Samson Wright's Applied Physiology

TWELFTH EDITION

SAMSON WRIGHT'S APPLIED PHYSIOLOGY

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REVISED BY

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§ 1. THE CELL AND BODY FLUIDS

THE CELL

Structure of the Cell

All living organisms are composed of cells, just as molecules are composed of atoms. The cell was recognized as the unit of structure in plants by Schleiden and in animal tissue by Schwann in 1839. Virchow, in 1859, confirmed the cellular hypothesis showing that all cells must necessarily be derived from pre-existing cells: *omnis cellula e cellula.*

The development of light microscopy showed that the cell possesses a membrane, a cytoplasm which seemed to possess a vague internal organization or cytoskeleton and which contained a nucleus and various inclusion bodies—such as the centrosome, the Golgi apparatus and various other ill-defined structures of granular nature [Fig. 1].

Two developments during the last 30 years have enormously advanced our knowledge. One was the use of the electron microscope. The resolution of the best light microscope is about 1μ (one thousandth of a millimetre). The cell boundary is of the order of 100 mμ ($\frac{1}{10}\mu$) and hence appeared only as a thin dense line at the outer boundary of the cytoplasm, forming a marginal zone between the cytoplasm and the extracellular region. The electron microscope has a thousand times the resolving power of the light microscope and its use has revealed the complexity of the cell

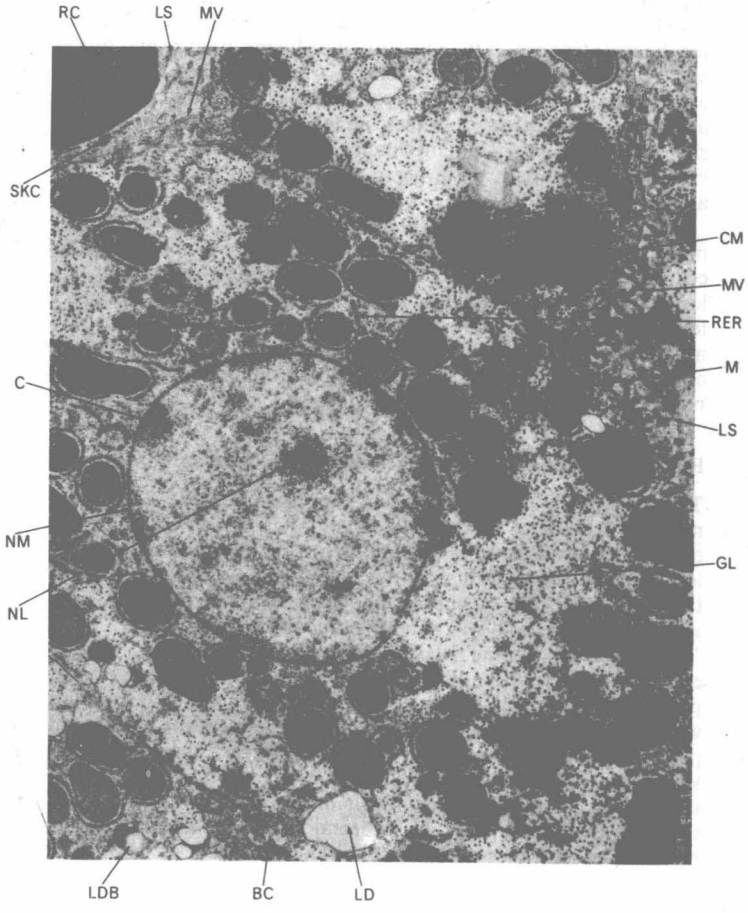

Fig. 1. Electron microscope picture of liver cell. Magnification × 12,000. [Kindly supplied by Mr. R. P. Gould.]

BC = Bile canaliculus
C = Chromatin
CM = Cell membrane
GL = Glycogen
LD = Lipid droplet
LDB = Lysosomal dense body
LS = Liver sinusoid
M = Mitochondrion between LS and MV
MV = Microvilli of liver cell projecting into the space of Disse
NL = Nucleolus
NM = Nuclear membrane
RC = Red cell in liver sinusoid
RER = Rough endoplasmic reticulum
SKC = Sinusoidal Kupffer cell

membrane. Similarly, for the cell inclusions—details of the nucleus and organelles now can be clearly defined. Just as the atoms were once thought to be the ultimate unit in chemistry and have since been shown to contain a positive nucleus with electron shells, so the cell can be redescribed in terms of its constituents.

The second development has lain in the elegant and sophisticated use of chemical and biochemical techniques in the study of the functional activities of the cell as a whole, and even more so of the working mechanisms of its different constituents. Thus, lysis of the cell, which destroys its membrane, causes the extrusion of its several constituents. 'Homogenization' of a tissue by mechanical means, such as grinding the tissue or disrupting its components ultrasonically, yields a broth of previously intracellular material. In this broth are suspended particles of different density and the use of ultracentrifuges with ever increasing gravitational power allows differential centrifugation of these various components of the cell. These can then be examined structurally by the electron microscopist and also studied chemically by the biochemist. As Brachet (1961) has said, the cell biologist now seeks to explain in molecular terms what he can 'see' with his electron microscope, whereas the biochemist has become a biochemical cytologist, as interested in the structure of the cell particles as in their biochemical activity. Neither discipline can ignore the other—the combination of efforts is required.

Even before these remarkable advances occurred and accelerated, there were nevertheless two clear points of understanding about cell characteristics. First, it was realized that cell function was epitomized by the ability of the cell to harness energy and transform it, and that this transformation of energy was necessary for the maintenance of the integrity of cellular structure and of the intracellular environment. Such energy transformations include the use of energy of the sunlight to produce chemical bond energy, as in plant cells. Other examples include the transformation of chemical bond energy into mechanical work, as in muscle cells, or into electrical energy, as in nerve cells, or even into visual light, as in the firefly tail.

Secondly, the cell interior was understood to contain macromolecules synthesized by energy mechanisms characterizing the cell. No macromolecule occurs in the non-living environment unless it represents the previous activity of cells now dead. Primitive life began with the spontaneous synthesis of complicated macromolecules at the expense of smaller molecules. It is the supreme ability of the cell to synthesize these larger molecules—deoxyribonucleic acid, ribonucleic acid, proteins, etc.

Electron Microscopic Details of Cellular Structure

The Cell Membrane. This measures approximately 75 Å in thickness. It exhibits a triple layer structure in which internal and external electron-dense layers are separated by a central layer of low density. The layered structure reflects the molecular organization and consists of a bimolecular layer of lipid molecules with associated protein layers on both surfaces [FIG. 2].

Until recently it was thought that the outer dense layer of the cell membrane and the inner dense layer were both composed of protein and lipid polar surfaces on the basis of fixation techniques which employed osmium tetroxide. The advent of potassium permanganate fixation has revealed, however, that the outer surface of the membrane is polysaccharide in nature, whereas the inner surface is indeed protein. Both inner and outer surfaces are of monomolecular thickness. The central clear area is formed of a bimolecular thickness of phospholipid arranged as shown in FIG. 2. The black dots represent the polar end of the molecule which is hydrophilic (attracted to water) and which consists of the phosphorus-carbon-nitrogen part of the phospholipid complex. The remainder of the molecule (a long chain hydrocarbon fatty acid) is hydrophobic (insoluble in water). The long chain molecule is arranged perpendicular to the membrane. To this non-polar chain is opposed another non-polar part of a second phospholipid molecule—oriented in the opposite direction and leading to the polar 'phosphate' group which abuts on the inner protein layer.

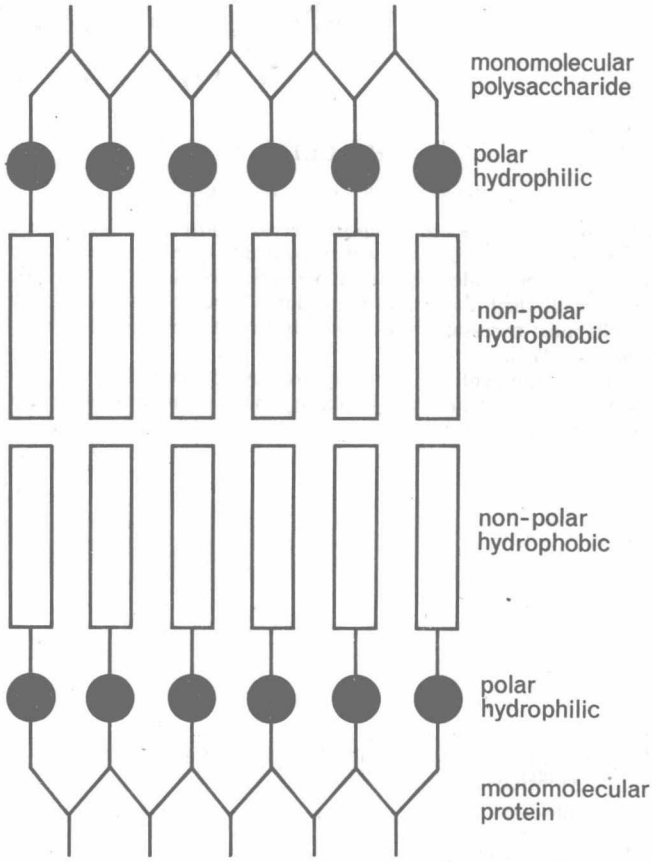

FIG. 2. Schematic representation of the molecular organization of a cell membrane or so-called 'unit membrane' (see text).
[After J. D. Robertson.]

The arrangement of the cell membrane certainly accounts for the lower permeability of the membrane to water-soluble substances and allows us to understand that the lipid-soluble gases oxygen and carbon dioxide can pass across the membrane with great ease. On the other hand, the explanation of how water-soluble substances do penetrate (as they undoubtedly do) was difficult. To circumvent the difficulty the hypothesis was proposed that there were pores in the membrane. Needless to say, such pores were understood to be necessarily small because of the outstanding difference in concentration between the intracellular concentration of sodium compared with that in the extracellular phase. $[Na^+]_{inside}$ is less than one-tenth of $[Na^+]_{outside}$. The intracellular concentration of potassium, on the other hand, is some thirty times that of the extracellular phase. The diameter of

the hydrated sodium ion is only 5·12 Å and that of potassium even less, 3·96 Å. Any pore in the cell membrane must therefore be of this order of size, and here of course the light microscope with its limited resolving power was quite useless as a tool. Even the advent of the electron microscope was of little use for electron microscopic techniques do not allow resolution of holes of 5–10 Å. Indirect techniques based on the measurement of the speed of transit of substances across the cell membrane (Solomon, 1960) have, however, yielded evidence of the existence of pores of 7–8 Å diameter in the membrane.

Secondly, it has been shown that phosphatide carrier molecules, which are lipid-soluble, exist in the membrane and combine preferentially with particular ions. Thus, an ion, which traverses the outer polysaccharide membrane layer, is picked up and carried by diffusion through the lipid layer to the inner protein surface, where it dissociates to gain access to the cytoplasm.

Long before the structural details of the cell boundary were known electrical measurements had shown that the interior of the cell was some 60–70 mV negative to the outside. Measurements of the capacity of the cell membrane showed this to be 1 microfarad/cm^2. The membrane may be considered as acting as the dielectric material of a charged condenser. The difference in charge across the membrane, 100×10^{-10} metres thick (75 Å), results in a potential difference of, say, 70 mV or 0·07 V. The electric field (stated in volts per metre) within the membrane is thus:

$$\frac{0{\cdot}07}{100 \times 10^{-10}} = 7 \text{ million volts/metre.}$$

This example, based on one given by Engleberg (1966), shows that the cell membrane has a very high insulating value indeed, for the highest dielectric strength (volts/metre) which a good commercial insulator (such as rubber) can stand without breaking down is about one million volts per metre.

The Endoplasmic Reticulum. Ribosomes and Golgi bodies. The 'interior of the cell' or cytoplasm is interlaced by a highly complicated arrangement of internal membranes forming tubules and vesicles—the endoplasmic reticulum. Through this network of canaliculi (each bounded by unit membrane) substances may be delivered from the outer membrane of the cell proper to the membrane of the nucleus or to other inclusion bodies of the cells, such as the mitochondria. Some authorities claim that the internal tubular system is continuous with the external cell membrane proper and is formed by the folding of this membrane. Robertson (1964), who has done much to clarify our thinking on the structural details of the cell, provides the accompanying illuminating diagram [FIG. 3]. He points out that such an arrangement would cause us to think of the cell as a three-phase system. 1. A cytoplasmic phase. 2. A phase constituted by the contents of the endoplastic reticular tubules. 3. A membrane phase which separates the first two. This concept would imply that any piece of membrane in the cell must have been formed from pre-existent membranes. The so-called nuclear membrane (*vide infra*) has indeed been shown to be simply a system of sacs of the endoplasmic reticular system arranged around the spherical bit of cytoplasm which contains the main genetic material (DNA and RNA) of the cell, i.e., the nucleus.

Robertson's views would suggest that the endoplasmic reticulum would provide an 'intracellular circulatory system'. It would also ensure a far greater surface of exposure of the cytoplasm to the 'extracellular' environment. In general the membranes of the endoplasmic reticular components are subdivided from their

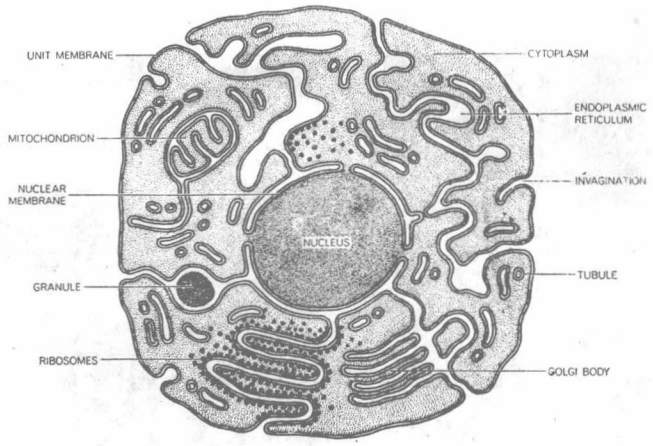

FIG. 3. Schematic diagram based on the author's concept of cell structure, shows the extensive distribution of membrane within the cell and some of the many membranous organelles so far identified. Since the membranes within cells always have the unit membrane pattern, it may be that most of these organelles are formed from the unit membrane structure bounding all cells.

[Robertson, J. D. (1962) *Scientific American*, Repr. 151. Copyright by Scientific American, Inc. All rights reserved.]

electron microscopic appearance into 'rough' and 'smooth'. Rough membranes appear so because they are studded with granules on their 'cytoplasmic' surface. These granules are the ribosomes, so-called because they are rich in ribonucleic acid (RNA). As will be seen [p. 461] their function lies in the synthesis of protein in which RNA plays a major role. In keeping with these findings, cells which produce large amounts of protein are packed with ribosomes. Thus, a liver cell may contain as many as 100 million ribosomes of about 150 Å diameter. The ribosome is *not* bounded by membrane.

The Golgi bodies (or complex) were first described by Golgi at the end of the nineteenth century. Found always in the vicinity of the nucleus, electron microscopic studies now reveal that they are part of the endoplasmic reticulum. Consisting of smooth vesicles they are credited with secretory function. Some have claimed that they are the site of production of new membrane material, but this remains uncertain.

Mitochondria. These occur in variable numbers from a few hundred to a few thousand in different cells, related often to the energy required for the functional activity of the cell. Rapidly acting skeletal muscle, which requires speedy replenishment of its energy, is rich in mitochondria and in its endoplasmic reticular apparatus. Heart muscle, which does not require such rapid replenishment, has only a moderately developed sarcoplasmic reticulum, but abounds in mitochondria 1–2 μ in length and 0·3–1·0 μ in diameter distributed primarily between and in close approximation to the myofibrils and comprising 40–50 per cent of the cell volume.

Mitochondria are variable in size, varying from 0·5 μ to as much as 12 μ in length, and in shape; many are filamentous, some are globular. They are highly structured organelles delimited from the cytoplasm by a double membrane [FIG. 4]. The inner aspect of this membrane gives rise to numerous infoldings, which form cristae composed of membrane pairs which project into the interior of the mitochondrion.

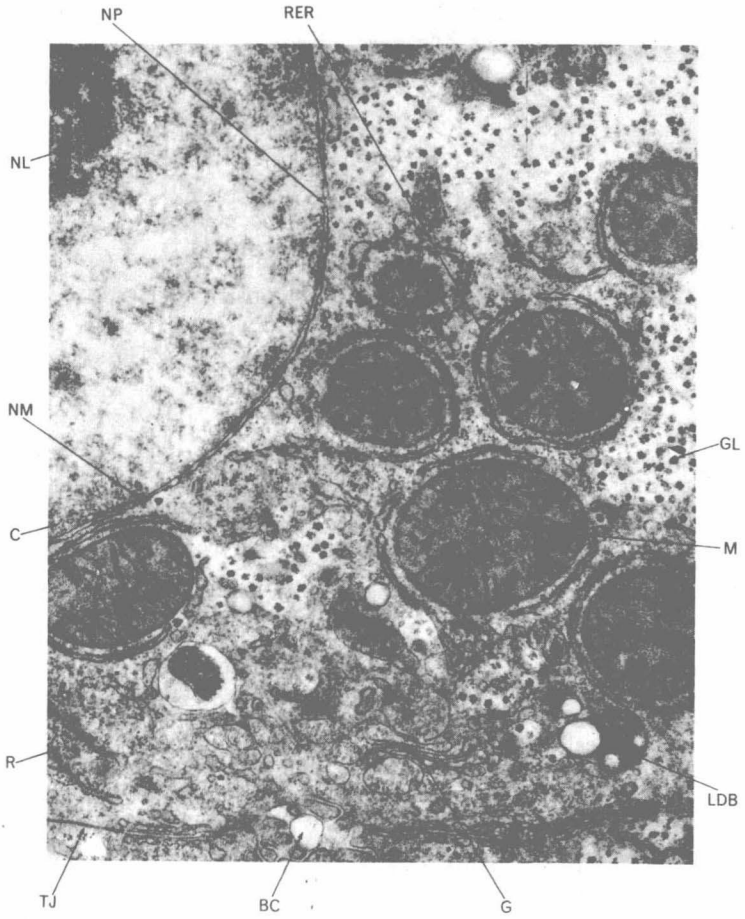

FIG. 4. Liver cell. Magnification × 41,000.

BC = Bile canaliculus
C = Chromatin
G = Golgi complex
GL = Glycogen granule
LDB = Lysosomal dense body
M = Mitochondrion
NL = Nucleolus
NM = Nuclear membrane
NP = Nuclear pore
R = Ribosomes
RER = Rough-surfaced endoplasmic reticulum
TJ = Tight junction

[Kindly supplied by Mr. R. P. Gould.]

Mitochondria play a vital role in cellular metabolism providing the fundamental respiration energy transformations by which, through oxidative phosphorylation, the energy contained in the nutritive material brought to the cell is converted to adenosine triphosphate (ATP), the prime medium of energy supply. Thus, the mitochondria, which serve as the 'power plants' of the cell, contain the enzymes of the tricarboxylic cycle [p. 436] enzymes for the catalysis of the oxidation of fatty acids and amino acids and the enzymes needed for coupling electron transport with the tricarboxylic acid cycle. The enzymes exist in a sterically ordered array along the cristae and surface membranes, thereby assuring stepwise aerobic biochemical reactions which result in the production of ATP. The evidence for this last statement is provided by the fact that a mitochondrial suspension prepared from cells can be broken up into fragments of the individual mitochondria and each fragment can conduct only a part of the complete reaction sequence.

Lysosomes. Also bounded by a membrane, but of entirely different function. 'The lysosome comes in a bewildering assortment of shapes and sizes even in a single type of cell; they cannot be identified solely on the basis of their appearance' (de Duve, 1963). Their function has been elucidated clearly by de Duve. Four types of lysosomes may be distinguished—'storage granules', 'digestive vacuoles', 'residual bodies' and 'autophagic vacuoles'.

The original form of the lysosome is the storage granule which consists of enzymic granules (formed by ribosomes) and wrapped up in a lipoprotein membrane (formed where ?) to form a roughly spherical organelle, some 250–750 mμ in diameter. The granules (about 60 Å diameter) consist of protein and are of a variety of types of hydrolytic enzymes. These include ribonuclease and deoxyribonuclease, phosphatases, proteolytic enzymes, glycosidases and sulphatases. Clearly such a conglomeration can have only one general function—a lytic or digestive one.

The membrane which surrounds this wasps' nest clearly has a vital function for if such a swarm were to escape indiscriminately, cell death would ensue quickly. Indeed lysosomes are responsible for the rapid post-mortem autolysis of, for example, the intestinal mucosa subsequent to the death of the host individual.

However, the discriminate function of the lysosomes is of great importance to the cell. When the cell ingests substances by pinocytosis a phagosome or food vacuole is formed. Several phagosomes may fuse together to form a single vacuole. A lysosome now fuses with the phagosome forming a digestive vacuole. The products of digestion diffuse through the lysosome membrane into the cell cytoplasm. The digestive vacuole continues its digestive activity until only indigestible material remains, whereupon it shows the final stage of a 'residual body'. In some cells, such as the amoeba, this may be extruded from the cell itself, but most cells of the

organized mammal cannot effect this and the residual body is used again and again in digestive activity.

The autophagic vacuole is a lysosome, in which can be identified remnants of cell debris (mitochondria or endoplastic reticulum) formed in the host cell.

White corpuscles in the blood live only for a short period. Their function is that of scavenging and they contain many lysosomes, which, at the time the cell is discharged from the bone marrow into the blood stream, are full of granules. When the white cell engulfs a bacterium for instance, the granular content of its lysosomes steadily disappears as they are used up in the digestive processes.

Cell damage due to oxygen lack or to cell poisons causes disruption of the lysosomes which not only wrecks the cell itself, but leads to escape of the digestive enzymes into the extracellular fluid and thence by diffusion to neighbouring structures.

Centrosomes (or centrioles). These paired structures become plainly visible under the light microscope only when the cell approaches its hour of division, a time at which these organelles function as the poles of the spindle apparatus that divides the chromosomes. Under the electron microscope the pair of centrosomes is seen clearly at each pole of the nucleus and all show a cylindrical structure made up of eleven fibres with two in the centre and nine arranged 'round the clock'.

Each pair of centrosomes gives rise to another when the cells divide. Their exact function during mitosis is still unknown. It is interesting that ciliated cells possess a structure called a kinetosome at the basis of the cilium. These are undoubtedly concerned with the motility of the cilium and have been described as 'monomolecular muscles'. They have a structure identical with that of the centrosome and also replicate when the cell divides. It has been suggested that the centrosomes pull the chromatin material apart on cell division.

The Nucleus. The cell nucleus is contained in or bounded by a nuclear membrane. This is composed of two unit membranes and shows large pores of 1000 Å diameter. It is probable that the nuclear membrane is composed of the terminal parts of the endoplasmic reticulum [FIG. 3] and that the pores which provide direct continuity between the nucleus and the cytoplasmic sap represent gaps between the 'end feet' of the endoplasmic reticulum.

The nucleus itself is spherical and approximately 10 μ in diameter; 80 per cent of it is water, but 80 per cent of its dry weight is protein. The remainder of the dry weight is made up by 18 per cent deoxyribonucleic acid (DNA) and 2 per cent ribonucleic acid (RNA). The nucleic acids have an affinity for the basic dyes toluidine blue and methyl green, and this has led to the location of nucleic acids in material examined by the light microscope. Additionally, the nucleic acids show intense absorption of light of wavelength 260 mμ (ultra-violet). Thus, microphotos taken in the ultra-violet microscope show the location of the nucleic acids as dark areas.

The dense staining network of the nucleus consists of DNA. This network is called chromatin and it gives rise to the chromosomes which can be identified prior to and during mitotic division of the cell. Densest of all the nuclear material is the nucleolus. Composed of a network of helically-coiled fibres it is rich in RNA and is probably the sole site of RNA in the nucleus. The nucleolus is most prominent when the cell is synthesizing protein. The relations of DNA and RNA to cell reproduction and protein are discussed on p. 460 and p. 461.

ENZYMES

Most chemical reactions occurring in the body are regulated by the catalytic action of enzymes. The compound which undergoes change in an enzymically-catalysed reaction is called a substrate for that enzyme. Enzymes are highly selective (specific) in their choice of substrate, and each will catalyse only a small range of chemically-related transformations. All the known enzymes are proteins; there seems no doubt that they function as catalysts by providing, within their macromolecular structure, 'active sites' of highly individual shape and arrangement, at which a particular chemical reaction can proceed with great ease. If the integrity of the active site is disturbed, or access of substrate to the site is restricted, then the catalytic power drops accordingly.

A cell can conduct hundreds of simultaneous chemical reactions, each specifically catalysed and therefore capable of rate regulation through the amount of enzyme available, the supply of substrate, or the removal of product. The intermeshing, and often self-adjusting, enzyme systems which can conduct so intricate an operation must account for most of the protein content of the cell. These intracellular enzymes are not easily studied, for any process of isolation tends to disrupt the system or remove it from the modulating influence of other systems. Some enzymes (e.g., the hydrolytic enzymes of the gut [pp. 403 *et seq.*]) are secreted by cells and fulfil their physiological role independently of other enzymes; these extracellular enzymes are therefore susceptible of purification and study, but it would be wrong to consider them as typical of the enzymes in general.

REFERENCES

BRACHET, J. (1961) The living cell, *Scientific American*, September.
DE DUVE, C. (1963) The lysosome, *Scientific American*, May.
ENGLEBERG, J. (1966) in *The Physiological Basis of Medical Practice*, ed. Best, C. H., and Taylor, N. B., Chap. 1, Edinburgh.
ROBERTSON, J. D. (1964) Unit membranes, in *Cellular Membranes in Development*, ed. Locke, M., New York.
SOLOMON, A. K. (1960) *Scientific American*, March.

IONS AND IONIC BALANCE

All acids, bases, and salts in aqueous solution are present, to varying degrees, as electrically-charged particles called *ions*. Compounds which give rise to ions in solution are called *electrolytes*. Some electrolytes (*strong* electrolytes) always exist in ionic form, even in the solid state, e.g., sodium chloride, Na^+Cl^-, sodium bicarbonate, $Na^+ HCO_3^-$; other electrolytes dissociate completely into ions when in solution, e.g., hydrogen chloride in water $\rightarrow H^+ + Cl^-$. On the other hand, there are *weak* electrolytes which are only partially dissociated into ions, and their solutions contain undissociated molecules as well as the corresponding ions. The application of these distinctions to acids and bases is discussed on p. 7.

If placed in an electrical field, positively-charged ions would move towards the negative pole or cathode; they are therefore called *cations*. Negatively-charged ions would move to the positive pole or anode; they are therefore called *anions*.

Some physiologically-important ions:

Cations: hydrogen ion, H^+; ammonium, NH_4^+; metallic ions like Na^+, K^+, Ca^{++}, Mg^{++}.

Anions: hydroxyl, OH^-; bicarbonate, HCO_3^-; chloride, Cl^-; sulphate, $SO_4^=$; dihydrogen phosphate, $H_2PO_4^-$; hydrogen phosphate, $HPO_4^=$; ionized protein, $(proteinate)^-$.

In all physiological fluids, electrical neutrality must be maintained. The total number of positive charges on cations must be balanced (or 'covered') by an equivalent number of negative charges on anions. It is important to consider the behaviour of the individual ions separately, and not in terms of the electrolytes from which they are derived; it is equally important to remember that a given solution cannot receive a supplement of additional cation or anion without this being accompanied by an equivalent coverage of anion or cation respectively.

Many of the organic solutes in the body fluids, e.g., glucose, and urea, are non-electrolytes, and exist in solution solely as the un-ionized molecules.

Equivalent Weights. The *gram-equivalent weight* (loosely referred to as the 'equivalent weight', the 'equivalent', or the 'gram-equivalent') of a reactive unit (atom, molecule, ion) is that weight in grams which combines with, displaces, or otherwise plays the part of 1 g of hydrogen ion in the reaction or situation being considered.

Thus the gram-equivalent weight (gEq) of a monovalent ion (Na^+, K^+, NH_4^+, OH^-, Cl^-, HCO_3^-, $H_2PO_4^-$) is the same as its ionic weight; the gEq of a divalent ion (Ca^{++}, Mg^{++}, $SO_4^=$, $HPO_4^=$) is half its ionic weight.

The equivalent weight of any ion combines with or replaces the equivalent weight of any other appropriate ion. Thus 23 g of Na^+, 39 g of K^+, or 18 g of NH_4^+ mutually replace one another in salts, or combine with 35·5 g of Cl^-, 48 g of $SO_4^=$, or 61 g of HCO_3^-; the weights mentioned above are *chemically* equivalent (hence the term equivalent). One equivalent of X will combine with or replace one equivalent of Y whatever the valencies or unit weights of X and Y.

Milli-equivalents. In biological solutions the gram-equivalent of an ion is an unnecessarily large unit, physiologically speaking. The unit of choice is the milli-equivalent (mEq). One mEq is one-thousandth of a gram-equivalent. 1 gEq = 1000 mEq. 1 mEq is the amount of material, measured in milligrams, which will combine with or play the part of 1 mg of H or H^+. 1 mEq of any ion will replace 1 mEq of any other appropriate ion.

By expressing amounts in terms of mEq we eliminate the factor of difference in relative weights of the ions, and can compare directly the relative number of ions and ionic charges concerned. The prerequisite for electrical neutrality of a fluid will be that the total number of mEq of its cations will be equal to the total number of mEq of its anions.

The importance of thinking in terms of milli-equivalents is being generally recognized, and it is now standard practice to express the electrolyte concentrations of body fluids in milli-equivalents per litre (mEq per litre). However, one must be able to convert concentrations expressed by the older 'grams per cent' method into concentrations expressed as mEq per litre. The procedure for this conversion is:

$$\text{value in mEq per litre} = \frac{\text{value in mg per 100 ml} \times 10}{\text{equivalent wt}}$$

$$\text{where equivalent wt} = \frac{\text{ionic wt}}{\text{valency (No. of charges)}}$$

For H^+ equiv. wt =	1 [for pH notation see below]
Na^+	23
K^+	39
NH_4^+	18 expressed as ammonium or 14 expressed as ammonium-N
Ca^{++}	40/2 = 20
Mg^{++}	24/2 = 12
OH^-	17
Cl^-	35·5
$SO_4^=$	96/2 = 48 expressed as sulphate or 32/2 = 16 expressed as sulphate-sulphur.

Phosphate presents a special case. In plasma at pH 7·4, 20 per cent of the phosphate is present as monovalent ion $H_2PO_4^-$ and 80 per cent as divalent ion $HPO_4^=$; this gives a virtual valency of 1·8 (=0·2 × 1 + 0·8 × 2) and an ionic wt of 31/1·8 = 17 expressed as phosphate-P.

It is customary to measure the plasma concentration of bicarbonate HCO_3^- not as a weight but as the volume of CO_2 which the HCO_3^- would give off after acidification. This volume of CO_2 (ml, measured at s.t.p. per 100 ml of plasma) is converted into mEq per litre by dividing by 2·226 (since 1 mEq of CO_2 has been found experimentally to occupy 22·26 ml).

$$\text{Thus} \qquad 60 \text{ ml } CO_2/100 \text{ ml plasma} = \frac{60}{2{\cdot}226}$$

$$= 26{\cdot}9 \text{ mEq } HCO_3^-/\text{litre}.$$

The total mEq of effective cation in a biological fluid can be measured by the increase in H^+ which occurs when a hydrogen-bearing cation-exchange resin is employed to replace all the cations with their equivalent in H^+. A hydroxyl-bearing anion-exchange resin can be used analogously to replace all anions by OH^-. Both approaches will obviously give the same result.

Normality

A *normal* solution of a substance has a concentration of 1 gEq of that substance per litre. Expressed another way, 1 ml of a normal solution contains 1 mEq of the solute. (Of course a *normal* solution is no more normal (usual) than any other solution —it is merely a formalized way of expressing concentrations involving equivalents.)

Example: If the concentration of a solution of sodium chloride (equiv. wt = 58·5) is 58·5 g/litre (= 58·5 mg/ml) the solution is normal (1 N) with respect to sodium chloride, and also, of course, with respect to sodium ion Na^+ and chloride ion Cl^-. If the concentration was only 5·85 g/litre, then the concentration would be 0·1 N, and so on.

Ionization of Water

Water consists almost entirely of unionized molecules, H_2O, but partial ionization (dissociation) into hydrogen ions H^+ and hydroxyl ions OH^- does occur:

$$H_2O \rightleftarrows H^+ + OH^-$$

In pure water, the number of hydrogen ions must equal the number of hydroxyl ions. The concentration of H^+, which is written $[H^+]$ and expressed as gEq per litre, in pure water at 23 °C is 10^{-7}. Since the equivalent weight of H^+ is 1, this means that there is 10^{-7} g of H^+ per litre of pure water. Likewise, the concentration of OH^- (written $[OH^-]$) of pure water is also 10^{-7} g

equivalents per litre (though this would represent 17×10^{-7} g of OH^-, equivalent weight = 17). Since we are interested mainly in the relative numbers of H^+ and OH^-, we shall express the concentrations $[H^+]$ and $[OH^-]$ in gEq per litre.

The product of the concentrations of H^+ and OH^- in pure water is called the *ionization constant* of water, K_w, and its value will obviously be 10^{-14}:

$$K_w = [H^+] \times [OH^-] = 10^{-7} \times 10^{-7} = 10^{-14}$$

This figure is a constant which applies to *all aqueous solutions, regardless of the presence of other ionic material.* The concentration of either H^+ or OH^- automatically fixes the concentration of the other.

All physiological reactions occur in aqueous solution and are profoundly affected by the inescapable presence of H^+ and OH^-. It is usual to consider the hydrogen ion as the significant and variable quantity.

Hydrogen Ion Concentration

A solution containing equal numbers of H^+ and OH^- is called a neutral solution. For a neutral solution, as for pure water, $[H^+] = [OH^-] = 10^{-7}$ gEq/litre.

If there are more H^+ than OH^- in a solution, then it is said to be acid with respect to water. If there are less H^+ than OH^-, then a solution is said to be alkaline with respect to water.

Examples:

1. If $[H^+] = 10^{-4}$, then $[OH^-]$ must $= 10^{-10}$, since K_w = constant 10^{-14}.

This means that $[H^+]$ is a million (10^6) times greater than $[OH^-]$ and the solution is acid.

2. If $[H^+] = 10^{-8}$, then $[OH^-]$ must $= 10^{-6}$. Thus $[H^+]$ is only one-hundredth of $[OH^-]$ and the solution is alkaline.

Solutions with $[H^+]$ greater than 10^{-7} gEq/litre are acid with respect to water. Solutions with $[H^+]$ less than 10^{-7} gEq/litre are alkaline with respect to water.

pH Notation

The above method of writing $[H^+]$, with negative indices is clumsy, and such concentrations are better expressed in the pH notation: $pH = -\log_{10}[H^+]$.
(The letter p signifies that the negative logarithm, to base 10, of the quantity is employed instead of the quantity itself.)

Examples:

If $[H^+] = 10^{-7}$ gEq/litre, then $pH = -\log 10^{-7} = 7$
$[H^+] = 10^{-4}$ $pH = -\log 10^{-4} = 4$
$[H^+] = 10^{-8}$ $pH = -\log 10^{-8} = 8$

A solution of pH 7 is neutral with respect to water. If the pH is less than 7, the solution is acid. If the pH is greater than 7, the solution is alkaline.

Lowering the pH by *one unit* (e.g., from 7 to 6, from 4 to 3, from 10 to 9, or other equivalent change) represents a 10-*fold increase* in $[H^+]$ from its previous value. Raising the pH by *one unit* (from 7 to 8, from 8 to 9, from 3 to 4, or other equivalent change) represents a *decrease* in $[H^+]$ to *one-tenth* its previous value.

The normal pH of blood is 7·35–7·40. Life is only possible within the range pH 7·0–7·7, corresponding to a change of $[H^+]$ from 10^{-7} to 2×10^{-8} (a 5-fold decrease). The following method of working out the $[H^+]$–pH relationship within this (or any other) range is useful and easy:

Let us begin with pH = 7·0. We know that this means $[H^+] = 10^{-7}$ gEq/litre. Let us express it as 10×10^{-8}. Now an increase in pH to say 8·0 means a fall in $[H^+]$ to 1×10^{-8}. If we remember:

1. That +0·3 is approximately the log of 2 and −0·3 is approximately the log of 1/2.
2. That an increase of pH represents a decrease in $[H^+]$, then the rest is quite simple.

Thus pH 7·0 = 10×10^{-8}, pH 7·3 = 5×10^{-8}, pH 7·6 = $2{\cdot}5 \times 10^{-8}$, and pH 7·9 = $1{\cdot}25 \times 10^{-8}$. Beginning on the other hand with pH = 8·0 = 1×10^{-8}, pH 7·7 = 2×10^{-8}, pH 7·4 = 4×10^{-8}, pH 7·1 = 8×10^{-8}. As pH 7·9 = $1{\cdot}25 \times 10^{-8}$, pH 6·9 = $12{\cdot}5 \times 10^{-8}$ and consequently pH 7·2 = $6{\cdot}25 \times 10^{-8}$, pH 7·5 = $3{\cdot}13 \times 10^{-8}$, and pH 7·8 = $1{\cdot}56 \times 10^{-8}$.

TABLE

pH	H^+ concn. g/litre	
7·0	10×10^{-8}	allowable range for human blood
7·1	8×10^{-8}	
7·2	$6{\cdot}25 \times 10^{-8}$	
7·3	5×10^{-8}	
normal 7·4	4×10^{-8}	
7·5	$3{\cdot}13 \times 10^{-8}$	
7·6	$2{\cdot}5 \times 10^{-8}$	
7·7	2×10^{-8}	
7·8	$1{\cdot}56 \times 10^{-8}$	
7·9	$1{\cdot}25 \times 10^{-8}$	
8·0	1×10^{-8}	

The hydrogen ion concentration of a fluid expressed as pH value is often called the *reaction* of that fluid.

Acids and Bases

An acid is a substance which provides hydrogen ions, and can thus increase the $[H^+]$ of a solution into which it is placed (i.e., it lowers the pH).

A base is a substance which accepts hydrogen ions, and can thus decrease the $[H^+]$ of a solution into which it is placed (i.e., it raises the pH).

Certain old established physiological terms involve the use of the words 'acid' and 'base' in senses different from those indicated above. In particular, 'acid' has been used to indicate anions like Cl^-, and 'base' has been used to indicate cations like Na^+. This usage is to be deprecated—neither Na^+ nor Cl^- can have any effect on $[H^+]$ by virtue of its *own* properties. It is true that either of them may indirectly influence $[H^+]$ through the other ion that must inevitably accompany it, but this only serves to emphasize the confusion caused by not analysing acid-base changes in terms of the central reactor—the hydrogen ion. More detailed assessment of these points will be found on p. 174 *et seq.*

Some acids in aqueous solutions are fully ionized at all times. They are called *strong* acids, e.g., hydrochloric acid.

$$HCl \rightarrow H^+ + Cl^-$$

Other acids, the *weak* acids, are only partially ionized, only a small proportion of their replaceable hydrogen being in the ionic form. An important physiological example is carbonic acid.

$$H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$
(dissociation in 0·1N soln. = 0·2 per cent)

Even this dissociation is reduced if H^+ or HCO_3^- are provided from elsewhere.

Some bases, the *strong* bases (alkalis), like sodium hydroxide, are completely ionized in solution at all times:

$$NaOH \rightarrow Na^+ + OH^-$$

On the other hand, *weak* bases owe their basicity to their power to capture H^+, e.g., ammonia $NH_3 + H^+ \rightleftharpoons NH_4^+$. A solution of ammonia (ammonium hydroxide) is alkaline for this reason:

$$NH_3 + \underbrace{H^+ + OH^-}_{\downarrow\uparrow\ H_2O} \rightleftharpoons NH_4^+ + \underline{OH^-}$$

Salts formed from a strong acid with a strong base are fully ionized (even in the solid state), e.g., sodium chloride Na^+Cl^-. Salts from either a strong acid with a weak base or a weak acid with a strong base are also fully ionized when dissolved in water, but this does not mean that their solutions will be neutral. In the case of the salt of a weak acid with a strong base, the anion will react partially with H^+ from the water to give the undissociated weak acid, leaving an excess of OH^-. The solution will be alkaline, with a pH above 7.

Example:

$$\text{Sodium bicarbonate in water} \rightarrow Na^+ + \underbrace{HCO_3^- + H^+}_{\downarrow\uparrow} + OH^- + H_2O$$

$$Na^+ + \quad H_2CO_3 \quad + \underline{OH^-} + H_2O$$

Similarly the salt of a weak base with a strong acid gives an acid solution because of the reversible loss of H^+ from the cation.

Example:

$$\text{Ammonium chloride in water} \rightarrow Cl^- + \underbrace{NH_4^+ + OH^-}_{\downarrow\uparrow} + H^+ + H_2O$$

$$Cl^- + \overbrace{NH_3 + H_2O}^{\downarrow\uparrow} + \underline{H^+} + H_2O$$

Buffer Systems

A buffer solution is one which can accommodate the addition to it of moderate amounts of acid or base without marked change in its hydrogen ion concentration.

To construct such a system requires an equilibrium mixture of ions and undissociated molecules which will resist, by changes in the equilibrium position, attempts to disturb the pH.

Consider the equilibrium from the dissociation of a weak acid HA into hydrogen ion and anion A^-:

$$HA \rightleftarrows H^+ + A^-$$

1. If OH^- is added, H^+ is removed as water; but then further ionization of undissociated acid occurs, and thus there is only a small rise in pH. Added OH^- is accommodated by the reserve HA.

$$2\,HA \rightleftarrows HA + H^+ + A^- \xrightarrow{+OH^-} HA + H_2O + A^- \quad (\downarrow\uparrow) \quad H^+ + A^- + H_2O + A^-$$

2. If H^+ is added, the ionization of the weak acid is depressed and the hydrogen ion concentration reduced.

$$H^+ + A^- \rightarrow HA$$

But the ionization of the acid is so small in any case that only a very small addition of H^+ can be accommodated before the pH falls. If, however, the weak acid HA was supplemented by additional anion A^-, then this anion would act as a reserve for 'mopping up' added H^+.

An adequate buffer system can thus be assembled from a *weak acid* mixed with the *salt* from that acid and a strong base, e.g., HA plus B^+A^- (where B^+ is the cation of a strong base, e.g., Na^+). This corresponds to a partially neutralized solution of weak acid, with some reasonable proportion of the acid converted to the anion; its pH will be higher than the pH of the weaker acid alone.

Examples of buffer pairs:

acetic acid/acetate $[CH_3COOH + CH_3COO^-(+B^+)]$
carbonic acid/bicarbonate $[H_2CO_3 + HCO_3^-(+B^+)]$ [p. 177]
oxyhaemoglobin (acid)/
oxyhaemoglobin anion $[H.HbO_2 + HbO_2^-(+B^+)]$ [p. 174]
dihydrogen phosphate/
hydrogen phosphate $[H_2PO_4^- + HPO_4^=(+B^+)]$ [p. 221]

The buffer anions of the blood are discussed on p. 174.

The pH of a buffer solution is determined by the chemical nature of the anion, and by the ratio of the amount of salt to weak acid

$$pH = pK_a + \log \frac{[salt]}{[acid]}$$

where K_a is the dissociation constant of the acid and pK_a = pH at which the acid is half neutralized and equal amounts of acid and ion (salt) are present. This is the Henderson-Hasselbalch equation; its application to the carbonic acid/bicarbonate system is discussed on p. 177.

The capacity of a buffer, i.e., the quantity of added acid or base that can be accommodated and yet retain the pH within small limits, is determined by the absolute concentration of the components and, of course, by the amount of buffer solution available.

For example, the phosphate buffer system contributes little to the total buffering capacity of blood because the blood phosphate concentration is so low compared with the amount of protein present; on the other hand, the phosphate buffer pair is a major outlet for H^+ via the urine which has a relatively high phosphate content [p. 221].

Fixed Ions and Buffer Ions

The ions of natural buffered fluids are of two types:

1. The fixed ions, whose concentrations are unaffected by moderate variation in H^+ concentration.

2. The buffer ions, whose concentrations are considerably affected by attempted changes in H^+ concentration.

The fixed cations (metallic cations), e.g., Na^+, K^+, Ca^{++}, are frequently, but wrongly, termed fixed base [see p. 178]. These fixed cations enter the body as such (with equivalent anion cover) and are seldom chemically reactive in normal metabolism. The ammonium ion NH_4^+ is in a special category of metabolically variable cation, because it can be obtained as 'required' from the normal metabolic production of ammonia [p. 223]. Below pH 7 it plays the part of a fixed cation; if excreted as cover for an excreted anion it will represent the loss of readily-available H^+ instead of the physiologically more valuable sodium cation.

The fixed anions, e.g., Cl^-, $SO_4^=$, $HPO_4^=$, are often wrongly termed fixed acid. All the chloride enters and leaves the body as such; on the other hand, most of the sulphate and phosphate are produced metabolically from the non-ionized sulphur of cystine and methionine [p. 447] and the less-ionized phosphate of phospho-

proteins. These fixed anions, phosphate and sulphate, have therefore gained access to the body, so to say, without fixed cation cover; their cover is provided by H^+ and the effects of this are then minimized by the buffer anions.

The buffer anions of blood are represented by bicarbonate HCO_3^-, haemoglobin anion, and other protein anions. Actually the carbonic acid/bicarbonate system is a poor buffer, in the normal sense, at the pH of blood. Its value lies in the ability of the lungs to eliminate undissociated carbonic acid as non-ionizable CO_2, so that HCO_3^- can be sacrificed without permanently increasing the H_2CO_3 concentration.

TRANSFER OF SUBSTANCES ACROSS MEMBRANES

The physiological activity of a cell is very dependent on the ease with which it can gain materials from, or lose materials to, its surrounding environment. A cell can only grow and function if its requirements for oxygen, ions, amino acids, vitamins, and a score of other chemical substances are met, and the waste or secretory products of its metabolism are removed. Even transport to or from the cell is not sufficient, because substances must pass through the cell boundary into or out of the cell. In many cases, the cell does not wait passively for these boundary interchanges to occur—it intervenes *actively* in the processes.

Passive Transport

This term refers to the movement of substances in the direction of the physical gradients; it is determined, for example, by gradients of concentration and potential, hydrostatic pressure, or osmotic forces [p. 11], and as movement occurs the gradient is diminished.

Diffusion

If the hydrostatic pressures on each side of a membrane are equal, then the movement is represented by diffusion—the movement of solute which occurs when a membrane or boundary separates two fluids differing in their concentration of solutes, i.e., when there is a concentration gradient across the membrane (or within a single solution). If all the solutes are freely diffusible through the membrane, an equilibrium position is reached with equal concentrations on each side, i.e., the gradient has disappeared. If one fluid also contains material which is not diffusible through the membrane, then passive diffusion to equilibrium may result in an *unequal* division of the freely-diffusible solutes also [the Donnan equilibrium with ions, see p. 10, and p. 11]. Passive diffusion is a consequence of the continuous and random movement of the molecules of solute and solvent.

If the hydrostatic pressures on the two sides of the membrane are unequal, a flow of water and dissolved solutes will occur by filtration [p. 79].

Active Transport

This refers to the movement of substances across membranes irrespective of the concentration gradient, and which therefore cannot be attributed solely to passive diffusion. Such movement requires the active expenditure of chemical energy (work) and establishes or maintains a gradient.

Active transport may take various forms:

1. *Acceleration* of movements along the gradient: thus when a glucose solution of higher concentration than that found in plasma is placed in the intestine, it tends to diffuse into the blood. It is, however, more rapidly absorbed through living intestinal mucosa than through dead, owing to the active intervention of the mucosal cells.

2. *Prevention* of movement with the gradient: thus Na^+ may be prevented from accumulating in tissue cells and red blood cells even when the extracellular Na^+ concentration far exceeds the intracellular. This does not mean that Na^+ cannot pass through the membrane—the use of radioactive ('labelled') Na^+ shows that it does. But the cell membrane and the cellular mechanism it encloses can maintain a steady state in which there is a higher concentration of Na^+ outside the cell than inside it. Just as a leaky boat can be kept afloat by continuous baling, so can the cell 'pump' Na^+ out by the continuous expenditure of chemical energy.

3. Movements of substances *against* the gradient: thus K^+ can be taken up by tissue cells or red cells even when the intracellular concentration of K^+ far exceeds the extracellular concentration. The cell can maintain, at equilibrium, a higher concentration of K^+ inside its membrane boundary than outside it; it 'pumps' K^+ in.

Cells which actively maintain concentration gradients by the expenditure of work are said to be *selectively-permeable.*

Secretion

The term secretion is used to include two consecutive processes: the formation of specific constituents and their subsequent transfer across a membrane. Thus secretion of saliva [p. 403] includes the formation of the specific enzymes and their transfer, together with water and electrolytes, into the gland lumen.

In some secretory tissue [e.g., the adrenal medulla, p. 390] passive diffusion may account sufficiently for the observed transference from producing cell to outside fluid; but more often than not, active transport has to be invoked. In the case of saliva, the secretion is hypotonic with respect to plasma and the gland does work overcoming osmotic forces [p. 403]. If the salivary duct is blocked the pressure within it rises owing to continued secretion; this pressure may exceed even the arterial blood pressure and therefore must greatly exceed the capillary blood pressure. In transferring fluid from the plasma via the interstitial fluid and gland cells into the duct, the cells of the salivary glands must overcome large hydrostatic pressure gradients.

Mechanisms

We know something of the enzyme and energy-systems concerned with the active transport of organic substances, particularly in the case of the renal tubule epithelium [p. 215], but little or nothing of those responsible for the active transport of water and ions.

It is important to realize that a chemical reaction cannot in itself cause diffusion against a concentration gradient. It can only create a fresh gradient so that passive diffusion can then occur in the desired direction. The difference between active transport and passive diffusion lies in the ability of the energy-requiring system of coupled chemical reactions to create favourable concentration gradients inside and across membranes.

Examples of active transport include:

1. Cation 'pumps' of red blood cells [p. 38].
2. Movement of Na^+, K^+, and Ca^{++} in nerve [p. 253].
3. Secretion of H^+ by gastric oxyntic cells [p. 404].
4. Alkaline secretion from pancreas [p. 408].
5. Absorption and secretion by kidney tubules [p. 220].
6. Secretion of hormones by ductless glands [p. 483 *et seq.*]

Permeability of Membranes

Membranes differ very much in permeability, i.e., in the size and kind of molecule which they will allow to pass through in solution. An *ideal* membrane would allow only water to pass through and would be impermeable to all dissolved substances; certain artificial membranes, e.g., copper ferrocyanide deposited across the pores of a porous pot, approach this ideal closely, but there are no ideal membranes in the body. Isolated cellular membranes are *partially-permeable*, i.e., they are fully permeable to water, electrolytes, and small crystalloid molecules but hold back the larger colloidal particles (macromolecules) such as those of proteins and polysaccharides. They are like molecular sieves, with penetration governed by molecular size and shape and, to a lesser extent, by solubility in the membrane lipids. Membranes (e.g., of collodion) can be made with varying degrees of porosity, a fine membrane retaining molecules of smaller molecular weight and size than a coarser membrane.

Permeability and Active Transport. As previously explained, what goes through a cell membrane, and at what rate, is profoundly modified by the processes of active transport. There is no predetermined relationship between the partial-permeability of an *isolated* membrane and its apparent permeability when forming the boundary of a living cell. The cell boundary is *selectively-permeable*, and what is selected at any time depends upon the metabolic condition and activity of the cell. An isolated cell membrane may be fully permeable to small ions, e.g., Na^+, yet the working cell may specifically maintain them at internal concentrations quite different from the external concentrations. If the state of the cell is changed, e.g., by cooling, poisoning, or altered composition, then the selective-permeability of its boundary may change also. If the cell metabolism ceases, the ions may penetrate freely at the sole dictate of the concentration gradients.

(The ambiguous description 'semi-permeable' is sometimes applied to natural and experimental membranes. It should be avoided.)

The movement of materials through the membrane occurs by diffusion, perhaps assisted by filtration.

Filtration

By filtration is meant the passage of fluid through a membrane owing to a difference of pressure on the two sides of the membrane; the fluid which passes through consists of water and any dissolved substances to which the membrane is permeable. Normal filtration through coarse membranes (e.g., filter paper) will remove only the larger suspended matter. Filtration through cell membranes is on a much finer scale, and is more appropriately termed ultrafiltration; colloidal material is retained; water, electrolytes, and crystalloids pass through in the ultrafiltrate. Ultrafiltration characteristically occurs in most of the capillaries, where capillary blood pressure drives a virtually protein-free plasma filtrate out of the blood vessels [pp. 79, 80]. Damage to the capillary wall increases its permeability to protein; in graver injuries, the suspended corpuscles may pass through as well.

Permeability to Protein. Although it is generally true that cell membranes are relatively impermeable to protein, this does not hold for specific proteins and specific types of cell, as the following examples show: placental membranes are permeable to maternal protein antibodies; foreign protein antigens have access to antibody-producing cells; protein enzymes are secreted into the lumen of the intestinal tract from specific cells; the enzyme ribonuclease can penetrate certain cells and destroy their RNA.

The walls of the minute blood capillaries of liver and muscle seem to have 'defects' which permit the passage of protein-rich plasma.

Donnan Membrane Equilibrium

Consider two ionized solutions *a* and *b* filling compartments of constant volume and separated by a partially-permeable membrane. It was shown theoretically (Gibbs), and confirmed experimentally (Donnan), that *at equilibrium*:

1. Each solution will be electrically neutral—its total charges on cations will equal those on anions

$$[\text{cations}]_a = [\text{anions}]_a \quad \text{and} \quad [\text{cations}]_b = [\text{anions}]_b$$

and 2. the product of the diffusible (penetrating) ions on one side of the membrane will equal the product of the diffusible ions on the other.

$$\left[\begin{matrix}\text{diffusible}\\ \text{cations}\end{matrix}\right]_a \times \left[\begin{matrix}\text{diffusible}\\ \text{anions}\end{matrix}\right]_a = \left[\begin{matrix}\text{diffusible}\\ \text{cations}\end{matrix}\right]_b \times \left[\begin{matrix}\text{diffusible}\\ \text{anions}\end{matrix}\right]_b$$

For example, with the freely diffusible ions of sodium chloride solutions, the equilibrium position will be reached when *a* and *b* have the same concentration of both ions (symmetrical distribution). But if one or more indiffusible ions are also present, the ionic distribution of the diffusible ions at equilibrium will be asymmetrical.

Consider that in our simple system sodium chloride (Na^+Cl^-) is present in solutions *a* and *b* but that only *a* contains a salt Na^+X^-, where X^- is an indiffusible anion unable to cross the membrane (as is the case with many protein and organic phosphate anions), thus:

soln. *a*	Na^+ Cl^- X^-	Na^+ Cl^-	soln. *b*

The penetrating ions (Na^+ and Cl^-) diffuse until equilibrium is attained. The two criteria established above will hold, namely

$$\left.\begin{matrix}[Na^+]_a = [Cl^-]_a + [X^-]_a\\ [Na^+]_b = [Cl^-]_b\end{matrix}\right\} \text{electrical neutrality}$$

and $$[Na^+]_a \times [Cl^-]_a = [Na^+]_b \times [Cl^-]_b \quad \text{products of } \textit{diffusible} \text{ ions}$$

From these relationships it follows that

$$[Na^+]_a > [Cl^-]_a$$

and therefore that

$$[Na^+]_a > [Na^+]_b$$
$$[Cl^-]_a < [Cl^-]_b$$

and $$[Na^+]_a + [Cl^-]_a > [Na^+]_b + [Cl^-]_b$$

Hence at *equilibrium*, the cation (Na^+) concentration on the side of the membrane containing the non-penetrating anion X^- is greater than the cation concentration on the other side; the opposite is true of the diffusible anion (Cl^-) concentration which will be greater on the side without the non-diffusible anion. This is known as the Gibbs-Donnan Membrane Equilibrium.

A further consequence is that there is a greater number of ions in *a* than in *b*. The osmotic consequences of this are considered later [p. 12].

It must be realized that the Gibbs-Donnan Effect is brought about by differential permeability and passive transport, but of course the effect can be magnified or opposed by active transport, which can maintain an intracellular ion at a constant concentration *as though it were* a non-penetrating ion.

Living cells contain an excess of non-penetrating anions, mainly organic phosphates and proteins. The effects of this intracellular anion can be summarized thus:

1. The distribution of the diffusible anions and cations between cells and extracellular fluids is unequal, even when active transport does not apply. This is responsible for the permanent electrical potential difference which exists across cell membranes.

2. The concentration of cation inside a cell is greater than outside. In the case of the red cell, the excess is about 10 mEq per litre of cell fluid, but the situation is complicated by active intake of K^+ and expulsion of Na^+. The intracellular $[H^+]$ is somewhat greater than that of the external medium, e.g., the pH of red cells is 7·2 in plasma of pH 7·4.

3. The diffusible anion concentration is lower inside cells than outside. In the case of human red cells, the ratio of intracellular chloride to plasma chloride is 0·7 [see p. 180 for the 'chloride shift']. The much lower ratios found in nerve are due to a high membrane potential arising from other causes [p. 253].

4. Animal cells are always tending to swell and burst due to the osmotic consequences of the higher cell concentrations. This tendency is normally opposed by active cation transport; if cell metabolism is depressed by cooling or poisoning, then active transport ceases and the unopposed Donnan Effect plus osmosis causes swelling and ultimate rupture.

OSMOSIS. OSMOTIC PRESSURE

A copper ferrocyanide pot acts as an ideal membrane, permeable to water but impermeable to solutes such as glucose. If a glucose solution is placed inside such a pot and the pot is placed upright in water, pure water diffuses from the exterior to the interior of the pot, and the pressure and volume within rise. Alternatively, the movement of water can be prevented by applying pressure to the glucose solution. A similar effect is shown when a partially-permeable bag (e.g., a cellophane sac, or a living cell) containing a non-diffusible colloid, is immersed in water. Unless sufficient pressure is applied from within the sac to establish equilibrium without net movement of water, water will diffuse in to dilute the colloidal solution.

This phenomenon is called *osmosis*. The osmotic pressure of a solution is the pressure increment that would have to be imposed on that solution to prevent entry of pure water through a boundary permeable only to water.

There is much misunderstanding about this. It is not strictly correct to talk of the 'osmotic pressure' exerted by glucose or plasma proteins; solute molecules do *not* attract water to themselves: they do *not* exert a 'negative pressure' across the membrane. In actual fact, osmosis concerns a property of water and the effect of dissolved substances such as glucose or proteins on that property. The chemical potential of water in an aqueous solution is lower than that of pure water. Thus, if a solution is separated from water by a membrane there is a 'potential difference' between the two phases, and equilibrium can only be established by:

1. Free distribution of solute on both sides of the membrane; this is only possible with diffusible solute and permeable membrane.

2. Passage of water through the membrane which eliminates the potential difference by diluting the solution—osmosis with an indiffusible solute.

3. Application of pressure to the solution to increase the potential back to that of pure water and thus prevent net movement of water—osmotic pressure.

If diffusible and non-diffusible solutes are present, only the non-diffusible ones will contribute permanently to the pressure increment required, since the other solutes will be equally distributed at equilibrium (if the Gibbs-Donnan Effect on ionic distribution is not applicable).

Osmosis is of great importance in physiology, especially in relation to the distribution of water between the blood and tissue spaces, but the idea has been so abused that it is difficult to re-establish its true meaning, and to distinguish theoretical calculation from actual measurement, ideal equilibrium from true cellular processes. We shall use the term 'osmotic pressure' of a given solution to mean the pressure difference which would be required between the solution and pure solvent, or other stated solution, to maintain the phases in equilibrium across the stated partially-permeable or selectively-permeable membrane. When dealing with solutes, colloids, and electrolytes we shall refer to their 'osmotic effect', i.e., the effect (measured as a restoring pressure) which they exert on the properties of pure water.

Osmolar Concentration

The osmotic pressure of a solution depends solely on the number of particles (undissociated molecules, ions, colloidal micelles) in solution, and not on their size or weight. The gram molecular weight of any substances represents the same (very large) number of molecules; it can be calculated that the aqueous solution of any undissociated substance at molar concentration (1gMol/litre) would exert an osmotic pressure of 22·4 atmospheres under ideal conditions and with an ideal membrane. For example: a molar solution of glucose (mol wt. = 180) exerts an ideal osmotic pressure of 22·4 atmos., while a glucose solution of 100 mg/100 ml (= 1 g/litre as in plasma) has an ideal osmotic effect equivalent to 22·4/180 atmospheres (= 95 mm Hg). In general terms, the pressure required for equilibrium with respect to water is proportional to the concentration of solute which cannot penetrate the membrane, provided that the solution is dilute; at higher concentrations, the simple proportionality no longer holds.

In view of the dependence on numbers of particles the concentration of osmotically-significant particles is often best expressed as an osmolarity (osmoles, or milli-osmoles, per litre). One osmole (= 1000 milli-osmoles) of a substance is that quantity of it which would have a calculated ideal osmotic effect equivalent to 22·4 atmos. when present in 1 litre of solution. An osmolar solution contains 1 osmole/litre, and therefore has an ideal osmotic effect of 22·4 atmos. For an undissociated non-electrolyte, the molar and osmolar concentrations are identical. In the case of substances which dissociate completely into ions, each ion has the same osmotic effect as an undissociated molecule; thus a molar solution of sodium chloride (Na^+Cl^-) has an ideal osmotic effect of $2 \times 22{\cdot}4$ atmos. and is therefore 2 osmolar (2 osmoles/litre). If substances dissociate only partially the appropriate multiplication factor must be found by experiment. If molecules associate, as with macromolecules in colloidal particles, the osmotic effect will be lower than that from the separate molecules.

The osmotic effect of a mixture of solutes is the sum of the effects from each component separately, and we refer to osmolarity, instead of to molarity or normality [p. 6] when we wish to show the additive osmotic effect of individual ions.

Solutions with the same ideal osmotic pressure are termed *isosmotic*. If separated by an ideal membrane (permeable only to water) no net movement of water would occur. The situation will be different with the 'real' membranes of cells, which are partially-permeable and allow water and certain small molecules and ions to pass. If two solutions separated by a partially-permeable membrane come to equilibrium without net transfer of water, they are termed *isotonic* solutions. For example, the intracellular fluid of human red blood cells is isotonic across the red-cell membrane with 0·92 per cent sodium chloride; this means that when 'living' red cells are suspended in this strength of sodium chloride solution, they neither gain nor lose water, though they will lose some diffusible glucose and urea to the solution. This situation, and the obvious isotonicity of red cells with plasma, is only maintained by active transport [see below].

An accepted physiological convention terms a fluid isotonic (without further qualification) when it is isotonic across the red cell membrane with cell fluid; hypertonic or hypotonic fluids have osmotic effects respectively greater or less than this, and would remove water from or add water to red cells suspended in them. Since we know so little about the permeability of membranes and the concentration gradients maintained by living cells, it is impossible to predict the actual 'tonicity' of any given solution; this can only be found by experiment.

Osmosis, Donnan Effect, and Active Transport

As explained previously [p. 10], the Gibbs-Donnan Effect results in an unequal distribution of diffusible ions across the two sides of a membrane if a non-diffusible ion is present on one side only. One consequence is a greater number of ions, and therefore a greater osmotic effect, on this side than on the other.

It was assumed in the figure on p. 10 that solutions *a* and *b* are confined in volume by rigid walls, as would be the case with vegetable cells. If the compartment walls are not rigid, then water will flow from *b* to *a*, *a* having the greater osmotic pressure. This will dilute *a* and concentrate *b* and disturb the Donnan equilibrium which can only be re-established by diffusible ion passing with the water from *b* to *a*, and so on. It is clear that, with no limitation on volume, true equilibrium cannot be established until all the water and ions on side *b* have passed to side *a*.

For animal cells, whose elastic walls are unable to resist an influx of water brought about by osmotic effects, rupture might seem inevitable; indeed this would be the case were it not for the occurrence of active transport, which is adjusted so as to oppose the Donnan Effect and prevent excess of cation, and therefore of water, entering the cell. Even a large difference in tonicity across the natural membrane of a metabolizing cell does not normally cause a large difference in hydrostatic pressure to develop, because the system is not in true equilibrium and metabolic work must be performed continuously to maintain it. The work done to maintain or increase concentration differences across a membrane by water transfer is called 'osmotic' work. For example, the osmotic work done by the kidneys in concentrating a urea solution from 0·03 per cent (as in blood filtrate) to 2 per cent (as in urine) is about 2·5 kcal/mol, regardless of whether this is a direct transfer of water or a transfer secondary to the movement of Na^+.

Osmotic Pressure of Plasma

The total osmotic pressure of plasma, calculated for an ideal membrane against pure water, would be about 6·5 atmos. (5000 mm Hg), i.e., its osmolarity is $6{\cdot}5/22{\cdot}4 = 0{\cdot}3$. This is due almost entirely to the dissolved ions and crystalloids, with a contribution of only 25 mm Hg from the plasma proteins. However, the capillary membranes of the body are freely permeable to ions and small molecules, though relatively impermeable to protein, and the only significant difference between plasma and interstitial fluids (intercellular tissue fluid) is in the protein content of the former. It is only this protein which can have an osmotic effect in promoting water movement from interstitial fluid to plasma.

Osmotic Effects of Plasma Ions

The hypothalamus contains cells known as osmoreceptors which are sensitive to changes in the osmolar concentration of non-penetrating Na^+ [p. 226]. These cells can exert nervous control over the secretion of a posterior pituitary hormone (antidiuretic hormone, ADH) which regulates the water-conserving systems of the kidney [p. 218]; the interplay of these mechanisms maintains the osmotic effect of the plasma ions, and therefore of the interstitial fluid, within narrow limits.

Osmotic Effects of Plasma Proteins

The osmotic effect of the plasma proteins causes water to flow from the protein-free interstitial fluid into the blood vessels. On the other hand the capillary blood pressure is a filtering force tending to drive protein-free ultrafiltrate into the interstitial spaces in the opposite direction to the osmotic effect of the colloids.

The osmotic effect of the plasma proteins depends mainly on the serum albumin and to a smaller extent on the serum globulins. Since albumin has a considerably smaller mol. wt. than globulin, a given weight of albumin contains more osmotically-effective particles than the same weight of globulin; 1 g/100 ml of albumin has an osmotic effect equivalent to 6 mm Hg, but of globulin only 1·5 mm. With the normal albumin level of 4·5 g/100 ml and globulin level of 2·5 g/100 ml (albumin/globulin ratio = 1·8), about 80 per cent of the protein osmotic effect is due to albumin. Even with these quantities of protein the osmotic effect is small (25 mm Hg) because of the high mol. wt. of these macromolecules.

By virtue of their osmotic effect, the plasma proteins tend to retain fluid in the blood capillaries and thus maintain the plasma volume [p. 153]. The opposing effects of osmosis and capillary pressure regulate the interchange of water between the plasma and the tissue spaces [p. 79]. The plasma proteins influence filtration in the glomeruli of the kidney [p. 215]. If the total plasma protein level is decreased below 3·5 g/100 ml by bleeding [plasmapheresis, p. 16] or by disease [e.g., nephrosis] the tissues may become waterlogged [oedema], the effect is accentuated if the protein lost is mainly the osmotically more useful albumin.

REFERENCES

BLAND, J. H., ed. (1963) *Clinical Metabolism of Body Water and Electrolytes*, Philadelphia.

CHRISTENSEN, H. N. (1964) *Body Fluids and the Acid-Base Balance*, Philadelphia.

THOMPSON, R. H. S., and KING, E. J. (1964) *Biochemical Disorders in Human Disease*, 2nd ed., London.

WOLF, A. V., and CROWDER, N. A. (1964) *Introduction to Body Fluid Metabolism*, Baltimore.