Preface

Another book on chemical pathology needs justification. Yet there has always been a notable gap in the range of textbooks available. At one end of the range the majority of books are written for clinicians with clinical knowledge taken for granted and with a minimum of working details given to guide the bench worker; at the other extreme there are many small books consisting of collections of technical methods with no guidance for their use.

This book is aimed at the trained technician in a medical laboratory. It is intended to guide him through the examinations of the Institute of Medical Laboratory Technology and to be a practical laboratory guide to the handling and analysis of biological samples.

The choice of methods for a book of this kind is not easy; all methods are to some extent a compromise between absolute specificity and 'accuracy, which can often be approached only with tedious and technically difficult methods, and practical convenience and speed, which may be at a premium in a clinical laboratory. There is a no single method for the estimation of a substance which can be called the 'best'; one may be best in a research setting, another much less precise, may nevertheless be the best for use in an understaffed hospital laboratory. All the methods included here are eminently suitable for a busy routine laboratory and all are known from personal experience to be reliable.

The author is fully aware of his indebtedness to various colleagues during the preparation of this work but would especially like to acknowledge the discussions with, and advice given by Dr F. E. Hytten and Mr G. R. Wilson of the Obstetric Medicine Research Unit.

G. A. C.

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CHAPTER 1

Introduction

Over the last 40 years chemical pathology has advanced to occupy
its present place as one of the important aids to clinical diagnosis.
The subject has developed slowly compared with some other lab-
oratory sciences, perhaps because of the inherent difficulties of
chemical technology. The basic techniques of histopathology, bac-
teriology and haematology depend mainly on observation by both
macro- and microscopic means, and the recognition of a patho-
genic bacterium or the differentiation of normal from abnormal
tissue is mainly a matter of training the senses. In chemical path-
ology, however, much less can be done by simple observation.
Blood has to be grossly disordered to be recognizably abnormal;
though Bright described the blood serum in nephritis as being
'fatty' from naked eye observation alone. No visual examination
can distinguish a high from a low serum sodium, or uraemic blood
from normal blood.

The excreta too were originally judged not by chemical criteria
but by gross visual differences. Urine for example was at first judged
only by differences of quantity, colour and sediment, and since it
was freely available and could demonstrate striking changes, it was
a frequent source of diagnostic information.

In medieval times, both diagnosis and prognosis were based on
star gazing and urine examination and in the early fifteenth cen-
tury diagnosis of disease by uroscopy or water casting was common.
C. H. Talbot* in a fascinating article tells of a medieval manu-
script consisting of six pieces of parchment, each piece devoted to
a single element in the making of a diagnosis. These elements were
a Calendar, Canons of eclipses of the sun and moon, a table of
Planets, Rules for phlebotomy and descriptions of urine. With
these the physician made his calculations and decided on treat-
ment. One part of the *vade mecum* shows a ring of flasks containing

* TALBOT C.H. (1961) *J. Hist. Med. allied Sci.* **16,** 213

urines drawn and coloured, with precise rules regarding signifi-
cance of colour and its interpretation.

'Then have the patient's urine brought to you so that the sick
man may know that you understand his illness not merely by his
pulse but also by his urine. In the urine look intently at the colour,
substance, quantity and content and afterwards promise the patient
health with the help of God.'*

The true foundations of chemical pathology were laid when
organic substances in the urine were first isolated in relatively pure
form and their concentration estimated by weight or titration. The
application of such tests to blood was more difficult, principally
because of the amounts of material required for test. Blood testing
did not become fully developed until micro-methods became avail-
able. These methods were originated by men whose names are
well known and much respected by those who spend their working
lives in laboratories. In the early part of the present century, Folin,
Benedict and Van Slyke opened the way to techniques suitable for
small samples, thus initiating quantitative chemical pathology in
its new r^{o}le as an aid to diagnosis.

The adoption of micro-techniques meant, for equal accuracy,
increasingly exacting technical manipulation and while methods
of measurement were improving technical accuracy, advances of
chemical knowledge were improving specificity. Both sources of
improvement continue to preoccupy the modern clinical chemist.
Today if one wishes to estimate glucose only, in a mixture of reduc-
ing substances, then the method of choice may be relied upon to
estimate 'true glucose' only. Nor need accuracy suffer, for with
modern techniques of biochemical analysis, examples of which are
electrophoresis and gas chromatography, specimens of a few micro-
litres volume may be used with complete confidence in the final
result. It is a far cry from the 1,000ml specimen to one of a thous-
andth of a millilitre, but in the early days of chemical pathology
an approximate result was no doubt considered better than no
result at all. As time passed and knowledge of technique increased,
blood tests became more accurate both quantitatively and specifi-
cally until at the present time the clinician may wholly rely on the
results produced by his laboratory colleagues.

Even so the results of an analysis however accurate are of value

* DE RENZIE S. *Collectio Salernitena* **II,** 74. Trans. in 'The rise of medicine at
Salerno in the twelfth century'. *Ann. med. Hist.* (1931) **3,** 6–7

only in the context of conditions under which the specimen was obtained. Blood constituents may vary not only in disease but in relation to such influences as food and exercise. Much remains to be learnt in this aspect of chemical pathology.

CHAPTER 2
Refresher Course in Basic Inorganic Chemistry

Before starting on the routines of chemical pathology it will be necessary to recall the basic rules of inorganic chemistry. In a book of this type it is neither possible nor desirable to provide a complete treatise on inorganic chemistry. An attempt will be made therefore to give only those basic laws which are directly concerned with calculations occurring in the chemical pathology laboratory.

The first law of chemistry, that of *conservation of mass*, states: 'Matter cannot be created or destroyed, but it may be changed from one form to another.' The mass of substances produced by any chemical reaction is equal to the mass of the reacting substances.

Three gross types of matter are dealt with in chemistry; these are elements, compounds and mixtures. An element is defined as a substance which from a chemical point of view cannot be obtained in simple form whilst compounds are more complex, being formed by the chemical combination of elements.

Mixtures on the other hand are composed of elements or compounds or both, which have not been chemically united. The different components retain their characteristics and may sometimes be separated by mechanical means.

Throughout the universe matter occurs in three main physical states: solids, liquids, or gases. For any single element or compound the solid form exists at a lower temperature than the liquid, the liquid at a lower temperature than the gas. Energy has to be supplied to turn a liquid into a gas or a solid into a liquid and energy is given up when the reverse change occurs. This leads to the second chemical law, that of *conservation of energy*, which may be stated thus: 'Energy cannot be created or destroyed but may be converted from one form to another.'

Mass and energy having been considered it is now necessary to recall the laws governing chemical combination. The first of these, the *law of fixed proportions*, states: 'Elements combine in definite proportions by weight and a particular chemical compound will always contain the same elements united in the same proportion by weight.'

The *law of multiple proportions* set down by Dalton in 1803 states that: 'When an element unites with another in more than one proportion these proportions bear a simple ratio to one another,' whilst the *law of reciprocal proportions* may be stated thus:

The proportions in which any two elements unite with a third element, are the proportions, or a simple multiple (or submultiple) of the proportions, in which they combine with each other.

All the foregoing laws are founded on experimental observation, and all are explained by the atomic theory postulated by John Dalton more than 150 years ago and which may be briefly summarized in three sentences.

1. All matter is composed of small indestructible particles called atoms.

2. All atoms contained in an element are identical but atoms of different elements differ in weight and properties.

3. Atoms combine to form compounds and this combination takes place in simple whole number ratios.

ATOMS AND MOLECULES

This brings us to consideration of the particles called atoms and molecules and further to the question of atomic and molecular weights of substances. An atom may be defined as 'the smallest unit which can take part in a chemical reaction', and the atomic weight of an element as 'the ratio of mass (or weight) of its atom compared with the mass of a standard element'. The usual reference is oxygen with an atomic weight of 16·00, this value being derived from experiments relating to the equivalent weights of other elements.

A molecule is 'the smallest part of an element or compound that can exist in the free state', and the molecular weight of a substance is 'the sum of the atomic weights of all the atoms combined in a molecule of the substance'.

THE ELECTRONIC THEORY OF THE ATOM

Great progress has recently been made in the study of the atom, and the experimental work of the last 40 years has considerably altered the original ideas on which the atomic theory was based. It is not sufficient at the present time to consider only the atom, for though minute in size atoms consist of a complex mixture of electrical units. The *electron* has no measurable mass and carries a unit negative electrical charge. The *proton* has a much greater mass than the electron and carries a unit positive charge. The *neutron* has a mass similar to the proton, but possesses no electrical charge, probably because it may in fact be composed of an electron and a proton, closely bound so that any electrical charge is neutralized.

More recently, it has been recognized that several more subatomic particles are present in the atom and these (positrons and mesons, the latter being negative or positive in electrical charge), are classified by weight into light, intermediate, and heavy particles, though each and every atomic particle carries the same unit amount of electrical charge. The atomic nucleus, consisting of protons and (usually) neutrons, is itself positively charged because it contains a number of free protons in addition to those bound to electrons, the latter forming neutrons. Free electrons are continuously spinning around the nucleus at a speed approximately the speed of light, though this varies, depending on the size of the atom and the distance of the electrons from the nucleus. Electrons in their movement may be envisaged as a miniature solar system arranged in concentric rings. Each ring is capable of holding only a certain number of electrons, two in the innermost ring, eight in the second, whilst in the third ring the number may vary from eight to eighteen. As the number of these electrons increases according to the type of element, so the electrons will be found in the fourth, fifth or sixth rings removed from the nucleus of the atom. The atom as a whole is electrically neutral, due to the equal number of free protons and electrons within the atom.

As an example of electron grouping, consider the atom of hydrogen which diagrammatically could be represented thus:
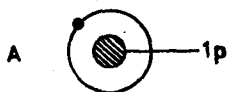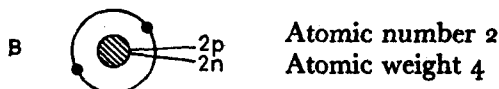

Atomic number 1
Atomic weight 1

in which the larger central dot represents the nucleus containing one proton and the smaller one, the electron, in the first ring or 'shell' surrounding the nucleus.

Similarly helium appears thus:

B — 2p
— 2n

Atomic number 2
Atomic weight 4

having two protons and two neutrons within the nucleus and two electrons in the first shell. It will also be seen that the atomic number in the two examples given is the same as the number of protons in the atom nucleus, and that the sum of both protons and neutrons constitutes the atomic weight of the element. This is so for all elements, the chemical properties of each being governed by the number and arrangement of the spinning electrons.

Thus the naturally occurring elements throughout the periodic table increase in atomic number as Table 1 shows. Eventually

TABLE 1

Examples from periodic table

Element	Atomic number	Nucleus contains		Atomic weight
		Protons	Neutrons	
Sodium	11	11	12	23
Aluminium	13	13	14	27
Sulphur	16	16	16	32
Argon	18	18	22	40

uranium is reached, which has an atomic number of 92; this indicates the presence of 92 protons in the nucleus of each of its atoms and a corresponding number of electrons spinning around each nucleus. The table does not in fact stop here, but extends to what are termed the 'transuranic elements', that is elements which have been artificially prepared. These have atomic numbers greater than 92 and examples of these are neptunium, plutonium and americium.

VALENCY

We can now consider the means by which one element holds atoms
of another element in combination. This ability of one element to
combine chemically with another is called valency and is demon-
strated in different ways. An element which unites with only one
atom of hydrogen is said to be monovalent or to have a valency of
1. Again, the element may be divalent, trivalent or tetravalent if
it is capable of combining with two, three or four hydrogen atoms
respectively. Elements do exist which are pentavalent but few have
a valency higher than this. Hydrogen is the standard used to assess
the valency of other elements with the combining power of hydro-
gen taken as 1. The reason for this is that a hydrogen atom can
only combine with one atom of any other element, and thus offers
a convenient starting point for assessing the valency of other ele-
ments. It is also possible to deduce the valencies of most elements
from the compounds which they form with elements or groups
other than hydrogen, for example with chlorine or bromine, since
these elements also have a valency of 1. Also, most elements com-
bine to form oxides and since oxygen is divalent the valency of an
element can be deduced from the formula of its oxide.

VALENCY AND THE ELECTRON THEORY

Certain factors must contribute towards the characteristic valency
of an element; why does one element have a greater combining
power than another? Modern theories of atomic structure go a
long way toward furnishing a simple explanation, and in fact the
valency of an element is decided by the number and arrangement
of the planetary electrons, particularly those in the outermost shell.
If the arrangement of these electrons within certain atoms is stable,
then the element formed of such atoms is chemically inactive and
combination with other elements is difficult. This can be illus-
trated by considering the electronic structure of a rare gas, for
example neon. In the neon atom the outer shell contains eight
electrons in a 'closed shell', and this indicates that chemical com-
bination for atoms of neon is not possible by ordinary means. In
sodium, the next element in the periodic table, the extra electron
within the atom orbits alone in an outer shell. Thus the atom has
two, eight and one electrons within the three shells, and is a good

example of an element which will combine chemically with ease. Sodium is in fact typical of the alkali metals, all of which have one electron in the outer shell, and are readily able to combine with other elements.

The valency of any element is decided by the possible number of electrons which can be gained or lost in the process of chemical combination. If therefore the valency of an element is known, it is possible to calculate the number of atoms of another element which can be held in chemical combination. This explanation however must be extended to consider other possible types of valency, electrovalency and covalency.

Electrovalency can best be described as an electrostatic attraction between two opposingly charged atomic nucleii, and can be demonstrated by considering the chemical combination of the elements sodium and fluorine which in the periodic table are placed on either side of neon thus:

Element	F	Ne	Na
Number of electrons in atom	9	10	11

Sodium has one electron in excess of the number (10), required to fill the first two rings. Neon possesses a stable grouping whilst fluorine has one electron less than this number. If however one electron can be lost from sodium, the remaining ten in two closed shells round a neon nucleus provide a stable arrangement. Also, if the fluorine atom can gain one electron providing a total of 10, it can be assumed that this number round a fluorine nucleus also conforms to a stable grouping. Thus the sodium atom can be expected to lose an electron and fluorine to gain one. One other detail remains to be considered—the behaviour of the electrical charge when the above combination occurs. The original sodium atom of nuclear charge +11 and with 11 negative extra-nuclear electrons was electrically neutral, but on reduction of the electrons to 10 the atom will no longer be neutral but will have a net electrical charge of +1, and will now be a positively charged particle, which is termed an 'ion'. The atom of fluorine with an original nuclear charge of +9 and 10 electrons now carries a net charge of −1 and is a negative ion. Thus the behaviour of sodium and fluorine atoms demonstrates the formation of the substance sodium fluoride, an electrovalent compound in which the combining ions are held by electrostatic attraction.

Electrovalency accounts for many examples of chemical combination but even so there are many occasions in which it does not. Compounds for example can be produced by chemical combination of elements which show no sign of ionization and this occurs with most organic substances. This type of chemical combination, called *covalency*, can be explained by again considering the element fluorine. Fluorine normally exists as molecules (F_2) and not as single atoms, and this compound cannot be represented in terms of electrovalency. It is not possible for electrons to transfer from one atom of fluorine to another to produce two ions, each having a group of 10 electrons corresponding to neon. If however it is assumed that a pair of electrons can be shared between the two fluorine atoms, and thus contribute towards the electron grouping of each, both atoms will have the required stable grouping of 10 electrons. In this covalency process the shared pair of electrons each belonging to an atom, are presumed to be attached to each other.

The short account of valency given above can be summarized as follows:

(a) Chemical combination occurs because of the formation of stable groupings by the extra-nuclear electrons of atoms. This may occur in the following ways.

(b) By the complete transference of electrons from atom to atom, the ions thus formed being held together by electrostatic attraction. This mode of formation is known as electrovalency.

(c) By the method of 'electron sharing' in which a pair of electrons are shared between two atoms. This process is termed covalency.

ISOTOPES

Whilst considering the various constitutents of the atom it will be convenient at this point to introduce the subject of isotopes, though this will be more fully dealt with later. Isotopes may be described as atoms of the same element, having the same atomic number but differing in atomic weight. As previously mentioned the units of the atom nucleus are the protons with positive electrical charge and the electrically neutral neutrons, both having approximately the same mass, this being about 1,850 times that of the individual electrons surrounding the nucleus. Two isotopes of the same ele-

ment however, have the same number of protons, equal to the number of surrounding electrons, but they differ in the number of neutrons in their nucleii. They therefore differ also in relative mass or atomic weight.

Most of the elements are known to have two or more isotopes occurring naturally, whilst others can be produced experimentally by means of the cyclotron. In this apparatus non-radioactive atoms are bombarded by high-speed subatomic particles (such as protons and neutrons) to produce artificially radioactive isotopes of the elements. Isotopes are also available from establishments such as the Atomic Energy Authority at Harwell and are products of the atomic pile. Many of the latter types of isotopes are unstable, with only temporary radioactivity, and are the materials used as tracer substances in medicine and biology.

THE GASEOUS STATE OF MATTER

Of the three chemical states of matter the gaseous state is that in which the simplest and most uniform behaviour is found when subjected to changing external influences such as temperature and pressure. The laws which govern the behaviour of gases are important in the chemical laboratory, and the first, which is concerned with the influence of pressure on gas volume, is Boyle's Law: 'The product of pressure and volume of a fixed mass of gas kept at uniform temperature is always the same,' i.e. $PV =$ Constant.

To illustrate this law consider 12 litres of a gas at atmospheric pressures of 1, 2, 3, 4 and 6 atmospheres. If the volumes of gas are measured the results are shown in Table 2.

TABLE 2

Pressure/volume of gases

Pressure (atmospheres)	1	2	3	4	6
Volume (litres)	12	6	4	3	2
Product (PV)	12	12	12	12	12

In general no two gases behave similarly and there are in fact deviations from Boyle's Law, but such deviations are insignificant. Assuming the truth of Boyle's Law, if the volume of a gas at a specific pressure is known, the volume it will occupy at another

pressure (provided temperature remains constant) may be calculated as follows.

Suppose the gas occupies 100ml at 500mm pressure, calculate the volume it will occupy at 760mm. The pressure is greater, consequently the volume will be less and may be calculated thus:

$$\frac{100 \times 500}{760} = 65 \cdot 7 \text{ml}$$

In the gas law of Charles, the influence of temperature on volume is considered and the law states 'The same rise in temperature produces in equal volumes of all gases, the same increase in volume if pressure be constant.' As temperature increases the donated energy causes increasingly vigorous movement of molecules and their intensified bombardment of the containing walls is recognized as increasing pressure. As temperature falls energy is taken from the system, the molecular movement becomes slower and less vigorous and pressure falls. Theoretically the temperature must eventually reach a point at which molecular movement stops; this point, $-273°C$, is the absolute zero and has been closely approached experimentally but has never been reached. All temperatures calculated from this nadir are termed 'absolute' and are signified by the symbol $°K$ in honour of Lord Kelvin (William Thomson). Charles' Law may be formulated as follows: if V is the volume of a gas when the absolute temperature is T, $°K$ and V_1 the volume at a temperature of T_1 $°K$ then Charles' Law may be written:

$$\frac{V}{T} = \frac{V_1}{T_1}$$

To complete this quick revision of the gas laws and to arrive at the general gas equation one more law must be mentioned. Dalton's Law, which deals with mixtures of gases, states 'In a mixture of gases having no chemical reaction upon one another, each gas exerts the same pressure as if it alone occupied the containing vessel and the total pressure is the sum of the various partial pressures.' As previously mentioned the various corrections incorporated in the foregoing gas laws are applied in the general gas equation. For practical use the laws mean that for comparison of metabolic measurements based on gasometric analysis, volumes must be corrected to standard conditions; these being that the gas is dry, and at sea level pressure (760mm Hg and 0°C (273 °K)).

The various factors derived from the laws of Charles and Boyle which must be incorporated in the general gas equation have been given, but it is necessary to consider one other factor derived from Dalton's Law. If the volume of gas for analysis has been collected over water, at room temperature and at the particular barometric pressure of the time of experiment, it is necessary for accuracy, to subtract the vapour pressure of water in mm Hg (sometimes called the 'aqueous tension'), from the barometer reading. This will give the pressure of *dry* gas at the particular experimental temperature, and the correction is incorporated in the general gas equation thus:

$$\frac{PV}{T} = \frac{V_1 \times (P_1 - p)}{T_1}$$

The volume of dry gas at 0°C and 760mm pressure is calculated as follows:

$$V = \frac{V_1 \times (P_1 - p) \times T}{T_1 \times P}$$

We have considered the various laws which govern the behaviour of gases, and the factors derived from these laws which must be introduced into the general gas equation for correction of gas volumes. It is now possible to build up the equation and to demonstrate how the various fractions are introduced to formulate the general gas equation.

Derived from Boyle's Law $\quad PV = \text{Constant}$

Derived from Charles' Law $\quad \dfrac{V}{T} = \dfrac{V_1}{T_1}$

Derived from Dalton's Law $\quad \dfrac{VP}{T} = \dfrac{V_1 \times (P_1 - p)}{T_1}$

As an example, if 185ml of a gas were collected over water at 18°C and 735mm Hg pressure, the volume of this gas at S.T.P. would be calculated as follows.

Corrected volume $(V) = \dfrac{V_1\,(185) \times (P_1\,(735) - p\,(15.5)) \times 273}{T_1\,(273 + 18) \times P\,(760)}$

i.e. $\qquad \dfrac{185 \times 719.5 \times 273}{291 \times 760}$

$\qquad = 164\text{ml}$