A - Cellular Physiology

(a) Cell membrane & its properties

- Thin
- Pliable
- Elastic Structure
- 7.5 to 10 nanometers thick

Composed of:

Lipids - phospholipid (25%)
- cholesterol (15%)
- other lipid (4%)

Protein (55%)

Carbohydrate (3%)

Lipid Bilayer

See diagram - Lipid bilayer

Hydrophobic internally (fatty acid)

Hydrophilic externally (phosphate)

Impermeable to H20 soluble substances:

ions

glucose

urea

Permeable to fat soluble substances:

O2

CO2

EtOH

Cell Membrane Proteins:

-Globular masses that float in lipid bilayer
-Mostly glycoproteins
-Peripheral - attached to one side of mem
-Integral - protrude through membrane

-enzymes

-carrier proteins

- H2O channels

- ion channels

- controllers of intracellular function

- receptors 4 neurotransmittes\ horm.

- structural

- immune function

Glycocalyx - membrane glycoprotein + glycolipids + proteoglycans

Functions:

1. negatively charged (thus repels other -ve charged objects)
2. provides way of attaching to other cells
3. receptor substances for hormones and drug binding
4. modulates immune function

Basement Membrane

- underlies most cells
- made from - collagens, fibronectins, lamins & proteoglycans - bind and regulate development & growth.

Tight Junctions

- attachments at apical of cells
- maybe tight (impermeable) or leaky (perm)
- help maintain polarity

Gap Junctions

- 6 subunit connexons
- low electrical resistance
- permit communication
- regulated by pH, voltage, Ca2+

Resting Membrane Potential

Determined by:

(1) K+ diffusion potential (most impt)
(2) Na+ diffusion potential
(3) Na-K ATPase
(4) Gibbs Donnan effect

- cell highly permeable to K+ (100x >Na+) via leak channels.
- Na-K ATPase creates RMP -70 to 90mV

Nerst Eqn - the potential level across the membrane that exactly opposes net diffusion of an univalent ion through a membrane @ 37°C.

K -94mV
Na -86mV

Goldman Eqn - the potential derived from the interactions between K, Na, Cl taking into account their conc. & permeabilities.

- the membrane potential will approach the Nerst Eqn if the permeability is greatly increased with respect to other ions.

- this explains the dominant influence of K on RMP.

(b) Functions of mitochondria, ER and other organelles

Mitochondria

See diagrams - Mitochondrial function

- "powerhouses" - produce energy
- 100 to several 1000/cell
- 2 lipid bilayer protein membranes
- self replicating (have own DNA)
- probable maternal inheritance

- oxidative enzymes sit on the outer membrane -> oxidation of nutrients (carbo, protein, fats) in respiratory chain
- substrate diffuses into mito cytoplasm where H+ is removed by dehydrogenase and pumped into intracristal space establishing proton gradient provides energy for formation of ATP
- H+ carried by NAD through the respiratory chain on outer membrane
- H+ ionizes and electrons pass along carrier molecules
    - flavoprotein
    - several iron sulphide proteins
    - ubiquinone
    - cytochrome B
    - cytochrome C & C1
    - cytochrome A & A3

- this provides substrate for reactions in the folds of the inner membrane (cristae) -> C02, H20 and ATP
- ATP transporter out of mitochondria into areas of cells that need energy

ATP

See diagram - ATP

- Nitrogenous base (adenine)
- Pentose surgar (ribose)
- Three PO4 radicals
- PO4 bonds = high energy + labile thus can be split easily

Uses:

(1) Membrane Transport
- Na+ through cell membrane
- Other ions

(2) Synthesis of Chemical Compounds
- proteins
- phospholipids
- cholesterol
- purines
- pyrimidines
- precursor for cAMP - intracellular secondary messenger

(3) Mechanical Work
- muscle contraction
- clilary + ameboid motion

Endoplasmic Reticulum

- lipid bilayer membrane with proteins
- network of tubular + flat vesicular structures
- between folds = ER matrix (watery fluid medium)
- multiple enzymes attached
- carries out major metabolic functions

- granular - ribosomes sit on top - synthesize and fold new peptides with disulfide bonds.
- agranular ER (called sarcoplasmic reticulum in muscle) -> synthesise lipid substances + detoxification

Functions:

(1) protein formation
(2) lipid formation - membrane, ER, transport vesicles
(3) enzyme provision for glycolysis
(4) enzyme provision for detoxification
- coagulation
- oxidation
- hydroxylation
- conjugation with glycuronic acid

Golgi apparatus

- closely related to ER
- transport vesicles pinch off and fuse with golgi forming:
    - lysosomes
    - secretory vesicles
    - or cyto components

- major function = provide additional processing of substances formed in ER
    - additional carbo moieties added
    - products compacted down
    - released in vesicles (lysosomes, replenish mem's, secretory)

Lysosomes

- provide an intracellular digestive system
    - damaged cellular structures
    - food particles
    - foreign matter

- 40 different hydrolase enzymes
- split organic compounds using H20 (hydroxylate)

Peroxisomes

- similar to lysosomes except:
- self replicate
- contain oxidases (form hydrogen peroxide - H_2O_2)
- contain catalase (important in detoxifying substances)

Secretory vesicles

- ER and Golgi -> vesicles/granules -> exocytose and act outside cell

Nucleus

- control centre
- DNA -> genes -> RNA
- RNA travels out through pores in nuclear membrane -> ER -> proteins

- determine xteristics of cell (proteins, enzymes, reproduction)

Filament + Tubular structures

- proteins from ribosome's -> polymerize

  - filaments
  - actin
  - myosin
  - tublin
  - cilia
  - centrioles
  - mitotic spindles
  - cytoskeleton

(c) Explain mechanisms of transport of substances across cell membranes

Diffusion (simple/facilitated)
Active transport (primary/secondary)
Bulk Flow or Ultrafiltration

See diagram - ICF & ECF Composition

	ICF	**ECF**
Na	10	142
K	140	4
Ca	0.0001	2.4
Mg	58	1.2
Cl	4	103
HCO3	10	28
Glucose	0-20	90mg/dL
Aa	200	30mg/dL
Protein	16	2g/dL
pH	7.0	7.4
pO2	20	35
pCO2	50	46
Osmolarity	285	285mosmol/L

How this difference is bought about…

Permeability increased by:

- increased lipid solubility
- low molecular weight
- low reflection co-efficient
- increased oil/water partition co-efficient
- decreased membrane thickness
- non-polar molecules

Diffusion

- all molecules in body fluid are in constant motion
- they bounce into each other transferring kinetic energy betwn each other
- particles move from areas of high to low concentration
- this continual movement = diffusion

Simple diffusion

- movement through a membrane without binding with a carrier protein
- rate of diffusion a
    - amount available
    - velocity of kinetic motion
    - number of openings
    - size of openings in membrane
    - lipid solubility - O2, N, CO2, R-OH - very lipid soluble

- water readily penetrates cell membranes via channels
- substances that are H2O soluble can pass through these channels in the same way as long as they are small (ie urea).

Selective permeability - results from diameter, shape, electrical charge on inside of channel

See diagram - Selective Permeability

Facilitated diffusion

- requires the interaction of carrier proteins with molecules/ions and then being shuttled through
- rate a rate @ which the carrier molecule can undergo conformational changes back and forth
- Vmax and saturation kinetics

See diagram - Facilitated Diffusion

- ie. glucose transport (facilitated diffusion increased 10-20x with insulin)

Gating - extensions of transport proteins that can close over or be lifted away by a conformational change in shape of protein.

(1) voltage-gated - open when electrical potential across membrane changes (Na+)

(2) chemical-gating or ligand gaiting - chemical binding -> conformational change -> gates open/close (ie Ach)

Factors that determine net rate of diffusion

(1) Concentration difference

(2) Membrane electrical potential - concentration the same, but +ve charge attracts -ve charge **then** particles move in **but** this creates a concentration gradient - these opposing force balance each other out (Nernst Eqn).

(3) Pressure differences - sum of all forces of molecules striking a unit surface area at a given instant.

Fick's Law of Diffusion

- see formula

Net Diffusion = change in P x A x S / d x (square root of MW)

Change in P = pressure difference
A = area
S = solubility of gas
D = distance of diffusion
MW = molecular weight of gas

- the rate of diffusion is proportional to the concentration gradient
- if more a substance is used up then diffusion will increase

Osmosis

- diffusion of H2O
- process by which solvent molecule diffuse to a region where there is a higher conc of solute to which the membrane is impermeable
- cell membranes are permeable to H2O via channels

Osmotic pressure - the hydrostatic pressure required to stop osmosis (**Van't Hoffs Eqn**)

See Equation

Osmole - concentration of a solution in terms of number of particles

Osmolarity - osmolar conc. Expressed as osmoles/L of solution

Normal osmolarity of ICF/ECF = 300 millosmoles/kg of H2O

Active Transport

- movement of ions/molecules 'uphill' against a concentration gradient.

Primary

- energy from breakdown of ATP (or other high energy compound)

Na-K ATPase

See diagram

- a subunit - 100,000 D
- b subunit - 50,000 D - anchors the a unit
- once 3 Na and 2 K bind, ATP activated
- conformational change
- Na extruded, and K goes inside cell

Functions:

(1) controls cell volume - w/out cells would swell and burst as Na would attract H2O

(2) maintains negative internal RMP (3 Na out and 2 K in)

Secondary

- energy from ionic concentration gradient created by primary active transport

Cotransport - gradient allows the pulling of other substances inside cell (Na-glucose, Na-aa)

Counter transport - uses gradient to power a molecule in the opposite direction (Na-H in PCT)

(d) Gibbs-Donnan Effect

- there is an alteration in the distribution of diffusible ions across any membrane where there is a NON-DIFFUSABLE charged species present on one side of the membrane.

- in the presence of a nondiffusable ion, the diffusible ions distribute themselves so that at equilibrium their concentration ratios are equal.

Effects

(1) causes cell swelling as there are proteins inside cells which attract H2O - thankfully the Na-K ATPase helps regulate cell volume by moving Na (and H2O) along with it.

(2) at equilib there is an asymmetric distribution of permeant ions across membrane -> electrical difference across the membrane (Nernst Eqn).

(3) plasma proteins > interstitial proteins -> Donnan effect on ion movement across capillary wall. This effect contributes an extra 9mmHg to osmotic pressure as attracts cations.

(e) Role of cellular receptors and function of secondary messengers within the cell

- chemical messengers bind to protein receptors on the surface of the cell (or cytoplasm or nucleus)
- triggers a sequence of intracellular changes
- physiological effect

- lots of messengers -> number of receptors decrease
- deficiency of messengers> number of receptors increases

Extracellular ligands = first messengers
Intracellular mediators = second messengers

Mechanisms by which first messengers bring about changes in cell function:

(1) Open/close ion channels in membrane - *Ach*
(2) Increase transcription of mRNA's - *Steroid hormones*
(3) Activate phosplipase C -> increase in DAG, IP3 and other inositol phosphates - *Vasopressin via V1 receptor*
(4) Effect adenyl cyclase -> production of cAMP changed - *Norad via b receptors*
(4) Increase cGMP - *ANP*
(5) Increase tyrosine kinase activity of cytoplasmic portions of transmembrane receptors - *Insulin*

Mechanism by which secondary messengers bring about changes is cell function (often via protein kinases):

(1) Alter enzyme function
(2) Trigger exocytosis
(3) Alter transcription of genes

Affinity constant - strength of binding between ligand and receptor.

As concentration of ligand increases - receptors exhibit:

- saturation

- desensitization - receptor chemically modified to become less responsive
- up regulation/down regulation (see above)

(f) Sources of energy available through metabolism

Diet

Carbohydrate - 50%
Protein - 17%
Fat - 33%

ATP

Energy is stored in **chemical bonds of organic molecules = ATP**

ATP produced and then used throughout the cell to energize almost ALL intracellular metabolic rxns.

See diagram - ATP

High energy PO4 bonds (releases 12,000 calories)

See above for uses of ATP

Production of ATP with Glucose

Aerobic conditions

See diagrams - Glycolysis, Citric Acid cycle, Oxidative phosphorylation

Glycolysis
Citric acid cycle
Oxidative phosphorylation via chemiosmotic mechanism

Anaerobic conditions

- no oxygen -> oxidative phosphorylation isn't an option so glycolysis is increased.
- increased pyruvate concentration which combines with H+ and produces lactate.
- the overall process produces one H+ for each lactate

Consequences:

- decrease pH
- decrease HCO3

- hyperventilation
- fatigue
- confusion
- stupor
- coma
- anorexia
- hyperkalaemia
- decreased myocardial contractility
- vasodilatation

After exercise O2 debt arises as O2 is needed to:

- remove lactic acid
- replenish ATP
- replenish phosphocreatinine O2 stores
- replenish myoglobin O2 stores
- replenish tissue O2 stores

Production of ATP with Fat

- dietary TCA's are hydrolysed to FFA and glycerol

- all cells (except brain) can use FFA interchangeably with glucose for energy
- carnitine transports the FFA into mitochondria where b-oxidation takes place and Acetyl Co A is progressively released

- glycerol -> glycerol-3-phosphate which can enter glycolysis

Production of ATP with Protein

- 25 aa's
- aa can enter the citric acid cycle at different levels and be used to make ATP

Ketogenic aa's - those that are converted to the ketone body acetoacetate and then into Acetyl CoA
Glucogenic aa's - those that give rise to compounds converted to glucose -> pyruvate…

(g) Composition of intracellular fluid and regulation including the role of the Na/K pump

The distribution of fluid betwn compartments is a osmotic effect of small solutes acting across the cell membrane.

Cell membrane is highly permeable to H2O

Cell membrane is impermeable to ions

See diagram - TBW distribution

TBW - measured by tritium marker (takes 6 hours to equilibrate)
ECF - measured by crystalloids (inulin, mannitol) that are large and cannot get in cells **or** by isotopes (Na, Cl)
ICF - measure indirectly (TBW-ECF)
Plasma - measured by radiolabelled albumin or Evans blue.
Interstitial fluid - measured indirectly (ECF-Plasma)

Small changes in the concentrations of impemeant solutes concentrations in ECF can cause tremendous change in cell volume.

Isotonic solutions (285 mosmol/L) - 0.9% NaCl, 5% glucose -> cell stays same volume
Hypotonic solutions (<285) - solutions < than 0.9% NaCl -> cells swell
Hypertonic solutions (>285) - solutions > than 0.9% NaCl -> cells shrink

Osmotic equilib attained in minutes throughout cells
Throughout compartments it takes 30min c/o absorption from gut -> blood -> transport

Na/K ATPase

See diagram - Na+\K+ ATPase

- 2 a subunits and 2 b subunits
- separation renders pump inactive
- pump dependent on Mg
- increased ICF Na or ECF K -> accelerates pump
- effects Ca transport in heart as intracellular Ca is exchanged for extracellular Na in sarcolemma

- a subunit contains the binding site for ATP

Process:

(1) Na binding to 3 receptor sites on inner aspect -> phosphorylation of protein
(2) Activation of ATPase near Na binding site
(3) Cleaving of 1 ATP -> ADP
(4) Conformational change which extrudes Na
(5) K then binds to receptors on external surface and is intruded -> dephosphorylation

(h) The role of G proteins

G protein coupled receptor

- most neurotranmitters act on both GPCR and ligand-gated channels
- examples:

    Muscarinic Ach receptor
    Adrenoceptors
    Dopamine receptor
    5-HT receptor
    Opiate receptor
    Purine receptors
    Chemoreceptors in olfactory & pheromone sensing

See diagram - Molecular Structure

3 families - catergorised by length of extracellular N terminal

(1) Rhodopsin (short)
(2) Secretin/glucagon (intermediate)
(3) Metabotrophic glutamate receptor/calcium sensor (long)

Receptor activation can take place by:

- binding to receptor
- snipping off of N-terminal via protease activity -> exposed residues bind to extracellular loops
- can be constitutively active in absence of agonist

G proteins

- the 'go between' proteins
- about 100 different ligand receptor complexes activate G protein
- 'universal transducers'
- 3 subunits (a,b,g)
- seven spanning (serpentine) receptors working on cytoplasmic face of membrane
- a unit anchored to membrane via fatty acid chain but can freely move in membrane
- interaction with guanine nucleosides (guanosine analogue of ATP)

Process:

See diagram - G proteins & their role

(1) Ligand binds to GPCR
(2) GTP replaces GDP in a subunit
(3) GTP and a subunit complex moves in membrane (some cases bg complex)
(3) Activation of protein kinases -> phosphorylation of aa on proteins
(4) Terminated by hydrolysis of GTP -> GDP by GTPase intrinsic to a unit.

Types: many!

Gi - activation **inhibits** adenylate cyclase -> effects cGMP -> protein phosphorylation

AT II receptors (angiotensin II)
A1 adenosine receptor (adenosine)
m receptor (morphine)
Muscarinic II receptor (atropine - competitively inhibits in heart)
a2 adrenoceptor
D2 receptor
Kappa, Mu, Kappa receptor

Gs - activation **stimulates** adenylate cyclase -> effects cGMP & cAMP -> protein phosphorylation

ANP receptor
ADH receptor
b1 & b2 adrenoreceptor
A2 adenosine receptor - vasodilatation
H2 receptor
Glucagon receptor
5HT 4, 6, 7 receptors
V2 receptor (vasopressin/ADH)
D1 & D5 receptor

Gp - activation stimulates phospholipase C -> hydolyses PIP2 to IP3 and diaglycerol (DAG)

(1) IP3 migrates to ER where it stimulates release of Ca2+ and influx of Ca2+ from ECF (contraction) -> Ca2+ + phosphatidylserine -> activates protein kinase C.

(2) DAG activates protein kinase C from membrane phospholipid

a1 adrenoceptor (norad)
H1 receptor
5HT2
5HT1
AT1
Glutamate 1 and 5 receptors
M1, M3, M5 receptors

See diagram in Faunce pg 10

Gt - stimulates cAMP phosphodiesterase in photoreceptors (transducin)

Go - involved in gating of ion channels

See diagram - Adenylate cyclase/cAMP system

See diagram - Phospliapse C/Inositol Phosphate system

(i) Describe the general response to injury

Inflammation

(1) vasodilation -> excess local blood flow
(2) increased permeability
(3) clotting of fluid in interstitial space
(4) migration of granulocytes and monocytes into tissue
(5) swelling of tissue cells

Acute phase products that cause these reactions:

- Histamine
- Bradykinin
- Serotonin
- Prostaglandins
- Complement - C3a, C5a
- Cytokines released by sensitized T cells

Cytokines:

- TNFa
- IL1 a & b
- IL 6, 8
- GM-CSF
- G CSF
- M CSF

NF-kB - released by endothelial cell -> initiates expression of leukocyte proinflammatory cytokines, chemokines and cell surface adhesion molecules.

Activated Protein C

- increases fibrinolysis
- inhibits coagulopathy by suppressing NF-kB
- inhibits apoptosis-1
- inhibits endothelial NOS

Fever

- common response to tissue injury
- causes:

    Infection
    Drugs
    Vasculitis
    Ischaemia
    Trauma
    Carcinoma
    Endocrine issues

- cytokines (IL 1, 6 and TNF a) -> preoptic anterior hypothalamic thermoregulatory receptors -> synthesis and release of PG E2 -> increase local cAMP -> resets hypothalamic set point.

SIRS

Requires 2 more of the following:

    Temp > 38 or <36
    HR >90
    RR > 20
    $PaCO_2$ < 32
    WBC >12 or < 4 or >10% neutrophils

Sepsis

SIRS caused by infection