1. Introduction to Physiology

Physiology is the science of the functions and phenomena of living things.

1.1 Cells

Mammalian cells are very small, of the order of 10^{-5} m in diameter. This is about midway between men (of the order of 1 m) and atoms (10^{-10} m). A man has about as many cells - about 10^{14}) - as a cells has molecules.

The detailed structure of cells was not revealed by the light microscope, with its limit of resolution of 0.2 microm. The electron microscope, however, with resolution down towards 1 nanom (10^{-9} m) has revealed a complex ultrastructure. The cells are tiny aquatic organisms - open systems that, like flames and waterfalls (and unlike crystals), need continuous supplies of matter and energy to maintain their form.

1.2 Water in the Body

The biological importance of water depends upon a number of peculiar properties:

1. It is a liquid at 'ordinary' temperatures.

2. It has a large heat of fusion.

3. It has a large heat capacity.

4. It has a large heat of vaporization.

The first of these is explained by hydrogen bonding; the others by the energy required to break bonds during melting, warming and vaporization.

5. It has a maximum density at 4 °C.

6. It has a large dielectric constant, which reduces electrostatic forces eighty times and makes water a superb solvent for ionic compounds. Moreover, water dipoles are strongly attracted to dissolved ions and to charged surfaces, coating these with relatively immobile layers of water molecules which greatly modify the properties of ions in solution.

About 60%, or two-thirds, of body weight is due to water. The 'average' 70 kg man has about 42 L; of this, 23 L is in his cells (intracellular) and 19 L outside the cells (extracellular). Three liters of the extracellular water is in the *blood plasma*, the rest (*interstitial fluid*) provides an aquatic habitat for the cells. The intracellular and the extracellular water have different solutes dissolved in them and so constitute two distinct kinds of fluid, the *intracellular fluid (ICF)* and the *extracellular fluid (ECF).* Muscle cell fluid and plasma are typical of ICF and ECF although the size and composition of ECF do to some extent differ in different parts of the body. The ICF has mainly *potassium* with organic anions. ECF has mainly *sodium* and *chloride*, rather like diluted sea water. The plasma

constituents are commonly expressed per litre of plasma rather than per kg of water and, because protein occupies a finite volume, the values are somewhat lower than the above values.

The ECF is a middle-man fluid, a medium for all exchanges between a cell and any other cell or the external environment.

1.3 Homeostatic Mechanisms

A homeostatic mechanism is a regulating mechanism triggered by alteration in some physiological property or quantity, which acts to produce a compensating change in the opposite direction.

Minimum requirements for homeostatic mechanisms are:

1. *Detectors,* often specialized to respond to particular variables.

2. *Effectors*, i.e., muscles and glands. The cardiovascular, respiratory, renal and alimentary systems can be considered as effector systems subserving homeostasis.

3. *Coordinating and integrating mechanisms*, linking 1 to 2. These are nervous and hormonal.

1.4 Exchange of Water and Solutes Within the Body

In tissues, *capillaries* bring blood within 5 to 10 microm of most cells. To get from blood to the interior of a cell, solutes like glucose and oxygen must:

1. cross the capillary wall,

2. cross a layer of interstitial fluid between capillary and cell, and

3. cross the plasma membrane, which separates the ICF from the ECF. The second is the simplest. Its mechanism is known as *diffusion.*

Diffusion

This occurs as the spontaneous result of random thermal motions which tend to disperse accumulations of molecules and make concentration uniform. Movements of molecules through the solution are called *fluxes. Net flux* of each species is down its own gradient from higher concentration to lower. The rate of net flux for uncharged molecules, that is, the amount moving per unit time, is given by *Fick's law.* The diffusion coefficient for each substance depends upon its relative molecular mass, the temperature and the viscosity of the solvent.

The driving force for diffusion is the gradient of concentration of the diffusing substance, and Fick's law states that the rate of transport is proportional to that driving force.

Distance is proportional to the square root of the number of steps, hence to the square root of time. This means it takes four times as long to go twice as far, but half the distance is covered in a quarter of the time. Diffusion is therefore *very rapid over short distances.*

Exchange Across Capillary Walls

Capillaries are minute vessels (diameter about 10 microm) specially adapted for the rapid exchange of water and solutes. Their walls are composed of a single layer of endothelial cells about 1 microm in thickness. In contrast to the plasma membrane, in most tissue the capillary wall is a very leaky membrane for it lets substances through whose relative molecular masses are less than about 70000. These include practically all the solutes in the plasma except the plasma proteins. In liver proteins pass through to a greater extent than elsewhere, while in brain, movement of water-soluble solutes is markedly retarded. In some specialized capillaries in the gut and kidney (renal glomerulus) exchange of solutes is facilitated by *fenestrations* - areas within the endothelial cells where little or no cytoplasm separates the plasma membranes on the two surfaces of the cell. In most vascular beds adjacent endothelial cells are attached to each other at their margins though the attachment offers relatively little resistance to solute exchange. In addition, a continuous *basement membrane* encircles the periluminal surface of all capillaries providing additional support to the endothelial cells. It is believed that with the exception of fenestrated capillaries, water and polar solutes do not pass through the endothelial cells but mainly diffuse through the gaps between them. In contrast, lipid-soluble substances including O_2 and CO_2 diffuse directly across the plasma membranes of endothelial cells.

Passive Movement Across Plasma Membranes

Cell membranes are thought to be composed of a mosaic of globular proteins embedded in a lipid matrix. The lipids are arranged as a bilayer with their hydrophilic ends orientated towards the outside of the membrane.

Generally, though there are many exceptions, most plasma membranes are more permeable to water than to solutes, and among solutes, far more permeable to gases, organic compounds and small anions than to cations.

Three possible mechanisms of permeation may be mentioned:

1. Permeation through water-filled pores in the membrane, i.e., water, urea, ions.

2. Permeation by dissolving and diffusing in the membrane lipid, i.e., O_2, CO_2, steroid hormones.

3. Permeation by temporary combination with a membrane component (carrier-mediated), i.e., glucose.

Carrier-mediated transport shows (a) specificity, (b) saturation kinetics, (c) competition between similar molecular species, (d) inhibition and (e) a large temperature coefficient. These are like properties of reactions catalysed by enzymes. Carrier-mediated downhill

transport is passive, in the same direction as diffusion, only faster for a given species in a given time, and so is often called *facilitated diffusion.*

Endocytosis and Exocytosis

This energy-dependent process differs from other modes of transport in that substances enter the cell by inclusion within vacuoles or vesicles. Such membrane-bound structures arise by invagination of the plasma membrane. Sensory and sympathetic neurones depend for their survival and development on the protein nerve growth factor, secreted by their target tissues and taken up by endocytosis at their axon terminals. This uptake is a high-affinity mechanism involving specific membrane receptors and is known as *adsorptive endocytosis.* There also exists a low-affinity non-specific mechanism of endocytosis at nerve terminals that permits uptake of exogenous proteins, i.e., horseradish peroxidase.

Pinocytosis and phagocytosis are forms of endocytosis. *Pinocytosis* ('cell drinking') refers to the endocytic uptake of soluble materials into cells, whereas *phagocytosis* ('cell eating') refers to the engulfing of particulate matter, i.e., bacteria and viruses, by neutrophils.

Exocytosis is the reverse of endocytosis. The release of peptide hormones from endocrine glands, of enzyme precursors from exocrine glands in the gut, and of neurotransmitters from nerve terminals. These processes appear to involve contractile proteins associated with the plasma membrane and are triggered by an influx of calcium ions in response to specific stimuli.

1.5 Active Transport Across Plasma Membranes

Cells accumulate potassium ions and keep the intracellular concentration of sodium ions low although they live in an ECF which is rich in sodium ions and poor in potassium ions. The membranes are permeable to both ions, and tracers show continuous exchange of both ions between ICF and ECF. To maintain their composition in a steady state cells need energy from metabolism to expel sodium ions that diffuses in and to take up potassium ions that diffuses out. Poorly metabolizing cells gain sodium and lose potassium down their concentration gradients. If the cells recover they take up potassium and expel sodium, both *against gradients.* This is '*uphill*' transport and it is known as *active transport.*

Criteria for Active Transport

These are:

(a) it is coupled directly to a continuous supply of energy,

(b) it is independent of the downhill movement of any other solute or of water, and

(c) it is uphill, which needs to be defined more precisely.

For uncharged molecules, uphill is *from lower to higher concentration,* i.e., against a chemical concentration gradient. For ions, uphill is *from lower to higher electrochemical*

potential since there is an electrical potential difference across the cell membranes as well as a chemical concentration gradient.

Nernst Equation

At equilibrium there is no electrochemical potential gradient across the membrane and delta mi = 0. Therefore the electrical potential difference (delta ksi) across the membrane at equilibrium, i.e., the equilibrium potential (E), is given by

$$\mathbf{E = (RT/zF) \ln (c1/c2).}$$

This is the *Nernst equation*. In effect it states either the maximum electromotive force that can be generated by a given ration of concentrations of an ion, or the maximum ratio of concentration that can be sustained by a potential difference imposed from an external source. For use it is often convenient to put in actual values for the constants and to convert the natural logarithms to log of 10. This gives for monovalent ions at 37 °C:

$$\mathbf{E = \pm \log (c1/c2)}$$

where E has units of mV and the sign is positive for cations and negative for anions.

Mechanism of Active Transport

Active transport shows the characteristics of carrier-mediate transport, as does facilitated diffusion. Whereas facilitated diffusion is downhill and passive, active transport is uphill and active. The mechanism of active transport involves carriers which probably have their affinity altered by reacting with adenosine triphosphate (ATP). The precise nature of these carriers and the mechanisms at the molecular level by which ATP reacts with them remain unclear. As well as the Na-K pump there is evidence for plasma membrane Ca++ and H+ pumps which involve a Ca-ATPase and a H-K-ATPase respectively.

An example of active transport is provided by the Na-K pump which moves sodium out and potassium in as ATP is split. It involves an ATPase, an enzyme in the membrane that is activated by sodium in the cell and potassium outside the cell. Because it is activated by both ions, the enzyme is often called the Na-K-activated ATPase, or simply Na-K-ATPase. It is inhibited by *cardiac glycosides*, of which *ouabain* is much used in experimental studies.

Co- and Counter-Transport

Inasmuch as the Na-K pump maintains the low cellular Na+ concentration and the membrane potential is negative on the inside, there is a favourable electrochemical potential gradient for Na+ entry to the cells. The energy in this gradient can be used to drive the net movements of other solvents against their electrochemical potential gradient by coupling their movement across the membrane to that of the passive downhill movement of Na+. If both the Na+ and the other solute move in the same direction this is termed *co-transport.* Examples include the coupled entry of glucose with Na+ and Cl- with Na+ into small intestinal epithelial cells from the gut lumen, the movement of glucose and Cl- being against their own

electrochemical potential gradients. If the Na+ and the other solute move in opposite directions across the membrane this is termed *counter-transport.* An example is provided by the coupling of Ca++ extrusion from cells with Na+ entry to cells. Co- and counter-transport are sometimes referred to as *secondary active transport* or as symport and antiport respectively. Note that metabolic energy is not coupled *directly* to these movements. Instead, some of the energy inherent in the Na+ gradient across the plasma membrane generated by the Na-K pump is dissipated by the coupled flow of Na+ with accompanying solute across the membrane.

Importance of Active Transport

1. In *all cells* active transport is important for maintaining normal ionic composition, in particular of K= and Ca++, which are essential for the regulation of many intracellular activities.

2. Active transport is important for moving ions, water and other substances that accompany ions by sharing common carriers (i.e., co- and counter-transport) across plasma membranes, particularly in kidney, stomach and intestine.

3. Active transport maintains the gradients of ionic concentration which are the basis of *resting potentials* and *action potentials* used for signalling in nerves and for the activation of muscles.

4. The active extrusion of Na+ is important for regulating cellular volume.

1.6 Resting Membrane Potential

A difference of electrical potential is found across plasma membranes between ICF and ECF. This difference, called the *resting membrane potential (RMP),* has a magnitude of up to -90mV with the internal electrode being negative with respect to that in the bathing fluid.

The RMP is considered to be predominantly a *diffusion potential* arising from the ionic concentration gradients across the membrane which has a selective permeability for ions. Generally plasma membranes are much more permeable to K+ than to Na+. Hence K+ ions tend to diffuse out down their concentration gradient faster than Na+ ions diffuse in. K+ ions carry positive charge out with them and leave the inside of the fibre negatively charged. If a simple membrane were permeable only to K+ this would go on until a sufficient potential difference was built up for the rate of re-entry due to the electrical gradient to balance the rate of loss down the concentration gradient. There would then be a dynamic equilibrium. The potential difference would be the *equilibrium potential for K+ (E_K),* with the magnitude given by the Nernst equation. The equilibrium in mV for monovalent ions at 37 °C is given by

$$\mathbf{E\ (inside) = \pm\ log\ (C_{outside}/C_{inside})}$$

where the sign is positive for cations and negative for anions. Therefore E_K (inside) is 61 log 5/150 = 61 x -1.8 = - 90 mV. However, this is somewhat larger than the measured

RMP of most cells, namely - 70 mV to -80 mV. The reason for this is that the plasma membrane is not totally impermeable to Na+, and Na+ ions diffusing in down their gradient carry some positive chargee into the cell and thus reduce the actual RMP below E_K.

The contribution of the diffusion of other ions to the RMP is expressed in the Goldman equation:

$$\textbf{delta ksi} = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o}$$

where delta ksi is the RMP, P is the membrane permeability to the ions denoted by its subscript, o indicates the outside concentration and i the inside concentration. Note that because Cl- is an anion the Cl- concentrations are reversed.

A simpler version of the above equation, which assumes no net Cl- diffusion, is the Hodgkin-Katz equation:

$$\textbf{delta ksi} = \frac{RT}{F} \ln \frac{K_o + bNa_o}{K_i + bNa_i}$$

where b is the ratio P_{Na}/P_K and is taken to be 0.01 for the resting axon. The equation predicts delta ksi = 61 log (5+1.5)/(150 + 0.1) = 61 X
-1.36 = -83 mV. Good agreement of this predicted value with the measured RMP supports the idea that the RMP is largely a *potassium diffusion potential* modified by the membrane's small permeability to Na+ ions. This is confirmed by the fact that the measured RMP in isolated fibres varies as the Hodgkin-Katz equation predicts if internal or external K+ concentration is changed experimentally. Note that if we calculated E_{Cl} using the Nernst equation it is -82 mV, which is not significantly different from the measured RMP. Since Cl- ions are apparently in electrochemical equilibrium, the omission of Cl- in the Hodgkin-Katz equation is justified.

Rather than writing these equations in terms of ionic permeabilities it is often convenient to express the membrane potential in terms of conductances (G) because the electrical measurements required are easier to perform experimentally. The membrane conductance (1/ohmcm^2) to an anion differs from the membrane permeability (1/cms) but, as an approximation, it is often used to represent ionic permeability. It can be shown that if the total current across the membrane is zero as it must be in the steady state, the membrane potential is given by

$$\textbf{delta ksi} = \frac{E_K G_K + E_{Na} G_{Na} + E_{Cl} G_{Cl}}{G_K + G_{Na} + G_{Cl}}$$

where E represents the equilibrium potential and G the conductance of the membrane to ions indicated by the subscripts.

A pump which moved one K+ in for each Na+ out would carry no net charge and, therefore, no current across the membrane. However, it is now realized that the pump is probably not electrically neutral but transfers three Na+ out for every two K+ carried in. Thus current is generated by the pump which is therefore termed *rheogenic* (a less satisfactory term is electrogenic). However, provided that the membrane is much more permeable to K+ than to Na+, this rheogenic ion transport contributes relatively little to the resting membrane

potential which is dominated by potassium diffusion potential. To take account of this rheogenic transport, the Hodgkin-Katz equation can be modified by introducing the term n, where n is the coupling ratio for the pump (i.e., number of K+ transported to Na+ transported in each pump cycle):

$$\text{delta ksi} = RT/F \ln (K_o + nbNa_o) / (K_i + nbNa_i)$$

1.7 Cell Volume Regulation

Cell volume reflects cell water content. Since plasma membranes are relatively permeable to water, water will be distributed between cells and interstitial fluid to maintain constant the *activity* of water in each compartment. Note that water activity in a solution is decreased as the total concentration of solute particles is increased and vice versa. A difference in water activity between compartments will result in a net movement of water across the membrane down its activity gradient - a process termed *osmosis.*

Osmosis

An *ideal semi-permeable membrane* prevents diffusion of solutes but not solvent. Net movement of water across such a membrane (osmosis) will occur from the more dilute to the more concentrated solution. It can be stopped by applying hydrostatic pressure to the more concentrated solution to raise its water activity. The hydrostatic pressure which just stops osmosis is the *osmotic pressure.* In other words it is the pressure required to equalize water activity in the two compartments. A greater hydrostatic pressure will cause water to leave the more concentrated solution and this is called *ultrafiltration.*

The osmotic pressure (delta pi) across an ideal semi-permeable membrane is calculated by the *van't Hoff equation:*

$$\text{delta pi} = RT \text{ sigma}(c_1 - c_2)$$

where R is the gas constant, T is the absolute temperature (K) and sigma(c_1-c_2) is the sum of the difference in molal concentrations (mol/kg water) of all osmotically active solutes on sides 1 and 2 of the membrane. Traditionally, delta pi has been expressed in atmospheres, in which case R = 0.082 kg atm/K mol. This equation predicts than an ideal 1 molal solution of a non-electrolyte should have an osmotic pressure of 22.4 atm at 0 °C. Osmotic pressure of actual solutions usually deviate from those expected from their molal concentrations because solute molecules interact with each other and with the solvent. Therefore, strictly speaking, in this equation c refers to the *osmotic activities* of the solutes rather than to their concentrations but in dilute solutions there are approximately the same. Also in dilute aqueous solutions molal concentrations (mol/kg water) closely approximate molar concentrations (mol/L water).

The osmolality (osmol/kg water) of a solution can be estimated from its osmotic pressure in atmospheres divided by RT. An electrolyte like NaCl is dissociated in solution into two osmotically active ions, so its osmolal concentration will be approximately twice the molal concentration. The osmolality of a solution can be measured in a commercial

osmometer which relies on the fact that the osmotic pressure is related to the depression of the freezing point. Mammalian fluids have an osmolality of about 0.3 osmol/kg water and thus exert an osmotic pressure across an ideal semi-permeable membrane of about RT x 0.3 = 7.6 atm or 5800 mmHg or 770 kPa at body temperature.

Effective Osmotic Pressure

Biological membranes are not ideal but in varying degree 'leaky'. The osmotic pressure difference across such a membrane (delta pi) is less than the ideal and diminishes with time as some solutes diffuse through and eliminate their difference in concentration. The *effective osmotic pressure* will therefore be less than the pressure calculated above and will depend on the degree to which these solutes cross the membrane. This is expressed by including the *reflexion coefficient, sigma,* in the van't Hoff equation:

$$\textbf{delta pi = sigmaRTSigma}(c_1 - c_2).$$

If none of the solute molecules colliding with the membrane cross it, sigma = 1, i.e., all molecules are reflected. If all the solute molecules pass through the membrane sigma = 0, none are reflected and there is no osmotic pressure. Thus the effective pressure of the cell fluid, often called its *colloid osmotic pressure,* is due to the large, reflected, non-penetrating solutes (i.e., proteins, organic phosphates). Some of these large solutes are negatively charged and attract small diffusible cations and repel small diffusible anions across the plasma membrane. This leads to an uneven distribution of small ions with a small excess inside the cell called the *Donnan excess,* which also contributes to the effective osmotic pressure of the cell fluid.

Net Flow of Water Across Membranes

The net flow of water across a membrane per unit time (J_v) is given by:

$$J_v = AL_p(\text{delta P - delta pi})$$

where A is the area of the membrane available for flow; L_p is the hydraulic conductivity which is a measure of the ease with which water flows through the membrane; (delta P - delta pi) is the driving force which is dependent on the differences in hydrostatic pressure (delta P) and effective osmotic pressure (delta pi) across the membrane.

Cell Volume

In the steady state cell volume remains constant and $J_v = 0$. This implies that delta P and delta pi are both zero or that the effective (colloid) osmotic pressure of the cell is offset by a greater hydrostatic pressure within the cell, i.e., delta pi = - delta P. The latter is not believed to be true and the determinants of cell volume are considered to be:

(a) the total number of osmotically active particles within the cell, and

(b) the effective osmolality of the interstitial fluid.

Although there is virtually no protein in the interstitial fluid and the cell has a considerable excess of colloid osmotic pressure, the cell does not swell. It is believed that the Na-K pump, by holding Na+ extracellularly and preventing its accumulation in the cell, effectively offsets the colloid osmotic swelling force of cell macromolecules. If the Na+ pump ceases to expel Na+, for example when cells are chilled, Na+ enters passively down its concentration gradient bringing Cl- with it to preserve electroneutrality; water then distributes to equalize water activities across the membrane and the cells swell.

Tonicity

The behaviour of cells in artificial bathing solutions cannot be predicted from the osmotic pressure or osmolality of these solutions as measured with an ideal semi-permeable membrane or by the depression of the freezing point. It depends also on the permeability of the membrane to the solutes. For example, when cells are placed in 300 mmol/L urea, which is approximately isosmotic with mammalian fluids, the cells will swell because urea enters and water follows. A new term is required to define the strength of a solution as it affects the volume of cells. This is *tonicity* and it can be defined operationally. Thus,

(a) if cells *shrink* in a solution, the solution is *hypertonic*,

(b) if cells *swell* in a solution, the solution is *hypotonic*, and

(c) if the cell volume is *unchanged* the solution is *isotonic*.

Tonicity may also depend upon the functional activity and metabolism of the cells. Most cells swell grossly if their metabolism is inhibited, even in isosmotic NaCl solutions, because the cells gain NaCl. Consequently, isolated tissues need substrates and oxygen as well as the appropriate inorganic ions in bathing solutions designed to be isosmolal with normal ECF.

1.8 Osmosis and Ultrafiltration Across Capillary Walls

The structure of the capillary wall allows free permeability to water and solutes of MW less than 70000. Small molecules diffuse through the capillary wall and therefore should achieve equal concentrations on both sides and make no contribution to the effective osmotic pressure. Plasma proteins with MW greater than 70000 cannot cross the capillary wall (i.e., proteins with sigma = 1 are reflected) and thus cause an effective osmotic pressure. Since plasma proteins are large enough to be classed as colloids, this effective osmotic pressure is known as thee *plasma colloid osmotic pressure.*

Plasma Colloid Osmotic Pressure

Plasma proteins amount to approximately 1 mmol/L of plasma and, as a simplification, there are almost no proteins in the interstitial fluid. This concentration difference for protein would cause an effective osmotic pressure for plasma of 16 mmHg (calculated using the van't Hoff equation). However, proteins are negatively charged and therefore attract diffusible cations and repel diffusible anions. This leads to an uneven distribution of small ions with a small excess (about 0.5 mmol/L) inside the capillary. This uneven distribution is referred to

as the *Gibbs-Donnan membrane distribution* and the small excess of diffusible ions in the plasma is known as the *Donnan excess.* This excess contributes a further 9 mmHg to the effective osmotic pressure of plasma. Hence the total plasma colloid osmotic pressure is 25 mmHg, first measured by E. H. Starling (1866-1927). This pressure is very small compared with the total osmotic pressure of all the solutes in plasma (5800 mmHg) measured when plasma is separated from pure water by an ideal semi-permeable membrane.

Starling Equilibrium

Starling realized that the plasma colloid osmotic pressure of 25 mmHg is very important because its value lies between that of the blood pressure in arterioles and venules. The blood pressure (hydrostatic pressure) at the arteriolar end of a capillary is about 32 mmHg and at the venular end about 15 mmHg. Hydrostatic pressure in the interstitial fluid varies from place to place but on average it is a few mmHg below atmospheric, say about -2 mmHg. The net hydrostatic pressure between blood and interstitial fluid forces fluid out of the capillaries by *ultrafiltration* and this force is greater at arteriolar than venular ends. This ultrafiltration is opposed by *osmosis* returning fluid into the capillaries, the osmotic force being the difference in colloid osmotic pressure between plasma and interstitial fluid. Note that this osmotic force remains constant along the length of the capillary. At the arteriolar end, the net balance of hydrostatic and osmotic forces causes ultrafiltration with fluid leaving the capillary. At the venular end, the net balance causes reabsorption with fluid returning to the capillary. This balancing of ultrafiltration and osmosis is referred to as the *Starling equilibrium.* The balance is not complete as there is slightly more ultrafiltration than osmosis. The Starling equilibrium provides an automatic control of circulating blood volume because alterations in capillary blood pressure change the rate of ultrafiltration leading to a redistribution of fluid between blood and interstitial fluid.

In some capillary beds, blood perfusion is intermittent and ultrafiltration when blood pressure is high is balanced by osmosis when blood pressure is low. In particular organs, special relations exist in capillary beds. For example, in the glomeruli of the kidneys blood pressure is high and there is only ultrafiltration. In the alveolar capillaries in the lungs, blood pressure is below the colloid osmotic pressure, hence there is only osmosis and liquid does not accumulate in the alveoli. In the liver, the interstitial fluid outside the capillaries is rich in protein and a very small capillary hydrostatic pressure is balanced by an equally small difference in colloid osmotic pressure.

Net flow of fluid per unit time (J_v) across the capillary walls of an organ will not only be altered by changes in hydrostatic pressures and colloid osmotic pressures but, since J_v = A L_p(delta P - delta pi), it will be affected by the number of capillary channels that are open and the hydraulic conductivity of the capillary walls. For example, during increases in metabolism more capillary channels are open and J_v will increase because of the increased area available for flow across the capillary walls.

Lymph

In fact, some protein escapes from most capillaries. If it accumulated in the interstitial fluid it would impede the return of fluid by osmosis. This problem is solved by the *lymphatic*

system which carries away this protein and any excess fluid resulting from an imbalance between ultrafiltration and osmosis. The lymphatic system consists of a network of blind-ended lymph capillaries lying in the interstitial fluid close to blood capillaries. The walls of lymph and blood capillaries are similar except that the gaps between endothelial cells in lymph capillaries are larger making them readily permeable to protein and fluid. Lymph capillaries collect into thin-walled lymph veins which eventually empty through two main vessels into the subclavian veins of the circulatory system. The lymphatic system carries *lymph* which is composed of cells (lymphocytes) and fluid (plasma). Lymph nodes along the course of the larger lymph vessels produce the lymphocytes and the interstitial fluid that enters lymph capillaries provides the plasma.

Hence the Starling equilibrium should be restated as:

Ultrafiltration (20 L/day) = Osmosis (16-18 L/day) + Lymph flow (4-2 L/day).

Since thee cardiac output amounts to 8000 L/day for adult man, or say 4000 L/day of blood plasma, the 20 L of fluid passing through blood capillary walls by ultrafiltration is less than 0.5 % of blood plasma volume flowing per day. *Hence the bulk movements of fluid through the blood capillary wall are of little importance for the exchange of nutrients, O_2 and CO_2, which move predominantly by diffusion.*

1.9 Movement of Water and Solutes Across Epithelia

Epithelia separate the internal from the external environment and regulate movement of solutes and water to and from the body. The epithelial cells, which may be one or more layers thick, are separated by their basement membranes from a variable amount of supporting connective tissue containing blood vessels, nerves and smooth muscle fibres. It is believed that the transport functions reside only in the epithelial cell layer.

All epithelial cells are separated from their neighbours by a space - the *lateral intercellular space* - whose size may vary. They are held to each other at their luminal edges by junctions - *'tight' junctions.* Tight junctions were originally thought to be impermeable, but it is now appreciated that to a variable extent solutes and water may diffuse across them. Substances passing between cells must cross tight junctions and pass through the lateral intercellular spaces - so-called paracellular or *shunt pathway.* The amount passing through this shunt pathway differs between epithelia, depending on the difference in permeability characteristics of the tight junctions. Epithelia in which the shunt pathway makes a significant contribution to total movements (i.e., proximal renal tubule, small intestine) are referred to as 'leaky' whereas those in which the cellular pathway predominates are referred to as 'tight' (i.e., renal collecting duct, salivary gland ducts, amphibian skin and urinary bladder). 'Tight' epithelia can generate and maintain, often under hormonal control, step salt gradients between lumen and interstitial fluid and thus cause some dissociation between ion and water movements. In contrast, 'leaky' epithelia cannot generate salt gradients and allow passage of isosmotic fluid.

The net movement of any solute across an epithelium is determined by:

1. The available surface area (since amount moved/unit time = area x flux).

2. The time in contact with the available area.

3. The electrochemical potential gradient, which will be influenced by blood and lymph flow.

4. The properties of the epithelium, i.e., 'tight' or 'leaky', together with the availability of specific transport mechanism.

'Tight' Epithelia

In general, epithelia which actively transport ions primarily move Na+. This transepithelial Na+ transport generates a potential difference across the epithelial layer. The transepithelial potential difference in 'tight' epithelia is high (> 20 mV) whereas in 'leaky' epithelia it is low (0 to 5 mV). Since electrical neutrality must be preserved, Cl- follows passively, as does water when water permeability of epithelia is sufficient. The detailed mechanisms by which Na+ crosses epithelia remain poorly understood but are summarized for a typical 'tight' epithelium. Unlike muscle, nerve and blood cells whose plasma membranes are symmetric, the plasma membranes of epithelial cells are asymmetric. The apical portion, which faces the lumen, has unusual permeability and transport characteristics. It has a high Na+ and low K+ permeability and no Na-K-ATPase. In contrast the basolateral portion consisting of the membrane beneath the 'tight' junctions and adjacent to the capillaries, is similar in its permeability and transport properties to other plasma membranes. In general, in 'tight' epithelia only Na+ readily crosses the apical membrane, probably by a passive carrier-mediated process.

'Leaky' Epithelia

In 'leaky epithelia', not only does Na+ cross the apical membrane but entry of Cl-, glucose and some amino acids may be coupled to Na+ entry. The electrochemical potential gradient for Na+ entry is used to drive the accompanying solute into the cells from the lumen (i.e., co-transport). The active transport of Na+ from cell to interstitial fluid is thought to involve the same Na-K pump as already described. The coupling of net water movement to net solute movement across 'leaky' epithelia may involve the generation within the lateral intercellular spaces of a local region of Na+ concentration, somewhat higher than that in interstitial fluid, reflecting the distribution and activity of the Na-K pump. This provides an osmotic gradient moving water, either through the cells or tight junctions, from the lumen to the lateral intercellular space. This movement in turn creates a local hydrostatic pressure gradient so that fluid flows from the lateral intercellular spaces to the interstitium. Note however that the magnitude of any local osmotic gradient in the lateral intercellular spaces is now thought to be only a few mmol/kg water.

1.10 Appendix: Gibbs-Donnan Distribution

Consider two compartments containing aqueous solutions of fixed volume separated by a membrane permeable to water and small MW solutes but impermeable to protein. Suppose that the solution in side 1 contains protein (Pr) with a net negative charge (z-) and that both solutions contain Na+ and Cl- ions. Because of the negative charge on the protein, diffusible cations, i.e., Na+, will be attracted to side 1 and diffusible anions, i.e., Cl-, repelled to side 2. As a consequence of the redistribution of ions, side 1 containing the protein will be slightly electrically negative with reference to side 2, that is, an electrical potential difference (delta ksi) will exist across the membrane. At equilibrium it will be equal to the equilibrium potential (E) for each ion which is given by the Nernst equation:

$$\mathbf{E = RT/F\ ln(Na^+_1/Na^+_2) = RT/F\ ln(Cl^-_2/Cl^-_1).}$$

Therefore:

$$\mathbf{(Na^+_1/Na^+_2) / (Cl^-_2/Cl^-_1) = r}$$

where r is the 'Donnan ratio', i.e.:

$$\mathbf{Na^+_1Cl^-_1 = Na^+_2Cl^-_2.}$$

The condition of bulk electroneutrality demands that the sum of all positive charges shall equal the sum of all negative charges in any solution, i.e.:

$$\mathbf{Na^+_1 = Cl^-_1 + Pr^{--}_1}$$

and

$$\mathbf{Na^+_2 = Cl^-_2.}$$

Therefore

$$\mathbf{Na^+_1Cl^-_1 = (Na^+_2)^2 = (Cl^-_2)^2.}$$

Now $Na^+_1Cl^-_1$ is not a square and since he sum of the sides of a rectangle is greater than the sum of the sides of a square of equal area,

$$\mathbf{Na^+_1 + Cl^-_1 > Na^+_2 + Cl^-_2}$$

or

$$\mathbf{Na^+_1 + Cl^-_1 = Na^+_2 + Cl^-_2 + e}$$

where e is called the 'Donnan excess'. This excess concentration of diffusible ions, e, contributes to the difference in effective osmotic pressure between the two compartments so that

$$\textbf{delta pi} = \textbf{RT}(\textbf{Pr}^{2-} + \textbf{e}).$$

Note that the system can only reach a steady state when the volumes of the two compartments are fixed, for the delta pi is offset by an increased hydrostatic pressure in compartment 1, i.e., delta pi = - delta P. Without this restriction an osmotic flow of water and a diffusion of permanent solutes from compartment 2 to 1 will continue until all the solution is found in compartment 1.

Note too that the resting membrane potential of cells is *not* a consequence of the Gibbs-Donnan distribution as the intracellular and extracellular concentrations of ions do not comply with the Donnan ratio. In the case of the capillary wall where a Gibbs-Donnan distribution does exist, it can be calculated that the potential difference across the wall is only a few millivolts.