Chapter One

Cell Membranes, Epithelial Barriers and Drug Absorption

INTRODUCTION

All living things are made of cells—small individually functional units which, in higher organisms, are organised into collections called *tissues*. A typical cell consists largely of *cytoplasm,* an aqueous liquid in which a wide range of biochemical processes occur. The cytoplasm is held as an intact unit by a *cell membrane,* which surrounds it and prevents it from mixing with its surroundings. Depending on the cell type and function, a number of other structures may be present, particularly a *nucleus,* in which the cell genetic information is stored in the form of DNA. Provision must be made for the supply and retention of substrates from extracellular sources and for the secretion of waste products that would otherwise accumulate in toxic amounts. The outer membrane of the cell must therefore allow penetration of some substances and not others, i.e. it must be selectively permeable. This is one of the most important features of the cell membrane.

Organs and tissues are collections of cells surrounded by specialised cell structures called *epithelia,* which can be thought of as the organ's 'outer membrane' in an analogous fashion to the membrane that surrounds the individual cell. Like cell membranes, they not only bound the organ, but also are the site for a wide range of transport, barrier and secretory processes which vary widely with the particular organ. Many epithelia protect organs from hostile environments (for example the skin or the contents of the stomach) and such cells generally have a rapid turnover and numerous barrier features.

In order for a drug to reach a site of action it must pass from an 'external' site (for example the surface of the skin or the small intestine) to an 'internal' site (the bloodstream or the cytoplasm of a particular cell group). In doing so it will have to pass through a number of tissues and epithelia, either by going through the cells themselves (and thus penetrating their plasma membranes) or by finding pathways between the cells. Overcoming these barriers to absorption is one of the most important considerations in the drug delivery process, and requires a detailed knowledge of the structure and behaviour of the cell membranes and epithelial tissues.

THE PLASMA MEMBRANE

The plasma membrane retains the contents of the cell and acts as a permeability barrier. That is, it allows only certain substances to enter or leave the cell, and the rate of entry is strictly controlled. Early researchers recognised that hydrophobic materials entered cells easily and proposed that an oily or 'lipoidal' layer was present at the cell surface. Gorter and Grendel in 1925 estimated the thickness of this layer by extracting the oily membrane from erythrocytes with acetone and spreading it as a monomolecular film in a Langmuir trough[1]. By measuring the film area and calculating the surface area of the original red cells (chosen since their geometry is reasonably constant), they concluded that exactly two layers of molecules were present at the interface, and proposed a lipid bilayer as the major cell membrane element. We now know that their experiment was subject to a considerable number of errors[2], but fortunately these cancelled out in the final analysis and hence they obtained the correct answer by the wrong route. Electron micrographs indicate a double layered lipid membrane with bands approximately 3 nm in width and an overall thickness of between 8 to 12 nm. Although this is consistent with the lipid bilayer view, electron micrographic evidence was held in doubt for many years due to the difficulty of preparing the samples and the possibility of artefacts at so small a scale.

Subsequent discovery of the incorporation of proteins and polysaccharides led to the fluid mosaic model of Singer and Nicholson[3]. This model tended to suggest that the membrane was a sea of tightly packed phospholipids interspersed with proteins, leading to a rather ill-defined mixed membrane. However, studies during the last decade have demonstrated that the membrane is a highly organised structure; proteins in specific

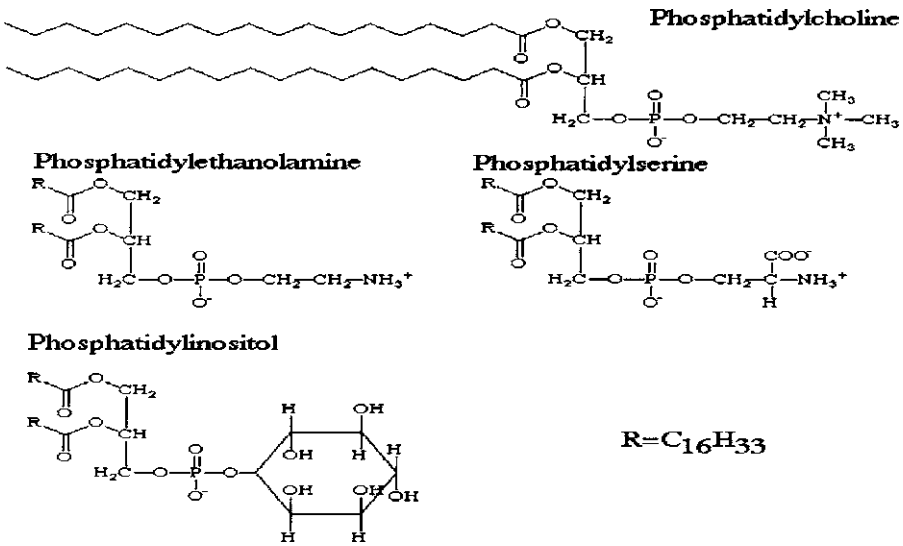

Figure 1.1 Common membrane phospholipids

conformations act as structural elements, transport nutrients, and sample the cell environment. The bilayer is not a lipid 'sea' but a carefully designed liquid crystal whose composition is controlled by the cell to achieve a specific degree of fluidity and an optimum environment for the processes which occur within it.

The phospholipid bilayer

The detailed chemistry of the cell membrane was not worked out for many years due to the very large number of components that occur in membranes from varying organs. The development of chromatography was pivotal in allowing the lipid mixtures to be separated into their numerous components for detailed analysis. We now know that the main 'scaffolding' of the bilayer consists of a range of surfactant molecules, of which *phospholipids* are the most important. Most membranes also contain other materials, most notably proteins and sterols, but the surfactant lipids themselves are sufficient to form the lipid bilayer.

Phospholipids are compounds of glycerol (propane-1, 2, 3-triol) in which two of the alcohol groups are joined to fatty acids, and the third to phosphoric acid (Figure 1.1). The phosphate group can additionally form a bond to a smaller organic molecule (generally a hydrophilic one). The resultant molecule thus has two oily tails, usually of 12–24 carbon atoms length, and a hydrophilic region around the charged phosphate ester, called the *headgroup*. Common headgroup molecules are choline, ethanolamine, serine and inositol, and the resulting phospholipids are termed phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol respectively. Due to the cumbersome names these are normally abbreviated to PC, PE, PS and PI. These molecules have a typical surfactant structure. In water surfactants usually aggregate to form *micelles,* small clusters in which the oily tails are turned towards a common centre, since it is energetically unfavourable for the oily tails to be surrounded by water molecules. However, in phospholipids and other membrane-forming surfactants, the molecules aggregate to a bilayer sheet in which the tails are in the centre of the bilayer and the polar headgroups are in contact with the external aqueous environment (Figure 1.2). Phospholipids are not the only surfactants that behave in this way; the distinction between whether a surfactant forms a closed micelle or a bilayer sheet depends purely on the

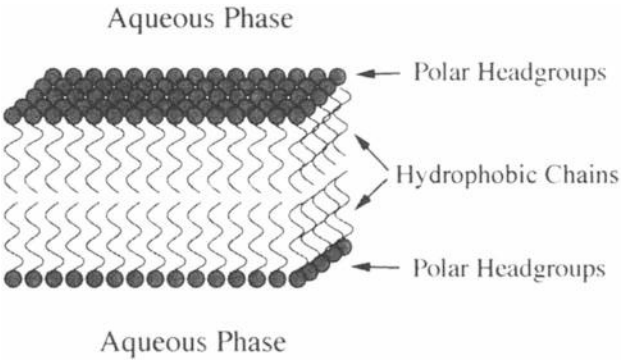

Figure 1.2 The phospholipid bilayer

geometry of the molecule. Phospholipids form bilayers spontaneously when dispersed in water, as this is their thermodynamically most stable configuration, that is, it has the lowest free energy. However the bilayers do not form infinite planar sheets, but generally close in on themselves to form spherical structures in which one layer is on the outside of the sphere, and the other on the inside, enclosing an aqueous space. Simply shaking phospholipids in water results in the formation of these microscopic structures, this are termed *liposomes*.

Dynamic behaviour of membranes

Although membranes are often depicted as regular structures, as in Figure 1.2, the reality is that the bilayer is much more disordered and dynamic. The most important dynamic processes are *lateral diffusion,* in which the lipid molecules can move in the plane of the bilayer, and *transverse diffusion* or *flip-flop,* where a lipid molecule switches from one side of the membrane to the other. Since this involves moving the headgroup through the oily core of the bilayer, this is an extremely slow process, and in natural systems is generally catalysed when required by specialised membrane proteins.

The most important factor in determining the dynamic behaviour of the membrane is the *transition temperature* of the bilayer. At low temperatures the lipid tails are held in a relatively ordered array in the bilayer core. As the temperature is raised, little movement takes place until the transition temperature is reached. At this point the lipid spacing increases slightly and the tails become much more disordered in arrangement. The transition is often thought of as a gel-liquid melting of the bilayer, and in the fluid state the lipid molecules are relatively mobile to lateral diffusion; they diffuse at a speed of several microns a second and can move around a typical cell membrane on a timescale of seconds. The transition temperature depends mainly on the structure of the fatty acid chains attached to the glycerol backbone, with unsaturated chains causing low transition temperatures (generally below 0°C) and saturated chains having higher transition temperatures. For example, when the fatty chains in PC are both formed from palmitic acid (C16 saturated fatty acid) the lipid bilayer melts at 42°C. It is thus evident that the cell can control the fluidity of its membrane by varying the fatty acid composition of the phospholipids.

Modulation of membrane fluidity by sterols

Although cell membrane fluidity can be regulated by altering the phospholipid fatty acid content, this is not the organism's only means of control. Most cell membranes contain varying amounts of sterols. In plants the primary membrane sterol is sitosterol; in animals, cholesterol, and in fungi ergosterol, although many other similar compounds also occur. Sterols alter the fluidity of the cell membrane by 'broadening' the melting transition so that the membrane melts over a much wider temperature range than that observed for the lipid alone. This is illustrated in Figure 1.3, which is a thermogram for the melting of a typical lipid membrane, in this case dipalmitoyl phosphatidylcholine. A thermogram is a plot of the energy absorbed as the temperature of the system is raised; the peak is caused by the absorption of energy required to melt the lipid bilayer. In the absence of sterols the bilayer melts over a small temperature range, causing a sharp peak in the thermogram. In the presence of cholesterol the melting transition is much broader, and the thermogram peak spans several degrees. The membrane begins to melt at a lower temperature than in the absence of sterol, and retains some structure up to a temperature above the transition temperature of the pure lipid. The effect of this is to 'smear out' the melting of the membrane, so that the fluidity is not so dependent on temperature. Obviously, this is of considerable importance in allowing the cell to function over a range of temperatures.

Models of cell membranes

Cell membranes are extremely complex structures, and it is difficult to untangle all the aspects of their behaviour by studying whole cells. Consequently, most of our understanding of membrane function has arisen from a study of membrane models, systems which display certain aspects of membrane behaviour without the complexity of the whole cell. We have already mentioned liposomes, which are the spherical structures formed when

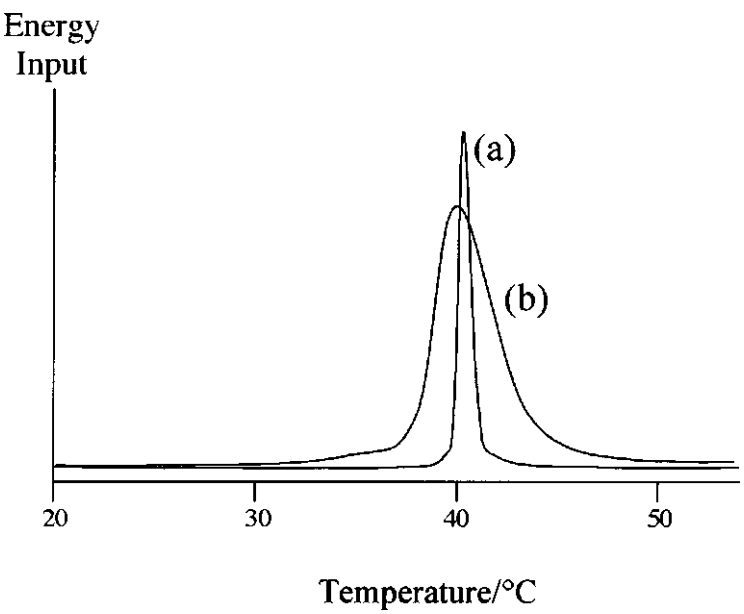

Figure 1.3 Melting transition in DPPC bilayers (a) DPPC (b) DPPC+cholesterol. Note the broadening of the peak due to the inclusion of the sterol.

phospholipids are dispersed in water. Liposomes made in this way are actually multilayered, in a concentric 'onion-like' structure, and unlike cells do not have a large central aqueous space. They are termed large multilamellar vesicles or MLV's. Exposure to shear fields (normally by ultrasound) breaks them into small unilamellar vesicles or SUV's which are, however, rather smaller than single cells. Better membrane models are provided by giant unilamellar vesicles (GUV's) which can be made by careful injection of ethanolic lipid solutions into water.

Although liposomes can be made from well-characterised lipid mixtures, it is often useful to study natural membranes which have a more 'natural' structure, without the complexity added by the cell contents. The most widely used model in this respect is the *erythrocyte ghost,* which is the membrane surrounding a red blood cell from which the contents have been removed. These are prepared by placing the cells in hypertonic saline, which causes pores to form in the membrane. The cell contents then equilibrate with the suspending medium, and since this is normally much larger in volume than the total cell interior, the cells effectively become washed clean. Markers such as dyes or radiolabels can then be added, and will equilibrate with the solution inside the cells. If the tonicity is then adjusted to normal, the cell pores will re-seal and the cells, now labelled in their inner space, can be washed free of unentrapped marker by repeated centrifugation. There have been several attempts to place drugs inside the cells which are then returned to the donor, the idea being that they will then not be recognised as foreign, the outer membrane having the donor's correct antigen profile[4]. This has proven only partly successful, the cell surfaces being easily damaged during the labelling process.

The most serious problem with these membrane models is that it is only possible to access the outside of the membrane, the interior being sealed, unless an invasive technique such as the insertion of a microelectrode is used. This problem can be avoided by the use of *black lipid films,* a technique in which a lipid bilayer is formed across a small hole in the partition of a two-compartment vessel (Figure 1.4). The technique allows access to both sides of the membrane so that electrical measurements can be made, and the composition of the fluid on either side of the membrane can be readily altered. Using this method it is, for example, possible to measure the ion current across the membrane caused by pore-forming antibiotics, and study the operation of ion pump proteins.

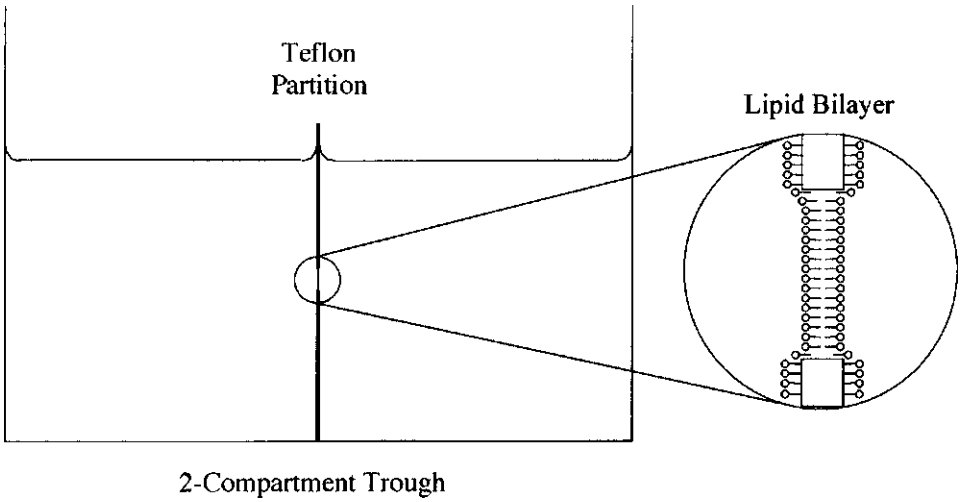

Figure 1.4 The black lipid film between 2 aqueous compartments

Membrane proteins

The cell membrane is home for a number of types of proteins, which are generally divided into integral proteins and peripheral proteins. Integral proteins contain a sequence of hydrophobic groups that are embedded in the lipid bilayer, while peripheral proteins are adsorbed on to the surface of the cell, generally attached to an integral protein. The majority of functional proteins are integral, the most important peripheral proteins being the spectrin and ankyrin proteins. These bind to the inside of the plasma membrane to form the cytoskeleton, a network of proteins which runs throughout the cell, and is involved in a range of structural and transport functions.

One of the most important groups of integral membrane proteins from a pharmacological viewpoint is the transport proteins. These are responsible for moving substances into and out of the cell; for example, ATPase proteins pump ions across the cell membranes to maintain the required Na^+/K^+ electrolyte imbalance, and secrete H^+ from the gastric parietal cells. Proteins also recognise and transport nutrients such as small carbohydrates and amino acids into the cell, each protein transporting a small group of structurally similar compounds.

A second important group of membrane proteins are the cell surface receptors. Although many biochemical receptors are present in the cytosol, a number of important materials are recognised by membrane proteins. These include a range of pituitary hormones, histamine receptors on mast cells, prostaglandins, and gastric peptides in the intestine.

Glycoproteins are a group of integral proteins carrying polysaccharide chains which are responsible for cell recognition and immunological behaviour. The segment of the protein chain, which is external to the cell, consists of hydrophilic protein residues, many of which carry small carbohydrate groups such as sialic acid. In many cells, these hydrophilic oligosaccharides form a continuous coat around the cell, together with polysaccharides attached to lipids (glycolipids). This layer is termed the *glycocalyx*. A common component of this layer is a peripheral protein called fibronectin, which contains binding sites for many membrane proteins, extracellular structural proteins such as collagen, and polysaccharides, and is thus an important component in intercellular binding and tissue formation.

Membrane asymmetry

Although liposomes are normally made with a similar lipid composition on both the inside and outside, living cells are much more asymmetric since they perform a range of processes which are obviously directional. The phospholipid composition of the inside and outside layers of the membrane is different in most cells; for example in the erythrocyte membrane phosphatidylcholine occurs predominantly on the outside of the cell and phosphatidylethanolamine predominantly on the inside. Glycolipids are normally oriented so that the polysaccharide segment is outside the cell, since it is responsible for immunogenicity and tissue adhesion. Integral proteins always have a specific orientation in the membrane that depends on their function; all molecules of the protein point in the same direction.

EPITHELIA

With a few exceptions, all internal and external body surfaces are covered with epithelium. This consists of a layer of structural protein, normally collagen, called the *basal lamina,* on which sit one or more layers of epithelial cells. There are several morphologically distinct common epithelial types (Figure 1.5):

a) Simple squamous epithelium. This forms a thin layer of flattened cells and consequently is relatively permeable. This type of epithelium lines most of the blood vessels.

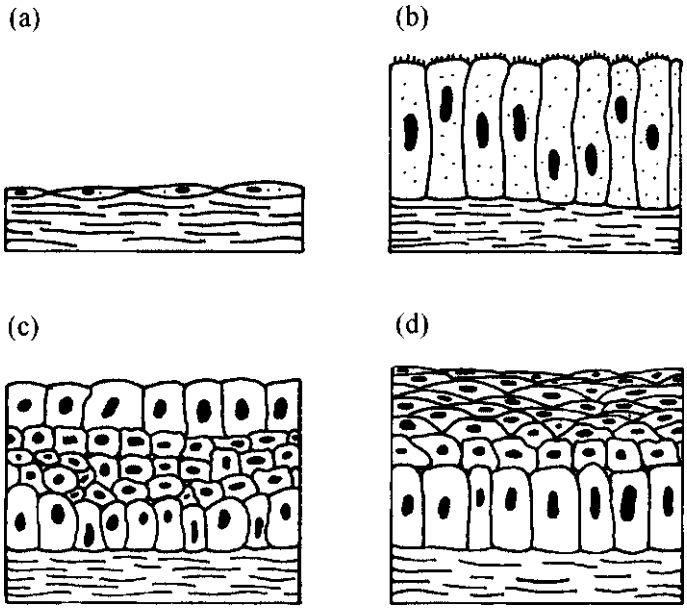

Figure 1.5 Common epithelial membrane types (a) simple squamous (b) simple columnar (c) transitional (d) stratified squamous epithelium

b) Simple columnar epithelium. A single layer of columnar cells is found in the epithelium of organs such as the stomach and small intestine.

c) Transitional epithelium. This is composed of several layers of cells of different shapes and it lines epithelia which are required to stretch.

d) Stratified squamous epithelium. These membranes are several cells thick and are found in areas which have to withstand large amounts of wear and tear, for example the inside of the mouth and oesophagus, and the vagina. In the skin the outer cells become filled with keratin, and then die and slough off from the outside. This type of epithelium is termed *keratinized* and provides a major permeability barrier as well as protection from the environment.

Epithelial cells are said to be polarised due to the asymmetric distribution of transport proteins on the opposite ends of their plasma membranes. This causes the transport activity of the apical membrane of the cell to be different to that of the basolateral membrane. For example, nutrients absorbed across the intestinal epithelium have to cross two types of barrier to enter the blood from the lumen. At the apex of the cell, nutrients are actively transported into the cell by carrier-mediated mechanisms. At the base of the cell they are resecreted out of the cell and into the bloodstream by different transport proteins.

Cell junctions

In the vast majority of tissues the cell membranes are not in close contact, but have an irregular intermembrane space of approximately 20 nanometres. Between this space lies the glycocalyx of the cells and a collection of glycoproteins and binding proteins such as fibronectin. In many tissues this space is bathed by the extracellular fluid and so is relatively permeable to small molecules. Drugs injected into a tissue of this type can diffuse with relative freedom. A typical example of this behaviour is the diffusion of drugs from an intramuscular injection, which rapidly spreads through the local muscle cells and into the

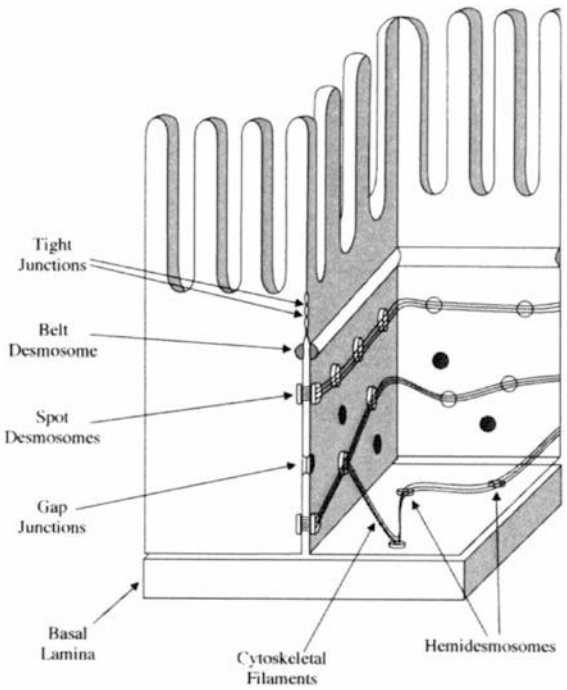

Figure 1.6 Intestinal epithelium illustrating the various types of junction

bloodstream. In epithelial tissues there is a need for a more effective chemical and physical barrier, and as a result the epithelial cells are bonded together by a number of different types of junctions which prevent diffusion of solutes around the cells. The primary types are (Figure 1.6):

a) tight junctions or zonulae occludens
b) gap junctions
c) desmosomes or zonulae adherens.

Tight junctions

Tight junctions are formed when specific proteins in two adjacent plasma membranes make direct contact across the intercellular space (Figure 1.7). A belt-like structure composed of many protein strands completely encircles each cell in the epithelium, attaching it to its neighbours and sealing the outer (luminal) space from the interior of the tissue or organ. At a tight junction, the interacting plasma membranes are so closely apposed that there is no intercellular space and the membranes are within 2Å of each other. As these junctions can be disrupted either by treatment with proteolytic enzymes or by agents that chelate Ca^{2+} or Mg^{2+}, both the proteins and divalent cations are thought to be required for maintaining their integrity. Beneath the tight junction, the spaces between adjacent cells are wider[5]. The structure of the epithelium has been likened to "a six-pack of beer extended indefinitely in 2 dimensions"[6].

Tight junctions play a critical part in maintaining the selective barrier function of cell sheets. For example, the epithelial cells lining the small intestine must keep most of the gut contents in the lumen. Simultaneously, the cells must pump selected nutrients across the cell

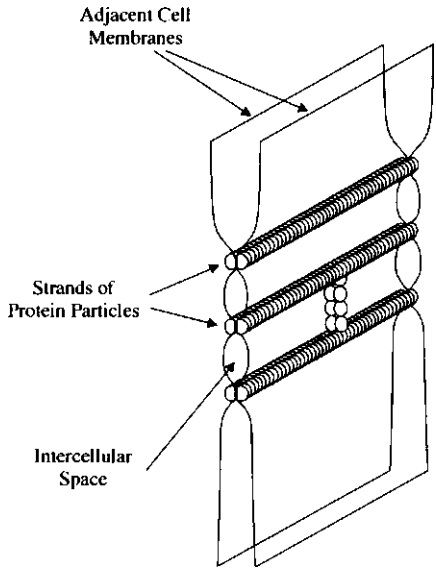

Figure 1.7 The tight junction

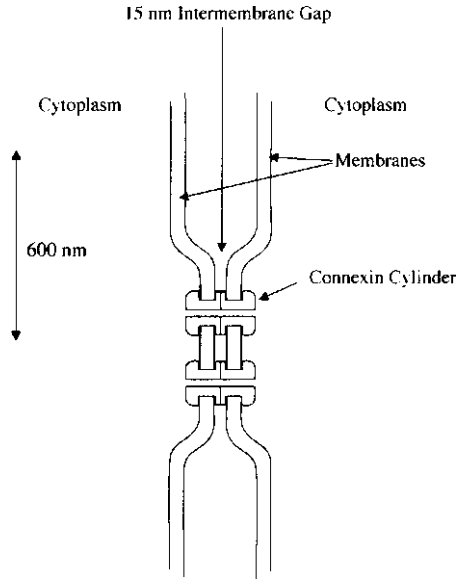

Figure 1.8 the gap junction

sheet into the extracellular fluid on the other side, from which they are absorbed into the blood. Studies have shown that the tight junctions are impermeable to colloidal particles, small molecules and ions, and possibly even to water. Electron microscopy shows that the junction consists of protein particles which are partly embedded in the membranes of both cells, so that the membranes become a single fused unit. As well as sealing the cells together, this prevents membrane proteins from the apical side of the cell diffusing to the basal side, maintaining the polarization of the cell.

Gap junctions

The commonest type of cell junction is the gap junction, which is widely distributed in tissues of all animals (Figure 1.8). It is not so much an adhesion point between cells as a means by which cells may communicate via the exchange of cytoplasmic materials. Gap junctions consist of regions in which the gap between adjacent cell membranes narrows to approximately 2 to 3 nm, over a cross-sectional area of several hundred square nanometres. In this region both cell membranes contain a specialised protein called connexin, which forms tubular hexameric clusters with a central pore. These clusters are aligned in both membranes so that they form a path from one cell to another, through which cytoplasm and its solutes can be transferred.

Molecules up to 1200 Daltons can pass freely through the gaps but larger molecules cannot, suggesting a functioning pore size for the connecting channels of about 1.5 nm. Coupled cells share a variety of small molecules (such as inorganic ions, sugars, amino acids, nucleotides and vitamins) but do not share their macromolecules (proteins, nucleic acids and polysaccharides). ATP can pass between the cells, as can cyclic AMP, which mediates many types of hormonal control. Consequently, hormonal stimulation in just one or a few epithelial cells will initiate a metabolic response in many cells. Gap junctions close in the presence of high concentrations of Ca^{2+} ions, so that if a cell is damaged, the influx of extracellular calcium will seal the cell's gap junctions and prevent the leak extending through the tissue.

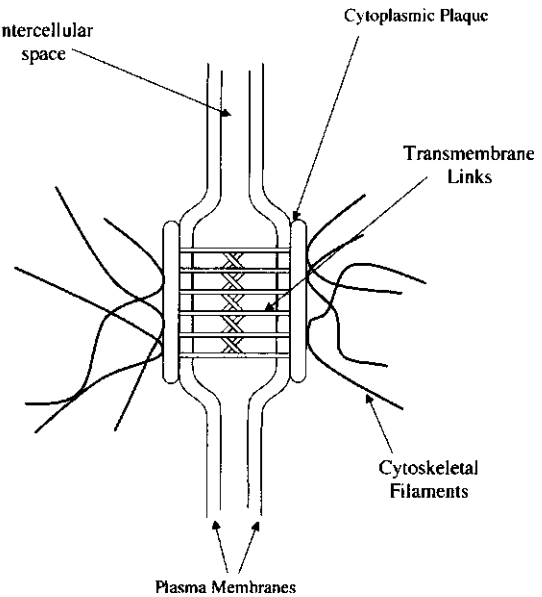

Figure 1.9 The desmosome

Desmosomes

Desmosomes are small structures which bond adjacent cells together, and are most abundant in tissues that are subject to severe mechanical stress, such as cardiac muscle, skin epithelium and the neck of the uterus (Figure 1.9). They are widely distributed in tissues, and enable groups of cells to function as structural units. Desmosomes can be divided into three different types: spot desmosomes, belt desmosomes and hemidesmosomes, all three of which are present in most epithelial cells.

Belt desmosomes form a continuous band around each of the cells in an epithelial sheet, near the cell's apical end, typically just below the tight junction. The bands in adjacent cells are directly apposed and are separated by a poorly characterised filamentous material (in the intercellular space) that holds the interacting membranes together. Within each cell, contractile bundles of actin filaments run along the belts just under the plasma membrane and connect the structure to the cytoskeleton.

Spot desmosomes act like rivets to hold epithelial cells together at button-like points of contact. They also serve as anchoring sites for actin filaments which extend from one side of the cell to the other across the cell interior, forming a structural framework for the cytoplasm. Since other filaments extend from cell to cell at spot desmosomes, the actin filament networks inside adjacent cells are connected indirectly through these junctions to form a continuous network of fibres across the entire epithelial sheet.

Hemidesmosomes or half-desmosomes resemble spot desmosomes, but instead of joining adjacent epithelial cell membranes together, they join the basal surface of epithelial cells to the underlying basal lamina. Together spot desmosomes and hemidesmosomes act as bonds that distribute any shearing force through the epithelial sheet and its underlying connective tissue.

TRANSPORT ACROSS CELL MEMBRANES

In order to function correctly a cell must be able to take up and release a wide range of materials; for drug therapy to be successful it must also be possible to get therapeutic substances into cells and across layers of cells such as epithelia. There are a number of possible mechanisms for transport across membranes; substances may simply diffuse across, or be carried by a range of more selective processes, depending on the substance involved.

Passive diffusion

Studies using model membranes have revealed that the phospholipid bilayer itself is remarkably impermeable to all but very small molecules such as water and ethanol, and gases such as oxygen and carbon dioxide. These compounds move across the membrane by *passive diffusion,* a process driven by the random motion of the molecules. Diffusion is described by Fick's law, which states that the diffusion rate R (in moles s^{-1}) is proportional to the concentration gradient $\Delta c/\Delta x$:

$$R=-DA\ \Delta c/\Delta x$$

Where Δc is the concentration difference between the outside and inside of the membrane, and Δx is the thickness of the membrane. A is the area of membrane over which diffusion is occurring, and D is a constant (for a specific molecule in a specific environment) called its *diffusion coefficient*. Since the area and thickness of the membrane are usually outside our control, it is evident that uptake of a molecule into a cell by passive diffusion can only be influenced by either increasing the external concentration of the drug, or by selecting our molecule so that D is large.

The diffusion coefficient of a drug is determined by a number of factors, but two are particularly important. These are the *solubility* of the drug, and its *molecular weight*. For a molecule to diffuse freely in a hydrophobic membrane it must be soluble in it, and conversely if it is to also diffuse in the extracellular fluid it must also be soluble in aqueous systems. The relative solubility of molecules in aqueous or oily environments is described by their *partition coefficient,* labelled P, which describes how the drug distributes itself between a pair of solvents (usually water and an oily solvent such as octanol). Hydrophobic molecules dissolve mainly in the oil and have a high partition coefficient, while hydrophilic molecules dissolve mainly in the water and have a low partition coefficient. Only for intermediate values of the partition coefficient will the drug be soluble in both the membrane and the extracellular fluid, and be free to diffuse from the extracellular fluid, across the membrane, and into the cell. Drugs which have a very low partition coefficient are poorly absorbed because they cannot dissolve in the oily membrane; conversely drugs which have a high partition coefficient cannot dissolve in the extracellular fluid and so cannot reach the membrane. Such drugs are said to be *solubility-limited*.

The diffusion coefficient, and hence rate of absorption, is also influenced by the molecular weight of the drug. Small molecules diffuse rapidly and so will cross the membrane more quickly than large, slowly-diffusing molecules. These concepts are illustrated in Figure 1.10, which shows how absorption depends on partition coefficient for drugs of different molecular weights. This is not based on specific drugs but is intended to illustrate the concepts. The drug with a molecular weight of 400 is rapidly absorbed for intermediate partition coefficients (P of approximately 100 or Log P=2. However, it is absorbed more slowly for larger values of P as its aqueous solubility falls, or for smaller values of P as its membrane solubility falls. The smaller drug with a molecular weight of 250 is subject to the same influence of P, but is generally absorbed more quickly due to its more rapid diffusion.

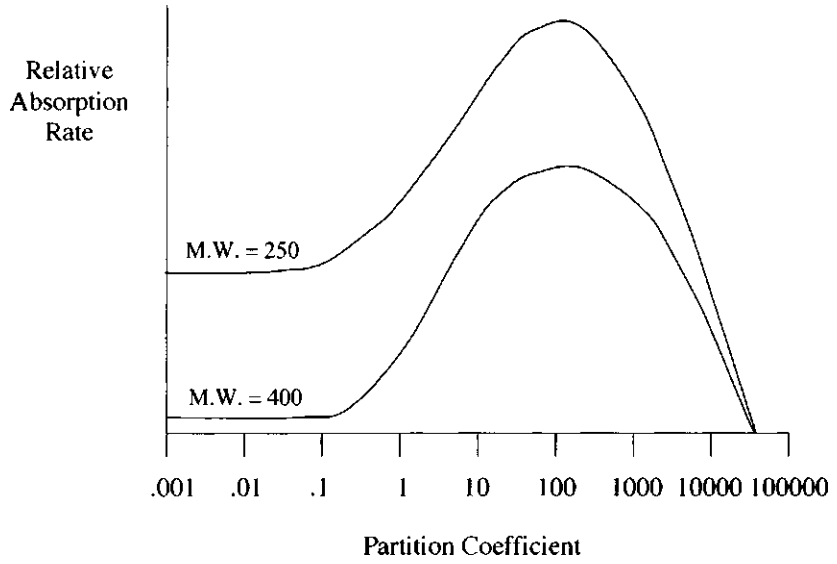

Figure 1.10 Absorption as a function of drug partition coefficient

Figure 1.10 shows how the diffusion of drug across a pure lipid membrane will vary depending on the properties of the drug. However, drug diffusion across real cell epithelia takes place not only through membranes, but also through small aqueous pores between cells (the *paracellular* route), and this enhances the absorption of hydrophilic molecules which are small enough to pass through the pores. The drug with a molecular weight of 250 can pass through these pores, but that with a higher molecular weight of 400 cannot.

The pH-partition hypothesis

Drug molecules are predominantly weakly ionizable species containing groups such as amine, carboxyl, phenyl, etc. These materials are absorbed across plasma membranes in their unionised forms, since these are non-polar; the ionised forms of the drug cannot pass through the membrane due to its hydrophobic character. Consequently, the pH of the extracellular environment is critical in determining the absorption across the membrane. Thus, for example, an acidic drug is absorbed from acidic solution if the pH is lower than the drug pK, since it will be in its unionised form. This is the basis of the pH-partition hypothesis, stressed in most classical texts of pharmacology, which discuss the absorption of drugs based on the relative degrees of ionization in the lumen and the blood.

The pH-partition hypothesis provides an indication of drug absorption, but suffers from many shortcomings. The most notable of these can be seen in the widely quoted example of the absorption of an acidic drug from the stomach, in which the drug is in its unionised state at pH 2 and so passes across the membrane. In the blood (at pH 7.4) the drug is ionized, and so cannot pass back across the membrane. This effect is referred to as ion-trapping. The conclusion is that pH and ionization are highly important in determining drug absorption. This example is logically correct but suffers from a number of errors. The gastric epithelium represents the most extreme example available of a pH gradient *in vivo*, but drug absorption from the stomach is minimal and most absorption takes place in the small intestine, which is normally close to pH 7. Here the ionisation of the drug in the lumen

is similar to that in the blood and little ion-trapping can occur. Gastric contents delivered into the duodenum make the first few centimetres of the intestine acidic, until the chyme has been neutralised by bicarbonate. The duodenal absorptive capacity is high, but transit through this region is extremely rapid and so no significant absorption occurs.

The biggest failing of this hypothesis is to attempt to calculate absorption from equilibrium drug distributions, when in practise the absorbed drug is swept away by the circulation. Absorption is a dynamic process involving dissolution, ionization, partition and blood flow, and consequently the correlation of pH-partition predictions with experiment is often poor.

Facilitated and carrier mediated diffusion

Despite the hydrophobic nature of the cell membrane, it is necessary for a number of hydrophilic materials to enter and leave the cell. Typical examples are small amino acids and carbohydrates, which the cell requires in quantity for metabolism. Ions are also required, as the cell maintains an ion imbalance with the surroundings, with the cell having substantially more potassium and less sodium than the extracellular fluid.

Since these molecules cannot diffuse freely across the cell membrane, they are transported by a range of membrane proteins collectively called *permeases*. These proteins fall into two broad groups, those which allow molecules to pass into the cell down a concentration gradient, and behave like passive but selective pores, and those which actively pump molecules into cells against a concentration gradient. The former group contains transporters that allow nutrients such as glucose into the cell; this *hexose transporter system* is present in most mammalian cells. Since the glucose is utilized inside the cell rapidly, the internal concentration is low and the diffusion always occurs down a concentration gradient. Consequently, no input of energy is required to drive this transport system.

The second group of proteins actively accumulate materials in cells even if their concentration is higher inside the cell than outside. This requires an input of energy, usually derived from the hydrolysis of intracellular ATP, and consequently the carriers are called ATPases. The best known examples are the Na^+/K^+ ATPase that pumps potassium into the cell and sodium out, and the H^+/K^+ ATPase which pumps hydrogen ions out of the gastric parietal cells, thus acidifying the stomach contents.

An important characteristic of carrier-mediated absorption is that it is *saturable*. If the external concentration of the molecule being transported is extremely high, the carrier will be fully utilized and will become rate limiting. Under these conditions, increasing the external concentration of the transported molecule will have no effect on the transport rate. The maximum transport rate will be determined by the concentration of carrier molecules and the speed with which they can shuttle material across the membrane, and not on the concentration of the molecules being transported.

A number of drugs are thought to be absorbed by carrier-mediated processed rather than passive diffusion. These include amoxycillin and cyclacillin[7], which show saturable kinetics, and cardioglycosides such as digitalis. The actual carrier mechanism is unclear since these materials are xenobiotics and are presumably being transported by a protein which normally serves some other purpose.

Cotransport

Energy must be expended in order to pump any molecule up a concentration gradient, and this is ultimately derived from ATP hydrolysis. The only transport systems that are directly coupled to ATP are those which pump ions such as Na^+ and Ca^{2+} However, cells often have to accumulate other materials, such as amino-acids and carbohydrates, at high concentrations. This is performed by cotransport, in which the cells' ion concentration

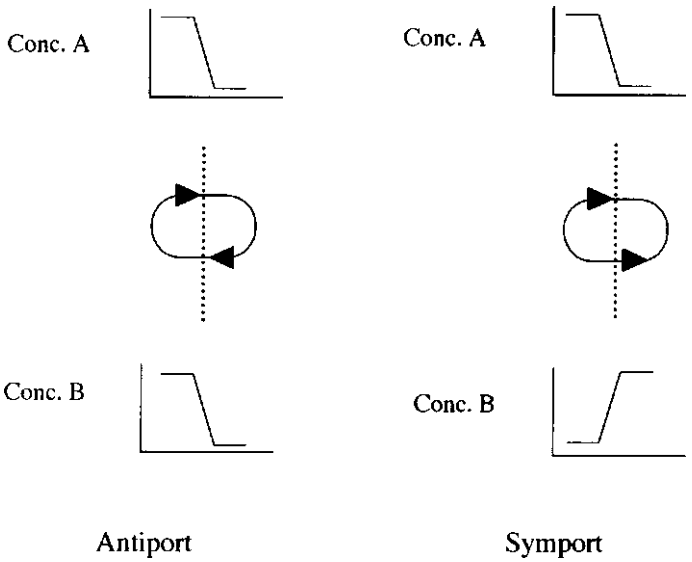

Figure 1.11 Cotransport across membranes

gradient is used as a secondary energy source (Figure 1.11). The transport proteins are systems which couple the transport of an ion to that of another molecule, so that, in allowing an ion to move out of the membrane to a lower concentration, the protein also moves a different molecule from lower to higher concentration. If the ion and molecule move across the membrane in the same direction, the process is called symport, while if they are exchanged in opposite directions it is called antiport.

An important example of this process is the absorption of glucose from the intestinal lumen by the intestinal epithelial cells. In most cells glucose is actively metabolized, so its concentration is low and it can be transported by passive transport. However, the intestinal epithelium is responsible for absorbing molecules like glucose, so it is often necessary to pump them up a concentration gradient into the epithelial cells before they can be passed into the bloodstream. This is accomplished by a sodium-glucose symport protein which couples the inward movement of a glucose molecule to that of a sodium ion. The intracellular sodium concentration is lower than in the intestinal lumen, so the inward movement of sodium is energetically favourable. A glucose molecule is simultaneously transported into the epithelial cell up a concentration gradient.

Uptake of macromolecules and particles

Membrane transport by diffusion or by transport proteins is only feasible for small molecules, since there is a limit to the size of pore that can be opened and closed by the conformational change of a membrane protein. Consequently, larger objects, such as macromolecules and particles, are internalized by a completely different mechanism, in which a portion of the membrane extends and envelops the object, drawing it into the cell to form a vacuole. This process is called *cytosis* and there are a number of variants which occur in different cells.

Endocytosis occurs when a small cavity forms on the membrane surface, which is gradually enclosed by membrane movement and finally taken within the cell (Figure 1.12). The process may be spontaneous in certain cells, and causes a small amount of extracellular

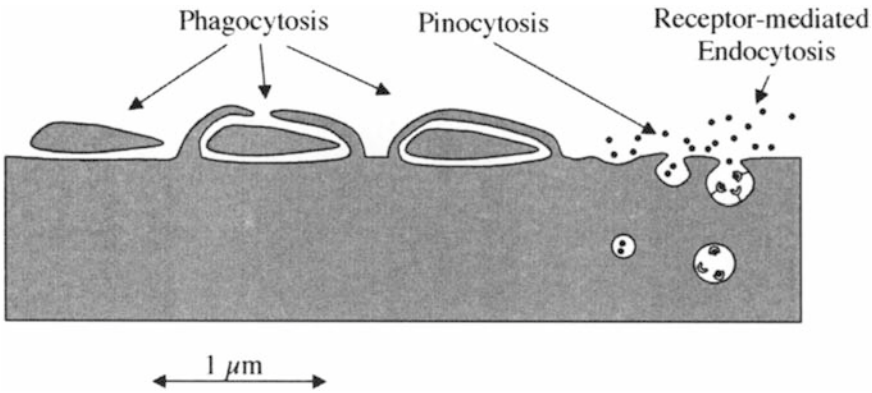

Figure 1.12 Uptake of particles and solutes by cytosis

fluid to enter the cell. This is called *pinocytosis*. More commonly the process is triggered by the binding of a particular macromolecule to a surface receptor on the membrane, a process termed *receptor-mediated endocytosis*.

Phagocytosis occurs when a particle is taken inside a cell. The most important example of this occurs when a white blood cell called a macrophage engulfs a foreign body such as a bacterium or virus. The foreign body first adheres to the cell membrane, which then gradually extends over it until it is internalized to form a vacuole within the cell. This then normally fuses with lysosomes and is degraded. The vacuoles formed in this process are much larger than those involved in endocytosis.

INTERCELLULAR ROUTES OF ABSORPTION

As well as being absorbed through the epithelial cells, molecules can pass through tissues via the intercellular or paracellular route through junctional gaps between cells. There has been much discussion regarding the importance of this process in transport across the gastrointestinal mucosa. There is considerable variation in the integrity of the tight junctions along the gastrointestinal tract, with the membranes of the stomach and large intestine having the highest transepithelial resistance. Norris and coworkers suggested that molecules with a greater molecular radius than 1.1 nm cannot permeate the intestinal paracellular space[8]. The pore size has been calculated to be 0.8 nm in the jejunum and 0.3 nm in the ileum and colon, so it unlikely that molecules of a significant size could be absorbed from the intestine by this route.

PERSORPTION

There is a special mode of permeation across the intestinal wall in which the cell membranes are not involved. Intestinal cells are continuously produced in the crypts of Lieberkühn and migrate towards the tip of the villus. During digestion the cells are sloughed off leaving a temporary gap at the cell apex, and through this gap large particles can slip into the circulation through the intercellular gaps. This process has been termed persorption. The observation that large objects such as starch grains can be found in the blood after a meal of potatoes or corn is often quoted as the prima facie evidence of persorption (Figure 1.13). Volkheimer and coworkers[9] hypothesised that a "kneading" action of the villus on the luminal contents allowed particles of up to 100 μm diameter to enter the lamina propria of

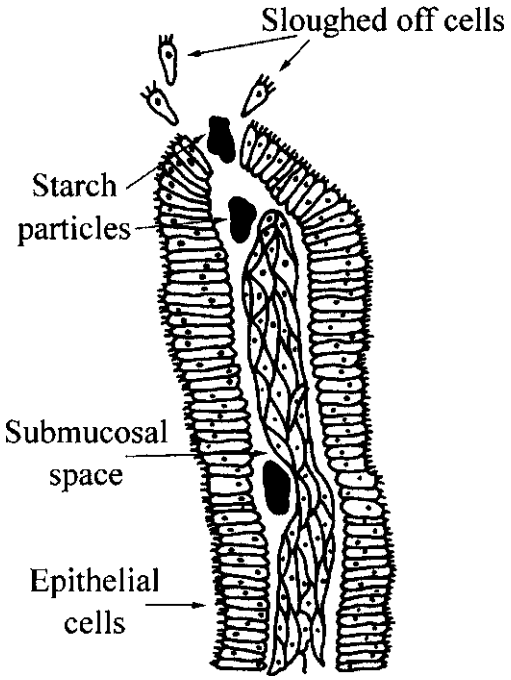

Figure 1.13 Persorption in the intestinal villus

the intestinal mucosa near the apex of the villus. Metallic iron particles of up to 52 μm were identified in both portal venous blood and thoracic duct lymph of dogs after the animals were fed 200 g of iron powder suspended in milk and cream. There is also some evidence that very small numbers of polymer particles can pass from the intestinal lumen into the bloodstream in this way[10]. Large foreign particles should be expected to enter intestinal lymph vessels in preference to mucosal blood vessels and it is remarkable that such large particles should appear at all in portal venous blood without finding their way to the lung capillary filter. It is possible that potentially harmful materials such as asbestos fibre can be absorbed in this way, but a detailed understanding of these effects is still lacking, and artefacts in many of the published experiments cannot be discounted.

MUCUS

Most epithelia consist of a number of different cell types with different functions. One cell that is common in many epithelia is the mucosal cell, which secretes mucus. An epithelium containing mucosal cells is called a mucosal epithelium or simply mucosa.

Mucus has several functions. It restricts the penetration of large molecules, and prevents the tissue from dehydrating. It keeps the tissue surface clean by its continuous removal, and lubricates the passage of materials such as food through the gastrointestinal tract. Its most important property is its viscoelasticity, which enables it to act as a locally rigid mechanical barrier which can flow under the influence of peristalsis. The primary component of mucus is a large polysaccharide called mucin built up in subunits of 500,000 Daltons or larger. It consists of a protein backbone approximately 800 amino acids long, rich in serine and threonine, which are hydroxylated amino acids. Most of the hydroxy-residues are linked to oligosaccharide side chains which serve to stiffen the backbone, and

which carry an extensive layer of water of hydration. The oligosaccharide chains are generally up to 18 residues in length and are composed of N-acetylgalactosamine, N-acetylglucosamine, galactose, fucose or N-acetylneuraminic acid.

Mucus is 95% water and so makes intimate contact with hydrophilic surfaces. Small particles less than ~600 μm (the average thickness of a mucus layer) may be buried in the surface and held securely due the stickiness of the mucus, but since the mucus is continually secreted the particles move further away from the mucosa and are ultimately sloughed off. Small molecules pass easily through mucus due to its high water content; larger molecules diffuse through the mucus more slowly and remain in contact for longer periods.

Many research groups have attempted to develop mucoadhesive materials, the idea being to bind a drug carrier to a mucous membrane in order to optimize drug delivery. However, mucus turnover can be rapid and there seems little point in attaching a drug to a surface which is to be sloughed off in a short time.

CONCLUSIONS

The absorption of drugs, although dependent on the site of absorption, is often controlled by similar types of barriers. These are mucus, hydrophobic membranes, transport processes and cell junctions. In the following chapters we will see how these barriers manifest themselves in different organs according to the form and function of the tissue involved, and how they determine the success or failure of drug delivery technology.

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