BIO 226N Study Guide
INTRODUCTION

LINNEAUS - Genus and species

KINGDOMS

EUCARYOTES - true nucleus
* ANIMALS
* PLANTS
* FUNGI - primitive plants
* PROTISTA - primitive animals (one cell)

EUBACTERIA - true bacteria
* TYPICAL BACTERIA
* PROCARYOTES
  No nucleus
  Single cells
  Contain both RNA and DNA

ARCHEOBACTERIA - unusual bacteria
PROCARYOTES
extreme environments, hot springs
high salt concentration
do not have typical Eubacterial cell walls
Ribosomes similar to those in higher organisms

BACTERIAL CLASSIFICATION

DIVISION - SECTION - FAMILY - GENUS - SPECIES
DIVISIONS ARE DIVIDED ON BASIS OF CELL WALL STRUCTURE

DIVISION:
I. - STAIN RED - GRAM NEGATIVE
II. - STAIN BLUE - GRAM POSITIVE
III. - WALL-LESS BACTERIA
IV. - BACTERIA WITH UNUSUAL CELL WALLS INCLUDING ARCHEOBACTERIA

ADDITIONAL CHARACTERISTICS WHICH FURTHER DIVIDE INCLUDE:

CELL MORPHOLOGY - SIZE, SHAPE
MOTILITY
COLONY COLOR
PATHOGENICITY
BIOCHEMICAL PROPERTIES

EXAMPLES:

DIVISION I - GRAM NEGATIVE
SECTION 1 - SPIROCHETES - SPIRAL SHAPE, FLEXIBLE
Treponema pallidum - syphilis

DIVISION II - GRAM POSITIVE
SECTION 16 - MYCOBACTERIAL - RODS, THICK WAXY WALLS
Mycobacterium tuberculosis
SECTION 12 - COCCI - GRAM POSITIVE SPHERES
FAMILY 1 - *MICROCOCCACEAE* - SPHERES IRREGULAR CLUMPS OR PACKETS OF 4 OR PACKETS OF 8
GENUS - *Staphylococcus* -
SPECIES
aureus - YELLOW COLONY; CAUSES BOILS
epidermidis - WHITE COLONY; NOT CAUSING DISEASE USUALLY
IMPORTANT GENUS (not in any family): *Streptococcus pyogenes* - STREP THROAT

BIO 226N Study Guide
LAB METHODS

I. CULTURE MEDIUM - MIXTURE OF NUTRIENTS

A. Composition of Media

1. Enriched:
extract of beef muscle, heart, brain
yeast cell extract
2. Defined - exactly known, pure chemicals
Carbon + Energy Source (glucose), H_20
N - NH_4Cl
S - $MgSO_4$
P - Na_2HPO_4
Common Minerals
Mg^{++}, K^+, Na^+, Cl^-
Trace Elements
Fe^{+++}, Ca^{++}

B. Liquid or Solid (semisolid) media

1. Liquid solutions
2. Semisolid - gelling agent - agar melts at 100°C; solidifies again at 41°C, Petri dishes

II. STERILIZATION

A. Dry Heat - 160-170°C - 2 hr - glassware
B. Autoclave - 121°C 15 lbs pressure/sq. inch, 15 min.
C. Filtration - sterile - 0.45 µm diameter
D. Flame - incineration of loop
E. Poison Gas - Ethylene oxide/plasticware
F. Radiation -Xrays, Gamma Rays

III. PURE CULTURE STREAK PLATE

IV. METRIC SYSTEM:

A. Distance Units -

Distance
1 meter = 39.37 inch = 3.28 Feet = 1.09 yards
1 decimeter = 1/10 m or 1 X 10^{-1}m
1 centimeter = 1/100 m or 1X 10^{-2}m
1 milimeter = 1/1000 or 1 X 10^{-3}m
1 micrometer (µm) = micron or 1 X 10^{-6}m
1 nanometer (nm) = 1 X 10^{-9} m
1 Angstrom (A) = 0.1 nm = 1 X 10^{-10}m
BACTERIA 1 X 3µm

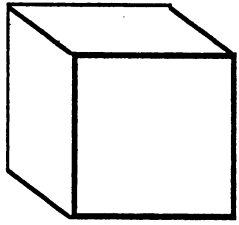
B. Volume Units -

Volume
1 liter = 1.06 quart — 1 quart = 0.946 liter
1deciliter = 1/10 liter — or 1X 10^{-1} liter
1 centiliter = 1/100 liter — 1X 10^{-2} liter
1 milliliter = 1/1000 liter — 1X 10^{-3} liter
1 microliter (µL) — 1X 10^{-6} liter

C. Mass Units -

Mass
1 gram = 0.036 ounce — 1 ounce = 28.4 grams
1 milligram = 1/1000 gram or 1X 10^{-3} gram
1 microgram =1X 10^{-6} gram
1 nanogram =1X 10^{-9} gram

VOLUME IS DISTANCE IN THREE DIMENSIONS



1 cubic centimeter = 1 cc = 1 milliliter (volume)

V. STAINS

A. Simple stain - fix, dye, crystal violet, methylene blue
B. Negative stain - background is colored
C. Gram stain - crystal violet & I_2, alcohol, counter stain with safranin
D. Acid fast stain - *Mycobacterium* - fix, dye & heat, decolorize with acid & alcohol

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CHEMICAL PRINCIPLES

I.

ATOMIC STRUCTURE

ATOMS; ELEMENTS

NUCLEUS - PROTONS (POSITIVE)

- NEUTRONS (NEUTRAL)

ELECTRONS (NEGATIVE)

ATOMIC NUMBER - # OF PROTONS

ATOMIC WEIGHT - # OF PROTONS PLUS NEUTRONS

ELECTRON SHELLS - ELECTRONIC CONFIGURATION

SHELLS GOING FROM NUCLEUS OUTWARD 2,8,8

NUMBER DONATED, ACCEPTED or shared ELECTRONS is called VALENCE

MOLECULE - at least 2 atoms, H_2, O_2, H_2O

COMPOUND - at least 2 different kinds of atoms, H_2O, CH_4

MOLECULAR WEIGHT - SUM OF ATOMIC WEIGHTS

H_2O	H	2 X 1 =	2
	O	1 X 16 =	16
Molecular Weight			18

MOLE - 6.023×10^{23} molecules; MOLECULAR WEIGHT in grams

II.

CHEMICAL BONDS

A. IONIC BONDS - donate electrons, receive electrons

B. COVALENT BONDS - sharing electrons

C. HYDROGEN BONDS - attraction of partial positive and partial negative charges
- unequal distribution of charges in the molecule
- polarity of water

III.

WATER, SOLVENT, SOLUTE, DISSOCIATION

A. ACIDS H^+ and ANION (OTHER THAN OH) (PROTON DONOR)
HCl $\rightleftharpoons$ $H^+ + Cl$

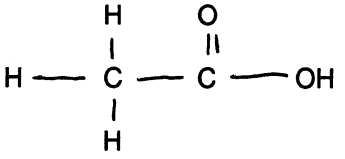
B. BASE CATION (OTHER THAN H^+) + OH^- (PROTON ACCEPTOR)
NaOH $\rightleftharpoons$ $Na^+ + OH$

C. SALT CATIONS + ANIONS OTHER THAN H^+ or OH
NaCl $\rightleftharpoons$ $Na^+ + Cl$

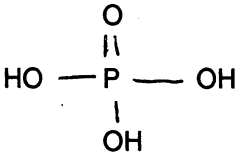
IV.

CLASSES of ORGANIC CHEMICALS - CARBON SKELETONS - FUNCTIONAL GROUPS

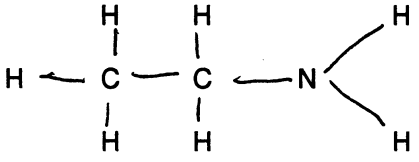
A. ACIDS

Ex. ACETIC ACID  - CARBOXYLIC ACID

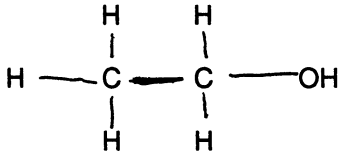
2C at 12 = 24
2O at 16 = 32
4H at 1 = 4
60 MOL WEIGHT

PHOSPHORIC ACID 

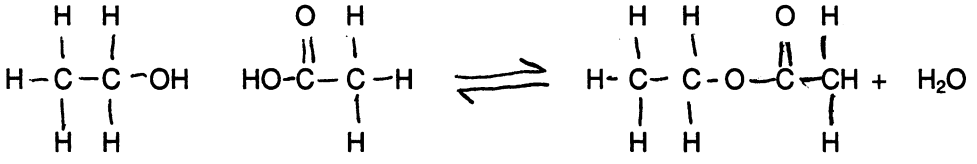
B. AMINES- NH_2; ETHYLAMINE



C. ALCOHOLS- OH; HYDROXYL GROUP

 ETHYL ALCOHOL

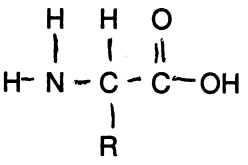
D. ESTERS ALCOHOL + ACID ESTER + H_2O



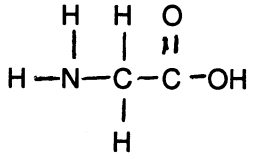
V.

LOW MOLECULAR WEIGHT BUILDING BLOCKS - PRECURSORS

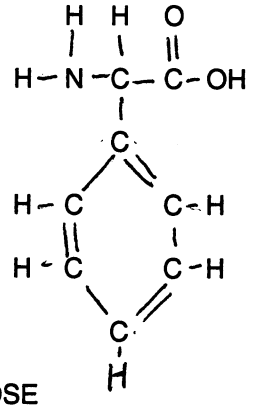
A. AMINO ACIDS



GLYCINE



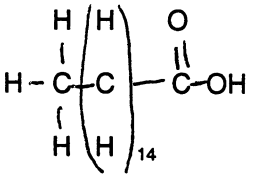
PHENYLALANINE



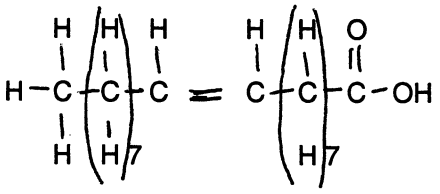
PEPTIDES, PROTEINS

B. SUGARS $(CH_2O)n$ - MONOSACCHARIDES - GLUCOSE
POLYSACCHARIDES (STARCH) DISACCHARIDES - SUCROSE

C. FATTY ACID - SATURATED - PALMITIC ACID



UNSATURATED - OLEIC ACID



LIPIDS (GLYCEROL PHOSPHATE, PHOSPHOLIPIDS)

D. NUCLEOTIDES - BASE: PURINES: ADENINE, GUANINE
PYRIMIDINES: THYMINE, URACIL, CYTOSINE
SUGARS: DEOXYRIBOSE, RIBOSE
PHOSPHATE; NUCLEOSIDE, NUCLEOTIDE

VI.

MACROMOLECULES - PROTEINS, POLYSACCHARIDES, LIPIDS, POLYNUCLEOTIDES, DNA, RNA.

A. PROTEINS: AMINO ACIDS, PEPTIDE BONDS

B. POLYSACCHARIDES: CHAINS OF MONO- OR DISACCHARIDES

C. LIPIDS/PHOSPHOLIPIDS
FATTY ACIDS PLUS GLYCEROL/ FATTY ACIDS PLUS GLYCEROL PHOSPHATE

D. NUCLEIC ACIDS (POLYNUCLEOTIDES)
RNA/DNA

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CELL STRUCTURE

I. CELL SHAPE

A. Coccus - (cocci) - spheres
   Single coccus: *Micrococcus luteus*
   Diplococcus (two cocci): *Neisseria gonorrhea*
   Neisseria meningitidis
   Clusters of cocci: *Staphylococcus aureus*
   Streptococcus (chains): *Streptococcus pyogenes*
B. Bacillus (bacilli) - cylinders, rods
   Bacillus anthracis - anthrax
   Corynebacterium diphtheriae - diphtheria - pleomorphic rods
C. Spiral shape
   1. Rigid -
      a. curved - (comma), *Vibrio cholerae* - cholera
      b. spirilla - *Rhodospirillum rubrum*
   2. Flexible - Spirochaetes: *Treponema pallidum* - syphilis
      Borrelia burgdorferi - Lyme Disease

II. COMPARISON OF PROCARYOTES & EUCAROYOTES

PROCARYOTES	EUCAROYOTES	
NUCLEOID	TRUE NUCLEUS	CHROMOSOMES IN MEMBRANE
FISSION	MITOSIS	
SEX - PRIMITIVE	SEXUAL REPRODUCTION	
	MEIOSIS	EGGS/SPERM
NONE (MESOSOMES)	MITOCHONDRIA	ENERGY
CHROMATOPHORES	CHLOROPLASTS	PHOTOSYNTHESIS
CELLS WALLS - PEPTIDOGLYCAN	CELL WALL	ANIMAL- NONE
		PLANT - CELLULOSE
		FUNGI - CHITIN
RIBOSOMES SMALLER, 70S	RIBOSOMES	LARGER, 80S

III. CELL COMPONENTS

A. GLYCOCALYX/CAPSULE
   Ex. *Streptococcus pneumoniae*
B. FLAGELLUM (Flagella): 10μm X 14nm
   MONOTRICHOUS - *Pseudomonas aeruginosa*
   AMPHITRICHOUS -
   PERITRICHOUS -
   LOPHOTRICHOUS -
   TAXIS; CHEMOTAXIS; ATTRACTANT & REPELLANTS;
C. PILUS (pili): 1μm X 7nm
D. FIMBRIAE: *Neisseria gonorrhoeae*
E. CELL WALL - (CELL ENVELOPE); SHAPE, OSMOTIC PROTECTION
   NAG: N-acetylglucosamine
   NAM: N-acetyl muramic acid (sugar with hydroxyls and acid group)
   NAG - NAM - disaccharide ; TETRAPEPTIDE SIDE-CHAIN; CROSSLINKING
   AMINO ACIDS - PENTAGLYCINE

COMPARISON OF CELL WALLS OF GRAM POSITIVE AND GRAM NEGATIVE

GRAM POSITVE	GRAM NEGATIVE
TEICHOIC ACID	LIPOPOLYSACCHARIDE OUTER
	LIPOPROTEIN MEMBRANE
	PHOSPHOLIPID
PEPTIDOGLYCAN (MANY LAYERS)	PEPTIDOGLYCAN (1-2 LAYERS)
	PERIPLASMIC SPACE

F. CYTOPLASMIC MEMBRANE
PHOSPHOLIPD BILAYER & PROTEINS
FLUID MOSAIC MODEL
SELECTIVELY PERMEABLE BARRIER

G. CYTOPLASM - 80% H_2O, PRECURSORS, ENZYMES, RIBOSOMES

H. NUCLEOID - CHROMOSOME - DNA - GENES, GENETIC INFORMATION
5×10^6 MONONUCLEOTIDE PAIRS
3×10^9 MOL. WEIGHT
2000 - 4000 GENES, GENETIC INFORMATION

I. ENDOSPORES (SPORES)
SPORULATION - DORMANT, RESISTANT, SPORE COAT
GERMINATION - OUT GROWTH OF CELLS (VEGETATIVE GROWTH AND CELL DIVISION)

Ex. *Clostridium botulinum*, - TOXIN - BOTULISM
Cl. perfringens - GAS GANGRENE
Cl. tetani - TETANUS
Cl. novyi - NON-PATHOGEN
Bacillus anthracis - ANTHRAX
Bacillus subtilis - NON-PATHOGEN

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NUTRITION and METABOLISM

I. INTRODUCTION

A. SYNTHESIS REACTIONS - ANABOLIC
ENERGY INPUT
B. BREAKDOWN REACTIONS - CATABOLIC
ENERGY USES
1. CHEMICAL WORK
2. MOTILITY
3. LIGHT PRODUCTION

II. NUTRIENTS - HOLOPHYTIC
CARBON AND ENERGY SOURCE;
NITROGEN, PHOSPHORUS, INORGANIC IONS, WATER;
OTHER ESSENTIAL COMPOUNDS

III. ENZYMES - BIOLOGICAL CATALYSTS; PROTEINS; ACTIVATION ENERGY;
TURN OVER NUMBER; SPECIFICITY;

A. INCREASE FREQUENCY OF COLLISIONS
B. HOLD REACTANTS IN PROPER ORIENTATION
C. LOWER ACTIVATION ENERGY

COENZYME (IONS, VITAMINS)
APOENZYME + COENZYME = HOLOENZYME

IV. ENERGY PRODUCTION AND STORAGE
OXIDATION OF FOOD; GLUCOSE OXIDATION

$$C_6H_{12}O_6 + 6CO_2 \rightarrow 6CO_2 + 6\,H_2O + \text{ENERGY (ATP)}$$

A. ATP PRODUCTION (GENERATION) FROM FOOD OXIDATION
1. SUBSTRATE LEVEL PHOSPHORYLATION
2. OXIDATIVE PHOSPHORYLATION

OXIDATION / REDUCTION REACTIONS
Loss of e^- electrons / Gain of e^- electrons

Ex. $NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+$

NICOTINAMIDE ADENINE DINUCLEOTIDE
ELECTRON TRANSPORT CHAIN
B. ATP PRODUCTION IN PHOTOSYNTHESIS / PHOTOPHOSPHORYLATION
C. CHEMIOSMOSIS - THEORY FOR ATP PRODUCTION
1. ETC (ELECTRON TRANSPORT CHAIN) - OXIDATION / REDUCTION

$NADH + H^+ \rightarrow NAD^+ + 2e^- + 2H^+$
(OR CHLOROPHYLL EXCITED)

2. $1/2\ O_2 + 2e^- + 2H^+ \rightarrow H_2O$
3. ETC SPLITS H_2O; PUMPS H^+ OUTSIDE
4. GRADIENT: H^+ OUTSIDE; OH^- INSIDE
5. ENERGIZED MEMBRANE - PROTON MOTIVE FORCE
6. H^+ ATP-ase PUMPS H^+ INSIDE
7. H^+ MOVEMENT ACROSS ENERGIZED MEMBRANE PROVIDES ENERGY TO CONVERT ADP TO ATP

V. GLYCOLYSIS - CATABOLIC BREAKDOWN OF COMPOUNDS
GLUCOSE $\rightarrow$ 2 PYRUVATES + ENERGY
INPUT: 2 ATP
YIELD: 4 ATP + 2NADH + $2H^+$
NET: 2 ATP + 2 NADH + $2H^+$ (EACH NADH GIVES 3 MOLECULES OF ATP IN ETC)

VI. RESPIRATION - CATABOLIC
PYRUVATE $\rightarrow$ $CO_2 + H_2O$ + ENERGY
ACETYL CoA; KREBS CYCLE
SUMMARY OF GLYCOLYSIS & RESPIRATION
GLUCOSE + $6O_2 \rightarrow 6CO_2 + 6H_2O$ + ENERGY
INPUT 2 ATP: YIELD 40 ATP

VII. FERMENTATION
YEAST: PYRUVATE $\rightarrow$ ETHANOL
LACTIC ACID BACTERIA: LACTIC ACID

VIII. ANABOLISM - SYNTHESIS
LOW MOLECULAR WEIGHT COMPOUNDS
POLYMERIZED INTO HIGH MOLECULAR WEIGHT COMPOUNDS

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BACTERIAL GROWTH

I. Physical Factors

A. Temperature

Psychrophiles 0-20°C

Mesophiles 25-40°C

Thermophiles 45-near 100°C

B. pH

Acid $\rightleftharpoons H^+$ + Anion

Base $\rightleftharpoons$ Cation + OH

Water $\rightleftharpoons H^+ + OH^-$

$pH = -Log_{10}[H^+]$ (Molar Conc.)

Molecular Weight

Molar Conc. = Moles/Liter

Water $pH = -Log[10^{-7}] = -(-7) = 7$

pH scale, water pH = 7 = neutral

Buffer:

$$NaH_2PO_4 \rightleftharpoons Na^+ + H_2PO_4^- \rightleftharpoons Na^+ + H^+ + HPO_4^{--} \rightleftharpoons Na^+ + 2H^+ + PO_4^{---}$$

C. Osmotic Pressure

Solution	Water Concentration Outside the Cells	Inside the Cells	Net Movement of Water Out	In
Isotonic	Normal	Normal	Out	In
Hypotonic	Higher	Normal	Out	In
Hypertonic	Lower	Normal	Out	In

Osmosis, Isotonic, (iso=equal), Hypotonic (hypo-under or less)
Hypertonic (hyper=above), Halophils, Solvent (H_2O) & Solute

II. Chemical Requirements

C; N; P; S

Nitrogen Fixation
Oxygen - aerobes, anaerobes, facultative anaerobes

III. Growth Cycle - Binary Fission

Generation Time (25-30 min); Doubling of Cells

IV. Dealing with Bacterial Numbers

1 liter = 1000 ml = 10^3 ml — metric system
1 milliliter = 1000 µl = 10^3 µl — For measuring volume
1 liter = 10^3 ml = 10^6 µl

V. Growth Curve - Lag; Exponential (Logarythmic); Maximum Stationary; Death

VI. Measuring Cell Numbers

A. Plate counts or colony counts

B. Filtration of Water: Coliforms, *Escherichia coli, Shigella, Salmonella, Campylobacter,* Hepatitis virus

C. Absorbance

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BACTERIAL GENETICS

I. GENETIC INFORMATION - STORAGE & USE

A. GENES: DNA - Chromosome, nucleotide, sequence in AGCT: gene products, enzymes, function

B. REPLICATION - synthesis of DNA (chromosome)
origin, replication form, terminus;
semiconservative replication - complementary daughter strand synthesized from each parent template - so DNA contains one parental strand an one new strand

C. TRANSCRIPTION - copying information from DNA (one coding strand) into mRNA (messenger RNA), mRNA synthesis. PROMOTER, TERMINATOR, mRNA - single stranded RNA

D. TRANSLATION - use of mRNA to synthesize proteins.
codons (Triplets). Reading frame. Start, stop
ribosomes; Amino Acids + tRNA + ATP.c Activated amino acids
INITIATION CODON = METHIONINE - AUG
STOP CODON, NONSENSE CODON - UAA, UAG, UGA

E. MUTATION - change in nucleotide sequence of DNA results in change in mRNA, results in change in amino acid sequence of protein, subsequent loss of enzyme function, altered 3D structure - usually has no enzyme acitivity.
wild type - mutant
genotype - gene sequence in nucleotides
phenotype - property one can see, observe,
mutagen - mutagenic

F. INFORMATION FLOW:

DNA c RNA c PROTEIN
REPLICATION c DNA c DNA
TRANSCRIPTION c DNA c RNA
TRANSLATION c RNA c PROTEIN

II. DNA EXCHANGES

A. TRANSFORMATION:
Streptococcus pneumoniae, smooth colonies; forms capsule, wild type, causes disease:
rough colonies, no capsule, nonpathogenic; recombination

B. CONJUGATION: PLASMID, MALE, F-FACTOR, R-FACTOR, TRANSPOSONS.
Contact between male and female bacteria, conjugation bridge, TRANSFER DNA from donor to recipient.

C. RECOMBINATION: BREAKING AND REJOINING STRANDS OF DNA
Transformation and Conjugation

D. PLASMID DNA TRANSFER BY TRANSFORMATION *IN VITRO*

E. RECOMBINANT DNA: Restriction enzymes, Target DNA, vector DNA; hybrid plasmid use

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VIROLOGY

I. Discovery – Ivanowski, 1892, Tobacco Mosaic Virus
Löffler & Frosch, 1898, Foot & Mouth Disease Virus
D'Herelle & Twort, 1915-17, Bacterial viruses, Bacteriophage, Phage.

II. Differences between bacterial viruses and Bacteria.

	Phages	Bacteria
A.	Size- smaller	larger
B.	RNA or DNA chromosome	DNA – chromosome RNA – [mRNA, tRNA, rRNA]
C.	Capsid	Cell wall and cytoplasmic membrane
D.	Grow only in living cells	Grow in growth medium
E.	Direct synthesis of components which then assemble	Binary Fission

III. Virulent Bacterial Viruses (e.g. T4)

A. Structure
B. Lytic growth cycle -
1. adsorption – attachment (receptors)
2. injection of genetic material, penetration (0-1 min)
3. Synthesis of components (1-20 min)
Transcription
Translation – host enzymes
Replication
Host energy
4. Maturation (20-30 min)/Assembly
5. Lysis – Lysozyme; release of ~200 phages
C. Growth in plaques – Enumeration
Host, Lawn, Confluent growth, plaques

IV. Temperate phages (lambda) λ

A. Lytic growth or Lysogeny;
B. Lysogeny – attachment, injection, integration, repressor
Prophage, passive replication with bacterial chromosome.
Lysogenic, Lysogen.
C. Induction of the prophage, inducing agents, excision of the prophage and lytic growth

V. Animal viruses

A. Differences between bacteriophage and animal viruses

1. Presence of envelope
2. Host Entry: a. Endocytosis, vesicle
   b. Fusion with host membrane & uncoating
3. Virus exit – budding of envelope viruses
4. Cytopathic effect
5. Long latent period
6. Tissue tropism
7. Some RNA viruses replicate through DNA intermediate and integrate DNA into host chromosome

B. Virus growth in the lab

1. Living Animals
2. Embryonated eggs
3. Tissue culture/cell culture; contact inhibition; plaques

C. Tumors & Viruses (Neoplasm)

Benign
Malignant (Metastasis)
Carcinoma – epithelial cells
Adenocarcinoma – epithelium of glands
Sarcoma – connective tissue
Leukemia – white blood cells

1. Causes – mutations/viruses
   Carcinogens, oncovirus, oncogene, proto-oncogene; growth hormones, hormone receptor proteins, cell cycle control proteins.
   Mouse mammary tumor virus (MMTV), Bittner 1936.
2. Transformation of cultures animal cells
   Rous sarcoma virus, cells growing in monolayers, contact inhibition;
   Retroviruses RNA → RNA/DNA hybrid → DNA → Provirus
3. Human Tumors & Viruses

   a. Epstein Barr virus (EBV) – Infectious Mononucleosis
      Burkitt's Lymphoma, Nasopharyngeal carcinoma.
   b. Herpes Simplex I & II –HHVI and HHVII
      Fever blister; stress; genital herpes (cervical carcinoma?)
   c. Human T-cell leukemia (Retrovirus)
      HTLV-I, HTLV-II

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Study Guide
Organisms Intermediate Between Viruses and Bacteria
"Intermediate" Bacteria

A. MYCOPLASMA – pleuropneumonia group - PPLO

BACTERIA without cell walls, pleomorphic
Need osmotic protection, size 0.1 to 0.25 mm diameter grow in serum containing media: have sterols in membranes
Resistant to antibiotics which act on cell walls.
Diseases: *Mycoplasma pneumoniae;* Pleuropneumonia; Primary atypical pneumonia.

NGU/non-gonococcal urethritis/ - *Mycoplasma hominis*, NGU also caused by *Ureaplasma urealyticum* – causes infertility
Patients with STD sometimes also carry *U. urealyticum.*

B. RICKETTSIA – 0.3 to 0.7 mm diam/ 1-2 mm length, Pleomorphic rods, obligate intracellular parasites of animal (human) cells; *Rickettsia rickettsii* – Ricketts, Rocky mountain spotted fever.

Rickettsia Prowazekii – Epidemic typhus
PERSON/LOUSE/PERSON/LOUSE/PERSON
person is the reservoir
louse is the vector

Rickettsia typhi – Endemic typhus
RAT/FLEA/RAT/FLEA/HUMANS

C. CHLAMYDIA – cocci 0.2 – 1.5 mm diameter

Obligate intracellular parasites of animal and humans grow in vertebrate hosts.
Transmission host to host
No ATP generating system, ATP dependence on the host
Chlamydia psittasci – psittacosis or Parrot Fever
Chlamydia trachomatis

1. trachoma – blindness
2. non – gonococcal urethritis
3. lymphogranuloma venereum (genital and anal regions)
   Chlamydia pneumoniae – mild pneumonia

BIO 226N
Study Guide
Mycology

1. General characteristics

Fungus, fungi (molds and yeasts); eukaryotes; primitive plants; chitin in cell wall; non motile; 5-10 mm diam., nucleus; mitosis.

Tolerate dryness, high osmotic pressure; acidity and alkaline pH in the environment.

2. Colony types

Mycelium – hypha, hyphae
Yeast cells – spheres, buds
Aerobic respiration; $CO_2 + H_2O$

3. Organisms - Examples

A. *rhizopus nigricans* – coenocytic hyphae, sporangium, sporangiophore, sporangiospores
B. *Aspergillus niger* – septate hyphae, conidiospores, conidiophores
C. *Penicillium notatum* – penicillin
D. Mushrooms – Basidiomycetes
E. Yeasts – *Saccharomyces cerevisiae*
F. Actinomycetes –Filamentous prokaryotes; antibiotic production–ex. *Streptomyces*

4. Diseases – mycosis, mycoses

A. Infection of skin, hair, nails – dermatophytes secrete enzyme called keratinase which degrades keratin. Examples – ringworm and athlete's foot
B. Systemic mycoses – deep organs
   1. *Histoplasma capsulatum* – Histoplasmosis endemic in Ohio River Valley, Mississippi River Valley, dimorphic fungus
   2. *Coccidioides immitis* – Coccidioidomycosis; desert fever; 95% mild respiratory disease; 5% chronic respiratory, TB-like generalized infection; Central, South America, Sacramento Valley, CA.
   3. *Cryptococcus neoformans* – Cryptococcosis; infects lungs, respiratory tract of humans; can be disseminated into the central nervous system; can cause meningitis – inflammation of the meninges.

C. Opportunistic pathogens (infections)
Candida albicans – thrush or moniliasis

Pneumocystis carinii – causes Pneumocystis pneumonia in immunosuppressed patients.

5. Toxins – Mycotoxins

Aspergillus flavus – produces toxic compounds called aflatoxins – contaminates peanuts, grain, cereal, corn, etc.; carcinogenic.

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STUDY GUIDE
CONTROL OF MICROBES

I. Physical Methods

A. High temperature

1. Dry heat - Sterilization - 160 - 170º C - 2 hours
2. Moist heat:
   a. Autoclave 121º C, 15 psi, 15-20 minutes
   b. Pasteurization - mild heating 63º C, 30 minutes
      milk, dairy food and cheese; kills most pathogens
      without damaging taste. Cannot kill endospores.
      72º C, 15 sec - HTST - high temperature, short time pasteurization
      140º C, 1 sec - UHT - ultra high temperature pasteurization

B. Low temperature 4º C - food preservation

C. Freeze-drying - lyophilization

D. Desiccation - drying

E. Osmotic pressure - high concentrates of salts & sugars can preserve food

F. Radiation: UV, x-ray, gamma rays used for sterilization of pharmaceutical and disposable dental & medical supplies; plastic syringes, surgical gloves - replacing gases.

G. Filtration can be used to sterilize liquid media

II. Chemical Methods

Sterilization, disinfection, antisepsis, bacteriocidal, bacteriostatic

A. Acids: Propionic acid, Glutamic acid, Benzoic acid:
   Calcium propionate and sodium glutamate

B. Alcohols: Ethanol, Isopropanol

C. Phenol (Carbolic acid): Phenolics (Cresol)

D. Halogens

1. Cl_2 - Chlorine

   $Cl_2 + H_2O \rightarrow H^+ + Cl^- + HOCl$ Hypochlorous acid

   $HOCl \rightarrow H^+ + OCl^-$ Hypochlorite ion

2. Iodine - 2% in alcohol is tincture of iodine

E. Heavy metals, $AgNO_3$ Silver Nitrate

F. H_2O_2 - Hydrogen Peroxide

G. Ethylene oxide

H. Formaldehyde

Formalin

CHEMOTHERAPY

Synthetic drugs, drugs produced by bacteria and fungi called antibiotics

Selective toxicity

I. Ehrlich 1906 - Chemotherapy idea

II. Sulfonamides 1930 - Sulfanilamide - PABA analog
PABA → FOLIC ACID (VITAMIN)
Streptococcus pyogenes and urinary tract infections

III. Antibiotics
A. Penicillin - Fleming - *Penicillium notatum*
Inhibitor of cell wall peptidoglycan synthesis
B. Streptomycin - Waksman - *Streptomyces griseus*
Mycobacterium tuberculosis
protein synthesis inhibitor on 70S ribosomes
others: Tetracycline, Erythromycin, Chloramphenicol

IV. Drug Mechanisms
A. Cell wall synthesis inhibition
B. Effects on membranes
C. Protein synthesis inhibition
D. Nucleic acid synthesis inhibition

V. Anti-viral Drugs

VI. Complications of Drug Use
A. Hypersensitivity - Penicillin
B. Toxicity - Streptomycin - Otic nerve damage
Chloramphenicol - pernicious anemia
C. Normal flora destruction -
Microbial antagonism
Prolonged use of antibiotic
Opportunistic pathogen
Candida albicans - Thrush, Vaginitis
D. Spread of multiple drug resistance
Pencillinase - *S. aureus*
Resistance plasmids - resistance factors
RTF (plasmid replication, plasmid transfer, drug resistance)
Multiple drug resistance - Japan - resistance to Sulfonamide, Streptomycin, Chloramphenicol, and Tetracycline

% Shigella with multiple drug resistance

	1954	1964
Japan	0%	50%

	1962	1965
London	3%	61%

VII. Transposons

Mobile genetic elements - jumping genes
Carry antibiotic resistance genes

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STUDY GUIDE
NORMAL FLORA, INFECTIONS

I. NORMAL FLORA
Symbiosis;
A. Skin - barrier - normal flora - diphtheroids - *Corynebacterium*
opportunistic pathogens *S. aureus; S. epidermidis; Candida albicans*
B. EYE - Lysozyme
C. RESPIRATORY TRACT
Nose + Nasopharynx - *Neisseria* species (non pathogenic)
Strep. pneumoniae
Strep. pyogenes; Hemophilus influenzae; Neisseria meningitidis
(carriers-adenoids, tonsils) *Pneumoncystis carinii* - Fungus; opportunistic pathogen
D. DIGESTIVE TRACT: Mouth + Oropharynx ; normal flora: Spirochetes, Lactobacilli, Diphtheroids; opportunistic pathogens *Candida albicans*; *Streptococcus mutants* - pathogen
Stomach - pH < 2.0 - acidic
Helicobacter pyloris - pathogen, ulcers
Intestine -
anaerobes & facultative anaerobes, coliforms,
Streptococcus fecalis, Klebsiella, Proteus,
Enterobacter, E. coli (pathogenic and non-pathogenic strains)
E. REPRODUCTIVE TRACT
Lactobacillus, Candida, Trichomonas vaginalis
F. UROGENITAL TRACT - opportunistic pathogens
Candida albicans - vaginitis
Trichomonas vaginalis - protozoan

II. INFECTION
A. DISEASE CLASSIFICATION - infectious, inherited, degenerative, neoplastic, nutritional deficiency, idiopathic
Pathology, Etiology, Virulence, Pathogenicity
B. RESERVOIR - source - animal body, food, water, soil, blood, human body
C. TRANSMISSION
1. Contact
a. Direct - person to person (example, sexual contact)
b. Indirect - contaminated object - Fomite (example, shared needle)
c. Droplets - sneezing, coughing
<1 meter travel (example, common cold)
2. Vehicles - food, water, airborne dust or droplet, nuclei, blood
Food - water
Shigella - Shigellosis
Salmonella - Salmonellosis
Vibrio - cholera
Hepatitis Virus A - infectious hepatitis
3. Airborne - dust, droplet nuclei -
travel more than 1 meter in the air
Mycobacterium tuberculosis
Histoplasma capsulatum
Measles, Chicken pox, Polio
Blood - Hepatitis B - serum hepatitis
3. Vectors - mechanical vector - flies
biological vector - example, Lyme disease
Deer tick, deer, mice, dogs, cats, people
Borrelia burgdorferi

D. NOSOCOMIAL INFECTIONS (Hospital Acquired)
Surgical wound infections
Catheters (urinary tract infections)
Immunosuppressed patients
E. SPREAD IN POPULATIONS
Endemic - always present - *Histoplasma capsulatum*
Epidemic - large number of cases in short time - cholera, typhus, influenza
Pandemic - world-wide epidemic AIDS
Sporadic - small number of isolated cases - Hanta virus
F. SPREAD IN INDIVIDUAL
Primary - influenza
Secondary - *Staph. aureus* pneumoniae

III. INVASIVE MECHANISMS
Pathogenicity, Virulence, Candida vs Pasteurella tularensis

A. CAPSULES - Strep pneumniae
B. EXOTOXINS - diptheria, botulism, gas gangrene
cholera, tetanus, scarlet fever

Corynebacterium diphtheria
Clostridium botulinum
Cl. perfringens
Vibrio cholerae
Cl. tetani
Strep pyogenes

C. ENDOTOXIN - cell walls of gram negative cells
phospholipic, lipoprotein, lipopolysaccharides (lipid A)

D. HEMOLYSINS - lyse RBC
Strep. pyogenes B hemolytic

E. LEUCOCIDINS - kills WBC (leucocytes)
Mycobacterium tuberculosis, Strep., Staph.

F. HYALURONIDASE - dissolve cementing substance
Clostridium - gass gangrene; *Streptococcus fasciatis*

G. STREPTOKINASE - dissolve blood clots
STAPHYLOKINASE

H. COAGULASE - clots blood, protects microbes inside the clot
Staph. aureus

IV. KOCH'S POSTULATES
A. Same organism present in every case of disease
B. Organism must be isolated from diseased host and grown in pure culture
C. Introducing pure culture into susceptible host causes same disease
D. Organism must be isolated from the deliberately infected host and grown again in pure culture

BIO 226N
Study Guide
Non Specific Resistance

A. SKIN, MUCOUS MEMBRANES, MUCUS, MECHANICAL 7 CHEMICAL BARRIERS

Epidermis – Keratin
Dermis – connective tissue
Epithelium layer – mucous membranes – mucus
Neisseria gonorrhoeae, Mycobacterium tuberculosis
Streptococcus pyogenes, Treponema pallidum
Tears – lacrimal apparatus – lysozyme
Sweat – flusing – lysozyme
Saliva; gastric juice (pH 1-3)
Urinary tract – flushing

B. PHAGOCYTOSIS (eat, cell)

White blood cells – leukocytes
Granulocytes (granules)
Neutrophils (red & blue) – phagocytic
Basophils (blue)
Eosinophils (red)
Agranulocytes (no granules)
Lymphocytes – lymphoid tissue
(specific defense)
Monocytes – mature into macrophages
Phagocytic
Phagocytic cells are called phagocytes
Blood = fluid (plasma) + cells: circulation
Blood flow: Heart – arteries – capillaries –
Tissue spaces – vein capillaries –
Vein – heart
Plasma in tissue space – interstitial fluid
Lymph = Name given to plasma which has become
interstitial fluid and then entered lymph capillaries
Lymph flow: Lymph capillaries – lymph vessels
- lymph nodes – vein (now part of blood)

Macrophages – Wandering – move to invasion
- Fixed – lungs, Liver, Lymph System

Phagocytosis Steps (Neutrophils & Macrophages)
1. Chemotaxis –
2. Adherence
3. Ingestion – phagosome – phagocytic vacuole
4. Digestion – lysosome, phagolysosome, digestive vacuole

C. INFLAMMATION –

Redness, Pain, Heat, Swelling

1. Vasodilation & Increased Permeability
2. Phagocyte Migration
   - Margination, Diapedesis
   - Pus
   - Abscess (pimple, boil, carbuncle)
3. Repair – heal

D. FEVER – Chapter 16 p. 417

hypothalamus –

E. INTERFERONS – antiviral proteins – Chapter 16 p. 421

Non-specific for viruses
Specific for animal

BIO 226N
STUDY GUIDE
IMMUNOLOGY LECTURES

SPECIFIC RESISTANCE

A. HUMORAL IMMUNITY

Antigens - provoke AB synthesis
Properties - foreign
- high molecular weight ≥ 10,000
- degradable by host

Examples - proteins on bacteria, viruses
- pollens, dust, dander, egg white
- transplanted tissue/organs

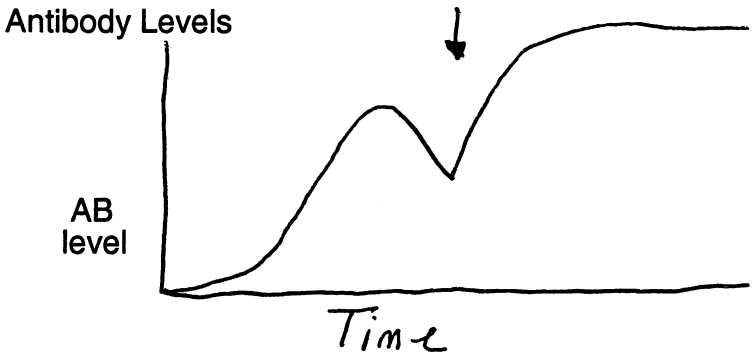
Antigenic Determinants

Antibodies = gamma-globulins = immunoglobulins = a certain class of serum proteins
[synthesized & secreted by some lymphocyte derivatives]
- plasma (circulate)
- bind to AG, help destroy
- specific; binding sites
- 2 heavy, 2 light chains
- constant and variable regions

Antibody Synthesis
gene → mRNA → translation
lymphocytes (T & B)
stem cells in bone marrow or liver T & B
B lymphocytes synthesize AB

STEPS OF ANTIBODY SYNTHESIS AFTER INJECTION OF T-DEPENDENT ANTIGEN

1. Macrophages ingest, digest, display antigenic determinants on macrophage surface
2. Now called antigen presenting cells (APC)
3. APC have self markers also on surface
4. APC + T helper cell binds
5. APC + T helper & B cell (pre-existing which can synthesize AB to that the AG)
6. Those B cells - stimulated to grow & divide and mature into plasma cells which produce and secrete AB
7. A few of these B cells become memory cells



Immunity to: Bacteria - *Bordetella pertussis*
Sal. typhi
Exotoxins - *Clostridium tetani* toxin
C. diphtheriae toxin
Viruses - Polio, Common cold, Hepatitis B, Influenza

B. CELL-MEDIATED IMMUNITY (CMI)

I. CMI involves T-lymphocytes
   a. Receptors
   b. React with foreign antigens on the surface of our own cells such as viruses budding through cytoplasmic membrane

II. Stem cells in the bone marow become many different kinds of cells
   a. Neutrophils, basophils, eosinophils, monocytes, etc.
   b. Some develop into Pre-B-lymphocytes
   c. Some migrate to thymus and become immature T-lymphocytes (T-cells)

III. T cells can react with a huge variety of antigens
   a. Surface proteins (receptors) that resemble immunoglobulins
   b. Antigen recognized on APC
   c. Self markers also on APC

IV. Antigen-stimulated T cells mature and divide (proliferate) and become:
   a. Cytotoxic T cells (T_C)
   b. Helper T-cells (T_H)
   c. Suppressor T-cells (T_S)
   d. Delayed type hypersensitivity (T_D)

Natural Killer Cells not really either T or B
Killer Cells

V. Cellular immunity combats:
   a. Intracellular viruses
   b. Multicellular parasites
   c. Cancer
   d. Some bacteria (Mycobacterium, Rikettsia)
   e. Transplanted tissues

C. DUALITY OF THE IMMUNE SYSTEM

I. Immune deficiencies
   a. Aggammaglobulinemia -- reduced (or no) circulating antibodies
   b. DiGeorge syndrome--no thymus & no CMI No T_C Lymphocytes

II. Both types of immunity of essential for health

D. VACCINES

I. Stimulate production of specific Antibodies or specific cytotoxic T-cells (T_C).

II. Bacterial vaccines
   a. *Bordetella pertussis*, a killed vaccine
   b. *Mycobacterium tuberculosis* strain BCG, an attenuated vaccine

III. Viral vaccines
   a. Polio
      1. First killed (Salk), then attenuated
      2. Grown in tissue cultures - monkey kidney cells
   b. Rabies -- killed or attenuated
   c. Smallpox
   d. Live, attenuated virus vaccines usually give better immunity than inactivated viruses

IV. Toxins/Toxoids
   a. Toxins often cause disease symptoms
   b. Antibodies against a toxin can neutralize it and prevent disease
      1. Toxoid = altered toxin
      2. DPT vaccine

V. Subunit Vaccines (Hepatitis B)

VI. Antiserum
   a. Pooled normal human serum
   b. Human with known antibody
   c. Purified human gamma globulin
   d. Serum from immunized animal

E. SEROLOGY

I. The study or use of antigen-antibody reactions in the laboratory

II. There are many types of antigen-antibody reactions and many ways to detect them
   a. Agglutination
   b. Hemagglutination
   c. Precipitation
   d. Toxin or virus neutralization

F. ACQUISITION OF IMMUNITY

I. Active immunity: body makes antibodies and/or specific T_C
   a. Natural -- infection and recovery with Ab production
   b. Artificial -- vaccination

II. Passive Immunity
   a. Natural -- fetus receives maternal antibodies while *in utero*
   b. Artificial -- injection of antiserum

G. IMMUNE DISORDERS OR HYPER-SENSITIVITIES (allergy) humoral or CMI: immediate or delayed

I. Anaphylaxis - humoral - IgE
   immune IgE binds basophils and mast cells surfaces and coats them; AG (e.g. pollen) bridges to adjacent IgE
   The cells release granules, includes mediators (histamine)
   Mediators cause inflammation, mucous secretion, smooth muscle contraction, breathing difficulty
   a. localized -
      digestive tract (food) vomit, diarrhea
      respiratory tract (pollen, house dust, fungal spores, dander)
      upper - itchy, watery eyes, cough, sneeze = hay fever
      lower - smooth muscle contraction, asthma
      Adrenalin = Epinephrine
   b. Systemic - Generalized
      Bee sting, penicillin 2%
      itch, rash, faint, dilation of blood vessels, blood pressure, drop, shock, death
      Adrenalin
      Desensitization

II. CYTOTOXIC REACTIONS
IgG or IgM react - AG on host blood or other tissue cell - lysis

a. transfusion reactions
ABO blood groups
AG, AB, genes
fever, prostration, kidney failure, shock, death

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determining blood type, donor cells & known serum
anti A or anti B agglutination with

known serum	group of donor
anti A	A
anti B	B
anti A & anti B	AB
no agglutination with anti A nor anti B	O

---

cross match (donor and recipient) to make sure there is no agglutination
major
donor RBC & recipient serum
minor
donor serum & recipient RBC
universal donor = O blood group
universal recipient = AB group, have no anti A or anti B

b. HEMOLYTIC DISEASE OF NEWBORN- RHESUS FACTOR
People 85% Rh+ and 15 % Rh-
Rh+ Father+Rh- Mother → Rh+ Child
Rh+ RBC from Fetus enter Mother, cause antibody ynthesis
subsequent pregnancy with Rh+Fetus
anti Rh antibody cross placenta; enter Fetus

anti Rh antibody & Rh+ RBC of Fetus → RBC Destruction

RESULT: Decrease in O_2 transport & increase in bilirubin level

AT BIRTH: Bilirubin cannot be metabolized by newborn baby's liver

TREATMENT
i. monitor expectant mother anti Rh
ii. fluorescent light on child
iii. monitor newborn bilirubin level
iv. blood exchange with Rh- blood after birth
v. passive immunize expectant mother
vi. infusion in utero in extreme cases

III. IMMUNE COMPLEX REACTIONS
Small Antigen-antibody complexes escape phagocytosis
Complexes deposited in tissues, cause inflammation
Phagocytes release digestive enzymes which damage host
a. Acute Post-Streptococcal Glomerulonephritis-inflammation of glomeruli in kidneys
b. Rheumatoid Arthritis - complexes in joints
c. Systemic lupus erythematosis - Antibodies to own nucleic acid

IV. DELAYED HYPERSENSITIVITY -CMI-T lymphocytes

24-48 hrs
contact dermatitis (poison ivy, cosmetics, metal)
tuberculin hypersensitivity
granulomatous hypersensitivity

H. TOLERANCE/AUTOIMMUNITY
body does not (normally) make AB to itself
sometimes we do
rheumatic fever - antistreptococcal AB react with heart valve

I. TRANSPLANTATION
major antigens on tissues differ in different individuals
tissue rejection

J. IMMUNOSUPPRESSION -
cyclosporin - suppresses CMI
heart or kidney transplant patients

K. IMMUNE DEFICIENCIES - SUMMARY
1. INHERITED
a. Hypogammaglobulinemia
b. Agammaglobulinemia
c. DiGeorge Syndrome

2. ACQUIRED – HIV/AIDS

metabolized by newborn baby's liver

BIO 226N
STUDY GUIDE
HIV/AIDS

A. History; Retroviruses

B. Transmission - Infected cells, secretion

C. Virus Growth

1. Receptors 2. Fusion of virus and cell 3. Reverse transcription 4. Provirus - integration into host chromosome 5. Latent 6. Sporadic activation 7. Kills T helper cells; depletes CMI + reduces humoral response 8. Fusion of infected and uninfected cells, syncytium, multiply killing effect 9. Humoral antibody is not protective - Antigen variation 10. Pathogen antigens activate HIV replication and wipe out T-helper cells when needed for defense.

D. Clinical Symptoms

1. Acute 1, 2 weeks; malaise; viremia
2. Seroconversion
3. Asymptomatic period 2-10 yrs (lymphadenopathy)
4. Progression to AIDS - T helpers depleted, weight loss, fever, oral thrush, diarrhea [ARC]
5. Aids - terminal HIV infection - no CMI, reduced humoral response - death due to opportunistic pathogen

E. Lab Diagnosis - test for antibodies to HIV

Elisa, Western blot, False Positive, False Negative

F. Treatment AZT - 3' azido - 2', 3' dideoxythymidine

G. Vaccine

H. Origin - HIV II, SIV, HIVI

I. Patterns of Transmission

KOCH'S POSTULATES

I. Same pathogen must be present in every case of that disease

II. The pathogen must be isolated from the diseased host and grown in pure culture

III. The pathogen from the pure culture must cause the disease when inoculated into healthy, susceptible animal

IV. The pathogen must then be isolated again from the diseased animal and shown to be the original organism