**AR-7486**
**B. Pharm. VI SEMESTER**
**EXAMINATION, 2013**
**(Paper fourth)**
**PHARMACOLOGY-IV**

*Time: Three Hours*] [Max. *Marks: 80*

**Note: Question no. 1 is compulsory. Attempt any four questions from section –B.**

**SECTION- A (Objective type questions)**

---------- 12 X 02 = 24

Q.No.1

Ans i): Misoprostol is a synthetic analog of prostaglandin $E_1$. Prostaglandin $E_2$ ($PGE_2$) and prostacyclin ($PGI_2$) are the major prostaglandins synthesized by the gastric mucosa. They bind to the $EP_3$ receptor on parietal cells and stimulate the $G_i$ pathway, thereby decreasing intracellular cyclic AMP and gastric acid secretion. $PGE_2$ also can prevent gastric injury by cytoprotective effects that include stimulation of mucin and bicarbonate secretion and increased mucosal blood flow.

Ans ii) : Loperamide has been shown to be effective against traveller's diarrhea, used either alone or in combination with antimicrobial agents (trimethoprim, trimethoprim-sulfamethoxazole, or a fluoroquinolone). It increases small intestinal and mouth-to-cecum transit times. Loperamide also increases anal sphincter tone, to prevent anal incontinence. In addition, loperamide has antisecretory activity against cholera toxin and some forms of E. coli toxin, presumably by acting on $G_i$-linked receptors and countering the increase in cellular cyclic AMP generated in response to the toxins.

Ans iii): Erythromycin mimick the effects of motilin. Motilin is a 22-amino acid peptide hormone found in the gastrointestinal M cells, as well as in some enterochromaffin cells of the upper small bowel. Motilin is a potent contractile agent of the upper GI tract. Motilin levels fluctuate along with the migrating motor complex and causes amplification of phase III activity. In addition, motilin receptors are found on smooth muscle cells and enteric neurons. Erythromycin induces phase III migrating motor complex activity and increases smooth muscle contractility. It has multiple effects on upper GI motility, increasing lower esophageal pressure and stimulating gastric and small-bowel contractility. By contrast, it has little or no effect on colonic motility.

Ans iv): Carbimazole inhibit the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin. It also inhibits the coupling of these iodotyrosyl residues to form iodothyronines. They interfere with the oxidation of iodide ion and iodotyrosyl groups. These drugs inhibit the peroxidase enzyme, thereby preventing oxidation of iodide or iodotyrosyl groups to the required active state.

Ans v): Regular insulins are solutions of crystalline zinc insulin (insulin injection) dissolved usually in a buffer at neutral pH. These are short and rapid-acting insulins. Short-acting insulin (i.e., regular or soluble) usually should be injected 30 to 45 minutes before meals. Regular insulin also may be given intravenously or intramuscularly.

Lente insulin (insulin zinc suspension) is a mixture of crystallized (ultralente) and amorphous (semilente) insulins in an acetate buffer, which minimizes the solubility of insulin. These are intermediate-acting insulins formulated to dissolve more gradually when administered subcutaneously and thus their durations of action are longer.

Ans vi): Acute adrenal insufficiency is a life-threatening disease, characterized by gastrointestinal symptoms (nausea, vomiting, and abdominal pain), dehydration, hyponatremia, hyperkalemia, weakness, lethargy, and hypotension. It is usually associated with disorders of the adrenal rather than the pituitary or hypothalamus, and sometimes follows abrupt withdrawal of glucocorticoids used at high doses or for prolonged periods.

Hydrocortisone possesses modest but significant mineralocorticoid activity. At doses used for replacement therapy in patients with primary adrenal insufficiency, it causes $Na^+$ retention, and has effects on carbohydrate metabolism (i.e., hepatic deposition of glycogen and gluconeogenesis), and antiinflammatory effects.

Ans vii): Bisphosphonates are analogs of pyrophosphate that contain two phosphonate groups attached to a geminal (central) carbon that replaces the oxygen in pyrophosphate. They form a three-dimensional structure capable of chelating divalent cations such as $Ca^{2+}$, thus have a strong affinity for bone, targeting especially bone surfaces undergoing remodeling. Bisphosphonates inhibit bone resorption. Due to negative charge, bisphosphonates are membrane impermeable but are incorporated into the bone matrix by fluid-phase endocytosis. Bisphosphonates remain in the matrix until the bone is remodeled and then are released in the acid environment of the resorption lacunae beneath the osteoclast as the overlying mineral matrix is dissolved. Their antiresorptive action is due to direct inhibitory effects on osteoclasts rather than strictly physiochemical effects. The antiresorptive activity apparently involves two primary mechanisms: osteoclast apoptosis and inhibition of components of the cholesterol biosynthetic pathway.

Ans viii). Sulfonamides are structural analogs and competitive antagonists of para-aminobenzoic acid (PABA), and prevent normal bacterial utilization of PABA for the synthesis of folic acid (pteroylglutamic acid). More specifically, sulfonamides are competitive inhibitors of dihydropteroate synthase, the bacterial enzyme responsible for the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid.

Ans ix): Cefotaxime is third-generation cephalosporins highly effective against meningitis caused by H. influenzae, penicillin-sensitive S. pneumoniae, and N. meningitides. Cefotaxime is highly resistant to many of the bacterial b-lactamases and has good activity against many gram-positive and gram-negative aerobic bacteria. It has poor activity against B. fragilis.
Cefuroxime is a second-generation cephalosporins that has broader gram-negative activity against some Citrobacter and Enterobacter spp. and have activity against anaerobes. It is active against H. influenzae but not against serratia or B. fragilis. The drug is effective for treatment of meningitis owing to H. influenzae (including strains resistant to ampicillin), N. meningitidis, and S. pneumoniae. Except for cefuroxime axetil, these drugs are not predictably active against penicillin-resistant pneumococci. The oral cefuroxime is active against B -lactamase-producing H influenzae or Moraxella catarrhalis and have been primarily used to treat sinusitis, otitis, or lower respiratory tract infections, in which these organisms have an important role. It is active against B-lactamase-producing H influenzae or K pneumoniae and penicillin-resistant pneumococci.

Ans x): **Urinary Tract Infections.** Gentamycin usually are not indicated for the treatment of uncomplicated urinary tract infections. In the seriously ill patient with pyelonephritis, an gentamycin alone or in combination with a β-lactam antibiotic offers broad and effective initial coverage.
**Pneumonia.** In combination with a β -lactam antibiotic may be used for hospital-acquired pneumonia. Ineffective for the treatment of pneumonia caused by anaerobes or S. pneumoniae. Gentamicin never should be used as the sole agent to treat community acquired pneumonia or nososchomial pneumonia acquired.
**Meningitis.** In infections caused by gram-negative organisms that are resistant to β -lactam antibiotics (e.g., species of Pseudomonas and Acinetobacter).
**Peritoneal Dialysis-Associated Peritonitis.** In patients with peritonitis, due to peritoneal dialysis.
**Bacterial Endocarditis.** "Synergistic" or low-dose gentamicin in combination with a penicillin or vancomycin is recommended in bacterial endocarditis and uncomplicated native-valve streptococcal endocarditis.
**Sepsis.**In febrile patient with granulocytopenia and for infections caused by P. aeruginosa. In non-urinary tract P. aeruginosa infections, particularly pneumonia with bacteremia.
**Topical Applications.** Gentamicin is absorbed slowly when it is applied topically in an ointment and somewhat more rapidly when it is applied as a cream.

Ans xi): Rifampin inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms by forming a stable drug-enzyme complex, leading to suppression of initiation of chain formation (but not chain elongation) in RNA synthesis. More specifically, the β subunit of this complex enzyme is the site of action of the drug, although rifampin binds only to the holoenzyme. High concentrations of rifamycin antibiotics can inhibit RNA synthesis in mammalian mitochondria, viral DNA-dependent RNA polymerases, and reverse transcriptases.
Ans xii): Enzyme involved in Transcription- RNA polymerase

Enzyme involved in Translation- aminoacyl-tRNA synthetase

**SECTION- B** **14X4= 56**

**Q2 to Q.7** (Descriptive type)

Ans 2 : **Dopamine-Receptor Antagonists:** Dopamine receptor antagonists are effective as prokinetic agents, however they have the additional advantage of relieving nausea and vomiting by antagonism of dopamine receptors in the chemoreceptor trigger zone. Ex; Metoclopramide and Domperidone.

**Metoclopramide: Mechanism of Action and Pharmacological Properties.** Metoclopramide is derivative of para-aminobenzoic acid and structurally related to procainamide. The mechanisms of action of metoclopramide are complex and involve 5-$HT_4$-receptor agonism, vagal and central 5-$HT_3$-antagonism, and possible sensitization of muscarinic receptors on smooth muscle, in addition to dopamine receptor antagonism. Its effects are confined largely to the upper digestive tract, where it increases lower esophageal sphincter tone and stimulates antral and small intestinal contractions.

**Pharmacokinetics.** Metoclopramide is absorbed rapidly after oral ingestion, undergoes sulfation and glucuronide conjugation by the liver, and is excreted principally in the urine, with a half-life of 4 to 6 hours. Peak concentrations occur within 1 hour after a single oral dose; the duration of action is 1 to 2 hours.

**Therapeutic Use.** Metoclopramide has been used in gastroesophageal reflux disease. It is less effective than proton pump inhibitors or histamine $H_2$-receptor antagonists. Metoclopramide is more often indicated in symptomatic patients with gastroparesis. Metoclopramide injection is used as an adjunctive measure in medical or diagnostic procedures such as intestinal intubation or contrast radiography of the GI tract. It has limited use in postoperative ileus. **Its greatest use is to ameliorate the nausea and vomiting that often accompany GI dysmotility syndromes.** Metoclopramide has also been used in the treatment of persistent hiccups. The usual initial oral dose range is 10 mg, 30 minutes before each meal and at bedtime. The onset of action is within 30 to 60 minutes after an oral dose. In patients with severe nausea, an initial dose of 10 mg can be given intramuscularly (onset of action 10 to 15 minutes) or intravenously (onset of action 1 to 3 minutes).

**Adverse Effects.** The major side effects of metoclopramide include extrapyramidal effects. Dystonias, usually occurring acutely after intravenous administration, and parkinsonian-like symptoms that may occur several weeks after initiation of therapy generally respond to treatment with anticholinergic or antihistaminic drugs and are reversible upon discontinuation of metoclopramide. Tardive dyskinesia also can occur with chronic treatment (months to years) and may be irreversible. Extrapyramidal effects appear to occur more commonly in children and young adults and at higher doses. Like other dopamine antagonists, metoclopramide also can cause galactorrhea by blocking the inhibitory effect of dopamine on prolactin release. Methemoglobinemia has been reported occasionally in premature and full-term neonates receiving metoclopramide.

**Domperidone; $D_2$ Receptor Antagonists**

In contrast to metoclopramide, domperidone predominantly antagonizes the dopamine $D_2$ receptor without major involvement of other receptors. It has modest prokinetic activity in doses of 10 to 20 mg three times a day. Although it does not readily cross the blood-brain barrier to cause extrapyramidal side effects, domperidone exerts effects in the parts of the CNS that lack this barrier, such as those regulating emesis, temperature, and prolactin release. Domperidone does not appear to have any significant effects on lower gastrointestinal motility.

**Dopamine-Receptor Antagonists**

Phenothiazines such as prochlorperazine, thiethylperazine, and chlorpromazine are among the most commonly used antiemetics. Their effects in this regard are complex, but their principal mechanism of action is dopamine $D_2$ receptor antagonism at the CTZ. Compared to metoclopramide or ondansetron, these drugs do not appear to be as uniformly effective in cancer chemotherapy-induced emesis. On the other hand, they also possess antihistaminic and anticholinergic activities, which are of value in other forms of nausea, such as motion sickness.

**$H_1$-receptor antagonists**

**Pharmacological Properties**

**Smooth Muscle:** $H_1$ antagonists inhibit most of the effects of histamine on smooth muscles, especially the constriction of respiratory smooth muscle. $H_1$ antagonists inhibit both the vasoconstrictor effects of histamine and vasodilator effects that are mediated by activation of $H_1$ receptors on endothelial cells (synthesis/release of NO and other mediators).

**Capillary Permeability:** $H_1$ antagonists strongly block the increased capillary permeability and formation of edema and wheal brought about by histamine.

**Flare and Itch:** $H_1$ antagonists suppress both.

**Exocrine Glands:** $H_1$ antagonists do not suppress gastric secretion, but they do suppress histamine-evoked salivary, lacrimal, and other exocrine secretions. The antimuscarinic properties of many of these agents, however, may contribute to decreased secretion in cholinergically innervated glands.

**Immediate Hypersensitivity Reactions: Anaphylaxis and Allergy:** Edema formation and itch are effectively suppressed.

**Central Nervous System:** The first-generation $H_1$ antagonists can both stimulate and depress the CNS. Stimulation causes restless, nervous, and unable to sleep. Central excitation results in convulsions, particularly in infants. Central depression, on the other hand, usually accompanies older $H_1$ antagonists with diminished alertness, slowed reaction times, and somnolence. The ethanolamines (e.g., diphenhydramine) are particularly prone to cause sedation.

The second-generation ("nonsedating") $H_1$ antagonists (e.g., loratadine, cetirizine, and fexofenadine) do not cross the blood-brain barrier. An interesting and useful property of certain $H_1$ antagonists is the capacity to counter motion sickness. This effect is observed with dimenhydrinate and subsequently with diphenhydramine, various piperazine derivatives, and promethazine.

**Anticholinergic Effects:** Many of the first-generation $H_1$ antagonists inhibit responses to acetylcholine that are mediated by muscarinic receptors. Promethazine has perhaps the strongest muscarinic-blocking activity among these agents and is among the most effective of the $H_1$ antagonists in combating motion sickness. The second-generation $H_1$ antagonists have no effect on muscarinic receptors.

**Local Anesthetic Effect.** Some $H_1$ antagonists have potent local anesthetic activity than procaine. Promethazine is especially active.

**Absorption, Fate, and Excretion.** The $H_1$ antagonists are well absorbed from the GI tract. Following oral administration, peak plasma concentrations are achieved in 2 to 3 hours, and effects usually last 4 to 6 hours; however, some of the drugs are much longer acting. Diphenhydramine, given orally, reaches a maximal concentration in the blood in about 2 hours, remains at about this level for another 2 hours, and then falls exponentially with a plasma elimination half-time of about 4 to 8 hours. The drug is distributed widely throughout the body, including the CNS. Peak concentrations of these drugs are achieved rapidly in the skin and persist after plasma levels have declined. $H_1$-receptor antagonists also induce hepatic cytochrome P450 enzymes (CYPs) and thus may facilitate their own metabolism.

The second-generation $H_1$ antagonist loratadine is absorbed rapidly from the GI tract and metabolized in the liver to an active metabolite by the hepatic CYPs. Cetirizine, loratadine, and fexofenadine are all well absorbed and are excreted mainly in the unmetabolized form. Cetirizine and loratadine are excreted primarily into the urine, whereas fexofenadine is excreted primarily in the feces.

**Side Effects.** The most frequent side effect in the first-generation $H_1$ antagonists is sedation. Concurrent ingestion of alcohol or other CNS depressants produces an additive effect that impairs motor skills. Other untoward central actions include dizziness, tinnitus, lassitude, incoordination, fatigue, blurred vision, diplopia, euphoria, nervousness, insomnia, and tremors.

The next most frequent side effects involve the digestive tract and include loss of appetite, nausea, vomiting, epigastric distress, and constipation or diarrhea. Other side effects apparently owing to the antimuscarinic actions of some of the first-generation $H_1$-receptor antagonists include dryness of the mouth and respiratory passages (sometimes inducing cough), urinary retention or frequency, and dysuria. These effects are not observed with second-generation $H_1$ antagonists.

**Other Adverse Effects:** Drug allergy may develop orally but results more commonly from topical application. Allergic dermatitis is not uncommon; other hypersensitivity reactions include drug fever and photosensitization. Hematological complications such as leukopenia, agranulocytosis, and hemolytic anemia are very rare. Because $H_1$ antihistamines cross the placenta, caution must be used when they are taken by women who are or may become pregnant. Several antihistamines (e.g., azelastine, hydroxyzine, and fexofenadine) showed teratogenic effects in animal studies, whereas others (e.g., chlorpheniramine, diphenhydramine, cetirizine, and loratadine) did not. Antihistamines can be excreted in small amounts in breast milk, and first-generation antihistamines taken by lactating mothers may cause symptoms in the nursing infant such as irritability, drowsiness, or respiratory depression.

**Available $H_1$ Antagonists.**

**Dibenzoxepin Tricyclics (Doxepin).** Doxepin, is a potent $H_1$ antagonist. It can cause drowsiness and is associated with anticholinergic effects.

**Ethanolamines (Diphenhydramine).** These drugs possess significant antimuscarinic activity and have a pronounced tendency to induce sedation. About half of those treated with conventional doses experience somnolence. They have less GI side effects.

**Ethylenediamines (Pyrilamine).** These agents causes somnolence. GI side effects are quite common.

**Alkylamines (Chlorpheniramine).** These drugs produces less drowsiness and are more suitable agents for daytime use, but patients do experience sedation. CNS stimulation is more common.

**First-Generation Piperazines.** The oldest member of this group, chlorcyclizine, has a more prolonged action and produces a comparatively low incidence of drowsiness. Hydroxyzine is a long-acting compound that is used widely for skin allergies; its considerable CNS-depressant activity may contribute to its prominent antipruritic action. Cyclizine and meclizine have been used primarily to counter motion sickness, although promethazine and diphenhydramine (dimenhydrinate) are more effective.

**Second-Generation Piperazines (Cetirizine).** It has minimal anticholinergic effects. It also has negligible penetration into the brain but is associated with a somewhat higher incidence of drowsiness than the other second-generation $H_1$ antagonists.

**Phenothiazines (Promethazine).** Most drugs of this class possess considerable anticholinergic activity. Promethazine, which has prominent sedative effects, and its many congeners are used primarily for their antiemetic effects.

**First-Generation Piperidines (Cyproheptadine, Phenindamine).** Cyproheptadine uniquely has both antihistamine and antiserotonin activity. Cyproheptadine and phenindamine cause drowsiness and also have significant anticholinergic effects.

**Second-Generation Piperidines (Terfenadine).** Current drugs in this class include loratadine, desloratadine, and fexofenadine. These agents are highly selective for $H_1$ receptors, lack significant anticholinergic actions, and penetrate poorly into the CNS.

**Therapeutic Uses**

**Allergic Diseases.** $H_1$ antagonists are most useful in acute types of allergy that present with symptoms of rhinitis, urticaria, and conjunctivitis. It can also be used in the treatment of systemic anaphylaxis and severe angioedema in which autacoids other than histamine play major roles. The best use lies in seasonal rhinitis and conjunctivitis (hay fever, pollinosis), in which these drugs relieve the sneezing, rhinorrhea, and itching of eyes, nose, and throat. $H_1$ antihistamines have been investigated for potential antiinflammatory properties.Certain allergic dermatoses (ex; acute urticaria, pruritus, atopic dermatitis and contact dermatitis) and in such diverse conditions as insect bites and poison ivy respond favorably to $H_1$ antagonists. Doxepin may be more effective in suppressing pruritus than are other antihistamines.

**Common Cold.** The weak anticholinergic effects of the older agents may tend to lessen rhinorrhea, but this drying effect may do more harm than good, as may their tendency to induce somnolence.

**Motion Sickness, Vertigo, and Sedation.** These are most effectively used for the prophylaxis and treatment of motion sickness. These drugs include dimenhydrinate and the piperazines (e.g., cyclizine and meclizine). Promethazine, is more potent and more effective antiemetic but exhibits sedation. Some $H_1$ antagonists, notably dimenhydrinate and meclizine, often are of benefit in vestibular disturbances such as Meniere's disease and in other types of true vertigo. Only promethazine has usefulness in treating the nausea and vomiting subsequent to chemotherapy or radiation therapy for malignancies. $H_1$-receptor antagonists produce somnolence and therefore used as hypnotics. $H_1$ antagonists, principally diphenhydramine, for insomnia is sold over the counter. Hydroxyzine and diphenhydramine are used as sedative and mild anxiolytics.

Ans 3: Five major types of alkylating agents are used in the chemotherapy of neoplastic diseases:

A. Nitrogen mustards: 1. Mechlorethamine hydrochloride 2. Cyclophosphamide 3. Chlorambucil 4. Melphalan 5. Ifosfamide

B. Alkyl sulfonates 1. Busulfan

C. Nitrosoureas 1. Carmustine 2. Lomustine 3. Semustine 4. Streptozocin

D. Ethylenimines 1. Thiotepa

E. Triazenes 1. Dacarbazine

**Mechanism of action:**

These drugs interfere with DNA integrity and function to induce cell death in rapidly proliferating tissues. Lethality of DNA alkylation depends on the recognition of the adduct, the creation of DNA strand breaks by repair enzymes, and an intact apoptotic response. In nondividing cells, DNA damage activates a checkpoint that depends on the presence of a normal p53 gene. Cells thus blocked in the $G_1$/S interface either repair DNA alkylation or undergo apoptosis. Malignant cells with mutant or absent p53 fail to suspend cell-cycle progression, do not undergo apoptosis, and exhibit resistance to these drugs. DNA is the ultimate target of all alkylating agents, however, in bifunctional agents, cytotoxic effects predominate, and in the monofunctional methylating agents (procarbazine, temozolomide), mutagenesis and carcinogenesis predominate. Cross-linking of DNA strands represents a much greater threat to cellular survival than do other effects, such as single-base alkylation and the resulting depurination and chain scission. On the other hand, the more frequent methylation may be bypassed by DNA polymerases, leading to mispairing reactions that permanently modify DNA sequence. These new sequences are transmitted to subsequent generations, and may result in mutagenesis or carcinogenesis. Some methylating agents, such as procarbazine, are highly carcinogenic. The DNA repair systems play an important role in removing adducts, and thereby determine the selectivity of action against particular cell types, and acquired resistance to alkylating agents.

**Nitrogen Mustards**

**Mechlorethamine.**

Mechlorethamine rapidly undergoes chemical degradation as it combines with either water or cellular nucleophiles, and the parent compound disappears within minutes from the bloodstream. Mechlorethamine HCl used primarily in the combination chemotherapy regimen of Hodgkin's disease. It also is used topically for treatment of cutaneous T-cell lymphoma as a solution that is rapidly mixed and applied to affected areas of skin. The major acute toxic manifestations of mechlorethamine are nausea and vomiting, lacrimation, and myelosuppression. Leukopenia and thrombocytopenia limit the amount of drug that can be given in a single course.

**Cyclophosphamide** The drug is not a vesicant, and produces no local irritation. Cyclophosphamide is well absorbed orally. The drug is activated by CYP2B to 4-hydroxycyclophosphamide. 4-Hydroxycyclophosphamide may be oxidized further by aldehyde oxidase, either in liver or in tumor tissue, and perhaps by other enzymes, yielding the inactive metabolites carboxyphosphamide and 4-ketocyclophosphamide, and ifosfamide is inactivated in an analogous reaction. The active cyclophosphamide metabolites such as 4-hydroxycyclophosphamide and its tautomer, aldophosphamide, are carried in the circulation to tumor cells where aldophosphamide cleaves spontaneously, generating stoichiometric amounts of phosphoramide mustard and acrolein. Phosphoramide mustard is responsible for antitumor effects, while acrolein causes hemorrhagic cystitis often seen during therapy with cyclophosphamide. Cystitis can be reduced in

intensity or prevented by the parenteral coadministration of mesna. Ample fluid intake is recommended and vigorous intravenous hydration is required during high-dose treatment. The half-life of parent drug in plasma is about 7 hours. As a single agent, a daily oral dose of 100 mg/$m^2$ for 14 days has been recommended as adjuvant therapy for breast cancer, and for patients with lymphomas and chronic lymphocytic leukemia. A higher dosage of 500 mg/$m^2$ intravenously every 2 to 4 weeks in combination with other drugs often is employed in the treatment of breast cancer and lymphomas. Gastrointestinal ulceration, cystitis (counteracted by mesna and diuresis), and less commonly pulmonary, renal, hepatic, and cardiac toxicities (a hemorrhagic myocardial necrosis) may occur after high-dose therapy with total doses above 200 mg/kg. The clinical spectrum of activity for cyclophosphamide is very broad. It is an essential component of many effective drug combinations for non-Hodgkin's lymphomas, ovarian cancers, and solid tumors in children. Cyclophosphamide is given as a single agent for Burkitt's lymphoma. It frequently is used in combination with methotrexate (or *doxorubicin*) and fluorouracil as adjuvant therapy after surgery for carcinoma of the breast. Because of its potent immunosuppressive properties, cyclophosphamide has been used to prevent organ rejection after transplantation. It has activity in nonneoplastic disorders associated with altered immune reactivity, including Wegener's granulomatosis, rheumatoid arthritis, and the nephrotic syndrome.

**Ifosfamide.** Ifosfamide, an analog of cyclophosphamide, also is activated by ring hydroxylation in the liver. Ifosfamide is approved for use in combination for germ cell testicular cancer and is widely used to treat pediatric and adult sarcomas. It may cause severe neurological toxicity, including hallucinations, coma, and death. This toxicity is thought to result from a metabolite, chloroacetaldehyde. The parent compound, ifosfamide, has an elimination half-life in plasma of approximately 15 hours after doses of 3.8 to 5 g/$m^2$ and a somewhat shorter half-life at lower doses, although its pharmacokinetics are highly variable from patient to patient due to variable rates of hepatic metabolism. Ifosfamide has the same toxicity profile as cyclophosphamide, although it causes greater platelet suppression, neurotoxicity, nephrotoxicity, and in the absence of mesna, urothelial damage.

**Melphalan**

Orally, melphalan is absorbed in an incomplete and variable manner, and 20-50% drug is recovered in the stool. The drug has a half-life in plasma of approximately 45 to 90 minutes, and 10% to 15% of an administered dose is excreted unchanged in the urine. Patients with decreased renal failure may develop unexpectedly severe myelosuppression. Oral *melphalan* for multiple myeloma is used in doses of 6 to 8 mg daily for a period of 4 days, in combination with other agents. Melphalan also may be used in myeloablative regimens followed by bone marrow or peripheral blood stem cell reconstitution. The clinical toxicity of melphalan is mostly hematological and is similar to that of other alkylating agents. Nausea and vomiting are less frequent. Alopecia does not occur at standard doses, and changes in renal or hepatic function have not been observed.

**Chlorambucil**

The cytotoxic effects of chlorambucil on the bone marrow, lymphoid organs, and epithelial tissues are similar to those observed with the nitrogen mustards. Nausea and vomiting may result from single oral doses of 20 mg or more. Oral absorption of chlorambucil is adequate and reliable. The drug has a half-life in plasma of approximately 1.5 hours, and it is almost completely metabolized to phenyl acetic acid mustard and to decomposition products. In treating chronic lymphocytic leukemia (CLL), the standard initial daily dosage of chlorambucil is 0.1 to 0.2 mg/kg, given once daily and continued for 3 to 6 weeks. It is a standard agent for patients with chronic lymphocytic leukemia and primary (Waldenstrom's) macroglobulinemia, and may be used for follicular lymphoma. In CLL, chlorambucil may be given orally for months or years, achieving its effects gradually and often without significant toxicity to a compromised bone marrow. Although it is possible to induce marked hypoplasia of the bone marrow with excessive doses of chlorambucil administered over long periods, its myelosuppressive action usually is moderate, gradual, and rapidly reversible. GI discomfort, azoospermia, amenorrhea, pulmonary fibrosis, seizures, dermatitis, and hepatotoxicity may rarely be encountered.

**Nitrosoureas**

Ex; Carmustine, Lomustine, and Semustine

The nitrosoureas are highly lipid soluble drugs. Carmustine, lomustine, and semustine are chemically unstable, forming highly reactive decomposition products.The chemical half-life of these drugs in plasma is only 5 to 15 minutes.Their marked lipid solubility facilitates distribution into the brain and cerebrospinal fluid (CSF).The chloroethyl moiety of these nitrosoureas is capable of alkylating nucleic acids and proteins and producing single-strand breaks and interstrand cross-linkageof DNA. *Both alkylation and carbamoylation* contribute to the therapeutic and toxic effects of the nitrosoureas. These agents can kill cells in *all* phases of the cell cycle. Oral absorption of lomustine and semustine is complete, but degradation and metabolism are so rapid that the parent drug cannot be detected after oral administration. Although the plasma half-lives of the parent drugs are only a few minutes, degradation products with antitumor activity may persist for longer periods. Carmustine and lomustine can produce remissions that last from 3 to 6 months in 40 to 50% of patients with primary brain tumors. Both drugs also are used as secondary treatment of Hodgkin’s disease and in experimental combination chemotherapy for various types of lung cancer. Other tumors in which remission rates of 10 to 30% have been obtained are non-Hodgkin’s lymphomas, multiple myeloma, melanoma, renal cell carcinoma, and colorectal cancer. The nitrosoureas produce severe nausea and vomiting in most patients 4 to 6 hours after administration.

The major site of dose-limiting toxicity is the bone marrow; leukopenia and thrombocytopenia occur after 4 to

5 weeks. Less frequent side effects include alopecia, stomatitis, and mild abnormalities of liver function. Pulmonary toxicity, manifested by cough, dyspnea, and interstitial fibrosis, is becoming increasingly recognized as a complication of long-term nitrosourea treatment. As alkylating agents, these drugs are potentially mutagenic, teratogenic, and carcinogenic.

**Streptozocin**

Streptozocin, a water-soluble nitrosourea produced by the fungus *Streptomyces achromogenes,* acts through methylation of nucleic acids and proteins. In addition, it produces rapid and severe depletion of the pyridine nucleotides nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH) in liver and pancreatic islets. Streptozocin is not well absorbed from the gastrointestinal tract and must be administered intravenously or intraarterially. In preclinical studies, the plasma half-life was 5 to 10 minutes. Streptozocin produces remission in 50 to 60% of patients with islet cell carcinomas of the pancreas. It is also useful in malignant carcinoid tumors. Almost all patients have nausea and vomiting. The major toxicity is *renal tubular damage,* which may be severe in 5 to 10% of patients taking streptozocin. Treatment of metastatic insulinomas may result in the release of insulin from the tumor and subsequent hypoglycemic coma. Less severe toxicities include diarrhea, anemia, and mild alterations in glucose tolerance orliver function tests.

**Alkyl Sulfonates**

**Busulfan** is a bifunctional methanesulfonic ester that forms intrastrand cross-linkages with DNA.

The drug is well absorbed after oral administration and has a plasma half-life of less than 5 minutes. Metabolites and degradation products are excreted primarily in the urine. Busulfan is used in the palliative treatment of chronic granulocytic leukemia. Daily oral therapy results in decreased peripheral white blood cells and improved symptoms in almost all patients during the chronic phase of the disease. Excessive uric acid production from rapid tumor cell lysis should be prevented by coadministration of allopurinol. At usual therapeutic dosages, busulfan is selectively toxic to granulocyte precursors rather than lymphocytes. Thrombocytopenia and anemia and less commonly, nausea, alopecia, mucositis, and sterility also may occur. Unusual side effects of busulfan include gynecomastia, a general increase in skin pigmentation, and interstitial pulmonary fibrosis.

**Ethylenimines**

**Thiotepa:**Although thiotepa is chemically less reactive than the nitrogen mustards, it is thought to act by similar mechanisms. Its oral absorption is erratic. After intravenous injection, the plasma half-life is less than 2 hours. Urinary excretion accounts for 60 to 80% of eliminated drug. Thiotepa has antitumor activity against ovarian and breast cancers and lymphomas. However, it has been largely supplanted by cyclophosphamide and other nitrogen mustards for treatment of these diseases. It is used by direct instillation into the bladder for multifocal local bladder carcinoma. Nausea and myelosuppression are the major toxicities of thiotepa. It is not a local vesicant and has been safely injected intramuscularly and even intrathecally.

**Triazenes**

**Dacarbazine** is activated by photodecomposition and by enzymatic *N*-demethylation. Eventual formation of a methyl carbonium ion results in methylation of DNA and RNA and inhibition of nucleic acid and protein synthesis. *As with other alkylating agents, cells in all phases of the cell cycle are susceptible to dacarbazine.* The plasma half-life of dacarbazine is biphasic, with a distribution phase of 19 minutes and an elimination phase of 5 hours. The drug is not appreciably protein bound, and it does not enter the central nervous system(CNS). Urinary excretion of unchanged drug is by renal tubular secretion. Dacarbazine metabolism and decomposition is complex. Dacarbazine is the most active agent used in metastatic melanoma, producing a 20% remission rate. It is also combined with doxorubicin and other agents in the treatment of various sarcomas and Hodgkin's disease. Dacarbazine may cause severe nausea and vomiting. Leukopenia and thrombocytopenia occur 2 weeks after treatment, with recovery by 3 to 4 weeks. Less common is a flulike syndrome of fever, myalgias, and malaise. Alopecia and transient abnormalities in renal and hepatic function also have been reported.

**General toxicity produced by cytotoxic drugs in cancer chemotherapy**

**Bone Marrow Toxicity**

The alkylating agents differ in their patterns of antitumor activity and in the sites and severity of their side effects. Most cause dose-limiting toxicity to bone marrow elements, and to a lesser extent, intestinal mucosa. Most alkylating agents, including nitrogen mustard, melphalan, chlorambucil, cyclophosphamide, and ifosfamide, cause acute myelosuppression. Both cellular and humoral immunity are suppressed by alkylating agents, which have been used to treat various autoimmune diseases. Immunosuppression is reversible at doses used in most anticancer protocols.

**Mucosal Toxicity**

In addition to effects on the hematopoietic system, alkylating agents are highly toxic to dividing mucosal cells, leading to oral mucosal ulceration and intestinal denudation. The mucosal effects are particularly significant in high-dose chemotherapy protocols associated with bone marrow reconstitution, as they predispose to bacterial sepsis arising from the gastrointestinal tract.

**Neurotoxicity**

CNS toxicity is manifest in the form of nausea and vomiting, particularly after intravenous administration of nitrogen mustard or BCNU. Ifosfamide is the most neurotoxic of this class of agents, producing altered mental status, coma, generalized seizures, and cerebellar ataxia.

Other **Organ Toxicities**
All alkylating agents have caused pulmonary fibrosis, usually several months after treatment. In high-dose regimens, particularly those employing busulfan or BCNU, vascular endothelial damage may precipitate veno-occlusive disease (VOD) of the liver, an often fatal side effect that is successfully reversed by the investigational drug *defibrotide*. The nitrosoureas and ifosfamide, after multiple cycles of therapy, may lead to renal failure. Cyclophosphamide and ifosfamide causes a severe hemorrhagic cystitis. Proximal, and less commonly distal, tubules may be affected, with difficulties in $Ca^{2+}$ and $Mg^{2+}$ reabsorption, glycosuria, and renal tubular acidosis. Most alkylating agents cause alopecia. Finally, all alkylating agents have toxic effects on the male and female reproductive systems, causing an often permanent amenorrhea, particularly in perimenopausal women, and an irreversible azoospermia in men.
**Leukemogenesis**
As a class of drugs, the alkylating agents are highly leukemogenic. Acute nonlymphocytic leukemia, often associated with partial or total deletions of chromosome 5 or 7, peaks in incidence about 4 years after therapy and may affect up to 5% of patients treated on regimens containing alkylating drugs. It often is preceded by a period of neutropenia or anemia, and bone marrow morphology consistent with myelodysplasia.

Ans : The drugs used in tuberculosis are as follows:
**Isoniazid**
Isoniazid is still considered the primary drug for the chemotherapy of tuberculosis. Isoniazid is bacteriostatic for "resting" bacilli, but is bactericidal for rapidly dividing microorganisms. The minimal tuberculostatic concentration is 0.025 to 0.05 µg/ml. The drug is remarkably selective for mycobacteria, and concentrations in excess of 500 µg /ml are required to inhibit the growth of other microorganisms. Among the various nontuberculous (atypical) mycobacteria, only *M. kansasii* is sometimes susceptible to isoniazid.
**Bacterial Resistance.** The most common mechanism of isoniazid resistance is mutations in catalase-peroxidase (*katg*) that decrease its activity, preventing conversion of the prodrug isoniazid to its active metabolite. Another mechanism of resistance is related to a mutation in the mycobacterial *inhA* and *KasA* genes involved in mycolic acid biosynthesis. Mutations in NADH dehydrogenase (*ndh*) also confer isoniazid resistance. Interestingly, isoniazid-resistant strains of *M. tuberculosis* appear to be less virulent in animal models.
**Mechanism of Action.** Isoniazid is a prodrug; mycobacterial catalase-peroxidase converts isoniazid into an active metabolite. A primary action of isoniazid is to inhibit the biosynthesis of mycolic acids□long, branched lipids that are attached to a unique polysaccharide, arabino galactan, to form part of the mycobacterial cell wall. Mycolic acids are unique to mycobacteria, explaining the high degree of selectivity of the antimicrobial activity of isoniazid. Isoniazid also inhibits mycobacterial catalase-peroxidase (the isoniazid-activating enzyme), which may increase the likelihood of damage to the mycobacteria from reactive oxygen species and $H_2O_2$. Exposure to isoniazid leads to a loss of acid-fastness and a decrease in the quantity of methanol-extractable lipids in the microorganisms.
**Absorption, Distribution, and Excretion.** Isoniazid is readily absorbed when administered either orally or parenterally. Aluminum-containing antacids may interfere with absorption. Peak plasma concentrations of 3 to 5 □g/ml develop 1 to 2 hours after oral ingestion of usual doses. Isoniazid diffuses readily into all body fluids and cells. The drug is distributerd in pleural and ascitic fluids. Isoniazid penetrates well into caseous material. 75% to 95% of a dose of isoniazid is excreted in the urine within 24 hours, mostly as metabolites. The main excretory products in humans result from enzymatic acetylation (acetylisoniazid) and enzymatic hydrolysis (isonicotinic acid). Small quantities of an isonicotinic acid conjugate (probably isonicotinyl glycine), one or more isonicotinyl hydrazones, and traces of *N*-methylisoniazid also are detectable in the urine. The half-life of the drug may be prolonged by hepatic insufficiency. The half-life of isoniazid varies from less than 1 to more than 4 hours.
**Therapeutic Uses.** Isoniazid is still the most important drug worldwide for the treatment of all types of tuberculosis. The commonly used total daily dose of isoniazid is 5 mg/kg, with a maximum of 300 mg; oral and intramuscular doses are identical. Isoniazid may be used as intermittent therapy for tuberculosis; after a minimum of 2 months of daily therapy with isoniazid, rifampin, and pyrazinamide, for sensitive strains of *M. tuberculosis,* patients may be treated with twice-weekly doses of isoniazid (15 mg/kg orally) plus rifampin (10 mg/kg, up to 600 mg per dose) for 4 months. Pyridoxine, vitamin $B_6$, (10 to 50 mg per day) should be administered with isoniazid to minimize the risks of peripheral neuropathy and central nervous system toxicity (*see* below) in malnourished patients and those predisposed to neuropathy (*e.g.,* the elderly, pregnant women, HIV-infected individuals, diabetics, alcoholics, and uremics).
**Untoward Effects.** The most prominent of these reactions were rash (2%), fever (1.2%), jaundice (0.6%), and peripheral neuritis (0.2%). Hypersensitivity to isoniazid may result in fever, various skin eruptions, hepatitis, and morbilliform, maculopapular, purpuric, and urticarial rashes. Hematological reactions also may occur (agranulocytosis, eosinophilia, thrombocytopenia, anemia). Vasculitis associated with antinuclear antibodies may appear during treatment but disappears when the drug is stopped. Arthritic symptoms (back pain; bilateral proximal interphalangeal joint involvement; arthralgia of the knees, elbows, and wrists; and the "shoulder-hand" syndrome) have been attributed to this agent. If pyridoxine is not given concurrently, peripheral neuritis (most commonly paresthesias of feet and hands) is the most common reaction to isoniazid and occurs in about 2% of patients receiving 5 mg/kg of the drug daily. Neuropathy is more frequent in slow acetylators and in individuals with diabetes mellitus, poor nutrition, or anemia. Muscle twitching, dizziness, ataxia,

paresthesias, stupor, and toxic encephalopathy that may be fatal are other manifestations of the neurotoxicity of isoniazid. A number of mental abnormalities may appear during the use of this drug, including euphoria, transient impairment of memory, separation of ideas and reality, loss of self-control, and florid psychoses.

## Rifampin

**Antibacterial Activity.** Rifampin inhibits the growth of most gram-positive bacteria as well as many gram-negative microorganisms such as *Escherichia coli, Pseudomonas,* indole-positive and indole-negative *Proteus,* and *Klebsiella.* Rifampin is very active against *Staphylococcus aureus* and coagulase-negative staphylococci. The drug also is highly active against *Neisseria meningitidis* and *Haemophilus influenzae*; minimal inhibitory concentrations range from 0.1 to 0.8 □g/ml. Rifampin inhibits the growth of *Legionella* species in cell culture and in animal models.

**Bacterial Resistance.** Microbial resistance to rifampin is due to an alteration of the target of this drug, DNA-dependent RNA polymerase, with resistance in most cases being due to mutations between codons 507 and 533 of the polymerase *rpoB* gene.

**Mechanism of Action.** Rifampin inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms by forming a stable drug-enzyme complex, leading to suppression of initiation of chain formation (but not chain elongation) in RNA synthesis. More specifically, the □ subunit of this complex enzyme is the site of action of the drug, although rifampin binds only to the holoenzyme. Nuclear RNA polymerases from a variety of eukaryotic cells do not bind rifampin, and RNA synthesis is correspondingly unaffected in eukaryotic cells. High concentrations of rifamycin antibiotics can inhibit RNA synthesis in mammalian mitochondria, viral DNA-dependent RNA polymerases, and reverse transcriptases. Rifampin is bactericidal for both intracellular and extracellular microorganisms.

**Absorption, Distribution, and Excretion.** The oral administration of rifampin produces peak concentrations in plasma in 2 to 4 hours; after ingestion of 600 mg, this value is about 7 □g/ml, but there is considerable variability. Aminosalicylic acid may delay the absorption of rifampin and cause a failure to reach adequate plasma concentrations. Following absorption from the gastrointestinal tract, rifampin is eliminated rapidly in the bile by enterohepatic circulation. Intestinal reabsorption is reduced by deacetylation (as well as by food), and thus metabolism facilitates elimination of the drug. The half-life of rifampin varies from 1.5 to 5 hours and is increased by hepatic dysfunction; the half-life may be decreased in patients receiving isoniazid concurrently who are slow inactivators of isoniazid. Up to 30% of a dose of the drug is excreted in the urine and 60% to 65% in the feces; less than half of this may be unaltered antibiotic. Rifampin is distributed throughout the body and is present in effective concentrations in many organs and body fluids, including the CSF.

**Therapeutic Uses.** Rifampin for oral administration is available alone and as a fixed-dose combination with isoniazid (150 mg of isoniazid, 300 mg of rifampin or with isoniazid and pyrazinamide (50 mg of isoniazid, 120 mg of rifampin, and 300 mg pyrazinamide). *Rifampin and isoniazid are the most effective drugs available for the treatment of tuberculosis.*

Rifampin, like isoniazid, should never be used alone for the treatment of tuberculosis because of the rapidity with which resistance may develop. Rifampin also is indicated for the prophylaxis of meningococcal disease and *H. influenzae* meningitis.

**Untoward Effects.** Rifampin generally is well tolerated. The most common are rash (0.8%), fever (0.5%), and nausea and vomiting (1.5%). Rarely, hepatitis and deaths due to liver failure have been observed in patients who received other hepatotoxic agents in addition to rifampin, or who had preexisting liver disease. However, chronic liver disease, alcoholism, and old age appear to increase the incidence of severe hepatic problems when rifampin is given alone or concurrently with isoniazid.

A flulike syndrome with fever, chills, and myalgias develops in 20% of patients so treated. The syndrome also may include eosinophilia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock. Gastrointestinal disturbances produced by rifampin (epigastric distress, nausea, vomiting, abdominal cramps, diarrhea) have occasionally required discontinuation of the drug. Various symptoms related to the nervous system also have been noted, including fatigue, drowsiness, headache, dizziness, ataxia, confusion, inability to concentrate, generalized numbness, pain in the extremities, and muscular weakness. Hypersensitivity reactions include fever, pruritus, urticaria, various types of skin eruptions, eosinophilia, and soreness of the mouth and tongue. Hemolysis, hemoglobinuria, hematuria, renal insufficiency, and acute renal failure have been observed rarely; these also are thought to be hypersensitivity reactions. Thrombocytopenia, transient leukopenia, and anemia have occurred during therapy. Since the potential teratogenicity of rifampin is unknown and the drug is known to cross the placenta, it is best to avoid the use of this agent during pregnancy.

## Ethambutol

**Antibacterial Activity, Mechanism of Action, Resistance.** Nearly all strains of *M. tuberculosis* and *M. kansasii* as well as a number of strains of MAC are sensitive to ethambutol. Ethambutol has no effect on other bacteria. It suppresses the growth of most isoniazid- and streptomycin-resistant tubercle bacilli. Bacterial resistance to the drug develops *in vivo via* single amino acid mutations in the *embA* gene when ethambutol is given in the absence of other effective agents.

**Absorption, Distribution, and Excretion.** About 75% to 80% of an orally administered dose of ethambutol is absorbed

from the gastrointestinal tract. A single dose of 25 mg/kg produces a plasma concentration of 2 to 5 µg/ml at 2 to 4 hours. The drug has a half-life of 3 to 4 hours. Within 24 hours, 75% of an ingested dose of ethambutol is excreted unchanged in the urine; up to 15% is excreted in the form of two metabolites, an aldehyde and a dicarboxylic acid derivative.

**Therapeutic Uses.** Ethambutol has been used with notable success in the therapy of tuberculosis of various forms when given concurrently with isoniazid. Because of a lower incidence of toxic effects and better acceptance by patients, ethambutol has essentially replaced aminosalicylic acid. The usual adult dose of ethambutol is 15 mg/kg given once a day. Ethambutol is not recommended for children under 5 years of age, in part because of concern about the ability to test their visual acuity.

**Untoward Effects.** The most important side effect is optic neuritis, resulting in decreased visual acuity and loss of ability to differentiate red from green. Ethambutol produces very few untoward reactions like visual acuity, rash, and drug fever. Other side effects that have been observed are pruritus, joint pain, gastrointestinal upset, abdominal pain, malaise, headache, dizziness, mental confusion, disorientation, and possible hallucinations. Numbness and tingling of the fingers owing to peripheral neuritis are infrequent. Anaphylaxis and leukopenia are rare.

## Streptomycin

**Antibacterial Activity.** Streptomycin is bactericidal for the tubercle bacillus *in vitro.* The vast majority of strains of *M. tuberculosis* are sensitive to 10 □g/ml. *M. kansasii* is frequently sensitive, but other nontuberculous mycobacteria are only occasionally susceptible.

**Bacterial Resistance.** Large populations of all strains of tubercle bacilli include a number of cells that are markedly resistant to streptomycin because of mutation. However, primary resistance to the antibiotic is found in only 2% to 3% of isolates of *M. tuberculosis.*

**Therapeutic Uses.** Since other effective agents have become available, the use of streptomycin for the treatment of pulmonary tuberculosis has been sharply reduced. Many clinicians prefer to give 4 drugs, of which streptomycin may be one, for the most serious forms of tuberculosis, such as disseminated disease or meningitis. For tuberculosis, adults should be given 15 mg/kg per day in divided doses given by intramuscular injection every 12 hours, not to exceed 1 g per day.

**Untoward Effects.** These involved the auditory and vestibular functions of the eighth cranial nerve. Other relatively frequent problems included rash (in 2%) and fever (in 1.4%).

## Pyrazinamide

Pyrazinamide is the synthetic pyrazine analog of nicotinamide.

**Antibacterial Activity.** Pyrazinamide exhibits bactericidal activity *in vitro* only at a slightly acidic pH. Activity at acid pH is ideal, since *M. tuberculosis* resides in an acidic phagosome within the macrophage. Resistance develops rapidly if pyrazinamide is used alone. The target of pyrazinamide appears to be the mycobacterial fatty acid synthase I gene involved in mycolic acid biosynthesis.

**Absorption, Distribution, and Excretion.** Pyrazinamide is well absorbed from the gastrointestinal tract and widely distributed throughout the body. The plasma half-life is 9 to 10 hours in patients with normal renal function. The drug is excreted primarily by renal glomerular filtration. Pyrazinamide is distributed widely□including to the CNS, lungs, and liver after oral administration. Penetration of the drug into the CSF is excellent. Pyrazinamide is hydrolyzed to pyrazinoic acid and subsequently hydroxylated to 5-hydroxypyrazinoic acid, the major excretory product.

**Therapeutic Uses.** Pyrazinamide has become an important component of short-term (6-month) multiple-drug therapy of tuberculosis. Pyrazinamide is available in tablets for oral administration. The daily dose for adults is 15 to 30 mg/kg orally, given as a single dose. The maximum quantity to be given is 2 g per day, regardless of weight. Children should receive 15 to 30 mg/kg per day; daily doses also should not exceed 2 g. Pyrazinamide has been safe and effective when administered twice or thrice weekly (at increased dosages).

**Untoward Effects.** Injury to the liver is the most serious side effect of pyrazinamide. When a dose of 40 to 50 mg/kg is administered orally, signs and symptoms of hepatic disease appear in about 15% of patients, with jaundice in 2% to 3% and death due to hepatic necrosis in rare instances. Elevations of plasma alanine and aspartate aminotransferases are the earliest abnormalities produced by the drug.

The drug inhibits excretion of urate, resulting in hyperuricemia in nearly all patients; acute episodes of gout have occurred. Other untoward effects that have been observed with pyrazinamide are arthralgias, anorexia, nausea and vomiting, dysuria, malaise, and fever.

## Quinolones

The fluoroquinolones are highly active against *M. tuberculosis* as well as nontuberculous mycobacteria and are important components of treatment regimens of multidrug-resistant tuberculosis The C-8-methoxy-fluoroquinolones, such as gatifloxacin and moxifloxacin, are the most active and therefore least likely to result in the development of quinolone resistance. Unfortunately, when resistance develops to one fluoroquinolone in mycobacteria, cross-resistance develops within this entire class of antibiotics.

## Linezolid

Linezolid is highly active *in vitro* against *M. tuberculosis* and some nontuberculous mycobacteria.

## Interferon- α

*Interferon-* **α** activates macrophages to kill *M. tuberculosis*. Aerosol delivery of IFN- **α** to the lungs of patients with multidrug-resistant tuberculosis results in wide pulmonary distribution and enhanced local immune stimulation.

## Ethionamide

**Antibacterial Activity, Resistance.** Resistance can develop rapidly when ethionamide is used as a single-agent treatment, and can include low-level cross-resistance to isoniazid .

**Mechanism of Action.** Ethionamide is also an inactive prodrug that is activated by a mycobacterial redux system. EtaA, an NADPH-specific, FAD-containing monooxygenase, converts ethionamide to a sulfoxide, and thence to 2-ethyl-4-aminopyridine. Ethionamide inhibits mycobacterial growth by inhibiting the activity of the *inhA* gene product, the enoyl-ACP reductase of fatty acid synthase II. inhibition of mycolic acid biosynthesis and consequent impairment of cell-wall synthesis.

**Absorption, Distribution, and Excretion.** The half-life of the drug is about 2 hours. Approximately 50% of patients are unable to tolerate a single dose larger than 500 mg because of gastrointestinal disturbance. Ethionamide is rapidly and widely distributed; the concentrations in the blood and various organs are approximately equal. Significant concentrations are present in CSF. Ethionamide is cleared by hepatic metabolism; like aminosalicylic acid, ethionamide inhibits the acetylation of isoniazid *in vitro.* Less than 1% of ethionamide is excreted in an active form in the urine.

**Therapeutic Uses.** *Ethionamide is a secondary agent, to be used concurrently with other drugs only when therapy with primary agents is ineffective or contraindicated.*

Ethionamide is administered only orally. The initial dosage of ethionamide for adults is 250 mg twice daily; it is increased by 125 mg per day every 5 days until a dose of 15 to 20 mg/kg per day is achieved.

**Untoward Effects.** The most common reactions to ethionamide are anorexia, nausea and vomiting, gastric irritation, and a variety of neurologic symptoms. Severe postural hypotension, mental depression, drowsiness, and asthenia are common. Convulsions and peripheral neuropathy are rare. Other reactions referable to the nervous system include olfactory disturbances, blurred vision, diplopia, dizziness, paresthesias, headache, restlessness, and tremors. Pyridoxine (vitamin $B_6$) relieves the neurologic symptoms and its concomitant administration is recommended. Severe allergic skin rashes, purpura, stomatitis, gynecomastia, impotence, menorrhagia, acne, and alopecia also have been observed. A metallic taste also may be noted. Hepatitis has been associated with the use of the drug in about 5% of cases. The signs and symptoms of hepatotoxicity clear when treatment is stopped. Hepatic function should be assessed at regular intervals in patients receiving ethionamide.

## Aminosalicylic Acid

**Antibacterial Activity.** Aminosalicylic acid is bacteriostatic. The antimicrobial activity of aminosalicylic acid is highly specific, and microorganisms other than *M. tuberculosis* are unaffected. Most nontuberculous mycobacteria are not inhibited by the drug. Aminosalicylic acid alone is of little value in the treatment of tuberculosis in humans.

**Mechanism of Action.** Aminosalicylic acid is a structural analog of *para*-aminobenzoic acid, and its mechanism of action appears to be very similar to that of the sulfonamides.

**Absorption, Distribution, and Excretion.** Aminosalicylic acid is readily absorbed from the gastrointestinal tract. The sodium salt is absorbed even more rapidly. The drug appears to be distributed throughout the total body water and reaches high concentrations in pleural fluid and caseous tissue but CSF levels are low. The drug has a half-life of about 1 hour. Over 80% of the drug is excreted in the urine; more than 50% is in the form of the acetylated compound; the largest portion of the remainder is made up of the free acid.

**Therapeutic Uses.** Aminosalicylic acid is a second-line agent. Its importance in the management of pulmonary and other forms of tuberculosis has markedly decreased since more active and better-tolerated drugs, such as rifampin and ethambutol, have been developed.

**Untoward Effects.** The incidence of untoward effects associated with the use of aminosalicylic acid is approximately 10% to 30%. Gastrointestinal problems□including anorexia, nausea, epigastric pain, abdominal distress, and diarrhea□are predominant and often limit patient adherence.

## Cycloserine

Cycloserine is a broad-spectrum antibiotic produced by *Streptococcus orchidaceus.* cycloserine is used in conjunction with other tuberculostatic drugs in the treatment of pulmonary or extrapulmonary tuberculosis when primary agents (isoniazid, rifampin, ethambutol, pyrazinamide, streptomycin) have failed. The drug is stable in alkaline solution but is rapidly destroyed when exposed to neutral or acidic pH.

**Mechanism of Action.** Cycloserine and D-alanine are structural analogs; thus, cycloserine inhibits reactions in which D-alanine is involved in bacterial cell-wall synthesis. The use of medium free of D-alanine reveals that the antibiotic inhibits the growth *in vitro* of enterococci, *E. coli, S. aureus, Nocardia* species, and *Chlamydia.*

**Absorption, Distribution, and Excretion.** When given orally, 70% to 90% of cycloserine is rapidly absorbed. Peak concentrations in plasma are reached 3 to 4 hours after a single dose. Cycloserine is distributed throughout body fluids

and tissues. There is no appreciable blood-brain barrier to the drug, and CSF concentrations are approximately the same as those in plasma. About 50% of a parenteral dose of cycloserine is excreted unchanged in the urine in the first 12 hours. Very little of the antibiotic is metabolized. The drug may accumulate to toxic concentrations in patients with renal insufficiency.

**Therapeutic Uses.** Cycloserine should be used only when retreatment is necessary or when microorganisms are resistant to other drugs. When cycloserine is employed to treat tuberculosis, it must be given together with other effective agents. Cycloserine is available for oral administration. The usual dose for adults is 250 to 500 mg twice daily.

**Untoward Effects.** Reactions to cycloserine most commonly involve the central nervous system. Among the central manifestations are somnolence, headache, tremor, dysarthria, vertigo, confusion, nervousness, irritability, psychotic states with suicidal tendencies, paranoid reactions, catatonic and depressed reactions, twitching, ankle clonus, hyperreflexia, visual disturbances, paresis, and tonic-clonic or absence seizures. Large doses of cycloserine or the concomitant ingestion of alcohol increases the risk of seizures. Cycloserine is contraindicated in individuals with a history of epilepsy and should be used with caution in individuals with a history of depression, as suicide is a risk.

**Dapsone:**

The sulfones are derivatives of 4,4□-diaminodiphenylsulfone (dapsone), all of which have certain pharmacological properties in common. Dapsone was found to be effective in suppressing experimental infections with the tubercle bacillus and for human leprosy.

**Antibacterial Activity, Mechanism of Action, and Resistance.** Dapsone is bacteriostatic, but not bactericidal, for *M. leprae,* and the estimated sensitivity to the drug is between 1 and 10 □g/ml for microorganisms recovered from untreated patients. *M. leprae* may become resistant to the drug during therapy.

The mechanism of action of the dapsone is the same as that of the sulfonamides: they are competitive inhibitors of dihydropteroate synthase and prevent the normal bacterial utilization of *para*-amino-benzoic acid. Both possess approximately the same range of antibacterial activity and both are antagonized by *para*-aminobenzoic acid. Dapsone-resistant strains of *M. leprae* are termed *secondary* if they emerge during therapy. Secondary resistance usually is seen in lepromatous (multibacillary) patients treated with a single drug.

**Therapeutic Uses.** Dapsone is available for oral administration. Daily therapy with 100 mg has been successful in adults. Therapy usually is begun with smaller amounts, and doses are increased to those recommended over 1 to 2 months. Therapy should be continued for at least 3 years and may be necessary for the lifetime of the patient.

**Untoward Effects.** The most common is hemolysis of varying degree. Doses of 100 mg or less in normal healthy persons and 50 mg or less in healthy individuals with a glucose-6-phosphate dehydrogenase deficiency do not cause hemolysis. Methemoglobinemia also is common, and Heinz-body formation may occur. A genetic deficiency in the NADH-dependent methemoglobin reductase can result in severe methemoglobinemia after administration of dapsone. Anorexia, nausea, and vomiting may follow the oral administration of sulfones. Isolated instances of headache, nervousness, insomnia, blurred vision, paresthesias, reversible peripheral neuropathy (thought to be due to axonal degeneration), drug fever, hematuria, pruritus, psychosis, and a variety of skin rashes have been reported. An infectious mononucleosis-like syndrome, which may be fatal, occurs occasionally. The sulfones may induce an exacerbation of lepromatous leprosy by a process thought to be analogous to the Jarisch-Herxheimer reaction. This "sulfone syndrome" may develop 5 to 6 weeks after initiation of treatment in malnourished people. Its manifestations include fever, malaise, exfoliative dermatitis, jaundice with hepatic necrosis, lymphadenopathy, methemoglobinemia, and anemia.

**Absorption, Distribution, and Excretion.** Dapsone is absorbed rapidly and nearly completely from the gastrointestinal tract. The di-substituted sulfones, such as sulfoxone, are absorbed incompletely when administered orally, and large amounts are excreted in the feces. Peak concentrations of dapsone in plasma are reached within 2 to 8 hours after administration; the mean half-life of elimination is about 20 to 30 hours. About 70% of the drug is bound to plasma protein. The sulfones are distributed throughout total body water and are present in all tissues. They tend to be retained in skin and muscle and especially in liver and kidney; traces of the drug are present in these organs up to 3 weeks after therapy is stopped. The sulfones are retained in the circulation for a long time because of intestinal reabsorption from the bile; periodic interruption of treatment is advisable for this reason. Dapsone is acetylated in the liver, and the rate of acetylation is genetically determined; the same enzyme carries out the acetylation of isoniazid. Daily administration of 50 to 100 mg results in serum levels exceeding the usual minimal inhibitory concentrations, even in rapid acetylators, in whom the serum half-life of dapsone is shorter than usual. Approximately 70% to 80% of a dose of dapsone is excreted in the urine. The drug is present in urine as an acid-labile mono-*N*-glucuronide and mono-*N*-sulfamate in addition to an unknown number of unidentified metabolites.

Ans 5. **Physiological actions of thyroid hormones**;

**Growth and Development.** Thyroid hormones seem to exert most of their effects through control of DNA transcription, and ultimately protein synthesis, profoundly influencing normal growth and development. Thyroid hormone plays a critical role in brain development. The appearance of functional, chromatin-bound thyroid hormone receptors coincides with neurogenesis in the brain. The absence of thyroid hormone during the period of active neurogenesis (up to 6 months postpartum) leads to irreversible mental retardation (cretinism) and is accompanied by multiple morphological alterations

in the brain. These severe morphological alterations result from disturbed neuronal migration, deranged axonal projections, and decreased synaptogenesis. Thyroid hormone supplementation during the first 2 weeks of life prevents the development of these disturbed morphological changes.
Myelin basic protein, a major component of myelin, is regulated by thyroid hormone during development, and decreased expression of myelin basic protein in the hypothyroid brain impairs myelinization. Altered expression of laminin likely alters neuronal migration and leads to the morphological abnormalities observed in the cretinous brain.
**Calorigenic Effects.** A characteristic response of homeothermic animals to thyroid hormone is increased $O_2$ consumption. Most peripheral tissues contribute to this response; heart, skeletal muscle, liver, and kidney are stimulated markedly by thyroid hormone. Several organs, including brain, gonads, and spleen, are unresponsive to the calorigenic effects of thyroid hormone. Thyroid hormone-dependent lipogenesis may constitute a quantitatively important energy sink. The observation that $T_3$ stimulates lipolysis provides a link between lipogenesis and thermogenesis. Further, thyroid hormone induces expression of several lipogenic enzymes, including malic enzyme and fatty acid synthase.
**Cardiovascular Effects.** Thyroid hormone influences cardiac function by direct and indirect actions; changes in the cardiovascular system are prominent clinical consequences in thyroid dysfunctional states. In hyperthyroidism, there is tachycardia, increased stroke volume, increased cardiac index, cardiac hypertrophy, decreased peripheral vascular resistance, and increased pulse pressure. In hypothyroidism, there is bradycardia, decreased cardiac index, pericardial effusion, increased peripheral vascular resistance, decreased pulse pressure, and elevation of mean arterial pressure.
Thyroid hormones directly regulate myocardial gene expression. $T_3$ regulates genes encoding the isoforms of the sarcomeric myosin heavy chains by increasing the expression of the a gene and decreasing the expression of the b gene. Interestingly, $T_3$ appears to have a direct, nongenomic vasodilating effect on vascular smooth muscle.
**Metabolic Effects.** Thyroid hormones stimulate metabolism of cholesterol to bile acids, and hypercholesterolemia is a characteristic feature of hypothyroid states. Thyroid hormones increase the specific binding of low-density lipoprotein (LDL) by liver cells, and the concentration of hepatic receptors for LDL is decreased in hypothyroidism.
Thyroid hormones enhance the lipolytic responses of fat cells to other hormones (*e.g.,* catecholamines) and elevated plasma free fatty acid concentrations are seen in hyperthyroidism. Postreceptor defects in the liver and peripheral tissues, manifested by depleted glycogen stores and enhanced glucogenesis, lead to insulin insensitivity. In addition, there is increased absorption of glucose from the gut. Compensatory increases in insulin secretion result in order to maintain euglycemia. Hypothyroidism results in decreased absorption of glucose from the gut, decreased insulin secretion, and a reduced rate of peripheral glucose uptake; however, glucose utilization by the brain is unaffected.

**Antithyroid drugs.** 1) *thioureylenes* (2) *aniline derivatives*and (3) *polyhydric phenols,* such as resorcinol. Antithyroid drugs inhibit the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin; they also inhibit the coupling of these iodotyrosyl residues to form iodothyronines. This implies that they interfere with the oxidation of iodide ion and iodotyrosyl groups. Drugs inhibit the peroxidase enzyme, thereby preventing oxidation of iodide or iodotyrosyl groups to the required active state. The antithyroid drugs bind to and inactivate the peroxidase only when the heme of the enzyme is in the oxidized state. Over a period of time, the inhibition of hormone synthesis results in the depletion of stores of iodinated thyroglobulin as the protein is hydrolyzed and the hormones are released into the circulation. Only when the preformed hormone is depleted and the concentrations of circulating thyroid hormones begin to decline do clinical effects become noticeable.
Propylthiouracil and methimazole: In addition to blocking hormone synthesis, propylthiouracil partially inhibits the peripheral deiodination of $T_4$ to $T_3$. *Methimazole* does not have this effect. The effect of a dose of 100 mg of propylthiouracil begins to wane in 2 to 3 hours, and even a 500-mg dose is completely inhibitory for only 6 to 8 hours. The half-life of propylthiouracil in plasma is about 75 minutes, whereas that of methimazole is 4 to 6 hours. The drugs are concentrated in the thyroid, and methimazole, derived from the metabolism of carbimazole, accumulates after carbimazole is administered. Drugs and metabolites appear largely in the urine. Propylthiouracil and methimazole cross the placenta equally and also can be found in milk. The use of these drugs during pregnancy is discussed below. The incidence of side effects from propylthiouracil and methimazole as currently used is relatively low. The adverse effect of propylthiouracil and methimazole, is the most serious reaction, agranulocytosis. The development of agranulocytosis with methimazole is probably dose-related, but no such relationship exists with propylthiouracil. Mild granulocytopenia, if noted, may be due to thyrotoxicosis or may be the first sign of this dangerous drug reaction.
**Therapeutic Uses.** The antithyroid drugs are used in the treatment of hyperthyroidism in the following three ways: (1) as definitive treatment, to control the disorder in anticipation of a spontaneous remission in Graves' disease; (2) in conjunction with radioactive iodine, to hasten recovery while awaiting the effects of radiation; and (3) to control the disorder in preparation for surgical treatment.

## Ionic Inhibitors

The *ionic inhibitors* interfere with the concentration of iodide by the thyroid gland. They are all monovalent, hydrated anions of a size similar to that of iodide. For example, *thiocyanate,* is not concentrated by the thyroid gland, but in large amounts may inhibit the organification of iodine. Thiocyanate is produced following the enzymatic hydrolysis of certain plant glycosides.
Among other anions, *perchlorate* ($ClO_4^-$) is ten times as active as thiocyanate. Perchlorate blocks the entrance of iodide

into the thyroid by competitively inhibiting the NIS. Although perchlorate can be used to control hyperthyroidism, it has caused fatal aplastic anemia when given in excessive amounts (2 to 3 g daily). Other ions, selected on the basis of their size, also have been found to be active; *fluoborate* ($BF_4^-$) is as effective as perchlorate.

## Iodide

**Mechanism of Action.** Iodide causes acute inhibition of the synthesis of iodotyrosines and iodothyronines known as the *Wolff-Chaikoff effect*. This transient, 2-day inhibition is observed only above critical concentrations of intracellular rather than extracellular concentrations of iodide. With time, "escape" from this inhibition is associated with an adaptive decrease in iodide transport and a lowered intracellular iodide concentration, most likely due to a decrease in NIS mRNA and protein.

**Therapeutic Uses.** Iodide is used in the treatment of hyperthyroidism, preoperative period in preparation for thyroidectomy, and in conjunction with antithyroid drugs and propranolol, in the treatment of thyrotoxic crisis.

**Untoward Reactions.** Angioedema is the outstanding symptom, and laryngeal edema may lead to suffocation. Multiple cutaneous hemorrhages may be present. Also, manifestations of the serum-sickness type of hypersensitivity, such as fever, arthralgia, lymph node enlargement, and eosinophilia may appear. Thrombotic thrombocytopenic purpura and fatal periarteritis nodosa attributed to hypersensitivity to iodide also have been described. The symptoms start with an unpleasant brassy taste and burning in the mouth and throat as well as soreness of the teeth and gums. Increased salivation is noted. Coryza, sneezing, and irritation of the eyes with swelling of the eyelids are commonly observed. Mild iodism simulates a "head cold." Irritation of the mucous glands of the respiratory tract causes a productive cough. Excess transudation into the bronchial tree may lead to pulmonary edema. In addition, the parotid and submaxillary glands may become enlarged and tender, and the syndrome may be mistaken for mumps parotitis. There also may be inflammation of the pharynx, larynx, and tonsils. Skin lesions are common and vary in type and intensity. They usually are mildly acneform and distributed in the seborrheic areas.

## Radioactive Iodine

**Chemical and Physical Properties.** Although iodine has several radioactive isotopes, greatest use has been made of $^{131}I$. It has a half-life of 8 days; therefore, more than 99% of its radiation is expended within 56 days. Its radioactive emissions include both αrays and β particles. The short-lived radionuclide of iodine, $^{123}I$, is primarily a γ-emitter with a half-life of only 13 hours. This permits a relatively brief exposure to radiation during thyroid scans.

**Effects on the Thyroid Gland.** The chemical behavior of the radioactive isotopes of iodine is identical to that of the stable isotope, $^{127}I$. $^{131}I$ is rapidly and efficiently trapped by the thyroid, incorporated into the iodoamino acids, and deposited in the colloid of the follicles, from which it is slowly liberated. Thus, the destructive  particles originate within the follicle and act almost exclusively upon the parenchymal cells of the thyroid, with little or no damage to surrounding tissue. The  radiation passes through the tissue and can be quantified by external detection. The effects of the radiation depend on the dosage. When small tracer doses of $^{131}I$ are administered, thyroid function is not disturbed. However, when large amounts of radioactive iodine gain access to the gland, the characteristic cytotoxic actions of ionizing radiation are observed. Pyknosis and necrosis of the follicular cells are followed by disappearance of colloid and fibrosis of the gland. With properly selected doses of $^{131}I$, it is possible to destroy the thyroid gland completely without detectable injury to adjacent tissues. After smaller doses, some of the follicles, usually in the periphery of the gland, retain their function.

**Therapeutic Uses.** Radioactive iodine finds its widest use in the treatment of hyperthyroidism and in the diagnosis of disorders of thyroid function. *Sodium iodide* $^{131}$ *I* is available as a solution or in capsules containing essentially carrier-free $^{131}I$ suitable for oral administration. Sodium iodide $^{123}I$ is available for scanning procedures.

***Hyperthyroidism.*** The use of stable iodide as treatment for hyperthyroidism, however, may preclude treatment and certain imaging studies with radioactive iodine for weeks after the iodide has been discontinued.

***Dosage and Technique.*** $^{131}I$, 7000 to 10,000 rads per gram of thyroid tissue, is administered orally.

***Disadvantages.*** The chief disadvantage of the use of radioactive iodine is the high incidence of delayed hypothyroidism that is induced. Another disadvantage of radioactive iodine therapy is the long period of time that is sometimes required before the hyperthyroidism is controlled.

***Contraindications.*** *The main contraindication for the use of* $^{131}$ *I therapy is pregnancy.* After the first trimester, the fetal thyroid will concentrate the isotope and thus suffer damage; even during the first trimester, radioactive iodine is best avoided because there may be adverse effects of radiation on fetal tissues.

6. Short notes on:

Ans a: Oral contraceptives are among the most widely used agents throughout the world and provide a convenient, affordable, and completely reliable means of contraception for family planning and the avoidance of unplanned pregnancies.

**Combination Oral Contraceptives.** The most frequently used agents are combination oral contraceptives containing both an estrogen and a progestin. Their theoretical efficacy generally is considered to be 99.9%. Ethinyl estradiol and mestranol are the two estrogens used (with ethinyl estradiol being much more frequently used); several progestins currently are used, with levonorgestrel probably being the most common worldwide. The progestins are 19-nor

compounds in the estrane or gonane series, and each has varying degrees of androgenic, estrogenic, and anti-estrogenic activities that may be responsible for some of their side effects. Compounds such as desogestrel and norgestimate are the most recently developed and have less androgenic activity than other 19-nor compounds.

Combination oral contraceptives are available in many formulations. **Monophasic, biphasic, or triphasic pills** are generally provided in 21-day packs. For the monophasic agents, fixed amounts of the estrogen and progestin are present in each pill, which is taken daily for 21 days, followed by a 7-day "pill-free" period. (Virtually all preparations come as 28-day packs, with the pills for the last 7 days containing only inert ingredients.) The biphasic and triphasic preparations provide two or three different pills containing varying amounts of active ingredients, to be taken at different times during the 21-day cycle. This reduces the total amount of steroids administered and more closely approximates the estrogen-to-progestin ratios that occur during the menstrual cycle. With these preparations, predictable menstrual bleeding generally occurs during the 7-day "off" period each month. Additional options include a once-monthly medroxyprogesterone-estradiol cypionate injectable, an ethinyl estradiol-*norelgestromin* (the active metabolite of norgestimate) patch applied weekly, and an ethinyl estradiol-*etonogestrel* (the active metabolite of desogestrel) flexible vaginal ring used for 3 weeks (followed by a removal for 1 week that leads to menstrual bleeding).

The estrogen content of current preparations ranges from 20 to 50 mg; the majority contain 30 to 35 mg. Preparations containing 35 mg or less of an estrogen are generally referred to as "low-dose" or "modern" pills. The dose of progestin is more variable because of differences in potency of the compounds used.

**Progestin-Only Contraceptives.** They are only slightly less efficacious than combination oral contraceptives, with reports of theoretical efficacy of 99%. Specific preparations include the "minipill"; low doses of progestins (*e.g.,* 350 mg of norethindrone or 75 mg of norgestrel) taken daily without interruption.

**Postcoital or Emergency Contraceptives.** High doses of diethylstilbestrol and other estrogens once were used for postcoital contraception (the "morning-after pill"). Two doses of the "minipill" (0.75 mg levonorgestrel per pill) can be taken separated by 12 hours. Two 2-pill doses of a high-dose oral contraceptive (0.25 mg of levonorgestrel and 0.05 mg of ethinyl estradiol per pill) can be taken separated by 12 hours. The first dose of such preparations should be taken anytime within 72 hours after intercourse, and this should be followed 12 hours later by a second dose. This treatment reduces the risk of pregnancy following unprotected intercourse by approximately 60% for the Yuzpe method and 80% for levonorgestrel alone. With either preparation, effectiveness appears to increase the sooner after intercourse the pills are taken.

## Mechanism of Action

**Combination of oral contraceptives.** Combination oral contraceptives act by preventing ovulation. Direct measurements of plasma hormone levels indicate that LH and FSH levels are suppressed, a mid-cycle surge of LH is absent, endogenous steroid levels are diminished, and ovulation does not occur. While either component alone can be shown to exert these effects in certain situations, the combination synergistically decreases plasma gonadotropin levels and suppresses ovulation more consistently than either alone. Progesterone clearly diminishes the frequency of GnRH pulses. Since the proper frequency of LH pulses is essential for ovulation, this effect of progesterone likely plays a major role in the contraceptive action of these agents. Estrogens also suppress FSH release from the pituitary during the follicular phase of the menstrual cycle, and this effect seems likely to contribute to the lack of follicular development in oral contraceptive users. Pharmacologically, the progestin component may also inhibit the estrogen-induced LH surge at mid-cycle. In the cervix, progestin effects also are likely to produce a thick, viscous mucus to reduce sperm penetration and in the endometrium to produce a state that is not receptive to implantation.

**Progestin-Only Contraceptives.** Progestin-only pills and levonorgestrel implants are highly efficacious but block ovulation in only 60% to 80% of cycles. Their effectiveness is thus thought to be due largely to a thickening of cervical mucus, which decreases sperm penetration, and to endometrial alterations that impair implantation.

**Emergency Contraceptive Pills.** Multiple mechanisms are likely to contribute to the efficacy of these agents, but their precise contributions are unknown. Some studies have shown that ovulation is inhibited or delayed, but additional mechanisms thought to play a role include alterations in endometrial receptivity for implantation; interference with functions of the corpus luteum that maintain pregnancy; production of a cervical mucus that decreases sperm penetration; alterations in tubular transport of sperm, egg, or embryo; or effects on fertilization. However, emergency contraceptives do not interrupt pregnancy after implantation.

## Untoward Effects

**Combination Oral Contraceptives.** Untoward effects of early hormonal contraceptives fell into several major categories: adverse cardiovascular effects, including hypertension, myocardial infarction, hemorrhagic or ischemic stroke, and venous thrombosis and embolism; breast, hepatocellular, and cervical cancers; and a number of endocrine and metabolic effects.

**Cardiovascular effects:** There is a 28% increase in relative risk for venous thromboembolism. In women who smoke or have other factors may predispose to thrombosis or thromboembolism. Early high-dose combination oral contraceptives caused hypertension in 4% to 5% of normotensive women and increased blood pressure in 10% to 15% of those with pre-existing hypertension.

**Cancer:** Given the growth-promoting effects of estrogens, there has been a long-standing concern that oral contraceptives might increase the incidence of endometrial, cervical, ovarian, breast, and other cancers. Overall there is no significant

difference in the cumulative risk of breast cancer between those who have ever used oral contraceptives and those who have never used them.

**Metabolic and Endocrine Effects:** High-dose oral contraceptives generally reported impaired glucose tolerance as demonstrated by increases in fasting glucose and insulin levels and responses to glucose challenge. The high-dose progestins in early oral contraceptives did raise LDL and reduce HDL levels, but modern low-dose preparations do not produce unfavorable lipid profiles. The estrogenic component of oral contraceptives may increase hepatic synthesis of a number of serum proteins, including those that bind thyroid hormones, glucocorticoids, and sex steroids.

The ethinyl estradiol present in oral contraceptives appears to cause a dose-dependent increase in several serum factors known to increase coagulation.

**Miscellaneous Effects:** Nausea, edema, and mild headache occur in some individuals, and more severe migraine headaches may be precipitated by oral contraceptive use in a smaller fraction of women. Some patients may experience breakthrough bleeding during the 21-day cycle when the active pills are being taken. Withdrawal bleeding may fail to occur in a small fraction of women during the 7-day "off" period, thus causing confusion about a possible pregnancy. Acne and hirsutism are thought to be mediated by the androgenic activity of the 19-nor progestins.

Ans b:**Physiological actions of estrogen:**

**Developmental Actions.** Estrogens are largely responsible for pubertal changes in girls and secondary sexual characteristics. They cause growth and development of the vagina, uterus, and fallopian tubes, and contribute to breast enlargement. They also contribute to molding the body contours, shaping the skeleton, and causing the pubertal growth spurt of the long bones and epiphyseal closure. Growth of axillary and pubic hair, pigmentation of the genital region, and the regional pigmentation of the nipples and areolae that occur after the first trimester of pregnancy are also estrogenic actions. Androgens may also play a secondary role in female sexual development.

Estrogens appear to play important developmental roles in males. In boys, estrogen deficiency diminishes the pubertal growth spurt and delays skeletal maturation and epiphyseal closure so that linear growth continues into adulthood. Estrogen deficiency in men leads to elevated gonadotropins, macroorchidism, and increased testosterone levels and also may affect carbohydrate and lipid metabolism and fertility in some individuals.

**Neuroendocrine Control of the Menstrual Cycle.**

The gonadotropins (LH and FSH) regulate the growth and maturation of the graafian follicle in the ovary and the ovarian production of estrogen and progesterone, which exert feedback regulation on the pituitary and hypothalamus. In the early follicular phase of the cycle: (1) the pulse generator produces bursts of neuronal activity with a frequency of about one per hour that correspond with pulses of GnRH secretion; (2) these cause a corresponding pulsatile release of LH and FSH from pituitary gonadotropes; and (3) FSH in particular causes the graafian follicle to mature and secrete estrogen. The effects of estrogens on the pituitary are inhibitory at this time and cause the amount of LH and FSH released from the pituitary to decline (*i.e.,* the amplitude of the LH pulse decreases), so gonadotropin levels gradually fall. At mid-cycle, serum estradiol rises above a threshold level of 150 to 200 pg/ml for approximately 36 hours. This sustained elevation of estrogen no longer inhibits gonadotropin release but exerts a brief positive feedback effect on the pituitary to trigger the preovulatory surge of LH and FSH.

The mid-cycle surge in gonadotropins stimulates follicular rupture and ovulation within 1 to 2 days. The ruptured follicle then develops into the corpus luteum, which produces large amounts of progesterone and lesser amounts of estrogen under the influence of LH during the second half of the cycle. In the absence of pregnancy, the corpus luteum ceases to function, steroid levels drop, and menstruation occurs. Estrogens act primarily on the pituitary to control the amplitude of gonadotropin pulses, and may also contribute to the amplitude of GnRH pulses secreted by the hypothalamus.

In the follicular phase of the cycle, estrogens inhibit gonadotropin release, but then have a brief mid-cycle stimulatory action that increases the amount released and causes the LH surge.

**Effects of Cyclical Gonadal Steroids on the Reproductive Tract.** The cyclical changes in estrogen and progesterone production by the ovaries regulate corresponding events in the fallopian tubes, uterus, cervix, and vagina. Physiologically, these changes prepare the uterus for implantation, and the proper timing of events in these tissues is essential for pregnancy. If pregnancy does not occur, the endometrium is shed as the menstrual discharge. The uterus is composed of an endometrium and a myometrium. These cell layers, the fallopian tubes, cervix, and vagina display a characteristic set of responses to both estrogens and progestins. The changes typically associated with menstruation occur largely in the endometrium. The overall endometrial response involves estrogen- and progesterone-mediated expression of peptide growth factors and receptors, cell cycle genes, and other regulatory signals. An important response to estrogen in the endometrium and other tissues is induction of the progesterone receptor (PR), which enables cells to respond to this hormone during the second half of the cycle. In later stages the placenta itself becomes the major site of estrogen and progesterone synthesis. Estrogens and progesterone have important effects on the fallopian tube, myometrium, and cervix. In the fallopian tube, estrogens stimulate proliferation and differentiation, whereas progesterone inhibits these processes. Also, estrogens increase and progesterone decreases tubal muscular contractility, which affects transit time of the ovum to the uterus. Estrogens increase the amount of cervical mucus and its water content to facilitate sperm penetration of the cervix, whereas progesterone generally has opposite effects. Estrogens favor rhythmic contractions of the uterine

myometrium, while progesterone diminishes contractions.

**Metabolic Effects.** It long has been recognized that estrogens have positive effects on bone mass. Estrogens directly regulate osteoblasts and increase the synthesis of type I collagen, osteocalcin, osteopontin, osteonectin, alkaline phosphatase, and other markers of differentiated osteoblasts. Estrogens also increase osteocyte survival by inhibiting apoptosis. However, the major effect of estrogens is to decrease the number and activity of osteoclasts. Much of the action of estrogens on osteoclasts appears to be mediated by altering cytokine (both paracrine and autocrine) signals from osteoblasts. Estrogens decrease osteoblast and stromal cell production of the osteoclast-stimulating cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α and increase the production of insulin-like growth factor (IGF)-1, bone morphogenic protein (BMP)-6, and transforming growth factor (TGF)-α, which are anti-resorptive. Estrogens also increase osteoblast production of the cytokine osteoprotegrin (OPG), a soluble non-membrane-bound member of the TNF superfamily. OPG acts as a "decoy" receptor that antagonizes the binding of OPG-ligand (OPG-L) to its receptor (termed RANK, or receptor activator of NF-kB) and prevents the differentiation of osteoclast precursors to mature osteoclasts. Estrogens increase osteoclast apoptosis, either directly or by increasing OPG. Estrogens have anti-apoptotic effects on both osteoblasts and osteocytes in animal models, and this action may be mediated by nongenomic mechanisms. Estrogens affect bone growth and epiphyseal closure in both sexes.

Estrogens have many effects on lipid metabolism; of major interest are their effects on serum lipoprotein and triglyceride levels. In general, estrogens slightly elevate serum triglycerides and slightly reduce total serum cholesterol levels. More important, they increase HDL levels and decrease the levels of LDL and Lp(a). This beneficial alteration of the ratio of HDL to LDL is an attractive effect of estrogen therapy in postmenopausal women. Estrogens also alter bile composition by increasing cholesterol secretion and decreasing bile acid secretion. This leads to increased saturation of bile with cholesterol and gallstone formation in some women receiving estrogens. Estrogen alone slightly decreases fasting levels of glucose and insulin but does not have major effects on carbohydrate metabolism. Estrogens affect many serum proteins, particularly those involved in hormone binding and clotting cascades. In general, estrogens increase plasma levels of cortisol-binding globulin, thyroxine-binding globulin, and sex hormone-binding globulin (SHBG), which binds both androgens and estrogens.

Estrogens alter a number of metabolic pathways that affect the cardiovascular system. Systemic effects include changes in lipoprotein metabolism and in hepatic production of plasma proteins. Estrogens cause a small increase in coagulation factors II, VII, IX, X, and XII, and decrease the anticoagulation factors protein C, protein S, and antithrombin III. At relatively high concentrations, estrogens have antioxidant activity and may inhibit the oxidation of LDL by affecting superoxide dismutase. Estrogen actions on the vascular wall include increased production of NO, which occurs within minutes *via* a mechanism involving activation of Akt (also known as protein kinase B), and induction of inducible NO synthase and increased production of prostacyclin. All of these changes promote vasodilation. Estrogens also promote endothelial cell growth while inhibiting the proliferation of vascular smooth muscle cells.

**Physiological and Pharmacological Actions of progestins**

**Neuroendocrine Actions.** As discussed above, progesterone produced in the luteal phase of the cycle has several physiological effects including decreasing the frequency of GnRH pulses, which is the major mechanism of action of progestin-containing contraceptives.

**Reproductive Tract.** Progesterone decreases estrogen-driven endometrial proliferation and leads to the development of a secretory endometrium, and the abrupt decline in progesterone at the end of the cycle is the main determinant of the onset of menstruation. Under normal circumstances, estrogen antecedes and accompanies progesterone in its action upon the endometrium and is essential to the development of the normal menstrual pattern. Progesterone also influences the endocervical glands, and the abundant watery secretion of the estrogen-stimulated structures is changed to a scant, viscid material. As noted previously, these and other effects of progestins decrease penetration of the cervix by sperm. The estrogen-induced maturation of the human vaginal epithelium is modified toward the condition of pregnancy by the action of progesterone, a change that can be detected in cytological alterations in the vaginal smear. Progesterone is very important for the maintenance of pregnancy. Progesterone suppresses menstruation and uterine contractility, but other effects also may be important.

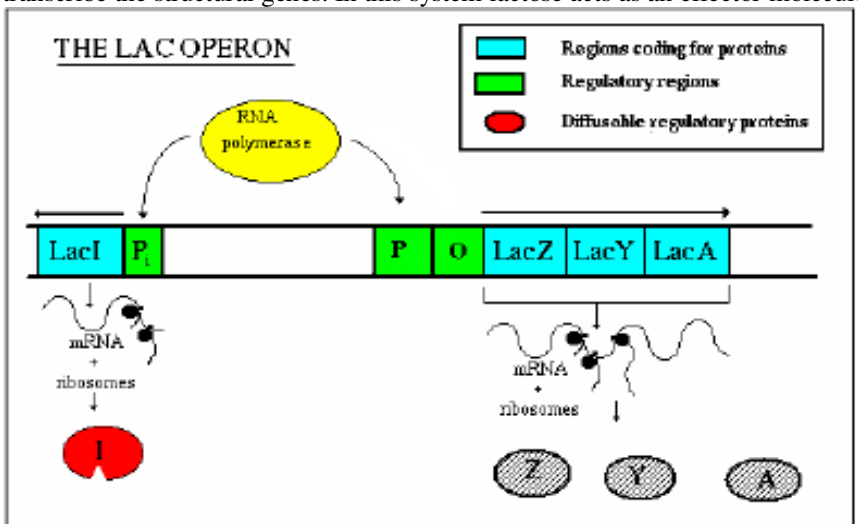
**Mammary Gland.** Development of the mammary gland requires both estrogen and progesterone. During pregnancy and to a minor degree during the luteal phase of the cycle, progesterone, acting with estrogen, brings about a proliferation of the acini of the mammary gland. Toward the end of pregnancy, the acini fill with secretions and the vasculature of the gland notably increases; however, only after the levels of estrogen and progesterone decrease at parturition does lactation begin.

During the normal menstrual cycle, mitotic activity in the breast epithelium is very low in the follicular phase and then peaks in the luteal phase. This pattern is due to progesterone, which triggers a *single* round of mitotic activity in the mammary epithelium. This effect is transient, however, and continued exposure to the hormone is rapidly followed by arrest of growth of the epithelial cells. As described above, progesterone may be responsible for the increased risk of breast cancer associated with estrogen-progestin use in postmenopausal women.

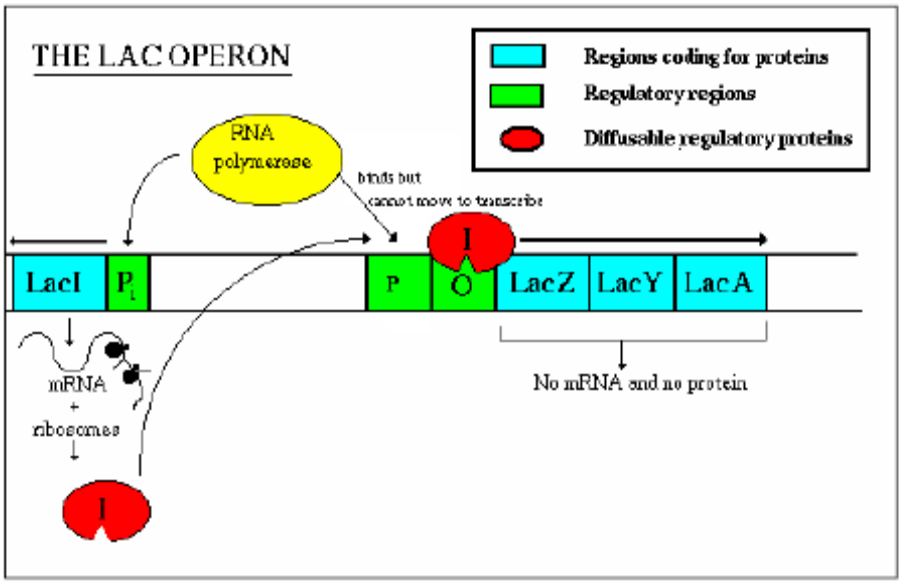
**CNS Effects.** During a normal menstrual cycle, an increase in basal body temperature of about 0.6□C (1□F) may be noted at mid-cycle; this correlates with ovulation. This increase is due to progesterone. Progesterone also increases the

ventilatory response of the respiratory centers to carbon dioxide and leads to reduced arterial and alveolar $PCO_2$ in the luteal phase of the menstrual cycle and during pregnancy. Progesterone also may have depressant and hypnotic actions in the CNS, possibly accounting for reports of drowsiness after hormone administration.
**Metabolic Effects.** Progesterone itself increases basal insulin levels and the rise in insulin after carbohydrate ingestion, but it does not normally alter glucose tolerance. Progesterone stimulates lipoprotein lipase activity and seems to enhance fat deposition. Progesterone increase LDL and cause either no effects or modest reductions in serum HDL levels. The 19-norprogestins may have more pronounced effects on plasma lipids because of their androgenic activity. Progesterone also may diminish the effects of aldosterone in the renal tubule and cause a decrease in sodium reabsorption that may increase mineralocorticoid secretion from the adrenal cortex.

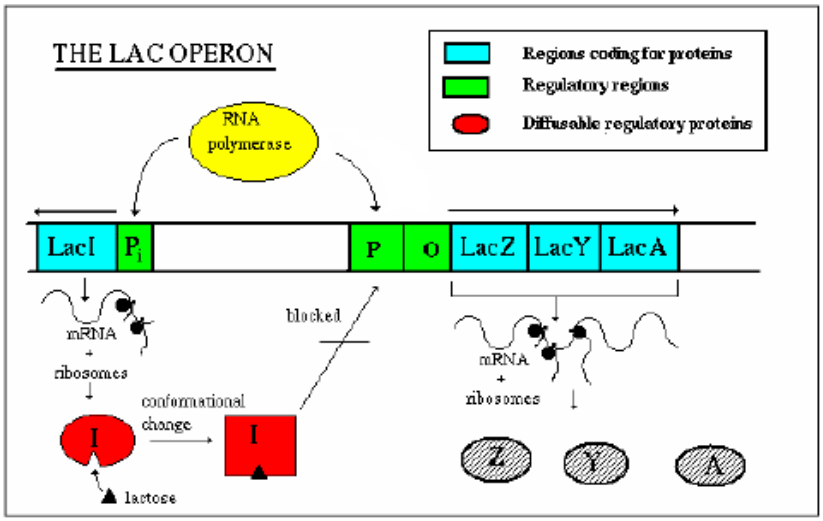
7.
Ans: Control of gene expression in prokaryotes occurs at the level of transcription.
**Induction** - the production of a specific enzyme (or set of enzymes) in response to the presence of a substrate
**Repression** - the cessation of production of a specific enzyme (or set of enzymes) in response to an increased level of a substrate
All of the genes which encode the enzymes necessary for the pathway are found next to each other on the E. coli chromosome. One key feature of both systems to be discussed is that a single mRNA is transcribed with multiple translation stop codons. The proteins that can be translated from the mRNA are the enzymes required for a specific pathway. This type of mRNA is called a polycistronic mRNA and is totally unique to prokaryotes.
Jacob and Monod were the first scientists to elucidate a transcriptionally regulated system. They worked on the lactose metabolism system in E. Coli. When the bacterium is in an environment that contains lactose, it should turn on the enzymes that are required for lactose degradation. These enzymes are:
**beta-galactosidase:**
This enzyme hydrolyzes the bond between the two sugars, glucose and galactose. It is coded for by the gene LacZ.
**Lactose Permease:**
This enzyme spans the cell membrane and brings lactose into the cell from the outside environment. The membrane is otherwise essentially impermeable to lactose. It is coded for by the gene LacY.
**Thiogalactoside transacetylase:**
The function of this enzyme is not known. It is coded for by the gene LacA.
**Operon -** a cluster of structural genes that are expressed as a group and their associated promoter and operator.
The components of lac operon
**Effector molecule** - a molecule that interacts with the repressor and affects the affinity of the repressor for the operator
**Repressor**- The regulator protein which can bind to the operator in the absence of inducer or effector blocking RNA polymerase to transcribe through the structural genes.
**Mechanism:** Without lactose in the cell, the repressor protein binds to the operator and prevents the read through of RNA polymerase into the three structural genes. With lactose in the cell, lactose binds to the repressor. This causes a structural change in the repressor and it loses its affinity for the operator. Thus RNA polymerase can then bind to the promoter and transcribe the structural genes. In this system lactose acts as an effector molecule.



## Lac operon in action: When lactose is not present.




## Lac operon in action- when lactose (inducer) is present

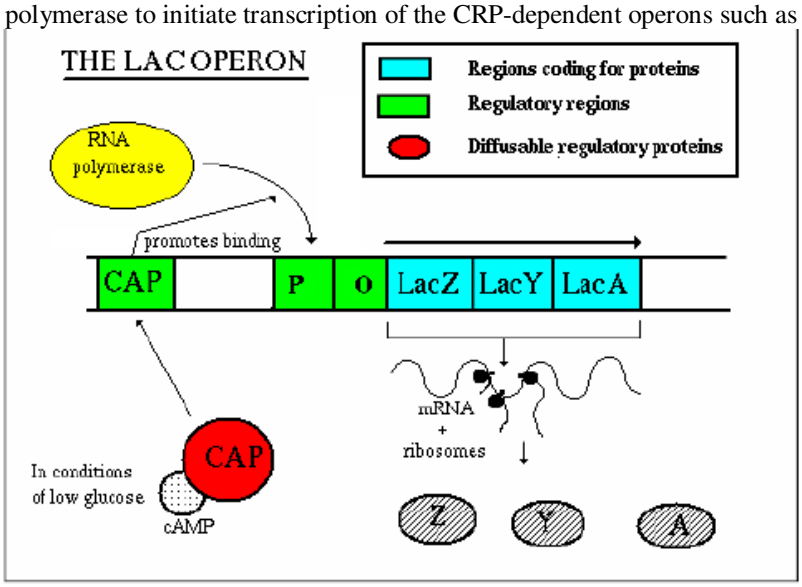



IPTG (isopropyl-beta-D-thiogalactoside) is a molecule that looks very much like lactose to the Lac repressor (Lactose). Thus, this molecule can be used as a **gratuitous inducer**, because it will induce the Lac operon by altering the conformation of LacI so that it can no longer block the promoter, but it is not a substrate for the lactose metabolism genes.

**Catabolic repression:**

The efficient initiation of transcription by RNA polymerase at the promoter of the lac operon requires not only the absence of bound lac repressor but also the presence of cyclic AMP and cyclic AMP receptor protein, which is called CAP or CRP protein. This auxiliary induction requirement is thought to result from the fact that the carbon and energy requirements of E. coli are most efficiently met by catabolizing glucose rather than other sugars. Consequently, cells have evolved a way to shut down the possibly inefficient use of other carbon utilization pathways if glucose is present. This phenomenon is known as the glucose effect or catabolite repression. The glucose effect is generated in two ways: by excluding inducers of some operons from the cell and by reducing the inducibility of some operons. When glucose is

present and is being metabolized, the concentrations of cAMP are low, and few CRP proteins contain bound cAMP. Only when CRP has bound cAMP, which occurs when glucose is absent, can the protein specifically bind to DNA and assist RNA polymerase to initiate transcription of the CRP-dependent operons such as lac.




**catabolite repression**

When levels of glucose (a *catabolite*) in the cell are high, a molecule called cyclic AMP is inhibited from forming. So when glucose levels drop, more cAMP forms. cAMP binds to a protein called CAP (catabolite activator protein), which is then activated to bind to the CAP binding site. This activates transcription, perhaps by increasing the affinity of the site for RNA polymerase. This phenomenon is called **catabolite repression**, a misnomer since it involves activation, but understandable since it seemed that the presence of glucose repressed all the other sugar metabolism operons. Because the CAP-cAMP complex is needed for transcription, the complex exerts a **positive control** over the expression of the *lac* operon.