CHAPTER 4
TRANSPORT OF SUBSTANCES THROUGH CELL MEMBRANES

BY FATIMA HAIDER

KGMC

- The extracellular fluid contains a large amount of sodium but only a small amount of potassium. The opposite is true for intacellular fluid.
- The extracellular fluid contains a large amount of chloride ions, whereas the intracellular fluid contains very little of these ions.
- The concentration of phosphorus and proteins in the intracellular fluid are considerably greater than those in the extracellular fluid
- Na+ concentration in extracellular fluid = 142 mEq/L
  Na+ concentration in intracellular fluid = 10 mEq/L
- K+ concentration in extracellular fluid = 4 mEq/L
  K+ concentration in intracellular fluid = 140 mEq/L

TRANSPORT THROUGH CELL MEMBRANE

- Lipid soluble substances diffuse directly through the lipid bilayer membrane
- The lipid solubility of oxygen, nitrogen, CO_2 and alcohols are high
- The proteins in cell membrane act as transport proteins. These includes channel proteins and carrier proteins

CHANNEL PROTEINS

The proteins which have watery spaces all the way through the molecule and allow free movement of water, as well as selected ions or molecules are called channel proteins.

Channel proteins allow molecules to flow in and out along their concentration gradient i-e a solute flow through channel protein is always passive

a) VOLTAGE-GATED CHANNELS : opened or closed by gates that are regulated by electrical signals
b) LIGAND-GATED CHANNELS : regulated by chemicals that bind to channel proteins

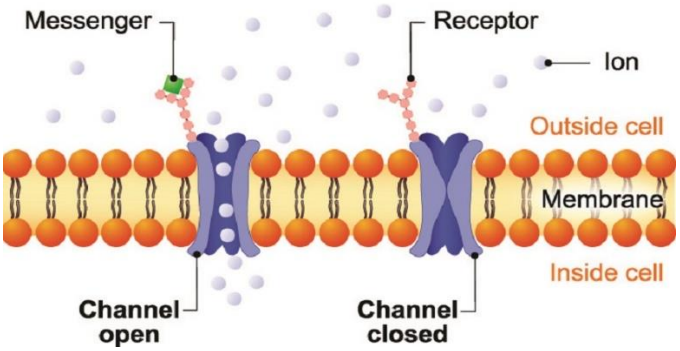
GATING OF PROTEIN CHANNELS

1. VOLTAGE-GATED ION CHANNELS

In case of voltage gating, the molecular conformations of the gate or its chemical bonds respond to the electrical potential across the cell membrane.

2. LIGAND-GATED ION CHANNELS

- Transmembrane ion channel open/close in response to binding of a chemical messenger (ligand)
- Only specific ligand can bind to specific channel
- EXAMPLE : EFFECT OF NEUROTRANSMITTER ACETYLCHOLINE ON ACETYLCHOLINE RECEPTOR
  Acetylcholine opens gate of channel, providing negatively charged pore about 0.65nm in diameter that allows uncharged molecules or positive ions smaller than this diameter to pass through. This gate is exceedingly important for transmission of nerve signal from one cell to another and from nerve cell to muscle cell to cause muscle contraction.



SELECTIVE PERMEABILTY OF PROTEIN CHANNELS

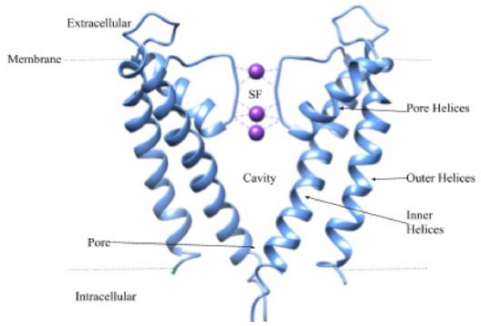
Many protein channels are highly selective for transport of one or more specific ions or molecules. This selectivity results from specific characteristics of the channel, such as its diameter, shape, and the nature of electrical charges and chemical bonds along its inside surfaces.

POTASSIUM CHANNELS STRUCTURE

Potassium channels have a tetrameric structure consisting of four identical protein subunits surrounding a central pore.

At the top of the channel pore are pore loops that form a narrow selectivity filter. Lining the selectivity filters are carbonyl oxygens.

When hydrated potassium ions enter the selectivity filter, they interact with the carbonyl oxygens and shed most of their bound water molecules, permitting the dehydrating potassium ions to pass through the channel. The carbonyl oxygens are too far apart, however, to enable them to interact closely with the smaller sodium ions, which are therefore excluded by the selectivity filter from passing through the pore.



SODIUM CHANNEL

- 0.3 to 0.5 nm in diameter
- The narrowest part of the sodium channel's open pore, the selectivity filter is lined with strongly negatively charged amino acid residues. These strong negative charges can pull small dehydrated sodium ions away from their hydrating water molecules into these channels, although the ions donot need to be fully dehydrated to pass through the channels.

CARRIER PROTEINS

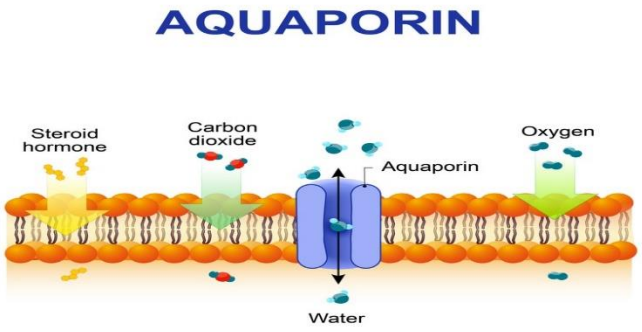
- Many carrier proteins are found in cell membrane, though they may also be found in the membranes of internal organelles such as mitochondria, chloroplasts, nucleolus and others.
- Carrier proteins are useful for active transport. However, carrier proteins can also be used for facilitated diffusion (a form of passive transport)
- Carrier proteins typically have a "binding site" which will only bind to the substance they are supposed to carry. Once the carrier protein has bound to a specific quantity of its target substance, the protein changes shape to "carry" the substance from one side of the membrane to the other.
- Channel proteins and carrier proteins are usually selective for the types of molecules or ions that are allowed to cross the membrane

AQUAPORINS

The aquaporins are highly specialized protein pores in the cell membrane that selectively permit rapid passage of water through the membrane.

The aquaporins are highly specialized and there are at least 13 different types in various cells of mammals.

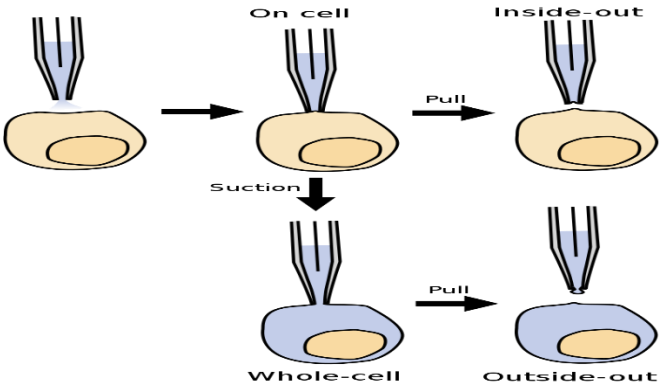
Aquaporins permit rapid passage of water through cell membranes but exclude other molecules. They have a narrow pore that permits water molecules to diffuse through the membrane in single file. The pore is too narrow to permit passage of any hydrated ions.



PATCH CLAMP METHOD FOR RECORDING ION CURRENT FLOW THROUGH SINGLE CHANNELS

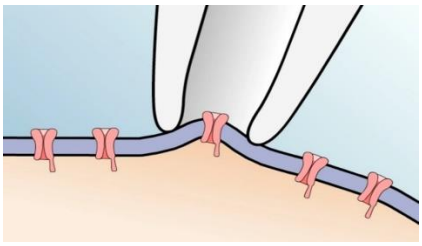
The Patch-clamp technique is a versatile electrophysiological tool for understanding ion channel behavior. Every cell expresses ion channels, but the most common cells to study with patch-clamp techniques include neurons, muscle fibers, cardiomyocytes, and oocytes overexpressing single ion channels

- To evaluate single ion channel conductance, a microelectrode forms a high resistance seal with the cellular membrane, and a patch of cell membrane containing the ion channel of interest is removed.
- Alternatively, while the microelectrode is sealed to the cell membrane, this small patch can be ruptured giving the electrode electrical access to the whole cell. Voltage is then applied, forming a voltage clamp, and membrane current is measured.
- Current clamp can also be used to measure changes in membrane voltage called membrane potential. Voltage or current change within cell membranes can be altered by applying compounds to block or open channels.
- These techniques enable researchers to understand how ion channels behave both in normal and disease states and how different drugs, ions, or other analytes can modify these conditions



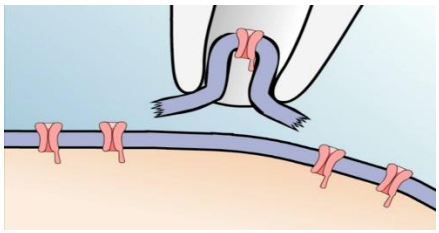
1. ON-CELL PATCH

- A micropipette with a tip diameter of only 1 or 2 micrometers is abutted against the outside of a cell membrane.
- Tight contact between pipette and cell membrane is formed
- Ions flow through the channel into the pipette
- The resulting electrical current is then recorded



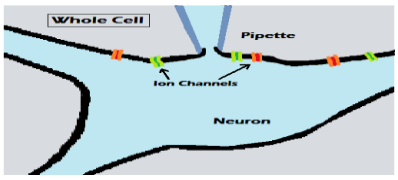
2. INSIDE-OUT PATCH

- In the **inside-out** method, a **patch** of the membrane is attached to the **patch** pipette, detached from the rest of the cell, and the cytosolic surface of the membrane is exposed to the external media, or bath.
- Cell membrane patch at the end of the pipette is torn away from the cell by mild suction.



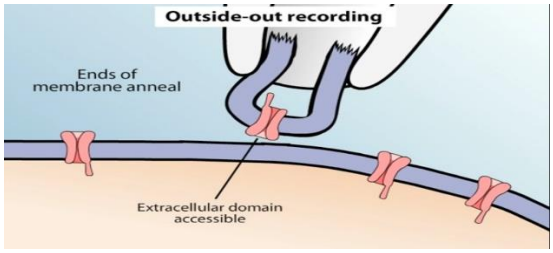
3. WHOLE CELL PATCH

Small pieces of membrane are pulled away from the cell without disrupting the seal.



4. OUTSIDE OUT PATCH

- In outside out patch, the extracellular membrane of the cell is exposed
- Optimum configuration for studying how channel activity is influenced by extracellular chemical signals such as neurotransmitters



FACILITATED DIFFUSION

- Carrier-mediated diffusion
- Involves the passive movement of molecules along their concentration gradient, guided by the presence of another molecule – usually an integral membrane protein forming a pore or channel
- Doesnot directly involve ATP

- In facilitated diffusion, there is some maximum rate of diffusion (V_{max}) when all the carrier proteins become saturated. Increasing the solute concentration further will not change the rate of diffusion.

EXAMPLES OF FACILITATED DIFFUSION

1. Glucose transporter
2. Ion channels
3. Aquaporins

GLUCOSE TRANSPORTER (GLUT)

- Atleast 14 members of a family of membrane proteins called GLUT that transport glucose molecules have been discovered in various tissues.
- Some of these GLUT proteins transport other monosaccharides that have structures similar to that of glucose, including galactose and fructose
- The glucose transporter that facilitates the movement of glucose is a carrier protein that has two major conformational changes.
- While the exact 3D structure is not known, the binding of glucose probably causes a conformational change that makes the binding site face the interior of the cell. When glucose is released into the cell, the transporter returns to its original conformation.
- GLUT 4 is activated by insulin which can increase the rate of facilitated diffusion of glucose as much as 10 to 20-fold in insulin sensitive tissues. This is the principal mechanism whereby insulin controls glucose use in the body.

NERNST/ REVERSAL POTENTIAL

In a biological membrane; the reversal potential (also known as Nernst potential) of an ion is the membrane potential at which there is no net flow of that particular ion from one side of the membrane to the other.

NERNST EQUATION

Calculates the voltage necessary to perfectly oppose the net movement of an ion down its concentration gradient

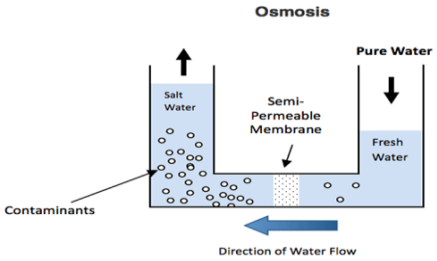
EMF (in mV) = ±61 log C1/C2

DIFFUSION ACROSS MEMBRANE

1. Ions move down concentration gradients
2. Ions move down electrical gradients
3. Molecules move from high pressure to low pressure

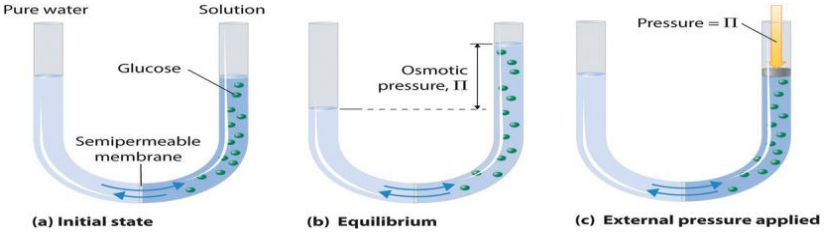
OSMOSIS

The net movement of water across a semipermeable membrane caused by a concentration difference of water is called osmosis.



OSMOTIC PRESSURE

The minimum pressure which needs to be applied to a solution to prevent the inward flow of its pure solvent across a semipermeable membrane



The osmotic pressure exerted by particles in a solution is determined by the number of particles per unit volume of fluid, not by the mass of particles

$$\pi = iMRT$$

π = osmotic pressure

i = van't Hoff's factor

M = Molar concentration of solution (mol/ L)

R = Ideal gas constant (0.08206 L atm mol^{-1} K^{-1})

T = Temperature in Kelvin (K)

The molar concentration is determined by dividing the number of grams of solute used to make the solution by the molecular weight of the solute.

van't Hoff's factor is a measure of the number of ions a solute will form when dissolved in water.

OSMOLALITY

- To express the concentration of a solution in terms of number of particles, a unit called the osmole is used in place of grams.
- One osmole is 1 gram molecular weight (1 mole) of osmotically active solute.
- 180g of glucose which is 1 mole of glucose, is equal to 1 osmole of glucose because glucose doesnot dissociate into ions.
- If a solute dissociates into two ions e.g NaCl, 1mol of the solute will become 2 osmoles because the number of osmotically active particles is now twice as great as for the nondissociated solute.

The normal osmolality of the extracellular and intracellular fluids is about 300 milliosmoles per kg of water

OSMOLARITY – A solution that has 1 osmole of solute dissolved in each litre of solution

OSMOLALITY – A solution that has 1 osmole of solute dissolved in each kg of solution

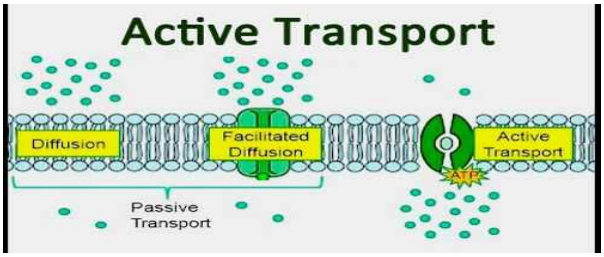
- The difference between osmolarity and osmolality is less than 1%
- Although osmolality is more accurate , we still use osmolarity as it is more practical
- Osmolality is more accurate because it is expressed in kg and mass doesnot change with increase or decrease in temperature.
- Osmolarity is more practical as fluids are easily measured in litres than kg.

OSMOTIC PRESSURE OF BODY FLUIDS

- Total calculated osmotic pressure of body fluids = 5790 mmHg
- Measured value = 5500 mmHg
- On average, the actual osmotic pressure of the body fluids is about 0.93 times the calculated value
- The reason for the difference between calculated and measured value is that many ions in the body fluids, such as sodium and chloride ions are highly attracted to one another so they cannot move entirely unrestrained in the fluids and create their full osmotic pressure potential

ACTIVE TRANSPORT

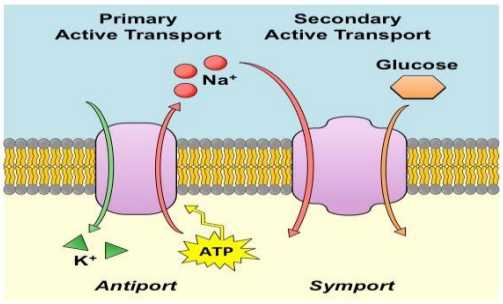
- **Active transport** is the movement of molecules across a cell membrane from a region of lower concentration to a region of higher concentration—against the concentration gradient.
- **Active transport** requires cellular energy to achieve this movement.



TYPES OF ACTIVE TRANSPORT

- Two types according to source of energy used to facilitate the transport :

1. **Primary Active Transport** – energy derived directly from breakdown of ATP or other high energy phosphate compound.
2. **Secondary Active Transport** - transport of molecules across the cell membrane utilizing energy in other forms than ATP. This energy comes from the electrochemical gradient created by pumping ions out of the cell.



PRIMARY ACTIVE TRANSPORT

- EXAMPLES

1. Sodium potassium pump
2. Primary active transport of calcium ions
3. Primary active transport of hydrogen ions

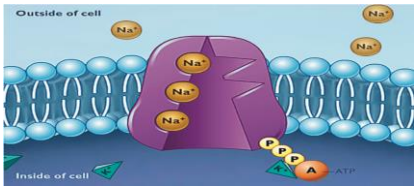
SODIUM-POTASSIUM PUMP

- The carrier protein for sodium-potassium pump is a complex of two separate globular proteins

1. alpha-subunit
    - The larger subunit
    - Molecular weight about 100,000
    - Important for functioning of pump
2. Beta subunit
    - Smaller subunit
    - Molecular weight about 55,000
    - Anchor the protein complex in the lipid membrane

ALPHA SUBUNIT

- Has three binding sites for sodium ions towards the intracellular fluid
- Has two binding sites for potassium ions towards the extracellular fluid
- ATP activity is observed near the sodium binding sites



WORKING

- When two potassium ions bind on the outside of the carrier protein and three sodium ions bind on the inside, the ATPase function of the protein becomes activated. Activation of the ATPase function leads to cleavage of one molecule of ATP, splitting it to adenosine diphosphate (ADP) and liberating a high-energy phosphate bond of energy. This liberated energy is then believed to cause a chemical and conformational change in the protein carrier molecule, extruding the three sodium ions to the outside and the two potassium ions to the inside.

- As with other enzymes, the Na+ -K+ ATPase pump can run in reverse. If the electrochemical gradients for Na+ and K+ are experimentally increased to the degree that the energy stored in their gradients is greater than the chemical energy of ATP hydrolysis, these ions will move down their concentration gradients and the Na+ -K+ pump will synthesize ATP from ADP and phosphate. The phosphorylated form of the Na+ -K+ pump, therefore, can either donate its phosphate to ADP to produce ATP or use the energy to change its conformation and pump Na+ out of the cell and K+ into the cell. The relative concentrations of ATP, ADP, and phosphate, as well as the electrochemical gradients for Na+ and K+ , determine the direction of the enzyme reaction. For some cells, such as electrically active nerve cells, 60 to 70 percent of the cells' energy requirement may be devoted to pumping Na+ out of the cell and K+ into the cell.

IMPORTANCE OF SODIUM-POTASSIUM PUMP

1. Control cell volume – the net loss of ions outside the cell initiates osmosis of water outside the cell preventing the cell to swell or burst

2. Creates electrical potential across cell membrane – for transmitting nerve and muscle signal

CALCIUM PUMP

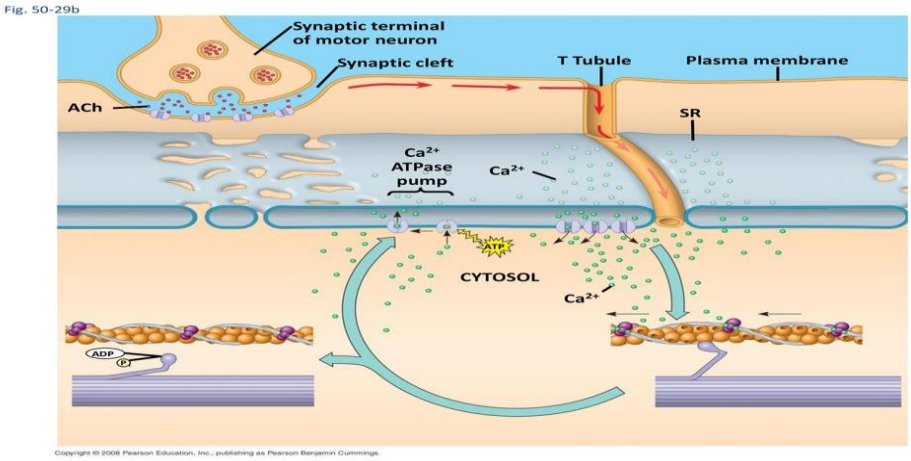
- Another important primary active transport mechanism is the calcium pump. Calcium ions are normally maintained at an extremely low concentration in the intracellular cytosol of virtually all cells in the body, at a concentration about 10,000 times less than that in the extracellular fluid.

- This level of maintenance is achieved mainly by two primary active transport calcium pumps.

    - One, which is in the cell membrane, pumps calcium to the outside of the cell.

    - The other pumps calcium ions into one or more of the intracellular vesicular organelles of the cell, such as the sarcoplasmic reticulum of muscle cells and the mitochondria in all cells.

- In each of these instances, the carrier protein penetrates the membrane and functions as an enzyme ATPase, with the same capability to cleave ATP as the ATPase of the sodium carrier protein. The difference is that this protein has a highly specific binding site for calcium instead of for sodium.
- Calcium channels are called slow channels, requiring 10 to 20 minutes as long for activation as sodium channels.
- Calcium channels are numerous in cardiac muscle and smooth muscle.
- In muscle cells, calcium pump is located in sarcoplasmic reticulum.
    - When calcium ions are pumped from cytoplasm to sarcoplasmic reticulum, muscle cell relaxes.
    - When calcium ions are pumped from sarcoplasmic reticulum to cytosol, muscle cell contracts.



HYDROGEN PUMP

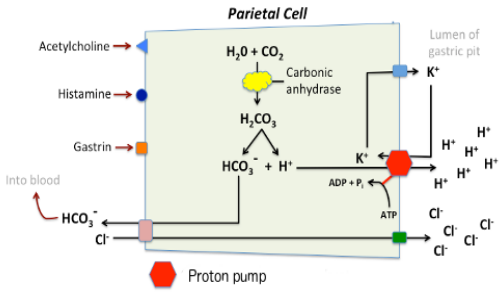
- Primary active transport of hydrogen ions is important at two places in the body:

(1) in the gastric glands of the stomach, and

(2) in the late distal tubules and cortical collecting ducts of the kidneys.

PROTON PUMP IN GASTRIC GLANDS

- At the secretory ends of the gastric gland parietal cells, the hydrogen ion concentration is increased as much as a million-fold and then is released into the stomach along with chloride ions to form hydrochloric acid.



PROTON PUMP IN RENAL TUBULES

- In the renal tubules, special intercalated cells found in the late distal tubules and cortical collecting ducts also transport hydrogen ions by primary active transport. In this case, large amounts of hydrogen ions are secreted from the blood into the urine for the purpose of eliminating excess hydrogen ions from the body fluids. The hydrogen ions can be secreted into the urine against a concentration gradient of about 900-fold

ENERGETICS OF PRIMARY ACTIVE TRANSPORT

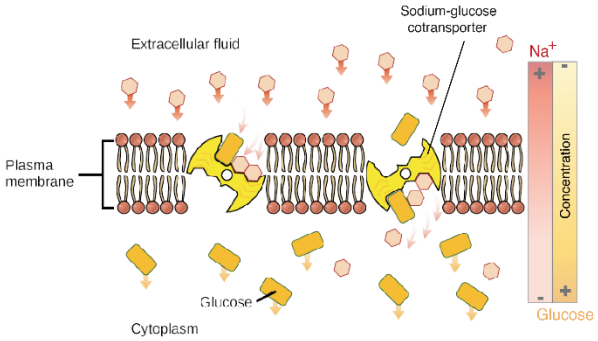
- The energy required to concentrate a substance 100 folds is twice as compared to concentrating a substance 10 times.
- Energy (in calories per osmole) = 1400 log C1/C2
- Energy required to concentrate 1 osmole of a substance 10 fold is about 1400 calories, whereas to concentrate it 100 fold, 2800 calories are required.

SECONDARY ACTIVE TRANSPORT

- The electrochemical gradients set up by primary active transport store energy, which can be released as the ions move back down their gradients. Secondary active transport uses the energy stored in these gradients to move other substances against their own gradients.

CO TRANSPORT

- Transport in same direction as the primary ion
- A carrier protein serves as an attachment point for both the ion and the substance to be co-transported. Once they are both attached, the energy gradient of the ion cause the ion and the other substance to be transported together to the interior of the cell.

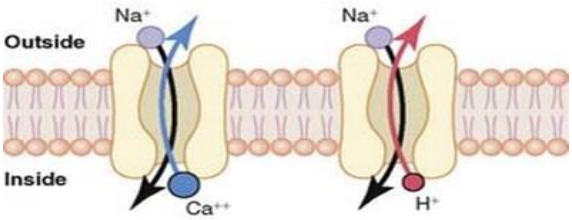


CO TRANSPORT EXAMPLES

1. Sodium-glucose co transporter
    - For transporting glucose across renal and intestinal epithelial cells to promote absorption of glucose into the blood
2. Sodium co-transport of amino acids
    - At least 5 amino acid transport proteins have been identified
    - Found in epithelial cells of intestinal tract and renal tubules of kidneys

COUNTER TRANSPORT

- Transport in a direction opposite to the primary ion

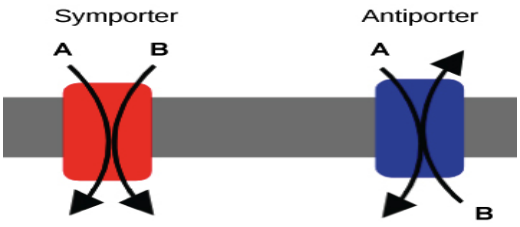


COUNTER TRANSPORT EXAMPLES

1. Sodium-calcium counter transport
    - Occurs through almost all cell membranes
    - Sodium ions move to interior and calcium moves to exterior
    - Sodium-hydrogen counter transport
    - Occurs in proximal tubules
    - Sodium ions move from lumen of the tubule to the interior of the tubular cell and hydrogen ions are counter-transported into the tubule lumen.

SYMPORT AND ANTIPORT

- In secondary active transport, the two molecules being transported may move either in the same direction (i.e., both into the cell), or in opposite directions (i.e., one into and one out of the cell). When they move in the same direction, the protein that transports them is called a **symporter**, while if they move in opposite directions, the protein is called an **antiporter**.



ACTIVE TRANSPORT THROUGH CELLULAR SHEETS

- Transport of this type occurs through the

  (1) intestinal epithelium

  (2) epithelium of the renal tubules

  (3) epithelium of all exocrine glands

  (4) epithelium of the gallbladder

  (5) membrane of the choroid plexus of the brain

BASIC MECHANISM

(1) active transport through the cell membrane on one side of the transporting cells in the sheet, and then

(2) either simple diffusion or facilitated diffusion through the membrane on the opposite side of the cell

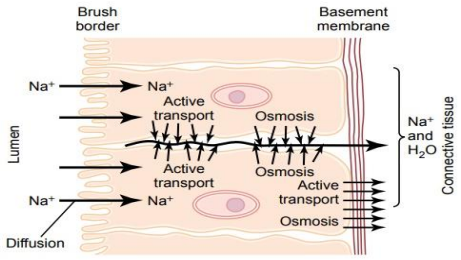


Figure 4–13

Basic mechanism of active transport across a layer of cells.

- This figure shows that the epithelial cells are connected together tightly at the luminal pole by means of junctions. The brush border on the luminal surfaces of the cells is permeable to both sodium ions and water. Therefore, sodium and water diffuse readily from the lumen into the interior of the cell. Then, at the basal and lateral membranes of the cells, sodium ions are

actively transported into the extracellular fluid of the surrounding connective tissue and blood vessels. This action creates a high sodium ion concentration gradient across these membranes, which in turn causes osmosis of water as well. Thus, active transport of sodium ions at the basolateral sides of the epithelial cells results in transport not only of sodium ions but also of water. It is through these mechanisms that almost all nutrients, ions, and other substances are absorbed into the blood from the intestine. These mechanisms are also the way the same substances are reabsorbed from the glomerular filtrate by the renal tubules.