely on the $P_{CO_2}$ in the blood at D (Fig. 10.16).

Fig. 10.15. The principle of the $P_{CO_2}$ electrode. A glass H$^+$-sensitive electrode (A) is immersed in an aqueous solution (B). C is a thin plastic membrane which is permeable to CO$_2$ but not to H$^+$ ions.
In practice, a micro-electrode is used for the pH measurement, and the fluid surrounding it is a thin film of sodium bicarbonate solution trapped in a spacer ('inner membrane') of special tissue paper ('Joseph paper') or nylon mesh. This spacer maintains this small volume of electrolyte between the thin 'outer' membrane, often made of Teflon (PTFE), and the H⁺-sensitive glass electrode. The scale on the pH meter at E is calibrated directly in $P_{CO_2}$ units and often read out in a digital display.

![Fig. 10.16. A $P_{CO_2}$ electrode. The CO$_2$ in the blood at D diffuses across the thin plastic membrane at C and equilibrates with the electrolyte at B. Hydrogen ions are generated and the meter at E records the pH detected by the glass H⁺-electrode at A.](image)

**CALIBRATION**

The electronics of the meter at E are set during calibration with a gas mixture of known composition. The gas is saturated with water vapour (otherwise the electrolyte at B would be evaporated) and flows through a sample chamber past the tip of the electrode at D. This sample chamber is devised so that an appropriate volume of blood (about 0.1 ml) can be drawn in anaerobically and kept in contact with the electrode membrane in anaerobic conditions until a steady reading is obtained. There is a time-lag while the CO$_2$ diffuses across the plastic membrane and equilibrates with the electrolyte in the electrode, but the reading should become stable within 1 or 2 minutes.

**The pH glass electrode**

The pH is also measured using a glass micro-electrode such as a capillary electrode. Many modern capillary electrodes require only about 25 μl (i.e. 0.025 ml) of blood for pH determination. Capillary glass electrodes function in exactly the same way as any other pH electrode, i.e. the potential across a thin piece of special glass which is sensitive to H⁺ ions is measured with respect to a reference electrode, usually a
calomel electrode. In a capillary electrode the glass is drawn into a capillary tube with the ion-sensitive surface on the inside (i.e. facing the lumen), instead of being blown into a bulb with the ion-sensitive surface on the outside as is the case with conventional macro-scale pH electrodes (Fig. 10.17). The capillary tube and reference electrode are mounted in a water jacketed chamber at 37°C; a salt bridge (saturated KCl) makes electrical continuity between the two. The physical organization of the components depends on the particular model of electrode, but Fig. 10.18 illustrates one typical layout.

Fig. 10.17. H⁺-ion sensitive electrodes. Capillary electrodes can be used with volumes as small as 25 µl. See also Fig. 10.18.

Fig. 10.18. A pH micro-electrode assembly (not to scale).

It is generally convenient to make the pH measurement on a further portion of the blood sample while the CO₂ electrode is equilibrating with the sample and approaching a steady state reading.

Several instruments have PO₂ and PCO₂ electrodes arranged so that they are simultaneously exposed to the same blood sample. The principle of the Clark oxygen electrode is briefly described in Appendix III.

An example of the use of the Siggaard–Andersen Alignment Nomogram to calculate acid–base parameters following direct measurement of PCO₂ and pH is given in Appendix IV.
Alveolar $P_{CO_2}$ measurement

Before measurement of $P_{CO_2}$ in arterial blood became feasible with micro-electrodes, it was common to estimate the $P_{CO_2}$ in end samples of expired air. If carefully taken these were fairly representative of alveolar air, which is assumed to be in equilibrium with the pulmonary arterial blood. Measurement of the $P_{CO_2}$ in the gas sample was generally made by a manometric method. This type of method was widely used by J. S. Haldane in his pioneering studies on the physiology of respiration.
The quality of any physical or chemical measurement on a blood sample is only as good as the sample itself. That is to say that no amount of skill and no sophisticated instrument can compensate for errors or carelessness in the taking and handling of the blood sample: this is true, of course, of all analytical procedures, but acid-base measurements are particularly vulnerable to errors at this stage.

**Venous, arterial or capillary blood?**

In the first place the correct choice of blood sample must be made. For accurate work *venous blood* is undesirable for $P_{CO_2}$, pH, base excess or standard bicarbonate determination; ordinary venous blood is totally valueless for $P_{O_2}$ measurement. The composition of venous blood in these respects is affected by the local activities of the organ drained by the vessels in question and by the rate of blood flow through these peripheral tissues; it is therefore not indicative of the acid-base status of the whole body, and the $P_{CO_2}$ and especially $P_{O_2}$ values in venous blood have little bearing on the functioning of the respiratory system. For approximate screening procedures entailing determination of pH and total $CO_2$ content venous samples have sometimes been considered adequate. Venous blood can be 'arterialized' by maintaining the patient completely at rest and with the forearm warmed to about 41°C for about 20 minutes. If venous blood is then taken without even temporary stasis from a vein on the dorsum of the hand, the acid-base parameters may be found to be similar to those in arterial blood. However this procedure is not often followed.

Some authorities have advocated the use of venous blood on the grounds of making acid-base (blood gas) determination a routine procedure. However, the indiscriminate proliferation of laboratory tests under the guise of 'screening tests' must be resisted. As with all tests the questions must be asked: What new or confirmatory information will this give about the patient? Will this information actually influence the management of the patient?

*Arterial blood* is the first choice for acid-base (or blood gas) measurements. In intensive-care patients who require frequent monitoring of acid-base parameters an indwelling arterial catheter will often be a convenient source of blood. Vessels with a good collateral circulation are preferable. The radial artery at the wrist is a frequent choice: it is easy to puncture and a complete collateral circulation is provided by the ulnar artery. Alternatively, the brachial artery at the antecubital fossa, the temporal artery (especially in infants) or the femoral artery may be used. About 5–10 ml of blood should be drawn into a 10 ml siliconized glass syringe. The syringe should
contain a metal ring washer or a drop of mercury for mixing, and the dead space must be filled with sodium heparin (1000 or 5000 u/ml) (see below).

Capillary blood is sometimes used as a convenient alternative to arterial blood, since the acid–base parameters are generally found to be closely similar to those of arterial blood. However Po2 values are somewhat low unless the region is warmed beforehand. Now that micro methods are readily available for determination of acid–base parameters on very small samples of blood capillary samples are often convenient, especially in paediatric work where it is not feasible to withdraw large volumes of blood frequently. Typical sites for capillary puncture are the richly vascular bed of a finger or thumb, of an ear-lobe, or of the back of the heel in infants.

The blood must be withdrawn only when the subject is relaxed and respiration is no longer affected by anticipation of the procedure. Obviously hysteria or breath-holding produces considerable changes in the Pco2.

Anaerobic sampling
The blood sample must be taken anaerobically and must be kept under such conditions. That is to say, contact with air must be strictly avoided. Thus if capillary blood is taken it must not be allowed to flow out slowly forming a thin film exposed to the air, as considerable loss of CO2 to the atmosphere can then occur. Instead, a good flow of blood should be obtained by warming the site and making a firm bold incision. If a suitably deep stab is made an adequate flow of blood will normally result and a capillary collecting tube should be held almost horizontally with the end into the centre of the drop of blood rather than at the edge of the drop. In this way air is not taken into the capillary collecting tube and the blood which is sampled is not allowed to come in contact with air. Local stasis and redistribution of tissue and vascular fluids by squeezing should be avoided.

If the blood is drawn into a syringe, a glass syringe rather than a plastic one should be used. Gases are soluble in plastic and therefore carbon dioxide and especially oxygen dissolve in the walls of the syringe and can diffuse across it. Although many argue that the error from this source is of academic interest only, the use of glass syringes is accepted practice. An additional advantage of glass syringes is that the plunger moves relatively easily: thus arterial pressure can be recognized when the needle is correctly in place and introduction of air bubbles by creating a negative pressure with a ‘sticky’ plunger is minimized. Air bubbles in the syringe must be strictly avoided; any air present should be expelled before the blood is mixed with the anticoagulant.

Site of blood sampling
In patients receiving an intravenous infusion samples for analysis should be taken from a limb remote from the one receiving the infusion. Although arterial blood should be unaffected by the intravenous infusion, the apparent composition of venous and possibly capillary blood will be grossly inaccurate and in principle this situation should be avoided habitually. The choice of artery for puncture has already been briefly discussed; the radial artery is often preferred.

Anticoagulant
Blood gas measurements require anticoagulated whole blood. As with other blood analyses, the correct choice of anticoagulant is vital. Heparin salt (either lithium or sodium heparin) is recommended; 1000 u/ml should be used. Fluoride, citrate,
EDTA, ammonium heparin and oxalate all change the pH and other acid-base parameters slightly and so should not be used. Blood sample bottles containing anticoagulant are generally unsuitable since the blood cannot be transferred to them anaerobically and kept in strictly anaerobic conditions. In practice the blood should be taken to the laboratory in the syringe. This may be capped by a luer hub cap or by placing a cork or bung over the end of the needle, or even by bending the needle.

Fine-bore glass tubes for collection of capillary blood generally have heparin salt dried on to the inside walls of the tubes. Thorough mixing of the blood sample with the anticoagulant is necessary and is generally achieved by the insertion into the tube of a small piece of wire about 5 mm long. Once the ends of the tube have been sealed with special wax or Plasticene a magnet is slid vigorously to and fro along the outside of the tube so that the wire stirrer mixes the blood thoroughly with the anticoagulant. If mixing at this stage is inadequate and part of the blood clots, there is no recourse apart from collecting a further blood specimen.

**Preservation of blood specimens and immediacy of analysis**

Analysis must be performed as soon as possible after the sample has been taken, i.e. within about 15 minutes. If the samples are chilled immediately after collection in a refrigerator (*not* a deep freeze since freezing and thawing of blood causes haemolysis) or in ice-water, then a delay of up to about 4 hours is probably acceptable.

Blood cells are continuously using O₂ (except the erythrocytes which use anaerobic glycolysis) and producing CO₂ and lactic acid. Therefore, unless the rate of cellular metabolism is reduced by cooling, the P_po₂, P_pco₂, pH and base excess, etc. values will change in the interval between collection and analysis of the blood specimen. In blood at 37°C the pH falls by about 0.01 units in 10 minutes while the P_co₂ rises by about 1 mmHg. In ice the rate of change is about one-tenth of that for unchilled blood.

The use of fluoride as a glycolytic inhibitor (as is exploited in the preservation of samples for blood glucose determination) is not recommended since fluoride itself significantly changes the pH of a blood sample and also because aerobic metabolism of substrates other than glucose is not inhibited.

**Temperature of patient**

The patient's temperature should be noted at the time of collection of the blood specimen. Values for acid-base parameters are normally reported as values at 37°C by international agreement. Corrections for small deviations in temperature from 37°C can be applied by reference to standard tables. Where large deviations are encountered, e.g. in surgical hypothermia, it must be borne in mind that the values regarded as normal at 37°C will no longer be valid.

If measurements are made on samples at temperatures other than 37°C correction values must be applied; the results obtained directly from nomograms such as the Siggaard-Andersen Curve Nomogram and the Alignment Nomogram are valid only at 37°C since the values for pK and the solubility coefficient of carbon dioxide (which are required for solution of the Henderson-Hasselbalch equation) are temperature-dependent. Appropriate corrections can be obtained from tables or else from the acid-base calculator slide-rule (see Appendix IV).

**Mixing of blood before analysis**

Blood samples must be thoroughly mixed before analysis in case the cells have sedimented. Immediately before introduction of a sample into an instrument,
thorough mixing must be effected either by use of the magnet and stirrer wire in the case of glass capillary tubes or by repeated inversion of a syringe. In the latter case some users find the inclusion of a metal washer or a blob of mercury in the syringe a convenience; the temptation to introduce an air bubble to allow effective agitation must, of course, be avoided altogether.
Appendix I

The Henderson–Hasselbalch Equation

The pH of any solution and the ratio of the concentrations of base to acid components of any buffer present are closely related. The Henderson–Hasselbalch equation is a very close approximation to this relationship.

Consider any weak acid HA dissociating as follows:

\[
\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-
\]

By definition the equilibrium constant, \( K \), which is constant at any given temperature is given by:

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

(this is the acid dissociation constant \( K_a \): see below for definition of the base dissociation constant, \( K_b \)).

(Strictly one ought to write activities rather than concentrations of all components in this equation. However, at fairly low concentrations activity coefficients are only very slightly less than unity, and therefore for practical purposes the concentration may be considered equal to the activity. This is convenient since true activities are difficult to determine.)

Then

\[
[H^+] = K \cdot \frac{[\text{HA}]}{[\text{A}^-]}
\]

(this is the general form of the Henderson equation).

or

\[
\frac{1}{[H^+]} = \frac{1}{K} \cdot \frac{[\text{A}^-]}{[\text{HA}]}
\]

Taking logs to base 10:

\[
\log \frac{1}{[H^+]} = \log \frac{1}{K} + \log \frac{[\text{A}^-]}{[\text{HA}]}
\]

By definition:

\[
pH = -\log [H^+] = \log \frac{1}{[H^+]}
\]
and similarly:

\[ pK = -\log K = \log \frac{1}{K} \]

So that:

\[ pH = pK + \log \frac{[A^-]}{[HA]} \]

i.e. in general terms:

\[ pH = pK + \log \frac{[\text{base}]}{[\text{acid}]} \]

this is the *Henderson-Hasselbalch equation*.

For the CO\textsubscript{2}/HCO\textsuperscript{−} buffer system:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

the overall pK for the equilibrium:

\[ \text{CO}_2 (+\text{H}_2\text{O}) \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]

(i.e. pK') is 6.1 at 37°C (N.B. one ignores the concentration of H\textsubscript{2}O in reaction since this value is always constant at 55.4 m).

Thus:

\[ pH = 6.1 + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2] + [\text{H}_2\text{CO}_3]} \]

where \([\text{HCO}_3^-], [\text{CO}_2] \) and \([\text{H}_2\text{CO}_3]\) are each in units of mmol/litre or mEq/litre.

In practice it is customary to use units of partial pressure of gaseous CO\textsubscript{2} (i.e. \(P_{\text{CO}_2}\)) since this is relatively easily measured. The conversion of units to mmol/litre and allowance for the solubility of CO\textsubscript{2} in water is accomplished by multiplying the \(P_{\text{CO}_2}\) by 0.03, the solubility coefficient at 37°C. Thus

\[ [\text{CO}_2] + [\text{H}_2\text{CO}_3] \text{ in mmol/litre} = 0.03 \times P_{\text{CO}_2} \text{ in mmHg} \]

If the new S.I. units are used then the solubility factor is 0.226:

\[ [\text{CO}_2] + [\text{H}_2\text{CO}_3] = 0.226 \times P_{\text{CO}_2} \text{ in kPa} \]

**Dissociation constants \(K_a\) and \(K_b\)**

It is general to define the pK (i.e. the p\(K_a\)) in terms of the \(K_a\), the acid dissociation constant, as discussed above:

\[ \text{HA} \rightleftharpoons \text{H}^+ + \text{A}^- \]

which gives:

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

However some books refer to values of \(K_b\), and p\(K_b\). These refer to the equilibrium between a base (proton acceptor) and water, viz.

\[ B + \text{H}_2\text{O} \rightleftharpoons \text{BH}^+ + \text{OH}^- \]

base conjugate acid
In this context the water is acting as an acid, i.e. a proton donor, viz.

\[ \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^- \]

The basic dissociation constant is then defined by:

\[
K_b = \frac{[\text{BH}^+][\text{OH}^-]}{[\text{B}]}
\]

It can easily be shown that \( pK_a \) and \( pK_b \) values are related thus:

\[ pK_a + pK_b = pK_w \]

where \( pK_w \) refers to the dissociation of water and has a value of 14.0 at 25°C, and 13.6 at 37°C. So:

\[ pK_b = 14 - pK_a \]

When no subscript is shown (viz. \( pK \)) the constant may be assumed to refer to the acid dissociation (i.e. \( pK_a \)); there is little practical benefit in the use of \( pK_b \) values.
Appendix II

Units and Normal Values of Acid-Base Parameters

The pH scale

Hydrogen ion concentration* is most frequently expressed according to the pH notation. pH is defined by:

\[ \text{pH} = \log \frac{1}{[H^+]} \quad \text{or} \quad \text{pH} = -\log [H^+] \]

i.e.

\[ \text{pH} = -\log cH^+ \quad cH \text{ in mol/litre} \]

so

\[ cH^+ = \text{antilog}(9 - \text{pH}) \quad cH \text{ in nmol/litre} \]

The advantages of this scale are that hydrogen ion concentration can be expressed as numbers within a convenient range, i.e. 0 to 14 pH units, rather than as extremely small numbers of the order of $10^{-7}$ mol/litre. For similar reasons dissociation constants for acids and bases are conveniently quoted in terms of pK values, where:

\[ \text{pK} = \log \frac{1}{K} = -\log K \]

However this advantage tends to be outweighed by the fact that non-chemists and non-mathematicians are not accustomed to thinking in logarithmic units. The Système Internationale (S.I.) of Units recommends the use of $[H^+]$ in nanomoles/litre (i.e. $10^{-9}$ moles/litre) rather than the use of the pH scale. A further disadvantage associated with pH is that it is equal to a negative logarithm, so that increasing hydrogen ion concentrations are represented by decreasing numbers on the pH scale and this concept is liable to confuse many. Furthermore the use of the logarithmic pH scale tends to conceal the real magnitude of changes in hydrogen ion concentration. For example, a 1.3 % change in pH from 7.45 to 7.35 corresponds to a very much larger percentage change (29 %) in the actual $H^+$ ion concentration from 35 to 45 nmol/litre.

Another problem concerns the calculation of mean or average values of pH: it is not strictly valid merely to add up all the pH values and divide by the number of

*Conventionally, square brackets are used to denote concentrations thus, $[H^+]$—also sometimes shown as $cH^+$. Strictly speaking one should use activities rather than concentrations, but in practice the distinction between the two is often ignored for convenience. Anyway, activity coefficients (the numerical factors relating activities to concentrations) are often sufficiently near to unity under physiological conditions as to make any distinction of theoretical interest only.
observations since the use of the logarithmic scale will weight the concentration values unequally. Instead each value should be converted into hydrogen ion concentration, and then all values should be averaged and the mean converted back into a pH value. Similar considerations should be taken into account before calculating a standard deviation. However since mean values are not often employed in clinical practice this problem is likely to affect mainly research workers.

Nevertheless the logarithmic pH notation is widely used and is likely to remain so for a long time yet. Therefore an easy familiarity with it will greatly aid understanding of much of the literature on acid-base balance as well as clinical laboratory data. From the definition of pH it can be seen that a doubling of hydrogen ion concentration is represented by a fall in pH of 0.3, since log 2 = 0.3. Conversely, an increase of 0.48 pH units corresponds to a decrease in hydrogen ion concentration by a factor of three, since log 3 = 0.48.

A semi-logarithmic graph relating pH and H⁺ ion concentration values is given in Fig. A.1.

![Fig. A.1. Semi-logarithmic relationship between pH and hydrogen ion concentration.](image)

**Metric units and S.I. units**

Over the past few decades metric units have been exclusively used for expression of clinical chemistry parameters, including those for acid–base and blood gas measurements.

The principal units encountered were mEq/litre and, for gas concentrations or partial pressures, mmHg. However at the time of writing there is a progressive transition to Système Internationale (S.I.) units. The transition will clearly be a gradual and slow one and there is unlikely to be any uniformity in the speed at which different
hospitals and clinical laboratories undergo the change-over. Therefore every clinician
must be aware of the two sets of units and must be prepared to think in terms of
whichever unit is favoured at any given time in his own particular environment. While
it is intended that S.I. units will become universally adopted, it will still be useful for
the newcomer to be familiar with the 'old' metric equivalents since the bulk of the
literature which he will read will use metric units. Furthermore he must be able to
communicate with and understand other clinicians and laboratory staff, etc., of the
'older school' who will inevitably think in 'old' units. Changes of units
are always difficult, but every clinician and laboratory worker has a clear responsibility
to adopt the recommended units with great precision. The half-hearted adoption of new
units will only lead to greater confusion, poorer communications, and less uniformity of
practice between doctors, and even accidents. Throughout this book both sets of
units have been used.

The recommended S.I. units for acid–base and blood-gas work are millimoles per
litre (mmol/litre) and kilopascals (kPa).

Inter-conversion of units

mEq/litre and mmol/litre

The equivalent weight of any molecule or ion is defined as the weight which will
combine with or release one equivalent of hydrogen (or one equivalent of any other
molecule or ion). In the case of a monovalent ion, the equivalent weight is equal to
the molecular weight.

\[
\text{HCl} = \text{H}^+ + \text{Cl}^-
\]

Thus 1 gram equivalent of hydrochloric acid is equal to 36.5 g. But in the case of
sulphuric acid which is divalent:

\[
\text{H}_2\text{SO}_4 = 2\text{H}^+ + \text{SO}_4^{2-}
\]

the equivalent weight is equal to half the molecular weight. In the case of bicarbonate:

\[
\text{HCO}_3^- + \text{H}^+ \rightleftharpoons \text{H}_2\text{CO}_3
\]

the equivalent weight is equal to the molecular weight. Hence 24 mEq HCO\textsubscript{3}\textsuperscript{-} per litre
is the same as 24 mmol/litre.* In this case (and for all other monovalent species) no
conversion factor is required from metric to S.I. units.

However calcium ions are divalent (Ca\textsubscript{2}+) and therefore the equivalent weight is
half the molecular weight (i.e. 1 gram molecule contains 2 gram equivalents). Therefore
a calcium concentration of 3 mEq/litre is expressed as 1.5 mmol/litre in S.I. units.
Note that while Ca\textsubscript{2}+ cannot be combined with or directly related to H\textsuperscript{+} ions, they can
combine with OH\textsuperscript{-} ions:

\[
\text{Ca}^{2+} + 2\text{OH}^- \rightarrow \text{Ca(OH)}_2
\]

One mole of OH\textsuperscript{-} ions is equivalent to one mole of H\textsuperscript{+} ions:

\[
\text{H}^+ + \text{OH}^- \rightleftharpoons \text{H}_2\text{O}
\]

so the equivalent weight of OH\textsuperscript{-} is equal to the molecular weight. Hence in:

\[
\text{Ca}^{2+} + 2\text{OH}^- \rightarrow \text{Ca(OH)}_2
\]

2 moles of OH\textsuperscript{-} (i.e. 2 gram equivalents) are combining with 1 mole (and hence 2
gram equivalents) of Ca\textsubscript{2}+. Therefore the equivalent weight of Ca\textsubscript{2}+ is equal to half
the molecular weight.

* The S.I. abbreviation 'mol' is short for mole or gram molecule. A gram molecule is a weight in
grams equal to the molecular weight.
MMHG and KPA

Gas concentrations (or tensions) are customarily expressed as partial pressures ($P_{CO_2}$ and $P_{O_2}$, sometimes written $p_{CO_2}$ etc., or when referred to arterial blood as $P_{acO_2}$, etc.). The metric unit for partial pressure is the millimetre of mercury (mmHg). At atmospheric pressure (e.g. 760 mm) the sum of the partial pressures of all gases in a mixture must add up to 760 mmHg by definition. Thus in a dry gas mixture containing 5% CO$_2$ at atmospheric pressure (760 mm), the partial pressure of carbon dioxide ($P_{CO_2}$) is $\frac{5}{100} \times 760 = 38$ mmHg. If barometric pressure is low at 740 mm, then $P_{CO_2} = \frac{5}{100} \times 740 = 37$ mmHg. If the gas mixture is not dry but is saturated with water, it must contain water vapour at a partial pressure equal to the saturated vapour pressure of water, which is 47 mmHg at 37°C. Thus a 5% CO$_2$ gas mixture saturated with water vapour at 37°C must exert a total pressure of 760 mm if this is atmospheric pressure; the sum of partial pressures exerted by all gases apart from the water vapour is $(760 - 47) = 713$ mm. Therefore the $P_{CO_2}$ of a 5% CO$_2$ mixture equilibrated with water is $\frac{5}{100} \times 713 = 35.65$ mmHg.

The S.I. unit for pressure (e.g. gas tension or partial pressure) is the pascal (Pa) or the kilopascal (kPa). One pascal is a newton per square metre.

One kPa $\equiv$ 7.5006 mmHg
1 mmHg $\equiv$ 133.32 Pa $\equiv$ 0.133 kPa

So that a $P_{CO_2}$ of 40 mmHg is the same as 5.3 kPa
or a $P_{O_2}$ of 40 mmHg is the same as 5.3 kPa

A conversion nomogram relating mmHg to kPa is shown in Fig. A.2.

![Fig. A.2. Interconversion of mmHg and kilopascals.](image)

**Normal values of acid-base parameters**

'Normal' values and their ranges may vary slightly according to criteria and methods. Therefore most clinical laboratories publish internally their own list of values which
they regard as ‘normal’. Normal values in infants and in hypothermic patients are different and are subject to some debate. Therefore the following list is given only for general guidance.

*Adults—Arterial Blood*

<table>
<thead>
<tr>
<th>Metric Unit</th>
<th>S.I. Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td><strong>H⁺ or cH</strong></td>
</tr>
<tr>
<td>= 7.40 (7.35—7.45)</td>
<td>= 40 (35—45)</td>
</tr>
<tr>
<td><strong>PcO₂</strong></td>
<td></td>
</tr>
<tr>
<td>40 (35—45) mmHg</td>
<td>5.33 (4.67—6.00) kPa</td>
</tr>
<tr>
<td>Actual bicarbonate</td>
<td>24 (22—26) mEq/litre</td>
</tr>
<tr>
<td>Standard bicarbonate</td>
<td>24 (22—26) mEq/litre</td>
</tr>
<tr>
<td>Base excess</td>
<td>0 (—2 to +2) mEq/litre</td>
</tr>
<tr>
<td>Total CO₂ content</td>
<td>25 (23—27) mEq/litre</td>
</tr>
</tbody>
</table>
Appendix III

Determination of $P_{O_2}$

Although oxygen tension measurements are not strictly relevant to acid–base balance, measurement of arterial $P_{O_2}$ frequently accompanies that of $P_{CO_2}$ in respiratory investigations. Indeed in a ventilation unit blood gases ($P_{O_2}$ and $P_{CO_2}$) are frequently determined while there may be little or no interest in the metabolic component.

The normal value of $P_{O_2}$ in arterial blood is 80–120 mmHg (10.7–16.0 kPa). Values in venous blood are enormously variable and are valueless as indicators of ventilatory sufficiency.

![Diagram of a Clark polarographic oxygen electrode.](image)

Fig. A.3. A Clark polarographic oxygen electrode.

The standard method for determination of $P_{O_2}$ utilizes the Clark polarographic electrode. Polarography entails the electro-chemical reduction of a substance in solution. The oxygen electrode consists of a very small platinum cathode and a silver anode across which a polarizing potential of about 0.6 volts is applied. At this potential, all oxygen molecules at the cathode are rapidly reduced:

$$O_2 + 4e^- \rightarrow 2O^2^-$$

At voltages around 0.6 volts it is found that the current flowing is almost independent
of voltage but is closely proportional (i.e. linearly related) to the \( P_O_2 \) at the cathode. The current flowing is typically of the order of \( 10^{-11} \) amp/mmHg.

In order to prevent poisoning of the electrode by deposition of blood proteins on the platinum cathode the electrode is immediately surrounded by a very small volume of an electrolyte such as KCl, and a thin plastic membrane which is permeable to oxygen. The volume of the electrolyte and the size of the cathode must be minimized in order to avoid inconsistencies in the oxygen diffusion gradient.

Fig. A.3 shows diagrammatically a typical Clark oxygen electrode. Calibration is achieved, as for the \( P_C_O_2 \) electrode, with standard gas mixtures. Alternatively since the characteristics of response to a gas and a blood sample are different, giving rise to a correction factor, the so-called 'blood gas factor', the calibration may be effected using either water which is saturated with air at 37°C or an \( O_2 \)-free reducing solution such as sodium dithionite to set the zero.

In practice, oxygen electrodes, as well as being supplied for use by themselves, are now frequently incorporated in blood gas analysers so that \( P_C_O_2 \) and \( P_O_2 \) electrodes are simultaneously in contact with the same small blood sample.
Appendix IV

Nomograms and Other Aids to Calculations

Nomograms

Nomograms and acid-base charts have three main functions:

1. **Educational**, in that they portray graphically and aid in understanding the relationships between the variables in the Henderson–Hasselbalch equation and the particular combinations of these parameters associated with particular disturbances.

2. **Computational**, since they allow the quick graphical solution of the Henderson–Hasselbalch equation: hence if any two variables are known, the remaining parameters can be calculated and so acid-base status can be assessed completely.

3. **Recording**, because the path of progressive changes in a patient’s acid-base status with time can be mapped and visualized, i.e. an acid-base path.

**In vivo (or whole-body) nomograms**

Additionally charts can be constructed using acid-base parameters obtained from patients in whom the pattern of acid-base balance was known with some certainty from clinical observations; such diagrams can then be applied to and used to interpret observations on patients where the clinical picture is less clear. They thus remove difficulties which may be caused by the fact that the titration curve of blood in vitro is significantly different from that in vivo (see Chapter 3). They also greatly facilitate the recognition and interpretation of mixed disturbances. It is argued that the in vivo response of the intact patient should provide the most appropriate reference point from which to assess mixed acid-base disorders.

A perplexing variety of different acid-base diagrams of various shapes and forms have been published and only a few examples will be briefly introduced here.

**Siggaard–Andersen curve nomogram**

The theoretical basis and the practical use of the log $P_{CO_2}$ versus pH coordinate plot have been discussed in Chapter 10 in connection with the Astrup equilibration technique. The Siggaard–Andersen Curve Nomogram is particularly useful for evaluating results obtained by the Astrup two-point interpolation method.

**Example**

Fig. A.4 shows a Siggaard–Andersen Curve Nomogram with a buffer line A–B entered thereon. The experimental observations and other data are recorded in the spaces at the foot of the chart. For this example, a sample of blood was tonometered (equilibrated) with a gas mixture containing 8.05% CO$_2$: this corresponds to a $P_{CO_2}$
Fig. A.4. Example of use of the Siggaard-Andersen Curve Nomogram (see text). (Reproduced by permission of Radiometer A/S.)
of 57 mmHg after allowance for barometric pressure of 755 mmHg and the saturated vapour pressure of water (47 mm) at 37°C. The pH after equilibration was found to be 7.16. Point A, corresponding to the coordinates (57; 7.16) was entered on to the nomogram. Likewise a second portion of blood tonometered with 4.08% CO2 (28.9 mmHg) had a pH of 7.32 and point B was entered on the nomogram. The straight line, the buffer line, is drawn to join points A and B. The actual pH of the blood sample, without any equilibration, was found to be 7.30 and this point was entered on to the buffer line at C.

The Pco2 (respiratory component) can then be read off directly by interpolation at D. Values for total buffer base, standard bicarbonate and base excess (metabolic component) are read on the respective scales at the point of intersection by the buffer line. Thus in the example in Fig. A.4 the Pco2 = 31 mmHg; total buffer base = 38 mmol/litre or mEq/litre; standard bicarbonate = 16.5 mmol/litre (mEq/litre); and base excess = −10 mmol/litre (mEq/litre).

These data are consistent with a metabolic acidosis with partial respiratory compensation.

CORRECTION FOR OXYGEN SATURATION

The equilibrating gases used in the Astrup technique contain a relatively high Po2 so that the tonometered blood samples will be 100% saturated with oxygen (i.e. So2 = 100%). If the blood sample from the patient has an oxygen saturation of less than about 90%, a correction should be applied to the buffer line before the Pco2 and base excess values are read off. The reason is that the pH of deoxyhaemoglobin is greater than that of oxyhaemoglobin since oxyhaemoglobin is a stronger acid than deoxyhaemoglobin. Hence the process of equilibrating the blood samples in the tonometer at an oxygen tension significantly higher than that already in the blood generates H+ ions which will therefore give rise to a smaller base excess (or greater base deficit) than actually existed in the patient. No correction is required for standard bicarbonate or total buffer base, since these values are defined conveniently in terms of fully oxygenated blood with So2 = 100%.

The magnitude of the correction factor, C, which has to be applied to significantly unsaturated blood (i.e. when So2 < about 90%) is given approximately by:

\[ C = 0.3 \times [\text{Hb}] \times \frac{100 - \text{So2}}{100} \text{ mmol/litre} \]

where [Hb] is haemoglobin concentration (g/100 ml), and So2 is the percentage oxygen saturation.

Strictly speaking this correction factor is pH-dependent; however over the pH range 7.0–7.8 this approximation is adequate for practical purposes. This correction value is added to both the apparent total buffer base and the apparent base excess values. The new corrected values are then marked on the two respective scales and the corrected buffer line is drawn in. The actual pH value is inserted and the true Pco2 read off by interpolation from this corrected buffer line.

An example may make this technique clearer. Consider the data already entered onto Fig. A.4 and now suppose that the patient's blood was only 50% oxygen-saturated. If the haemoglobin concentration is 14.5 g% (as in the previous example) then the correction factor:

\[ C = 0.3 \times 14.5 \times \frac{100 - 50}{100} = 2.2 \text{ mmol/litre} \]
Fig. A.5. Example of correction for oxygen saturation on Siggaard-Andersen Curve Nomogram (see text). (Reproduced by permission of Radiometer A/S.)
Fig. A.6. Example of use of the Siggaard-Andersen Alignment Nomogram (Reproduced by permission of Radiometers A/S.)
The apparent value of total buffer base as determined from the uncorrected buffer line was 38 mmol/litre: the corrected value therefore is \((38 + 2\cdot2) = 40\cdot2\) mmol/litre. Similarly the apparent value for base excess which was \(-10\) mmol/litre (i.e. base deficit = 10 mmol/litre) gives a corrected value of \((-10 + 2\cdot2) = -7\cdot8\) mmol/litre (i.e. base deficit = 7-8 mmol/litre). The corrected buffer line is then drawn through these points at X and Y respectively, and the actual pH of the unequilibrated sample (7-30 in this example) inserted at Z (Fig. A.5). This corresponds to \(Pco_2\) = 36 mmHg (N on Fig. A.5).

**Siggaard-Andersen alignment nomogram**

This linear nomogram allows immediate solution of the Henderson–Hasselbalch equation provided any two out of the following variables are known: pH, \(Pco_2\), base excess (and \([Hb]\)), actual bicarbonate, or total \(CO_2\)-content. In practice the alignment nomogram is particularly convenient when values of \(Pco_2\) and pH have been obtained electrometrically, or when the total \(CO_2\) content and either the pH or \(Pco_2\) are known.

**Example**

The nomogram is shown in Fig. A.6, together with an example. The pH and \(Pco_2\) of a blood sample were found to be 7-35 and 70 mmHg respectively. The haemoglobin concentration is also known to be 15 g% (i.e. 15 g Hb/100 ml of whole blood). The points pH = 7-35 and \(Pco_2\) = 70 are marked at A and B on the respective scales and the straight line is drawn through these two points and right across the diagram. The actual bicarbonate concentration is read at C (38 mmol/litre) and the base excess value is read at D where the straight line cuts the \([Hb]\) = 15 g% line on the base excess scale (this gives base excess = +9 mmol/litre).

If the standard bicarbonate concentration is required this may be evaluated by drawing a second straight line on to the nomogram as the dotted line from E to F. The line is drawn so as to pass through \(Pco_2\) = 40 mmHg (5-33 kPa), i.e. respiratory factor normal, and the point D on the base excess scale. The point of intersection on the bicarbonate scale, i.e. at G, gives the standard bicarbonate, that is, what the bicarbonate concentration would be if the respiratory component were normal. In this example the value is 33 mmol/litre.

The data of this example are consistent with a well compensated (renal compensation) respiratory acidosis as in a chronic, i.e. prolonged, condition such as chronic bronchitis.

**Alignment Nomogram for Astrup Equilibration Method**

This alignment nomogram may also be used to calculate full results from data obtained using the Astrup equilibration method. An example is shown in Fig. A.7, using the same data as those already plotted on the curve nomogram in Fig. A.4. The blood sample tonometered at \(Pco_2\) = 57 mmHg (7-58 kPa) had a pH of 7-16. These values are marked at A and B respectively (Fig. A.7) and joined by a straight line. Since the base excess value is unaffected by the tonometry and must be the same as that in the patient the value can be used to read off from the base excess scale if the haemoglobin concentration is known.

However if the haemoglobin concentration is not known or if one is using the conventional two-point equilibration technique advocated by Astrup then one tonometers a second portion of the blood sample and draws in a second line. In the previous example the second sample was equilibrated at \(Pco_2\) = 29 mmHg (3-86 kPa) and its pH was then 7-32. These values are marked at C and D respectively and are joined by a straight line. The two lines intersect at E from which the base excess
Fig. A.7. Example of use of the Siggaard-Andersen Alignment Nomogram to evaluate results obtained by the Astrup equilibration technique. (Reproduced by permission of Radiometer A/S.)
value is read and also an approximate value for the haemoglobin concentration. The latter should tally within about ± 3 g% with an independent estimate, and serves as a check on the analytical procedure.

Finally the actual pH of the unequilibrated blood sample (7.30) is noted on the pH scale at F. A line drawn through E and F (the dotted line in Fig. A.7) enables the actual PCO₂ in the original blood sample to be read off at point G on the PCO₂ scale.

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![Blood Acid-Base Nomogram](image)

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**Fig. A.8.** The I.L. triangular blood acid-base diagram. *(Reproduced by permission of Instrumentation Laboratory (U.K.) Ltd.)*
which shows \( P_{\text{CO}_2} = 31.5 \text{ mmHg} \) (4.19 kPa). Note that these values agree with those obtained using the Curve Nomogram.

Supplies of both the Siggaard-Andersen nomograms are commercially available from the Radiometer Company, Copenhagen, who manufacture acid-base analytical apparatus.

**The triangular blood acid-base nomogram**

This nomogram (Fig. A.8) is one of a large number of different styles of diagram relating \( P_{\text{CO}_2} \) and \( \text{pH} \), etc. If total \( \text{CO}_2 \) content and \( \text{pH} \) values are known then the \( P_{\text{CO}_2} \) can be read off directly. This type of diagram (and alternatives such as the Davenport \( \text{pH} \) versus \( \text{HCO}_3^- \) diagram) is useful for drawing 'acid-base paths', that is for mapping the time-course of changes in acid-base disturbances, both for interpretation of the progress of the patient's status and for record purposes.

Obviously blood from a patient with \( \text{pH} \) in the range 7.35 to 7.45 and \( P_{\text{CO}_2} \) in the range 35–45 mmHg is normal, and so falls within the hexagon in the centre of the diagram. A pure metabolic acidosis will be accompanied by a fall in \( \text{pH} \) so that the point must lie along, or in the immediate vicinity of, the line marked 'mean metabolic control line' at, say, A. Note that if there is no respiratory compensation, or respiratory disorder, the \( P_{\text{CO}_2} \) will be 40 mmHg (or at least within the range 35–45 mmHg). Therefore all uncompensated metabolic disturbances must be represented by points lying along the 'mean metabolic control line'.

When respiratory compensation occurs the \( P_{\text{CO}_2} \) must fall and the \( \text{pH} \) rise; hence the status moves in the direction of point B on this diagram. Note that the line AB is approximately parallel to the line marked 'mean respiratory control line'.

It can be seen that each zone in this diagram corresponds to a unique combination of respiratory and metabolic factors (Fig. A.9). Thus a point in the region around the point B can only be achieved in a metabolic acidosis with respiratory compensation (or a mixed disturbance involving a primary respiratory alkalosis as well as the primary metabolic acidosis). Similarly a point lying on (or in the immediate vicinity of) the 'mean respiratory control line' can only be obtained if there is a respiratory disturbance but no metabolic disturbance including no compensation. A point in zone C, for example, can only be achieved in the presence of a mixed respiratory plus metabolic acidosities.

**The Flenley diagram**

It has been pointed out several times, especially in Chapter 3 (p. 21), that the \( \text{CO}_2 \)-titration curve of blood is different \textit{in vivo} from that \textit{in vitro}. Fig. 3.3 showed typical \( \text{CO}_2 \)-titration curves of blood: the upper line was drawn from titration data obtained \textit{in vitro} after the blood had been taken, while the lower one was obtained in the intact humans by allowing them to breathe relatively high concentrations of carbon dioxide. It is clear that the two curves are different and that the differences are particularly marked at \( P_{\text{CO}_2} \) values above about 55 mmHg (7.3 kPa). Thus the body fluids \textit{in vivo} are seen to be less effective than blood \textit{in vitro} is. The main reason is that the bicarbonate generated from \( \text{H}_2\text{CO}_3 \) \textit{in vivo} is distributed throughout the considerable volume of weakly buffered ECF as well as the blood. The implications of this difference are that values such as base excess and standard bicarbonate which are evaluated from \textit{in vitro} \( \text{CO}_2 \)-titration data can be inaccurate indicators of the buffer status \textit{in vivo}. It can therefore be said that 'the acid-base status of the patient's ECF is more important to the clinician than is that of his blood equilibrated with \( \text{CO}_2 \) \textit{in vitro}'.

In order to avoid such difficulties of interpretation, some authorities prefer to use nomograms constructed from such \textit{in vivo} 'whole-body' titration curves. Furthermore,
Appendix IV

C02ct. 'Respiratory Acidosis with Metabolic Compensation' 'Combined Metabolic and Respiratory Acidosis' Metabolic Acidosis with Respiratory Compensation

pH

Fig. A.9. Explanation of the I.L. triangular blood acid–base diagram. (Reproduced by permission of Instrumentation Laboratory (U.K.) Ltd.)

\[ \text{[HCO}_3\text{]} (\text{mmol/litre}) \]

\[ \text{pH} \]

Fig. A.10. The Flenley P\text{CO}_2 \text{ vs } [\text{H}^+] \text{ acid–base diagram. Acid–base diagram of arterial blood, the shaded rectangle indicating normal values of [H\\text{+}] and P\text{CO}_2; the significance bands for acute and chronic respiratory acidosis in vivo, with linear extrapolation of the acute band to include respiratory alkalosis; and the significance band for in vivo metabolic acidosis and alkalosis.} \text{ (Reproduced by permission of the author and Editor from Flenley 1971.)} \]
such whole-body titration curves should provide a good reference point from which the presence of mixed acid–base disorders can be judged. Compensation in chronic acid–base disorders is often not complete (see Chapter 9), and reference data from patients whose acid–base status is well characterized allow one to assess whether the observed pH in a patient can be accounted for by the normal compensatory processes or whether other factors (such as a complicating mixed disturbance) must be considered.

<table>
<thead>
<tr>
<th>Patient identification</th>
<th>Sample No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date and hour</td>
<td></td>
</tr>
<tr>
<td>pH in plasma</td>
<td></td>
</tr>
<tr>
<td>pCO₂ in blood</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>excess concentration of</td>
<td>(APo)</td>
</tr>
<tr>
<td>BASE in extracellular fluid</td>
<td>(mmol/l)</td>
</tr>
<tr>
<td>excess concentration of</td>
<td></td>
</tr>
<tr>
<td>BASE in extracellular fluid</td>
<td>(mmol/l)</td>
</tr>
<tr>
<td>pH in arterial plasma</td>
<td></td>
</tr>
<tr>
<td>pCO₂ in arterial blood</td>
<td>(mmHg)</td>
</tr>
</tbody>
</table>

Fig. A.11. The Siggaard-Andersen acid-base chart. (Reproduced by permission of Radiometer A/S.)
The Flenley PCO₂ vs [H⁺] acid–base diagram is an important example of a whole-body nomogram (Fig. A.10).

It is noteworthy that all the cases on which Flenley based his diagram indicated that the empirical relationships between PCO₂ and [H⁺] in primary unmixed disturbances lie along straight lines (Fig. A.10).

The zones indicated on the Flenley diagram have been constructed as the 95% confidence limits for uncomplicated (unmixed) primary disturbances. Clinical information about the subjects whose data contributed to this diagram gave firm grounds for belief that mixed disturbances were not included. Therefore if the observed data for a patient fall outside these confidence limits for a simple unmixed disturbance it is probable that a mixed disturbance (i.e. more than one primary acid–base disturbance) is present: this is often impossible to detect from other types of nomogram. However the converse is not true, and so it is not possible to state that a patient whose parameters lie within one of the marked bands does not have a mixed disorder.

Thus it should be noted that this type of diagram is not derived mathematically or theoretically but embodies and summarizes clinical experiences over large numbers of patients. The interested reader is referred to the original account of this diagram which includes a set of eight interesting case histories with acid–base paths (Flenley 1971).

Siggaard–Andersen acid–base chart
An alternative version of a whole-body acid–base chart is that of Siggaard–Andersen as shown in Fig. A.11. Copies of this diagram are available through Radiometer A/S.

Acid–base slide rules
Several manufacturers produce slide rules which are designed to solve the Henderson–Hasselbalch equation and to perform related calculations. These are simply mechanical versions of the alignment nomogram and are used extensively by those who routinely handle blood gas data. Probably the most comprehensive acid–base slide rule is that designed by Severinghaus (1966) and marketed by the Radiometer Company. This rule has scales for solution of the Henderson–Hasselbalch equation, computation of base excess, calculations on oxygen dissociation curves, temperature corrections and for gas–volume corrections for temperature, pressure and water vapour, etc.

Electronic computers
A number of instrument manufacturers offer small computers which can either be interfaced with existing acid–base analyser equipment or built into automatic acid–base apparatus. These have the advantage that they can be used by relatively unskilled personnel, although their expense currently puts them out of reach of small hospitals and laboratories. There is an increasing trend to produce semi-automatic equipment which is self-calibrating, and takes electronic readings of PCO₂, PO₂ and pH from a single small sample of blood. Measured and derived parameters are then displayed on digital displays or are printed out. Such equipment may make it possible to perform complete acid–base (or blood-gas) analysis in the intensive care unit rather than in a remote laboratory. Nevertheless stringent quality control checks must be carried out frequently to ensure accuracy.
Appendix V

Brief Summaries of Main Contents of Each Chapter

Chapter 2. The need for acid–base regulation

Blood pH must be kept within range 7.35–7.45 in spite of large normal metabolic production of acids. Volatile acid (CO₂) excreted via lungs, and non-volatile acids (notably H₂SO₄ from S-containing amino acids) excreted via kidneys. Plasma bicarbonate is depleted in buffering non-volatile acids (fixed acids) and must be regenerated by the kidneys. Normally all filtered bicarbonate must be conserved by renal reabsorption. The amounts of acid or base to be excreted depend on the diet, physiological circumstances (e.g. exercise) and normal or pathological functioning of metabolism. In pathological conditions metabolizable acids may accumulate faster than they can be metabolized: bicarbonate is depleted and Na⁺ and K⁺ counter-ions may also be lost. Carbon dioxide has important effects on the cardiovascular and central nervous system, so PCO₂ also must be regulated.

The main defence mechanisms are (1) chemical buffers which minimize pH changes and (2) physiological mechanisms (respiratory system and kidneys) which regulate the composition of the body fluids.

Chapter 3. Physiological buffers

Buffers are physicochemical mechanisms which minimize pH change on addition of acid or base. A buffer consists of a mixture of a weak acid and its conjugate base, and has maximum buffering capacity when pH = pK. An acid is a proton (H⁺) donor, and a base is a proton acceptor. Strength of an acid is expressed by pK value (pK = −log K where K is the dissociation constant). The lower the pK value the stronger is the acid. Polybasic (polyprotic) acids and bases have one pK for each dissociating and associating group. Relationship between pK, pH and buffer components is given by Henderson–Hasselbalch equation:

\[ \text{pH} = \text{pK} + \log \left( \frac{[\text{base}]}{[\text{acid}]} \right) \]

Bicarbonate/CO₂ buffer. The principal buffer of extracellular fluids. Volatile CO₂ is regulated by lungs, while bicarbonate is regulated (more slowly) by kidneys. The facts that CO₂ is volatile and is continually produced and that PCO₂ kept fairly constant by ventilation makes this a good buffer in spite of its unfavourable buffer ratio (20:1 at pH 7.4 since pK' = 6.1).

Haemoglobin. Many imidazole side chains with pKs near 7 make this a fairly good buffer. There is a large quantity of Hb in blood and also the fact that oxyhaemoglobin
is a stronger acid than deoxyhaemoglobin greatly increases the physiological significance.

*Plasma proteins and phosphate.* Relatively unimportant as buffers in extracellular fluid because of their low concentration.

**Bone.** Ca$^{2+}$, Na$^+$ and other ions in bone can exchange with extracellular H$^+$ ions thus ‘buffering’ them, especially in prolonged acidosis.

*Ammonia.* This is an important buffer in urine.

The buffering properties of blood *in vitro* overestimate those of the intact human, especially at very high PCO$_2$ values.

**Chapter 4. Acidosis and alkalosis**

An acidosis is either a deficit in buffer base or a gain in buffer acid with respect to normal levels. Acidemia and alkalosis indicate deviations from normal pH. Any disturbance directly associated with abnormal PCO$_2$ values is classed as a respiratory disturbance, while one associated directly with abnormal bicarbonate levels (due to ingestion or metabolic production or loss of acids or bases or to renal malfunctioning) is classified as a metabolic or non-respiratory disturbance. Changes in the actual bicarbonate concentration which result inevitably from respiratory changes in PCO$_2$ are not metabolic disturbances. Compensatory mechanisms (respiratory and renal) tend to bring pH towards normal by altering the normal buffer component in the same direction as the abnormality in the other component, so that the buffer ratio is restored towards 20:1.

**Chapter 5. Regulation of PCO$_2$: the respiratory factor**

PCO$_2$ is normally maintained at 40 (35–45) mmHg or 5.33 (4.67–6.00) kPa. Ventilation controlled by respiratory centre and central (medulla) and peripheral (carotid and aortic bodies) chemoreceptors, which are sensitive to both pH and PCO$_2$. Central receptors are much more sensitive to PCO$_2$ and pH than the peripheral ones. In hypoxia the importance of peripheral chemoreceptors increases owing to depression of the central receptors. The peripheral (but not central) receptors are sensitive to PO$_2$ and so provide the ‘hypoxic drive’.

An increase in PCO$_2$ of 10 mmHg increases ventilation four-fold, while a decrease in pH of 0.1 unit increases ventilation two-fold.

Carbon dioxide is carried in blood as dissolved CO$_2$ (in plasma and RBC), H$_2$CO$_3$ and HCO$_3^-$ (both in plasma and RBC) and carbamino compounds. Deoxyhaemoglobin has greater capacity for formation of carbamino compound than oxyhaemoglobin (Haldane effect); this facilitates the release of carbamino-CO$_2$ to the lungs.

Normal excretion of CO$_2$ requires normal functioning of ventilation (chemoreceptors, respiratory centre, mechanical aspects such as chest muscles and ribs), diffusion (across semipermeable alveolar membrane) and pulmonary perfusion.

Examples of causes of respiratory acidosis (hypercapnia) include depression of respiratory centre owing to injury, drugs, very high PCO$_2$ or low PO$_2$, some antibiotics; and mechanical injuries to lung or chest or airway obstruction; and inflammation, oedema, fibrosis or accumulation of mucus in the lungs; and perfusion defects such as caused by infarction, destruction of lung tissue and compression of vessels. Ventilation–perfusion abnormalities are often reflected by hypoxia which may lead to hypocapnia by stimulation of ventilation. Respiratory acidosis causes depression of the central nervous system.

Examples of causes of respiratory alkalosis include stimulation of the respiratory
centre by nervous stimuli (e.g. hysteria), drugs or damage to the pons, incorrect adjustment of an artificial ventilator, or by the hypoxic drive at high altitudes. Respiratory alkalosis causes a number of non-specific effects, but its major threat is cerebral hypoxia secondary to cerebral vasoconstriction.

Chapter 6. The kidney as a regulatory organ
Renal tubular reabsorption and secretion control the output from the body of waste materials, and the conservation of valuable electrolytes and other solutes, and so the kidney regulates the composition of the body fluids. Normally all bicarbonate in the glomerular filtrate is conserved by reabsorption; additionally bicarbonate must be regenerated to replace that lost in buffering the relatively strong fixed (non-volatile) acids and H⁺ excreted. These processes are effected by tubular secretion of H⁺ ions by active transport into the tubular luminal fluid in exchange for Na⁺ ions. The H⁺ ions are formed within the tubule cells by hydration of CO₂ (catalysed by carbonic anhydrase) and subsequent dissociation of carbonic acid. The HCO₃⁻ ions formed simultaneously are passed into the peritubular fluid (thence to renal venous plasma) accompanied by Na⁺ ions. Any HCO₃⁻ in the tubular luminal fluid is displaced by the secreted H⁺ ions and the CO₂ diffuses into the tubule cells where it is available for hydration.

Potassium competes with H⁺ for excretion (potassium/sodium exchange). The rate of H⁺ and K⁺ exchange for Na⁺ is increased by aldosterone and is also dependent on the concentrations of these ions and the PCO₂.

Water follows Na⁺ osmotically; the permeability of the tubule cells to water being increased by vasopressin (ADH). Plasma levels of ADH and of aldosterone are regulated by plasma [Na⁺] and osmotic pressure. Extracellular volume maintenance probably takes precedence over acid–base homeostasis.

Chapter 7. The buffering of urine
Glomerular filtrate contains the non-protein buffers of blood, but normally all the HCO₃⁻ is reabsorbed (indirectly by the process of tubular H⁺ ion secretion). The main buffers in urine are phosphate and ammonium ion, the latter being produced from NH₃ produced by deamination and deamidation of amino acids especially glutamine within the tubule cells. Titration of urinary phosphate (H⁺ + HPO₄²⁻ ⇌ H₂PO₄⁻), acidification of urine and reabsorption of filtered bicarbonate occur simultaneously; they are not stepwise processes.

The high pK (about 9) of NH₃ + H⁺ → NH₄⁺ means that NH₃ acts as a ‘sink’ for H⁺ ions completely removing an equivalent amount. When the minimum pH of urine (about 4.5) is reached H⁺ ions can still be excreted if NH₃ is also being excreted. Thus the importance of ammonium buffering increases with the severity of an acidosis, and the kidney regulates the urinary output of NH₃ according to the amount of acid to be excreted. Ammonium ion excretion also ‘spares’ other cations (Na⁺, K⁺); this is important when large amounts of ionized strong acids have to be excreted in the urine.

Chapter 8. Metabolic disturbances of acid–base balance
All disturbances of non-respiratory origin are classed as metabolic disorders. In the absence of other primary disturbances the plasma [HCO₃⁻] is lowered. Since actual [HCO₃⁻] is also affected by the respiratory component it cannot be used by itself as
an index of the metabolic factor. In conjunction with $P_{CO_2}$ or pH the actual [HCO$_3^-$] may be used; alternative indices are standard bicarbonate (i.e. [HCO$_3^-$]) at a fixed 'normal' $P_{CO_2}$ of 40 mmHg (5.33 kPa) and base excess (i.e. the amount of $H^+$ which would have to be added to return the pH to 7.40 at $P_{CO_2} = 40$ mmHg (5.33 kPa) at 37°C—this is the same as the deviation from the normal buffer base concentration (i.e. the sum of all buffer bases) for that particular Hb concentration. Both standard bicarbonate and base excess are independent of the respiratory status, but are derived from the in vitro titration curve of blood and do not necessarily accurately reflect the overall in vivo buffer status of the whole body. Normal values are: Actual [HCO$_3^-$] = 22–26 mmol/litre; standard bicarbonate = 22–26 mmol/litre; base excess = $-2$ to $+2$ mmol/litre. In metabolic acidosis the standard bicarbonate is less than 22 mmol/litre and base excess is negative and is less than (i.e. more negative than) $-2$ mmol/litre (i.e. there is a base deficit). Conversely in metabolic alkalosis standard bicarbonate is greater than 26 mmol/litre, and base excess is positive, greater than $+2$ mmol/litre.

Typical causes of metabolic acidosis include:

1. Excessive metabolic production or ingestion (or infusion) of $H^+$ ions, e.g. diabetic ketoacidosis; tissue catabolism in starvation, trauma, fever and thyrotoxicosis; lactic acidosis in tissue hypoxia, shock, acute respiratory failure, acute myocardial infarction, pancreatitis, also in acute alcohol intoxication, phenformin therapy, glycogen storage disease and in unexplained 'spontaneous lactic acidosis'; transfusion of large quantities of 'banked' blood.

2. Impaired renal excretion of $H^+$ ions. In many chronic or acute renal diseases, also in aldosterone deficiency (e.g. in Addison's disease) or in the presence of an aldosterone antagonist (such as spironolactone), the active transport system which pumps $H^+$ (and $K^+$) ions into the tubular luminal fluid in exchange for $Na^+$ ions will be inhibited. Diuretics which are carbonic anhydrase inhibitors also restrict the intracellular production of $H^+$ (for excretion) and of HCO$_3^-$ (for 'reabsorption' into the plasma).

3. Direct loss of base. In impaired renal function, since $H^+$ excretion is impaired (see 2 above) reabsorption of filtered HCO$_3^-$ is not complete. Hence bicarbonate is lost into the urine. Direct loss of bicarbonate-rich intestinal fluid (e.g. diarrhoea, cholera, fistulae) or ion exchange across the colon in ureterosigmoidostomy patients leads to direct loss of base.

Typical causes of metabolic alkalosis include: loss of bicarbonate-poor fluid in vomiting, excessive renal $H^+$ (and $K^+$) ion excretion in hyperaldosteronism (adrenal tumour, cirrhosis, burns or cardiac failure); contraction alkalosis (where water is suddenly lost, e.g. under potent diuretics); infusion or ingestion of excess base in conjunction with an elevated renal threshold. Metabolic alkalosis is often associated with $K^+$ depletion.

**Chapter 9. Compensation and repair mechanisms**

Compensation: a physiological mechanism producing a secondary acid–base disturbance which tends to restore the buffer ratio, hence pH, to normal.

Repair: correction of primary acid–base disturbance so that pH, metabolic factor and respiratory factor are all normal.

Changes in pH and $P_{CO_2}$ are sensed by peripheral and especially central chemoreceptors which regulate ventilatory rate accordingly via respiratory centre. Respiratory compensation is often incomplete. Renal (metabolic) compensation is governed largely by $P_{CO_2}$ which regulates $H^+$ excretion and HCO$_3^-$ reabsorption, and is slower than respiratory compensation.
Repair entails the removal of the fundamental cause of the primary disturbance. Therapeutically \( \text{PCO}_2 \) may be regulated by artificial ventilation; the metabolic component can be adjusted by administration of potential acids (e.g. ammonium chloride, lysine or arginine hydrochloride—together with \( K^+ \) supplementation if necessary) or of bases or potential bases (e.g. \( \text{NaHCO}_3 \), sodium acetate, lactate or citrate or 'Tham'). The amount of base (or acid) required (mmol) can be estimated approximately from formulae such as \( 0.3 \times \text{base deficit (or excess)} \) in mmol/litre \( \times \) body weight in kg, although such theoretical estimates are not always valid.

**Chapter 10. Measurement of acid–base status**

Full assessment of acid–base status requires knowledge of pH, metabolic factor (standard \( \text{HCO}_3^- \), base excess, or actual \( \text{HCO}_3^- \)) and respiratory factor (\( \text{PCO}_2 \)).

Total \( \text{CO}_2 \) content \( (\text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{[carbamino compounds]}) \) can be determined gasometrically by Van Slyke or Natelson method or colorimetrically with an AutoAnalyzer as \( \text{CO}_2 \) following acidification. It is an approximate index of the metabolic factor but is also affected by respiratory factor (\( \text{PCO}_2 \)). The \( \text{CO}_2 \) combining power (i.e. the \( \text{PCO}_2 \) content of plasma already artificially equilibrated at \( \text{PCO}_2 = 40 \text{ mmHg (5.3 kPa)} \)) is similarly determined.

**Astrup equilibration technique:** Simple theory and practical measurements show that the titration curve (or ‘buffer line’), \( \log \text{PCO}_2 \) vs pH, of whole blood in vitro is a straight line. The position of the line depends on the plasma \( \text{[HCO}_3^- \) and the slope (about -1) depends on the \([\text{Hb}] \). Portions of a blood sample are tonometered (equilibrated) at two \( \text{PCO}_2 \) values, and their pHs determined so that the buffer line can be plotted on a Siggaard–Andersen Curve Nomogram (or an Alignment nomogram). Total buffer base, standard bicarbonate and base excess values are read off where the buffer line intersects the respective scales. The actual pH of the blood without artificial equilibration is also entered on the line, and the \( \text{PCO}_2 \) read off by interpolation. Agreement between the \([\text{Hb}] \) predicted from the slope of the buffer line and the true \([\text{Hb}] \) can be used to check accuracy.

**Direct electrometric \( \text{PCO}_2 \) and pH determination:** Capillary glass electrodes allow pH measurement on micro-samples. A pH electrode surrounded by an aqueous electrolyte which is separated from the blood sample by a \( \text{CO}_2 \)-permeable (but \( \text{H}^+ \) and \( \text{HCO}_3^- \)-impermeable) plastic membrane acts as a \( \text{PCO}_2 \) electrode. Knowledge of \( \text{PCO}_2 \) and pH allows calculation of actual bicarbonate, and (if \([\text{Hb}] \) is known) standard bicarbonate and base excess from the Henderson–Hasselbalch equation, e.g. using the Siggaard–Andersen Alignment Nomogram or an acid–base slide rule or computer.

**Chapter 11. Blood sampling and general precautions**

Valid sample handling is crucial. Generally, arterial blood is preferred; capillary blood if taken anaerobically generally gives closely similar values; venous blood is not favoured (especially for \( \text{PO}_2 \) measurements), although venous blood can sometimes be ‘arterialized’ acceptably. All samples must be strictly kept out of contact with air; glass syringes should be used. Sodium heparin is the normal anticoagulant, and mixing must be complete (both immediately after collection and before analysis). Analysis should be as rapid as possible, although a 2–4 hour delay is permissible provided the sample is chilled to minimize metabolic changes, e.g. increase in \( \text{PCO}_2 \) and decrease in \( \text{PO}_2 \) and base excess.
The literature on acid–base balance and related topics is enormous. No attempt is made here to refer either to the literature of historical importance or to contemporary research work. However the following list of selected references may be useful to readers who wish detailed information on specific topics, or to see other approaches to the material covered in this book, or to trace other original references. Numerous other textbooks contain useful information.


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