GATE Material for Pharmaceutical Sciences

- Natural Products
- Pharmacology
- Medicinal Chemistry
- Pharmaceutics
- Pharmaceutical Analysis
- Biochemistry
- Microbiology
- Clinical Pharmacy

I.NATURAL PRODUCTS

1.Steroids

The term ‘steroid’ is applied to a group of naturally occurring or synthetic fat-soluble organic compounds (lipids), whose structure is chemically based on a steroid nucleus - a hydrogenated cyclopentanoperhydrophenanthrene ring system (seventeen carbon atoms arranged in four rings). Many hormones, body constituents and drugs are steroids. All the corticosteroid hormones of the adrenal cortex (glucocorticoids or mineralocorticoids), all the sex hormones (sex hormones are found in higher quantities in one sex than in the other; male sex hormones are androgens, which include testosterone; female sex hormones are estrogens and progesterone), all vitamins of the Vitamin D group (calciferol), the bile acids (ursodeoxycholic acid and analogues), cardiac aglycones, sterols like cholesterol, toad poison, saponins, some carcinogenic hydrocarbons, some corticosteroid drugs like prednisone are all steroids. Synthetic chemical analogues of many of the naturally occurring steroids are vital in medicine. Both natural and synthetic steroids are used to treat many disorders and play a vital role in the normal functioning of the body.

Types of Steroids :

Steroidal drugs may be of three types – anabolic, androgenic and cortico steroids. Anabolic steroids are chemically derived from testosterone. Many attempts were made to separate the anabolic effects of the hormones from their androgenic effects, but with little success. Thus anabolic compounds may cause androgenic side effects, especially when used for extended periods.

Androgenic Steroids :

Anabolic effects are seen as the growth or thickening of the body's non-reproductive tract tissues including the skeletal muscles, bones, the larynx and vocal chords and a decrease in body fat. Androgenic steroid effects are seen in the growth of the male reproductive tract and the development of male secondary sexual characteristics. Medically, anabolic steroids were given for osteoporosis in women but it not recommended nowadays. They can also be used to treat the vascular manifestations of Behçet's disease, for hereditary angioedema, to treat aplastic anemia and to reduce the itching of chronic biliary obstruction.

Cortico Steroids :

Cortico steroid is a generic name for the group of hormones that have a Cortisone-like action. They are man-made steroids that mimic the activity of cortisone. Cortisone is produced naturally in the body and is involved in regulating inflammation, thus dealing with injury. Thus corticosteroids are not the same as anabolic steroids. Corticosteroids are used in the treatment of many diseases like asthma, eczema, allergies, arthritis, colitis and kidney disease.

Anabolic Steroids :

The large muscle mass of males is because of the nitrogen-retaining effects of androgen. They may have a property of protein building and when taken lead to an increase in muscle bulk and strength. The term 'anabolic' is derived from a Greek word that means to 'build up'. Though the proper term is ' anabolic-androgenic' steroids, anabolic steroids is most familiar to people. Street names: roids, juice, Arnolds,hype, weight trainers, gym candy, or pumpers.

Anabolic steroids were developed in the late 1930s to treat hypogonadism. Hypogonadism is a condition in which the testes do not produce sufficient testosterone for normal growth, development and sexual functioning. The primary medical uses of anabolic steroids are to treat delayed puberty, some types of impotence, and wasting of the body caused by HIV-infection or other diseases. Around the same time, scientists discovered that these compounds could facilitate the growth of skeletal muscles in laboratory animals, which led to their use first by bodybuilders and weightlifters, then by athletes in other sports.

While anabolic steroids are illegal without a prescription, steroidal supplements can be bought over-the-counter legally. Such supplements are more commonly called dietary supplements, though they are not food products. Steroidal supplements contain dehydroepiandrosterone (DHEA) and/or androstenedione – street name 'andro'. If large quantities of steroidal supplements substantially increase testosterone levels in the body, they might most likely produce the same side effects as anabolic steroids. It is important to know that the Food and

Drug Administration (FDA) does not regulate these substances. Hence, they are not held to the same strict standards as drugs.

Effects of Steroids :

Medically, anabolic steroids may be used for many purpose, including:

Stimulating protein anabolism in debilitating illness and in acute renal failure

Promoting growth in children with pituitary dwarfism and other growth disorders

The retention of nitrogen and calcium may benefit patients with Osteoporosis and patients receiving corticosteroid therapy

May stimulate bone marrow function in hypoplastic anaemia

However, when abused, anabolic steroids can have serious side effects. Athletes and body builders aiming to improve their strength, stamina, speed or body size have always abused them. Steroids appear to work by decreasing the amount of time necessary for recovering between bouts of exercise. Because of this, trainees can exercise more often, or more intensely, without overwhelming the body's ability to adapt, or over-training.

Over a period of time, the gland that is responsible for the natural production and release of the hormone atrophies in steroid users. This is because their abundant presence in the bloodstream sends signals to various organs and tissues, just like the hormones. Glands also become weak if not used, just like our muscles.

Side Effects of Steroids :

Some side effects of steroids (anabolic) are irreversible and they may not be noticed until it is too late.

Hormonal system

Men

Infertility

Breast development

Shrinking of the testicles

Women

Enlargement of the clitoris

Excessive growth of body hair

Both sexes

Male-pattern baldness

Musculoskeletal system

Short stature

Tendon rupture

Cardiovascular system

Heart attacks

Enlargement of the heart’s left ventricle

Liver

Cancer

Peliosis hepatis

Skin

Acne and cysts

Oily scalp

Infection

HIV/AIDS

Hepatitis

Psychiatric effects

Homicidal rage

Mania

Delusions

Anabolic Steroid Abuse :

Steroids in Sport

Athletes take steroids to push their bodies past their limits. Athletes, young or old, professional or amateur, always look to gain an advantage over their opponents. Taking steroids gives them an edge, increasing muscle mass and body strength.

Steroid use is most commonly seen in:

Football or baseball players, wrestlers – players of sports that depend on strength and size

Track and field athletes and swimmers – those who need endurance

Bodybuilders

Any person interested in building and developing their muscles

It is important to understand that using steroids does not increase skill, agility and performance. These are determined by many factors, including genetics, body size, age, sex, diet, and how hard the athlete trains.

Anabolic steroids are not legal in organized sports. Most professional and amateur sports organizations and medical associations ban anabolic steroids. Athletes who test positive for steroids will be suspended or disqualified and may lose their chance to compete in their sport. Barry Bonds, the San Francisco Giants slugger is the latest sports celebrity involved in the federal probe of steroids used in baseball.

Other reasons for steroid abuse

Many buy steroids on the black market without a doctor's prescription. One reason people give for taking steroids is to increase their muscle size and / or reduce body fat. This group includes people who have muscle dysmorphia, a behavioral syndrome in which people have distorted images of their bodies. Sometimes, people who have experienced physical or sexual abuse take steroids to boost muscle size. Adolescents abuse steroids as a part of a pattern of high-risk behaviors. Despite these commonly cited reasons, researchers agree that most steroid abusers do not have psychological problems when they start abusing the drugs.

Commonly abused steroids :

Oral steroids

Anadrol (oxymetholone)

Oxandrin (oxandrolone)

Dianabol (methandrostenolone)

Winstrol (stanozolol)

Injectable steroids

Deca-Durabolin (nandrolone decanoate)

Durabolin (nandrolone phenpropionate)

Depo-Testosterone (testosterone cypionate)

Equipoise (boldenone undecyclenate)

2.Flavonoids

The term flavonoid refers to a class of plant secondary metabolites. According to the IUPAC nomenclature,[1] they can be classified into:

flavonoids, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure

isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure

neoflavonoids, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure.

Flavonoids are most commonly known for their antioxidant activity. However, it is now known that the health benefits they provide against cancer and heart disease are the result of other mechanisms.[2][3] Flavonoids are also commonly referred to as bioflavonoids in the media – the terms are largely equivalent and interchangeable, for most flavonoids are biological in origin.

Biosynthesis :

Flavonoids are synthesized by the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA[3]. This can be combined with malonyl-CoA to yield the true backbone of flavonoids, a group of compounds called chalcones, which contain two phenyl rings (see polyphenols). Conjugate ring-closure of chalcones results in the familiar

form of flavonoids, the three-ringed structure of a flavone. The metabolic pathway continues through a series of enzymatic modifications to yield flavanones → dihydroflavonols → anthocyanins. Along this pathway, many products can be formed, including the flavonols, flavan-3-ols, proanthocyanidins (tannins) and a host of other polyphenolics.

Biological effects :

Flavonoids are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects. The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans, ingest significant quantities in their diet. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory[4] , anti-microbial and anti-cancer activity.

Consumers and food manufacturers have become interested in flavonoids for their medicinal properties, especially their potential role in the prevention of cancers and cardiovascular disease. The beneficial effects of fruit, vegetables, and tea or even red wine have been attributed to flavonoid compounds rather than to known nutrients and vitamins.

Health benefits aside from antioxidant values :

In 2007, research conducted at the Linus Pauling Institute and published in Free Radical Biology and Medicine indicates that inside the human body, flavonoids themselves are of little or no direct antioxidant value.[5] Unlike in the controlled conditions of a test tube, flavonoids are poorly absorbed by the human body (less than 5%), and most of what is absorbed is quickly metabolized and excreted from the body.

The huge increase in antioxidant capacity of blood seen after the consumption of flavonoid-rich foods is not caused directly by the flavonoids themselves, but most likely is due to increased uric acid levels that result from expelling flavonoids from the body.[2] According to Frei, "we can now follow the activity of flavonoids in the body, and one thing that is clear is that the body sees them as foreign compounds and is trying to get rid of them. But this process of gearing up to get rid of unwanted compounds is inducing so-called Phase II enzymes that also help eliminate mutagens and carcinogens, and therefore may be of value in cancer prevention... Flavonoids could also induce mechanisms that help kill cancer cells and inhibit tumor invasion."[2]Their research also indicated that only small amounts of flavonoids are necessary to see these medical benefits. Taking large dietary supplements provides no extra benefit and may pose some risks.

Diarrhea :

A study done at Children's Hospital & Research Center Oakland, in collaboration with scientists at Heinrich Heine University in Germany, has shown that epicatechin, quercetin and luteolin can inhibit the development of fluids that result in diarrhea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl– transport inhibiting cAMP-stimulated Cl– secretion in the intestine.

3.Purines

One of the important specialized pathways of a number of amino acids is the synthesis of purine and pyrimidine nucleotides. These nucleotides are important for a number of reasons. Most of them, not just ATP, are the sources of energy that drive most of our reactions. ATP is the most commonly used source but GTP is used in protein synthesis as well as a few other reactions. UTP is the source of energy for activating glucose and galactose. CTP is an energy source in lipid metabolism. AMP is part of the structure of some of the coenzymes like NAD and Coenzyme A. And, of course, the nucleotides are part of nucleic acids. Neither the bases nor the nucleotides are required dietary components. (Another perspective on this.) We can both synthesize them de novo and salvage and reuse those we already have.

Nomenclature:

Nitrogen Bases

There are two kinds of nitrogen-containing bases - purines and pyrimidines. Purines consist of a six-membered and a five-membered nitrogen-containing ring, fused together. Pyridmidines have only a six-membered nitrogen-containing ring. There are 4 purines and 4 pyrimidines that are of concern to us.

Purines :

Adenine = 6-amino purine

Guanine = 2-amino-6-oxy purine

Hypoxanthine = 6-oxy purine

Xanthine = 2,6-dioxy purine

Adenine and guanine are found in both DNA and RNA. Hypoxanthine and xanthine are not incorporated into the nucleic acids as they are being synthesized but are important intermediates in the synthesis and degradation of the purine nucleotides.

Pyrimidines :

Uracil = 2,4-dioxy pyrimidine

Thymine = 2,4-dioxy-5-methyl pyrimidine

Cytosine = 2-oxy-4-amino pyrimidine

Orotic acid = 2,4-dioxy-6-carboxy pyrimidine

Cytosine is found in both DNA and RNA. Uracil is found only in RNA. Thymine is normally found in DNA. Sometimes tRNA will contain some thymine as well as uracil.

Nucleosides:

If a sugar, either ribose or 2-deoxyribose, is added to a nitrogen base, the resulting compound is called a nucleoside. Carbon 1 of the sugar is attached to nitrogen 9 of a purine base or to nitrogen 1 of a pyrimidine base. The names of purine nucleosides end in -osine and the names of pyrimidine nucleosides end in -idine. The convention is to number the ring atoms of the base normally and to use l', etc. to distinguish the ring atoms of the sugar. Unless otherwise specificed, the sugar is assumed to be ribose. To indicate that the sugar is 2'-deoxyribose, a d- is placed before the name.

Adenosine

Guanosine

Inosine - the base in inosine is hypoxanthine

Uridine

Thymidine

Cytidine

Nucleotides:

Adding one or more phosphates to the sugar portion of a nucleoside results in a nucleotide. Generally, the phosphate is in ester linkage to carbon 5' of the sugar. If more than one phosphate is present, they are generally in acid anhydride linkages to each other. If such is the case, no position designation in the name is required. If the phosphate is in any other position, however, the position must be designated. For example, 3'-5' cAMP indicates that a phosphate is in ester linkage to both the 3' and 5' hydroxyl groups of an adenosine molecule and forms a

cyclic structure. 2'-GMP would indicate that a phosphate is in ester linkage to the 2' hydroxyl group of a guanosine. Some representative names are:

AMP = adenosine monophosphate = adenylic acid

CDP = cytidine diphosphate

dGTP = deoxy guanosine triphosphate

dTTP = deoxy thymidine triphosphate (more commonly designated TTP)

cAMP = 3'-5' cyclic adenosine monophosphate

Polynucleotides :

Nucleotides are joined together by 3'-5' phosphodiester bonds to form polynucleotides. Polymerization of ribonucleotides will produce an RNA while polymerization of deoxyribonucleotides leads to DNA.

Hydrolysis of Polynucleotides :

Most, but not all, nucleic acids in the cell are associated with protein. Dietary nucleoprotein is degraded by pancreatic enzymes and tissue nucleoprotein by lysosomal enzymes. After dissociation of the protein and nucleic acid, the protein is metabolized like any other protein.

The nucleic acids are hydrolyzed randomly by nucleases to yield a mixture of polynucleotides. These are further cleaved by phosphodiesterases (exonucleases) to a mixture of the mononucleotides. The specificity of the pancreatic nucleotidases gives the 3'-nucleotides and that of the lysosomal nucleotidases gives the biologically important 5'-nucleotides.

The nucleotides are hydrolyzed by nucleotidases to give the nucleosides and Pi. This is probably the end product in the intestine with the nucleosides being the primary form absorbed. In at least some tissues, the nucleosides undergo phosphorolysis with nucleoside phosphorylases to yield the base and ribose 1-P (or deoxyribose 1-P). Since R 1-P and R 5-P are in equilibrium, the sugar phosphate can either be reincorporated into nucleotides or metabolized via the Hexose Monophosphate Pathway. The purine and pyrimidine bases released are either degraded or salvaged for reincorporation into nucleotides. There is significant turnover of all kinds of RNA as well as the nucleotide pool. DNA doesn't turnover but portions of the molecule are excised as part of a repair process.

Purine and pyrimidines from tissue turnover which are not salvaged are catabolized and excreted. Little dietary purine is used and that which is absorbed is largely catabolized as well. Catabolism of purines and pyrimidines occurs in a less useful fashion than did the catabolism of amino acids in that we do not derive any significant amount of energy from the catabolism of purines and pyrimidines. Pyrimidine catabolism, however, does produce beta-alanine, and the endproduct of purine catabolism, which is uric acid in man, may serve as a scavenger of reactive oxygen species.

II. PHARMACOLOGY

1.Basic Pharmacology:

HENDERSON-HESSELBACH EQUATION:

Weak Acid

pKa:

If its pKa < pH of the environment, then the conjugate base (anion) form of the species will predominate. Example = CH3COO-

If its pKa > pH of the environment, then the environment is more acidic, so its acidic (neutral) form will predominate. Example CH3COOH

Weak acids tend to be absorbed in acidic environments, like the stomach.

Weak Base

pKa

If its pKa < pH of the environment, then the environment is more basic, so the species will remain in the neutral form. Example = NH3

If its pKa > pH of the environment, then the environment is more acidic, so it will give up its extra H+ to the base, and the base will exist in its cation form. Example = NH4+

Weak bases tend to be absorbed in basic environments, like the duodenum.

DRUG PERMEATION:

Partition Coefficient: The ratio of lipid solubility to aqueous solubility. The higher the partition coefficient, the more membrane soluble is the substance.

Kidney Glomeruli have the largest pores through which drugs can pass ------> drug filtration.

Blood Brain Barrier (BBB): Only lipid-soluble compounds get through the BBB.

Four components to the blood-brain barrier:

Tight Junctions in brain capillaries

Glial cell foot processes wrap around the capillaries

Low CSF protein concentration ------> no oncotic pressure for reabsorbing protein out of the plasma.

Endothelial cells in the brain contain enzymes that metabolize, neutralize, many drugs before they access the CSF.

MAO and COMT are found in brain endothelial cells. They metabolize Dopamine before it reaches the CSF, thus we must give L-DOPA in order to get dopamine to the CSF.

Exceptions to the BBB. Certain parts of the brain are not protected by the BBB:

Pituitary, Median Eminence

Supraventricular areas

Parts of hypothalamus

Meningitis: It opens up the blood brain barrier, due to edema. Thus Penicillin-G can be used to treat meningitis, despite the fact that it doesn't normally cross the BBB.

Penicillin-G is also actively pumped back out of the brain once it has crossed the BBB.

Routes of Administration:

ORAL

FIRST-PASS EFFECT: Alteration of drugs in liver via protal circulation. Some drugs have a high first-pass effect and thus a lower bioavailability. Know these:

Morphine

Imipramine

Propanolol

Gastric Emptying: Generally, anything that slows gastric emptying will slow the absorption of drugs.

Things that slow gastric emptying: Fats, acidic pH, bulk, anticholinergics, hypothyroidism, Al(OH)3

Faster gastric emptying is beneficial for the absorption of most drugs

Tetracycline chelates calcium and should therefore not be given with milk.

TOPICAL: Lipophilic drugs absorbed through skin.

Examples: Nicotine patch, nitroglycerine, scopolamine = anti-histamine given for motion-sickness.

VOLUME OF DISTRIBUTION: The apparent amount of volume that a drug seems to distribute to.

Sites of Concentration: They can affect the Volume of Distribution

FAT: Drug concentrates in fat lower concentration of drug in the plasma high Vd

BONE: Drug concentrates in bone lower concentration of drug in the plasma high Vd

TISSUE: Drug concentrates in tissue - lower concentration of drug in the plasma high Vd

PLASMA PROTEINS: Drug binds to plasma protein higher concentration of drug in the plasma -- low Vd.

The Vd is based on the total amount of drug in the plasma not just the amount of free drug!

TRANSCELLULAR: Drug concentrates in non-plasma locations lower concentration of drug in the plasma high Vd

Apparent Vd	Apparent Vd		
#Liters in 70kg man	% Total Body Weight	Example, Explanation	
Plasma Water	0.045 L/kg	3 L	4.5% Plasma-Protein-bound drugs, and large drugs that stay in plasma. Concentrates in blood and thus has a small Vd.
Example = Heparin			
Extracellular Water	0.2 L/kg	14 L	20% Large water soluble drugs.
Example = Mannitol			

Total Body Water 0.6 L/kg 42 L 60% Small water soluble drugs; rapid equilibration between body compartments.

Example = Ethanol

Tissue

Concentration

>0.7 L/kg >42 L ----- Drugs that bind to tissue

Example = chloroquine, which intercalates with DNA intracellularly.

Vd may be greater than TBW volume, hence some drug must be bound to plasma.

This is very common and occurs with many drugs.

Enterohepatic Circulation: Drugs that are recycled through the enterohepatic circulation will have a lower concentration of drug in the plasma, and therefore a higher Vd.

Plasma Protein Binding : Two Main Plasma Protein Carry Drugs in the Blood

ALBUMIN	**alpha$_1$-Acid Glycoprotein** **OROSOMUCOID**
Negatively Charged, hence it binds primarily to **weak acids**.	Positively Charged, hence it binds primarily to **weak bases**.
Negative acute-phase protein: its synthesis decreases during time of body insult.	**Positive acute-phase protein**: its synthesis increasesduring times of body insult.
Examples:**Phenytoin, Salicylates**	Examples: **Quinidine, Propanolol**

2. Autocoids

HISTAMINE

Histamine Release:

Mast Cells and Basophils

Hypothalamus

GI mucosa

Histamine Effects:

H1 RECEPTORS: Phosphoinositol pathway (constrictive) or cGMP (vasodilate)

Allergic: increased vascular permeability and vasodilation

CV: Decreased blood pressure and reflex tachycardia

Local anesthetic effects

Smooth Muscle: bronchoconstriction, stimulation of GI muscle. No stimulation of uterine muscle.

2-methylhistamine is an endogenous H1 agonist.

H2 RECEPTORS: cAMP pathway

Allergic: increased vascular permeability and vasodilation

GI: Stimulate gastric-acid secretion

4-methylhistamine is an endogenous H2 agonist.

H3 RECEPTORS: Little known about them. Probably located centrally.

Anti-Cholinergic: Dried secretions, blurry vision, urinary retention, constipation, sedation.

Histamine Metabolism:

Histidine → Histamine (Histidine Decarboxylase)

DOPA Decarboxylase will also create histamine, due to similar structure of substrate.

Histamine → Imidazole Acetic Acid (Diamine Oxidase, or MAO)

Imidazole Acetic Acid → Methyl Imidazole Acetic Acid (Histamine Methyltransferase) (excreted in urine)

HISTAMINE LIBERATORS: Things that degranulate histamine granules.

Amines and Diamines

Morphine

Tubocurarine (curare)

Large molecules: certain long-chain polymers and dyes.

Bacterial endotoxin

Compounds that cause general tissue damage: Trypsin, venoms, detergents.

ANTI-HISTAMINES:

EFFECTS: Antagonize H1 receptors reduce inflammation, redness, swelling. They do not affect H2 receptors.

Treat motion sickness.

Local anesthetic properties; pain relief.

Indicates:

Allergies: Pollinosis, urticaria

Motion sickness.

Of little value in the common cold; anti-cholinergic properties may improve rhinorrhea.

Sedation, anti-anxiety: centrally acting agents only.

Adverse Effects:

Sedation: May be considered adverse effect or primary effect.

Centrally acting drugs (3) cause sedation; peripheral drugs (4) don't.

GI: Anorexia, nausea, vomiting, constipation, diarrhea, dry mouth and throat.

TOXICITY: Hallucinations, ataxia, athetosis, convulsions. Can especially in children taking 20-30 tablets. Symptoms resemble atropine poisoning.

CV: When injected IV, antihistamines can mimic anti-arrhythmics and cause heart block.

RENIN-ANGIOTENSIN SYSTEM:

Prorenin: There is more prorenin in the blood than renin.

Renin: Renin secretion occurs in the kidney Macula Densa cells. It is stimulated by:

Reduced tubular fluid flow reduced delivery of salt to macula densa (lower tubular Na+) stimulate release of Renin.

beta-receptors stimulate release of Renin.

Most drugs which decrease blood pressure also increase Renin, via reflex sympathetic activation.

Diuretics also have a direct effect on Renin secretion: lower tubular Na+ in Macula Densa

higher Renin secretion.

Angiotensin: Renin converts Angiotensinogen Angiotensin I

Structure: It is a decapeptide.

Estrogen increases levels of angiotensinogen in blood (increased liver enzymes) potential hypertension.

Angiotensin Converting-Enzyme (ACE) (dipeptidyl peptidase): In the lung, it converts Angiotensin I Angiotensin II

Angiotensin II:

Actions:

Intense vasodilation (G-Protein; phosphoinositol)

Promote the release of aldosterone in adrenal gland promote Na+ reabsorption in distal tubule higher blood volume.

Regulatory negative feedback on the release of Renin.

CNS: Stimulate thirst in hypothalamus, stimulate sympathetic outflow.

Structure: It is an octapeptide.

Angiotensin Receptors:

AT1 Receptor: The principle receptor, found in vascular smooth muscle and in adrenal cortex (to release aldosterone). Acts by both IP3 and cAMP pathways.

AT2 Receptor: Actions largely unknown.

Angiotensin III: Breakdown product of Angiotensin II

Structure: Heptapeptide

Actions: Promotes aldosterone release. Probably no significant vasoconstrictive effects.

SEROTONIN:

EFFECTS:

CV: Mixed vasoactive effects. Direct vasoconstriction, plus vasodilation in skeletal muscle. Also causes platelet aggregation.

GI: Diarrhea, as is often seen with Carcinoid Tumor.

RELEASE: Primarily released from enterochromaffin cells of the GI tract.

VASOPRESSIN (ADH):

STRUCTURE: Nonapeptide, stored in hypothalamus and released through posterior pituitary.

RECEPTORS:

V1 Receptor: Vascular receptor, causing vasoconstriction.

Associated with phosphoinositol / Ca+2 increase.

V2 Receptor: Tubular receptor. Increased permeability to water in distal tubule increased water reabsorption.

Associated with cAMP increase.

Diabetes Insipidus: Congenital lack of secretion of ADH no water retention in kidneys profuse fluid loss. Vasopressin is used to treat Diabetes Insipidus.

ERGOT ALKALOIDS: Methysergide, LSD, Ergonovine, Ergotamine

ACTIONS: Prolonged vasospasm, hallucination, uterine smooth muscle contraction.

ERGOTISM: Prolonged vasospasm caused by ergot alkaloids which, if untreated, may lead to ischemia and gangrene.

PHARMACOLOGY:

5HT1-Receptor agonist and/or antagonist, depending on the drug.

alpha-agonist.

Dopamine agonist.

KININS: Kallidin, Bradykinin

METABOLISM: Ultimate product is bradykinin

Kallikreins convert Kininogen (alpha2-globulin) Bradykinin or Kallidin, depending on tissue.

Kallidin Bradykinin

ACTION: Wide variety of actions:

Pain, vasodilation. Responsible for the flushing associated with carcinoid tumors.

Some responses mediated by prostaglandins and/or NO.

EICOSANOIDS:

CYCLOOXYGENASE PRODUCTS: Inhibited by NSAID's.

Prostaglandin E1 (PGE1): Wide variety of effects.

Maintain patent ductus arteriosus before birth.

Prostaglandin E2 (PGE2):

CV: Potent vasodilator.

GI: Cytoprotective effects in gastric mucosa (increased mucous, HCO_3^-)

Causes pain and sensitized nerve endings.

Prostaglandin F2alpha (PGF2alpha): Essential promotor of uterine contractions during labor. Also stimulates GI muscle and vascular smooth muscle.

CV: Vasoconstrictor

Bronchoconstriction

Prostacyclin (PGI2): Works via cAMP.

CV:

It inhibits platelet aggregation and elicits vasodilation.

Antagonizes the effects of Thromboxane A2.

It is released by vascular endothelial cells.

GI: Cytoprotective effects in gastric mucosa (increased mucous, HCO_3^-)

Thromboxane A2 (TXA2): Works via IP3 (increase Ca+2).

It is produced by platelets -- not endothelial cells.

CV: It promotes vasoconstriction and is essential for platelet aggregation.

LIPOXYGENASE PRODUCTS:

Leukotriene B4 (LTB4):

It causes intense bronchoconstriction. Receptors are antagonized by Zafirlukast and Zileuton.

Chemotaxis

Allergic reactions.

Hydroxyeicosatetaraenoic Acid (HETE's): Implicated in inflammation, chemotaxis.

ADVERSE EFFECTS: Nausea, vomiting, diarrhea. Fever, bronchoconstriction.

ASPIRIN (NSAID): It inhibits cyclooxygenase

LOW DOSE: It is more specific for thromboxane derivatives (TXA2), thus low doses are given for prophylaxis for CAD.

HIGH DOSE: It is more specific for prostaglandin derivatives, thus high doses are used for anti-inflammatory properties. High doses are not as effective for CAD prophylaxis.

3.Electrical Conduction of The Heart :

MYOCARDIUM DEPOLARIZATION:

Phase 0: Initial upswing of action potential.

Na+ Channels open until threshold is reached.

Phase 1: The potential may repolarize slightly before starting the plateau phase.

Na+ Channels are inactivated.

Outward Rectifier K+ Channels open transiently, causing slight repolarization.

Membrane potential remains near zero.

Phase 2: Plateau Phase -- This stage is responsible for prolonging the cardiac action potential, making it longer than a nerve action potential.

Ca+2 Channels open, to keep the cells depolarized.

Phase 3: Repolarization

Ca+2 Channels close.

Delayed Rectifier K+ Channels open to effect normal repolarization.

Phase 4: Diastolic membrane potential.

Inward Rectifier K+ Channels (different than the ones above) are open, to maintain resting potential.

They are open at highly negative membrane potentials (i.e. hyperpolarization-activated).

SA-NODE DEPOLARIZATION: It is similar to depolarization in the myocardium, except for the following differences:

Depolarization results from influx of Ca+2 rather than Na+

There is no plateau phase (no Phase 1 and 2).

Automaticity: Hyperpolarization-activated cation current is activated at low potentials, resulting in automaticity of the SA-Node.

Epinephrine increases the rate of rise and acetylcholine decreases the rate of rise of Phase-4 depolarization.

REFRACTORY PERIOD: Cardiac muscle cells have prolonged refractory periods, to prevent tetany of cardiac muscle.

AUTONOMIC REGULATION of HEARTBEAT:

Acetylcholine slows heart rate by increasing K+ permeability.

Norepinephrine speeds heart rate by increasing the rate of rise of the cardiac action potential during phase 0.

PROPAGATION of ACTION POTENTIAL:

ATRIAL CONTRACTION: It takes about 70 msec to get from the SA-Node ------> depolarize the atria ------> to the AV-Node.

AV-NODAL DELAY: There is a delay in depolarization of about 90msec, once the impulse reaches the AV-Node.

The function of this delay is to separate the contraction of the atria (i.e. atrial systole) from that of the ventricles (ventricular systole), so that more blood has a chance to fill into the ventricles.

The AV-Node depends on slow-conducting Ca+2 Channels for depolarization, which helps to explain its slow rate of depolarization.

A smaller cell-size also helps to explain the slow rate of conductance.

BUNDLE OF HIS

BUNDLE-BRANCHES: Two continuing branches of the Bundle of His.

Left Bundle Branch: It depolarizes first. Depolarization goes from the left side of the ventricular septum to the right side, accounting for the Q-Wave.

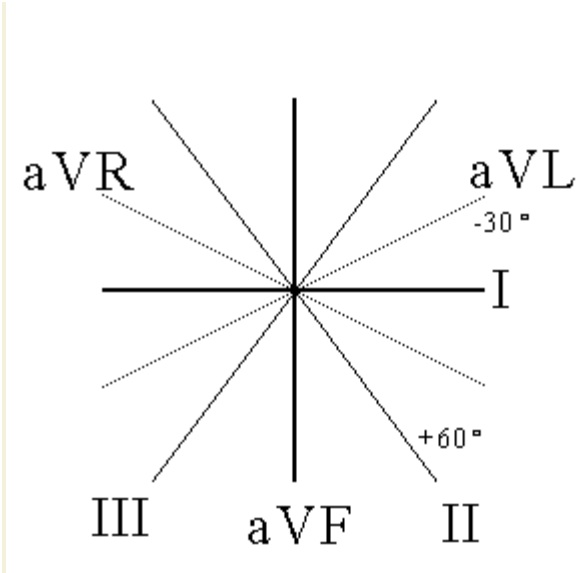
Right Bundle Branch: It depolarizes after the left side.

PURKINJE SYSTEM: Very fast conduction.

VENTRICULAR MUSCLE

As depolarization proceeds in the ventricles, it moves from endocardium ------> epicardium.

EKG LIMB LEADS:



Depolarization occurs toward the positive side (the positive sides are labelled to the right, and the respective negative sides are unlabeled).

HEXAXIAL SYSTEM: The positive end of each limb lead is as follows:

I: 0

II: +60

III: +120: In a normal ECG, Lead III should have a net-zero QRS-Complex, as it is perpendicular to aVR.

aVR: -150: In a normal ECG, the aVR lead should have a completely negative QRS Complex.

aVL: -30

aVF: +90

DIRECTION OF ECG DEFLECTION: A positive deflection on an ECG represents a depolarization that is traveling toward the positive side of a particular lead.

Maximal Positive Deflection: Occurs when depolarization vector is in the exact same direction as the limb lead.

Zero net deflection: Occurs when depolarization vector is exactly perpendicular to limb lead.

Maximal Negative Deflection: Occurs when depolarization vector is in the exact opposite direction as the limb lead (i.e. in the direction of the negative end).

ELECTROCARDIOGRAM:

P-WAVE: Atrial depolarization. P-Wave duration is normally 80 msec.

PR-INTERVAL: The distance from the beginning of the P-Wave to the beginning of the Q-Wave.

PR-Interval is the period from beginning of atrial depolarization to the beginning of ventricular depolarization.

PR-Interval is normally 180-220 msec.

PR-SEGMENT: The distance from the end of the P-Wave and the beginning of the Q-Wave.

QRS-COMPLEX: Ventricular Depolarization. QRS Duration is normally 30-100 msec.

Individual Components:

Q-WAVE: Depolarization of the septum. On most leads (except III and aVR) the Q-Wave points downward if it can be seen at all. Septum depolarization goes from the left side of the septum to the right side.

R-WAVE: Depolarization of the ventricles. Sharp upward turn.

S-WAVE: Return of volt-potential to zero, because all the ventricular muscle has depolarized and is therefore once again isoelectric.

Sharp downward turn back to isoelectric point. The S-Wave may go slightly negative before return back to isoelectric point.

QT-INTERVAL: From beginning of Q-Wave to end of T-Wave. QT-Interval is normally 260-490 msec. This is the period from beginning of ventricular depolarization to the end of repolarization.

ST-SEGMENT: Short segment from end of S-Wave to beginning of T-Wave.

ST-INTERVAL: From end of S-Wave to end of T-Wave.

RR-INTERVAL: Distance between QRS-Complexes, or the distance between heart beats in a normal sinus rhythm.

T-WAVE: Repolarization of Ventricles. Atrial repolarization masked by QRS-Complex.

Repolarization occurs in the opposite direction as depolarization, but the vector still points in the same direction because the change in voltage also has an opposite sign.

In the ventricles, the first tissue to depolarize is the last tissue to repolarize.

READING THE ECG:

Vertical Direction: 10 mm = 2 big boxes = 1 mV deflection.

Horizontal Direction:

1 mm = 40 msec.

At standard speed, there are 25 mm, or 5 big boxes, in each second.

Speeds:

Standard Speed = 25 mm/sec

Extra-Sensitivity Speed = 50 msec, at which point all values above must be doubled.

PRECORDIAL LEADS: V1 thru V6 are placed to specific places on the chest, for advanced ECG diagnostics. V1 is right-most, near the SA-Node, while V6 is leftmost, past the apex of the heart.

MEAN ELECTRICAL AXIS OF THE HEART:

Two ways to graphically determine mean electrical axis:

SHORT WAY: This is only accurate when there is a net QRS-Deflection of virtually zero (i.e. the R deflection is equal and opposite to the S deflection).

Determine the lead that has a net zero QRS-Deflection.

On the hexaxial system, the mean electrical axis points in the direction that is perpendicular to that lead.

LONG WAY: This is longer but more accurate.

Consider any two of the six hexaxial leads. Determine again the Net QRS-Deflection for each lead.

Plot that deflection along the appropriate axis on a hexaxial chart.

Draw a dotted line perpendicular to each of the above plots, and extend the two lines until the intersect each other.

The Mean Electrical Axis is the vector that points from the center to the intersection of those two lines.

LAB: Different physiological effects on the mean electrical axis:

INSPIRATION: The diaphragm moves down ------> It pulls the apex of the heart toward the right (i.e. in a more vertical direction) ------> the mean electrical axis is more positive (+ more degrees).

FORCED EXPIRATION: The exact opposite of above. The apex of the heart gets pushed upward and toward the left horizontal axis ------> the mean electrical axis is less positive or even negative.

PREGNANCY: The mean electrical axis would deviate to the left, within normal limits. The physical presence of the fetus would push up the diaphragm ------> heart leans toward left.

LEFT VENTRICULAR HYPERTROPHY: Mean axis deviation toward the left.

Pulmonary Valve Stenosis: If we assume that it leads to Right Ventricular Hypertrophy ------> Then we get (potentially severe) right axis deviation.

INFANCY: Right Axis Deviation, because the infant's right ventricle and left ventricle musculature are about the same size at birth. Left ventricle becomes larger within a couple months.

NORMAL MEAN AXIS: Anywhere between -30 and +110.

Anything negative of -30 is left axis deviation, as occurs from left ventricular hypertrophy.

Anything positive of +110 is right axis deviation, as occurs from right ventricular hypertrophy.

ECG ABNORMALITIES:

SINUS BRADYCARDIA: A heart rate slower than 60 SA-Nodal depolarizations per minute. "Sinus" indicates that the cardiac impulse is originating from the SA-Node as normal.

SINUS TACHYCARDIA: Heart rate faster than 100 bpm, originating as normal from the SA-Node.

Tachycardia generally means you'll see a shorter RR-Interval (i.e. faster heart rate).

SINUS ARREST: No SA-Node depolarization.

This can be artificially induced by carotid massage, which results in overstimulation of the Vagus SA-Node hyperpolarized.

ATRIAL PAROXYSMAL TACHYCARDIA: Faster heart rate resulting from an ectopic pacemaker in the atrial muscle.

In the example the P-Wave points downward because the atrial depolarization starts in the LA, because that is where the tissue is leaky.

BUNDLE-BRANCH BLOCKS: There is some conduction block in the Bundle of His (Left or Right Bundle branches), with results as below:

1 BLOCK: Partial block. The PR-Interval is longer than normal because it takes longer to conduct the impulse from SA-Node to AV-Node.

2 BLOCK: A QRS-Complex occurs only after every other P-Wave. In other words, it takes two P-Waves to sufficiently excite the AV-Node to conduct the impulse to the ventricles.

3 BLOCK: There is no temporal relationship between the P-Wave and QRS-Complex. Atrial and ventricular depolarizations are being controlled by their own independent pacemakers (the SA-Node and AV-Node respectively).

AV-NODAL TACHYCARDIA: Tachycardia, plus the P-Wave is insignificant or absent.

This is tachycardia, where the impulse originates from the AV-Node. The inherent pacemaker of the AV-Node is faster than the SA-Node.

PREMATURE VENTRICULAR CONTRACTION (PVC): A premature QRS-Complex, or one that occurs without being preceded by a P-Wave.

That means that the P-Wave didn't start the impulse, but it started somewhere else.

Ectopic Pacemaker: With PVC, the impulse originates in the ventricular muscle itself, due to leaky membranes in the muscle.

VENTRICULAR FIBRILLATION: Waves of depolarization traveling in multiple directions all over the ventricular muscle. The pacemaker activity is lost.

ATRIAL FIBRILLATION: Fibrillation in the atria is not serious in children, but it is serious in old people.

That's because in old people, atrial systole contributes a greater relative blood volume to cardiac output than in children.

CLINICAL LECTURE: WOLF-PARKINSON-WHITE SYNDROME

Normally, the AV-Node is the only pathway for conduction of the impulse from the atria to the ventricles.

Bachman's Bundle: Normally conducts the impulse from Right Atrium to Left Atrium during atrial systole.

Moderator Band: Normally conducts the impulse from the right ventricular septal wall to the right free wall during ventricular systole.

Lupus Erythematosus: Rare condition associated with pediatric bradycardia. Usually pediatric heart problems result in Tachycardia -- not bradycardia.

PEDIATRIC TACHYCARDIAS: They are divided into two types

Supraventricular Tachycardia (SVT): One where the problem originates somewhere in the AV-System.

Ventricular Tachycardia (VT): Problem originates in the ventricular system.

Wolf-Parkinson-White Syndrome: Extra conductive tissue in the myocardium, creating an accessory pathway for conduction from atria to ventricles.

This accessory pathway ultimately results in a Reentry Tachycardia, or a conduction loop between the normal and accessory pathways.

The Wolf-Parkinson-White ECG: Shorter PR-Interval due to rapid conduction of signal to ventricles through accessory pathway.

This is the ECG when the patient is healthy and no problems are going on.

The P-Wave and the QRS-Complex are scrunched together, creating the appearance of a delta-wave (hump right before QRS), and a longer overall QRS Complex.

Reentry Tachycardia: You get it from a unidirectional block in one pathway, coupled with slowed conduction of an alternative pathway. This results in continuous impulse conduction, or circus dysrhythmia.

With WPW, the accessory pathway can get blocked because it hasn't had the time to repolarize, then the normal pathway provides a mean for retrograde conduction of depolarization.

This results in a conduction loop and severe tachycardia.

TREATMENT: Slow down the conduction through one pathway or the other.

Use Ca+2-Channel Blockers (such as Verapamil)

Use Digoxin to increase AV-Nodal sensitivity to ACh.

Use beta-Blockers to block the normal NorE sympathetic receptors on the AV-Node and cardiac muscle.

In severe cases, surgically remove the conductive tissue from the myocardium.

III. MEDICINAL CHEMISTRY

1.Introduction To Drug Design

The need for ongoing development of new drugs needs no emphasis in light of the current global situation of health and disease. Traditionally, the process of drug development has revolved around a screening approach, as nobody knows which compound or approach could serve as a drug or therapy. Such almost blind screening approach is very time-consuming and laborious.The shortcoming of traditional drug discovery; as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational drug design" (Kuntz 1992).

Nobody could design a drug before knowing more about the disease or infectious process than past. For "rational" design, the first necessary step is the identification of a molecular target critical to a disease process or an infectious pathogen. Then the important prerequisite of "drug design" is the determination of the molecular structure of target, which makes sense of the word "rational".

In fact, the validity of "rational" or "structure-based"drug discovery rests largely on a high-resolution target structure of sufficient molecule detail to allow selectivity in the screening of compounds.When the above information are available, the "rational" design of drug will be more possible.

2. Concept: how to design a drug TOP

In the real work, the researchers will exploit all of the possible approaches to design or find good candidates for drug. The following figure briefly shows the flowing of drug design.

3. The useful tools developed in our lab TOP

Structure-based drug design

Solely based on a fine structure of target molecule,one whole new ligand could be constructed. This is just De novo of a ligand. Ligbuild is a rather powerful tool to build a ligand just based on a protein structure in Brookheaven format.

When building a new ligand, or screening the ligand from a database, it is critical to evaluate the bind energy of the complex. This is so-called scoring approach. SCORE is a tool to evaluate the binding affinity of protein-ligand complex with known three-dimensional structure.

QSPR/QSAR TOP

After obtaining a number of candidate molecules of drug from database screening or De novo design, there are several criteria for further screening out appropriate molecules to perform the experiment test.

Permeation across the biomembrane is a major limitation of many compounds to serve as drug. At present, logP, is widely used to evaluate the hydrophilic and hydrophobicity, which represents the permeation across the biomembrane of the given molecules.

XLOGP can calculate logP of the common organic compounds. By using a large number of compounds (1853 altogether) as training set, it can give rather accurate logP values of the most interesting molecules with known structures. Furthermore, XLOGP can provide detailed hydrophobicity distribution information of the molecule.

To complement the inefficient calculation of peptides, another package, PLOGP, has been developed to meet this need. This package calculates the logP values of peptides particularly. But the present version can not deal with the analogues of peptides. On the other hand, PLOGP can produce Molecular Lipophilicity Potential (MLP) profile of a protein with known structure.

Of cause, one compound with rather high toxicity could not become a drug, even when it may meet all of the other criteria required for a potential drug. To prevent a "toxicant" to be chosen for the experimental or even clinical evaluation, our laboratory is actively developing a database-based predictive system to assess the risk of the chemicals in the early stage of drug design. The methodology combines activity prediction with the exploration of chemical database with structural diversity, which mainly includes three parts:

Fast and efficient clustering of molecules based on molecular shape.

Field-based similarity computation of molecular structure based on shape cluster.

Flexible QSAR analysis of molecules based on shape cluster.

In the past ten years, CoMFA, as a kind of 3D QSAR method, has been applied widely. But with more and more reasearch work around it, some limitations in CoMFA have been found. Here we provide a program(AOS/APS)to solve some of these limitations .

2.Stereochemistry Of Drug Molecules

A New Effective Algorithm for the Unambiguous Identification of the Stereochemical Characteristics of Compounds During Their Registration in Databases

Introduction:

Chemical structures represent separate entities, which exist in nature or can be synthesized, and are widely studied and described in publications. A variety of methods have been devised to impart information on the structural representation of compounds. These methods include the use of molecular formulae, various line notations [1], trade/trivial names, registry numbers, structure diagrams, and systematic nomenclature [2].

For the practising chemist, the most acceptable visual representation of a chemical compound is a two-dimensional plan of the three-dimensional structure, and this usually gives an adequate view of the structure, and is easily understood, drawn and copied. Used in reaction sequence it shows clearly the course of the reaction, with display of transient intermediates if required. If it is necessary to show the third dimension, the use of special bond configurations representing lines going above or below the plane can accomplish this. This diagrammatic representation is usually referred to as a structure diagram or structure graph. The structure diagram conventions are established as an international standard and so far are the only unique method of communicating information on a chemical compound. The diagrams, for computer processing, are transformed (manually or automatically) into linear string of characters or into two-dimensional matrices listing all the atoms (nodes) and their mutual interconnections (bonds, edges). These concise representations are referred to as connection tables (CT). Although important only for computer storage and processing, connection tables have, in recent times, been the main means of communication of the information on chemical compounds [3]. Uniquely derived from the standardised structure diagrams, the CT-s became the most complete structural representation and, in the same time, the most visually unrecognisable for the human and the most computer-friendly.

One of the main purposes of the various representations of chemical structures is their unambiguous identification and unique registration for storage in databases of chemical substances as well as for their effective and fast retrieval from the databases. The issue of reliable identification is more and more crucial as database sizes grow steadily. Thus the two biggest and most important databases i.e., CAS and Beilstein contain enormous number of structures (CAS over 18,1 million organic and inorganic substances as of June 2001 [4], and Beilstein File over 8 million organic compounds and 1.4 million inorganic and organometallic compounds as of June 2001 [5]) together with much bigger numbers of accompanying records describing chemical, physical, and recently, also biological characteristics of the corresponding compounds (for example the Beilstein File contains over 35 million associated chemical property and bioactivity records as of June 2001).

The data stored in the databases come from two main sources namely from scientific journals as well as from published patents. For both sources - if they contain structural diagrams

representing the compounds - usually some sort of an “offline” graphical structure editing software package is used. The structural diagram of the new compound selected for registration in a database is drawn using a structure editor (ISIS/Draw, Beilstein SE, ChemDraw ACD/ChemSketch, etc.), the resulting - initial - representation of the structural diagram as a connection table is sent as input to a dedicated program for normalisation and afterwards, also as connection table, is stored as a record in the database. The connection table - as referred to in this paper – is understood as a labelled graph for which nodes are stored either as atom adjacency matrices of bond order labels (single, double, triple, single up, single down, etc.) or as an atom adjacency lists which, for each atom separately, contain bond order labels only to directly connected atoms (neighbours). For both representations each atom is specified by the atom property vector containing such information as atomic number, atomic mass (if non-standard), charge, valency (in non standard), etc. Such connection tables are then canonicalized, i.e., a canonical numbering of atoms is generated by special algorithms [6,7]. The only purpose of the canonicalization is to generate - independent of the used structure coding method – a numeric or alphanumeric identifier (strictly numeric for the Beilstein File, for example) which uniquely and unambiguously labels the structural diagram of the input compound. Such an identifier – specific within a particular database - is called a registry number (CRN for CAS or BRN for Beilstein).

An important property of a chemical compound is its stereoisomerism, i.e., the differentiation of the compounds exclusively due to the arrangement of their atoms in space. Different stereoisomers of the same compound should have different registry numbers. This is not always the case since the translation of the stereoisomers into unique registry numbers is a very complex.

This paper describes a new algorithm for analysis and processing of a chemical structure represented as structural graphs in scientific journal and/or patent publications. Correct and unique interpretation of the steric information contained in graphs of such structures – by the computer programs - provides for reliable registration of different stereoisomers of the same compound in databases of chemical compounds.

3.Analgesic

What Are Analgesics?

Simply put, analgesics are a class of drugs used to relieve pain. The pain relief induced by analgesics occurs either by blocking pain signals going to the brain or by interfering with the brain's interpretation of the signals, without producing anesthesia or loss of consciousness. There are basically two kinds of analgesics: non-narcotics and narcotics.

It should be noted that some references include aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) in the class of analgesics, because they have some analgesic properties. Aspirin and NSAIDS primarily have an anti-inflammatory effect, as opposed to being solely analgesic.

Non-Narcotic Analgesics:

Acetaminophen is the most commonly used over-the-counter, non-narcotic analgesic. Acetaminophen is a popular pain-reliever because it is both effective for mild to moderate pain relief and relatively inexpensive. It must be emphasized though that the safety of acetaminophen is tied to proper use of the drug (use according to specific prescribing instructions). If acetaminophen is not used according to the directions on the label, serious side effects and possible fatal consequences can occur. For example, taking more than 4000 mg/day or using it longterm can increase the risk of liver damage. The risk of liver damage with acetaminophen use is also increased by ingesting alcohol. Make sure you discuss with your doctor the maximum allowable dose of acetaminophen and any other guidelines for its use.

Many people do not realize that acetaminophen is found in more than 600 over-the-counter drugs. It can be found in combination with other active ingredients in many cold, sinus, and cough medications. The cumulative effect of acetaminophen must be considered if you are talking multiple drugs which contain acetaminophen.

How can acetaminophen damage the liver? Acetaminophen changes into metabolites which are eliminated from the body. By taking more than the recommended maximum daily dose of acetaminophen, more toxic metabolites are produced than can be eliminated.

Narcotic Analgesics:

There are two types of narcotic analgesics: the opiates and the opioids (derivatives of opiates). Opiates are the alkaloids found in opium (a white liquid extract of unripe seeds of the poppy plant).

Opioids are any medication which bind to opioid receptors in the central nervous system or gastointestinal tract. According to Wikipedia, there are four broad classes of opioids:Endogenous opioid peptides (produced in the body: endorphins, dynorphins, enkephalins)

Opium alkaloids (morphine, codeine, thebaine)

Semi-synthetic opioids (heroin, oxycodone, hydrocodone, dihydrocodeine, hydromorphone, oxymorphone, nicomorphine)

Fully synthetic opioids (pethidine or Demerol, methadone, fentanyl, propoxyphene, pentazocine, buprenorphine, butorphanol, tramadol, and more)

Opioids are used in medicine as strong analgesics, for relief of severe or chronic pain. Interestingly, there is no upper limit for the dosage of opioids used to achieve pain relief, but the dose must be increased gradually to allow for the development of tolerance to adverse effects (for example, respiratory depression). According to eMedicine, "Some people with intense pain get such high doses that the same dose would be fatal if taken by someone who was not suffering from pain."

Side Effects / Adverse Reactions Of Opioids:

Common side effects and adverse reactions:

nausea

vomiting

drowsiness

dry mouth

miosis (contraction of the pupil)

orthostatic hypotension (blood pressure lowers upon sudden standing)

urinary retention

constipation and/or fecal impaction

Less common side effects and adverse reactions:

confusion

hallucinations

delirium

hives

itch

hypothermia

bradycardia (slow heart rate)

tachycardia (rapid heart rate)

raised intracranial pressure

ureteric or biliary spasm

muscle rigidity

flushing

Most severe side effects and adverse reactions:

respiratory depression

fatal overdose.

4.NSAIDS(Non Steroidal Anti-Inflammatory Drugs

Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are medications which, as well as having pain-relieving (analgesic) effects, have the effect of reducing inflammation when used over a period of time.

Uses:

NSAIDs can be used as simple pain killers (analgesics), but paracetamol is usually preferable, as it is likely to have less unwanted effects, and costs less. They are most useful in conditions which cause inflammation. The anti-inflammatory effects may take from a few days to three weeks to come on, so it is worth persevering for a while before deciding that a NSAID is not going to help.

NSAIDs are used as follows:

The commonest use of these drugs is for arthritis. Paracetamol is often adequate for osteoarthritis, but NSAIDs are particularly useful in the inflammatory forms of arthritis (eg rheumatoid arthritis) and, sometimes, in the more severe forms of osteoarthritis.

Back pain and sciatica. Ibuprofen has been clearly demonstrated to be helpful, and the other NSAIDs are also helpful.

Sprains, strains, and rheumatism.

Dental pain.

Post-operative pain.

Period pain (dysmenorrhoea) and heavy periods (menorrhagia).

Pain from kidney stones (renal colic).

To help reduce temperature in someone with a fever.

Migraine.

Other painful conditions, especially where there is inflammation.

Properties:

The NSAIDs work by affecting some chemicals in the body which cause inflammation, the prostaglandins. Unfortunately the same group of chemicals are involved in the stomach, and so the NSAIDs tend to cause indigestion, and may even cause duodenal or stomach ulceration.

As a result of this side-effect they cannot be used in someone with a history of peptic ulcer, except in exceptional circumstances, under close medical supervision. Also they would rarely be used and, if used, only with extra care, in somebody with heartburn or indigestion.

In general, the more effective a NSAID is at reducing inflammation, the more likely it is to cause indigestion. Sometimes your doctor will prescribe them along with something to cut down the risk of ulceration. There is even one medication that contains both components together.There have been recent advances, in that some NSAIDs are said to be more specific in dealing with inflammation and less likely to irritate the digestive (gastro-intestinal) system, but nothing has yet overcome this problem altogether.

The drugs vary in strength and side effects. Usually, as with other medications, the more effective they are, the more side-effects they are likely to have. Aspirin, which originated from willow bark, has been around for a long time and is in many people's medicine chests. This is an anti-inflammatory analgesic. Most NSAIDs also reduce the temperature in someone with a fever.

Of the newer medications in this group, the one in widest general use is Ibuprofen, which is available over the counter in many countries. There are a large number of other NSAIDs, most of which have to be obtained on prescription in the UK.The one in most extensive use in UK, after Ibuprofen, is Diclofenac.

Adverse reactions:

There are many other potential side effects, but these vary according to the drug chosen and the individual taking it. The other side effects are far less likely to occur.

Specific allergy to a NSAID can be quite dramatic, with rash, swelling of the face, and sometimes difficulty breathing. (If this happens you should call for help urgently.) As with other drugs, if you get a reaction which you suspect to be a side effect, stop taking it and check with your doctor. Sometimes, if the reaction involves an itchy rash and or swelling, it is worth trying an antihistamine in the meantime.

Some people with asthma react to the NSAIDs by getting more wheezy. If this happens you should stop the drug, and use your usual asthma medication, calling the doctor if this does not work.

Warnings:

NSAIDs cannot be used (are contraindicated) in the following cases:

Allergy to aspirin or any NSAID

Aspirin should not be used under the age of 16 years

During pregnancy

During breast feeding

On blood thinning agents (anticoagulants)

Suffering from a defect of the blood clotting system (coagulation)

Active peptic ulcer

Care is needed if you have:

Asthma

Kidney impairment

Heart impairment

Liver impairment

Forms

These drugs are available in nearly every form:

By mouth, they come in tablets, capsules or medicines. This is the most widely used form.

Some NSAIDs are available as injections. This form is used for the pain after surgical operations and also is very effective for the treatment of pain produced by kidney stones (renal colic).

Suppositories are available. These are often used for post operative pain and sometimes in chronic pain when the patient is unable to take medication by mouth.

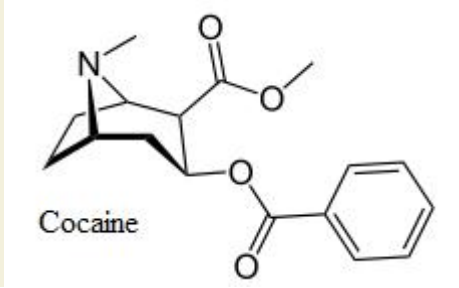
Creams, gels and foams to apply to the skin. These are not felt to be as effective, but some people do get considerable relief from their use.

Although non-oral routes of administration avoid the direct irritation of the stomach, they do not avoid the indigestion and ulcer risks, as these are caused by the chemical once it is in the blood stream.

5.Local Anaesthetics

Local anesthetic agents and their history:

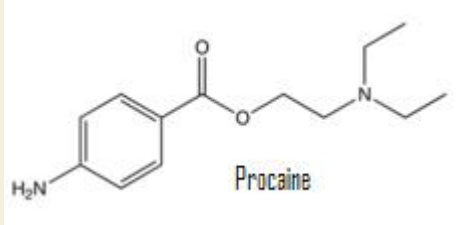
Surprisingly, the first local anesthetic was Cocaine which was isolated from coca leaves by Albert Niemann in Germany in the 1860s. The very first clinical use of Cocaine was in 1884 by (of all people) Sigmund Freud who used it to wean a patient from morphine addiction. It was Freud and his colleague Karl Kollar who first noticed its anesthetic effect. Kollar first introduced it to clinical ophthalmology as a topical ocular (eye) anesthetic. Also in 1884, Dr. William Stewart Halsted was the first to describe the injection of cocaine into a sensory nerve trunk to create surgical anesthesia. Halsted was an eminent surgeon who had been trained in Britain. He was the first to establish formal surgical training for physicians in America. Prior to that time, surgery was a self taught discipline among US physicians. He also invented and pioneered the use of rubber gloves. Unfortunately, much to his own regret, he began to use cocaine himself and became highly addicted to it. At that time, there was no stigma attached to the recreational use of cocaine, and it gained a following among the elites of the day. Arthur Conan Doyle's Sherlock Holmes was supposed to be an addict, and Holmes kept Dr Watson around as a source for his drugs, as well as for the comic relief he provided.



Cocaine

It became fairly obvious fairly quickly that while the anesthetic characteristics of cocaine were desirable, the euphoria and subsequent addiction it produced were not! The turn of the century was a tremendous time of scientific progress, and the new discipline of organic chemistry enabled the synthesis of the first analog of cocaine in 1905. (An analog of a chemical molecule is one in which the original molecule is progressively modified to retain and enhance certain holistic characteristics of the original The first synthetic local anesthetic was procaine, better remembered today by its trade name, "Novocain".

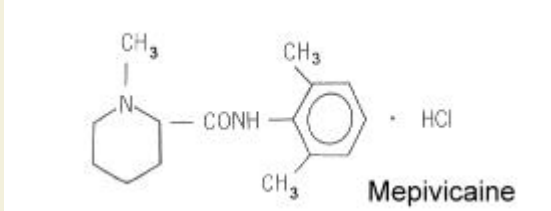
Novocain was not without its problems. It took a very long time to set (ie. to produce the desired anesthetic result), wore off too quickly and was not nearly as potent as cocaine. On top of that, it is classified as an ester. Esters have a very high potential to cause allergic reactions. It is estimated that about one third of all persons who received it developed at least minor allergic reactions to it. Faced with the legal and ethical difficulties associated with the use of cocaine as a local anesthetic, and with the inefficiencies and allergenicity associated with the use of procaine, it is not surprising that most dentists of the day worked without any local anesthetic at all. (Nitrous oxide gas was available during this period.) Today, procaine is not even available for dental procedures.



The first modern local anesthetic agent was lidocaine (trade name Xylocaine®). It was invented in the 1940s. Prior to its introduction, Nitrous oxide gas and procaine (plus alcohol in the form of whiskey) were the major sources of pain relief during dental procedures. Lidocaine proved to be so successful that during the 1940s and 1950s the use of procaine and nitrous oxide gas as primary anesthetic agents all but vanished. (Whiskey somehow survived, but it is no longer used on patients.) Today, nitrous oxide is used principally as an anti-anxiety palliative, and Novocaine is no longer available.

Lidocaine (along with all other injectable anesthetics used in modern dentistry) is in a broad class of chemicals called amides, and unlike ester based anesthetics, amides are hypoallergenic. It sets quickly and when combined with a small amount of epinephrine (adrenalin), it produces profound anesthesia for several hours. Lidocaine is still the most widely used local anesthetic in America today.Over the next thirty years, a number of other amide local anesthetics were invented, most not differing significantly from lidocaine. The major problem with lidocaine and

its analogs is that they cause vasodilation, or the tendency of the local blood vessels to open wider increasing the blood flow in the area. This causes the anesthetic to be absorbed too quickly to take effect.The opposite effect (ie vasoconstriction) and closes the blood vessels down to keep the anesthesia in position long enough to produce long lasting numbness.



Mepivicaine

(Carbocaine®) and prilocaine (Citanest®) have much less vasodilative qualities and hence can be used without the epinephrine vasoconstrictor. The advantage to this is that these anesthetics can be used more safely in patients who are taking medications which may interact negatively with the vasoconstrictor. These drugs include certain blood pressure medications (most notably non selective beta blockers) and tricyclic antidepressants (Elevil® and imipramine are two examples). Carpules that do not contain the vasoconstrictor also do not contain a preservative. This eliminates a possible source of allergic reaction.

Bupivicaine (Marcaine®):

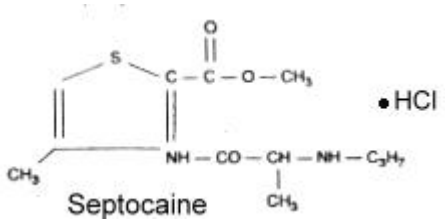
Bupivicaine is a special case in dental anesthesia. It is used mostly by surgeons who want to produce very long acting anesthetic effects in order to delay the post operative pain from their surgery for as long as possible. Bupivicaine comes in 0.5% solution with a vasoconstrictor. It is the most toxic of all the anesthetic agents and this toxicity is reflected in its low concentration in the carpules. As noted in the PKa table, it is has a very alkaline (basic) PKa which means that a relatively low percentage of the uncharged base radical (RN) is available for immediate diffusion through the cell membrane. Thus it takes a fairly long time to set. However, once inside the cell membrane, over 80% of the radicals that do diffuse become available for binding to the sodium channel proteins. This high protein binding ability causes the drug to remain active for a long time once it has diffused through the cell membrane. (PKa and its relationship with cell membrane permeability is a concept explained later in this course.)

Articaine (Septocaine®):

Articaine is the newest addition to the local anesthetic arsenal and was approved by the Food and Drug Administration in April 2000. It has been in use in Europe since 1976 and in Canada since 1983. Its approval in the US has been delayed by the FDA due to the presence of a preservative which the agency said was unnecessary in single use carpules and was a potential allergen. It was approved when the French company Septodent finally removed the preservative from American shipments.

Articaine has the same PKa and toxicity as Lidocaine, however it is metabolized differently. It has a half life in the body less than 1/4 as long as that of lidocaine and only 1/5 as long as mepivicaine. This means that more of the drug can be injected later in the dental procedure with less likelihood of blood concentrations building to toxic levels. Articaine is formulated in a 4.0% solution with vasoconstrictor. The presence of the vasoconstrictor retards the systemic absorption of the anesthetic allowing higher concentrations of the drug to remain in the area of injection and slowing the absorption into the bloodstream. The higher local concentration of the drug produces a high level of the uncharged radical (RN) to be present at the membrane which brings about very rapid absorption of the drug. (The concept of membrane permeability is discussed on page 4 in this course.) In addition, the benzene ring on the left end of the molecule has been replaced with a thiophene ring. This modification allows for faster and more complete absorption through the nerve cell membrane. The ability of this drug to penetrate barriers is so great that it has been used to penetrate thick bone to produce anesthesia in a way that other anesthetics cannot. Articaine has become the local anesthetic of choice in most countries into which it has been introduced. I have found that it produces profound anesthesia (in most patients) when used as an infiltration (field block) for mandibular premolars and anterior teeth instead of the traditional mandibular nerve block..

With clinical reports of profound anesthesia, fast onset, and success in difficult-to-anesthetize patients, Septocaine has become the most used dental anesthetic brand name in the US, although lidocaine still remains the most used type of anesthetic. Recently, the same articaine formulation became available from a second company under the name Cook-Waite Zorcaine.Because of its bone penetrating ability, articaine has become popular for producing profound anesthesia in lower premolars and lower anterior teeth using localized field blocks (infiltrations) without resorting to mandibular blocks.



Septocaine

Articaine and prolonged numbness and paresthesia:

Unfortunately, one complication concerning the use of articaine has arisen. There have been persistent reports of unexplained paresthesia (burning, tingling, ans sometimes sharp shooting pains in tissues previously anesthetized with this anesthetic) in a low percentage of patients. This effect has been noted only when articaine is used in major nerve blocks such as the mandibular block. It has not been noted in field blocks. So far, no common factor has been

found to explain the link between articaine and persistent paresthesia, however the higher concentration of anesthetic molecules in the anesthetic solution (4% for articaine instead of 2% for lidocaine or 3% for mepivicaine without vasoconstrictor) probably is a factor. The statistics produced for this phenomenon so far have been quite inconsistent. The incidence for persistent paresthesia have ranged between 1 in 5,286 for 2002 to 1 in 45,900 in 2004. The incidence for persistent paresthesia for other types of dental local anesthetic solutions ranged between 1 in 25,850 to 1 in 68,675 during the same timeframe (all statistics approximate; CRA June 2005; vol 29, issue 6).

10% of cases of paresthesia lasted for 24 hours or less.

52% of cases of paresthesia lasted 1 to 4 weeks.

29% of cases of paresthesia lasted 1 month to 1 year.

10% of cases of paresthesia lasted for over a year.

The most common link with articaine and paresthesia was administration of mandibular nerve block injections. For this reason a number of dentists have abandoned the use of articaine for mandibular nerve blocks, but still use it for infilatration anesthesia (field blocks) of mandibular anterior teeth and bicuspids.

Treatment of anesthesia and paresthesia:

I am not aware of any specific way to treat the numbness caused by Articaine (other than waiting out the effect), however the disruptive and often painful paresthesia, including the shooting pains in the distribution of the affected nerves may be controlled with certain anti convulsant drugs. One such drug is Lyrica (Pregabalin) 50 mg. three times a day. Two older drugs in this category are Neurontin (gabapentin) and Tegretol (carbamazepine).

6.Cardio Vascular Drug

Basic cardiovascular physiology and pathology:

what you need to know and where to find information on the control of heart rate, cardiac output, blood pressure, blood volume, ionic composition, renin / angiotensin system, vascular endothelium, regulation of tissue perfusion, hypertension, dislipidaemias, atherosclerosis, blood clotting, ischaemic heart disease, cardiomyopathies, cardiac arrhythmias and cardiac faCardiovascular drugs: inotropic agents, b blockers, calcium antagonists, organic nitrates, anti-arrhythmics, ACE inhibitors, ATII (=AT1) antagonists, diuretics, cholesterol lowering drugs, clot-busters, anti-coagulants, anti-platelet dExamples and opportunities for rational drug design in

relation to renin, angiotensin, aldosterone, cytokines, vasoactive peptides and other cardiovascular targets.

Basic Cardiovascular :

The right side of the heart (pumping blood to the lungs) is a low pressure pump, while the left side of the heart (serving the rest of the body) operates at a much higher blood pressure. This is reflected in the chamber dimensions and wall thickness.

The Starling mechanism. If the heart chambers are distended with blood, the ensuing beat is much more forceful than if the chambers were initially empty. It is essential that hearts should respond in this way, since otherwise Laplace's Law would make cardiac pumping impossible if the ventricles ever became overfull. The molecular explanation is that calcium ion release from the sarcoplasmic reticulum is much greater when the SR is mechanically stretched. This should be considered in conjunction with the next point.

About 70% of the blood volume resides in the great veins, which have muscular walls. Contraction of venous smooth muscle, or an expansion in blood volume, raises central venous pressures and transfers some of this reserve blood supply into the heart chambers, stretching the heart and greatly increasing the cardiac output. Ventricular filling is often decribed as the PRELOAD applied to the heart.

Most of the resistance to blood flow arises from smooth muscle compressing the walls of the arterial tree. The main effects are in the small arterioles, and in the pre-capillary sphincters which ration blood flow into the capillary beds. The aortic pressure depends on the cardiac output and the peripheral vascular resistance, and is often called the AFTERLOAD applied to the heart.

Local control of blood flow relies mainly on nitric oxide and endothelin produced by vascular endothelial cells and adenosine released by ischaemic tissues. Capillary loops seem to be folded back on themselves so as to bring the inflow and outflow vessels into close juxtaposition, and thereby facilitate this local signalling.

Local blood flow regulation helps to keep the cellular oxygen concentration low. Oxygen is a very toxic substance, and cells are easily damaged by high concentrations. Local regulation directs the limited cardiac output to the areas where it is most needed.

The cardiac cycle:

Each beat is initiated by spontaneous depolarisation of pacemaker cells in the sino-atrial (SA) node. These cells trigger the neighbouring atrial cells by direct electrical contacts and a wave of depolarisation spreads out over the atria, eventually exciting the atrio-ventricular (AV) node.

Contraction of the atria precedes the ventricles, forcing extra blood into the ventricles and eliciting the Starling response. The electrical signal from the AV node is carried to the ventricles by specialised conducting tissue (Purkinje fibres) in the interventricular septum, formed from modified cardiac muscle cells. The heart does not require innervation in order to beat, but the autonomic nerve supply can vary the force and frequency of cardiac contraction.

Control of cardiac output: Aortic output varies over a twenty-fold range between sleeping and vigorous exercise, and is also increased during pregnancy. The Starling mechanism is very important, but in addition circulating adrenalin, and noradrenalin released by cardiac nerve terminals, have inotropic effects, enhancing cardiac contractility via cyclic AMP and protein kinase A. Increased contractility means that the same peak systolic pressure can be achieved with a lower end diastolic pressure, i.e. with a lower degree of ventricular stretch. Increased contractility does not necessarily produce a rise in arterial pressure, but may produce a fall in the central venous pressure. Ion channel phosphorylation increases calcium entry during the action potential, calcium release from the SR during systole (contraction), and calcium uptake by the SR during diastole (relaxation). [Direct electrical connections between adjacent cardiac muscle cells preclude the progressive fibre recruitment seen in voluntary muscles: in the heart every muscle cell depolarises every beat.] Catecholamines from the sympathetic nerves acting on cardiac b1 adrenoceptors make the beats more rapid and forceful, while acetylcholine from the parasympathetic nerves acting on muscarinic M2 receptors has the opposite effect.

Congestive heart failure (CHF): This term is often misunderstood. It does NOT mean the heart has stopped beating, it means that output is insufficient to meet the needs of the body. The autonomic nervous system detects the inadequate tissue perfusion, and reacts as though the blood volume were too low. It leads to vasoconstriction, salt and water retention, reduced parasympathetic activity, increased sympathetic activity and cytokine production. The low inherent cardiac contractility requires excessive venous pressures to maintain ventricular output through the Starling mechanism. Permanently high venous pressures are a serious problem: if the systemic venous pressures are high (right side failure) it may cause disabling lower limb oedema, while if the pulmonary venous pressures are raised (left side failure) it will cause lung congestion (fluid accumulation) breathlessness (dyspnoea) and coughing. Both sides of the heart may be affected at classical heart failure symptoms such as dyspnoea (breathlessness) and swollen ankles. [The cardiac index is the output in litres/minute divided by the body surface area in square metres.]

Traditional therapy was based on diuretics, vasodilators and inotropic agents, which relieved symptoms and improved cardiovascular status, without improving overall mortality! The later introduction of loop diuretics (frusemide), potassium-sparing diuretics (amiloride) aldosterone antagonists (spironolactone), b-blockers, and particularly ACE inhibitors and angiotensin receptor antagonists has greatly reduced the morbidity and mortality associated with CHF.

Angina:

This is a pain in the chest caused by insufficient blood flow through the coronary arteries, leading to cardiac ischaemia. It may be a chronic condition persisting for many years, and often exacerbated by excitement, smoking and over-eating. Reduced coronary blood flow sometimes results from coronary artery spasm, but most commonly follows atherosclerotic damage to the blood vessel endothelial lining. This inflammatory process leads to blood clot formation and obstruction of the vessel lumen. See Staels (2002) Nature 417, 699 - 701 for a recent brief review. Angina may be associated with other manifestations of atherosclerosis, such as strokes and intermittent claudication in skeletal muscle. Prolonged or severe angina may progress to necrosis of cardiac cells, release of the cell contents into the bloodstream and myocardial infarction (MI).Blood osmolarity is monitored by osmoreceptor cells in the hypothalamus which control overall water balance through the pituitary peptide vasopressin (= anti-diuretic hormone, or ADH). These sensors are also connected to the behavioural systems controlling thirst and salt craving. Stretch receptors in the right atrium and the arterial tree monitor total blood volume, and this neurally transmitted information is also used to modulate ADH release.

There is interaction between the control of blood volume and blood osmolarity. Stretching the right atrium stimulates the release of atrial natriuretic peptide (ANP) from the atrium, which promotes sodium excretion by the kidney, ultimately reducing blood volume and salt content. Ventricles produce brain natriuretic peptide or BNP. Conversely, low salt concentration in the distal convoluted tubule, or inadequate perfusion of the kidney, both stimulate release of renin into the bloodstream. This short half-life protease cleaves circulating angiotensinogen producing angiotensin I. Subsequent proteolysis by the lung endothelium yields angiotensin II which has a powerful vasoconstrictor effect on arteriolar smooth muscle. This raises systemic blood pressure, restores kidney perfusion, and stimulates release of the salt-retaining hormone aldosterone from the adrenal cortex, which promotes renal sodium retention and potassium loss.

Nitroglycerine tablets dissolved under the tongue remain the classical treatment for anginal attacks. The drug is rapidly absorbed through the buccal epithelium, and is metabolised in the tissues to nitric oxide, which has vasodilator and hypotensive effects. [Hypotension was noted many years ago among workers in munitions factories who absorbed organic nitrates through their skin.] A variety of polyol nitrates, calcium channel antagonists and b adrenergic blocking

agents may also be used to achieve the desired effect. A new treatment for unstable angina without infarction is abciximab - a chimeric mouse-human monoclonal antibody directed against the platelet glycoprotein IIb/IIIa fibrinogen receptor. This treatment interferes with blood clotting, and delays the extension of the arterial plaque.

Myocardial infarction: The heart is uniquely sensitive to lack of oxygen because it operates a highly aerobic, lipid-based metabolism, with up to 95% arteriovenous oxygen extraction. Many tissues have arterio-venous shunts in their capillary network and extract only about 30% of the available oxygen in arterial blood. The high cardiac oxygen extraction may arise because the capillaries are squeezed during systole, so that blood flow only occurs during diastole.

If the coronary arteries are partially or completely blocked, then downstream muscle cells may die, releasing their enzymes into the extracellular space. The enzymes continue to leak into the bloodstream over several hours. This release is non-specific but can be useful in the diagnosis of suspected myocardial infarction from peripheral blood samples. Not all heart attacks are painful enough to be immediately recognised, although many are. Other signs of myocardial infarction are changes in the ECG, and under favourable conditions these can show the precise location of the damage. A really big transmural infarct carries the risk that the ventricle may eventually rupture with disastrous results, but normally the most serious risks are cardiogenic shock and ventricular fibrillation provoked by the abnormal electrical properties of partially ischaemic cells at the edge of the infarct. If the patient survives the first few days the damaged area is invaded by macrophages and fibroblasts.

medical condition	**angina pectoris**	**congestive heart failure**
physiological problem	cardiac oxygen demand exceeds supply	low contractility (more rarely, atrial fibrillation) causes high venous blood pressure
treatment strategy	reduce cardiac work output, try to improve oxygen supply	increase cardiac volume output, reduce cardiac workload, reduce venous congestion
drug 1	nitroglycerine causes arterial vasodilation, lowers afterload	ACE inhibitors or AT_1blockers produce arterial vasodilation
drug 2	β-blocker (e.g. metoprolol) but care needed in diabetics	diuretics (e.g. furosemide plus amiloride) remove excess fluid
drug 3	calcium channel blocker (e.g. nifedipine or verapamil)	β-blockers (e.g. carvedilol) in hemodynamically stable patients
drug 4	lipid lowering and anti-clotting drugs, aspirin or clopidogrel	digitalis (especially for atrial fibrillation, or severe failure)

The cells are replaced by scar tissue, leaving the heart usable, but permanently weakened. Infarcts affecting the cardiac impulse conducting system may produce partial or complete heart block, where synchronisation is lost between atria and ventricles. This can be treated with an artificial pacemakeIn many tissues there is a local angiotensin metabolism superimposed on the whole body mechanisms described above. Angiotensin is also concerned with tissue growth.

The whole ensemble is monitored and regulated by the autonomic nervous system. There are blood pressure receptors (baroreceptors) in the great veins and in the aorta and the arterial tree. The baroreceptor signals are integrated with information on body position from the system controlling voluntary movements, and with information from chemical sensors, ultimately regulating renin production, cardiac contractility and arterial and venous smooth muscle tone. The physical position of the body is important, because the hydrostatic pressures associated with an upright posture are greater than arterial blood pressures, and very much greater than the pressure in the veins.

7.Cholinergic drugs

Cholinergic Stimulating Agents:

Acetylcholine is the chemical transmitter for nerves of the parasympathetic, somatic, preganglionic sympathetic, and parts of the central nervous system. Acetylcholine is synthesized by the transfer of an acetyl group from acetyl CoA to choline, a normal constituent of the diet.Acetylcholine is concentrated in large amounts in presynaptic vesicles, which release their contents into the synapse when voltage-gated calcium channels open in response to membrane depolarization.

Upon interaction with the receptor, acetylcholine produces an influx of sodium through a ligand-gated ion channel which sends the impulse.

After acetylcholine interacts with the cholinergic receptor it is very rapidly hydrolyzed by the enzyme acetylcholinesterase. The hydrolysis reaction is the reverse of the synthesis reaction except that choline and acetic acid are products. The choline is retaken up by the nerve ending where it is reused for synthesis of new molecules of acetylcholine.

Acetylcholine acts on two vastly different classes of receptors - nicotinic receptors (with two subtypes, one at the neuromuscular junction of skeletal muscle, the other within ganglia and the CNS), and muscarinic receptors (widely distributed within both peripheral and central nervous systems). Muscarinic receptors originally were distinguished from nicotinic receptors by the selectivity of the agonists muscarine and nicotine respectively. Notice the similarities in structure for all three of these compounds.

Although there appears to be at least two cholinergic receptor sites, they are similar enough to be considered as one. The acetylcholine interacts with the receptor site through ionic attraction of the positive nitrogen, polar attraction of the ester group, and through hydrophobic interactions with the methyl groups.

Stimulation:

Stimulation of cholinergic nerves is achieved either directlyor indirectly. Direct acting agents (agonists) activate the receptor site by mimicking the effects of acetylcholine. Cholinesterase inhibitors act indirectly by preventing the enzyme from hydrolyzing (inactivating) acetylcholine at the receptor site. This inhibition permits the buildup of acetylcholine and results in more intensive and prolongedactivation of the receptor site. The effects of cholinergicstimulation include: vasodilation of blood vessels; slower heart rate; constriction of bronchioles and increased secretion of mucus in the respiratory tract; intestinal cramps; secretion of salvia; sweat and tears; and constriction of eye pupils.

Direct Acting Cholinergic Agents - Agonists:

Direct acting cholinergic agents act as agonists and initiate stimulant type responses at the receptor site. Directstimulation of acetylcholine receptors is achieved by: Arecholine, Pilocarpine, Urecholine(Betanechol), Carbachol, Choline, Metacholine, Mushrooms (Boletus sp., Clitocybe sp. , Inocybe sp.)

Drugs: Urecholine and philocarpine are direct acting drugs. Urecholine is used to restore parasympathetic tone to smooth muscles of the intestinal tract and bladder following abdominal surgery. Pilocarpine is used to constrict pupils and reduce pressure caused by glaucoma. Pilocarpine contracts the ciliary muscle with causes the iris to be withdrawn. This action permits drainage of the aqueous humor and thus relieves the pressure due to a glaucoma condition.

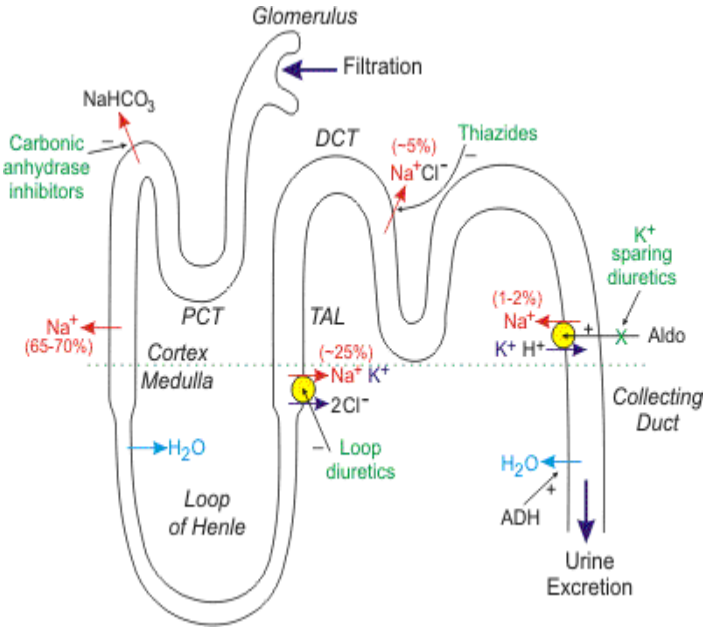
Cholinergic Poison agents which mimic the structure of acetylcholine include two poisons: muscarine - an alkaloid present in poisonous mushrooms and nicotine from cigarettes. Muscarinic effects are those of parasympathetic overactivity and include bradycardia, pinpoint pupils, sweating, blurred vision, excessive lacrimation, excessive bronchial secretions, wheezing, dyspnoea, coughing, vomiting, abdominal cramping, diarrhea, and urinary and fecal incontinence.

Nicotine: Nicotinic effects are those of sympathetic overactivity and neuromuscular dysfunction and include tachycardia, hypertension, dilated pupils, muscle fasciculation and muscle weakness.Accidental ingestion of these poisons may produce death from heart failure unless treated with a suitable antidote. Atropine blocks the receptor site to decrease the stimulant effects produced by the muscarine type poisons, but has no effect on nicotine receptors.

8.Diuretics

Renal handling of sodium and water. To understand the action of diuretics, it is first necessary to review how the kidney filters fluid and forms urine. As blood flows through the kidney, it passes into glomerular capillaries located within the cortex (outer zone of the kidney). These glomerular capillaries are highly permeable to water and electrolytes. Glomerular capillary

hydrostatic pressure drives (filters) water and electrolytes into Bowman's space and into the proximal convoluting tubule (PCT). About 20% of the plasma that enters the glomerular capillaries is filtered (termed filtration fraction). The PCT, which lies within the cortex , is the site of sodium, water and bicarbonate transport from the filtrate (urine), across the tubule wall, and into the interstitium of the cortex. About 65-70% of the filtered sodium is removed from the urine found within the PCT (this is termed sodium reabsorption). This sodium is reabsorbed isosmotically, meaning that every molecule of sodium that is reabsorbed is accompanied by a molecule of water



As the tubule dives into the medulla, or middle zone of the kidney, the tubule becomes narrower and forms a loop (Loop of Henle) that reenters the cortex as the thick ascending limb (TAL) that travels back to near the glomerulus. Because the interstitium of the medulla is very hyperosmotic and the Loop of Henle is permeable to water, water is reabsorbed from the Loop of Henle and into the medullary interstitium. This loss of water concentrates the urine within the Loop of Henle. The TAL, which is impermeable to water, has a cotransport system that reabsorbs sodium, potassium and chloride at a ratio of 1:1:2. Approximately 25% of the sodium load of the original filtrate is reabsorbed at the TAL. From the TAL, the urine flows into the distal convoluting tubule (DCT), which is another site of sodium transport (~5% via a sodium-chloride cotransporter) into the cortical interstitium (the DCT is also impermeable to water). Finally, the tubule dives back into the medulla as the collecting duct and then into the renal pelvis where it joins with other collecting ducts to exit the kidney as the ureter. The distal segment of the DCT and the upper collecting duct has a transporter that reabsorbs sodium (about 1-2% of filtered load) in exchange for potassium and hydrogen ion, which are excreted into the urine. It is important to note two things about this transporter. First, its activity is dependent on the tubular concentration of sodium, so that when sodium is high, more sodium is reabsorbed and more potassium and hydrogen ion are excreted. Second, this transporter is regulated by aldosterone, which is a mineralocorticoid hormone secreted by the adrenal cortex. Increased aldosterone stimulates the reabsorption of sodium, which also increases the loss of potassium and hydrogen ion to the urine. Finally, water is reabsorbed in the collected duct through special pores that are regulated by antidiuretic hormone, which is released by the posterior pituitary. ADH increases the permeability of the collecting duct to water, which leads

to increased water reabsorption, a more concentrated urine and reduced urine outflow (antidiuresis). Nearly all of the sodium originally filtered is reabsorbed by the kidney, so that less than 1% of originally filtered sodium remains in the final urine..

Mechanisms of diuretic drugs. Diuretic drugs increase urine output by the kidney (i.e., promote diuresis). This is accomplished by altering how the kidney handles sodium. If the kidney excretes more sodium, then water excretion will also increase. Most diuretics produce diuresis by inhibiting the reabsorption of sodium at different segments of the renal tubular system. Sometimes a combination of two diuretics is given because this can be significantly more effective than either compound alone (synergistic effect). The reason for this is that one nephron segment can compensate for altered sodium reabsorption at another nephron segment; therefore, blocking multiple nephron sites significantly enhances efficacy.

Loop diuretics inhibit the sodium-potassium-chloride cotransporter in the thick ascending limb (see above figure). This transporter normally reabsorbs about 25% of the sodium load; therefore, inhibition of this pump can lead to a significant increase in the distal tubular concentration of sodium, reduced hypertonicity of the surrounding interstitium, and less water reabsorption in the collecting duct. This altered handling of sodium and water leads to both diuresis (increased water loss) and natriuresis (increased sodium loss). By acting on the thick ascending limb, which handles a significant fraction of sodium reabsorption, loop diuretics are very powerful diuretics. These drugs also induce renal synthesis of prostaglandins, which contributes to their renal action including the increase in renal blood flow and redistribution of renal cortical blood flow.Thiazide diuretics, which are the most commonly used diuretic, inhibit the sodium-chloride transporter in the distal tubule. Because this transporter normally only reabsorbs about 5% of filtered sodium, these diuretics are less efficacious than loop diuretics in producing diuresis and natriuresis. Nevertheless, they are sufficiently powerful to satisfy most therapeutic needs requiring a diuretic. Their mechanism depends on renal prostaglandin production.

Because loop and thiazide diuretics increase sodium delivery to the distal segment of the distal tubule, this increases potassium loss (potentially causing hypokalemia) because the increase in distal tubular sodium concentration stimulates the aldosterone-sensitive sodium pump to increase sodium reabsorption in exchange for potassium and hydrogen ion, which are lost to the urine. The increased hydrogen ion loss can lead to metabolic alkalosis. Part of the loss of potassium and hydrogen ion by loop and thiazide diuretics results from activation of the renin-angiotensin-aldosterone system that occurs because of reduced blood volume and arterial pressure. Increased aldosterone stimulates sodium reabsorption and increases potassium and hydrogen ion excretion into the urine.

There is a third class of diuretic that is referred to as potassium-sparing diuretics. Unlike loop and thiazide diuretics, some of these drugs do not act directly on sodium transport. Some drugs in this class antagonize the actions of aldosterone (aldosterone receptor antagonists) at the distal segment of the distal tubule. This causes more sodium (and water) to pass into the collecting duct and be excreted in the urine. They are called K+-sparing diuretics because they do not produce hypokalemia like the loop and thiazide diuretics. The reason for this is that by inhibiting aldosterone-sensitive sodium reabsorption, less potassium and hydrogen ion are exchanged for sodium by this transporter and therefore less potassium and hydrogen are lost to the urine. Other potassium-sparing diuretics directly inhibit sodium channels associated with the aldosterone-sensitive sodium pump, and therefore have similar effects on potassium and hydrogen ion as the aldosterone antagonists. Their mechanism depends on renal prostaglandin production. Because this class of diuretic has relatively weak effects on overall sodium balance, they are often used in conjunction with thiazide or loop diuretics to help prevent hypokalemia.Carbonic anhydrase inhibitors inhibit the transport of bicarbonate out of the proximal convoluted tubule into the interstitium, which leads to less sodium reabsorption at this site and therefore greater sodium, bicarbonate and water loss in the urine. These are the weakest of the diuretics and seldom used in cardiovascular disease. Their main use is in the treatment of glaucoma.

Cardiovascular effects of diuretics. Through their effects on sodium and water balance, diuretics decrease blood volume and venous pressure. This decreases cardiac filling (preload) and, by the Frank-Starling mechanism, decreases ventricular stroke volume and cardiac output, which leads to a fall in arterial pressure. The decrease in venous reduces capillary hydrostatic pressure, which decreases capillary fluid filtration and promotes capillary fluid reabsorption, thereby reducing edema if present. There is some evidence that loop diuretics cause venodilation, which can contribute to the lowering of venous pressure. Long-term use of diuretics result in a fall in systemic vascular resistance (by unknown mechanisms) that helps to sustain the reduction in arterial pressure.

9.Coagulants

Platelet activation:

Damage to blood vessel walls exposes subendothelium proteins, most notably ollagen, present under the endothelium. Circulating platelets bind collagen with surface collagen-specific glycoprotein Ia/IIa receptors. The adhesion is strengthened further by the large, multimeric circulating proteins von Willebrand factor (vWF), which forms links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. This adhesion activates the platelets.

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet activating factor (PAF), vWF, platelet factor 4 and thromboxane A2 (TXA2) which in turn activate additional platelets. The granules contents activate a Gq-linked protein receptor cascade resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C which in turn activates phospholipase A2 (PLA2). PLA2 then modifies the ntegrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets changed shape from spherical to stellate and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets.

The coagulation cascade:

The coagulation cascade of secondary hemostasis has two pathways, the contact activation pathway (formerly known as the intrinsic pathway) and the tissue factor pathway (formerly known as the extrinsic pathway) that lead to fibrin formation. It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the tissue factor pathway. The pathways are a series of reactions, in which a ymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase a appended to indicate an active form.The coagulation factors are generally serine proteases (nzymes). There are some exceptions. For example, FVIII and FV are glycoproteins and Factor XIII is a transglutaminase. Serine proteases act by cleaving other proteins at specific sites. The coagulation factors circulate as inactive zymogens.The coagulation cascade is classically divided into three pathways. The tissue factor and contact activation pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

Tissue factor pathway:

The main role of the tissue factor pathway is to generate a "thrombin burst", a process by which hrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released instantaneously. FVIIa circulates in a higher amount than any other activated coagulation factor.

Following damage to the blood vessel, endothelium Tissue Factor (TF) is released, forming a complex with FVII and in so doing, activating it (TF-FVIIa).

TF-FVIIa activates FIX and FX.

FVII is itself activated by thrombin, FXIa, lasmin, FXII and FXa.

The activation of FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).

FXa and its co-factor FVa form the prothrombinase complex which activates prothrombin to thrombin.

Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which in turn activates FIX), and activates and releases FVIII from being bound to vWF.

FVIIIa is the co-factor of FIXa and together they form the "enase" complex which activates FX and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.)

Contact activation pathway:

The contact activation pathway begins with formation of the primary complex on ollagen by high-molecular weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to allikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the enase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder.

Final common pathway:

Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it activates Factors VIII and V and their inhibitor rotein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

Following activation by the contact factor or tissue factor pathways the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the enase complex, until it is down-regulated by the anticoagulant pathways.

Cofactors:

Various substances are required for the proper functioning of the coagulation cascade:

Calcium and phospholipid (a latelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets as well as procoagulant microparticles or microvesicles shedded from them. Calcium is also required at other points in the coagulation cascade.

Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as rotein S, Protein C and rotein Z. In adding the gamma-carboxyl group to glutamate residues on the immature clotting factors Vitamin K is itself oxidized. Another enzyme, Vitamin K epoxide reductase, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target for anticoagulant drugs arfarin and related coumarins such as acenocoumarol, phenprocoumon and icumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g. in malabsorption), or disease (epatocellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (proteins formed in vitamin K absence) this causes partial or non gamma carboxylation and affects the coagulation factors ability to bind to expressed phospholipid.

Regulators:

Five mechanisms keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:Protein C is a major physiological anticoagulant. It is a vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin binds these proteins in such a way that it activates Protein C. The activated form, along with protein S and a phospholipid as cofactors, degrades FVa and FVIIIa. Quantitative or qualitative deficiency of either may lead to thrombophilia (a tendency to develop thrombosis). Impaired action of Protein C (activated Protein C resistance), for example by having the "Leiden" variant of Factor V or high levels of FVIII also may lead to a thrombotic tendency.

Antithrombin is a serine protease inhibitor (erpin) that degrades the serine proteases; thrombin, FIXa, FXa, FXIa and FXIIa. It is constantly active, but its adhesion to these factors is increased by the presence of heparan sulfate (a glycosaminoglycan) or the administration of eparins (different heparinoids increase affinity to FXa, thrombin, or both). Quantitative or

qualitative deficiency of antithrombin (inborn or acquired, e.g. in proteinuria) leads to thrombophilia.

Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF). It also inhibits excessive TF-mediated activation of FIX and FX.

lasmin is generated by proteolytic cleavage of plasminogen, a plasma protein synthesized in the liver. This cleavage is catalyzed by tissue plasminogen activator (t-PA) which is synthesized and secreted by endothelium. Plasmin proteolytically cleaves fibrin into fibrin degradation products which inhibits excessive fibrin formation.

Prostacyclin (PGI2) is released by endothelium and activates platelet Gs protein linked receptors. This in turn activates adenylyl cyclase which synthesizes cAMP. cAMP inhibits platelet activation by counteracting the actions that result from increased cytosolic levels of calcium and by doing so inhibits the release of granules that would lead to activation of additional platelets and the coagulation cascade.

Fibrinolysis:

Eventually, all blood clots are reorganised and resorbed by a process termed fibrinolysis. The main enzyme responsible for this process (lasmin) is regulated by various activators and inhibitors.

10.Anti Coagulants

Definition:

Compounds that do not allow blood to clot are called anticoagulants. These include drugs such as heparin and coumarin. Drugs that dissolve pre-formed clot including streptokinase are not referred to as anticoagulants.

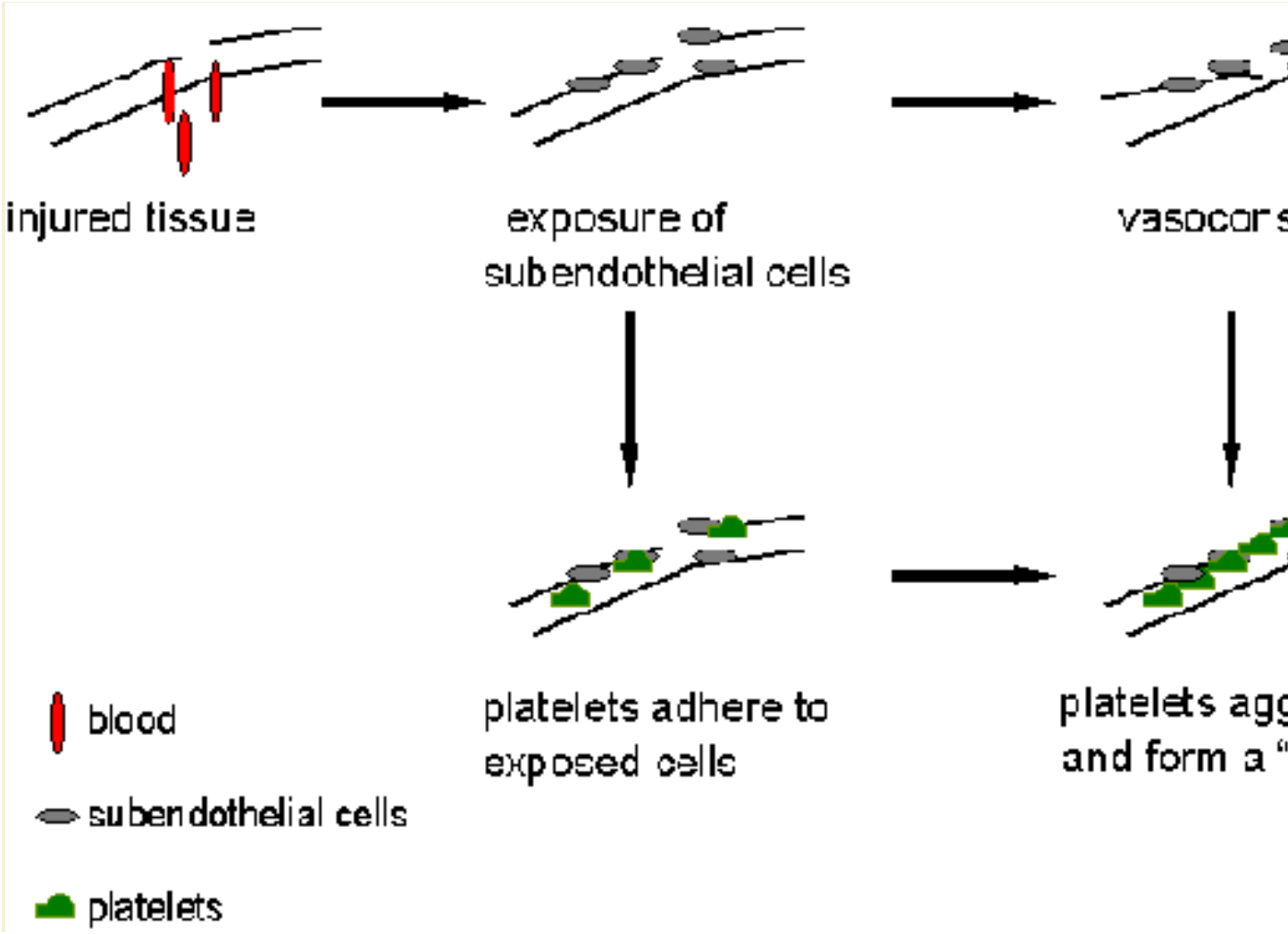
Anticoagulants are usually administered to patients with myocardial infarction, venous thrombosis, peripheral arterial emboli and pulmonary emboli. They have been used to prevent transient ischemic attacks and to reduce the risk of recurrent myocardial infarction.

Process of clotting:

The circulatory system has to be self-sealing, otherwise continued blood loss from even the smallest injury would be life threatening. Normally, all but the most catastrophic bleeding is rapidly stopped, a process known as hemostasis. This process occurs through a progression of several. Hemostasis is a combination of events that occur due to physical and chemical forces.

The initial steps lead to a reduction in the blood flow due to the formation of a cellular plug. The later steps utilize chemical energy to form a blood clot, medically known as thrombus.

The Physical Process:



Mechanism of clotting

A blood clot forms as a result of concerted action of some 20 different substances, most of which are plasma glycoproteins (Table).

Coagulation Factors		
Factor	**Name**	**Plasma half-life (h)**

I	Fibrinogen	72 - 96
II	Prothrombin	60
III	Tissue Factor or thromboplastin	--
IV	Ca^{++}	--
V	Proaccelerin	15
VII	Proconvertin	5
VIII	Antihemophilic A factor	10
IX	Antihemophilic B factor or Christmas factor	25
X	Stuart or Stuart-Prower factor	40
XI	Plasma thomboplastin antecedent	45-65
XII	Hageman factor, contact factor	60
XIII	Fibrin stabilizing factor	150
	Prekallikrein factor	--
	High-molecular-weight kininogen	156

The phenomenon of blood coagulation is traditionally distinguished into two pathways. These pathways are the intrinsic and the extrinsic pathways (Figure below). The intrinsic pathway is defined as a cascade that utilizes only factors that are soluble in the plasma, whereas the extrinsic pathway consists of some factors that are insoluble in the plasma, e.g., membrane-bound factors (factor VII). However, the boundary differentiating these two is becoming more and more blurred.

11.Vitamins

A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. A compound is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and the particular organism. For example, ascorbic acid functions as vitamin C for some animals but not others, and vitamins D and K are required in the human diet only in certain circumstances.Vitamins are classified by their biological activity, not their structure. Thus, each "vitamin" actually refers to a number of vitamer compounds, which form a set of distinct chemical compounds that show the biological activity of a particular vitamin. Such a set of chemicals are grouped under an alphabetized vitamin "generic descriptor" title, such as "vitamin A," which (for example) includes retinal, retinol, and many carotenoids. Vitamers are often inter-convertible in the body. The term vitamin does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids, nor does it encompass the large number of other nutrients that promote health but are otherwise required less often.

Vitamins have diverse biochemical functions, including function as hormones (e.g. vitamin D), antioxidants (e.g. vitamin E), and mediators of cell signaling and regulators of cell and tissue

growth and differentiation (e.g. vitamin A). The largest number of vitamins (e.g. B complex vitamins) function as precursors for enzyme cofactor bio-molecules (coenzymes), that help act as catalysts and substrates in metabolism. When acting as part of a catalyst, vitamins are bound to enzymes and are called prosthetic groups. For example, biotin is part of enzymes involved in making fatty acids. Vitamins also act as coenzymes to carry chemical groups between enzymes. For example, folic acid carries various forms of carbon group – methyl, formyl and methylene - in the cell. Although these roles in assisting enzyme reactions are vitamins' best-known function, the other vitamin functions are equally important.Until the 1800s, vitamins were obtained solely through food intake, and changes in diet (which, for example, could occur during a particular growing season) can alter the types and amounts of vitamins ingested. Vitamins have been produced as commodity chemicals and made widely available as inexpensive pills for several decades, allowing supplementation of the dietary intake.

History:

The value of eating a certain food to maintain health was recognized long before vitamins were identified. The ancient Egyptians knew that feeding a patient liver would help cure night blindness, an illness now known to be caused by a vitamin A deficiency. The advancement of ocean voyage during the Renaissance resulted in prolonged periods without access to fresh fruits and vegetables, and made illnesses from vitamin deficiency common among ship's crew.

In 1749, the Scottish surgeon James Lind discovered that citrus foods helped prevent scurvy, a particularly deadly disease in which collagen is not properly formed, causing poor wound healing, bleeding of the gums, severe pain, and death. In 1753, Lind published his Treatise on the Scurvy, which recommended using lemons and limes to avoid scurvy, which was adopted by the British Royal Navy. This led to the nickname Limey for sailors of that organization. Lind's discovery, however, was not widely accepted by individuals in the Royal Navy's Arctic expeditions in the 19th century, where it was widely believed that scurvy could be prevented by practicing good hygiene, regular exercise, and by maintaining the morale of the crew while on board, rather than by a diet of fresh food. As a result, Arctic expeditions continued to be plagued by scurvy and other deficiency diseases. In the early 20th century, when Robert Falcon Scott made his two expeditions to the Antarctic, the prevailing medical theory was that scurvy was caused by "tainted" canned food.

The discovery of vitamins and their structure:

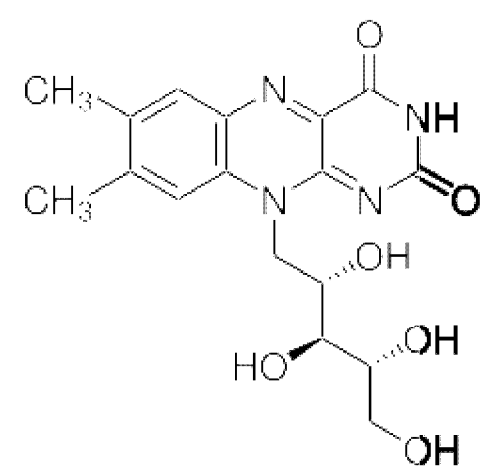
Year of discovery	Vitamin	Source
1909	Vitamin A (Retinol)	Cod liver oil
1912	Vitamin B_1 (Thiamin)	Rice bran
1912	Vitamin C (Ascorbic acid)	Lemons
1918	Vitamin D (alciferol)	Cod liver oil
1920	Vitamin B_2 (Riboflavin)	Eggs
1922	Vitamin E (Tocopherol)	Wheat germ oil
1926	Vitamin B_{12} (Cobalamin)	Liver
1929	Vitamin K (Phyllochinone)	Luzerne
1931	Vitamin B_5 (Pantothenic acid)	Liver
1931	Vitamin B_7 (Biotin)	Liver
1934	Vitamin B_6 (Pyridoxine)	Rice bran
1936	Vitamin B_3 (Niacin)	Liver
1941	Vitamin B_9 (Folic acid)	Liver

In 1881, Russian surgeon Nikolai Lunin studied the effects of scurvy while at the University of Tartu in present-day Estonia. He fed mice an artificial mixture of all the separate constituents of milk known at that time, namely the proteins, fats, carbohydrates, and salts. The mice that received only the individual constituents died, while the mice fed by milk itself developed normally. He made a conclusion that "a natural food such as milk must therefore contain, besides these known principal ingredients, small quantities of unknown substances essential to life." However, his conclusions were rejected by other researchers when they were unable to reproduce his results. One difference was that he had used table sugar (sucrose), while other researchers had used milk sugar (lactose) that still contained small amounts of vitamin B.

In the Orient where polished white rice was the common staple food of the middle class, beriberi resulting from lack of vitamin B was endemic. In 1884, Takaki Kanehiro, a British trained medical doctor of the Japanese Navy observed that beriberi was endemic among low ranking crew who often ate nothing but rice but not among crews of Western navies and officers who were entitled to a Western-style diet. Kanehiro initially believed that lack of protein was the chief cause of beriberi. With the support of Japanese navy, he experimented using crews of two battleships, one crew was fed only white rice, while the other was fed a diet of meat, fish, barley, rice, and beans. The group that ate only white rice documented 161 crew with beriberi and 25 deaths, while the latter group had only 14 cases of beriberi and no deaths. This convinced Kanehiro and the Japanese Navy that diet was the cause of beriberi. This was confirmed in 1897, when Christiaan Eijkman discovered that feeding unpolished rice instead of

the polished variety to chickens helped to prevent beriberi in the chickens. The following year, Frederick Hopkins postulated that some foods contained "accessory factors"—in addition to proteins, carbohydrates, fats, et cetera—that were necessary for the functions of the human body. Hopkins was awarded the 1929 Nobel Prize for Physiology or Medicine with Christiaan Eijkman for their discovery of several vitamins.

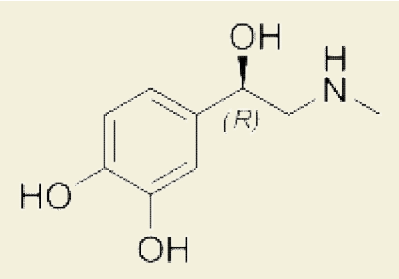
In 1910, Japanese scientist Umetaro Suzuki succeeded in extracting a water-soluble complex of micronutrients from rice bran and named it aberic acid. He published this discovery in a Japanese scientific journal. When the article was translated into German, the translation failed to state that it was a newly discovered nutrient, a claim made in the original Japanese article, and hence his discovery failed to gain publicity. Polish biochemist Kazimierz Funk isolated the same complex of micronutrients and proposed the complex be named "Vitamine" (a portmanteau of "vital amine") in 1912. The name soon became synonymous with Hopkins' "accessory factors", and by the time it was shown that not all vitamins were amines, the word was already ubiquitous. In 1920, Jack Cecil Drummond proposed that the final "e" be dropped to deemphasize the "amine" reference after the discovery that vitamin C had no amine component.



Riboflavin (vitamin B2)

Throughout the early 1900s, the use of deprivation studies allowed scientists to isolate and identify a number of vitamins. Initially, lipid from fish oil was used to cure rickets in rats, and the fat-soluble nutrient was called "antirachitic A". The irony here is that the first "vitamin" bioactivity ever isolated, which cured rickets, was initially called "vitamin A", the bioactivity of which is now called vitamin D. What we now call "vitamin A" was identified in fish oil because it was inactivated by ultraviolet light. In 1931, Albert Szent-Györgyi and a fellow researcher Joseph Svirbely determined that "hexuronic acid" was actually vitamin C and noted its anti-scorbutic activity. In 1937, Szent-Györgyi was awarded the Nobel Prize for his discovery.

12.Hormones



Epinephrine (adrenaline), a catecholamine-type hormone

A hormone (from Greek ὁρμή - "impetus") is a chemical messenger that carries a signal from one cell to another. All multicellular organisms produce hormones; plant hormones are also called phytohormones. Hormones in animals are often transported in the blood. Cells respond to a hormone when they express a specific receptor for that hormone. The hormone binds to the receptor protein, resulting in the activation of a signal transduction mechanism that ultimately leads to cell type-specific responses.Endocrine hormone molecules are secreted (released) directly into the bloodstream, while exocrine hormones (or ectohormones) are secreted directly into a duct, and from the duct they either flow into the bloodstream or they flow from cell to cell by diffusion in a process known as paracrine signalling.

Hierarchical nature of hormonal control:

Hormonal regulation of some physiological activities involves a hierarchy of cell types acting on each other either to stimulate or to modulate the release and action of a particular hormone. The secretion of hormones from successive levels of endocrine cells is stimulated by chemical signals originating from cells higher up the hierarchical system. The master coordinator of hormonal activity in mammals is the hypothalamus, which acts on input that it receives from the central nervous system.

Other hormone secretion occurs in response to local conditions, such as the rate of secretion of parathyroid hormone by the parathyroid cells in response to fluctuations of ionized calcium levels in extracellular fluid.

Hormone signaling :

Hormonal signalling across this hierarchy involves the following:

Biosynthesis of a particular hormone in a particular tissue.Storage and secretion of the hormoneTransport of the hormone to the target cell(s)

Recognition of the hormone by an associated cell membrane or intracellular receptor protein.

Relay and amplification of the received hormonal signal via a signal transduction process: This then leads to a cellular response. The reaction of the target cells may then be recognized by the original hormone-producing cells, leading to a down-regulation in hormone production. This is an example of a homeostatic negative feedback loop.

Degradation of the hormone.

As can be inferred from the hierarchical diagram, hormone biosynthetic cells are typically of a specialized cell type, residing within a particular endocrine gland (e.g., the thyroid gland, the ovaries, or the testes). Hormones may exit their cell of origin via exocytosis or another means of membrane transport. However, the hierarchical model is an oversimplification of the hormonal signaling process. Cellular recipients of a particular hormonal signal may be one of several cell types that reside within a number of different tissues, as is the case for insulin, which triggers a diverse range of systemic physiological effects. Different tissue types may also respond differently to the same hormonal signal. Because of this, hormonal signaling is elaborate and hard to dissect.

Interactions with receptors:

Most hormones initiate a cellular response by initially combining with either a specific intracellular or cell membrane associated receptor protein. A cell may have several different receptors that recognize the same hormone and activate different signal transduction pathways, or alternatively different hormones and their receptors may invoke the same biochemical pathway.

For many hormones, including most protein hormones, the receptor is membrane associated and embedded in the plasma membrane at the surface of the cell. The interaction of hormone and receptor typically triggers a cascade of secondary effects within the cytoplasm of the cell, often involving phosphorylation or dephosphorylation of various other cytoplasmic proteins, changes in ion channel permeability, or increased concentrations of intracellular molecules that may act as secondary messengers (e.g. cyclic AMP). Some protein hormones also interact with intracellular receptors located in the cytoplasm or nucleus by an intracrine mechanism.

For hormones such as steroid or thyroid hormones, their receptors are located intracellularly within the cytoplasm of their target cell. In order to bind their receptors these hormones must cross the cell membrane. The combined hormone-receptor complex then moves across the nuclear membrane into the nucleus of the cell, where it binds to specific DNA sequences, effectively amplifying or suppressing the action of certain genes, and affecting protein

synthesis. However, it has been shown that not all steroid receptors are located intracellularly, some are plasma membrane associated.

An important consideration, dictating the level at which cellular signal transduction pathways are activated in response to a hormonal signal is the effective concentration of hormone-receptor complexes that are formed. Hormone-receptor complex concentrations are effectively determined by three factors:

The number of hormone molecules available for complex formation

The number of receptor molecules available for complex formation and

The binding affinity between hormone and receptor.

The number of hormone molecules available for complex formation is usually the key factor in determining the level at which signal transduction pathways are activated. The number of hormone molecules available being determined by the concentration of circulating hormone, which is in turn influenced by the level and rate at which they are secreted by biosynthetic cells. The number of receptors at the cell surface of the receiving cell can also be varied as can the affinity between the hormone and its receptor.

IV.PHARMACEUTICS

1.New Drug Delivary system

What are Drug Delivery Systems?

The term "drug delivery systems" refers to both the construct of the drug – its delivery vehicle – and its method of administration.

New Drug Delivery Vehicles:

Some of the major vehicles being researched for delivering drugs are:

Organic and synthetic polymers, and other chemical constructs that can release drugs at a sustained rate, or release them only in certain environments; and, Liposomes.

New Methods of Administration :

Medicated skin patches,Implanted devices that can release drugs with an external remote control; and,Powder forms of traditional drugs which can be inhaled and absorbed through the lungs.The main focus of this document will be on the new drug delivery vehicles.

The Science – How do Drug Delivery Systems Work?

Liposomes:

Liposomes encapsulate active drugs to improve their delivery. With liposomes, the active drug is carried within its layers or in the hallow space in the middle of the liposome. The immune system, which seeks out foreign material for destruction, can be a major obstacle to liposomes. In 1992, researchers discovered that coating liposomes with inactive polymers, such as polyethylene glycol (PEG), drastically increased the liposome's ability to evade recognition by the immune system.

The main mechanism of a liposome is simply fusing to the cell membrane or through endocytosis.

Polymers:

Polymers are made in different shapes and sizes. They can come in a rod-like shape, with the drug attached to it, or be circular with the drug inside the polymer construct. They can also be biodegradable or non-biodegradable. Biodegradable polymers like PLA and PLGA are commonly used since they do not have to be surgically removed from the body after the drug is released.Polymers can deliver drugs through dissolution, diffusion, or osmosis.In dissolution, the drug is released over time as the polymer dissolves in the gastrointestinal tract. Mixing and layering polymers with varying dissolutions rates controls the rate of

release.In diffusion, the release of the drug is controlled by its rate of diffusion out of the polymer.

In osmosis, the drug is contained in a polymer consisting of two compartments: one compartment contains the drug and the other contains a biologically inactive agent that can push out the drug under certain conditions – a push layer. When an individual takes the drug in pill form, water penetrates the pill through the membrane of the polymer. This step activates the push layer, which then drives the active ingredient into the gastrointestinal tract through one or more tiny holes on the other side of the pill.Upon swallowing, the biologically inactive parts of the pill remain intact during its voyage through the gastrointestinal tract and are eliminated in the feces as an insoluble shell.

Biotechnology Drug Delivery Systems:

Biotechnology is improving the mechanism of two drug delivery vehicles called liposomes and polymers.

Liposomes:

Liposomes are fatty droplets made artificially in the lab by the addition of a water solution to a phospholipid gel. Liposomes encapsulate active drugs to improve their delivery. Depending on the construction of the liposome, the active drug can be carried within its layers or in the hallow space created by the encapsulation. Liposomes mimic the natural phospholipid cell membranes in the human body.Liposomes are currently being used for intravenous delivery of small molecules and are being investigated for oral, transdermal (through skin), and sustained-release delivery of drugs. Liposomes are often attached to other molecules, like polyethylene glycol (PEG), that will prevent or lessen detection by the immune system.

Polymers:

Polymers are formed by the linkage of a large number of smaller molecules, called monomers. Natural polymers include proteins, DNA, and latexes, such as rubber. Synthetic polymers include glass, concrete and plastics. The polymers used to deliver drugs are usually lactic acid based, such as polylactic acid (PLA) and polylactic-co-glycolic acid (PLGA).Lactic acid is a compound that is naturally produced when glucose is metabolized in the body. PLA and PLGA have been used as bioabsorbable sutures for more than 20 years, so their safety is well

established.Polymers can be designed to either release a drug at a steady rate for a long time, or it can release the drug after a prolonged interval. Polymers can also be attached to antibodies to deliver drugs to a specific target.

The Role of Biotechnology:

Biotechnology is contributing to advances in drug delivery through gene and protein discovery, and the resulting knowledge of human biological systems. This allows researchers to create synthetic systems that mimic the already existing biological processes in the body. Also, as researchers learn more about certain diseases, they can discover drug delivery targets that are more specific to the particular disease.

The current development of delivery systems as well as methods of administration are the result of chemical, technical, and biological advances and the subsequent understanding of the body.

Drug Delivery Systems in Development:

The next step in drug delivery will be the creation of vehicles or constructs that can target the disease tissue more accurately. There are several developments currently underway:

Smart Drugs – Also known as "prodrugs," these compounds are designed to work only when activated by certain components in the body. For example, a smart drug designed to be activated by a certain enzyme will be activated only in tissues that produces that specific enzyme.

Monoclonal Antibodies – These are antibodies made in the lab that can target antigens with extreme specificity. They are attached to a drug in order to guide it to a specific cell. For example, cancer drugs can be attached to monoclonal antibodies made against tumour cells, which helps the drug target only tumour cells. This reduces the toxic effects of cancer drugs.

2.BioPharmaceutics And Pharmacokinetics

Introduction:

A young child given an im injection might ask "How will that 'ouch' get from there to my sore throat"? The answer to this question is the basis of pharmacokinetics.

That is, how drugs move around the body and how quickly this movement occurs. During this semester many of the processes which control the absorption, distribution, metabolism, and excretion of drugs will be discussed.

1.Routes Of Drug Administration:

An alternate method of classifying these routes of administration is ENTERAL and PARENTERAL. Enteral means to do with the GI tract and includes oral, buccal, and rectal. Parenteral means not through the alimentary canal and commonly refers to injections such as IV, IM, and SC; but could also include topical and inhalation. We can also distinguish IV from the rest, as with all others at least one membrane must be crossed, thus an absorption process is involved in the administration and the pharmacokinetics.

2.Metabolism

Objectives:

To describe the various processes by which a drug may be metabolized in the body.To understand the relationship between the parameters hepatic clearance, hepatic blood flow, fraction unbound, and free intrinsic clearance

The body has another way of deactivating drugs in the body. This method of elimination is metabolism or biotransformation. Metabolic processes, in general, have the overall effect of converting drug molecules into more polar compounds. Again, in general, the effect of this should be to decrease tubular re-absorption in the kidney and thus increase drug elimination. Generally, it also means an immediate loss of pharmacological activity because transport into the site of action is hindered (less lipid soluble) or the molecule no longer fits into the receptor site. There are exceptions however, and a number of `new' drugs have been discovered as active metabolites.

Metabolism takes place by enzymatic catalysis. Most metabolism occurs in the liver although other sites have been described, such as intestinal wall, kidney, skin, blood.

3.Drug distribution

Objectives: To understand and describe the processes by which drugs are distributed throughout the body

To understand the effect of protein binding on drug distribution and methods by which protein binding is measured

Drug distribution means the reversible transfer of drug from one location to another within the body. Once a drug has entered the vascular system it becomes distributed throughout the various tissues and body fluids in a pattern that reflects the physiochemical nature of the drug and the ease with which it penetrates different membranes. The ONE COMPARTMENT model assumes rapid distribution but it does not preclude extensive distribution into various tissues.

4.Routes Of Excretion

Objectives:

To describe the various routes by which a drug may be excreted from the body

To understand the parameter renal clearance and its relationship with excretion processes in the kidney

To understand the influence of renal disease on drug elimination

To calculate suitable drug dosage regimen for patients with impaired renal function

The major routes included will be renal, biliary, pulmonary, and salivary.

5.Clinical application of Pharmacokinetics

Objectives:

To understand the basics of a Therapeutic Drug Monitoring service

To understand the principles of the analytical methods, HPLC, GLC, RIA, and EMIT

To describe and understand how changes in physiology effect the pharmacokinetics of drugs in the very young and the elderly

We can start this topic by talking about the Clinical Pharmacy service, Therapeutic Drug Monitoring. This involves the measurement and interpretation of plasma/serum/blood concentrations in patients.

Therapeutic Drug Monitoring:

Therapeutic drug monitoring (TDM) becomes important when:

a) the drug has a narrow therapeutic-toxic range,

b) there is a large variability in pharmacokinetic parameter values between patients,

c) the therapeutic effect is not readily assessed (e.g. antibiotics) or clinical symptoms are to be avoided (e.g. seizure). Not as useful for blood pressure lowering (can measure B.P. directly) or anticoagulants (again measure clotting time directly),

d) there is a direct relationship between Cp or concentration in other biological sample (e.g. saliva) and pharmacological effect,

e) an appropriate (accurate, short turn around, inexpensive) analytical method is available for the drug,

f) the expected or desired therapeutic effect is not observed (may be absorption or compliance problem),

g) a drug with high first pass effect is involved, or

h) a patients has altered and/or variable renal state and the drug is eliminated mostly as unchanged drug in urine (fe less than 1)

Typical drugs:

Table XXI-1 Therapeutic Concentration Ranges

Drug	Therapeutic Concentration Range
Aminoglycoside (gentamicin, tobramycin)	0.5 <-> 8 mg/L
Digoxin	0.5 <-> 8 2.0 ug/L
Phenytoin	10 <-> 8 20 mg/L
Theophylline	10 <-> 8 20 mg/L

Procedure:

Pharmacist and physician develop initial dosing recommendations

Information required:

Patient - Age, weight, sex, height, smoker

Clinical - Drug requirements, clinical status (renal - serum creatinine; cardiac - cardiac output, liver, etc.)

Calculate initial loading dose or maintenance regimen and make recommendations

Organize sample collection and analysis

Accurate Timing in necessary

Evaluate pharmacokinetically the analytical result and recalculate dosing regimen recommendations

Organize further samples if necessary, repeat as necessary

Calculations:

Computer or calculator programs can be used to help the bedside development of dosing regimen. Other more sophisticated programs are available to calculate values for the drug pharmacokinetics and make further recommendations.

6.Multiple Over Dose Administration

Objectives:

To recognize and use the integrated equations used to describe plasma concentrations versus time after multiple oral doses

To calculate appropriate multiple dose drug regimen

To define, use, and calculate the parameter

So far we have looked at multiple IV bolus administration. In an analogous fashion, equations can be developed which enable you to calculate the plasma concentration achieved following multiple oral administration. To start the plasma concentration achieved following a single oral dose can be given by:-

$$Cp = \frac{F \cdot DOSE \cdot ka}{V \cdot (ka - kel)} \cdot \left[e^{-kel \cdot t} - e^{-ka \cdot t} \right]$$

Equation XV-1 Cp after a Single Oral Dose:

This can be converted to an equation describing plasma concentration at any time following n equal doses with constant dosing interval t.

$$Cp = \frac{F \cdot Dose \cdot ka}{V \cdot (ka - kel)} \cdot \left[\frac{1 - e^{-n \cdot kel \cdot \tau}}{1 - e^{-kel \cdot \tau}} \cdot e^{-kel \cdot t} - \frac{1 - e^{-n \cdot ka \cdot \tau}}{1 - e^{-ka \cdot \tau}} \cdot e^{-ka \cdot t} \right]$$

Equation XV-2, General Equation

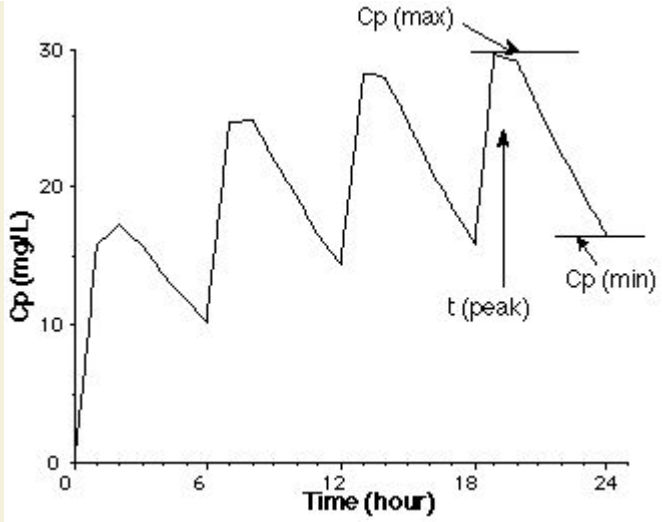


Figure XV-1, Plot of Cp Versus Time for Multiple Oral Doses showing Cpmax and Cpmin

Using a JAVA aware browser you can create your own version of Figure XV-1.

Plasma Concentration versus Time Plots

The plasma concentration versus time curve described by this equation is similar to the IV curve in that there is accumulation of the drug in the body to some plateau level and the plasma concentrations fluctuate between a minimum and a maximum value.

The Cpmax value can be calculated at the time t = tpeak after many doses (n ->) but it is complicated by the need to determine the value for tpeak.

3.Formulation and Preparation Of Cosmetics

1.Lipsticks

1.Before starting

2.Typical Ingredients / Usual Method & Tips

3.Sample Recipe

4.To go further

1.Before starting...

A little bit of history...

We're tracking the origin of lip coloration within the high antiquity. Egyptian women used to apply on their lips some dyes based on red blood stone or henna. However the origin of the lipstick such as we know it today begins within the end of the XIXth century. At this time, actresses used to use mixtures of beeswax, butter, vine grape extracts or other kinds of natural dyes to be applied on their lips. Synthetic dyes discovery at the beginning of the XXth century allowed for a considerable pallet of coloring nuances as well as the emancipation of the lipstick.

Required qualities :

Consistency must be stable whatever the variations in temperature

Must be applied easily on lips

Must leave a mark as soon as lightly applied

Musn't break during the application

Typical Ingredients		Usual Method
Must Use Consistency factors/ Waxes	20-55%	Waxes and emollients are mixed and melted until 80°C. Pigments may often have to be premixed separately in liquid emollients mixture. Premixed pigments dispersions should preferably be used, otherwise pigment dispersion must be as homogeneous as possible. The mixture can be added to the first hot phase or once the mix has cooled. Then pour the warm mixture (temperature must be 10°C above its melting point) into the appropriate moulds. Moulds must be placed into the refrigerator.
Emollients (liquid)	25-70%	
Pigments (color)	0.5-10%	
Usual Thickeners/ stabilizers	0-1%	Tips

Preservatives	0-1 %
Antioxidants	0.05-1%
Pigments (special effects)	5-25%
Optional Sunscreens/ UV filters	0-2%
Fragrances/ Flavoring agents	0-1%
Active agents	0-1%

How to obtain the wanted mattifying/ shining effect in your lipstick?

Mixing the correct amount of pigments every time and getting the desired color and effect is an art of its own. Pigments particles size and nature is varied in order to obtain more shine or more matt effect. The more the distribution is large, the more the mattifying effect is high. Mattifying effect is associated with adhesion (long-lasting). Cosmeticians would like to obtain lipsticks which join the brilliance with the long-lasting effect to satisfy consumers. These two properties are contradictory.

To go further

Consistency agents/ waxes	Lipsticks are made of a mixture of waxes ("white paste or body"), oils and pigments. Waxes are chosen considering their melting point (which varies from 50°C to 90°C) and their specific properties. They bring hardness and shining. They enhance stability and consistency, and provide richness to formulations. Most animal origin waxes were used in the past. They're now widely replaced by synthetic or vegetal origin waxes (carnauba, candelilla..).
Pigments are non-soluble components	Thanks to their non-solubility neither in water nor in oil pigments are permitted in decorative cosmetics. Moreover they are considered to be non-irritant to mucous membranes. TiO2 is commonly used as an opacifier. Pigments derived from mica are used and often coated with titanium dioxide to enhance the range of colors available
What kind of lipstick was called the kiss of death?	The ancient Egyptians used for lip rouge a reddish purple mercuric plant dye. They didn't know it was potentially poisonous . Talk about the kiss of death!

2.Shampoo

1.Before starting

2.Typical Ingredients / Usual Method & Tips

3.Sample Recipe

4.To go further

1.Before starting...

A shampoo is a colloidal dispersion of various surfactants in water. Base components of a classical shampoo are cleansing agents, thickeners and water. Most of the time additives and adjuvants are added to the formula in order to help cleansing, enhance aesthetics properties, foaming or make hair shine. Indeed, beauty brought by the product is as important as cleanliness: As long as it is an hygiene product, a shampoo has to maintain a pleasant feel of the hair. Moreover, in the mind of many consumers cleanliness is often associated with foam quantity...So...Shampoos have to foam!

2.Required qualities:

Cleansing properties without eliminating all surface lipids

Foaming properties

Make hair shine and soft

Make hair easy to comb

Antistatic

Pleasant odor

Non-irritant, non-stinging

Typical Ingredients		Usual Method
Must Use Surfactants	15-30%	Disperse hydrophilic thickener(s) in water under intensive stirring, until a homogeneous gel is formed. Then add the surfactants and the other ingredients and mix gently until the product becomes homogeneous. Oil-soluble ingredients (some preservatives, perfume..) should beadded by pre-mixing them with a small amount of surfactants.
Foam Stabilizers	1-4 %	
Thickener	0-5%	
Deionized Water	Q.S.	
Usual Preservatives	0,1-1 %	
Humectants	1-5 %	
PH Buffers	Q.S. neutral pH	

Tips

How to significantly reduce the overall irritation of your mixture?

By increasing the betaïne surfactants %. When these ones are combined with fatty alcohol sulfates, they form large anionic-cationic complex which lowers irritation.

Chelating Agents	0-0,02 %	Part of anionic surfactants can also be replaced by non-ionic surfactants like alkylpolyglucosides.
Optional Pearlescing/ opacifying agents	0,2-2%	
Perfume	0,5-1 %	
Active agents	0-2%	
Colouring agents	Q.S.	
Conditioning agents	Q.S. desired effect	
UV Filter	0.01-0.1%	

Specific substances may be incorporated in order to provide a restoring and protecting effect on hair like natural and modified lipids, amino acids and silicones. They may also have reconstituting effect on the integrity and health of the hair and scalp - such as preventing dandruff and excessive sebaceous secretion.

1.Baby Shampoos: They should be based on amphoteric surfactants (imidazolinic derivatives or betaïnes, 75 to 100% of total surfactants)

2.Greasy Hair Shampoo

Its first mission is to eliminate excessive sebum without eliminating all surface lipids. Following actives may be used:

- sulfur in order to regulate sebum secretion

- clays to absorb fats

3.Dry Hair Shampoo

A dry scalp leads to a dry aspect of the hair because of lack of sebum. Cleansing base is composed with a ternary mixture of surfactants (anionic, amphoteric and non-ionic). Formulation is completed by superfatting agents: castor oil, lecithins..

Antidandruff Shampoos Formulae contain antimicrobial and keratolytic actives (zinc pyrithione, piroctone olamine)

2-in-1 Shampoos Some conditioning agents (polyquaterniums, silicones..) are added in order to help combability

3.Creams

1.Before starting

2.Typical Ingredients / Usual Method & Tips

3.Sample Recipe

4.To go further

1.Before starting...

Facial care creams formulations include many kinds of formulations, depending on the nature of the skin or on the desired effects. Either protection against pollution and oxidation is needed or anti-wrinkle activity. Actives may also be added in order to correct an oily/ greasy skin, a dry or a sensitive skin.. Many formulations are basically Oil in Water emulsions or Water in Oil emulsions. The typical skin care emulsion is now more likely to be o/w than w/o. Technology has advanced to the point where w/o stable emulsions can be prepared at room temperature.

2.Required qualities :

Neutral or pleasant odor and color

Easy to spread, pleasant feeling during application

Easy penetration

Non-oily/ non-greasy after application

Non comedogenic

Well tolerance/ non-allergenic

Bring hydration

Typical Ingredients	
Must Use Emulsifiers	2-6%
Emollients	10-35 %
Thickener	0.1-1%
Deionized Water	Q.S.
Usual Preservatives	0.2-1 %
Humectants	1-8 %
Consistency factors	1-6%
Antioxidants	0.01-0.05%
UV filters	0.01-0.5%
Optional Chelating Agents	0-0.02 %
Fragrance	0.1-1 %
Active agents	0.1-2%
Colouring agents	Q.S.
Aesthetic enhancers	0.1-5%

Usual Method

Aqueous phase: Thickener is dispersed in cold or warm water at 75-80°C (depending on the recommandations of the producer) under intensive stirring, until a homogeneous gel is formed. This first phase is combined with the oily phase (Lipophilic components : emulsifiers + emollients + consistency factors) that has also been melted and heated to the same temperature. Mix under intensive stirring until emulsion is formed. Then mix gently while emulsion is being cooled. Sensitivecomponents like actives, special additives and preservatives are added after the mixture has been cooled (40-30°C) to keep their properties intact.

Tips

How to vary the consistency of your cream?

- Changing the % of the oily phase allows variations in the final viscosity of your preparation. For Oil in Water emulsion the greater the oily phase the higher the viscosity. Inversely for Water in Oil or Water in Silicone emulsion : the greater the aqueous phase the higher the viscosity.

- Vary the % of thickeners (gelling agents or consistency agents like waxes). However some stabilizers can prevent settling without increasing the viscosity.

4.Pharmaceutical Calculations

Accurately performing pharmaceutical calculations is a critical component in providing patient care in every pharmacy practice environment. Consequently, pharmaceutical calculations are a vital part of any pharmacy curriculum. Although most pharmaceutical calculations are not 'difficult,' it is a topic that deserves attention because it requires flawless accuracy. Before students are able to become optimally proficient at performing pharmaceutical calculations and using them to contribute to optimal patient care, they must understand approaches to pharmaceutical calculations that help minimize error and maximize accuracy. The objectives of this introduction to pharmaceutical calculations are:

1. Openly address common student perceptions so that these perceptions do not hinder students' focus on pharmaceutical calculations.

2. Review the two main approaches to pharmaceutical calculations, proportion and dimensional analysis, and describe advantages and shortcomings of each.

3. Provide recommendations for improving efficiency and accuracy while avoiding errors and misinterpretations when completing pharmaceutical calculations.

4. Describe methods for double-checking calculations.

Frequent Perceptions:

Like any class, students come to a pharmaceutical calculations class with perceptions and expectations. Some of these are accurate and some require refinement or explanation. The following issues often come up when students first come to the calculations course."I already know how to do this - it is just simple math."-Although some pharmaceutical calculations require basic science knowledge, such as interpreting a chemical formula to determine the number of equivalents per mole or knowing that 1ml of water weighs 1g, a large part of performing pharmaceutical calculations requires math skills learned prior to high school. Students quickly recognize this fact and some may perceive that pharmaceutical calculations should be easy. The fact of the matter is that pharmaceutical calculations are NOT easy. No student in 8th grade was expected to be correct 100% of the time. The major difficulty in pharmaceutical calculations is not the math, it is the fact that the margin for error is non-existent.

Some of the content of the remaining discussion will provide mathematical reviews that will be quite basic to some students. This level of detail is purposely provided so that all students fully understand these basic mathematical concepts, as their future patients' doses, dosage forms, and lives will depend on it.

"I can do this in my head."-As students and later as practitioners, there are occasions when calculations are done without a paper and pen. Most students and pharmacists do not need a paper and pen to determine that if 1 tsp contains 250 mg of amoxicillin, one half tsp will contain 125 mg. Students need to show

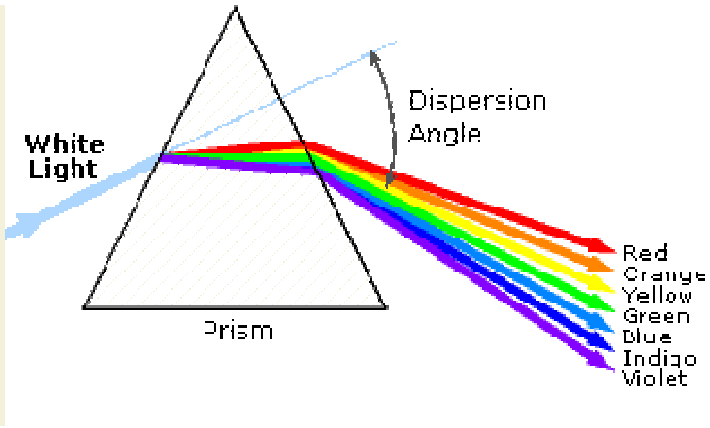
their work for even the simplest calculations, however. The obvious reason for this (grading) is not the most important. Showing their work allows a student to visualize the problem and slow down their thought process, making it less likely for errors to occur. It also develops good habits for practice, when documentation allows other practitioners to double-check work and easily see the method of calculation used.

"It is boring - let's get to the exciting stuff."-Pharmaceutical calculations are not glamorous. In fact, one could argue that they are somewhat mundane. This can be particularly true early in a lecture series on pharmaceutical calculations, when the topic focuses on understanding the basic concepts of proportions and dimensional analysis (before diving into the slightly more glamorous calculations themselves). The challenge lies in the fact that many students (and instructors alike) would rather spend time discussing new and innovative therapies for a condition than basic concepts in pharmaceutical calculations, and consequently, it is tempting to rush through introductory material. Calculations can be occasionally mundane, particularly the repetition necessary to develop confidence as well as accuracy. Nevertheless, deliberate and undivided attention to detail is required to clearly understand the basic concepts that will serve as building blocks during later coursework. There can be no misunderstanding of the basic concepts among students.

V. PHARMACEUTICAL ANALYSIS

1.Absorption Spectroscopy

Visible and Ultraviolet Spectroscopy:

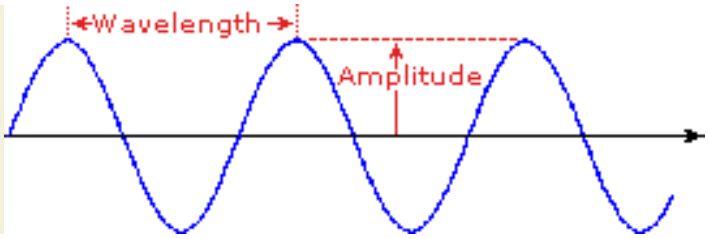


1. Background

An obvious difference between certain compounds is their color. Thus, quinone is yellow; chlorophyll is green; the 2,4-dinitrophenylhydrazone derivatives of aldehydes and ketones range in color from bright yellow to deep red, depending on double bond conjugation; and aspirin is colorless. In this respect the human eye is functioning as a spectrometer analyzing the light reflected from the surface of a solid or passing through a liquid. Although we see sunlight (or white light) as uniform or homogeneous in color, it is actually composed of a broad range of radiation wavelengths in the ultraviolet (UV), visible and infrared (IR) portions of the spectrum. As shown on the right, the component colors of the visible portion can be separated by passing sunlight through a prism, which acts to bend the light in differing degrees according to wavelength. Electromagnetic radiation such as visible light is commonly treated as a wave phenomenon, characterized by a wavelength or frequency.

Wavelength is defined on the left below, as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10-9 meters). Frequency is the number of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or hertz (Hz). Visible wavelengths cover a range from approximately 400 to 800 nm. The longest visible wavelength is red and the shortest is violet. Other common colors of the spectrum, in order of decreasing wavelength, may be remembered by the mnemonic: ROY G BIV. The wavelengths of what we perceive as particular colors in the visible portion of the spectrum are displayed and listed below. In horizontal

diagrams, such as the one on the bottom left, wavelength will increase on moving from left to right.



Violet: 400 - 420 nm

Indigo: 420 - 440 nm

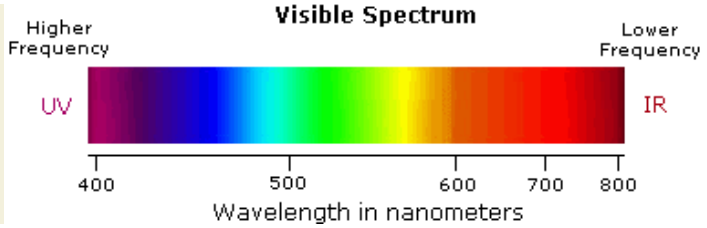
Blue: 440 - 490 nm

Green: 490 - 570 nm

Yellow: 570 - 585 nm

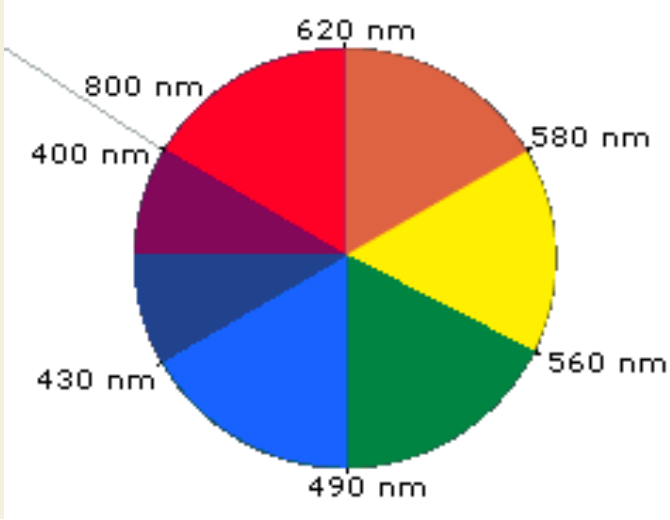
Orange: 585 - 620 nm

Red: 620 - 780 nm



When white light passes through or is reflected by a colored substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary color to the wavelength(s) absorbed. This relationship is demonstrated by the color wheel shown on the right. Here, complementary colors are diametrically opposite each other. Thus, absorption of 420-430 nm light renders a substance yellow, and absorption of 500-520 nm light makes it red. Green is unique in that it can be created by absoption close to 400 nm as well as absorption near 800 nm.

Early humans valued colored pigments, and used them for decorative purposes. Many of these were inorganic minerals, but several important organic dyes were also known. These included the crimson pigment, kermesic acid, the blue dye, indigo, and the yellow saffron pigment, crocetin. A rare dibromo-indigo derivative, punicin, was used to color the robes of the royal and wealthy. The deep orange hydrocarbon carotene is widely distributed in plants, but is not sufficiently stable to be used as permanent pigment, other than for food coloring. A common feature of all these colored compounds, displayed below, is a system of extensively conjugated pi-electrons.



2.Fluorimetry

Quantitative Fluorimetry

Introduction:

Light emission from atoms or molecules can be used to quantitate the amount of the emitting substance in a sample. The relationship between fluorescence intensity and analyte concentration is:

F = k * QE * Po * (1-10[-epsilon*b*c])

where F is the measured fluorescence intensity, k is a geometric instrumental factor, QE is the quantum efficiency (photons emitted/photons absorbed), Po is

the radiant power of the excitation source, epsilon is the wavelength-dependent molar absorptivity coefficient, b is the path length, and c is the analyte concentration (epsilon, b, and c are the same as used in the Beer-Lambert law).

Expanding the above equation in a series and dropping higher terms gives:

$F = k * QE * Po * (2.303 * epsilon * b * c)$

This relationship is valid at low concentrations (<10-5 M) and shows that fluorescence intensity is linearly proportional to analyte concentration.

Determining unknown concentrations from the amount of fluorescence that a sample emits requires calibration of a fluorimeter with a standard (to determine K and QE) or by using a working curve.

Limitations:

Many of the limitations of the Beer-Lambert law also affect quantitative fluorimetry. Fluorescence measurements are also susceptible to inner-filter effects. These effects include excessive absorption of the excitation radiation (pre-filter effect) and self-absorption of atomic resonance fluorescence (post-filter effect).

3.Flame Photometry

Flame photometry is an atomic emission method for the routine detection of metal salts, principally Na, K, Li, Ca, and Ba. Quantitative analysis of these species is performed by measuring the flame emission of solutions containing the metal salts. Solutions are aspirated into the flame. The hot flame evaporates the solvent, atomizes the metal, and excites a valence electron to an upper state. Light is emitted at characteristic wavelengths for each metal as the electron returns to the ground state. Optical filters are used to select the emission wavelength monitored for the analyte species.

Flame photometry is a simple, relatively inexpensive, high sample throughput method used for clinical, biological, and environmental analysis. The low temperature of the natural gas and air flame, compared to other excitation

methods such as arcs, sparks, and rare gas plasmas, limit the method to easily ionized metals. Since the temperature isn't high enough to excite transition metals, the method is selective toward detection of alkali and alkali earth metals. On the other hand, the low temperatures renders this method susceptible to certain disadvantages, most of them related to interference and the stability (or lack thereof) of the flame and aspiration conditions. Fuel and oxidant flow rates and purity, aspiration rates, solution viscosity, concomitants in the samples, etc affect these. It is therefore very important to measure the emission of the standard and unknown solutions under conditions that are as nearly identical as possible.

This experiment will serve as an introduction to sodium analysis by flame emission photometry and will demonstrate the effects of cleanliness and solution viscosity on the observed emission intensity readings. The instrument is calibrated with a series of standard solutions that cover the range of concentrations expected of the samples. Standard calibrations are commonly used in instrumental analysis. They are useful when sample concentrations may vary by several orders of magnitude and when the value of the analyte must be known with a high degree of accuracy. This experiment does not produce hazardous waste.

Procedure :

Consult your Teaching Assistant for operating instructions for the Buck PFP-7 Flame Photometer. Allow a sufficient warm-up period. Be sure to aspirate deionized-distilled water between samples to clean out the sample tube and aspirator. Sodium is ubiquitous. It is imperative that you use scrupulously cleaned glassware to obtain good results.

Standard Preparations:

Prepare sodium chloride standard solutions by volumetric dilution of the stock solution. The following approximate concentrations should be made: 5, 10, 25, 50, 75, and 100 mg/mL as Na. Be sure to use clean methods. Use ultra-pure deionized-distilled water to clean your glassware and for dilution of the 1000 mg/mL standard. Prepare these standards in scrupulously clean volumetric glassware and transfer the solutions to plastic bottles. Glass often is made from high sodium glass. Allowing extremely high or low pH solutions to stand in glass could alter the sodium concentrations in solution. Prepare 25 mg/mL Na solutions in other solvents, 10% Ethanol, 50% Ethanol, 50% Glycerin. Standard solutions may be pre-prepared by the laboratory instructor or may be made up as a class or group project.

Unknown Preparation:

Obtain a sodium unknown from your instructor in a scrupulously clean 50 mL volumetric flask. Dilute to the mark with distilled water.

Instrument Calibration:

Set the readout to zero using distilled water as a blank. Set the peak reading according to the instrument instructions using the most concentrated sodium solution (100 mg/mL). Measure the emission intensity of each of the remaining sodium standard solutions, and of the sodium unknown solution. Check for accuracy and repeatability by measuring the standards several times. Be sure to aspirate deionized distilled water between measurements.

Cleanliness Check:

Dip two fingers into a clean beaker containing about 20 mL of distilled water. Measure and record the sodium emission intensity. Measure and record the sodium emission intensity of tap water. Be sure to aspirate deionized distilled water between measurements.

Viscosity Variations :

Measure and record the sodium emission intensity of each of the 25 mg/mL Na solutions in various solvents. Be sure to aspirate deionized distilled water between measurements.

Reproducibility Check:

Remeasure the emission intensity of two or three of the standard solutions. If a significant change has occurred, READJUST the zero and peak readings, and RE-MEASURE the emission intensity of the standards and the unknown.

Results :

Plot a working curve from the data obtained in the Instrument Calibration step. Calculate and report the sodium concentration of the unknown, "fingered" solution, and tap water in units of mg/ml.

Plot intensity reading as a function of viscosity for the data of Viscosity Variation step.

Turn in the two graphs and the sodium concentrations of the unknown, "fingered" solution, and tap water. Grades will be based 80% on the reported unknown concentration and 20% on graphs. Tap water and "fingered" solution data is for our future reference.

Reference:

Sawyer, Heineman, Beebe, Chemistry Experiments for Instrumental Methods, Wiley, New York, 1984.

D. C. Harris Quantitative Chemical Analysis 4th Ed., W. H. Freeman and Company, New York 1995 Chapter 21

4.Potentiometry

Introduction:

Potentiometry is the field of electroanalytical chemistry in which potential is measured under the conditions of no current flow. The measured potential may then be used to determine the analytical quantity of interest, generally the concentration of some component of the analyte solution. The potential that develops in the electrochemical cell is the result of the free energy change that would occur if the chemical phenomena were to proceed until the equilibrium condition has been satisfied.

$$\Delta G_{rxn} = -nFE_{rxn}$$

This concept is typically introduced in quantitative analysis courses in relation to electrochemical cells that contain an anode and a cathode. For these electrochemical cells, the potential difference between the cathode electrode potential and the anode electrode potential is the potential of the electrochemical cell.

$$E_{cell} = E_{cathode} - E_{anode}$$

If the reaction is conducted under standard state conditions, this equation allows the calculation of the standard cell potential. When the reaction conditions are not standard state, however, one must utilize the Nernst equation to determine the cell potential.

$$E_{cell} = E^{o} - \frac{RT}{nF}\ln(K_{eq})$$

Physical phenomena which do not involve explicit redox reactions, but whose initial conditions have a non-zero free energy, also will generate a potential. An example of this would be ion concentration gradients across a semi-permeable

membrane. This can also be a potentiometric phenomena, and is the basis of measurements that use ion-selective electrodes.

$$E_{mem} = (\text{constant}) - \frac{RT}{z_i F} \ln(a_i)$$

5. Nuclear Magnetic Resonance Spectroscopy

Over the past fifty years nuclear magnetic resonance spectroscopy, commonly referred to as nmr, has become the preeminent technique for determining the structure of organic compounds. Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. Although larger amounts of sample are needed than for mass spectroscopy, nmr is non-destructive, and with modern instruments good data may be obtained from samples weighing less than a milligram. To be successful in using nmr as an analytical tool, it is necessary to understand the physical principles on which the methods are based.

The nuclei of many elemental isotopes have a characteristic spin (I). Some nuclei have integral spins (e.g. I = 1, 2, 3 ....), some have fractional spins (e.g. I = 1/2, 3/2, 5/2 ....), and a few have no spin, I = 0 (e.g. 12C, 16O, 32S, ...). Isotopes of particular interest and use to organic chemists are 1H, 13C, 19F and 31P, all of which have I = 1/2. Since the analysis of this spin state is fairly straightforeward, our discussion of nmr will be limited to these and other I = 1/2 nuclei.

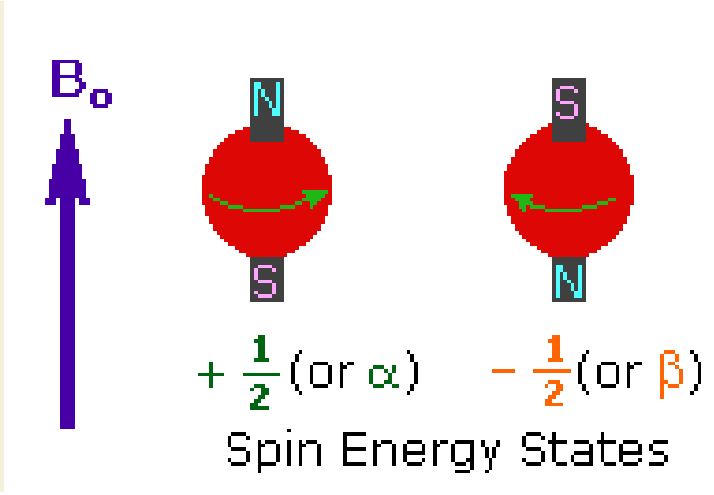
The following features lead to the nmr phenomenon:

1. A spinning charge generates a magnetic field, as shown by the animation on the right.

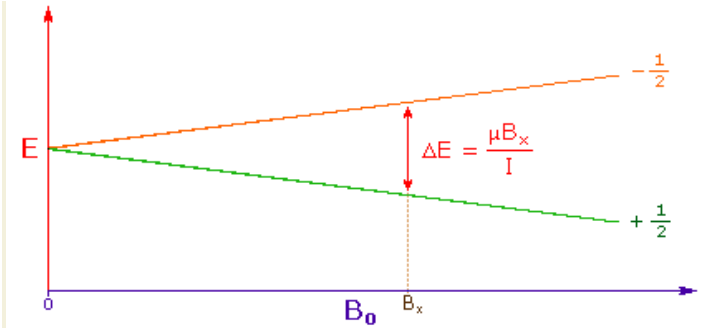
The resulting spin-magnet has a magnetic moment (μ) proportional to the spin.

2. In the presence of an external magnetic field (B0), two spin states exist, +1/2 and -1/2.

The magnetic moment of the lower energy +1/2 state is alligned with the external field, but that of the higher energy -1/2 spin state is opposed to the external field. Note that the arrow representing the external field points North.



1. The difference in energy between the two spin states is dependent on the external magnetic field strength, and is always very small. The following diagram illustrates that the two spin states have the same energy when the external field is zero, but diverge as the field increases. At a field equal to Bx a formula for the energy difference is given (remember I = 1/2 and μ is the magnetic moment of the nucleus in the field).

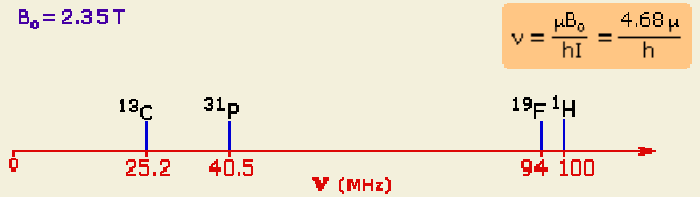


Strong magnetic fields are necessary for nmr spectroscopy. The international unit for magnetic flux is the tesla (T). The earth's magnetic field is not constant, but is approximately 10-4 T at ground level. Modern nmr spectrometers use powerful magnets having fields of 1 to 20 T. Even with these high fields, the energy

difference between the two spin states is less than 0.1 cal/mole. To put this in perspective, recall that infrared transitions involve 1 to 10 kcal/mole and electronic transitions are nearly 100 time greater. For nmr purposes, this small energy difference (ΔE) is usually given as a frequency in units of MHz (106 Hz), ranging from 20 to 900 Mz, depending on the magnetic field strength and the specific nucleus being studied. Irradiation of a sample with radio frequency (rf) energy corresponding exactly to the spin state separation of a specific set of nuclei will cause excitation of those nuclei in the +1/2 state to the higher -1/2 spin state. Note that this electromagnetic radiation falls in the radio and television broadcast spectrum. Nmr spectroscopy is therefore the energetically mildest probe used to examine the structure of molecules.

The nucleus of a hydrogen atom (the proton) has a magnetic moment μ = 2.7927, and has been studied more than any other nucleus. The previous diagram may be changed to display energy differences for the proton spin states (as frequencies) by mouse clicking anywhere within it.

2. For spin 1/2 nuclei the energy difference between the two spin states at a given magnetic field strength will be proportional to their magnetic moments. For the four common nuclei noted above, the magnetic moments are: 1H μ = 2.7927, 19F μ = 2.6273, 31P μ = 1.1305 & 13C μ = 0.7022. These moments are in nuclear magnetons, which are 5.05078•10-27 JT-1. The following diagram gives the approximate frequencies that correspond to the spin state energy separations for each of these nuclei in an external magnetic field of 2.35 T. The formula in the colored box shows the direct correlation of frequency (energy difference) with magnetic moment (h = Planck's constant = 6.626069•10-34 Js).



6.ESR Spectroscopy

EPR Spectroscopy. Introduction.

Objective: You will learn what kind of information ESR can provide both, spectroscopic and kinetic, and investigate some organic and inorganic radicals and ion radicals and gather information anot only bout their hyperfine structure but also what may affect it (e.g. concentration, oxygen etc.)

Electron Paramagnetic Resonance (EPR), often called Electron Spin Resonance (ESR), is a branch of spectroscopy in which electromagnetic radiation (usually of microwave frequency) is absorbed by molecules, ions, or atoms possessing electrons with unpaired spins, i.e. electronic spin $S > 0$. EPR is similar to Nuclear Magnetic Resonance (NMR) which you will get acquainted with in the Lab 8. The NMR technique deals with nonzero nuclear spins, $I > 0$.

In both EPR and NMR, the sample material is immersed in a strong static magnetic field and exposed to an orthogonal low- amplitude high-frequency field. ESR usually requires microwave-frequency radiation (GHz), while NMR is observed at lower radio frequencies (MHz). With ESR, energy is absorbed by the sample when the frequency of the radiation is appropriate to the energy difference between two states of the electrons in the sample, but only if the transition satisfies the appropriate selection rules.

Most of materials in a bulk form at normal conditions have net zero electronic spin and, thus, are EPR silent, but some are can provide an EPR signal. In this lab will analyse some representative species from the three groups below:

Transition-metal and rare-earth species which contain unpaired nd and/or mf electrons. This experiment will offer you to examine liquid solutions of Mn2+, Fe3+ and Cu2+ ions with some ligands, as well as Cr3+ as a dilute impurity in a solid Al2O3 host (ruby crystal).

A small number of organic molecules are called free radicals because they contain a single unpaired electron, 1,1'-Diphenyl-2-picryl-hydrazyl (DPPH) and 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) are the examples; the latter will be used in a first part of this experiment. All but one of the electrons of these molecules are paired so there is only the orbital and spin motion of one electron present per molecule.

Organic ion-radicals, very reactive species which are created along a course of redox reactions. They can be stabilized at certain conditions. You will make and analyze perylene+ cation-radical and benzosemiquinone anion-radical.

Triplet-state organic molecules and biradicals.

7.Mass Spectrometry

In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. The three essential functions of a mass spectrometer, and the associated components, are:

1. A small sample of compound is ionized, usually to cations by loss of an electron. The Ion Source

2. The ions are sorted and separated according to their mass and charge. The Mass Analyzer

3. The separated ions are then detected and tallied, and the results are displayed on a chart. The Detector

Because ions are very reactive and short-lived, their formation and manipulation must be conducted in a vacuum. Atmospheric pressure is around 760 torr (mm of mercury). The pressure under which ions may be handled is roughly 10-5 to 10-8

torr (less than a billionth of an atmosphere). Each of the three tasks listed above may be accomplished in different ways. In one common procedure, ionization is effected by a high energy beam of electrons, and ion separation is achieved by accelerating and focusing the ions in a beam, which is then bent by an external magnetic field.

The ions are then detected electronically and the resulting information is stored and analyzed in a computer. A mass spectrometer operating in this fashion is outlined in the following diagram. The heart of the spectrometer is the ion source. Here molecules of the sample (black dots) are bombarded by electrons (light blue lines) issuing from a heated filament. This is called an EI (electron-impact) source. Gases and volatile liquid samples are allowed to leak into the ion source from a reservoir (as shown), but non-volatile solids and liquids may be introduced directly.

Cations formed by the electron bombardment (red dots) are pushed away by a charged repellor plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments. When the ion beam experiences a strong magnetic field perpendicular to its direction of motion, the ions are deflected in an arc whose radius is inversely proportional to the mass of the ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum).

When a high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). This leaves behind a molecular ion (colored red in the following diagram). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces (colored green) and smaller fragment ions (colored pink and orange). The molecular ion is a radical cation, but the fragment ions may either be radical cations (pink) or carbocations (orange), depending on the nature of the neutral fragment. An animated display of this ionization process will appear if you click on the ion source of the mass spectrometer diagram.

8.X-Ray Diffraction Analysis

General Description.

X-Ray powder Diffraction analysis is a powerful method by which X-Rays of a known wavelength are passed through a sample to be identified in order to identify the crystal structure. The wave nature of the X-Rays means that they are diffracted by the lattice of the crystal to give a unique pattern of peaks of 'reflections' at differing angles and of different intensity, just as light can be diffracted by a grating of suitably spaced lines. The diffracted beams from atoms in successive planes cancel unless they are in phase, and the condition for this is given by the BRAGG relationship.

nl = 2 d Sin q

l is the wavelength of the X-Rays

d is the distance between different plane of atoms in the crystal lattice.

q is the angle of diffraction.

The X-Ray detector moves around the sample and measures the intensity of these peaks and the position of these peaks [diffraction angle 2q]. The highest peak is defined as the 100% * peak and the intensity of all the other peaks are measured as a percentage of the 100% peak.

1. J.C.P.D.S. [Joint Committee on Powder Diffraction Standards]

This organisation produced standard diffraction patterns for many of the minerals and inorganic structures suitable for analysis by X-Ray Diffraction Spectroscopy, and published these data as standards. The reference standard for Calcium Hydroxylapatite is 9-432. The procedure was carried out by Hodge.cs. The reference standards for other phosphates and for other possible impurities is given in Table 2. There is no specified method for sample preparation quoted in the J.C.P.D.S. handbook, consequently we have outlined below the sample preparation method to be used.

2. Sample preparation.

The method preferred for the analysis of Hydroxylapatite powders is the method by which all possible crystal orientations are presented simultaneously to the beam of X-Rays. Some crystallites will be correctly orientated to fulfil the Bragg equation for each value of d, and the detector will measure the intensity of the beams of diffracted X-rays at all locations given by the theoretical values of q. Any impurities will give additional lines which can be identified by looking at the pattern expected from likely impurities such as a Tri-Calcium Phosphate or b Tri-Calcium Phosphate etc. The material should be removed from the test plate by bending the mild steel plate in a vice, the flaked material then should be ground by hand in a pestle & mortar sufficiently fine so that it will just pass through a 40 micron sieve. The quantity of powder required to fill the sample holder used is 0.35 grams approx and this should be packed into the holder using a standard back fill method sufficiently firmly such that it will not fall out during the 90ø tilt test.

The standard backfill method is as follows:-

Place the sample holder on a glass microscope slide and hold in place with sticky tape. Fill the recess with the ground and sieved sample, tap the holder lightly to ensure the corners are filled, draw another glass microscope slide over the recess to remove excess sample. place the second slide on the sample surface and hold in place with sticky tape. Turn the whole unit over such that the bottom is now the top and remove the first glass slide. The sample is now ready to be inserted into the X-Ray machine.

3. Operation method of the X-Ray diffractometer.

The Xrays used are of the Copper k a wavelength 1.54056 x 10-10m, the scan is taken between 2 theta of 10° and 2 theta of 45° at increments of 0.04° with a count time of 4 seconds for each step. These angles have been selected as this is where the important reflections lie for Hydroxylapatite and other relavent impurities. The count time is selected as 4 seconds to give a good signal to noise

ratio and yet to enable the analysis to take place over a reasonable period. The data are collected by computer and stored on floppy discs for later evaluation and hard copy production. See graph 2 enclosed. The intensity of the Xrays are measured on the Y axis, and increasing values of 2 theta are shown on the X axis. The sample is run on the machine and then the Hydroxylapatite standard is run for comparison.

4. Evaluation of data:

4a. Relative Crystallinity

Determination of the relative crystallinity for the Hydroxylapatite sample under test utilises an XRD software package and involves integrating the area under the curve on the graph printout between 2 theta of 30°and 2 theta of 35° , the result is then divided by a value obtained similarly from a control sample. (standard P120 hydroxylapatite powder supplied by Plasma Biotal and X-rayed on the same day (under the same scan parameters). The ratio expressed in percentage terms gives the relative crystallinity of the sample tested. The Standard P120 hydroxylapatite powder is compared with the international standard for a Alumina [Reference Standard material 674A available from US National Institute of Standards and Commerce. Gaithersburg, MD. 20899.USA.]

N.B. The height of the peaks (intensity) depends upon the number of crystallites diffracting the X-Rays, thus a sample more finely ground will give higher but narrower peaks than the same sample coarsely ground. The area under the graph (as described above) measuring crystallinity will yield the same result in each case whether the sample is finely or coarsely ground.

4b. Quantification of beta Ortho Tricalcium phosphate (Whitlockite).

When equal proportions 50% Hydroxylapatite and 50% beta Whitkockite by weight are mixed then upon analysis the 100% peaks for Hydroxylapatite and for beta Whitlockite are of equal intensity. Graphs have been produced for 10%, 5%, 2%, 1%. mixtures of beta Whitlockite with Hydroxylapatite by weight. these are used to check that the estimate produced for whitlockite by the method below is in the correct range.

The assessment of % by weight of beta Whitlockite is found by integrating the area under the 100% whitlockite peak (2 theta of 31.027) and the 100% Hydroxylapatite peak (2 theta of 31.774). this fraction when multiplied by the overall relative crystallinity give the percentage (weight by weight) of whitlockite present in the sample (to the nearest significant percentage.)

4c. Unit Cell Dimensions.

The Unit cell dimensions are not measured on a regular basis however from many analyses performed (1990-1993) it has become clear that the unit cell dimensions are consistently different in Hydroxylapatite analysed from coating samples, from those obtained by analysing the starting material, this is indicative of minor microstructural changes. These are likely to have occurred during the heating and cooling stages of the process.

4d. Preferred Orientation.

* [When analysing Hydroxylapatite it may be possible under certain circumstances for the `100%' peak, normally at Interplanar spacing 2.814 angstroms units to be reduced height and the `40%' peak at Interplanar spacing 3.440 angstroms units to be of increased height such that the 40% peak is higher than the 100% peak. This is thought to arise due to 'preferred orientation' which is described as follows:-

The crystallites of Hydroxylapatite can be regular 'needle shaped' particles which tend to all lie in a similar direction in the sample holder, this is especially true of the Hydroxylapatite which has been thermally processed by the coating procedure. The X-Ray powder diffraction method requires that the crystallites be randomly packed to give the correct intensity of reflections, thus on occasions the sample may need to be reground and the holder repacked

9.Chromatography Methods

Introduction: Chromatography is a separations method that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the the components in a mixture.

A column (or other support for TLC, see below) holds the stationary phase and the mobile phase carries the sample through it. Sample components that partition strongly into the stationary phase spend a greater amount of time in the column and are separated from components that stay predominantly in the mobile phase and pass through the column faster.As the components elute from the column they can be quantified by a detector and/or collected for further analysis. An analytical instrument can be combined with a separation method for on-line analysis. Examples of such "hyphenated techniques" include gas and liquid chromatography with mass spectrometry (GC-MS and LC-MS), Fourier-transform infrared spectroscopy (GC-FTIR), and diode-array UV-VIS absorption spectroscopy (HPLC-UV-VIS).

Specific chromatographic methods:

1.Gas chromatography (GC)

Applied to volatile organic compounds. The mobile phase is a gas and the stationary phase is usually a liquid on a solid support or sometimes a solid adsorbent.

2.High-performance liquid chromatography (HPLC)

A variation of liquid chromatography that utilizes high-pressure pumps to increase the efficiency of the separation.

3.Liquid chromatography (LC)

Used to separate analytes in solution including metal ions and organic compounds. The mobile phase is a solvent and the stationary phase is a liquid on a solid support, a solid, or an ion-exchange resin.

4.Size-exclusion chromatography (SEC)

Also called gel-permeation chromatography (GPC), the mobile phase is a solvent and the stationary phase is a packing of porous particles.

5.Thin-layer chromatography (TLC)

A simple and rapid method to monitor the extent of a reaction or to check the purity of organic compounds. The mobile phase is a solvent and the stationary phase is a solid adsorbent on a flat support.

VI.MICROBIOLOGY

Unit-1 : fundamentals Of Microbiology

1.Introduction to Microbiology

Microbiology is the study of microorganisms which must be viewed with the aid of a microscope or electron microscope.

The importance of microbiology includes: used in biomedical research, creation of medicines, environmental applications and new research tools.

Disease causing organisms include: protists, bacteria, viruses and other microorganisms.

Bacteria are important for fixing N2 in a usable form for plants.

Bacteria and some fungi are important in decomposition and recycling of raw materials.

Industry applications of microbiology: waste management, food industry, mining, medicine, research and biotechnology.

1660's Robert Hooke observed microorganisms for the first time with a microscope and coined the term "cell".

1632-1723 Anton van Leeuwenhoek credited with having observed the first bacteria.

1828-1898 Ferdinand Cohn developed the first classification scheme based on bacteria shape. Cohn detailed and described the life cycle of Bacillus.

Cohn's Classification System:

Sphaerobacteria are spherically shaped.

Microbacteria are rod shaped

Desmobacteria are filamentous

Spirobacteria are spiral shaped

1822-1895 Louis Pasteur Defined pasteurization to prevent spoilage of food by bacteria, develop vaccines and disproved the scientific dogma of "Spontaneous Generation". He defined "Germ Theory" and demonstrated that germs were responsible for disease.

1843-1910 Robert Koch identified anthrax and developed agar growth medium. Koch's postulates was a systematic method to establish the microbial cause of disease.

Ignaz Semmelweis was the first to recognize the need for good hygiene during medical procedures. The first to identify nosocomial infections.

1827-1912 Joseph Lister developed antiseptic methods for use in surgery and medicine.

1854-1915 Paul Ehrlich developed chemotherapy to cure infectious diseases and discovers antibiotics to treat sleeping sickness and syphilis.

1881-1951 Alexander Fleming discovered penicillin and lysozyme.

1864-1920 Dmitri Ivansvski discovered the first virus which is known as the tobacco mosaic virus (TMV).

1952 Hershey & Chase Experiments identified that DNA was the genetic material of bacteriophages.

Hershey Case Experiment: using phage radioactively labeled with P32 (DNA) or S35 (protein) they infected bacteria cells. They found the P32 inside the bacteria not S35.

Recommendations for Success in Microbiology:

1.Successful completions of general biology 2 semesters

2.Knowledge of basic chemistry and biochemistry

3.Review layout of text book used in the course.

4.Read and master learning objectives laid out in the text book.

5.Basic understanding of classification

6.Master definitions presented in the course.

2. Techniques in Microbiology

Microscopy:

Electron Microscopy: uses electrons to create an image of the specimen.

Brightfield Microscopy: specimen is visualized after the light has passed through it.

Dark Field Microscopy: used when staining a sample is difficult or impossible.

Inverted Microscope: lenses are under the stage point upward used for thick samples.

Upright Light Microscope: lenses are above the specimen point down.

Slide Preparation and Cell Stains:

Wet Mount slide preparation: can be done with specific dyes or water.

Common Laboratory Stains: Gram-Negative stain with safranin and appear red.

Gram-Positive: crystal violet sometimes methylene blue.

Aseptic Techniques: Method to collect, grow and preserve a sample such that no other microbes are introduced or lost from the original culture.

Agar Plates:

Streak Plate Procedure: plate is streaked in a zig zag patter to dilute a sample spread out sample for the purpose of isolating individual colonies.

Selective Agar: growth medium with chemicals mixed in to select bacteria based on specific physiological characteristics e.g. antibiotic resistance.

Quantification: Colony forming units (CFU) method to determine the number of bacteria in ample.

Dilution Plating: successive dilution of a sample to quantify and isolate single colonies.

Isolation of pure bacteria colonies: method to create a pure culture.

Colony Characteristics: Shape, size, morphology and growth patterns.

Phage Quantification: PFU, plaque forming units, clear areas of growth on a bacterial lawn plated on agar which indicate the presence and numbers of phage present.

DNA Methods:

PCR: Polymerase Chain Reaction, method used to amplify specific DNA sequences for ease of identification and sequencing.

Electrophoresis: DNA sample place in a gel substrate between two electrodes. The charge and size of the DNA determines how fast the DNA migrates in the electrical field.

DNA sequencing: Method to determine the order of which nucleotides are arranged on a strand of DNA.

3. Prokaryotic and Eukaryotic Cells:

Prokaryote:

- Do not have membrane bound organelles. The DNA of prokaryotes is simple in structure and floats freely in the cell. The cell walls typically consist of peptidoglycan molecules.

- The cell wall of prokaryotes is a fluid phospholipid bilayer having no carbohydrates on the membraneand typically no sterols. Although the membranedoes have hopanoids (which are sterol like)molecules.

Eukaryote:

- Eukaryotes have membrane bound organelles. Their DNA is complex and typically associated with structural and regulatory proteins and it is contained within a membrane bound nucleus. The cells are about ten times larger then those of prokaryotes. Some eukaryotes (e.g. plants) have cell walls but are not made up of peptidoglycan molecules.

- Replication in prokaryotes involves mitosis and meiosis. Meiosis occurs in sex cells like sperm and egg. The eukaryote cell member is a fluid phospholipid bilayer containing sterols andcarbohydrates. The membranes can endocytose, phagocytose, pinocytose and exocytose.

- In animal cells the plasma membrane is the onlyseparation between the cell's interior and the environment.

- In fungi, bacteria and plants there is an additional cell wall as the outer most boundary.

- The plasma membrane is about 10 nm thick and is a selective barrier.

Cell wall*:* A cell membrane is a lipid bilayer that usually has proteins associated with its surface, interior or even transmembrane.

The membrane is selectively permeable.
Microbe Evolution: The concept of common evolutionary ancestor is introduced.
Cell Mobility and Locomotion:
The motion of eukaryotes and prokaryotes when done using flagella is fundamentally different. Structurally the flagella are different and eukaryotes typically used ATP (myosin) as an energy source to drive the motion of the flagella.

4. Microbial Metabolism

Metabolism

1.Metabolism is the sum of all chemical reactions within an organism.

2.The chemical processes in a living cell or organism.

3.In metabolism some substances are broken down to yield energy while others are synthesized which requires energy.

4.All metabolisms have some common features. These features include a requirement for the nutrient to get into the cell and that the nutrient be available to be metabolized.

5.The complementary process of catabolism and anabolism create energy and building materials. This energy and building materials are used in biosynthesis, assemble, polymerization and general cell survival.

6.The process of catabolism and anabolism may utilize different pathways depending on the cell, nutrient and current environmental pressures the cell is currently experiencing.

Energy Production

Nutrient molecules have energy electrons associated with their chemical bonds.

Catabolic processes oxidize the electrons (removeelectrons) and store them in other accessible high energy bonds like ATP.

ATP (adenosine triphosphate) is an energy form that is retrievable by the cell.

ATP and ADP (adenosine diphosphate) have high energy phosphate bonds with negative fee energy of around 7 Kcal per mole.

Oxidation and reduction reactions are couples (redox).

Oxidation is the removal of electrons from a molecule.

Reduction is the acquisition of electrons.

Oxidation reaction release energy. In the celloxidation usually is accompanied by the loss of hydrogen atoms from a complex substrate molecule in the process electrons are usually lost as well. When a molecule is oxidized it gives up energy.

Reduction reactions grab or trap chemical energy. The substrate or complex bimolecular gains electronsand or hydrogens. A molecule that has been reduced (e.g. accepted an electron) is energy rich.

Substrate level phosphorylation is the transfer of phosphate to ADP from anotherphosphorylated organic compound.

Oxidative phosphorylation is the process in which energy from redox reactions of respiration is used to attach an inorganic phosphate to ADP.

Photophosphorylation is the process in which light energy is used to phosphorylate ADP with inorganic phosphate.

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are cofactors in cells and act as electron carriers. NAD+ is reduced to NADH and NADPH is reduced to NADP+.

Flavin adenine dinucleotide FADH and FADH2 are reduced forms of FAD. FADH2 is produced in the citric acid cycle.

Metabolic Cycles

Glycolysis: a single molecule of glucose yields twomolecules of pyruvic acid (pyruvate). There are 4 ATPs formed and two used in the cycle. Therefore there is a net of 2 ATPs. Two molecules of NAD+ are reduced to NADH.

Krebs Cycle: also known as TCA Cycle (tricarboxylic acid cycle). Pyruvic acid is decarboxylated to acetate. The acetate is attached to coenzyme A in the process a molecule of NAD+ is reduced to NADH. For every two molecules of acetyl-CoA in the Krebs cycle two molecules of ATP are generated. In redox reactions for every two molecules of acetyl-CoA entering Krebs cycle six molecules of NADH and two of FADH2 are made. The NADH and FADH2 are the most important products of the Krebs cycle.

Electron Transport: The largest amounts of ATP produced are in the Electron Transport chain. This is a series of membrane bound carrier molecules that pass or transfer electrons from one to the next and ultimately to a final electron acceptor. The electrons' energy is used to pump protons across the membrane. Electron transport chain in eukaryotes are in the inner mitochondrial membranes in prokaryotes it is in the cytoplasmic membrane. Electron carrier molecules are diverse and in prokaryotes can change depending on environmentalfactors. These carriers include: flavoproteins, ubiquinones, metal containing proteins and cytochomes. Electrons carried by NADH enter the chain at a flavoprotein, FADH2 are introduced by a ubiquinone. Final electron acceptors include oxygen atoms which can make H2O upon the addition of hydrogen ions. The later is aerobic respiration. In anaerobic respiration use other inorganic molecules(rarely organic molecules) instead of oxygen as the final electron acceptor. In aerobic electron transport chain 34 ATP are produced.

Fermentation: the partial oxidation of metabolites releasing energy using an organic molecule as an electron acceptor instead of the electron transport chain. Fermentation oxidizes NADH to NAD+ while reducing organic molecules which are the final electron acceptors.

Anabolic Processes: are reactions that synthesize or make something and require energy. The energy is provided by ATP. There are many anabolic pathways that are catabolic pathways running in revere. Example of anabolic process is the production of cell membrane and cell wall components such as cellulose and peptidoglycan.

5.Microbial Genetics And Biotechnology

DNA Replication:

DNA replication is semi conservative process. DNA is copied from the 5' to the 3' direction.

The process starts at the Origin (of replication)

Chromosomal proteins are released exposing the DNA.

DNA helicase unwinds or unzips a local region of DNA breaking hydrogen bonds and exposing the two separate strands.

This opening is called the replication fork (think of a fork in the road).

DNA polymerase (an enzyme that matches a complementary base to the one exposed) binds to the open strand.

Primase synthesizes a short complementary RNA molecule. The RNA primer provides a 3'hydroxyl group for the DNA polymerase to bind.

The polymerase moves along the strand in the 5' to 3' direction.

Because there are two strands that are antiparellel cells synthesize new strand in two ways.

One strand is called the leading strand is synthesized continuously as a single long chain of nucleotides.

Lagging strand is synthesized in short segments that are later joined.

RNA:

Three kinds of RNA that are transcribed from DNA: messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (rRNA).

Initiation of transcription RNA polymerase attaches nonspecifically to DNA and slides along until it reaches a promoter site.

Upon recognition of the promoter RNA polymerase unzips the DNA.

RNA polymerase links the triphosphate ribonucleotides with their DNA complement. The high energy nucleotides produce the energy for this reaction.

Transcription terminated when the RNA polymerase falls off the DNA as a result of ether a stop code on the DNA or another enzyme kicking it off.

Mutations:

DNA can be damaged from a variety of mechanisms, including radiation, chemical and mistakes made by the polymerase. This damage can lead to mutation of the DNA. Mutation is a change in the nucleotide base sequence of a genome.

Mutations are usually deleterious but are sometimes neutral and rarely improvements.

Biotechnology:

Horizontal gene transfer donor cell contributes part of genome to a recipient cell.

Transduction a virus that has infected one cell, can upon its lysing the host cell take a part of the host genome with it. When the virus then subsequently infects another cell this DNA gets transferred.

Bacterial conjugation can transfer several traits that are carried on plasmids. These may include the ability to form pili, antibiotic resistance and other metabolic advantageous traits.

PCR, polymerase chain reaction is a method for copying a specific DNA sequence. A PCR reaction mixture includes: the DNA template, excess amount of DNA primer, polymerase and DNA nucleotides. A thermal cycler mixes, heats and cools the reaction mixture repeatedly allowing for multiple successive DNA replication cycles.

Unit-2 : Microorganisms

1.Classification of Microorganisms

Traditional classification schemes were based on overt morphology of an organism, its habitat, method of getting energy, nutrition and method of replication. With the advent of DNA and protein sequencing many of the old categories were modified and new ones created. In the 18th century when classification was first formalized all living things were placed into two kingdoms, Plants or Animals likewise there were two Domains: Prokaryota and Eukaryota.

The three-domain system was introduced by Carl Woese in 1970's. Based on 16S rRNA gene sequences he split the Prokaryota domain into Eubacteria and Archaebacteria. Their genetic sequences indicated that each of these Domains arose separately from an ancestor. The Domains were renamed: Bacteria, Archaea and Eukarya.

The biochemistry of Archaea and Bacteria is a significant parameter in their classification. The microbes that are currently in the kingdom archaea was originally grouped with Bacteria. The gross morphology of these two Kingdoms are similar. However the creation of a new kingdom (Archaea) was necessary when information from gene and protein sequencing was combined with the unique demands of the niche these organisms occupy. One of the fundamental functions of classification and taxonomy is to determine how organisms are related both physically and in time. One way to do this is to monitor the rate of change in a shared trait, like ribosome sequences.

A major goal of classification and taxonomy is describe how organisms are related physically and evolutionarily. One method to do this is to monitor the rate of change in a shared trait, like ribosome sequences.

2. The Prokaryotes: Domains Bacteria and Archaea

Three basic prokaryote shapes: cocci, spiral and bacilli.

Cocci are roughly spherical to oval shaped.

Bacilli are shaped like a prolate ellipsoid, but can appear filamentous or spindly.

Spiral shaped prokaryotes.

There are some variations of these shapes but these are predominant.

Cell arrangements:

The final arrangement that the cells take depends on plane in which the cell divides. In binary fission the planes in which the cell divide determinearrangement.

Diplococci are cocci pairs that remain attached create long chains called streptococci.

Cocci that divide in two planes and stay attached form tetrads.

Division in three planes form cuboidal packets called sarcinae.

Clusters of cocci are called staphylococci and they resemble a bunches of grapes.

Bacilli have less variability in arrangement because they divide perpendicular to the long axis.

The diphtheriae bacteria divide by snapping and they form a V shape or side by side stacking called palisade.

In hostile environments some bacteria can form Endospores. Endospores are not the same as reproductive spores.

Steps of endospore formation:

DNA is replicated.

DNA aligns with the long axis of the cell.

The cytoplasmic membrane invaginates forming the forespore.

The cell membrane continues to grow and the forespore is enveloped within a second membrane. The DNA of the vegetative cell disintegrates.

A spore coat is formed around the endospore.

The endospore matures by the completion of the spore coat.

The endospore is released.

Extremophiles

Microbes that need extreme conditions of: temperature, pH, salinity or atmosphere to survive.

DNA Facts

The DNA of a cell is made up of guanine, cytosine, thymine and adenine. Guanine pairs with cytosine in DNA. The G+C ratio refers to the amount, in percent, of guanine and cytosine in a cells DNA. G+C ratio below 50% are considered log G+C bacteria. Above 50% G+C ratio is considered high. Gram-positivebacteria with low G+C ratios have similar 16s rRNA sequences and those with high G+C have rRNA sequences in common.

Taxonomists have used the G+C ratio as a criteria for placing bacteria in phyla.

Gram positive high G+C bacteria include bacteria that have rod shaped cells and filamentous bacteria. The latter look like fungi in how they grow and reproduce and sporulate.

3. The Eukaryotes: Fungi, Algae, Protozoa and Helminths

The DNA in eukaryotes is complexed with histones which are proteins. Eukaryote chromosomes and complexed proteins form chromatin and is located in the cells nucleus.

Some eukaryotic DNA can be found in chloroplasts and mitochondria. These organelles reproduce by binary fission.

Eukaryote asexual reproduction includes: binary fission, budding, fragmentation, spore formation and schizogony.

Sexual reproduction involves the formation of gametes. Fusions of gametes and formation of a zygote.

Algae, fungi and some protozoa reproduce both sexually and asexually

In meiosis a diploid parent cell creates four haploid daughter cells. The DNA has usually undergone some crossing over so the chromosomes are not only halved but are also changed through rearrangement.

Mitosis: Cells have two main stages in the life cycle. Interphase: the cells grow and duplicate their DNA, in the second stage the cell's nucleus divides. In mitosis nuclear division starts after the cell has duplicated its DNA. The result is two exact copies of the DNA.

One of each copy goes to the new cells nucleus. Mitosis maintains the ploidy of a cell. That is a haploid nucleus undergoing mitosis creates two identical cells each with a haploid nucleus. A diploid nucleus undergoing mitosis creates two identical cells each with a haploid nucleus.

Asexual reproduction, in which a cell undergoes multiple mitoses creating a multinucleate cell is called a schizont. This multinucleate cell then simultaneously releases many uninucleate daughter cells called merozoites. Plasmodium, which causes malaria, reproduces this way inside red blood cells and within the liver. The huge release of merozoites results in the cyclic fever and chills associated with malaria.

Modern (early) 21st century classification is based on DNA and protein sequence information. This information combined with existing taxonomic information has resulted in new divisions and classifications. The kingdom Protista was reclassified into several new kingdoms: Alveolata, Euglenozoa, Diplomonadida and Parabasala. In the current classification scheme Algae are distributed in the kingdoms: Stramenophila and Rhodophyta and Plantae. Water molds belong to the kingdom of Stramonophila and slime molds are in the kingdom Mycetozoa.

4.Viruses and Other Non-living Infectious Agents

A virus is an acellular agent that is infectious, it is small and has one or many pieces of nucleic acid. It can infect humans, animals, plants and bacteria and cause disease. Viruses have a much simpler structure then a bacteria. They do not have cell membranes and are made up of only a few organic molecules. Viruses and viroids do not carry out metabolism, such as transport of nutrients across a cell membrane.

The major viral characteristics: type of genetic material (DNA or RNA, single or double stranded), viral size, capsid structure and target host are used to determine how best to classify a virus.

Viral genomes can be linear, one piece or several molecules of nucleic acid (similar to eukaryotic chromosomes).

Not all viruses are as specific to a host (although most are). Some can infect many different hosts and different tissues within a host. An example of a "generalist" virus is rabies. Rabies can infect many different mammals from humans to dogs to bats.
Viruses have three basic capsid shapes: helical, polyhedral and complex.

An envelope surrounds certain viruses. Enveloped viruses acquire their envelope from the host cell during viral replication or when the viral is released from the cell.

A virion without an envelope is called a nonenveloped or naked virion.

Unit-3 Microorganisms And Humans

1.Principles of Disease and Epidemiology

Epidemiology is the study of the frequency, distribution and causes of diseases in a given population.

Definitions:

Infection: the growth of a pathogen in a hostorganism. Infection depends on exposure to the pathogen and host susceptibility.

Disease: the response by a host to an infection, which when bad evokes a recognizable pattern of clinicalsymptoms.

Incidence is the number of new cases divided by time.

Incidence rate is the number of new cases divided by the total population at risk.

Prevalence is the number of cases existing at any moment in time.

Prevalence rat is the number of cases divided by the total population at risk.

Etiology: study of disease causing pathogen.

Symbiosis: interactive relationship between two species.

Residents: colonizing microbes.

Transients: temporary microbes.

Opportunists: normal flora which become pathogenic under certain circumstances.

Pathogens: disease causing microbes.

Immuno compromised: a organism with a weakened immunity.

Commensalism: the interaction of two organisms in which one benefits and the other is neither harmed nor helped by the interaction.

Mutualism: relationship where both the host and microbe are metabolically dependent on each other e.g. lichen have a symbiotic association with a fungus.

Parasitism: the interaction of two (or more) organisms where one is benefited and the other harmed by the relationship.

Disease Severity: Acute: short duration, Chronic: lasts for a longer time period, Sub-acute: duration between acute and chronic, Latent: causative agent lies dormant within the body and suddenly become active resulting in disease.

Reservoir: Also known as an asymptomatic infection carrier it is an organisms that carries and spreads the pathogen but is unharmed itself.

Types of Epidemiology:

Descriptive Epidemiology

Analytical Epidemiology

Retrospective Epidemiology

Prospective epidemiology

Experimental Epidemiology

Serological Epidemiology

Application of Epidemiology

Tracking of health problems occurring in a community.

Establish the clinical picture of the disease or health problem in a community.

Estimating risk of specific diseases or syndromes.

Identify syndromes, precursors and treatments for diseases.

Investigate epidemiology of unknown etiology.

Establish the history of a disease in a defined population.

Koch's Postulates & Exceptions

Pathogen must be present in every instance of disease.

Pathogen must be grown in pure culture.

Pathogen must be capable of being re-isolated from an inoculated animal expressing the disease.

Exception: Some bacteria and viruses cannot be grown in the laboratory.

Exception: Some diseases are caused by several microbes.

Exception: Some pathogens are responsible for causing different diseases.

Exception: Some pathogens can not be grown in animals and exist only in humans.

Distribution Formats:

Sporadic: Few cases

Endemic: Cases in a local region or area.

Epidemic: Wide spread outbreak of a disease in excess of what is normally expected. disease like syndrome.

Pandemic: Spreading throughout the globe.

Disease:

Disease is an interaction between a Host, Pathogen (agent of infection) and Environment.

Classification of Disease: Communicable Diseases, Contagious Diseases and non-Communicable Diseases.

Stages of Disease Development:

Incubation period: pathogen is multiplying.

Prodromal period: symptoms being to appear

Illness: visible signs of disease.

Period of decline: pathogen is under control

Period of convalescence: patient begins to recover.

Iceberg Concept of Infection

Cell Response: Exposure no cell entry®Incomplete viral maturation®Cell transformation®Cytophatic effect®Fatal

Host Response: Exposure but no infections®Infections with no clinical illness®Mild illness®Severe disease®Fatal

Mechanisms of Transmission: Aerosolizing, touch, insect bite, animal bite fomites.

Ecological factors of infections: altered environment, changes in food production, deforestation, global warming, increased use of antibiotics and bacteria in the air.

Factors Influencing Outbreaks and Disease Spread

Factors effecting outbreak: presence of an infected host, adequate number of potential hosts and an effective methods of transmission by contact.

Factors affecting spread: stability of virus within it's the environment, number of virion particles releases, virulence and invasiveness of pathogen, availability of proper vector or medium for spread.

Normal Flora

Can be residents or transients. Resident population remains constant and prevents invagination by pathogens and raises overall immunity. Residents can in rare situations become opportunists and cause infections. Transient population number varies.

In the body normal biota can inhabit: skin, respiratory tract, intestine, mouth, nose, throat and vagina.

Benefits of Normal Flora include: resistance to some pathogens, release of bacteriocins and colicins, production of vitamin K, continued antigenic stimulation from commensals.

Disadvantages of Normal Flora: commensal bacteria may cause localized infections, can become pathogenic if they acquire virulence factors or are introduced to sterile sties, contribute to body odor.

2. Innate Immunity

Is the basic resistance to disease that an organisms or individual has as a function of birth. It is the initial immune defense system.

Is non-specific.

At the species level it refers to the immune response that all members show to a pathogen.

Within a species there exists subgroups such as race, gender etc. that may exhibit differences in immunity to pathogens.

Individual immunity refers to differences in an individual's response to a pathogen within a given race.

Four Types of barriers to infections: anatomic, physiologic, phagocytic and inflammatory.

Examples of these barriers: temperature, pH, enzymes and chemical, skin, mucous membranes, phagocytic or endocytic barriers, inflammatory barriers.

Organs Involved in Innate Immunity :

Eyes: tears wash away pathogens and have bacteriocidal enzymes.

Skin: Difficult for a pathogen to penetrate, sweat creates high salt conditions, oil layer makes an inhospitable environment.

Stomach: acid kills pathogens and sterilizes food.

Nose: Mucus traps pathogens which are swallowed or blown out.

Mouth: Natural microbiota prevents growth of opportunistic pathogens.

Lungs: mucus lining of lungs traps pathogens and cilia move particles out to throat and it is swallowed.

Large intestine: Natural microbiota prevents growth of opportunistic pathogens.

Reproductive system: acid conditions and natural microbiota.

Innate Immunity Influences

Age :

Nutrition

Endocrine functions: disorders including diabetes, hyperthyroidism, adrenal dysfunction and stress.

Mechanisms of Innate Immunity

Non-specific broad spectrum response.

No lasting immunological memory.

Has limited flexibility and repertoire.

Responses are evolutionarily ancient.

3.Immunologic Disorders

Components of the Immune System:

Lymph, bone marrow, thymus, spleen, leukocytes, antibodies, complement, tonsils and adenoids are among the major components of the immune system.

Antibodies-1:

IgG is the most abundant antibody in blood. Only antibody to cross the placenta.

IgA is the second most abundant antibody in blood. Is the main antibody found in bodily secretions: tears, saliva, mucous, respiratory, intestinal and others.

IgM third most abundant antibody in the blood and the largest.

Cytokines:

Interleukins are modifiers of a body's immune response and modulate inflammation. They are produced by cells such as lymphocytes, macrophages and monocytes.

Growth factors are proteins that are able to stimulate cellular proliferation and differentiation. They typically act as signaling molecules between cells promoting cell differentiation and maturation.

Interferons are a group of proteins which inhibit viral replication in the host's body.

Hypersensitivity – Type 1: (Also known as Immediate Hypersensitivity)

An allergic response due to re-exposure to a specific antigen.

Is an inflammatory response e.g. asthma, rhinitis.

Exposure can be a result: ingestion, inhalation, injections or direct contact.

Mediated by IgE antibodies.

Results in an immediate release of histamine, tryptase, arachidonate and derivatives by basophils and mast cells.

Hypersensitivity – Type 2: (Also known as Cytotoxic Hypersensitivity)

Antibody mediated generally by IgG and to a lesser extent IgM.

Process typically involves K-cells rather than mast cells.

May involve complement that binds to cell-bound antibodies.

Antibodies react with “self” antigens.

Tissue damage may result.

Antibodies bind to antigens forming complexes that activate the complement for eliminating cells presenting foreign antigens.

Acute inflammation created at the site were Ag-Ab complexes causing cell lysis and death.

Example is Erythroblastosis Fetalis the mother produces antibodies that attack the red blood cells of the fetus. This can occur when the mother and baby have different blood types.

Hypersensitivity – Type 3: (Also known as Immune complex Hypersensitivity)

Circulating antibodies react with free antigen.

The complexes can be deposited on tissue which may trigger complement reaction resulting in tissue damage.

Aggregations of antigens and IgG and IgM form in the blood.

The reaction can take hours to days to develop.

Systemic Lupus Erythematosus is an example of this. Antinuclear antibodies generate circulating immune complexes that activate complement. Results in systemic inflammation of body tissues.

Hypersensitivity – Type 4: (Also known as Delayed Hypersensitivity)

Cell mediated immune response.

CD8 cytotoxic T cells and CD4 helper T cells are key molecules involved.

Antigen presenting cell are macrophages and they release Interleukin 1 stimulating CD4 cell proliferation.

Activated CD8 cells and macrophages destroy target cells.

Memory TH1 cells release cytokines that recruit and activate macrophages.

Examples of this is contact dermatitis i.e. Poison Ivy.

Immunodeficiency Disorders – 1 :

Immunodeficiency disorders are a malfunction of the immune system that result in the development and frequent reoccurrence of disease that are also more severe and longer lasting then typical.

One or more components is defective or missing from the immune system.

Immunodeficiency disorders can be inherited.

Temporary immune deficiencies can develop as a function of disease.

Immunodeficiency Disorders – 2 :

Depressed immune response due to smoking, stress, surgery transfusions etc.

Inherited poorly functioning immune system.

B-cell system not functioning correctly so unable to make antibodies.

Immunodeficiency Disorders – 3 :

Thymus missing, small or defective, lacking T-cells.

Severe Combined Immunodeficiency Disease: birth defect of several immune system organs or defenses.

Autoimmune Diseases:

The immune system looses the ability to discriminate between self and other.

T-cells and antibodies (called auto-antibodies) are made and directed against "self" cells. Rheumatoid factor is an auto-antibody.

Causes of auto immune diseases include: heredity, infections, certain drugs, sunlight and hormones.

Type 1 diabetes is caused by the immune system attacking the pancreas cell.

AIDS: The HIV virus attacks and destroys T-cells. Can also be a latent infection.

Rheumatoid arthritis: The body does not distinguish correctly between self and non-self. The body produces an antibody known as Rheumatoid Factor which is directed against the body's own immune system.

Definitions:

Hypersensitivity: an exaggerated immune response to a foreign agent.

Inflammation: the first response of the immune system it involves leukocytes entering a site of injury or infection.

Anaphylaxis: is a rapid and severe allergic response.

Auto-immune disease: the failure of the immune system to recognize "self" from other cells. This results in an immune response against its own cells and tissues.

Asthma: is a disease of the respiratory system often resulting from an immune response resulting in inflammation.

Rheumatoid arthritis is a chronic inflammatory autoimmune disease in which the joints are attacked.

Mast cells: contain histamine and heparin and are involve in allergy and anaphylaxis. They are similar to basophils and granulocytes.

Cytokines: peptides and proteins that are used as cell to cell signals.

Macrophages.

5.Antibiotics

Introduction of Antibiotics their mechanisms of action and cellular targets.

Antibiotics are antimicrobials that can kill or inhibitgrowth of susceptible organisms.

Antibiotics are targeted antimicrobials that usually affect a metabolic pathway.

Beta lactam-A beta-lactam ring or penam: is a lactam consisting of a heteroatomic ring structure containing three carbons and one nitrogen atom.

Beta Lactams Antibiotics bind and inhibit enzymes involved in cell wall synthesis. Little effect on non replicating bacteria. Lethal to dividing bacteria.

Penicillin: is an antibiotic used to treat bacterialinfections (usually Gram-positive). Originally derived from the blue-green mold Penicillium.

Penicillin binds irreversibly to transpeptidase and prevents it from cross linking the peptidoglycan units of the cells wall.

Cephalosporin: beta-lactam family of antibiotics, bactericidal, prevents cell wall synthesis.

Tetracycline: interfere with protein synthesis by binding to ribosomes.

Glycopeptides are molecules that work by interfering with the synthesis of bacterial cell walls.

Polymyxin: antibiotic damages the cytoplasmic membrane of bacteria. Polymyxin is produced by thebacteria Bacillus polymyxa. Increase the permeability of bacterial cell membranes causing a loss of equilibrium.

Aminoglycosides: is a collections of antibiotics that target the cells ribosome and causeerror prone reading of the mRNA inhibiting protein synthesis. Aminoglycosides include: amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin, tobramycin and apramycin.

Rifampin is derived from rifamycin and interferes with RNA synthesis by binding to RNA polymerase.

Antifungal drugs inhibit the growth of fungi. Typically toxic to humans because both organisms are eukaryotic. Examples include: imidazole, clotrimazole, ketoconazole, miconazole and others. They extract membrane sterols or prevent theirsynthesis.

Antibiotics can be synthetically produced.
Alternatively organisms producing antibiotics can be grown in fermentation flasks and the products separated.

Antibiotic resistance to beta lactams is a result of an enzyme called beta lactamase which break the beta lactam ring. The gene for the enzyme can be transferred between bacteria resulting in resistance.

Antibiotic Targets

Cell Wall which provides the bacteria a outer protection and mediates the amount oliquid that can enter and leave the cell.

Cell Membrane: is a lipid bilayer and functions as a permeability barrier.

Cellular Proteins: these targets can include ribosomes and other proteins vital for cell metabolism and reproduction.

Cellular Nucleic Acids: interfere with DNA production.

Many antibiotics, fungicides, antimalarials and antivirals act on the DNA or RNA of a cell.

Gene function may be suppressed by inhibiting nucleic acid or protein biosynthesis.

Enzyme Inhibition: irreversible or reversible binding or competitive inhibition of cell enzymes.

Antimetabolites

Chemotherapy exploits the biochemical differences between host and pathogen.

Metabolites are substances used or produced by biochemical reactions.

Antimetabolite is a drug or chemical that has chemical similarity (is a mimic) to a natural metabolite.

Sulfa Drugs and it analogs were the first ant metabolite successfully used against microbes.

Folic Acid analogs block the final conversion of PABA to folic acid which results in nucleotide and proteinsynthesis inhibition in bacteria.

Gram-Positive bacteria have multiply layers of peptidoglycan stacked to form the cell wall. The peptidoglycan portion of the cell wall consists of repeating units of N-acetylglucosamine linked b-1,4 to N-acetylmuramic acid (NAG-NAM).

Unit-4 Medical Microbiology

1.Skin and Eye Infections

There are three major regions of the skin, epidermis, dermis and hypodermis. Each of the three regions have a distinctive structure and function.

Infective agents favor specific regions of the skin and exploit those regions.

Learn to regard the great diversity of visual symptoms associated with different pathogens.

Epidermis has 5 layers of cells, each layer forming a "stratum". The layers of the epidermis include: Stratum Corneum, Stratum Lucidum, Stratum Granulosum and Stratum Spinosum.

Dermis has two distinct layers:

Papillary layer with bumps called papillae

Reticular layer under the papillae layer

Blood vessels in the dermis do not enter the epidermis. Nutrients and wastes of the epidermis move by diffusion between the layers. The papillae increase the contact surface area between the epidermis and dermis this enhances adhesion between the layers as well as diffusion of nutrients and wastes.

Hypodermis Structure: Lies under the dermis, is not part of the skin, connects the skin to underlying tissues and stores energy.

Sweat and sebaceous glands are the two primary glands of the skin. Both glands create their secretions within the dermis then dump them onto the surface of the skin. The skins surface is typically covered with salt that is left aft sweat evaporates and sebum. Sebum is an oily lipid secreted by sebaceous glands in the dermis. Chemicals in sweat and sebum are antimicrobial

Although the sweat is formed in the deepest coiled portion of the sweat gland, the tube that lead to the surface of the skin actively refines the

concentrations of ions and that will ultimately reach the surface. In the case of cystic fibrosis, the function of ion channels has been altered and therefore also the concentration of ions that appear in the sweat.

There are four nerve sensors in the skin. Of which only one is located in the epidermis and is called the merkel cell.

Bacterial Infections Described: Acne, Rocky Mountain Spotted Fever, Impetigo, Necrotizing Fasciitis (Flesh Eating Bacteria), Cat Scratch Fever, Cutaneous Anthrax, and Pseudomonas.

Viral Infections of the Skin: Poxviruses, Herpes, Warts, Rubella, Rubeola, Chicken Pox, Shingles and Varicella-zoster.

Fungal infections of the skin are categorized by the regions of the body they infect. The categories of Mycoses: Superficial, Cutaneous, Subcutaneious and Systemic (affecting internal organ systems).

The Protozoa infection, Leishmaniasis is profiled as an example of this type of infection.

Basic Eye Structure:

The cornea is the first structure crossed as light enters the eye; it offers the greatest degree of bending of the light. Next the light passes through the iris which through the motions of two muscles, a sphincter and a dilator, controls the amount of light that enters the eye. Next the light passes through the lens of the eye which controls the fine focusing of the eye. Interesting, when the focusing muscles of the lens relax, the eye focuses on close objects. The light exits the lens, transits the clear jelly-like vitreous humor to strike the optically active retina where the energy of the photons will be converted into neural signals. The neural signals are conducted to the brain via the optic nerve (CN II). The sclera is very tough fibrous exterior structure which is the site of attachment for all of the muscles that move the eye. One can imagine that the sclera is pretty special, because it must not change shape when the muscle attached to it move the eye.

2. Nervous System Infections

The Central Nervous System (CNS) consists of the brain and spinal cord. The brain has three large regions called: cerebrum, cerebellum and brain stem. Cerebrum is responsible for the control of involuntary muscles, perception and thinking.

Cerebellum controls involuntary movements. The CNS is an axenic environment. That means the CNS is a closed system and has no normal microbiotaBrain stem connects the brain to the spinal cord. It also controls breathing, heart rate and blood pressure.

Spinal Cord:

7 cervical vertebrae

12 thoracic vertebrae

3 lumbar vertebrae

Sacrum

Coccyx

Cauda equine are a bundle of nerves that extends from the spinal cord below the lumbar region.

Meninges :

The meninges are three layers of tissue that surround the brain and spinal cord.

Dura mater: Lies next to the bones (e.g. skull), is a tough and fibrous sheath that is strong and flexible. It covers the soft organs of the CNS. The dura mater provides a barrier against the spread of infections from the bones.

Arachnoid mater: going inward from the dura mater the next layer is the arachnoid mater. It has numerous branching fibers that look like a spider's web. The area between the fibers are called subarachnoid space.

Pia mater: the internal meninx is closest to the spinal cord and brain. The blood vessels that supply the CNS are on top of the pia mater. These blood vessel walls are made up of very tightly joined cells and collectively are called the blood-brain barrier.

Peripheral Nervous System (PNS):

Consists of nerves that transfer messages from the CNS to the muscles and glands in the body. It also serves to provide information to the CNS about the body. Cranial nerves come from the brain through holes in the cranial

bones. Spinal nerves come from the spinal cord through gaps in the vertebrae. There are three types of nerves (based on function): sensory, motor and mixed nerves.

The PNS is made up of nerves and neuron that are outside the CNS and not protected by bone or the blood brain barrier. The PNS has a greater exposure to toxins and mechanical injuries. The PNS is subdivided into the somatic nervous system and the autonomic nervous system.

Structure of a Neuron:

There are two types of fingerlike projections from neurons: axons and dendrites. Many small short projections coming from the cell body are called dendrites. A single long projection is called an axon.

An axon generates an electrical potential also called a nerve impulse. Within an axon the cytoskeleton transports substances by a process called axonal transport.

Synapses are at the terminal ends of axons and form junctions with other neurons. The synapse is responsible for transfer of a signal to a neighboring postsynaptic cell.

In most cases there is a space between the synapses called the synaptic cleft. The cleft stops the electrical signal from passing so the neuron releases chemicals called neurotransmitters. The neurotransmitter can pass a signal to either stimulate or inhibit.

Motor neurons:

Motor neurons forms a synapse with a muscle cell. This synapse is called a neuromuscular junction. There is a space between the neuron and the muscle cell (synaptic cleft). The motor neuron releases a neurotransmitter acetylcholine (Ach) which binds to the surface of the muscle cell and sends the signal for the muscle to contract.

A diverse set of pathogens and toxins that exploit vulnerabilities in the nervous system are presented.

3. Cardiovascular, Lymphatic and Systemic Infections

1.Blood Structure:

Blood consists of several different types of cells.

Red blood cells are also called erythrocytes.

The cells are suspended in plasma.

Red blood cells carry oxygen.

White blood cells are also called leukocytes and are used to fight disease.

Platelets are involved in blood clotting.

2.Cardiovascular System

The heart is a muscle that acts like a pump.

Consists of a closed system where blood is pumped by the heart.

The heart pumps blood into arteries, veins carry blood back to the heart.

The major arteries are the pulmonary arteries which carry blood to the lungs and the aorta that take the blood to the remaining parts of the body.

The major veins are: pulmonary veins which carry blood from the lungs to the heart, superior vena cava head neck and arms to heart and inferior vena cava from the rest of the body to the heart.

The heart itself is supplied by coronary arteries which come from the aorta and cardiac veins which drain into the left atrium.

3.Human Heart:

The human heart is a muscle with four chambers.

The heart is enclosed in a pericardial sac that is lined with a serous membrane. The pericardial sac is an important target for infection or inflammation by microbes.

The wall of the heart is made up of an outer, fibrous pericardium, muscular myocardium and inner endocardium.

Chambers of the Heart: The internal cavity of the heart is divided into four chambers: right & left atrium, right & left ventricle.

The right atrium receives deoxygenated blood from systemic veins; the left atrium receives oxygenated blood from the pulmonary veins.

The valves of the heart are another target for microbial infection. The valves keep the fluid flowing in one direction.

The heart acts like two pumps, one on the right and one on the left.

Blood goes from the right atrium to the right ventricle, and then is pumped to the lungs where it is oxygenated.

From the lungs the blood goes to the left atrium and then to the left ventricle. From there it is pumped to the body for systemic circulation.

4.Lymphatic System :

The lymphatic system is a network of lymphoid organs, lymph nodes, ducts, tissues, vessels and fluid.

The lymphatic system is an important component of the immune system.

There are three functions of the lymphatic system: removes excess fluid from body tissues, absorbs fatty acids and takes them to the circulatory system and finally make immune cells such as lymphocytes, monocytes and antibody producing cells called plasma cells.

The lymphatic system is made up of thin vessels that branch throughout the body similar to the blood system. The vessels carry a clear liquid called “lymph”. Blood plasma leaks from the capillaries of the blood circulatory system and fill the spaces between the cells of tissue, this is called interstitial fluid. The fluid accumulates slowly and is similar to the blood plasma. Most is returned to the circulatory system via the capillaries. Lymph has a large number of white blood cells. The remaining fluid, about 10% is collected as lymph (or lymphatic fluid which is colorless) by the lymphatic system. It is processed by the lymph nodes before it returns to the circulatory system.

Lymph nodes are bean shaped and function as filters. They are filled with lymphocytes (white blood cells) which increase rapidly when fighting an infection.

Pathogens and toxins that target vulnerabilities of the cardiovascular and lymphatic systems can spread throughout the body to cause what is referred to as systemic diseases. Several different pathogens are profiled that demonstrate these concepts.

4.Respiratory System Infections

The structures of the upper respiratory system: pharynx, middle ears, sinuses, nasal cavity, auditory tube opening.

Function of the upper respiratory system is to collect and filter air. Including filtering of: pollens, microbes and other contaminants in the air.

Structures of the lower respiratory system include: larynx, trachea, pleura, bronchus, bronchiole.

Auditory (eustachian tubes) from the ears to the pharynx allow air pressure to equalize.

Tonsils or adenoids are aggregations of lymphoid tissues and located near the junction of the nasal cavity and pharynx. Tonsils have cells and chemicals to fight microbes.

The lower respiratory system does not normally have microorganism and is referred to as axenic. The cilia, secretary antibodies and phagocytic cells keep the organs clear of contaminants.

The upper regions of the pharynx is home to several organisms including: some relatively harmless Gram negative cocci, diphtheroids, opportunistic Staphylococcus and alpha-hemolytic streptococci including Streptococcus pneumoniae. The latter is an opportunistic pathogen and causes most cases of pneumonia. The organisms that live in the pharynx can lead to an infection of the middle ears and sinuses.

Bacterial diseases of the upper respiratory system, sinuses and ears can be caused by bacteria.

Pneumonia is an inflammation of the lungs the alveoli and bronchioles may become fluid filled.

Empyema the name of the condition if the fluid filling a lung is pus.

The most common infection of the upper respiratory system is commonly called "the common cold". However the common cold is not caused by a single virus but as many as 900 viruses can cause a cold.

Flue virus structure and evolution

Antigenic drift and antigenic shift

Fungal infections.

5.Digestive System Infections

1.The digestive system is often divided into two sections: gastrointestinal (GI) system is the tubular path from the mouth to the anus.

2.The second section is referred to as the accessorydigestive organs.
The accessory digestive organs areresponsible for either grinding the food (teeth) or injecting digestive secretions (pancreas).

3.The internal surface of the small intestine has millions of hair like projections called villi.

4.Intestinal peristalsis moves undigested and unabsorbed food from the small intestine into the large intestine and colon.

5.The colon finishes the absorption of nutrients and water.

6.Feces is the remaining undigested material which is eliminated via the anus. As much as 40% of the fecal volume is bacteria.

7.Antigenic drift and antigenic shift primary mechanism for production of new strains of flu.

8.The membrane covering most of the GI tract and protects it is called the peritoneum.

9.Accessory digestive organs include tongue, teeth, liver, gallbladder and pancreas.

10.The esophagus, stomach and small intestine (duodenum) are almost free of microbes.

11.Gastroenteritis is an inflammation or irritation of the lining in the: stomach or intestines.

12.Bacteria growing on the tooth's surface make acid which then destroys the enamel.

13.Gingivitis is an inflammation of the gums surrounding the teeth.

14.Bacterial gastroenteritis is an inflammation of the stomach and intestines caused by bacteria or bacterial toxins.

15.Bacterial pathogens of the gastrointestinal tract, attach and bind to the surface, proliferate and transmit disease. These microbes develop mechanisms that optimize these characteristics while minimizing the host's ability to destroy them.

16.Viral gastroenteritis is an intestinal infection caused by several different viruses.

17.Hand washing is the single most important way to avoid infection of the digestive system

18.Fecal Oral route is a major cause of digestive diseases due to infection.

19.The human heart is a muscle with four chambers.

Unit-5 : Microbiology Outside The Human Body

1.Microbiology and the Environment

Microorganisms in Ecosystems:

An ecosystem is an area of nature that includes biotic and abiotic components. These components have processes that make up and define a certain section of the environment. The biotic and abiotic components are linked by nutrient cycling and the flow of energy.

Microorganisms in the ecosystem decompose organic substrates called mineralization and are a source of food for some chemoheterotrophic microbes.

Some organisms produce antimicrobial substances such as antibiotics.

Adaptations of a microbe to its environment can include modifying its metabolism to optimize its survival in a given place.

Fungi play an important role in decomposition of organic matter.

Algae are very important in converting atmospheric CO2 into organic matter.

Protozoan have adapted the ability to move and acquire digestible organic food.

Bacteria and Archaea are involved in virtually all the cycles of essential elements.

Ecosystem and its components: Abiotic Substances, Producers, Consumers, Decomposers.

Ecosystems consist of: living organisms which interact with inanimate substances, other organisms and each other resulting in changes in the abiotic environment. The interactions of organisms is called ecology.

Microenvironment is a subset of the larger ecosystem. It is characterized by the presence of overlapping gradients of resources, toxic material and limiting factors.

The most important aspect of microbes on their environment is the ability to recycle essential elements that make up cells.

Essential Cycles :

Biogeochemical cycle – is chemical interactions between the atmosphere, lithosphere, hydrosphere and biosphere.

Recycling of elements (CHONSP):

Essential elements must be converted from one form to another.

Microbial cells are crucial to the transformation of these elements.

The elements include, carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus.

Carbon Cycle Geological :

CO2 in the atmosphere is recycles via the oceans and land.

Carbon Cycle – Biological:

The combined processes of photosynthesis, decomposition, and respiration in which carbon is recycled.

Sulfur Cycle:

The process were by sulfur that is a part of organic molecules, as in proteins, are reduced by fungi or bacteria.

The reduced H2S is then oxidized the H2S and lithotrophic bacteria further oxidizes it to SO4.

Plants and bacteria then incorporate it into organic molecules.

Phosphorus Cycle:

Phosphorus cycle is a biogeochemical cycle in which the atmosphere does not play a significant role in its movements.

Plants absorb phosphates from the soil and can then be eaten herbivores that may then be eaten by carnivores. Upon death the animal or plant will decay and the phosphate is returned to the soli.

Phosphates are important components of nucleotides, energy storage molecules such as ATP, bones and phospholipids.

Iron Cycle:

Is a biogeochemical cycle through landforms, atmosphere and oceans.

Bacteria such as Pseudomonas and Thiobacillus ferroxidan can reduce and oxidize (respectively) iron to make it bioavailable.

Nitrogen Cycle:

Nitrogen exists in the atmosphere as a gas (mostly N2).

Nitrogen is often a limiting nutrient for plant growth.

Atmospheric nitrogen become a part of biological matter primarily by the actions of bacteria and algae in a process called nitrogen fixation.

Nitrogen fixing bacteria form nodules on the roots of legumes and take nitrogen from the air and convert it into ammonia NH3. The NH3 is further converted by other bacteria into NO2- and then into NO3-. Plants use the nitrate ion as a nutrient or fertilizer to grow.

Nitrogen is incorporated into amino acids.

Definitions :

Microenvironment: small spaces deep in the earth with varying environmental factors.

Food Chain is a series of organisms each feeding on a preceding one.

Food Web a complex interlocking series of food chains.

Liebig's Law of the Minimum: this principle says that the growth of organisms is not controlled by the total amount of resources available but rather by the least or scarcest resource.

Biogeochemical cycling is the cycling of essential elements by microbes.

Nitrification is the process were by ammonia NH4+ is oxidize to (NO3-) by autotrophic bacteria.

Habitat is the physical place that an organism lives.

Niche the habitat and biological adaptations an organism posses to live.

Population – the number of like organisms in a place at any given time.

Community – a collection of organisms of different species that interact with each other.Biological Oxygen Demand: BOD is an indirect measure of organic matter in an aquatic environment. The amount of dissolved oxygen needed for microbial oxidation of biodegradable organic matter. BOD levels in sewage and waste water have been set.

Bioremediation is the use of microorganisms to clean up an environment. By chemotaxis and other mechanisms microbes move around environments that have the contaminate that is broken down or recycled.

2. Microbiology and Industry

1.Industrial Microbiology and the Food Industry:

Yeast is used make breads, baked goods, alcohol, yogurt and other foods and drink items.

Today's yeast are specially engineered to work in large scale industrial applications.

Specialized bacteria and molds are used to make cheeses of different types.

Biofertilizers include bacteria such as Rhizobia that fix nitrogen.

Food additives increase nutritional value, retard spoilage, change consistency and enhance flavor. These may be natural compounds such as guar gum and xanthan gum or flavor enhancers and vitamins.

2.Industrial Microbiology and Medicine:

Biosensors are monitors used in the detection of specific targets in the environment, human body or other organisms.

Antibiotic production is a capacity that many microbes have naturally.

Microbes have been developed as a drug delivery system.

Lactic acid bacteria (LAB) has been exploited to make and deliver vaccines and other bioactive materials.

Microbes have been developed that degrade oil so that they it may be more easily extracted.

Microbes are involved in the production of paper.

3.Industrial Microbiology and Economics:

In the cosmetic industry the botulism toxin derived from Clostridium botulinum is utilized.

Biopesticides have been developed for the control of insect, nematodes and other pathogens that effect plants.

Synthetic energy fuels such as ethanol, methane, hydrogen and hydrocarbons are produced by microbes.

Gasohol which is a 9:1 blend of gasoline and ethanol is a popular fuel alternative. The ethanol is produced as a by product of yeast fermentation.

Microbes have been used in mining. An example of this is the recovery of metals is facilitated by bacteria by helping to solubilize it making it more easily extracted.

Microorganisms have been used to clean up the environment in a process called bioremediation. In bioremediation a microbe is introduced into an environment where its natural metabolism results in the detoxification or break down of hazardous chemicals or pollutants.

4.Specialized Microbes:

Rhizobia are bacteria that fix nitrogen and make it available for plant nutrition and growth. They form nodules on the roots of legumes.

Azolla is a fee floating water plant that fixes nitrogen in association with cyanobacteria. It acts as a renewable biofertilizer.

Azotobacter are nitrogen fixing bacteria that do not form nodules on plant roots or associate with legumes. They are free living and in addition to fixing nitrogen they can produce antibiotics and beneficial growth substances.

Azospirillum fix nitrogen inside plant roots. They produce beneficial compounds for plant growth and can survive in wetland conditions as well as soils.

Mycorrhiza are fungi that form symbiotic relationships with plant roots. Vesicular arbuscular mycorrhiza (VAM) is the most important member of this group. VAM colonies take up nutrients and water which is available for the plant and they act as root extensions.

Definitions:

Probiotics are live microbes that may have a beneficial affect on a host eating them.

Biofertilizers are living microbes that enrich the nutrient quality of soil.

Biopesticides are microbes which are used to manage pests including insects, nematode or other organisms.

Food additives are substances used to enhance the nutritional value, stabilize or increase the palatability of a food.

VII. CLINICAL PHARMACY

INTRODUCTION

This guidance describes a basic framework for designing and conducting PK/PD studies in pregnant women. It provides recommendations to sponsors on how to assess the influence of pregnancy on the pharmacokinetics (PK), and where appropriate, the pharmacodynamics (PD) of drugs or biologic products.[2] Additionally, this guidance provides recommendations to primary investigators, clinical researchers, and clinical pharmacologists about issues to consider when designing and conducting PK studies in pregnant women.

The Agency recommends using this guidance in conjunction with other FDA and ICH guidances, and pharmacological and clinical literature, on the design, conduct, and interpretation of pharmacokinetic studies. Because the conduct of studies in pregnant women requires specialized knowledge in a variety of areas, investigators designing such studies are encouraged to obtain advice from experts in fields such as obstetrics, pediatrics, pharmacology, clinical pharmacology, pharmacometrics, statistics, and other applicable disciplines.Although this guidance provides recommendations on when PK studies in pregnant women are appropriate, it does not address ways to assess efficacy of a drug in pregnancy or how to assess whether the drug causes adverse pregnancy or neonatal outcomes.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in Agency guidances means that something is suggested or recommended, but not required.

2.Study Design

Study design considerations are important when conducting a study in pregnant women to determine if the PK and/or PD are altered enough to require an adjustment from the established dosage. Ideally, PK studies in pregnancy would be done pre-pregnancy (for baseline comparison) and during all three trimesters, especially for chronically administered drugs. Given the constraints of a study design that enrolls women prior to pregnancy, an alternative can be to determine PK/PD in the second and third trimesters, with the baseline assessment for comparison to the pregnant state done in the postpartum period.

The Agency recommends care be taken to select the most appropriate postpartum time for PK/PD determination, if known. Cardiovascular and renal changes do not return to the pre-pregnancy state until 3 months postpartum. Optimally, postpartum PK/PD assessments for comparative purposes to PK/PD in pregnancy would be done when the woman is neither pregnant nor lactating.

The PK and/or PD study can also be nested within a larger clinical study on safety, efficacy, and outcomes of interest (e.g., Prevost et al. performed a study on the PK of nifedipine on a small subset of patients who were participating in a larger clinical study to assess treatment for pregnancy-induced hypertension (Prevost 1992)).

A. Longitudinal Design :

For drugs that are administered chronically or given for several treatment cycles during pregnancy, a longitudinal study design is most clinically meaningful. This allows for intensive PK studies in pregnant women conducted serially so that each woman serves as her own control, avoiding the common criticism that PK/PD studies in pregnant women are flawed because of the comparison group employed (Reynolds 1991; Little 1999). Such a study would focus on comparing each pregnant woman enrolled at one trimester of pregnancy to the same patient at a different trimester as well as during the postpartum period. We recommend that the rationale for which trimesters are chosen be stated clearly in the study protocol. This longitudinal design minimizes interindividual variability across gestational ages; however, intraindividual variability would be taken into account when determining the sample size. It is important that the analytical plan take into consideration the repeat measures characteristics of a longitudinal design.

Because physiologic changes are continuous throughout pregnancy, and abrupt changes do not necessarily coincide with each trimester shift, the Agency recommends that investigators consider narrowing the time of sampling from trimester to a window of time during each trimester. For example, 4-week windows can be selected for second trimester (e.g., 24-28 weeks) and third trimester assessments (e.g., 34-38 weeks).

The Agency recommends that each woman serve as her own control and have PK/PD determinations performed at different trimesters and in the postpartum period. For certain drugs that are given acutely (e.g., single dose or short course of therapy) it can be difficult to implement a longitudinal design using the same subjects throughout and after pregnancy. For example, in certain circumstances drug therapy may no longer be medically essential in the postpartum period. In these situations, a multi-arm study can be designed to compare different pregnant subjects at different trimesters and in the postpartum period.

B. Population PK Design :

A population PK approach with nonlinear mixed effects modeling techniques can be used as an alternate way to enroll pregnant women in PK studies and minimize the number of blood draws and PD assessments. The population PK approach can assess the impact on the PK of a drug on various covariates, such as maternal characteristics (e.g., age, gravity, parity, race, weeks or trimester of gestation), concomitant medications, and underlying medical conditions. For example, a measure of pregnancy status such as weeks gestation can be one of the covariates, making it possible to model the relationship between gestational age of pregnancy and PK parameters such as the apparent clearance of the drug (CL/F).

In principle, a population PK study design and analysis might detect PK differences large enough to warrant dosage adjustment if the study has enough pregnant and nonpregnant women enrolled with sufficient representation of second and third trimesters (with a continuum of gestational ages from 13 to 40 weeks). Typically, each patient is only sparsely sampled to obtain plasma drug concentration data and/or PD data. Due to the intrinsic characteristics of a population PK study, the controls for this study design can differ from other study designs and can potentially include matched healthy nonpregnant female volunteers. To ensure the ability to determine the inter-occasion variability and prevent a parallel group trial design, a cohort of study subjects would have data collected from all trimesters and the postpartum period.

Some investigators have proposed conducting a population PK study as a preliminary step and to subsequently conduct a standard intensive PK/PD study if the population PK study suggests changes between the pregnant and nonpregnant women (Stika 2000). For further information about the population PK approach, see the Guidance for Industry

3.Other Design Consideration

A. Study Participants :

Study participants should be representative of a typical patient population for the drug to be studied including race, ethnicity, and trimester of pregnancy. Factors with significant potential to affect the PK of a drug to be studied (e.g., age, weight, diet, smoking, concomitant medications, ethnicity, renal function, other medical conditions) can be considered depending on the pharmacologic properties of the drug. The FDA recommends that uniform diagnostic measures be applied to all pregnant women to ensure similarity of diagnosis for the treatment being given and to reduce disease-specific variability in PK. The FDA recommends that measures used for dating the pregnancy be stated clearly in the study protocol and consistently applied throughout the study. Inclusion and exclusion criteria can be tailored to the study.

For drugs that are metabolized by enzymes known to exhibit genetic polymorphism (e.g., CYP2D6 or CYP2C19), the FDA recommends that the investigator consider the metabolic status of the enrolled subjects when analyzing the results of the study. Genotype has been shown to have an effect on pregnancy-related changes in metabolism (Wadelius 1997).

B. Postpartum Assessments :

Physiology changes rapidly at delivery but can take from weeks to months to return to the pre-pregnancy state. The Agency recommends that drugs used only during the peripartum period (e.g., labor and delivery) be studied only at that time. In the peripartum period, PK and receptor sensitivity related to PD can change, so PK/PD studies for drugs used in the peripartum period are important.

A woman's own postpartum PK/PD assessments can serve as a control or comparator for the pregnant state. For women to whom drugs are administered chronically and for whom a pregnancy on the medication of interest is planned, the pre-pregnancy PK/PD assessment can serve as the comparison. For drugs used throughout pregnancy and the postpartum period, PK studies can be performed during the postpartum period to serve as the comparator or control group. Postpartum assessments can potentially be done longitudinally (e.g., at 2, 4, 6, and 8 weeks postpartum) to determine the time course for PK changes to return to the nonpregnant state. Some pregnancy-related medical conditions rapidly improve after delivery such that pharmacologic therapy is no longer needed in the postpartum period (e.g., some cases of pregnancy-induced hypertension or gestational diabetes). In this scenario women can participate in a single-dose PK/PD study in the postpartum period. If a drug possesses linear kinetics, the single-dose PK data can be extrapolated to the multiple-dose steady state kinetics and then compared with steady state kinetics obtained during pregnancy when the drug was administered chronically.

If subjects are breast-feeding during the postpartum portion of the study, the FDA recommends that the study incorporate appropriate safety precautions concerning drug excretion into breast milk and the effects of the drug on the breast-fed infant. The study design should take into account data concerning the pediatric pharmacology and adverse effects of the drug. A lactation study might be performed in conjunction with postpartum sampling.

C. Sample Size :

The objective and design of a study are determining factors in deciding adequate sample size. The number of subjects enrolled in a study should be sufficient to detect PK differences large enough to warrant dosage adjustments. Sample size considerations include PK and PD variability for the drug being studied, the study design (i.e., single-dose versus multiple-dose), and the physiologic changes inherent in pregnancy. For a population PK approach sparse sampling with a larger number of subjects that span the gestational time periods of interest is encouraged.

As a practical matter, it is prudent that the final number of subjects enrolled be in excess of that originally determined by standard sample size calculations to take into account withdrawal of subjects from the study. Even if data for a subject are missing for one trimester, the Agency suggests that the subject be retained in the study for the postpartum assessments.

D. Drug Administration :

In single-dose studies, the same dose can usually be administered to all women in the study. Lower or less frequent doses can be considered to minimize fetal risk in pregnant women who volunteer to take the medication for study purposes, even if it is expected to pose minimal risk at standard doses. The dosage regimen can be adjusted based on the best available pre-study estimates of the PK of the drug and its active metabolites and what is known about drug elimination. A concentration-controlled study design or a dosage adjustment based on the patient's response are alternative methods to consider. For example, the study might be conducted to achieve a specific target concentration using therapeutic drug monitoring procedures. When studying pregnant patients who need the study drug, the dose can be modified, either increased or decreased as pregnancy progresses, to achieve the appropriate response (e.g., lowering of blood pressure, or to decrease adverse events such as hypotensive episodes with antihypertensive therapy).

E. Sample Collection and Analysis :

The Agency recommends that plasma or whole blood samples and urine samples be analyzed for the parent drug and any metabolites with known or suspected activity, therapeutic or adverse. It is recommended that the frequency and duration of plasma sampling and urine collection be sufficient to estimate accurately the relevant PK parameters for the parent drug and its active metabolites (see Section VI, Data Analysis).

Plasma protein binding, like renal function, is often altered in pregnancy.[9] For example, albumin and alpha-1-acid glycoprotein levels are reduced in pregnancy, consequently the protein binding of drugs can be affected. With systemically

active drugs and metabolites, the unbound concentrations are generally believed to determine the rate and extent of delivery to the sites of action. For drugs and metabolites with a relatively low extent of plasma protein binding (e.g., the extent of binding is less than 80 percent), alterations in binding due to pregnancy are small in relative terms. In such cases, description and analysis of the PK in terms of total concentrations would be sufficient. For drugs where the extent of protein binding is greater than 80 percent, primarily to albumin, it is recommended that the PK be described and analyzed with respect to the unbound concentrations of the drug and active metabolites. Although unbound concentrations should be measured in each plasma sample, if the binding is concentration-independent and unaffected by metabolites or other time-varying factors, the fraction unbound can be determined using a limited number of samples or even a single sample from each patient during each trimester. The unbound concentration in each sample should then be estimated by multiplying the total concentration by the fraction unbound for the individual patient.

F. Studies with No Intended Therapeutic Benefit :

It is possible to study drugs that have no intended direct therapeutic benefit to the pregnant woman provided that the risk to the fetus is minimal (45 CFR 46). For example, probe substrates can be used to investigate drug metabolism (e.g., cytochrome P-450 activity) or drug transporter status (e.g., p-glycoprotein). Data from these studies offer generalizable information to other pregnant women but do not offer direct therapeutic benefit to study participants. The Agency encourages sponsors or investigators to explore additional safeguards for human subject protection for this type of study. To minimize exposure to a nontherapeutic drug, each pregnant woman can be exposed to the drug once during pregnancy and in the postpartum period employing a nonlongitudinal design (e.g., one cohort of women sampled in second trimester and postpartum and another cohort of women sampled in third trimester and postpartum). Examples of additional safeguards include administering only products with a long or known record of safety in pregnancy, administering products using only a single dose of the drug, using lower doses of the drug, decreasing the number of

drugs (probe substrates) used in any study subject, and limiting study participants to pregnant women only in second or third trimester.

G. Pharmacodynamic Assessments :

PK studies are usually enhanced by including PD assessments as part of the study. The Agency encourages sponsors to discuss the selection of the PD endpoints with the appropriate FDA review staff. Endpoints would be based on the pharmacological characteristics of the drug and metabolites (e.g., the behavior of other drugs in the same pharmacological class), and include consideration of relevant biomarkers.

4.Data Analysis

The primary intent of the data analysis is to assess whether dosage adjustment is needed for pregnant patients, and, if so, to develop dosing recommendations for such patients based on gestational age or trimester. The analysis, specifically modeling and dosing recommendations, will depend on the study design characteristics. The categorization of pregnancy status, either as nominal (e.g., trimester) or continuous (e.g., week of gestation) data will direct the type of analysis performed. The Agency encourages giving special analytical considerations to longitudinal study designs and the baseline (e.g., postpartum) comparisons. The data analysis typically consists of the following steps:

· Estimation of PK parameters

· Development of dosing recommendations

A. Parameter Estimation :

The Agency recommends that total and unbound plasma concentration data (and urinary excretion data if collected) be used to estimate PK parameters of the parent drug and metabolite(s). Standard PK parameters of a drug include the area under the plasma concentration curve (AUC), peak concentration (Cmax), plasma clearance (CLT) or apparent oral clearance (CL/F), renal clearance (CLR), apparent volume of distribution (VZ/F or Vss/F), and terminal half-life (t1/2). It is

recommended that PK parameters be expressed in terms of total and unbound concentrations and when applicable (e.g., oral and renal clearance, expressed in terms of body weight, L/hr/kg). For drugs and metabolites with a relatively low extent of plasma protein binding (e.g., extent of binding less than 80 percent), description and analysis of the PK in terms of total concentrations can be sufficient. Noncompartmental and/or compartmental modeling approaches to parameter estimation can be employed.

B. Development of Dosing Recommendations :

Specific dosing recommendations should be constructed based on study results. Typically the dose should be adjusted to produce a comparable range of unbound plasma concentrations of drug or active metabolites in both controls and pregnant patients. Simulations are encouraged as a means to identify doses and dosing intervals that achieve that goal for pregnant patients at different trimesters or gestational ages.

One approach might be for the sponsor to recommend, prior to the conduct of the studies, specific no effect boundaries for the ratio of a PK measurement from pregnant patients and controls, such as (AUCu,pregnant/AUCu,control) or (Dpregnant/Dcontrol). If the 90 percent confidence interval for the ratio of PK measurements falls within these boundaries, the sponsor might claim no effect of pregnancy on PK, and it would be reasonable to conclude that no dosage adjustment is needed for pregnancy. The sponsor might determine no effect boundaries from population or individual PK/PD relationships, dose-finding studies and/or dose-response studies which are conducted as part of drug development.

Another approach might be for the sponsor to assume no effect boundaries of 80-125 percent for Cmax and AUC without further justification, recognizing that the small sample sizes in pregnancy studies coupled with high intersubject variability can preclude meeting the 80-125 percent no effect boundaries.

For some drugs, pregnancy may not alter PK sufficiently to warrant dosage adjustment. A sponsor might make this claim by providing an analysis of the study data to show that the PK measurements most relevant to therapeutic outcome in pregnant patients are similar or equivalent to those in the comparator group.

5.Labelling

1.Development of Dosing Recommendations

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For some drugs, pregnancy may not alter PK sufficiently to warrant dosage adjustment. A sponsor might make this claim by providing an analysis of the study data to show that the PK measurements most relevant to therapeutic outcome in pregnant patients are similar or equivalent to those in the comparator group.

2. Special Populations Subsection :

It is recommended that this section recapitulate, in brief, the PK changes found in pregnancy and, if needed, dosing adjustments for pregnant patients. This information should be based on the studies performed as described in this guidance. Reference should be made to the PRECAUTIONS/PREGNANCY and the DOSAGE AND ADMINISTRATION sections. The following text provides examples of possible wording for these sections.

The simplest situation involves drugs for which pregnancy has little or no effect on PK:

The disposition of [Drug X] was studied in [number of] pregnant patients [in y trimester or from a through b weeks gestation]. Pregnancy has little or no influence on [Drug X] pharmacokinetics and no dosing adjustment is needed.

This should be followed by a brief summary of the PK/PD data (e.g., mean, range). Similarly, for drugs whose PK is influenced by pregnancy, the statement similar to the following can be modified as appropriate and in accordance with what is known about the drug (e.g., active or toxic metabolite) and from the studies performed in accordance with this guidance:

The disposition of [Drug X] was studied in [number of] pregnant patients [in y trimester or from a through b weeks gestation]. Elimination of the drug (and metabolite, if applicable) is significantly changed during pregnancy. Total body clearance of (unbound, if applicable) [Drug X]/metabolite was reduced/increased in pregnant patients compared to [healthy postpartum women, the same women prior to pregnancy or c weeks postpartum]. The terminal half-life of [Drug X]/metabolite is [prolonged/decreased] by Y-, and Z- fold in second and third trimesters, respectively. Protein binding of [Drug X]/metabolite [is/is not] affected by pregnancy. The [drug/metabolite accumulates/does not accumulate] in pregnant patients on chronic administration resulting in increased/decreased plasma levels of drug/metabolite. The pharmacologic response [is/is not] affected by pregnancy. The dosage/dosing interval should be [decreased/increased] in pregnant patients receiving [Drug X] (see DOSAGE AND ADMINISTRATION).

B. Precautions/Pregnancy :

In addition to standard labeling for use in pregnancy, including Pregnancy Category, a brief statement regarding PK/PD in pregnancy would be included in the PRECAUTIONS/PREGNANCY section with cross

reference to DOSAGE AND ADMINISTRATION and CLINICAL PHARMACOLOGY sections. If PK studies in pregnancy were not conducted, the Agency recommends that the labeling indicate that.

C. Dosage and Administration :

As appropriate, the following information could be included:

· A statement describing the relationship between the drug's clearance and pregnancy

· A statement describing how the dose would be adjusted during pregnancy, for example:

The dose of [Drug X] should be [increased/decreased by _____%] during pregnancy.

· A statement describing how the dose would be adjusted in the postpartum time period in nonlactating women, specifying the time period studied (e.g., 2 weeks postpartum)

· The dosing adjustment regimen can alternatively be represented in tabular format, for example:

Group	Dosage (mg)	Frequency
1st trimester	x	Every y hours
2nd trimester		
3rd trimester		
Postpartum (specify time)		
Standard adult dose		

The influence of pregnancy on [Drug X] pharmacokinetics is sufficiently small that no dosing adjustment is needed.