BIOL 152 CELL FUNCTION

LECTURER:
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AKANWARIWIAK
Professor of Biological Sciences

ATTENDANCE AT LECTURES

- You should be reminded that attendance at lectures forms an integral part of the course assessment.
- 10 % will be awarded to students at the end of the semester for 100 % attendance at lectures.

ATTENDANCE AT LECTURES CONT'D.

However, if any student absents herself/himself for a cumulative total of three (3) lecture periods before mid-semester, she/he will not be eligible to write both the mid-semester and end of semester examinations or will not be eligible to write the end of semester examination if this occurs after the mid-semester examination.

ATTENDANCE AT LECTURES CONT'D.

- Any student(s) affected by this rule will however, be scored deferred (Df) and not zero (0%) for the course.
- Any student who absents herself or himself from lectures with my express permission or for any reason acceptable to me will not be adversely affected.
- Pass mark for the course is 40%. Mid-Semester is 30% and end of Semester is 70%.

READING OR REFERENCE MATERIAL.

- Cell Biology, 1st Edition by C. A. Smith and E. J. Wood.
- Molecules of Life, Structure and Function at a glance, 2nd Edition, by J. P. Adjimani.

READING OR REFERENCE MATERIAL CONT'D.

- Biology, Concepts and Connections, 2nd Edition, by Neil A. Campbell, Lawrence G. Mitchell and Jane B. Reece.

READING OR REFERENCE MATERIAL

- Principles and techniques of practical Biochemistry. Fourth Edition and edited by Keith Wilson and John Walker.
- Techniques used in Bioproduct Analysis. Published by Butterworth-Heinemann Ltd.
- Structure and Function of cells, by Colin R. Hopkins.

READING OR REFERENCE MATERIAL CONT'D.

- Cell Biology, Structure, Biochemistry and function, by Philip Sheeler and Donald E. Bianchi etc
- Microbiology: An Introduction (8th Edition) by Gerard J. Tortora, Berdell R. Funke and Christine L. Case.

READING OR REFERENCE MATERIAL CONT'D.

- Cell and Molecular Biology, Concepts and Experiments, 3rd Edition, by Gerald Karp.
- Biology, 8th Edition, Pearson International, by N. A. Campbell, J. B. Reece, L. A. Urry, M. L. Cain, S. A. Wasserman, P. V. Minorsky and R. B. Jackson.

READING OR REFERENCE MATERIAL CONT'D.

- Cell and Molecular Biology, Concepts and Experiments, 3rd Edition, by Gerald Karp.

What Industry looks for in potential employees: Employability Skills

- Some examples of employability skills are:

i. Communication and Interpersonal Skills

ii. Problem Solving skills

iii. Initiative

iv. Working under pressure

v. Organisational skills

What Industry looks for in potential employees: Employability Skills Cont'd

vi. Team working

vii. Adaptability

viii. Numeracy

ix. Valuing diversity and difference

x. Critical thinking

COURSE ASSESSMENT

- The course assessment will be in two components:

1. Mid-Semester – This will carry 30 % of the total End of Semester Examination marks.

Mid-semester Examinations is from Monday, 19th March 2018 to Friday, March 23rd 2018.

COURSE ASSESSMENT CONT'D.

- The Examination will either be wholly MCQs or wholly fill in the blanks. It shall be of 1 hour duration.
- The instruction shall be; Answer as many questions as you can.

COURSE ASSESSMENT CONT'D.

2. The end of Semester Examination shall carry 70 % of the total marks.

- This Examination shall be made of 100 MCQs. The end of semester Examination will take place from Monday, April 30, 2018 to Friday, May 11, 2018.

BROAD COURSE OBJECTIVES.

- At the end of the Semester, you should be able to:
- Define and explain with examples the various transport phenomena in biological systems such as passive or simple diffusion, facilitated diffusion, active transport, osmosis, filtration,

BROAD COURSE OBJECTIVES CONT'D.

exocytosis, endocytosis etc.

- Define enzymes and enzyme systems.
- State the characteristics of enzymes and enzyme catalysed reactions.

BROAD COURSE OBJECTIVES CONT'D.

- Classify enzymes.
- Understand the usefulness of the Michaelis-Menten equation as in Enzyme kinetics.
- Explain competitive, non-competitive and uncompetitive inhibition.

BROAD COURSE OBJECTIVES CONT'D.

- Plot graphs and compare the characteristics of normal, competitive, non-competitive and uncompetitive inhibited reactions.

BROAD COURSE OBJECTIVES CONT'D.

- Understand the usage of terminologies such as metabolism, catabolism and anabolism.
- Explain glycolysis, TCA and electron transport system as applied to carbohydrate metabolism.

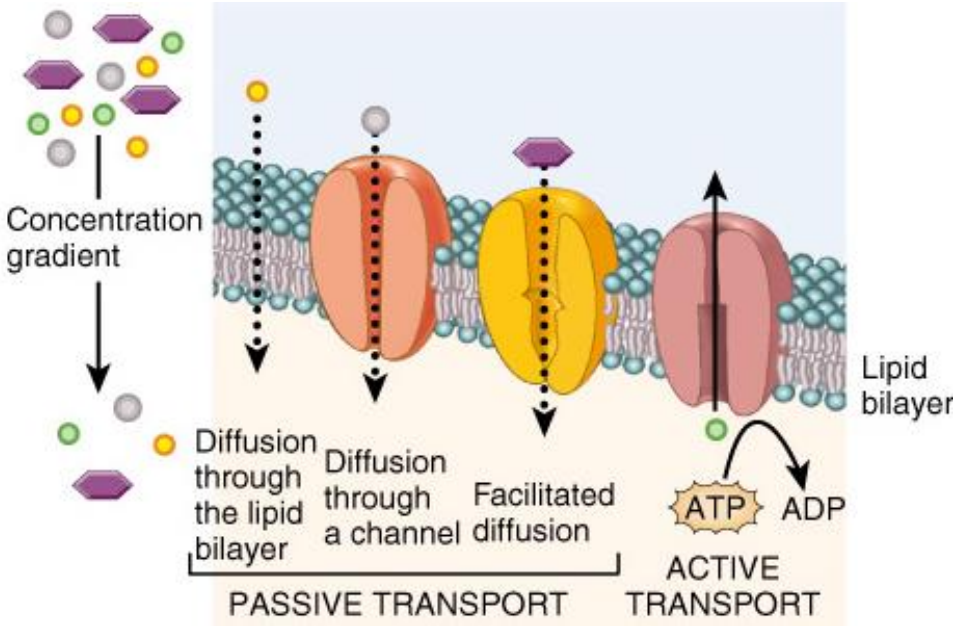
BROAD COURSE OBJECTIVES CONT'D.

- Understand lipid and protein metabolism and how energy is generated from these.
- Explain the various metabolic pathways and the importance of the various by-products.

- **TRANSPORT ACROSS MEMBRANES:**
  - ***–Mediated transport*** moves materials with the help of a transporter protein
  - ***–Non-mediated transport*** does not use a transporter protein
  - ***–active transport*** uses ATP to drive substances ***against*** their concentration gradients
  - –passive transport moves substances ***down their concentration gradient*** with only their kinetic energy
  - ***–vesicular transport*** move materials across membranes in small vesicles - either by exocytosis or endocytosis

Transport Across the Plasma Membrane

- Substances cross membranes by a variety of processes:
  - mediated transport moves materials with the help of a transporter protein
  - nonmediated transport does not use a transporter protein
  - active transport uses ATP to drive substances against their concentration gradients



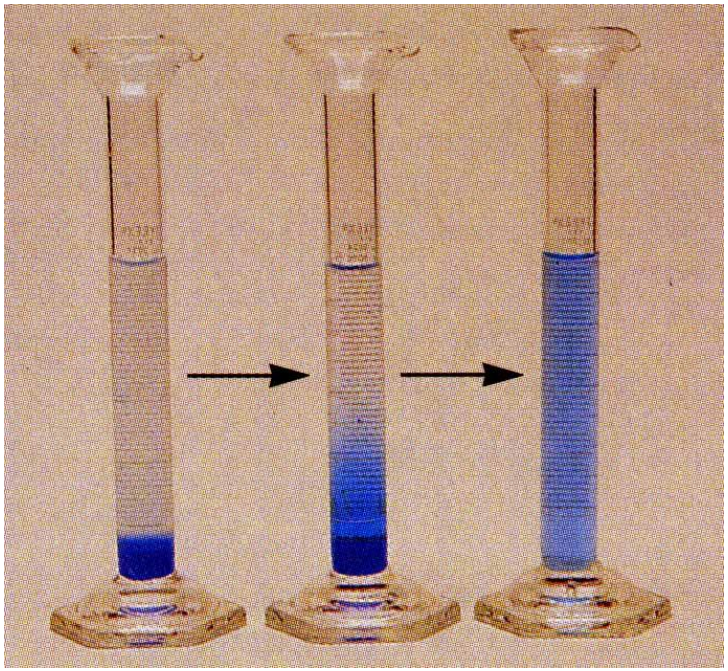
  - passive transport moves substances down their concentration gradient with only their kinetic energy
  - vesicular transport move materials across membranes in small vesicles -- either by exocytosis or endocytosis

PRINCIPLES OF DIFFUSION

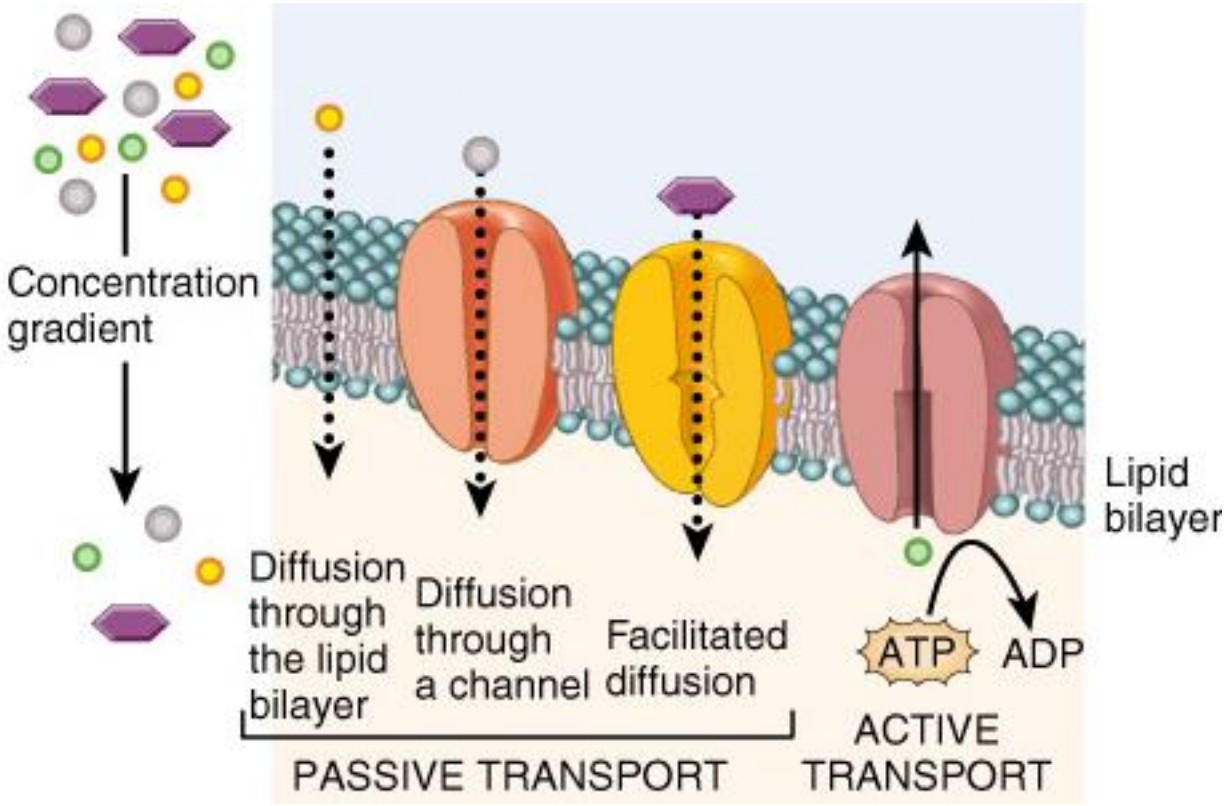
- Random mixing of particles in a solution as a result of the particle's kinetic energy
  - **more molecules move away from an area of high concentration to an area of low concentration**
    - the greater the difference in concentration between the 2 sides of the membrane, the faster the rate of diffusion
    - the higher the temperature, the faster the rate of diffusion
    - the larger the size of the diffusing substance, the slower the rate of diffusion
    - an increase in surface area, increases the rate of diffusion
    - increasing diffusion distance, slows rate of diffusion
- Equilibrium will be reached when the molecules are evenly distributed.

DIFFUSION

- A crystal of a dye placed in a cylinder of water
- Net diffusion from the higher dye concentration to the region of lower dye (upper portion of the cylinder)
- Equilibrium has been reached in the far right cylinder (uniform colour)



Diffusion Through the Lipid Bilayer



- Important for absorption of nutrients -- excretion of wastes
- Nonpolar, hydrophobic molecules
  - oxygen, carbon dioxide, nitrogen, fatty acids, steroids, small alcohols, ammonia and fat-soluble vitamins (A, E, D and K)

SIMPLE DIFFUSION

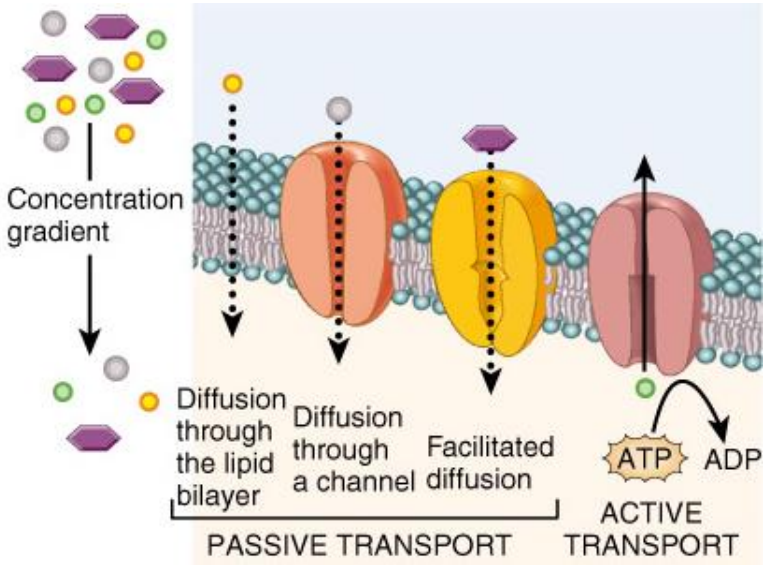
It refers to a process whereby a substance passes through a membrane without the aid of an intermediary such as an integral membrane protein.

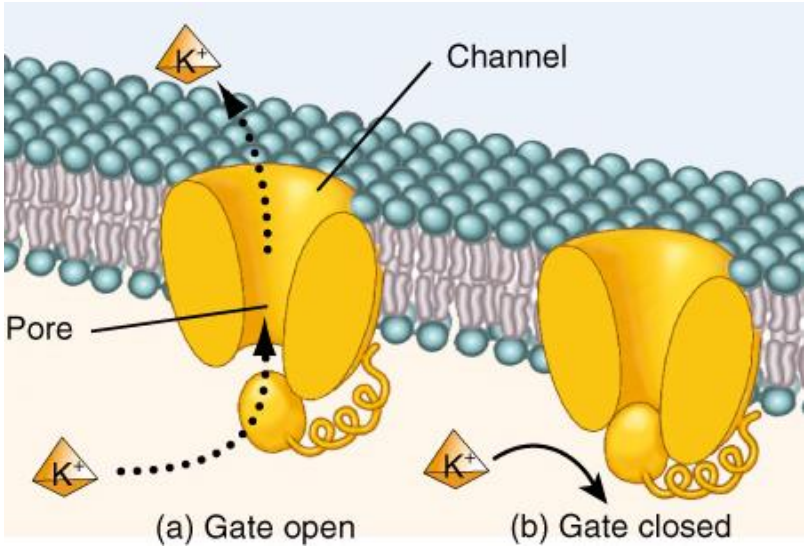
- Lipid/non polar soluble substances cross cell membranes readily than water soluble substances

Simple Diffusion Cont'd.

- Cell membranes are impermeable to large water soluble substances
- The direction of movement of solutes by diffusion is always from a higher to a lower concentration.
- It refers to a process whereby a substance passes through a membrane without the aid of an intermediary such as an integral membrane protein.

Diffusion Through Membrane Channels





- Each membrane channel is specific for particular ion eg K^+, Cl^-, Na^+ or Ca^{+2}
- Slower than diffusion through membrane but still 1million K^+ through a channel in one second
- Channels may be open all the time or gated (closed randomly or as ordered)

Facilitated Diffusion: Channels

- Ions are relatively lipid insoluble. Ionic diffusion is via protein channels in plasma membranes.
- Channels:

- are selectively permeable to certain substances
- may be open or closed by gates

- Gated channels may be
  - voltage-gated
  - ligand (chemical)-gated

Channels Cont'd.

- mechanically-gated
- Stretch - gated

Movement of ions is also affected by their electrical charge.

- The net transmembrane flux of an ion is proportional to the combined effect of the electrical and concentration/chemical gradients

Ligand-gated ion channels

- Many ion channels open or close in response to a small signalling molecule or “ligand”. Some ion channels are gated by extracellular ligands; some by intracellular ligands. In both cases, the ligand is not the substance that is transported

External Ligands

- External ligands bind to a site on the extracellular side of the channel
- Examples:

  Acetyl choline – Binding at certain synapses opens channels that admit Na^+ and initiate a nerve impulse or

External and Internal Ligands

muscle contraction.

- Internal ligands: Bind to a site on the channel protein to the cytosol.
- Example:

  ATP is needed to open the channel allowing Cl^- ions out of the cell.

Internal ligands

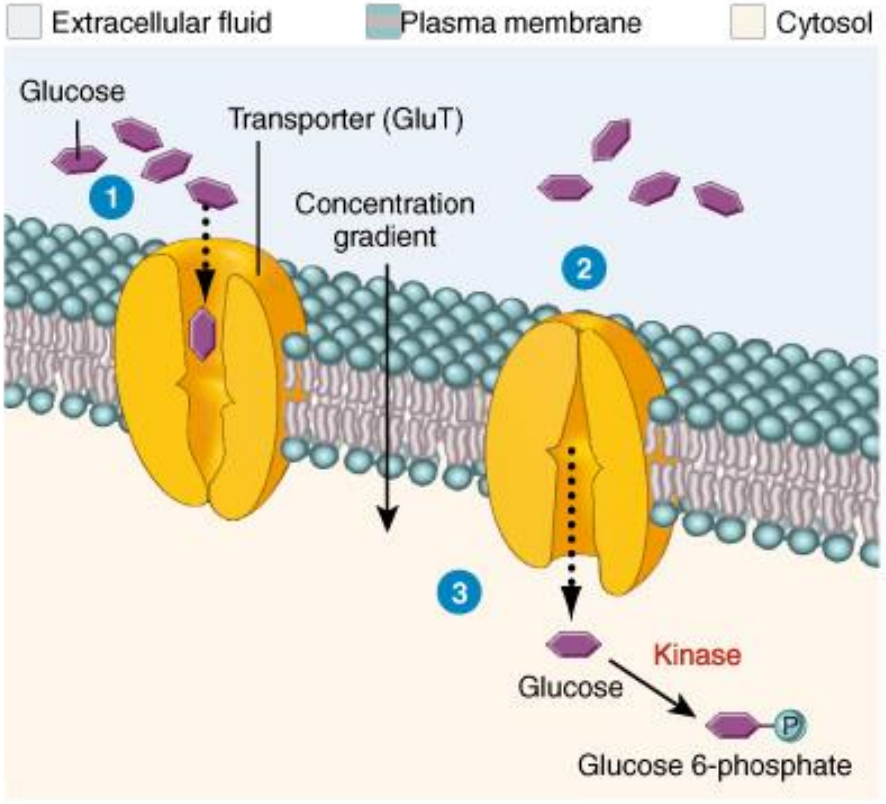
- This channel is defective in patients with cystic fibrosis.
- Voltage-gated channels are found in excitable tissues eg nerves and muscles. They open in response to changes in the charge across the plasma membrane.

Facilitated Diffusion

- Substance binds to specific transporter protein
- Transporter protein conformational change moves substance across cell membrane
- Facilitated diffusion occurs down concentration gradient only
  - if no concentration difference exists, no net movement across membrane occurs
- Rate of movement depends upon
  - steepness of concentration gradient
  - number of transporter proteins (transport maximum)

Facilitated Diffusion of Glucose

- Glucose binds to transport protein
- Transport protein changes shape
- Glucose moves across cell membrane (but only down the concentration gradient)
- Kinase enzyme reduces glucose concentration inside the cell by transforming glucose into glucose-6-phosphate
- Transporter proteins always bring glucose into cell

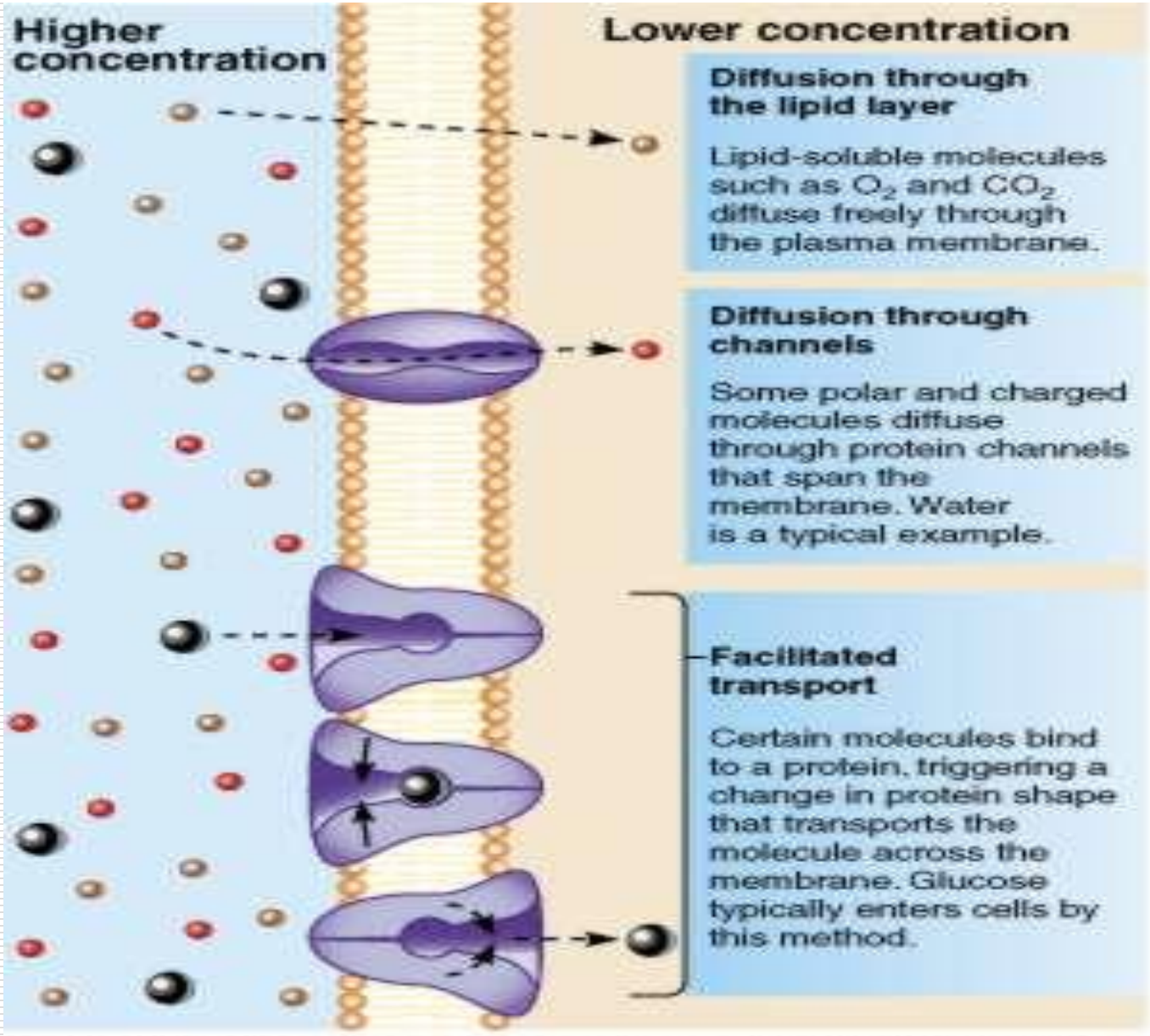


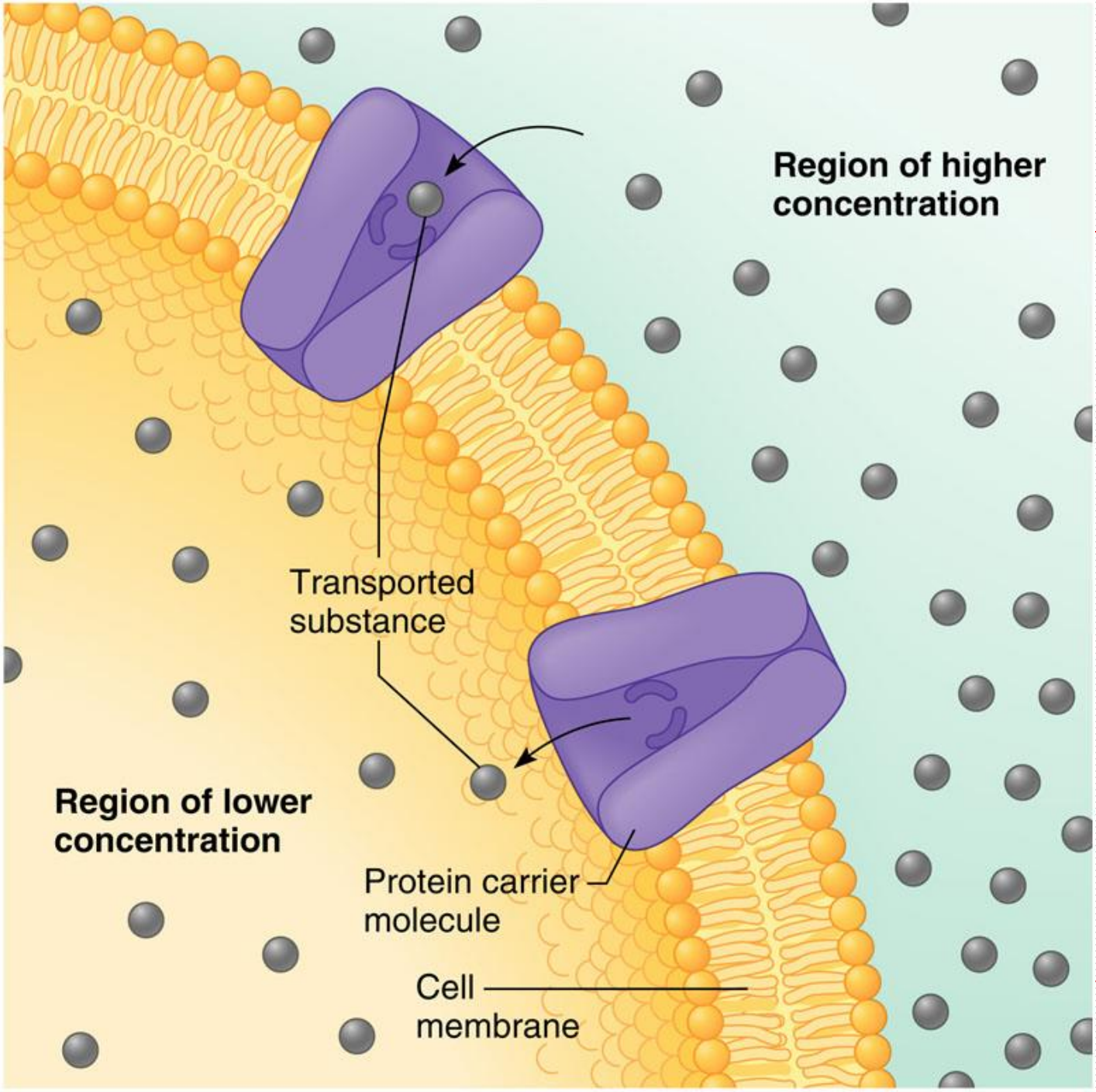
Facilitated diffusion

- Facilitated diffusion eg glucose, amino acids
  - Like simple diffusion net flux is down an electrochemical gradient
  - requires a membrane carrier that spans the thickness of the membrane
  - requires no metabolic energy input
  - exhibits characteristics of carrier-mediated transport

Facilitated diffusion Cont'd.

- more rapid than simple diffusion
  - saturation kinetics
  - substrate
  - stereo specificity
  - competition
  - inhibition



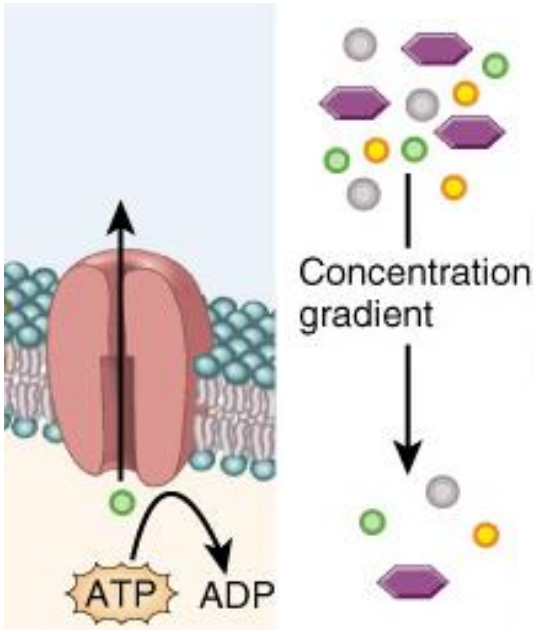


Active Transport

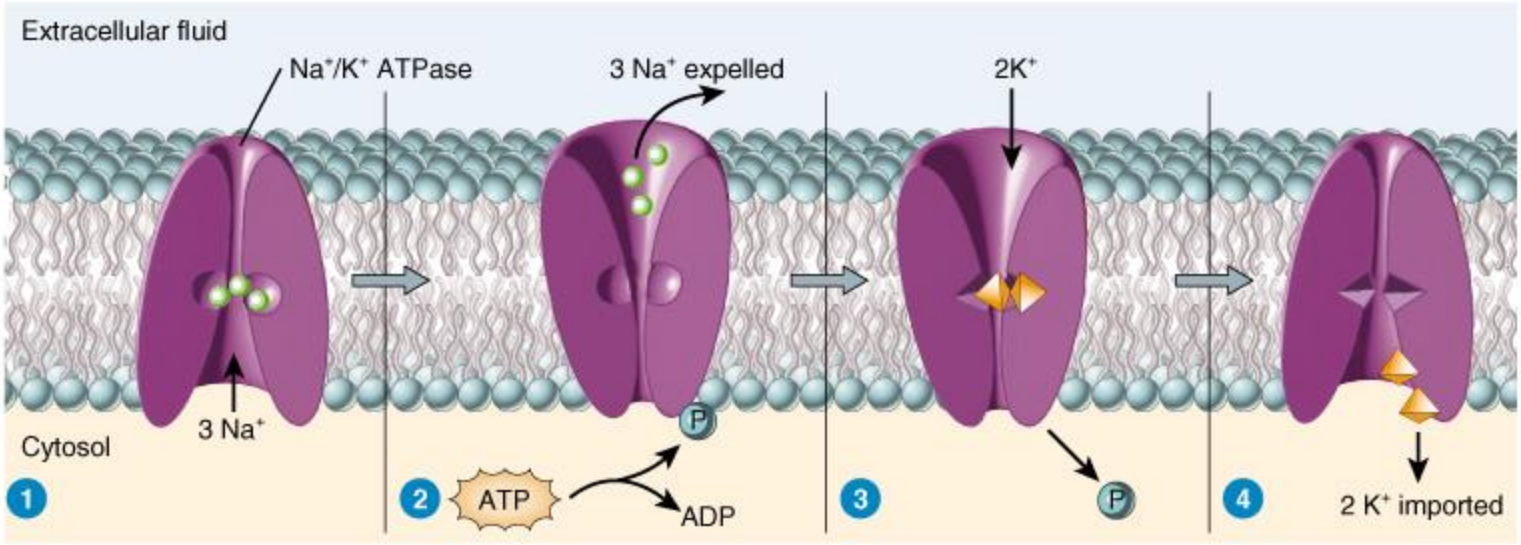
- Movement of polar or charged substances against their concentration gradient
  - energy-requiring process
    - energy from hydrolysis of ATP (primary active transport)
    - energy stored in an ionic concentration gradient (secondary active transport)
- Exhibits transport maximums and saturation
- Na^{+}, K^{+}, H^{+}, Ca^{+2}, I^{-} and Cl^{-}, amino acids and monosaccharides

Primary Active Transport

- Transporter protein called a "pump"
  - works against concentration gradient
  - requires 40% of cellular ATP
- Na^+/K^+ ATPase pump
  most common example
  - all cells have 1000s of them
  - maintains low concentration of Na^+ and a high concentration of K^+ in the cytosol
  - operates continually
- Maintenance of osmotic pressure across membrane
  - cells neither shrink nor swell due to osmosis & osmotic pressure
    - sodium continually pumped out as if sodium could not enter the cell (factor in osmotic pressure of extracellular fluid)
    - K^+ inside the cell contributes to osmotic pressure of cytosol



Na^+/K^+ Pump & ATP As Its Energy Source



1. Na^+ binding
2. ATP split
3. Na^+pushed out
4. K^+ binding
5. Phosphate release
6. K^+ is pushed in

3 Na^+ ions removed from cell as 2 K^+ brought into cell.

Active transport

❖Active transport

- In active transport substances are moved against their electrochemical gradient
- requires a carrier protein
- requires energy
- exhibits characteristics of carrier-mediated transport
- Two types – Primary and secondary active

Active transport Cont'd

- Primary Active Transport
- Utilizes metabolic energy in the form of ATP

  eg Na^+-K^+ATPase, Ca^{2+}ATPase,

  H^+-K^+ATPase etc

Direct Active Transport

1. The Na^+/K^+ ATPase

   The cytosol of animal cells contains a concentration of K^+ as much 20 times higher than that in the extracellutar fluid. Conversely, the extracelluar fluid contains a concentration of Na^+ as much as 10 times greater than

Direct Active Transport Cont'd.

that within the cell.

- These concentration gradients are established by the active transport of both ions. And, in fact, the same transporter, called the Na^+/K^+ ATPase, does both jobs. It uses energy from the hydrolysis of ATP

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Direct Active Transport Cont'd.

to:

- Actively transport 3 Na^+ ions out of the cell, for each 2 K^+ ions pumped into the cell.
- This accomplishes several vital functions:

Direct Active Transport Cont'd.

- It helps establish a net charge across the plasma membrane with the interior of the cell being negatively charged with respect to the exterior. This resting potential prepares nerve and muscle cells for the propagation of action potentials

Direct Active Transport Cont'd.

leading to nerve impulses and muscle contraction.

- The accumulation of Na^+ ions outside of the cell draws water out of the cell and thus enables it to maintain osmotic balance.

Direct Active Transport Cont'd.

- The gradient of Na^+ ions is harnessed to provide the energy to run several types of indirect pumps.

2. The H^+/K^+ ATPase

   The parietal cells of the stomach use this pump to secrete gastric

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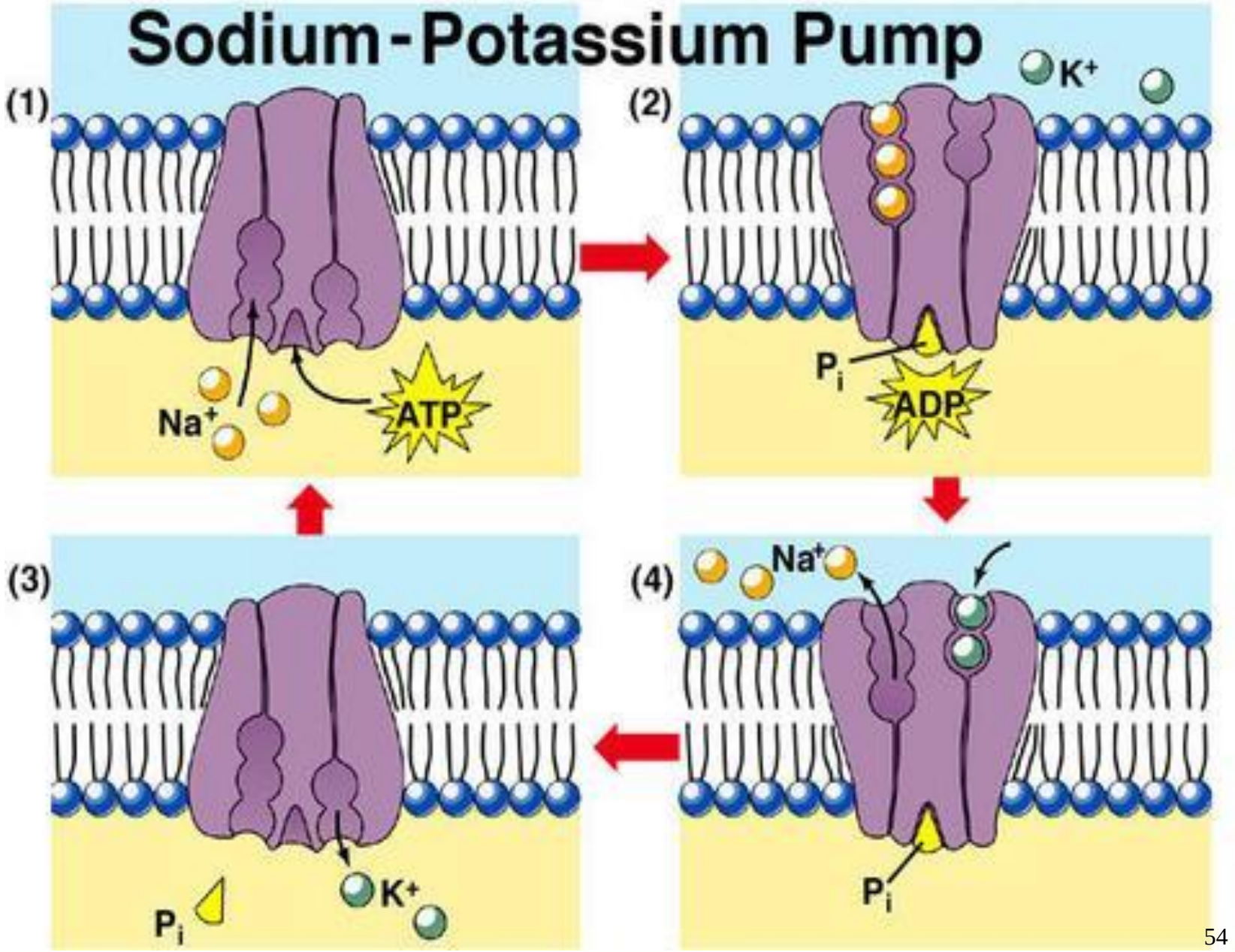
Direct Active Transport Cont'd.

juice. These transport H^+ ions from a concentration of about $4x10^{-8}$ M within the cell to a concentration of about 0.15 M in the gastric juice giving it a pH close to 1.0.



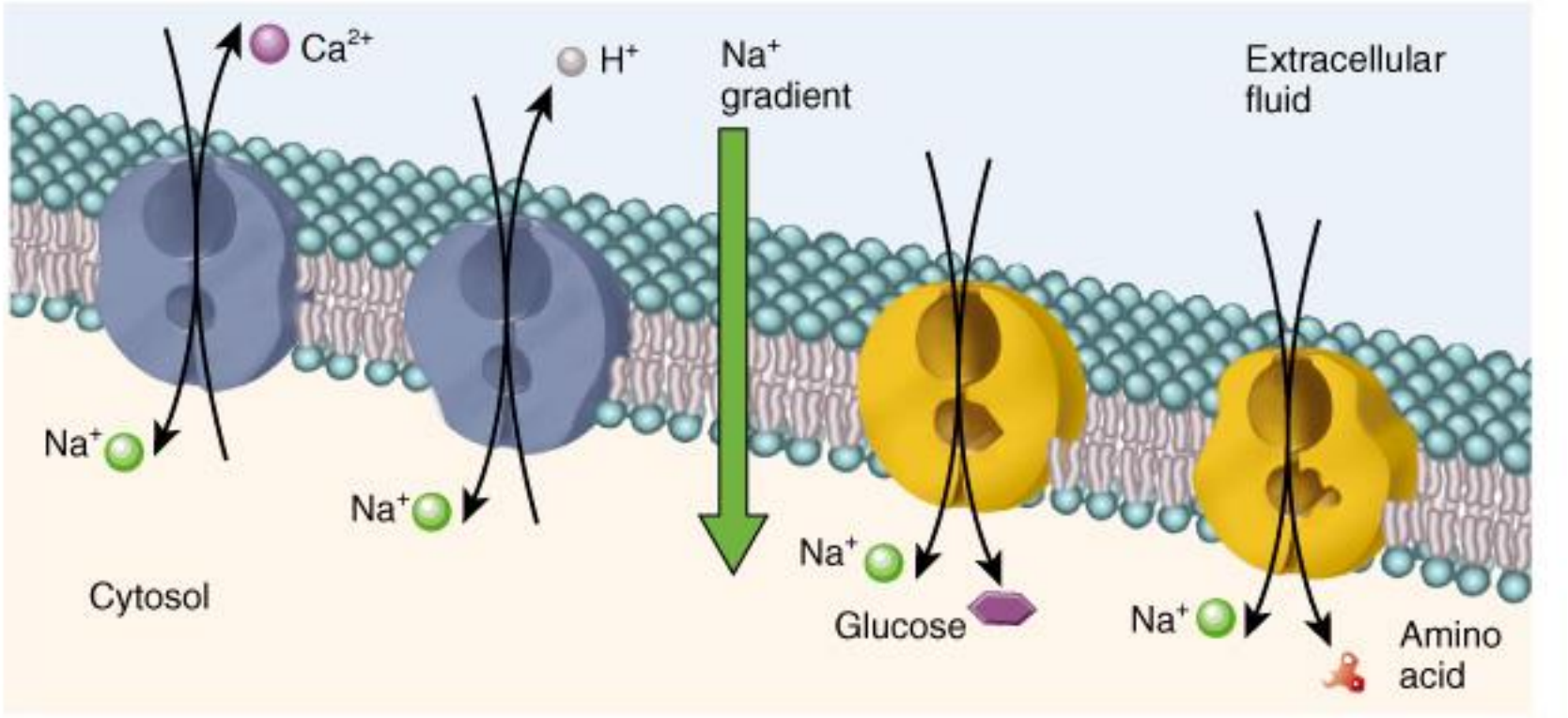
❑ Secondary Active Transport

- Indirect utilization of metabolic energy
- This utilizes the energy released during the passive movement of one substance down its electrochemical gradient to transport another substance against a concentration gradient eg co-transport of Na^+ and glucose, co-transport of Na^+ and amino acids

Secondary Active Transport

- Uses energy stored in an ion concentration gradient to move other substances against their own concentration gradient
- Na^+/K^+ pump maintains low concentration of Na^+ inside of cells
  - provide route for Na^+ to leak back in and use energy of motion to transport other substances
  - Na^+ symporter proteins
    - glucose or amino acids rush inward with Na^+ ions
  - Na^+ antiporters protein
    - as Na^+ ions rush inward, Ca^{+2} or H^+ push out

Antiporters and Symporters



One in & one out.

Both going in

Symports

- In this type of indirect active transport, the driving ion (Na^+) and the pumped molecule pass through the membrane pump in the same direction.

Symports Cont'd.

- Examples:

1. The Na^+/glucose transporter

   This trans-membrane protein allows Na^+ ions and glucose to enter the cell together. The Na^+ ions flow down their concentration gradient while the

Symports Cont'd.

glucose molecules are pumped up theirs. Later the Na^+ is pumped back out of the cell by the Na^+/K^+ ATPase.

- The Na^+/glucose transporter is used to actively transport glucose out of the intestine and also out of the

Symports Cont'd.

kidney tubules and back into the blood.

- All the amino acids can be actively transported eg out of the kidney tubules and into the blood by sodium-driven transporters.

Symports Cont'd.

- The Na^+/iodide transporter

  This symporter pumps iodide ions into the cells of the thyroid gland and also into the cells of the mammary gland.

Antiports Pumps

- In antiport pumps, the driving ion (usually Na^+) diffuses through the pump in one direction providing the energy for the active transport of some other molecules or ion in the opposite direction.

Antiports Pumps Cont'd

- Examples:

1. Mg^{2+} ions are pumped out of cells by a sodium-driven antiport pump.
2. The Na^{+}/K^{+} ATPase is also an antiport using the energy of ATP.

Antiports Pumps Cont'd

to pump Na^+ out of the cell; K^+ in.

- Some inherited ion-channel diseases

1. Chloride –channel diseases:
   - Cystic fibrosis

Inherited ion-channel diseases

- Inherited tendency to kidney stones

2. Potassium-channel diseases:

- Some inherited life-threatening defects in the heartbeat
- A rare, inherited tendency to epileptic seizures in the newborn

Inherited ion-channel diseases Cont'd

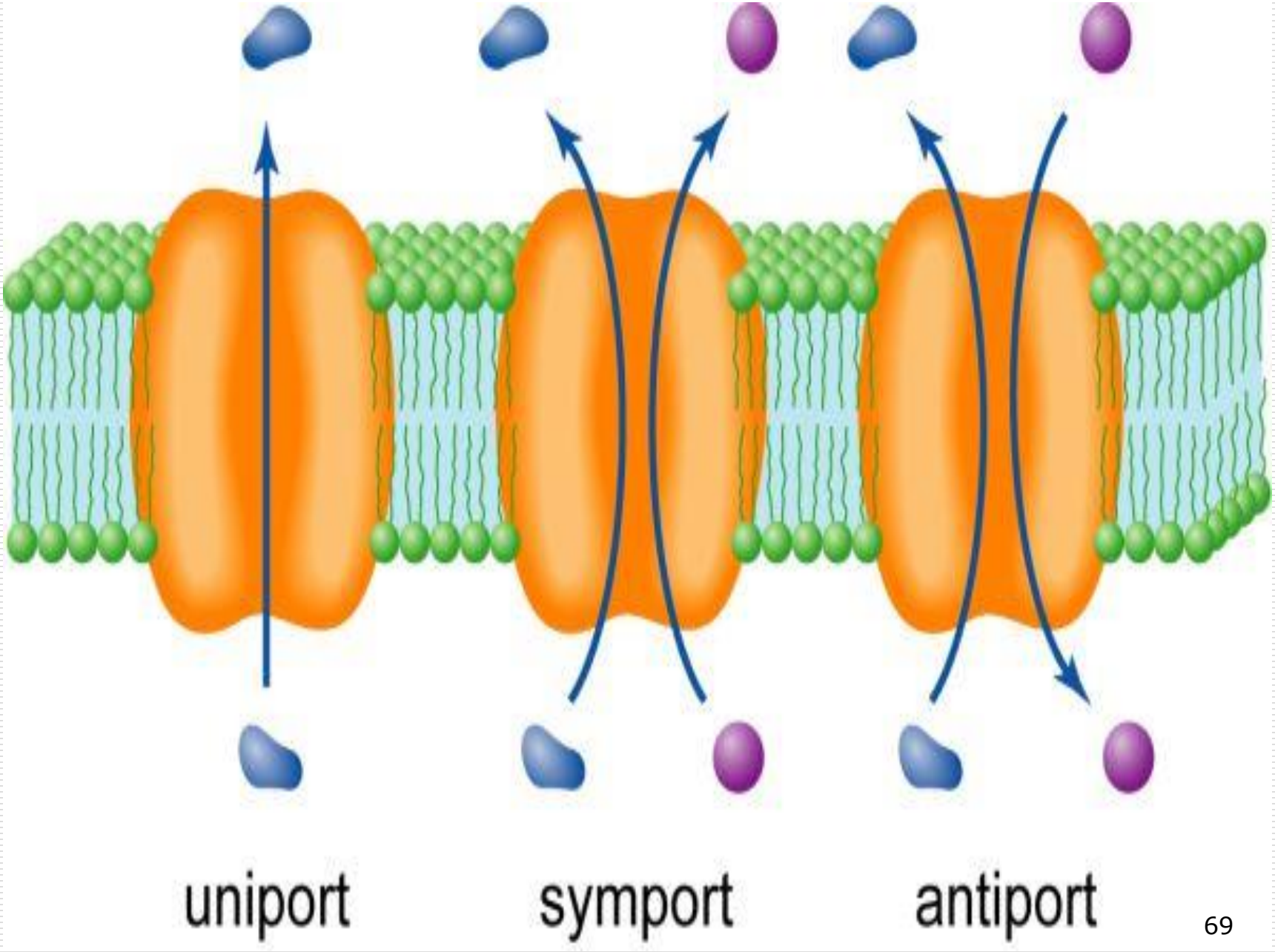
- Several types of inherited deafness

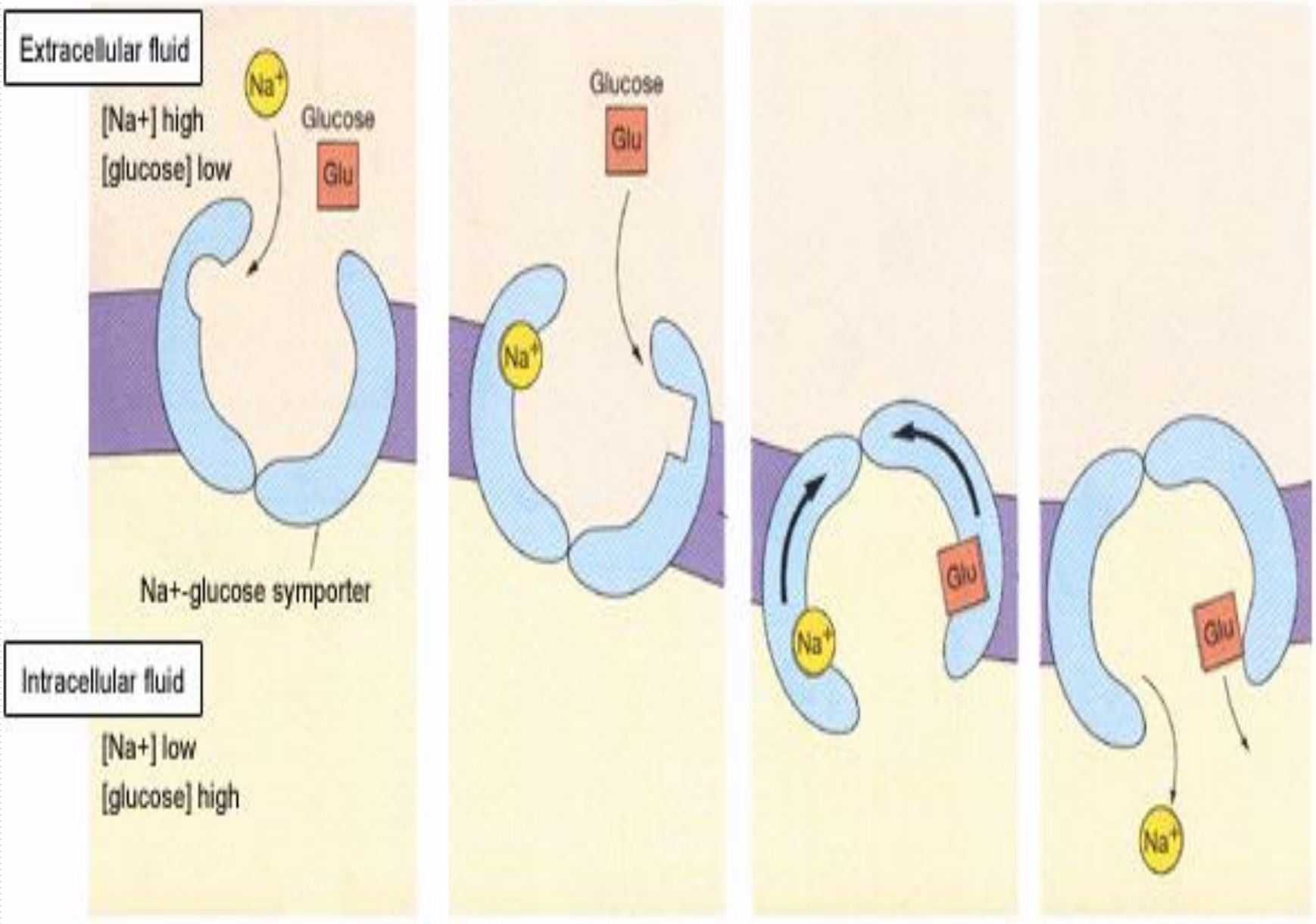
3. Sodium-channel diseases

- Inherited tendency to certain types of muscle spasms
- Liddle's syndrome: Inadequate Na^+ transport out of the kidneys,

Inherited ion-channel diseases Cont'd

because of a mutant sodium channel, leads to elevated osmotic pressure of the blood and resulting in hypertension (high blood pressure).


uniport
symport
antiport


Extracellular fluid
[Na+] high
[glucose] low
Na+
Glucose
Glu
Na+-glucose symporter
Intracellular fluid
[Na+] low
[glucose] high
Glucose
Glu
Na+
Glu
Na+
Glu
Na+

Osmosis

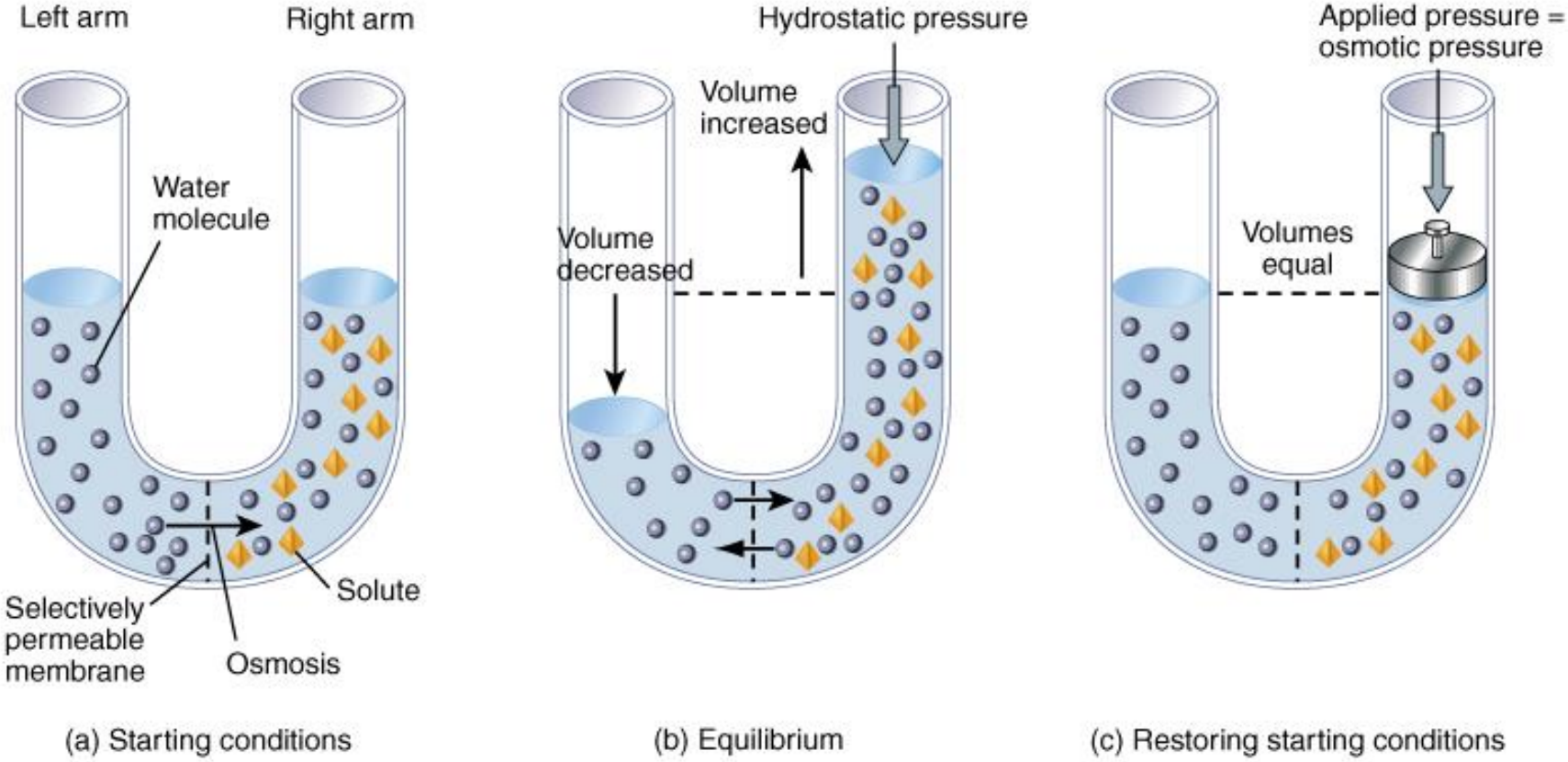
- Net movement of water through a selectively permeable membrane from an area of high water concentration to an area of low water concentration
  - diffusion through lipid bilayer
  - aquaporins (transmembrane proteins) that function as water channels
- Only occurs if membrane is permeable to water but not to certain solutes

❖ Osmosis

The passive diffusion of water across a semipermeable membrane from a region of low solute concentration to a region of high solute concentration ie the passage of water from a region of high water concentration through a semi-permeable membrane to a region of low water concentration.

- Presence of solute results in a decrease in the chemical potential of water
- osmosis generates a pressure called osmotic/ hydrostatic ('water-stopping') pressure.

Osmosis of Water Through a Membrane

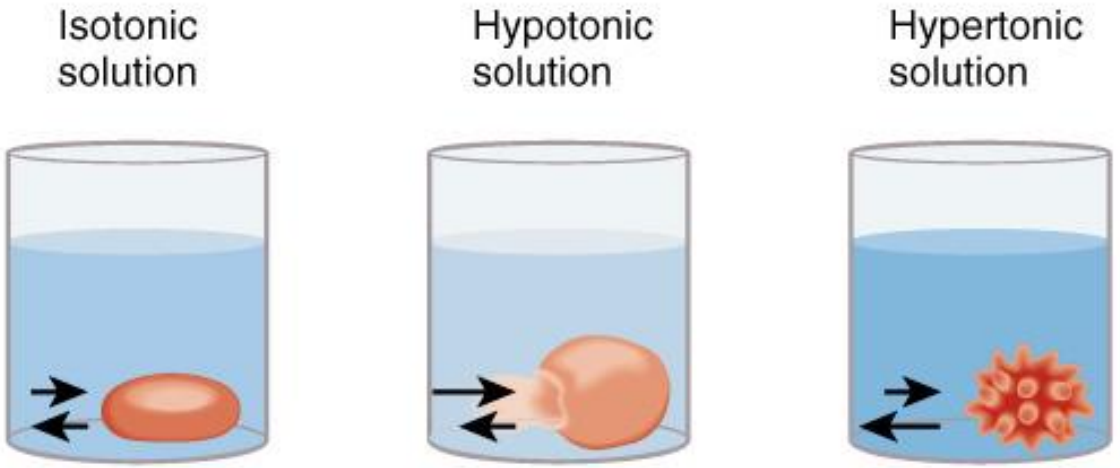


- Pure water on the left side & a membrane impermeable to the solute found on the right side
- Net movement of water is from left to right, until hydrostatic pressure (osmotic pressure) starts to push water back to the left

Effects of Tonicity on RBCs in Lab

- Normally the osmotic pressure of the inside of the cell is equal to the fluid outside the cell
  - cell volume remains constant (solution is isotonic)
- Effects of fluids on RBCs in lab
  - water enters the cell faster than it leaves
  - water enters & leaves the cell in equal amounts
  - water leaves the cell

Effects of Tonicity on Cell Membranes



- Isotonic solution
  - water concentration the same inside & outside of cell results in no net movement of water across cell membrane
- Hypotonic solution
  - higher concentration of water outside of cell results in haemolysis
- Hypertonic solution
  - lower concentration of water outside of cell causes crenation

Digitalis

- Slows the sodium pump, which lets more Na+ accumulate in heart muscle cells.
- Less Na+ concentration gradient across the membrane
- Na+/Ca+2 antiporters slow down so more Ca+2 remains inside the cardiac cells
- Strengthening the force of contraction
- Balance between concentration of Na+ and Ca+2 in cytosol & extracellular fluid is important

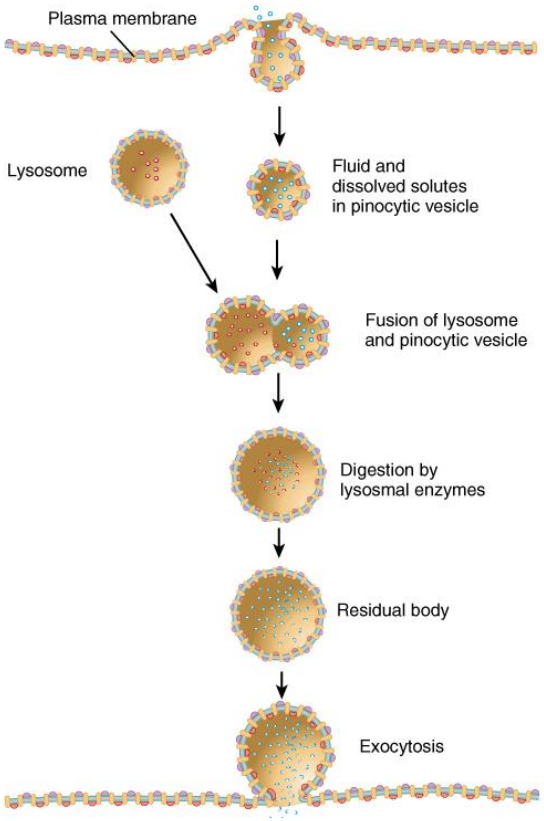
Vesicular Transport of Particles

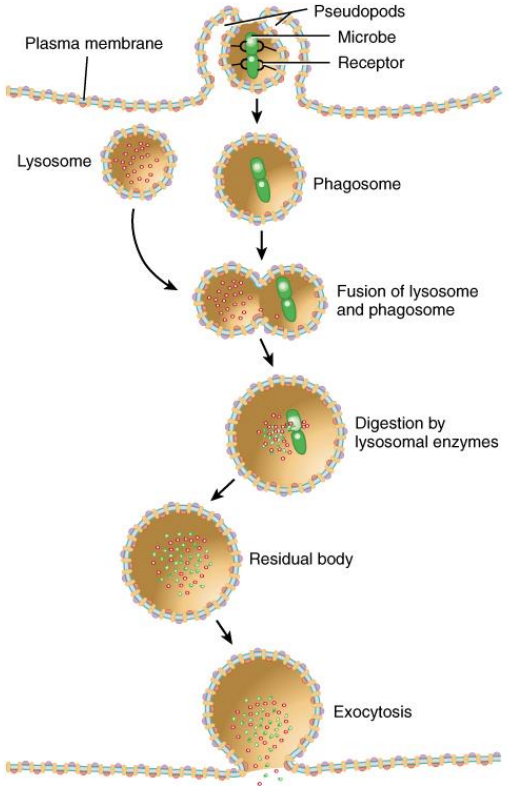
- Endocytosis = bringing something into cell
  - phagocytosis = cell eating by macrophages & WBCs
    - particle binds to receptor protein
    - whole bacteria or viruses are engulfed & later digested
  - pinocytosis = cell drinking
    - no receptor proteins
  - receptor-mediated endocytosis = selective input
    - mechanism by which HIV virus enters cells
- Exocytosis = release something from cell
  - Vesicles form inside cell, fuse to cell membrane
  - Release their contents
    - digestive enzymes, hormones, neurotransmitters or waste products
  - replace cell membrane lost by endocytosis

Receptor-Mediated Endocytosis

- Mechanism for uptake of specific substances -- ligands
- Desired substance binds to receptor protein in clathrin-coated pit region of cell membrane causing membrane to fold inward
- Vesicles become uncoated & combine with endosome
- Receptor proteins separate from ligands and return to surface
- Ligands are digested by lysosomal enzymes or transported across cell -- epithelial cell crossing accomplished

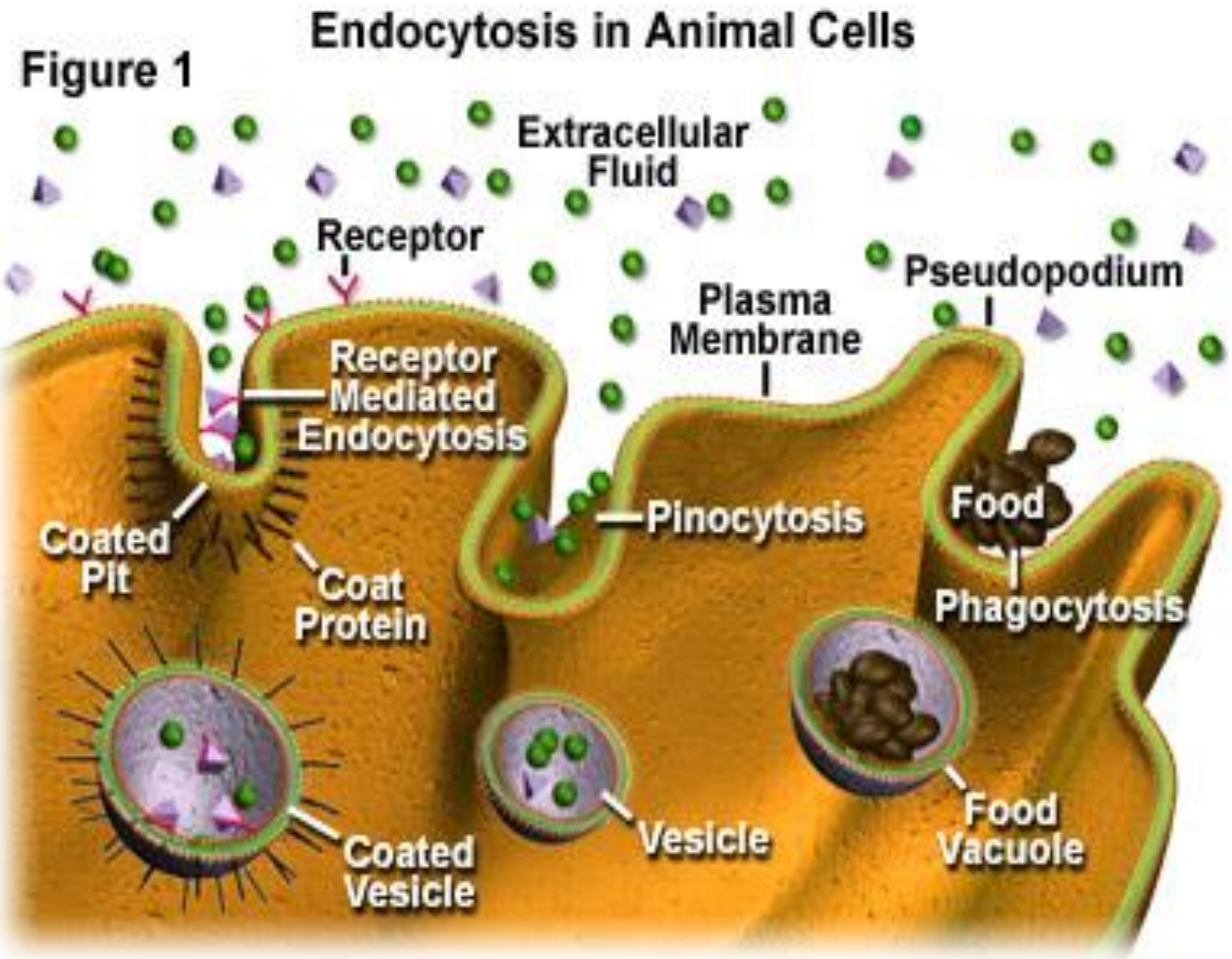
Pinocytosis and Phagocytosis





- No pseudopods form
- Nonselective drinking of extracellular fluid

- Pseudopods extend to form phagosome
- Lysosome joins it


Endocytosis in Animal Cells
Figure 1
Extracellular Fluid
Receptor
Pseudopodium
Plasma Membrane
Receptor Mediated Endocytosis
Pinocytosis
Food
Coated Pit
Coat Protein
Phagocytosis
Coated Vesicle
Vesicle
Food Vacuole

❖ Filtration

The process by which fluid is forced through a membrane/barrier because of differences in pressure on the two sides. The amount of fluid filtered is proportionate to

- the pressure difference
- surface area of the membrane
- permeability of the membrane

ENZYMES

- Enzymes are protein in nature
- Are produced by living cells of plants and animals
- Are capable of functioning within or without the cell
- Act as biological catalysts and so, usually speed up the rate of biochemical reactions

Characteristics of enzymes

- **Characteristics of Enzymes**
  - 1) They are proteins.
  - 2) They are highly specific in their reactions.
  - 3) They reduce the need for activation energy in bringing about chemical reactions.

Characteristics of enzymes cont'd.

- Enzymes speed up the rate of biochemical reactions without affecting the equilibrium composition of the mixture.
- Enzymes remain unchanged at the end of the reaction.
- They are needed in minute quantities

Characteristics of enzyme catalysed reactions

Characteristics of enzyme action:

- How enzymes function, how fast they cause reactions to go, when they start working and when they stop are all influenced by intricate control mechanisms within the cell.

Characteristics of enzyme catalysed reactions Cont'd.

- Many of these factors affecting enzymes are "environmental" in nature (i.e. factors in the surroundings). While others are specific control mechanisms of the cell and the enzyme itself.

Characteristics of enzyme catalysed reactions Cont'd.

Factors Affecting Rate of Reaction:

- Enzyme reactions are fast. Eg Catalase can repeat its reaction 600,000 times per second.

1) The higher the concentration of substrate the more rapid the reaction (to a point).

2) Heat will affect the rate of reaction

Characteristics of enzyme catalysed reactions Cont'd.

- Increasing the temperature will increase the rate of reaction.10^0C up doubles reaction rate **but** eventually you reach a temperature when the enzyme (protein) **denatures** and the reaction stops.

Characteristics of enzyme catalysed reactions Cont'd.

- Every enzyme has a temperature range of optimum activity. Outside that temperature range the enzyme is rendered inactive and is said to be totally inhibited. This occurs because as the temperature changes this supplies enough energy to break some of the

Characteristics of enzyme catalysed reactions Cont'd.

intramolecular attractions between polar groups (Hydrogen bonding, dipole-dipole attractions) as well as the Hydrophobic forces between non-polar groups within the protein structure. When these forces are disturbed and changed, this causes a change in the secondary and tertiary levels of protein

Characteristics of enzyme catalysed reactions Cont'd.

- structure, and the active site is conformation beyond its ability to accomodate the substrate molecules it was intended to catalyze.

Characteristics of enzyme catalysed reactions Cont'd.

P^H Change and Reaction Rate

- Each enzyme operates at an optimum pH. Some perform best at high (basic) pH others at lower (acid) pH.

Characteristics of enzyme catalysed reactions Cont'd.

- Some enzymes like many of the hydrolytic enzymes in the stomach such as Pepsin and Chymotrypsin effectively operate at a very low acidic pH. Other enzymes like alpha amylase found in the saliva of the mouth operate most effectively at near neutrality.

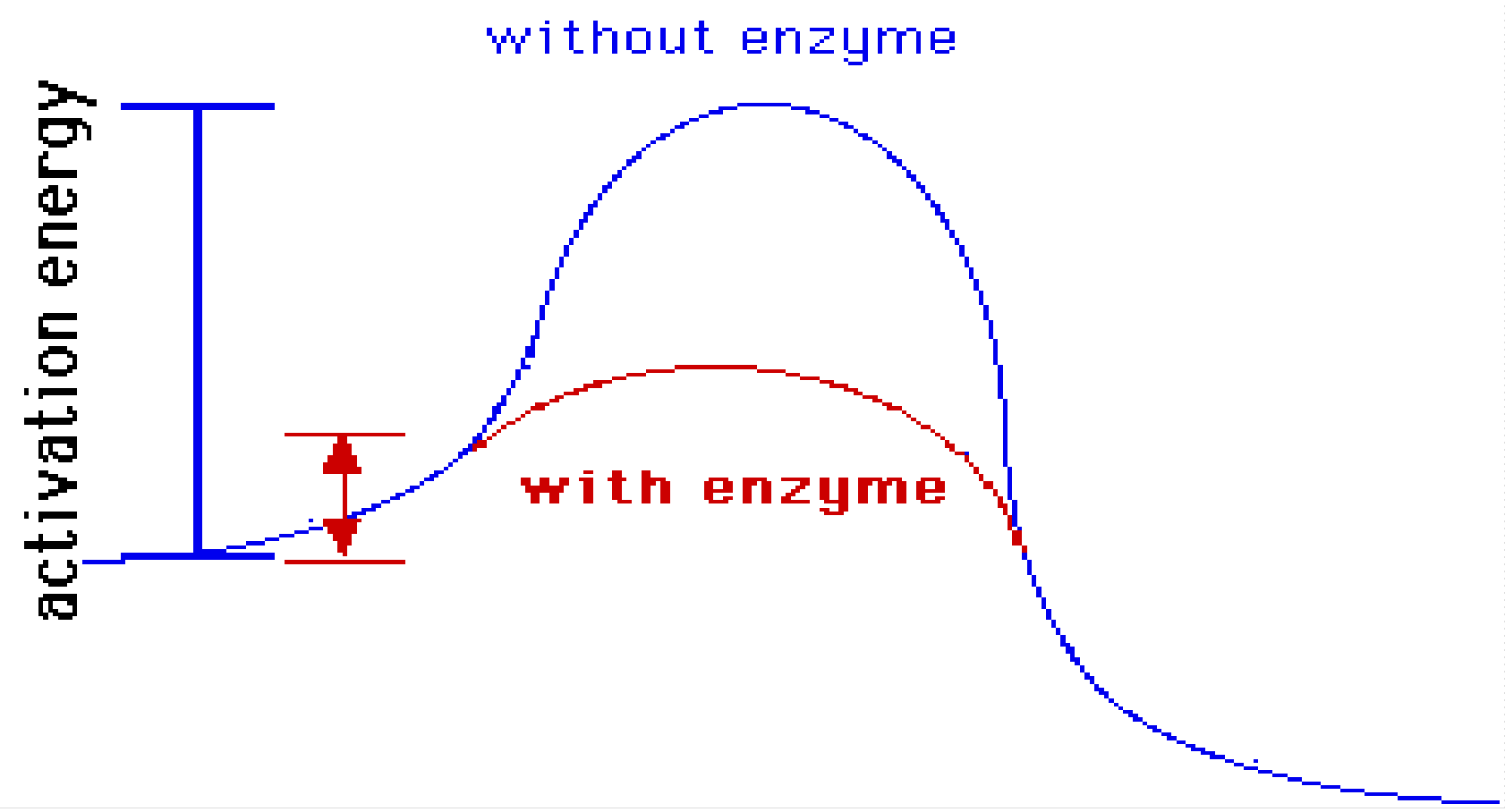
Characteristics of enzyme catalysed reactions Cont'd.

- Still other enzymes like the lipases will function most effectively at basic pH values.

Characteristics of enzyme catalysed reactions Cont'd.

- Enzymes do not initiate biochemical reactions in the body but speed up the rate of a thermodynamically feasible reaction by lowering the energy of activation.

Characteristics of enzyme catalysed reactions Cont'd.



Characteristics of enzyme catalysed reactions Cont'd.

- Catalysts are substances that increase product formation by (1) lowering the energy barrier (activation energy) for the product to form and (2) increases the favorable orientation of colliding reactant molecules for product formation to be successful.

Characteristics of enzyme catalysed reactions Cont'd.

- There are two types of catalysts:
  - Heterogeneous Catalysts
  - Homogeneous Catalysts
- Heterogeneous catalysts are those that provide a surface for the reaction to proceed upon. The catalyst and the reactant molecules are not in the same

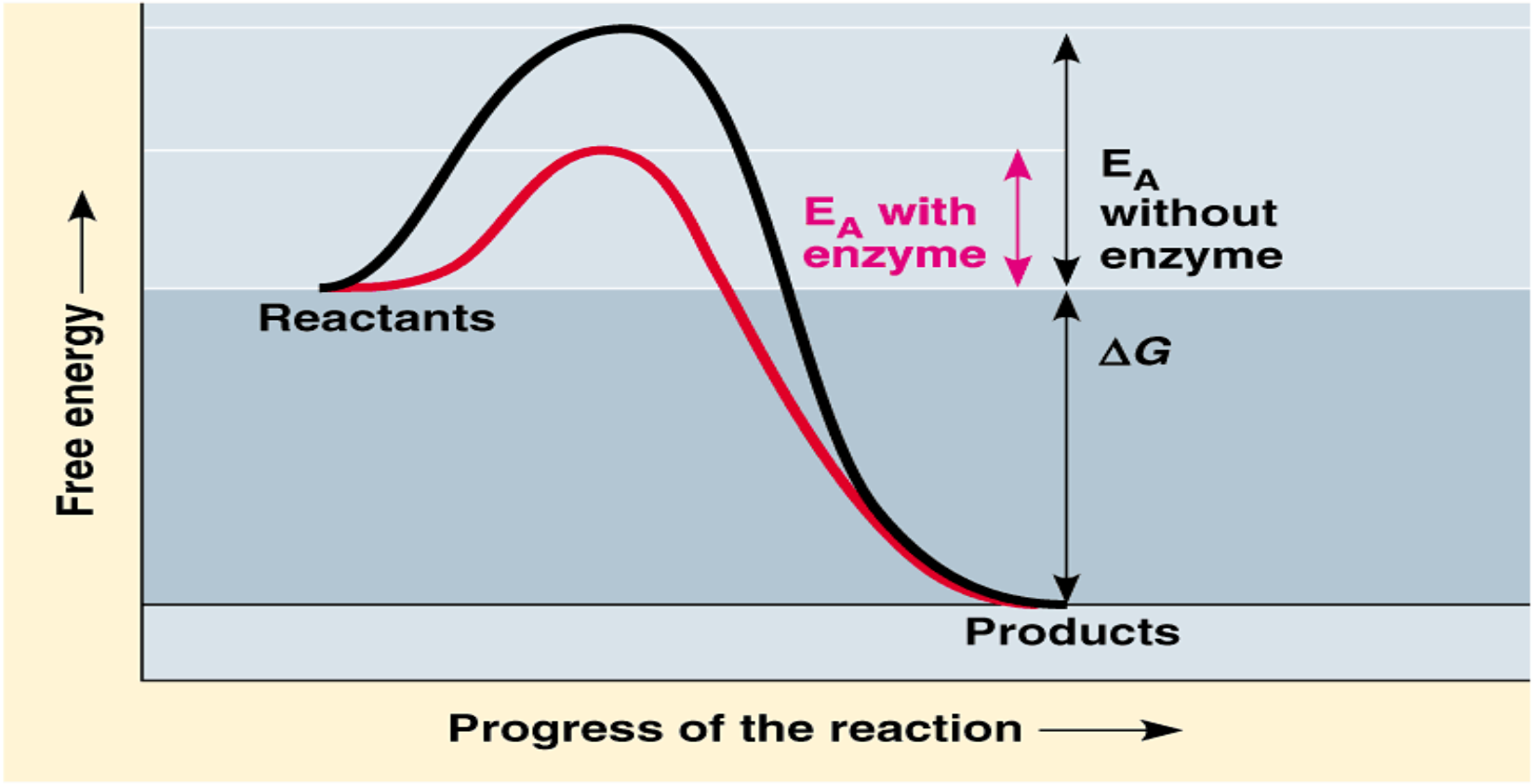
Characteristics of enzyme catalysed reactions Cont'd.

phase. This is sometimes referred to as surface catalysts. Certain transition state metals like Pladium, Platinum, Nickel, and Iron serve as industrial catalysts that catalyze a wide variety of reactions such as Hydrogenation.

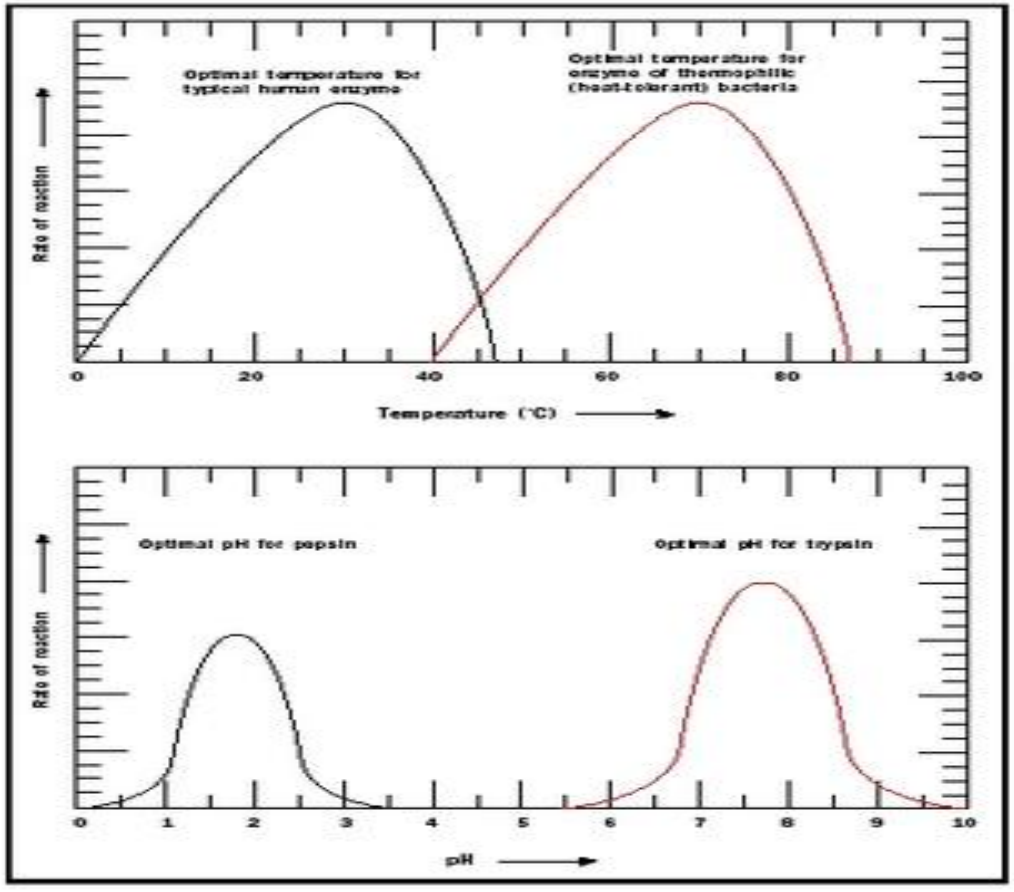
Characteristics of enzyme catalysed reactions Cont'd.

- Homogeneous catalysts are catalysts that exist in the same phase as the reactant molecules usually in a solution. Acids and Bases in solution serve as catalysts in a wide variety of Organic reactions.
- Enzymes belong to this category.

Characteristics of enzyme catalysed reactions Cont'd.



Effect of Temp. and pH.



PARTS OF AN ENZYME

- Some enzymes such as trypsin, consists of only protein. Most enzymes, however, contain a protein part called an apoenzyme that is inactive without a non-protein component called the cofactor. Together, the apoenzyme and cofactor are an activated holoenzyme.

PARTS OF AN ENZYME CONT'D.

- Many cofactors are metallic ions eg. Fe^{2+}, Mn^{2+}, Zn+, Mg^{2+}, Ca^{2+} etc.
- Many other enzymes require more complex organic molecules to produce their catalytic activity. These are called **coenzymes**.
- Many of these coenzymes are derived from the so-called **vitamins**.

Mechanism of Enzyme Action.

- The basic mechanism by which enzymes catalyze chemical reactions begins with the binding of the **substrate** (or substrates) to the active site on the enzyme. The **active site** is the specific region of the enzyme which combines with the substrate.

Mechanism of Enzyme Action Cont'd..

- The **active site** has a unique geometric shape that is complementary to the geometric shape of a substrate molecule, similar to the fit of puzzle pieces. This means that enzymes specifically react with only one or a very few similar compounds.

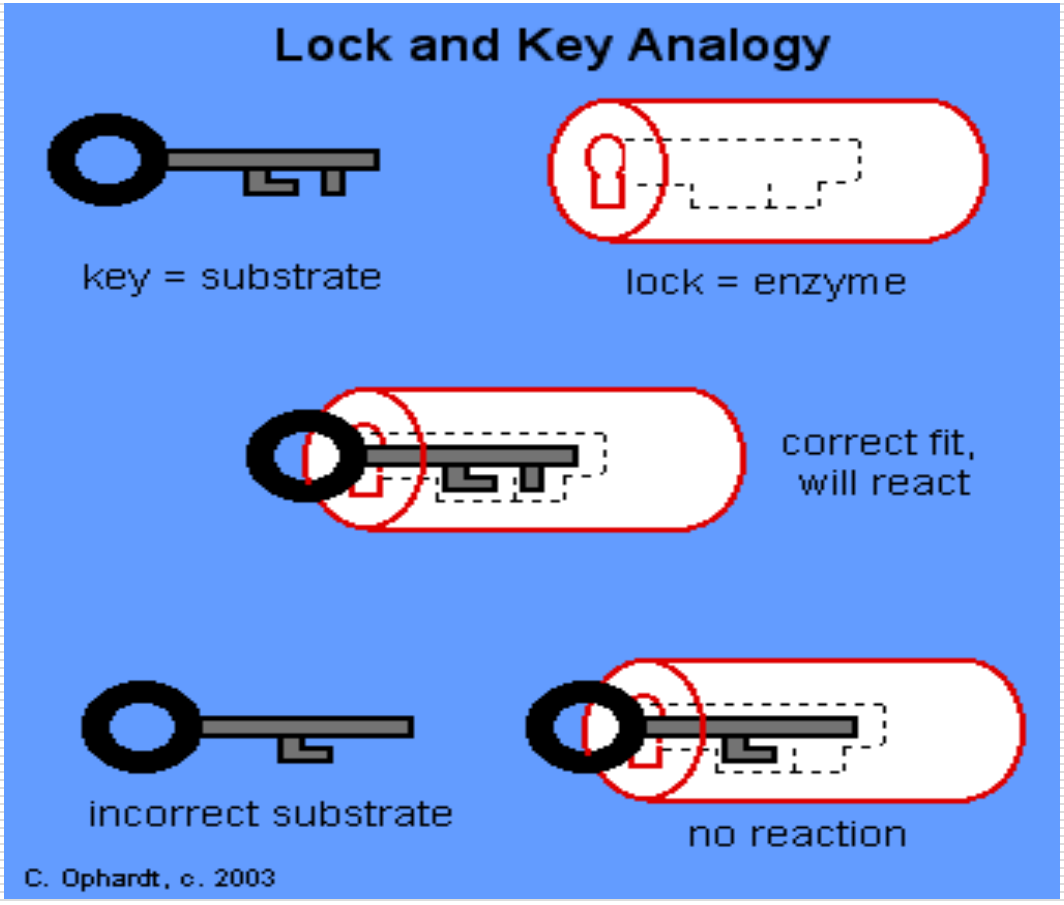
Lock and Key Theory.

- The specific action of an enzyme with a single substrate can be explained using a Lock and Key analogy. In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized key (substrate) fits into the key hole (active site) of the lock (enzyme).

Lock and Key Theory Cont'd.

- Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized substrate molecules) do not fit into the lock (enzyme). Only the correctly shaped key opens a particular lock.

Lock and Key Theory Cont'd.



Induced Fit Theory.

- Not all experimental evidence can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theory. For this reason, a modification called the induced-fit theory has been proposed.

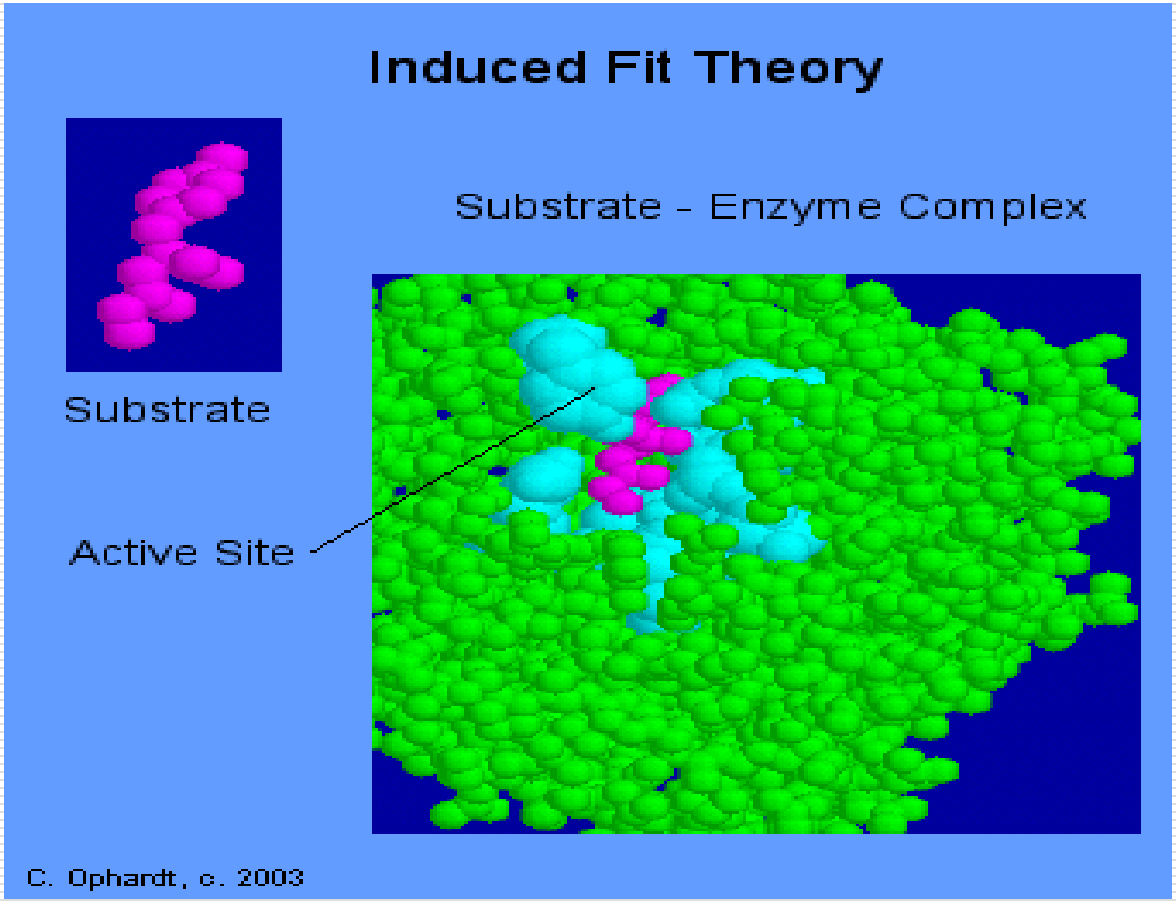
Induced Fit Theory Cont'd.

- The induced-fit theory assumes that the substrate plays a role in determining the final shape of the enzyme and that the enzyme is partially flexible. This explains why certain compounds can bind to the enzyme but do not react because the enzyme has been distorted too much.

Induced Fit Theory Cont'd.

- Other molecules may be too small to induce the proper alignment and therefore cannot react. Only the proper substrate is capable of inducing the proper alignment of the active site.

Induced Fit Theory



CLASSIFICATION OF ENZYMES.

Classes of Enzymes

Enzyme Commission (E.C.) 4.1.1.32

1. Oxidoreductases- lactate dehydrogenase
2. Transferases- glucokinase
3. Hydrolases- chymotrypsin, G6Pase
4. Lyases- fumarase
5. Isomerases- phosphoglucoisomerase
6. Ligases- Acyl CoA synthetase

Oxidoreductase

- In biochemistry, an **oxidoreductase** is an enzyme that catalyzes the transfer of electrons from one molecule (the reductant, also called the hydrogen acceptor or electron donor) to another (the oxidant, also called the hydrogen donor or electron acceptor).

Oxidoreductase Cont'd

- For example, an enzyme that catalyzed this reaction would be an oxidoreductase:
  - $A^- + B \rightarrow A + B^-$
- In this example, A is the reductant (electron donor) and B is the oxidant (electron acceptor).

TRANSFERASE

- In biochemistry, a **transferase** is an enzyme that catalyzes the transfer of a functional group (e.g. a methyl or phosphate group) from one molecule (called the donor) to another (called the acceptor). For example, an enzyme that catalyzed this reaction would be a transferase:

TRANSFERASE CONT'D.

- $A–X + B \rightarrow A + B–X$

- In this example, A would be the donor, and B would be the acceptor. The donor is often a coenzyme.

HYDROLASE.

- In biochemistry, a **hydrolase** is an enzyme that catalyzes the hydrolysis of a chemical bond. For example, an enzyme that catalyzed the following reaction is a hydrolase:
  - $A–B + H_2O \rightarrow A–OH + B–H$

LYASE.

- In biochemistry, a **lyase** is an enzyme that catalyzes the breaking of various chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure. For example, an enzyme that catalyzed this reaction would be a lyase:
  - ATP → cAMP + PPi

LYASE CONT'D.

- Lyases differ from other enzymes in that they only require one substrate for the reaction in one direction, but two substrates for the reverse reaction.

ISOMERASE

- A major class of enzymes comprising those that catalyze the process of isomerization, such as the interconversion of aldoses and ketoses.

- 1,5 disulfide i. — an enzyme that catalyzes disulfide bond formation by

ISOMERASE CONT'D.

- cross-linking certain cystine residues of polypeptides; occurs as a post-translational modification.
- Isomerases thus, catalyze reactions of the form:
  - $A \rightarrow B$

LIGASE.

- In biochemistry, a **ligase** (from the Latin verb *ligāre* — "to bind" or "to glue together") is an enzyme that can catalyse the joining of two large molecules by forming a new chemical bond, usually with accompanying hydrolysis of a small chemical group pendant to one of the larger molecules.

LIGASE CONT'D.

- Generally ligase catalyses the following reaction:
  - $Ab + C \rightarrow A–C + b$

ENZYME KINETICS

- To explain the saturating effect of high substrate concentrations, Michaelis and Menten proposed that enzymes take part in reactions as ff:

Where E stands for enzyme, S for substrate, P for products and ES for the

ENZYME KINETICS CONT'D.

enzyme-substrate complex. In this two step reaction, the enzyme rapidly combines with substrate to form the complex, which can either dissociate again into unchanged substrate and enzyme or go on to the second step and form products and unchanged enzyme.

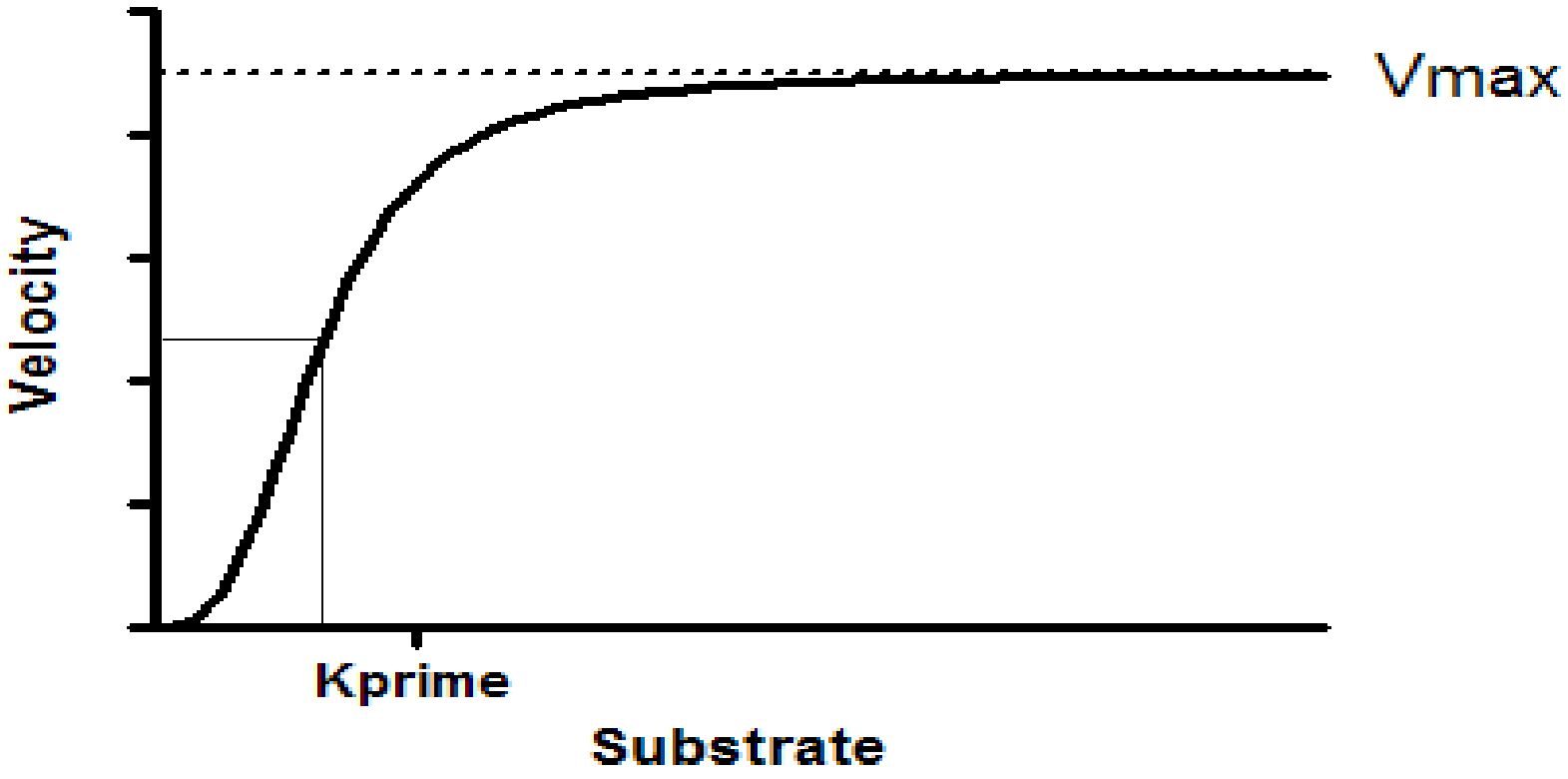
ENZYME KINETICS CONT'D.

The reverse of the second step would lead to the synthesis of ES from enzyme and products, but this process can generally be ignored unless the products are allowed to accumulate. The formation of an enzyme-substrate complex as an intermediate in the reaction provides an explanation for the

ENZYME KINETICS CONT'D.

saturation effect of increasing substrate concentration. The saturation point is reached when the substrate concentration is high enough to ensure that virtually all the enzyme is converted into ES. No further increase in rate can be obtained unless more enzyme is added.

ENZYME KINETICS CONT'D



THE MICHAELIS-MENTEN EQUATION.

- Michaelis and Menten used the reaction in equation (1) to derive a general equation for the relationship between velocity and [S].
- The separate reactions in equation (1) can be assigned rate constants as indicated in equation (2).

THE MICHAELIS-MENTEN EQUATION CONT'D.

- $E + S \underset{k_2}{\overset{k1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k3}{\rightleftharpoons}} E + P$ (2)

  As indicated earlier, the formation of ES from E and P can generally be ignored unless the products accumulate, and on that assumption we can say that the initial rate of formation of the products will be given by the rate equation:

THE MICHAELIS-MENTEN EQUATION CONT'D.

- $V = K_3[ES]$ (3)

  Where, V is equal to the velocity of product formation and [ES] is the concentration of the enzyme-substrate complex.

  Although, we cannot measure [ES]directly, it is clear that the total concentration of enzyme, [E], will be the sum of the concentration of the complex, [ES], and of the free enzyme.

THE MICHAELIS-MENTEN EQUATION CONT'D.

This can be expressed in the form of a conservation equation,

[E] = [ES] + free enzyme (4)

From this it is clear that the concentration of free enzyme is [E] – [ES]. If we also assume that the total substrate concentration [S] is much greater than [E], then we can ignore the amount of S which is present in the ES complex. Now, as long as the formation of

THE MICHAELIS-MENTEN EQUATION CONT'D.

ES from E and P is negligible, the rate of formation of ES is given by:

Rate of ES formation = $k_1([E] - [ES])[S]$ (5)

while the rate at which the complex breaks down is given by:

Rate of breakdown = $k_2[ES] + k_3[ES]$ (6)

When the overall process shown in equation (2) is in a steady state, the rate of formation of the ES complex will equal its rate of

THE MICHAELIS-MENTEN EQUATION CONT'D.

breakdown and this can be expressed in a steady-state equation.

$$k_1([E] - [ES])\,[S] = (K_2 + K_3)\,[ES] \quad (7)$$

or

$$\frac{([E] - [ES])\,[S]}{[ES]} = \frac{K_2 + K_3}{K_1} = k_m \quad (8)$$

THE MICHAELIS-MENTEN EQUATION CONT'D.

where k_m is a new constant called the Michaelis constant. It should be noted that since [E] – [ES] is the concentration of the free enzyme, k_m has the same form as an equilibrium constant. Where k_3 is much smaller than k_1 and k_2, the right side of equation (8) simplifies to:

THE MICHAELIS-MENTEN EQUATION CONT'D.

$$\frac{k_2}{k_1} = k_m = k_s \quad (9)$$

$$V_0 = k_3 \frac{[E_T][S]}{km + [S]} \quad (10)$$

When the [S] is so high that essentially all the enzyme in the sysem is present as the [ES]complex, ie when the enzyme is saturated, we reach the maximum initial

THE MICHAELIS-MENTEN EQUATION CONT'D.

velocity, V_{max}, given by:

$$V_{max} = k_3[E_T] \quad (11)$$

in which $[E_T]$ is the total enzyme concentration. Now, substituting for $k_3[E_T]$, its value from equation (10) will be:

$$V = \frac{Vmax[S]}{km + [S]} \quad (12)$$

THE MICHAELIS-MENTEN EQUATION CONT'D.

The Michaelis-Menten equation relates the initial velocity (V_0), the maximum velocity and the initial [S] through the Michaelis-Menten constant. It is important to note that although the Michaelis-Menten equation appears to have no term for [E], it is actually contained in the term V_{max} which we have seen is equal to $k_3[E]$ (11).

THE MICHAELIS-MENTEN EQUATION CONT'D.

An important numerical relationship emerges from the Michaelis-Menten equation in the special case when the initial reaction rate is exactly ½ the maximum velocity, ie when $V_0 = ½ V_{max}$.

$$\frac{Vmax}{2} = \frac{Vmax[S]}{Km + [S]} \quad (13) \text{ from } (12).$$

THE MICHAELIS-MENTEN EQUATION CONT'D.

If we divide by $V_{max,}$ we obtain,

$$½ = \frac{[S]}{Km + [S]} \qquad (14)$$

On rearranging, this becomes

$K_m + [S] = 2[S]$

$K_m = [S]$

Thus, we see that $K_{m,}$ the Michaelis-Menten

THE MICHAELIS-MENTEN EQUATION CONT'D.

constant, is equal to the [S] at which the initial reaction velocity is half maximal.

LINEWEAVER-BURK TRANSFORMATION OF THE MICHAELIS-MENTEN EQUATION

- In biochemistry, the **Lineweaver-Burk plot** (or **double reciprocal plot**) is a graphical representation of the Lineweaver-Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934

LINEWEAVER-BURK TRANSFORMATION OF THE MICHAELIS-MENTEN EQUATION

- The plot provides a useful graphical method for analysis of the Michaelis-Menten equation:

$$V = V_{max} \frac{[S]}{K_m + [S]}$$

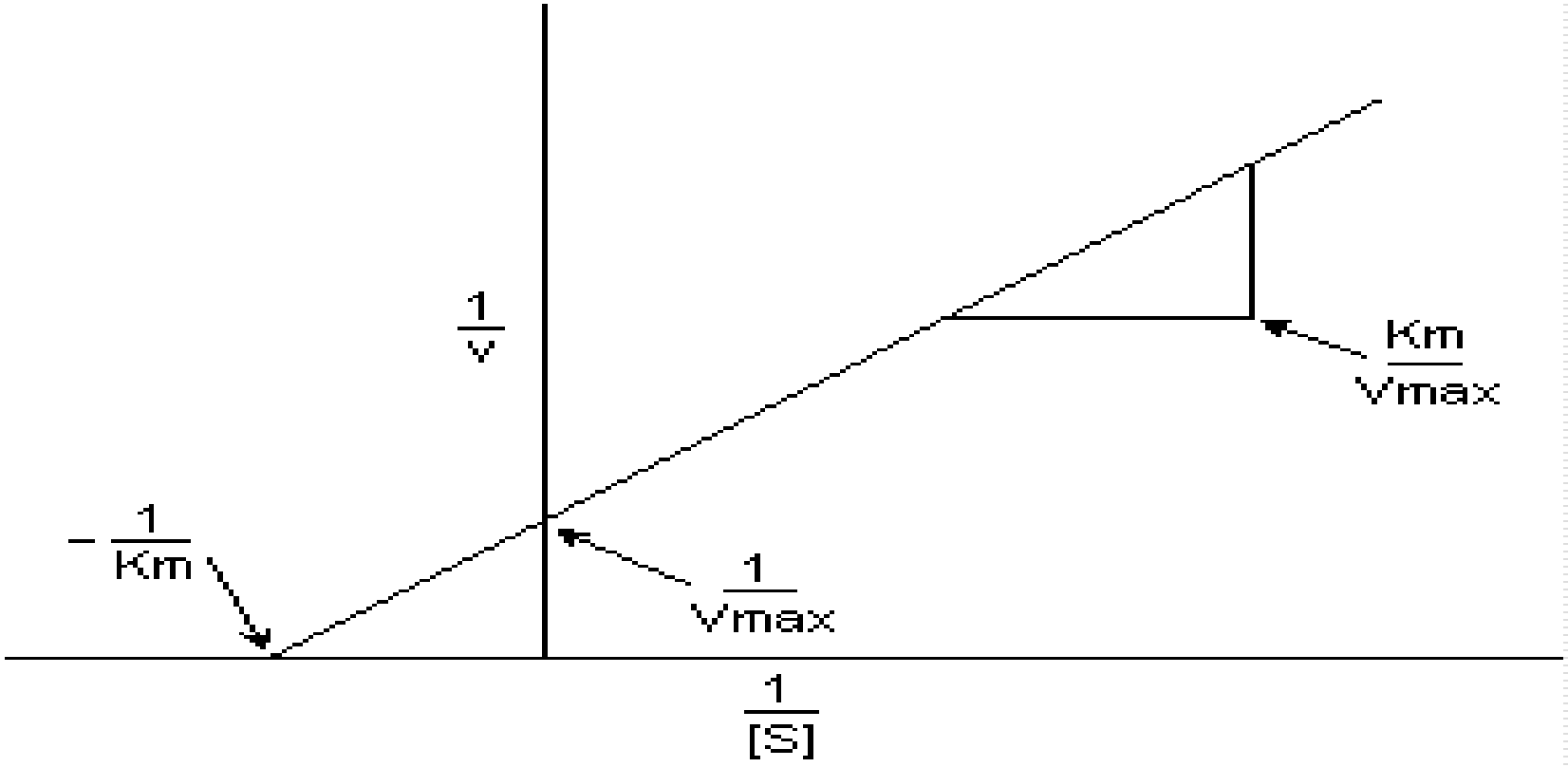
LINEWEAVER-BURK TRANSFORMATION OF THE MICHAELIS-MENTEN EQUATION

- Taking the reciprocal gives:

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

- where *V* is the reaction velocity, *Km* is the Michaelis-Menten constant, *Vmax* is the maximum reaction velocity, and [*S*] is the substrate concentration.

LINEWEAVER-BURK TRANSFORMATION OF THE MICHAELIS-MENTEN EQUATION



LINEWEAVER-BURK TRANSFORMATION OF THE MICHAELIS-MENTEN EQUATION

- **Use:**

- The Lineweaver-Burk plot was widely used to determine important terms in enzyme kinetics, such as K_m and V_{max} before the wide availability of powerful computers and non-linear regression software, as the y-intercept of such a graph is equivalent to the inverse of V_{max}; the x-intercept of the graph represents $-1/K_m$.

LINEWEAVER-BURK TRANSFORMATION OF THE MICHAELIS-MENTEN EQUATION

- It also gives a quick, visual impression of the different forms of enzyme inhibition.
- When used for determining the type of enzyme inhibition, the Lineweaver-Burk plot can distinguish competitive, noncompetitive and uncompetitive inhibitors.

ENZYME INHIBITION

- Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used as herbicides and pesticides. Not all molecules that bind to enzymes are inhibitors; *enzyme activators* bind to enzymes and increase their enzymatic activity.

Types of reversible inhibitor

- Reversible inhibitors bind to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding. In contrast to substrates and irreversible inhibitors, reversible inhibitors generally do not undergo chemical reactions when bound to the enzyme and can be easily removed by dilution or dialysis.

Types of reversible inhibitor Cont'd.

- There are three kinds of reversible enzyme inhibitors. They are classified according to the effect of varying the concentration of the enzyme's substrate on the inhibitor.
- In competitive inhibition, the substrate and inhibitor cannot bind to the enzyme at the same time, as shown in the figure on the left.

Types of reversible inhibitor Cont'd.

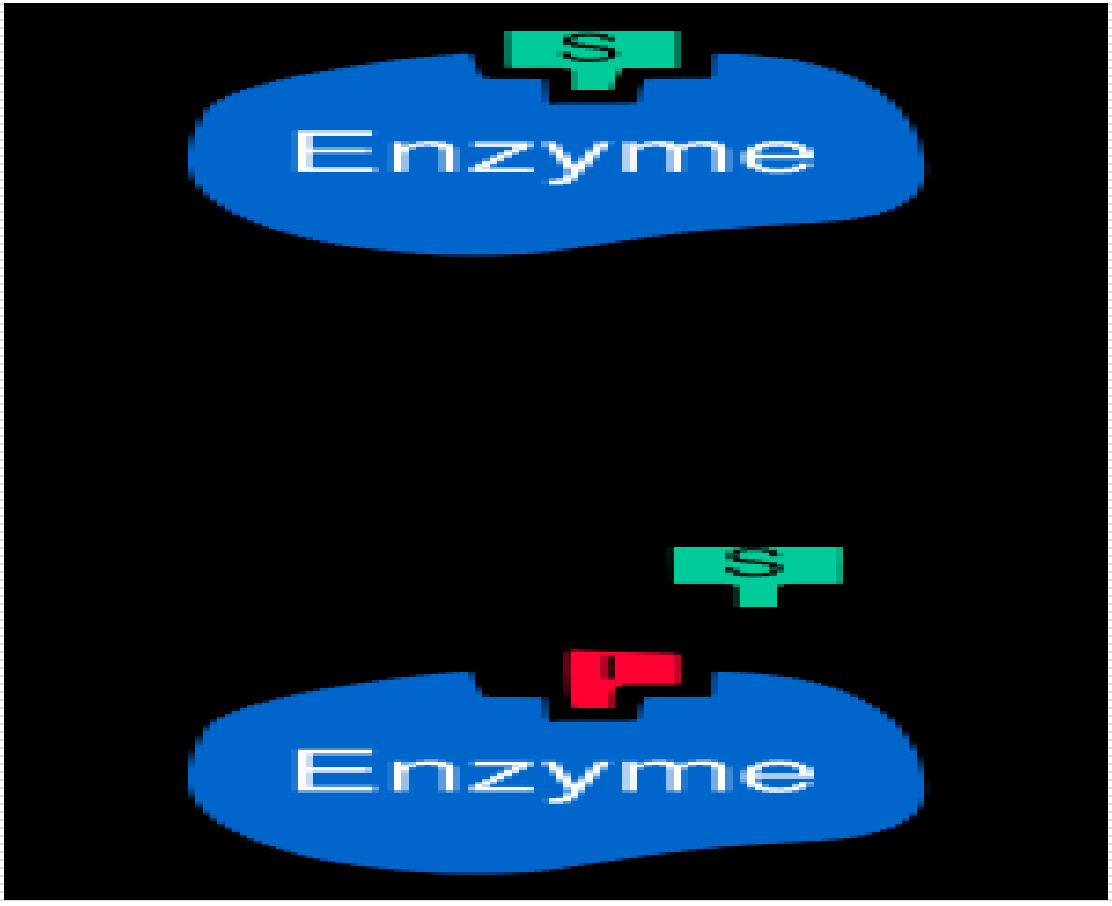
- This usually results from the inhibitor having an affinity for the active site of an enzyme where the substrate also binds; the substrate and inhibitor *compete* for access to the enzyme's active site. This type of inhibition can be overcome by sufficiently high concentrations of substrate, i.e., by out-competing the inhibitor.

Types of reversible inhibitor Cont'd.

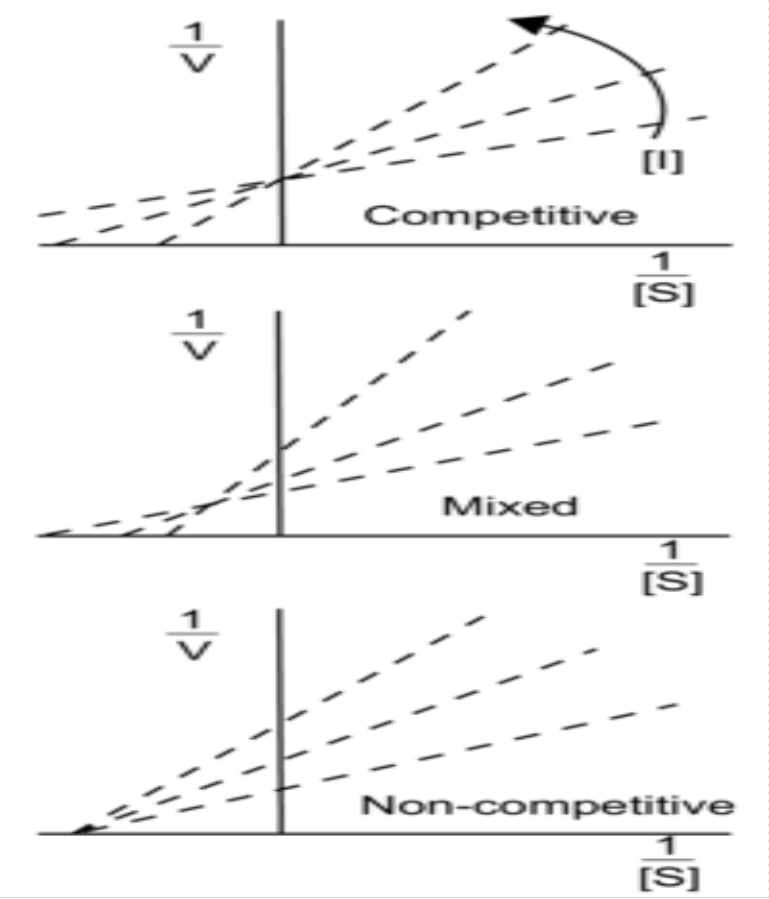
Competitive inhibitors are often similar in structure to the real substrate.

- Competitive inhibitors can bind to E, but not to ES. Competitive inhibition increases *K*m (i.e., the inhibitor interferes with substrate binding), but does not affect V_{max} (the inhibitor does not hamper catalysis in ES because it cannot bind to ES).

Competitive inhibition: substrate (S) and inhibitor (I) compete for the active site.



Lineweaver-Burk plots of different types of reversible enzyme inhibitors. The arrow shows the effect of increasing concentrations of inhibitor.



Non-Competitive Inhibition

- **Non-competitive inhibition** is a form of mixed inhibition where the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate. As a result, the extent of inhibition depends only on the concentration of the inhibitor.

Non-Competitive Inhibition Cont'd.

- Non-competitive inhibitors have identical affinities for E and ES. Non-competitive inhibition does not change K_m (i.e., it does not affect substrate binding) but decreases V_{max} (i.e., inhibitor binding hampers catalysis).

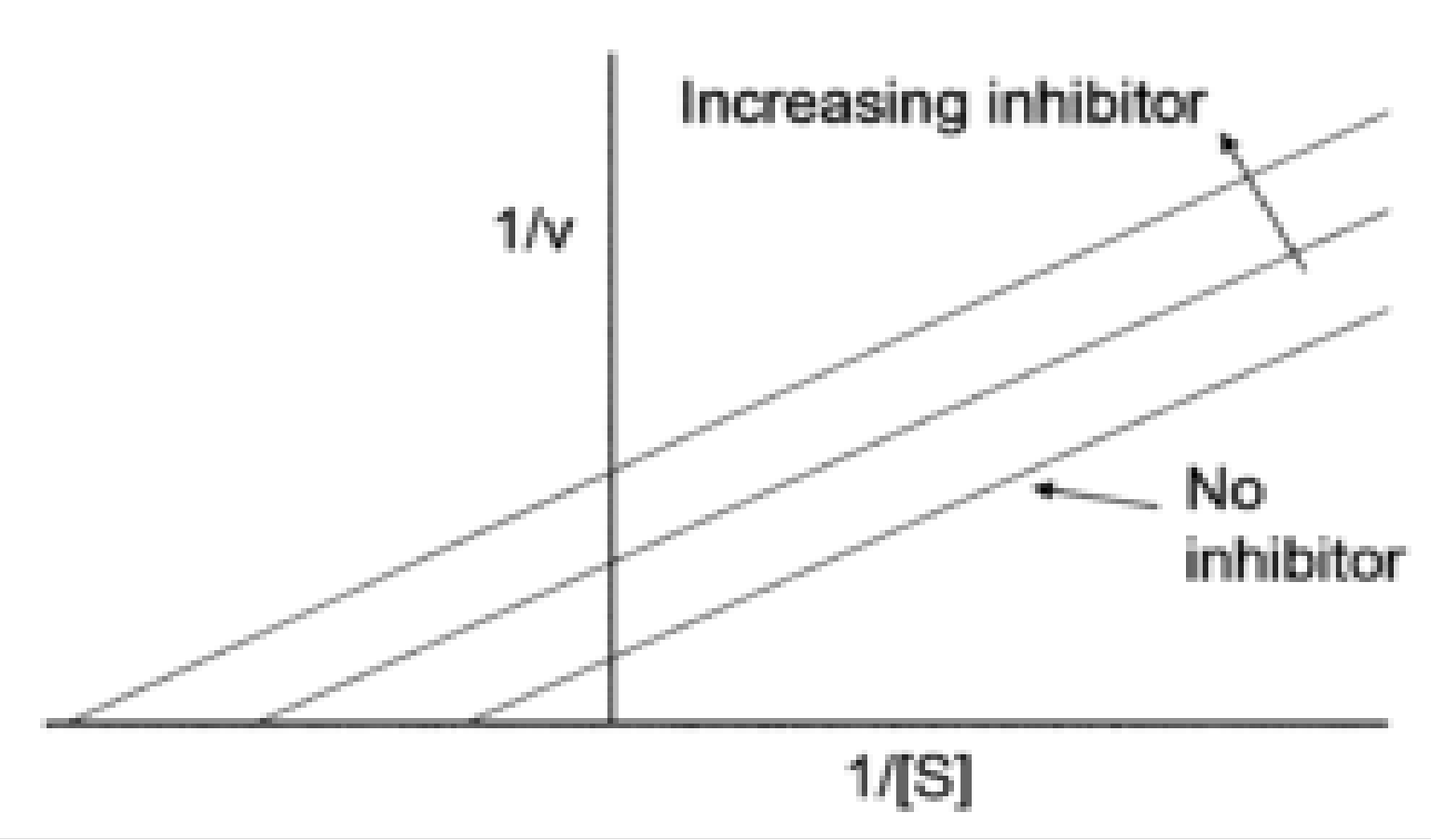
Uncompetitive Inhibition

- Uncompetitive inhibition takes place when an enzyme inhibitor binds only to the complex formed between the enzyme and the substrate (the E-S complex). This reduction in the effective concentration of the E-S complex increases the enzyme's apparent affinity for the substrate Chatelier's principle (*K*m is lowered) and decreases the maximum enzyme activity (Vmax),

Uncompetitive Inhibition Cont'd.

as it takes longer for the substrate or product to leave the active site. Uncompetitive inhibition works best when substrate concentration is high.

Uncompetitive Inhibition



METABOLISM

- In its broadest sense, metabolism refers to the sum of all the biochemical reactions that occur within a living organism. Since chemical reactions either release or require energy, the body's metabolism may be thought of as an energy balancing act. Metabolism has two phases:
  - Catabolism, an energy-generating process and anabolism, an energy-requiring process.

METABOLISM CONT'D.

- Catabolism:

  It is the term used for breakdown processes. When large, complex molecules (eg carbohydrates, proteins and fats) are taken into the body cells, they are systematically broken down to produce even smaller and simpler molecules such as CO_2, NH_3, and urea. Catabolism is an energy-releasing

METABOLISM CONT'D.

process. A considerable portion of the energy released is captured and stored in the form of a high-energy molecule known as ATP.

- Anabolism:

  It refers to the process that build up or synthesize new, more complex molecules from simpler molecules. For example, nucleic acids, protein and polysaccharides

METABOLISM CONT'D.

are built from nucleotides, amino acids and monosaccharides respectively. The synthesis of these bio-organic molecules require the expenditure of cellular energy furnished by the ATP produced from the cell's catabolic activities.

- Catabolism and anabolism are performed simultaneously in living cells, but each is regulated independently of the other.

CARBOHYDRATE METABOLISM

- During digestion, carbohydrates are hydrolysed into simpler sugars – Glucose, fructose and galactose – that are then absorbed into capillaries of the villi of the small intestine and carried through the hepatic portal vein to the liver. The liver is the only organ that converts fructose and galactose into glucose. Thus, the description of carbohydrate metabolism that occurs in

CARBOHYDRATE METABOLISM CONT'D.

all other cells is really a description of the metabolism of glucose. Glucose is the body's most direct source of energy and the fate of any absorbed glucose molecule depends on the energy need of the body's cells. If the cells require immediate energy, they transport glucose from blood plasma into the cell. The liver, in turn, releases stored glucose into blood plasma and

CARBOHYDRATE METABOLISM CONT'D.

restores the blood sugar level to its normal constant value. The glucose not needed for immediate use is handled in several ways. First the liver converts excess glucose to glycogen (glycogenesis) and stores it in this compact form. Skeletal muscles can also store excess glucose as glycogen, but unlike the liver, they can never release glucose back into the blood plasma. The glycogen

CARBOHYDRATE METABOLISM CONT'D.

remains within the muscle fibre as its own private source of glucose that can be drawn on during periods of intense muscle activity. Sometimes intense muscle activity will breakdown glucose to lactate which is released into circulation and taken to the liver where it is reconverted to glucose. Second, if the glycogen storage areas are

CARBOHYDRATE METABOLISM CONT'D.

filled, the adipocytes can transform the glucose to fat (lipogenesis), which can be stored in adipose tissue. Later if the cell's need for energy continues, the stored glycogen and fat can be converted back to glucose for cellular use by reversing the storage processes. Glycogen is hydrolysed to produce glucose by a process of glycogenolysis. Third, if the level of blood

CARBOHYDRATE METABOLISM CONT'D.

glucose reaches very high concentrations and there are no storage areas available, it will be excreted in the urine. Normally, this happens only when a meal contains mostly carbohydrates and no fats. Without the inhibiting effects of fats, the stomach empties its contents quickly and the carbohydrates are digested very rapidly. As a result, large numbers of monsaccharides

CARBOHYDRATE METABOLISM CONT'D.

since the liver is unable to process all of them simultaneously, the blood glucose level rises, causing hyperglycaemia. When the blood glucose level rises above a certain limit, glucose is excreted in the urine, a condition called glucosuria or glycosuria.

GLYCOLYSIS

- The sequence of enzymatic steps that degrades glucose to pyruvate is known as glycolysis or the Embden-Meyorhof pathway. There is very little free glucose in cells. Intracellular glucose exists as a phosphorylated molecule, glucose-6-phosphate. The process of glucose phosphorylation is powered by ATP molecule as follows:

GLYCOLYSIS CONT'D.

ATP + glucose $\longrightarrow$ ADP + glucose-6-phosphate

- The next step in the sequence is the conversion of glucose-6-phosphate to fructose-6-phosphate. This is an enzymatic reaction that does not add to or remove any atom from the molecule but rearranges the internal bonding of the atom. Thus:

Glucose-6-phosphate $\rightleftharpoons$ fructose-6-phosphate

GLYCOLYSIS CONT'D.

- In the 3rd step, also powered by ATP, a 2nd phosphate is added to the fructose-6-phosphate on the first carbon as follows:

  ATP + fructose-6-phosphate → ADP + fructose-1,6-diphosphate

- During the 4th step, the six carbon fructose-1,6-diphosphate is broken down into 2 x three-carbon (triose) pieces. Each piece has one phosphate. The triose phosphates are called

GLYCOLYSIS CONT'D.

dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.

- The 5th step is crucial for understanding the amount of ATP and pyruvate produced by the glycolytic process. Of the 2 triose phosphates present at this point, only glyceraldehyde-3- phosphate can be metabolised further. An enzyme present in all cells converts dihydroxyacetone

GLYCOLYSIS CONT'D.

phosphate into glyceraldehyde-3-phosphate.

This is an enzyme isomerization that does not add or remove atoms but only rearranges the bonding of the atoms thus:

Dihydroxyacetone phosphate → glyceradehyde-3-phosphate

Both glyceraldehyde-3- phosphate molecules proceed through the succeeding steps of glycolysis.

GLYCOLYSIS CONT'D.

- The 6th step is one of the most important steps in the glycolytic pathway. In this step, each glyceraldehyde-3- phosphate is provided with a 2nd phosphate group. Simultaneously, it loses 2H atoms, which associate with the coenzyme NAD^+. The NAD^+ is reduced in the process, and the reduced form is designated $NADH + H^+$.

GLYCOLYSIS CONT'D.

The phosphate that is employed by the enzyme in this reaction is not high-energy phosphate, but inorganic phosphate (H_3PO_4), abbreviated as P_i. Thus:

Glyceraldehyde-3- phosphate + NAD^+ + P_i ⟶⟵

1,3-diphosphoglycerate + NADH + H^+

- In the 7th step ATP molecules are produced. The enzyme for this step removes the

GLYCOLYSIS CONT'D.

phosphate from carbon-1 and transports it to an ATP molecule, thus putting a 3rd phosphate on the nucleotide.

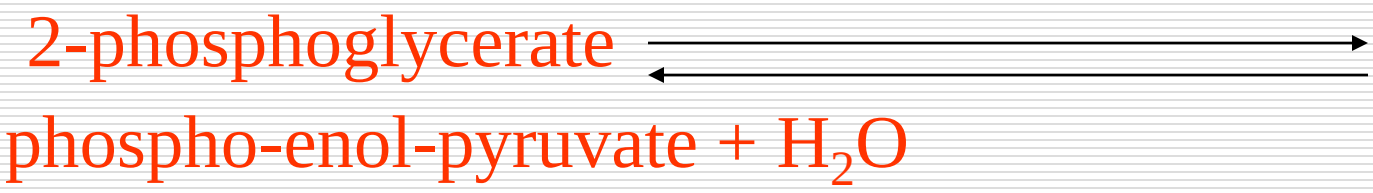
1,3-diphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP

- The 8th step is a simple one. The phosphate on carbon-3 is moved to carbon-2. The reaction is:

3-phosphoglycerate ⇌ 2-phosphoglycerate

GLYCOLYSIS CONT'D.

- The 9th step is the removal of a molecule of H_2O from the 2-phosphoglycerate. The resultant molecule is called phospho-enol-pyruvate:



- In the 10th step, another ATP molecule is produced. The enzyme for this step removes the phosphate from phospho-enol-pyruvate and adds it to ADP.

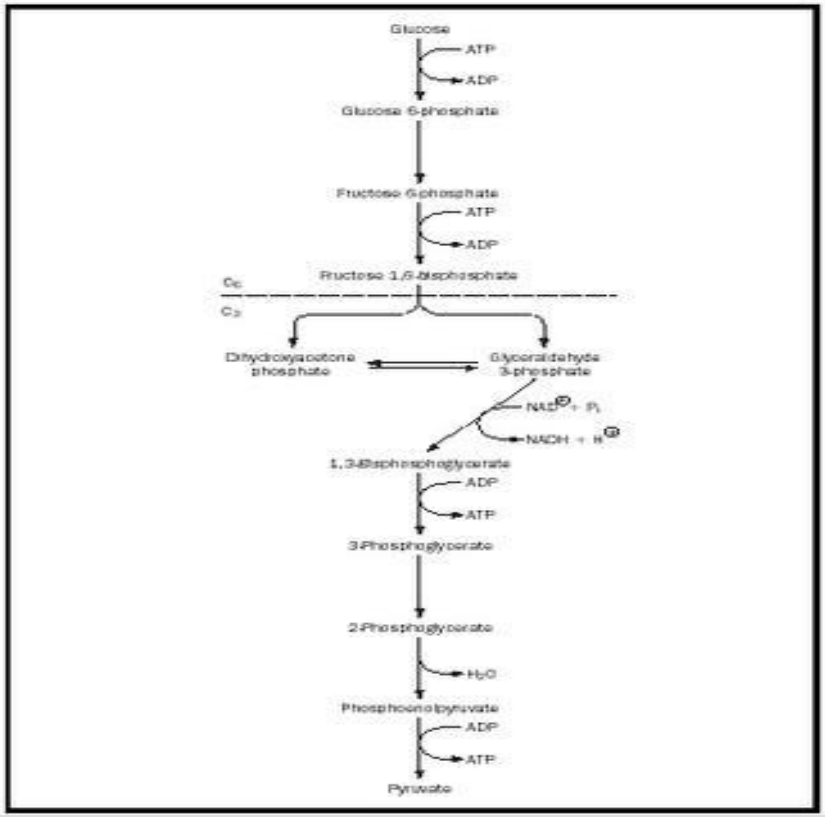
GLYCOLYSIS CONT'D.

Phospho-enol-pyruvate + ADP $\rightleftharpoons$ pyruvate + ATP

- The 11th step is an alternative step chosen only when anaerobic conditions exist within the cell. When O_2 is in short supply to the cell, an enzyme is used that adds 2H atoms to pyruvate to form lactate. The Hs are provided by the reduced nucleotide; NADH + H^+.

Pyruvate + NADH + H^+ $\rightleftharpoons$ lactate + NAD^+

Glycolytic Sequence



Tricarboxylic Acid Cycle (TCA)/Krebs Cycle

- In the presence of O_2, pyruvate molecules diffuse from the cytoplasm into the mitochondrion where it undergoes oxidative decarboxylation to yield a 2-carbon compound called acetate. This acetate then combines with coenzyme A (CoA) to yield acetyl coenzyme A (Acetyl CoA).

Krebs Cycle Cont'd.

- The Krebs cycle is a series of enzymatic reactions that catalyzes the aerobic metabolism of fuel molecules to carbon dioxide and water, thereby generating energy for the production of adenosine triphosphate (ATP) molecules. The Krebs cycle is so named because much of its elucidation was the work of the British biochemist Hans Krebs. Many types of fuel molecules can be drawn into and

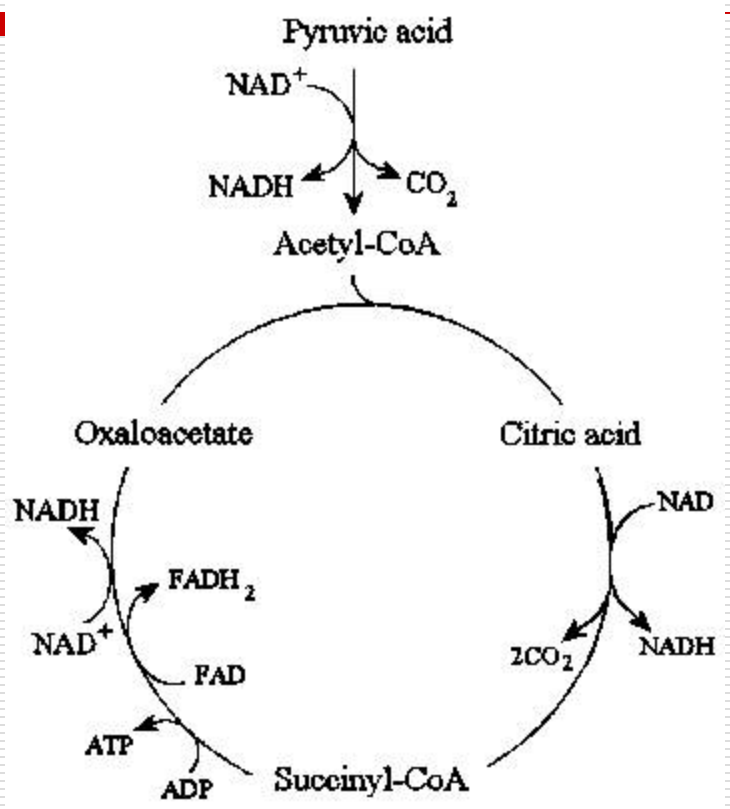
Krebs Cycle Cont'd.

utilized by the cycle, including acetyl coenzyme A (acetyl CoA), derived from glycolysis or fatty acid oxidation. Some amino acids are metabolized via the enzymatic reactions of the Krebs cycle. In eukaryotic cells, all but one of the enzymes catalyzing the reactions of the Krebs cycle are found in the mitochondrial matrixes. Because the Krebs cycle is responsible for the ultimate oxidation of metabolic **intermediates** produced during the metabolism of fats, proteins, and carbohydrates, it is the central mechanism for metabolism in the cell. In the first reaction

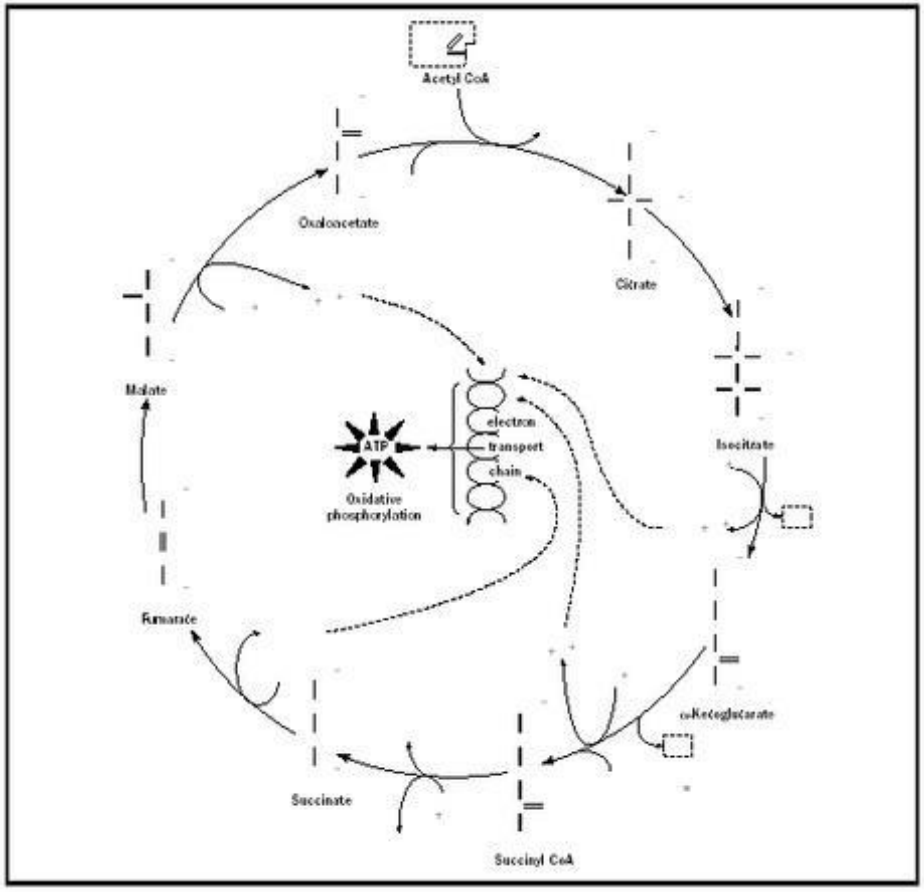
Krebs Cycle Cont'd.

of the cycle, acetyl CoA condenses with oxaloacetate to form citric acid. Acetyl CoA utilized in this way by the cycle has been produced either via the oxidation of fatty acids, the breakdown of certain amino acids, or the oxidative decarboxylation of pyruvate (a product of glycolysis). The citric acid produced by the condensation of acetyl CoA and oxaloacetate is a tricarboxylic acid containing three carboxylate groups.

Krebs Cycle


Pyruvic acid
NAD+
NADH
CO2
Acetyl-CoA
Oxaloacetate
Citric acid
NADH
NAD
FADH2
NAD+
FAD
2CO2
NADH
ATP
ADP
Succinyl-CoA

Krebs Cycle


Acetyl CoA
Oxaloacetate
Citrate
Isocitrate
α-Ketoglutarate
Succinyl CoA
Succinate
Fumarate
Malate
ATP
Oxidative phosphorylation
electron transport chain

Krebs Cycle Cont'd.

After citrate has been formed, the cycle machinery continues through seven distinct enzyme-catalyzed reactions that produce, in order, isocitrate, α-ketoglutarate, succinyl coenzyme A, succinate, fumarate, malate, and oxaloacetate. The freshly produced oxaloacetate, in turn, reacts with yet another molecule of acetyl CoA, and the cycle begins again. Each turn of the Krebs cycle produces two molecules of carbon dioxide, one guanosine triphosphate molecule (GTP),

Krebs Cycle Cont'd.

and enough electrons to generate three molecules of NADH and one molecule of FADH2. The Krebs cycle is present in virtually all eukaryotic cells that contain mitochondria, but functions only as part of aerobic metabolism (when oxygen is available). This oxygen requirement is owing to the close relationship between the mitochondrial electron transport chain and the Krebs cycle. In the Krebs cycle, four oxidation–reduction reactions occur. A high energy phosphate bond in the form of GTP is also generated.

Krebs Cycle Cont'd.

(This high energy phosphate bond is later transferred to adenosine diphosphate [ADP] to form adenosine triphosphate [ATP]). As the enzymes of the Krebs cycle oxidize fuel molecules to carbon dioxide, the coenzymes NAD+, FAD, and coenzyme Q (also known as ubiquinone) are reduced. In order for the cycle to continue, these reduced coenzymes must become reoxidized by transferring their

Krebs Cycle Cont'd.

electrons to oxygen, thus producing water. Therefore, the final acceptor of the electrons produced by the oxidation of fuel molecules as part of the Krebs cycle is oxygen. In the absence of oxygen, the Krebs cycle is inhibited.

SUMARRY OF KREBS CYCLE

- 1. Prior to entering the Krebs Cycle, pyruvate must be converted into acetyl CoA (pronounced: acetyl coenzyme A). This is achieved by removing a CO_2 molecule from pyruvate and then removing an electron to reduce an NAD^+ into NADH. An enzyme called coenzyme A is combined with the remaining acetyl to make acetyl CoA which is then fed into the Krebs Cycle. The steps in the Krebs Cycle are summarized below:

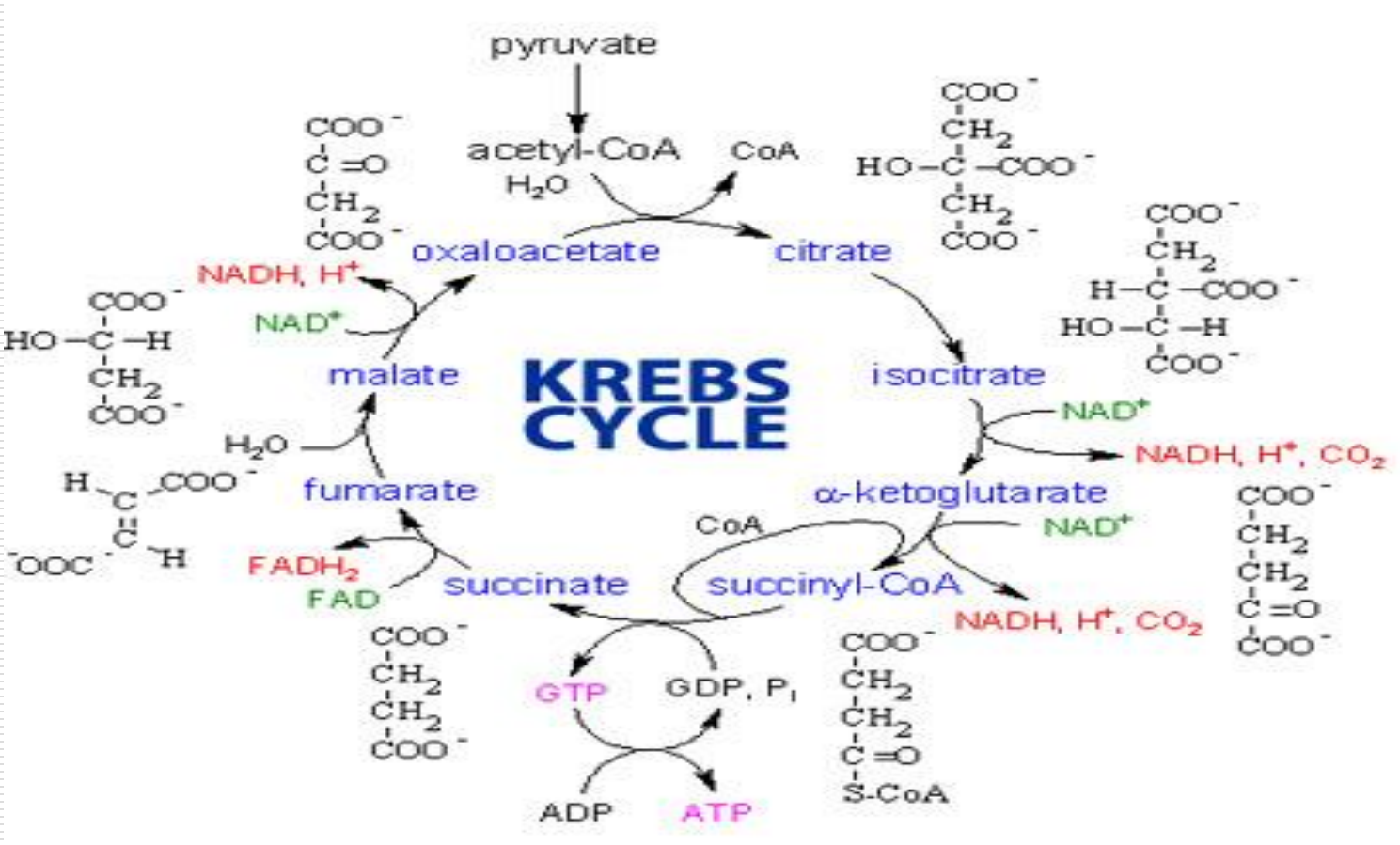
SUMARRY OF KREBS CYCLE CONT'D.

- 2. Citrate is formed when the acetyl group from acetyl CoA combines with oxaloacetate from the previous Krebs cycle.
- 3. Citrate is converted into its isomer isocitrate.
- 4. Isocitrate is oxidized to form the 5-carbon α-ketoglutarate. This step releases one molecule of CO_2 and reduces NAD^+ to $NADH_2^+$.

SUMARRY OF KREBS CYCLE CONT'D.

- 5. The α-ketoglutarate is oxidized to succinyl CoA, yielding CO_2 and $NADH_2$+.
- 6. Succinyl CoA releases coenzyme A and phosphorylates ADP into ATP.
- 7. Succinate is oxidized to fumarate, converting FAD to $FADH_2$.
- 8. Fumarate is hydrolized to form malate.
- 9. Malate is oxidized to oxaloacetate, reducing NAD^+ to $NADH_2^+$.

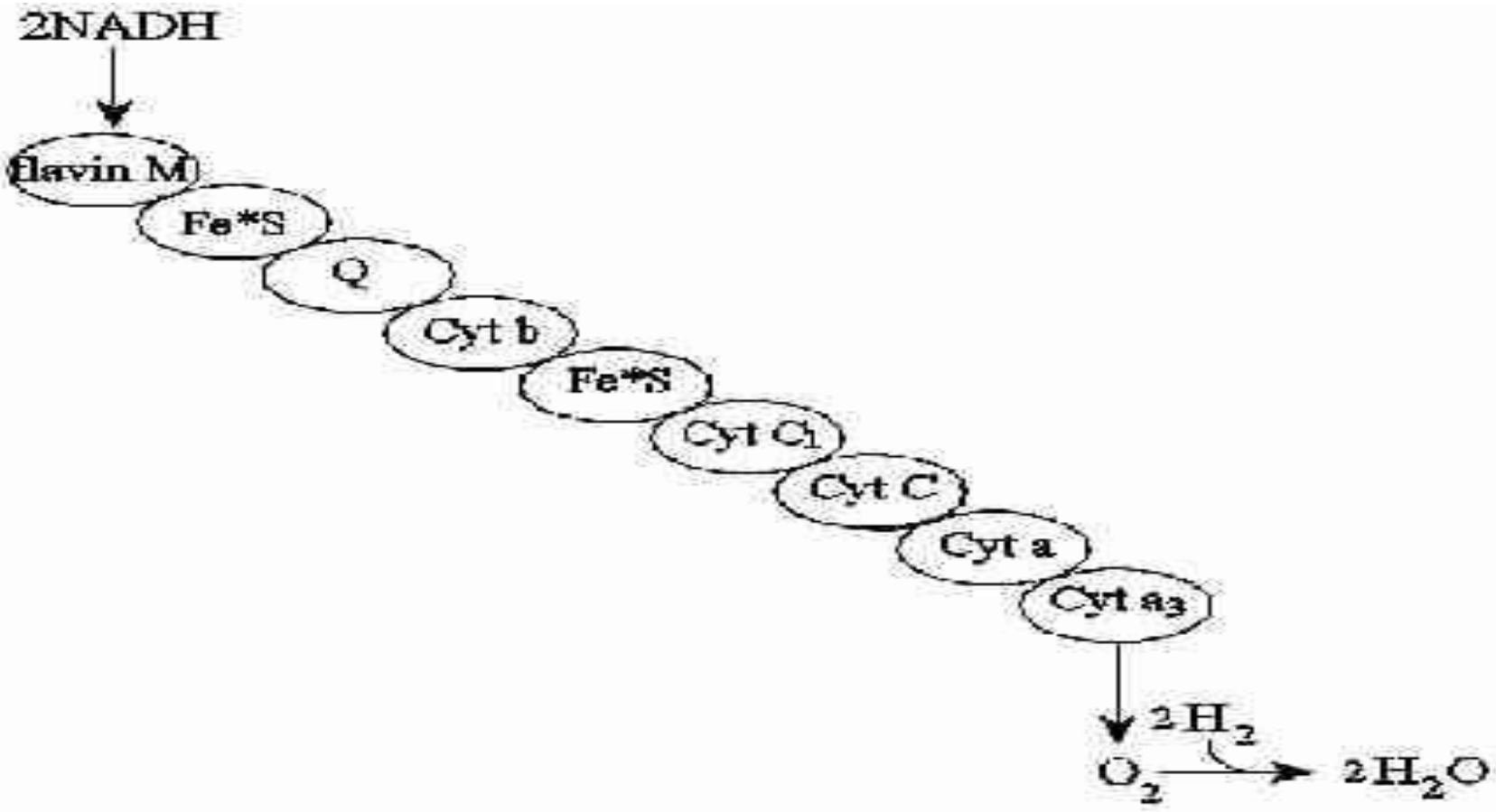
Krebs Cycle


pyruvate
acetyl-CoA
H_2O
CoA
oxaloacetate
citrate
NADH, H^+
NAD^+
malate
KREBS CYCLE
isocitrate
NAD^+
$NADH, H^+, CO_2$
H_2O
fumarate
α-ketoglutarate
CoA
NAD^+
$FADH_2$
FAD
succinate
succinyl-CoA
$NADH, H^+, CO_2$
GTP
GDP, P_i
ADP
ATP
S-CoA

Electron transport chain

- What happens to the $NADH_2^+$ and $FADH_2$ produced during glycolysis and the Krebs cycle? The molecules have been reduced, receiving high energy electrons from the pyruvic acid molecules that were dismantled in the Krebs Cycle. These carrier molecules transport the high energy electrons and their accompanying hydrogen protons from the Krebs Cycle to the electron transport chain in the inner mitochondrial membrane.

Electron transport chain



Electron transport chain Cont'd.

In a number of steps utilizing enzymes on the membrane, $NADH_2^+$ is oxidized to NAD^+, and $FADH_2$ to FAD. The high energy electrons are transferred to ubiquinone (Q) and cytochrome c molecules, the electron carriers within the membrane. The electrons are then passed from molecule to molecule in the inner membrane of the mitochondrion, losing some of their energy at each step. The final transfer involves the combination of electrons and H_2 atoms with oxygen to form water.

Electron transport chain Cont'd.

The molecules that take part in the transport of these electrons are referred to as the electron transport chain. The process can be summarized as follows: the electrons that are delivered to the electron transport system provide energy to "pump" hydrogen protons across the inner mitochondrial membrane to the outer compartment. This high concentration of hydrogen protons produces a free energy potential that can do work. That is, the hydrogen protons tend to move down the concentration gradient from the outer compartment to the inner compartment.

Electron transport chain Cont'd.

However, the only path that the protons have is through enzyme complexes within the inner membrane. The protons therefore pass through the channel lined with enzymes. The free energy of the hydrogen protons is used to form ATP by phosphorylation, bonding phosphate to ADP in an enzymatically-mediated reaction. Since an electrochemical osmotic gradient supplies the energy, the entire process is referred to as chemiosmotic phosphorylation.

Electron transport chain Cont'd.

Once the electrons (originally from the Krebs Cycle) have yielded their energy, they combine with oxygen to form water. If the oxygen supply is cut off, the electrons and hydrogen protons cease to flow through the electron transport system. If this happens, the proton concentration gradient will not be sufficient to power the synthesis of ATP. This is why we, and other species, are not able to survive for long without oxygen.

LIPID METABOLISM: Key Concepts

Stored lipids is the primary source of energy in most organisms.

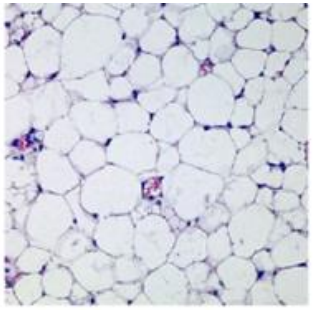
The three sources of triacylglycerols in animals are dietary lipids, stored triacylglycerols in adipose tissue, and fatty acids synthesized in the liver.

oxidation is the mitochondrial process by which fatty acids are oxidized to yield NADH, FADH2, and acetyl-CoA.

Ketogenesis takes place in liver mitochondria when acetyl-CoA levels are high and oxaloacetate levels are low.

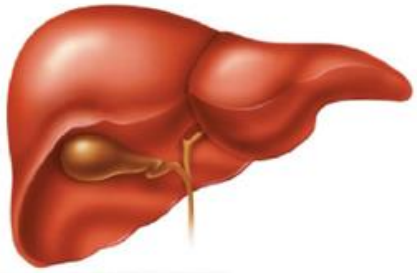
Overview of Lipid Transport in Animals

Dietary triacylglycerols



Triacylglycerols stored in adipose tissue and released in response to hormones

Glucagon
Epinephrine



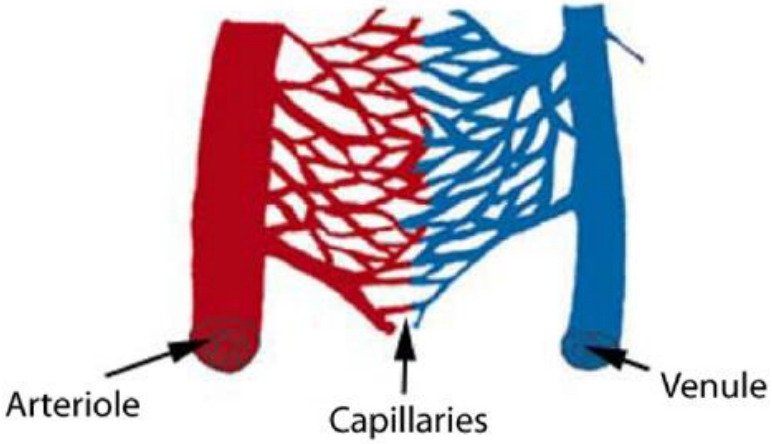
Triacylglycerols synthesized in the liver from carbohydrates and exported

Chylomicrons

Fatty acids

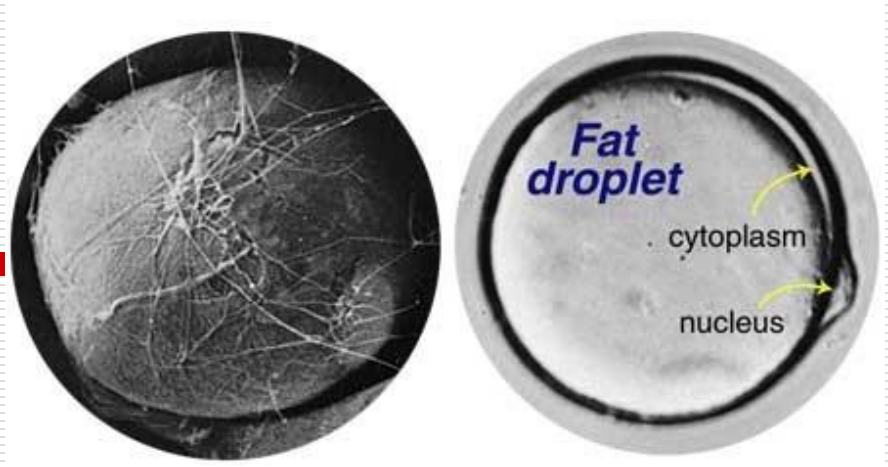
Albumin

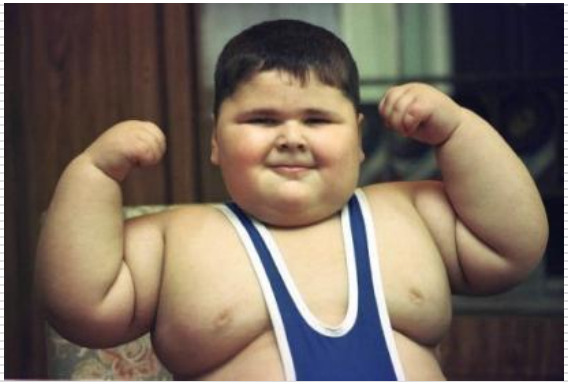
VLDL particles

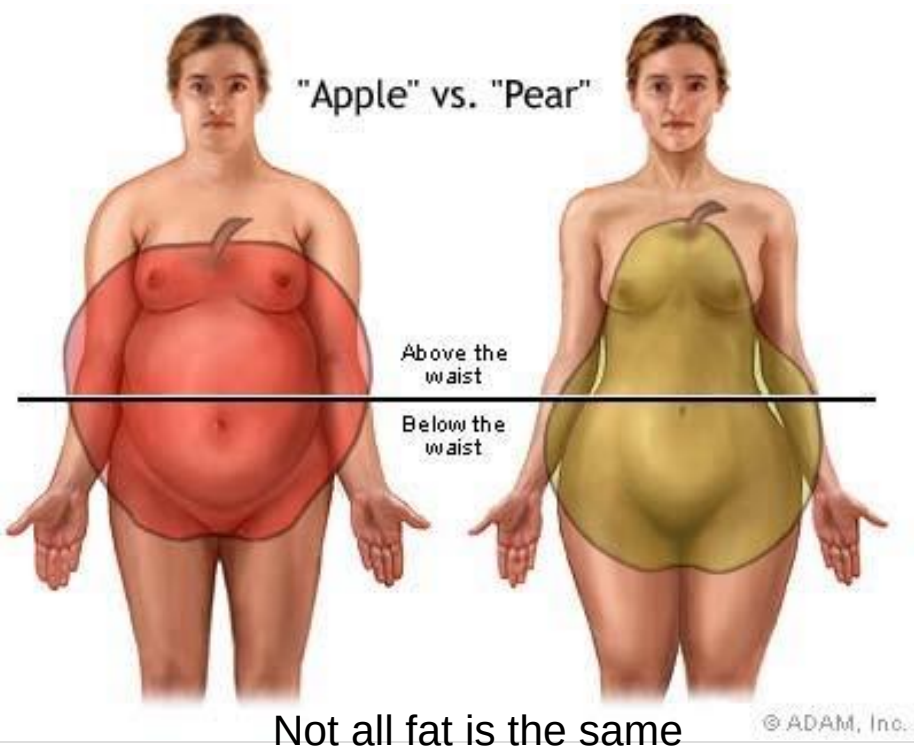


VLDL stands for very low density lipoprotein. Lipoproteins are made up of cholesterol, triglycerides, and proteins. They move cholesterol, triglycerides, and other lipids (fats) to around the body. **VLDL** is one of the three main types of lipoproteins. **VLDL** contains the highest amount of triglycerides.

Fat is stored in fat cells (**adipocytes**). Obesity, especially childhood obesity, can be due to both more fat storage per cell, and to a larger number of adipocytes.







Not all fat is the same

LIPID METABOLISM CONT'D.

- The biologically important lipids are the fatty acids and their derivatives, the neutral fats (triglycerides), the phospholipids and their related compounds, and the sterols.
- Fat stored in the body's depots constitutes the largest reserve of energy. The body can store much more fat than glycogen. Moreover, the energy yield per molecule of fat is more than twice that of carbohydrate.

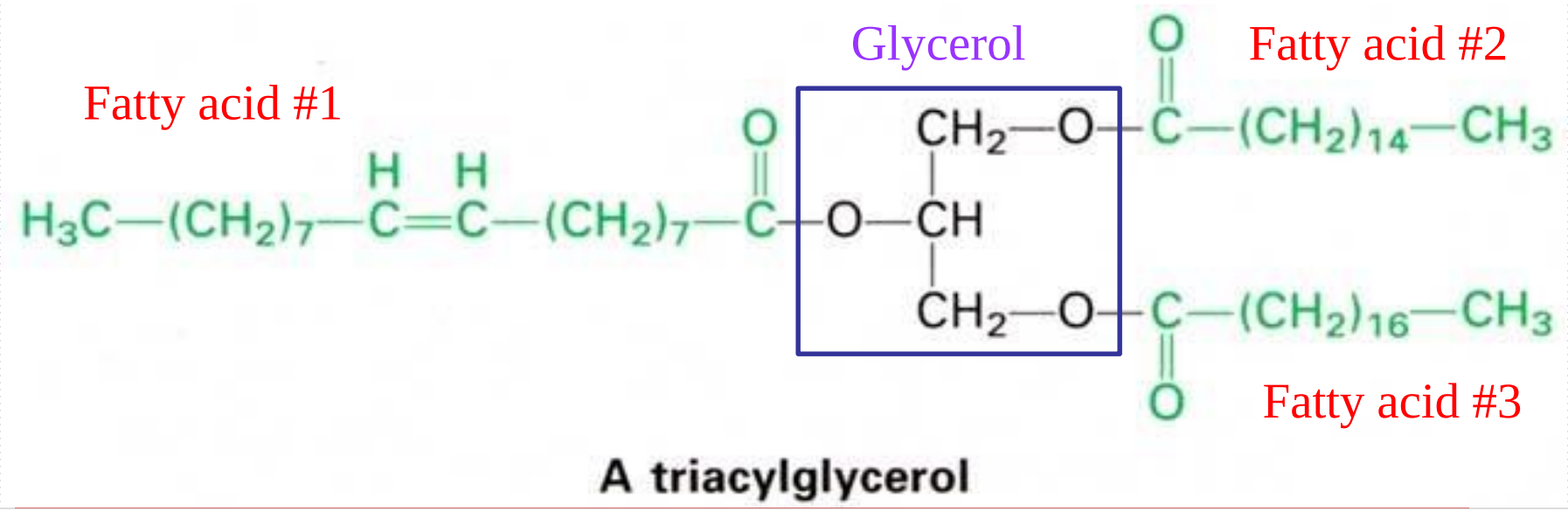
LIPID METABOLISM CONT'D.

Nevertheless, fats are usually the body's second-most-utilized source of energy because they may be more difficult to mobilize than carbohydrates.

- Triglycerides are the lipid-type molecules that are used mainly for energy storage. Other lipid-type molecules – phospholipids and cholesterol – are used intracellularly for the production of hormones and other functional molecules.

LIPID METABOLISM CONT'D.

The triglyceride molecules consist of a molecule of glycerol and three fatty acid molecules. They are linked together by an ester bond to form the triglyceride.



LIPID METABOLISM CONT'D.

The first step in the breakdown of triglycerides stored in fat or adipose tissue is their separation into one molecule of glycerol and three molecules of fatty acids by the action of hormonally controlled enzymes called lipases.

LIPID METABOLISM CONT'D.

The glycerol is converted by many other tissues immediately into glyceraldehyde, which then enters the phosphogluconate pathway for glucose metabolism, or the glyceraldehyde can be converted to glucose by gluconeogenesis. Glycerol is not taken up by adipocytes.

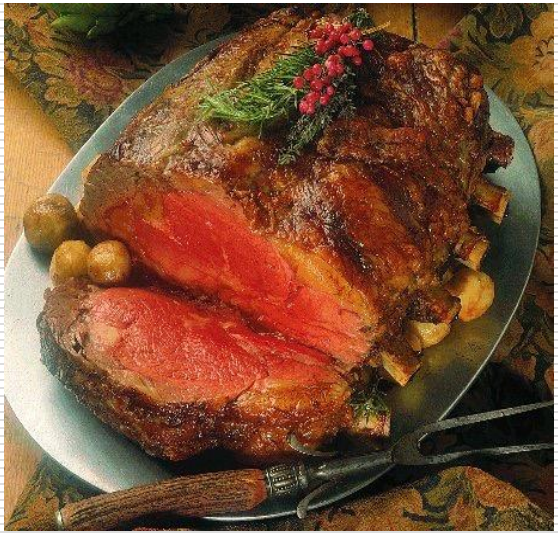
LIPID METABOLISM CONT'D.

The fatty acids released from fat deposits are transported, bound to albumin, to many other tissues that can metabolize fatty acids. The long-chain fatty acid molecules are oxidized completely to CO_2 and H_2O by all tissues except the nervous tissue. There are times when even the brain can utilize certain fatty acid breakdown products such as β-hydroxybutyrate.

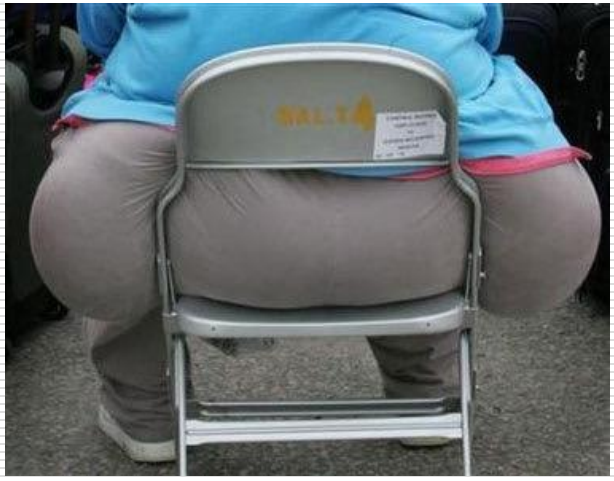
LIPID METABOLISM CONT'D.

Heart tissue is somewhat unusual in that, it always obtains most of its energy from fatty acid metabolism.

Lipid transport in animals, fatty acid oxidation, ketogenesis in liver mitochondria



Prime rib contains large amounts of saturated fats in the form of triacylglycerols



Adipose tissue is the primary triacylglycerol storage depot in animals, fats are an excellent form of redox energy

Stored fat comes from the conversion of carbohydrates into fatty acids in the liver

FATTY ACID OXIDATION

The metabolic degradation of fatty acids is carried out in the matrix of the mitochondria. There, a series of soluble enzymes catalyze a process referred to as beta oxidation.

FATTY ACID OXIDATION CONT'D.

In this process, the long-chain molecules are reduced to two-carbon units of acetic acid bound to coenzyme A, producing acetyl CoA. Since the vast majority of fatty acids contain an even number of carbons, the number of acetyl CoAs produced is easily calculated by dividing the number of carbons in the fatty acid by two.

FATTY ACID OXIDATION CONT'D.

- The first step of fatty acid oxidation requires the transport of the fatty acid from the cytoplasm of the cell into the mitochondrial matrix. This step is a complex reaction that requires much energy. An ATP molecule is hydrolysed to AMP and the energy is used to activate the fatty acid by bonding the fatty acid to coenzyme A.

FATTY ACID OXIDATION CONT'D.

$$R.COOH + ATP + CoA \rightarrow R.COCoA + AMP + PP_i$$

fatty acid fatty acyl CoA

Once activated, the fatty acid is then transferred to the transport molecule, carnitine, located in the inner mitochondrial membrane. The reaction is catalysed by carnitine acyl transferase I to

FATTY ACID OXIDATION CONT'D.

Produce the acyl carnitine complex, which then crosses the membrane. The availability of carnitine is regulated by insulin, and the activity of the carnitine acyl transferase I is regulated by manonyl CoA.

Acyl CoA + carnitine Acyl carnitine + CoA.SH

A second enzyme located in the inner surface of the cristae causes the release of the fatty acyl group from carnitine and its transfer to mitochondrial coenzyme A.

FATTY ACID OXIDATION CONT'D.

Acyl carnitine + CoA acyl CoA + carnitine

This fatty acyl CoA complex presents the fatty acid to the enzymes of the β-oxidation pathway.

The mitochondrial fatty acyl CoA undergoes an enzymatic attack that removes two hydrogen atoms, one each from the α and β carbons.

Dehydrogenation of the α and β carbons is carried out by an enzyme that contains a tightly bound FAD coenzyme molecule.

FATTY ACID OXIDATION CONT'D.

$R\text{-}CH_2\text{-}CH_2\text{-}$

The $FADH_2$ transfers its hydrogen atoms to the CoQ molecules of the electron transport system so that the hydrogens can travel the rest of the electron transport system to form water and two ATP molecules.

The next step is the addition of water across the double bond between the α and β carbons (OH^- is added to the β carbon and H^+ to the α carbon).

FATTY ACID OXIDATION CONT'D.

R-C=C-C-sCoA + H_2O R-C-CH_2-C-sCoA

Another dehydrogenation step removes two hydrogen atoms from the β carbon. The hydrogens removed are accepted by NAD^+ and passed down the electron transport system to form H_2O and three ATP molecules.

R-C-CH2-C-sCoA + CoA R-C-sCoA + CH_3-C-CoA

The acetyl CoA is then available for terminal

FATTY ACID OXIDATION CONT'D.

Oxidation in the TCA cycle.

Beta Oxidation

It is made up of five (5) stages: 1. Activation

2. Dehydrogenation 3. Hydration

4. Dehydrogenation and 5. Cleavage as described above.

Fatty acids with odd numbers of carbon atoms form propionyl-CoA instead of acetyl CoA as the final step in fatty acid catabolism. This is converted to

FATTY ACID OXIDATION CONT'D.

succinyl–CoA and enters the TCA cycle. Mitochondrial acetyl CoA can enter several metabolic pathways, including the TCA cycle for the generation of ATP, lipid synthesis or ketone body synthesis. It is important to note that fatty acids cannot be used in the synthesis of glucose.

FATTY ACID OXIDATION CONT'D.

ATP Yield in Fatty acid metabolism

For example if palmitic acid (16C):

Since this fatty acid contains 16 carbons, it will yield 8 acetyl CoA molecules after complete hydrolysis. The five (5) steps of β-oxidation will be carried out only 7 times. Thus, 8 acetyl CoAs produced can enter the Krebs cycle for complete oxidation, where they will produce:

FATTY ACID OXIDATION CONT'D.

1. 8 x 3 NADH + H^+ = 24 NADH + H^+
2. 8 x 1 $FADH_2$ = 8 $FADH_2$
3. 8 x 1 ATP = 8 ATP

Recall that the steps of β-oxidation also produce reduced coenzymes. Each operation of the β-oxidation sequence yields one $FADH_2$ and one NADH + H^+. Thus, 7 operations of β-oxidation will produce:

FATTY ACID OXIDATION CONT'D.

1. 7 x $NADH + H^+$ = 7 $NADH + H^+$
2. 7 x $FADH_2$ = 7 $FADH_2$

Addition of reduced coenzymes and ATP shows that hydrolysis of palmitic acid (16C) yields:

31 $NADH + H^+$ + 15 $FADH_2$ + 8 ATPs

The reduced coenzymes, in turn, channel the hydrogen atoms into the electron transport system. Each $NADH + H^+$ can produce 3 molecules, where each $FADH_2$ yields 2 ATP molecules.

FATTY ACID OXIDATION CONT'D.

1. 31 NADH + H^+ produce 3 x 31 = 93 ATPs
2. 15 $FADH_2$ produce 2 x 15 = 30 ATPs
3. 8 ATPs from Krebs cycle = 8 ATPs

Total = 131 ATPs

However, the initial activation process that enabled the fatty acid to enter the mitochondrial matrix required ATP AMP + PP_i. To repay the energy spent in this reaction requires 2 ATP molecules be paid back. Therefore, the net yield of ATP is 129 ATPs.

Summary of stages in the production of energy from oxidation of fatty acids