المستخلصات الحيوية

Bio extracts

natural products

هي المركبات التي يتم استخلاصها من الكائنات الحية باستخدام طرق استخلاص مختلفة

ماهي هذه المركبات؟

1-مركبات الايض الاولية Primary Metabolism

2-مركبات الايض الثانوية Secondary Metabolism

-Construct common **biological macromolecules** from simple building blocks found within every cell

- Typically a process of **polymerization**, stringing monomers together into a macromolecule that performs a cellular function

sugars	polysaccharides
amino acids	proteins
fats	phospholipid bilayers

- Block production: cell **dies** (primary metabolites are essential

Natural Products: Folk to Modern Medicines

- Humans have long used chemicals in plant and animal extracts for many purposes: medicines, poisons, recreational stimulants

- Since the 1800's, chemists have characterized and synthesized such **natural products**, uncovering the basis for folk remedies

- Identifying the mechanism of action of natural products was the genesis of modern drug discovery

- The active ingredients in many ancient curatives are still used medicinally today

- Synthesize compounds that are **unique to a particular species**
  or genus (unlike common proteins, lipids, etc.)
  - Molecules may have extremely complex structures

- These molecules typically have **no** effect on the producing organism, but are often highly biologically active against **other** organisms (competitors, pathogens, predators)
  - Often present at extraordinary concentrations, >10% of the dry weight of the organism

inference is, they must do <u>something</u>

some terms:-

pharmacognocy ,

Pharmacological activity , medical activity , pharmaceutical , pharmacopeia , Extract , extract , crud extract , pure extract , active compounds , isolation techniques

Alkaloids

Definition: the term “alkaloid” (alkali-like) is commonly used to designate basic heterocyclic nitrogenous compounds of plant origin that are physiologically active.

Deviation from Definition:

- Basicity: Some alkaloids are not basic e.g. Colchicine, Piperine, Quaternary alkaloids.
- Nitrogen: The nitrogen in some alkaloids is not in a heterocyclic ring e.g. Ephedrine, Colchicine, Mescaline.
- Plant Origine: Some alkaloids are derived from Bacteria, Fungi, Insects, Frogs, Animals.

Classification:

- **True (Typical) alkaloids** that are derived from amino acids and have nitrogen in a heterocyclic ring. e.g Atropine
- **Protoalkaloids** that are derived from amino acids and do not have nitrogen in a heterocyclic ring. e.g Ephedrine
- **Pseudo alkaloids** that are not derived from amino acids but have nitrogen in a heterocyclic ring. e.g Caffeine
- **False alkaloids** are non alkaloids give false positive reaction with alkaloidal reagents.

New Definition: Alkaloids are cyclic organic compounds containing nitrogen in a negative state of oxidation with limited distribution among living organisms.

Distribution and occurrence:

- Rare in lower plants.
- Dicots are more rich in alkaloids than Monocots.
- Families rich in Alkaloids: Apocynaceae, Rubiaceae, Solanaceae and Papaveracea.
- Families free from Alkaloids: Rosaceae, Labiatae

Distribution in Plant:

- All Parts e.g. Datura.
- Barks e.g. Cinchona
- Seeds e.g. Nux vomica
- Roots e.g. Aconite
- Fruits e.g. Black pepper
- Leaves e.g. Tobacco
- Latex e.g. Opium

Forms of Alkaloids:

- Free bases
- Salts with Organic acids e.g. **Oxalic, acetic acids**
- Salts with inorganic acids e.g. **HCl, H_2SO_4**.
- Salts with special acids:
  e.g. **Meconic acid** in Opium
  Quinic acid in *Cinchona*
- Glycosidal form e.g. Solanine in *Solanum*.

Function in Plants

- They may act as **protective** against insects and herbivores due to their bitterness and toxicity.
- They are, in certain cases, the final **products of detoxification (waste products)**.
- **Source of nitrogen** in case of nitrogen deficiency.
- They, sometimes, act as **growth regulators** in certain metabolic systems.
- They may be utilized as a **source of energy** in case of deficiency in carbon dioxide assimilation.

Nomenclature:

Trivial names should end by **"ine"**. These names may refer to:

- The **genus** of the plant, such as Atropine from *Atropa belladona*.
- The plant **species**, such as Cocaine from *Erythroxylon coca*.
- The **common name** of the drug, such as Ergotamine from ergot.
- The name of the **discoverer**, such as Pelletierine that was discovered by Pelletier.
- The **physiological action**, such as Emetine that acts as emetic, Morphine acts as narcotic.
- A prominent **physical character**, such as Hygrine that is hygroscopic.

Physical Properties:

I- Condition:

- **Most** alkaloids are crystalline **solids**.
- Few alkaloids are amorphous solids e.g. emetine.
- **Some** are **liquids** that are either:

  Volatile e.g. nicotine and coniine, or

  Non-volatile e.g. pilocarpine and hyoscine.

II- Color:

The **majority** of alkaloids are **colorless** but **some** are **colored** e.g.:

- Colchicine and berberine are yellow.
- Canadine is orange.
- The salts of sanguinarine are copper-red.

Physical Properties:

III- Solubility:

- Both **alkaloidal bases** and their **salts** are **soluble in alcohol**.
- Generally, the **bases** are **soluble in organic solvents** and **insoluble in water**

Exceptions:

- Bases **soluble in water**: caffeine, ephedrine, codeine, colchicine, pilocarpine and quaternary ammonium bases.
- Bases **insoluble or sparingly soluble in certain organic solvents**: morphine in ether, theobromine and theophylline in benzene.

- **Salts** are usually **soluble in water** and, **insoluble or sparingly soluble in organic solvents.**

Exceptions:

- Salts **insoluble in water**: quinine monosulphate.
- Salts **soluble in organic solvents**: lobeline and apoatropine hydrochlorides are soluble in chloroform.

Chemical Properties:

I- Nitrogen:

Primary amines	$R\text{-}NH_2$	e.g.	Norephedrine
Secondary amines	$R_2\text{-}NH$	e.g.	Ephedrine
Tertiary amines	$R_3\text{-}N$	e.g.	Atropine
Quaternary ammonium salts	$R_4\text{-}N$	e.g	*d*-Tubocurarine

II- Basicity:

- $R_2\text{-}NH > R\text{-}NH_2 > R_3\text{-}N$
- Saturated hexacyclic amines is more basic than aromatic amines.

Qualitative test for Alkaloids:

- **Precipitation Reagents:**

They are used to:

1- Indicate the absence or presence of Alkaloids

2- Test for complete of extraction

Disadvantages: Some non alkaloids interfere such as Proteins, lactones, coumarins

Classification of Alkaloidal precipitating agents:

1- Reagents that form double salts:

a- Mayer’s Reagent: Potassium Mercuric Iodide.

b- Dragendorff’s Reagents: Potassium Iodobismethate.

c- Gold Chloride.

2- Reagents Containing Halogens:

a- Wagner’s Reagent: Iodine/ Potassium Iodide.

3-Organic Acids:

a- Hager’s Reagent: Picric Acid

b- Tannic Acid.

4- Oxygenated High Molecular Weight Acids:

a- Phosphomolybdic acid

b- Phosphotungestic acid

c- Silicotungestic Acid

➢Purification of the Crude Alkaloidal Fractions:

- **Repeated Acid-Base procedures:**

  Render extract **Acidic**, extract with **organic solvent** (dissolve non alkaloidal impurities), **Alkalinize** and extract again with **organic solvents** (Dissolve Alkaloids).
- Precipitation with alkaloidal precipitating agent.
- Convert to crystalline salts.

GLYCOSIDES

- Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in the same molecule.

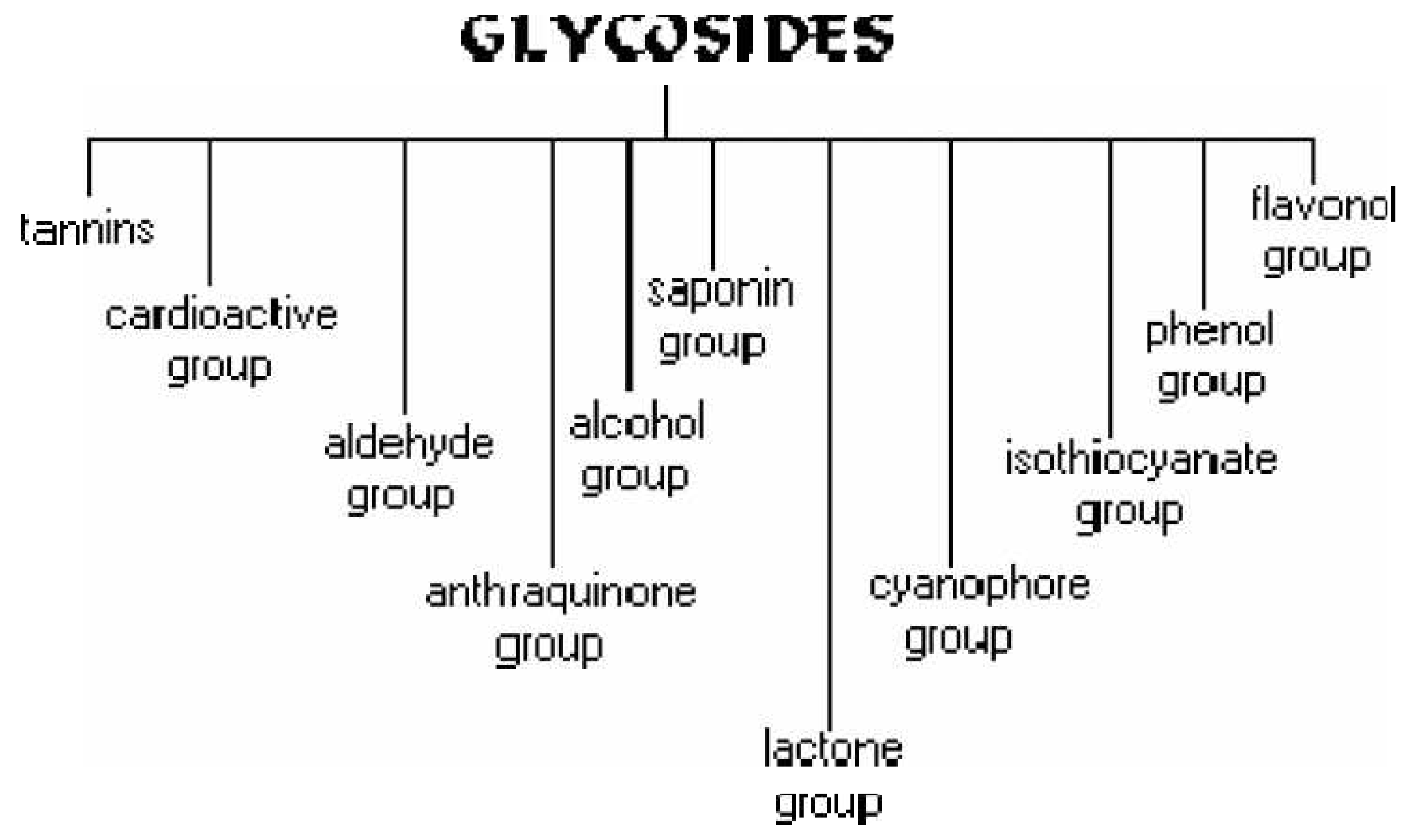
- The carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a noncarbohydrate residue or AGLYCONE.

- The nonsugar component is known as the AGLYCONE. The sugar component is called the GLYCONE.

Definition:

- Glycosides are non-reducing organic compounds that on hydrolysis with acids, alkalis or enzymes yield:
  - A sugar part (or glycone, formed of one or more sugar units).
  - A non-sugar part (or aglycone, also called genin).

THE CHEMICAL NATURE OF THE AGLYCONE GROUP IS USED AS THE BASIS OF SYSTEMATIZATION



GLYCOSIDES

- tannins
- cardioactive group
- aldehyde group
- anthraquinone group
- alcohol group
- saponin group
- lactone group
- cyanophore group
- isothiocyanate group
- phenol group
- flavonol group

CLASSIFICATION

- Atom from the aglycone involved in the glycosidic linkage:
    - Aglycone- O- Sugar O-glycosides
    - Aglycone- C- Sugar C-glycosides
    - Aglycone- S- Sugar S-glycosides
    - Aglycone- N- Sugar N-glycosides
- Number of sugars:
    - One sugar monosides e.g. Salicin.
    - Two sugar Biosides e.g. Diosmin.
    - Three sugars Triosides e.g. Digoxin.
- Nature of the glycoside:
    - Primary glycosides: Originally present in the plant e.g. Purpurea A
    - Secondary glycosides: Resulted from removal of one sugar from the primary glycosides e.g. Digitoxin

- Type of the glycosidic linkage:
  - α- glycosides
  - β- glycosides
- Botanical source:
  - Digitalis glycosides
  - Senna glycosides.
- Therapeutic use:
  - Analgesic glycosides.
  - Purgative glycosides.
  - Cardiac glycosides.
- Chemical nature of the aglycone:
  - Flavone glycosides.
  - Steroidal glycosides.
  - Aldehydic glycosides.

PHYSICAL CHARACTERS

- Solids either amorphous or crystalline.
- Non volatile.
- Usually bitter in taste.
- Soluble in water and polar organic solvents.
- Reduce Fehling’s solutions only after hydrolysis.

THE FUNCTION OR THE ROLE OF GLYCOSIDES IN THE PLANT ORGANISM

- Converting toxic materials to non or less toxic.
- Transfer water insoluble substances by using monosaccharide.
- Source of energy (sugar reservoir).
- Storing harmful products such as phenol.
- Regulation for certain functions(growth).
- Some have beautiful colours(pollenation process).

- Some glycosides have antibacterial activity, so they protect the plants from bacteria and diseases

EXTRACTION AND ISOLATION

- Because of the wide range of physical and chemical properties of glycosides and other constituents associated with them, no common general method for their isolation is recommended.
- Water, methanol, water-ethanol and ethanol are the most common solvents for extraction of glycosides.

- <u>Precautions before extraction</u>
  - <u>Deactivation of enzymes:</u>
- Drying for 15-30 min at 100 oC followed by slow drying at a low temperature.
- Dipping the fresh material into boiling water or boiling alcohol for 10-20 min.
- Boiling the fresh plant material with acetone.
- Carrying out the extraction at very low temp.
- Freeze-drying of the plant material before extraction (lyophilization).
- Carrying the extraction in the presence of $(NH_4)_2SO_4$.

CARDIAC GLYCOSIDES

- **A large group of unrelated plants with toxic compounds that have a direct effect on the heart and blood vessels and can cause the death of animals that consume them**

- **Cardiotoxic compounds found in plants such as yew (*Taxus* spp.), rhododendrons (*Rhododendron* spp.) are responsible for poisoning in animals and humans**

- There are 2 basic groups of glycosides in plants, cardenolides and bufadienolides, both of which have direct effects upon cardiac function

- The best known cardiac glycosides (cardenolides) are digoxin and digitoxin, derived from the parent glycoside digitalis

- Cardenolide type:

  Digitalis lanata and Digitalis purpurea – digoxin, digitoxin

- Bufadienolide type:

  Urginea maritima

- Examples of animals producing cardiac glycosides:
- Bufadienolide type:
  - Bufo toads

- Cardiac glycosides are found in all plant parts, especially the leaves.
- Generally, only very small quantities of the plants must be ingested to produce poisoning
- In cattle and horses, as little as 0.005 percent body weight of green oleander leaves is reportedly lethal

- They inhibit the cellular membrane sodium-potassium pump (Na+K+ adenosine triphosphatase [ATPase] enzyme system) with resulting depletion of intracellular potassium and an increase in serum potassium
- This results in a progressive decrease in electrical conductivity through the heart causing irregular heart activity and eventually completely blocking cardiac activity.

- **Toxic doses of the glycosides cause a variety of severe dysrhythmias and conduction disturbances through the myocardium that results in decreased cardiac output and death.**

- In low doses, the glycosides have a beneficial therapeutic effect on the heart by increasing the force of contraction, slowing the heart rate, and increasing cardiac output.

- Cardiac failure is described as the inability of the heart to pump blood effectively at a rate that meets the needs of the metabolizing tissues.
- This occurs when the muscles that perform contraction and force the blood out of heart are performing weakly.
- Reduced contraction of heart leads to reduced heart output but new blood keeps coming in resulting in the increase in heart blood volume.
- The heart feels congested and the term congestive heart failure

- Increasing the force of contraction of the heart (positive inotropic activity) is very important for most heart failure patients
- The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart

- The mechanism whereby cardiac glycosides cause a positive inotropic effect and electrophysiologic changes is still not completely clear.
- Several mechanisms have been proposed, but the most widely accepted involves the ability of cardiac glycosides to inhibit the membrane bound Na+-K+-ATPase pump responsible for Na+-K+ exchange

- The Na+ / K+ exchange requires energy which is provided by an enzyme Na+-K+-ATPase.
- Cardiac glycosides are proposed to inhibit this enzyme with a net result of reduced sodium exchange with potassium that leaves increased intracellular Na+.
- This results in increased intracellular [Ca++].

- Elevated intracellular calcium concentration triggers a series of intracellular biochemical events that ultimately result in an increase in the force of the myocardial contraction or a positive inotropic effect

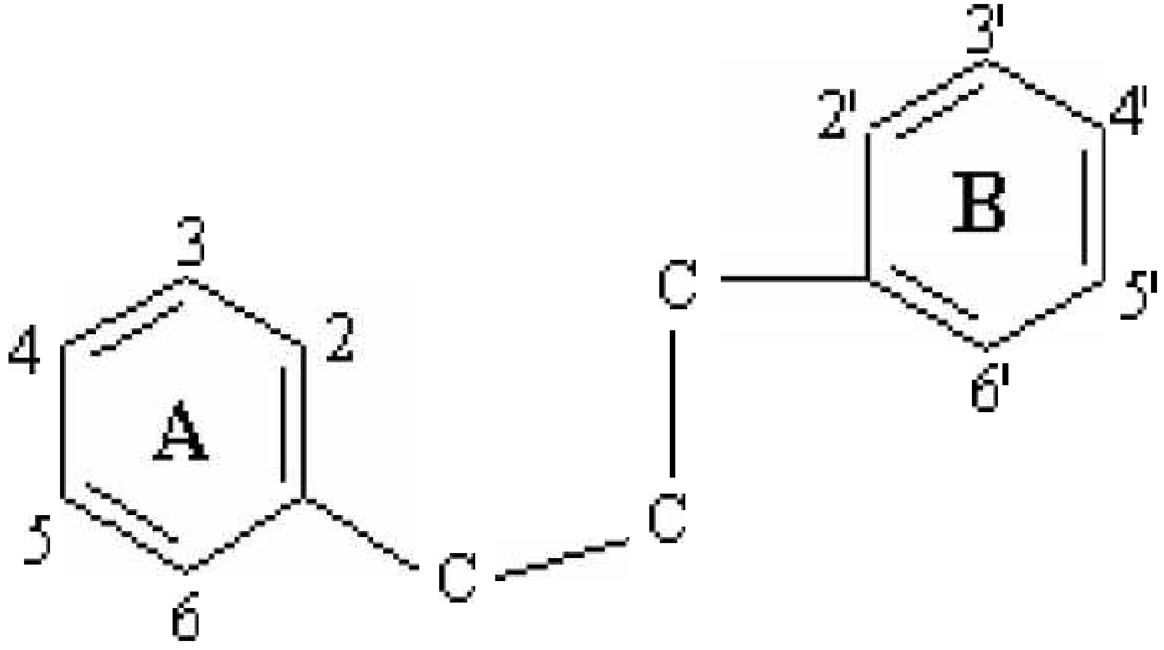
Flavonoid glycoside

- The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain having the carbon skeleton C6 - C3 - C6 and they are the plant pigments and they are having polar in nature and soloube in methanol and water.

- Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants).

- However, their occurence is not restricted to flowers but include all parts of the plant.

- They are secondary mettabolite and effective in CNS disorders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Flavonoids

- **Over 4,000 flavonoids**
- **Easily recognized as flower pigments in most angiosperm plants but are not always flower pigments**
- **In plants they repair damage and shield from environmental toxins**

The Most Important Classes of Flavonoids and their Biological Significance

class	number of known members	biological significance (so far as known)
anthocyanin(s)	250	red and blue pigments
chalcons	60	yellow pigments
aurones	20	yellow pigments
flavones	350	cream-coloured pigments of flowers
flavonols	350	feeding repellents (?) in leaves
dihydrochalcons	10	some taste bitter
proanthocyanidins	50	astringent substances
catechins	40	some have properties like those of tannins
isoflavonoids	15	oestrogen effect, toxic for fungi

Flavone:- Luteolin, Apigenin, Tangeritin
Flavonol:- Quercetin, Kaempferol, Myricetin,
Fisetin, Isorhamnetin,
Pachypodol, Rhamnazin
Flavanone:- Hesperetin, Naringenin,
Eriodictyol, Homoeriodictyol
Flavanonol- Taxifolin, Dihydrokaempferol

- CHALCONE

- Chalcone is derived from three acetates and cinnamic acid

- Anthocyanidin is an extended conjugation made up of the aglycone of the glycoside anthocyanins. Next to chlorophyll, anthocyanins are the most important group of plant pigments visible to the human eye.

GLYCOSIDE = AGLYCONE + GLYCONE

anthocyanin anthocyanidin sugar

- The anthocyanodins constitute a large family of differently coloured compounds and occur in countless mixtures in practically all parts of most higher plants. They are of great economic importance as fruit pigments and thus are used to colour fruit juices, wine and some beverages.
- The anthocyanidins in Hydrangea, colours it RED in acid soil and BLUE in alkali soil.

Flavonoids

- Act like antioxidants. How effective they are depends on their molecular structural characteristics
- Some flavonoids in hops and beer have been found to have better antioxidant effects than tea or red wine; most flavonoids are found in fruits, vegetables, teas, and other drinks.
- Flavonoids have been known to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities

1. Diuretic.
2. Antispasmodic.
3. Diaphoretic.
4. Increase tensile strength of capillary walls.
5. Free radical scavengers
6. inhibit coagulation, thrombus formation or platelet aggregation
7. modify blood lipid levels

- A teaspoon of dried parsley contains 0.5g of flavones.
- green tea and bananas, all citrus fruits(bioflavonoids) , dark chocolate

SAPONIN GLYCOSIDES

- "*Sapo*" is Latin name for soap (soap-like)
- -Group of organic compounds that form persistent froth when shaken with water even in dilute solution.
- -Saponins cause haemolysis of red blood cells.

Properties:

- - Soluble in water, alcohol and mixture of them.
- Form persistent froth with water.
- - Used as detergent (surfactant) and emulsifying agents.
- - Aglycones are called Sapogenin (acid hydrolysis).
- - Cause haemolysis of RBC's if reach the blood (injection).
- - Only small part absorbed when taken orally.
- - Enhance the absorption of other drugs.
- - Form precipitate with $Ba(OH)_{2}$.

Classification of saponins:

- According to the nature of the aglycone saponins are classified into Steroidal and Triterpenoidal saponins.
- According to sugar: monodesmoside (one sugar chain), Bidesmoside (two sugar chains)

Steroidal Sapogenins

Less distributed in nature compared to triterp.

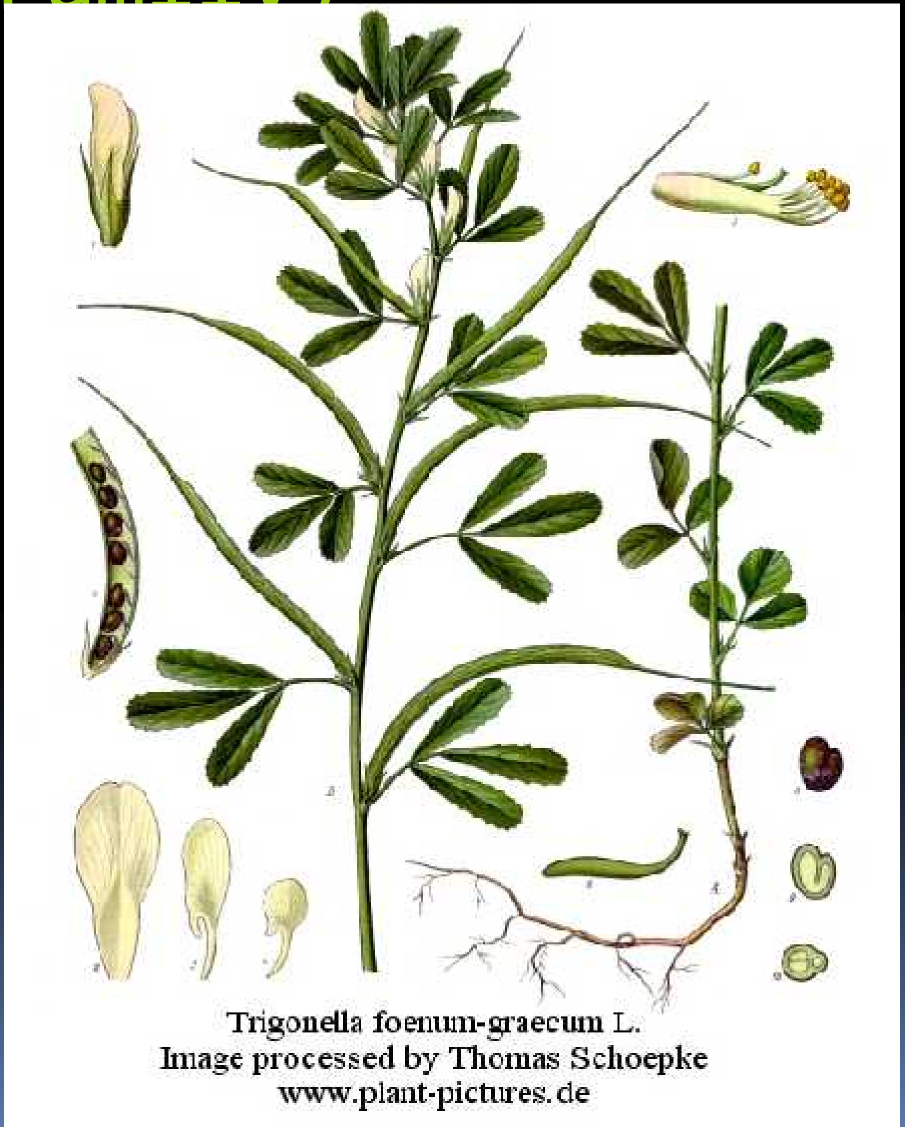
saponins (found in Monocots, Dioscoraceace& Liliaceae).

- They are C-27 sterol with modified side chain .

- - Used mainly as precursors for the partial synthesis of sex hormones and corticosteroids.
- - Drugs containing Steroidal saponins such as:

Trigonella foenum-graecum – Fabaceae (Legume Family)

- Definition: Fenugreek consists of the dried seeds of *Trigonella foenum-graecum.*



Trigonella foenum-graecum L.
Image processed by Thomas Schoepke
www.plant-pictures.de

Trigonella

- Active Constituents
  - Steroidal saponins (diosgenin, yamogenin), B vitamins, alkaloids, glycosides, flavonoids, courmarin, mucilage.

- NOTE: Should not be used to treat diabetes without professional supervision.

- Medicinal Actions
  - Anti-inflammatory, carminative, antiseptic, emollient, expectorant, hypoglycaemic, phytoeostrogenic.

- Medicinal Uses:
  - Stabilize blood glucose levels, anorexia, catarrh, cough, sore throat. Topically for boils, burns, cellulitis, gargle for sore throats, eyewash for inflammed eyes and a facial wash.

Triterpenoidal Saponins

Very common in Dicot plants

- Contain 30 carbon atoms
- Ginseng: ginsenosides, panaxosides,….
- Horse chestnut seed: aescin
- Liquorice root: glycyrrhizin

Glycyrrhiza glabra - Leguminosae

- Definition: Liquorice consists of the dried unpeeled roots and stolons of *Glycyrrhiza glabra.*

Glycyrrhiza glabra - Constituents

- Glycrrhizin – responsible for the sweet taste.

- Glycerrhizic acid – triterpenoid saponin.

- Flavonoids – responsible for yellow colour.

- Liquiritin – a chalcone

- Sugars (glucose and sucrose), protein & starch.

- Glycyramarin – bitter principles (mostly in outer tissue – removed in peeled varieties)

Glycyrrhiza glabra – Actions & Uses

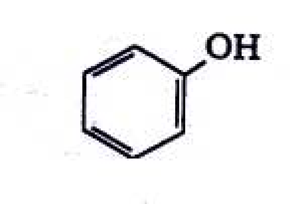
- Actions
    - Demulcent
    - Expectorant
    - Anti-microbial/anti-viral
    - Anti-spasmodic
    - Anti-Ulcerogenic

- Uses
    - Flavouring agent
    - Addison's disease
    - Inflammatory conditions
    - Peptic ulcers

Phenoles

Phenolics

- Plants produce a variety of compounds that contain one or more phenol groups - called phenolics
- Thousands of phenolics occur in plants

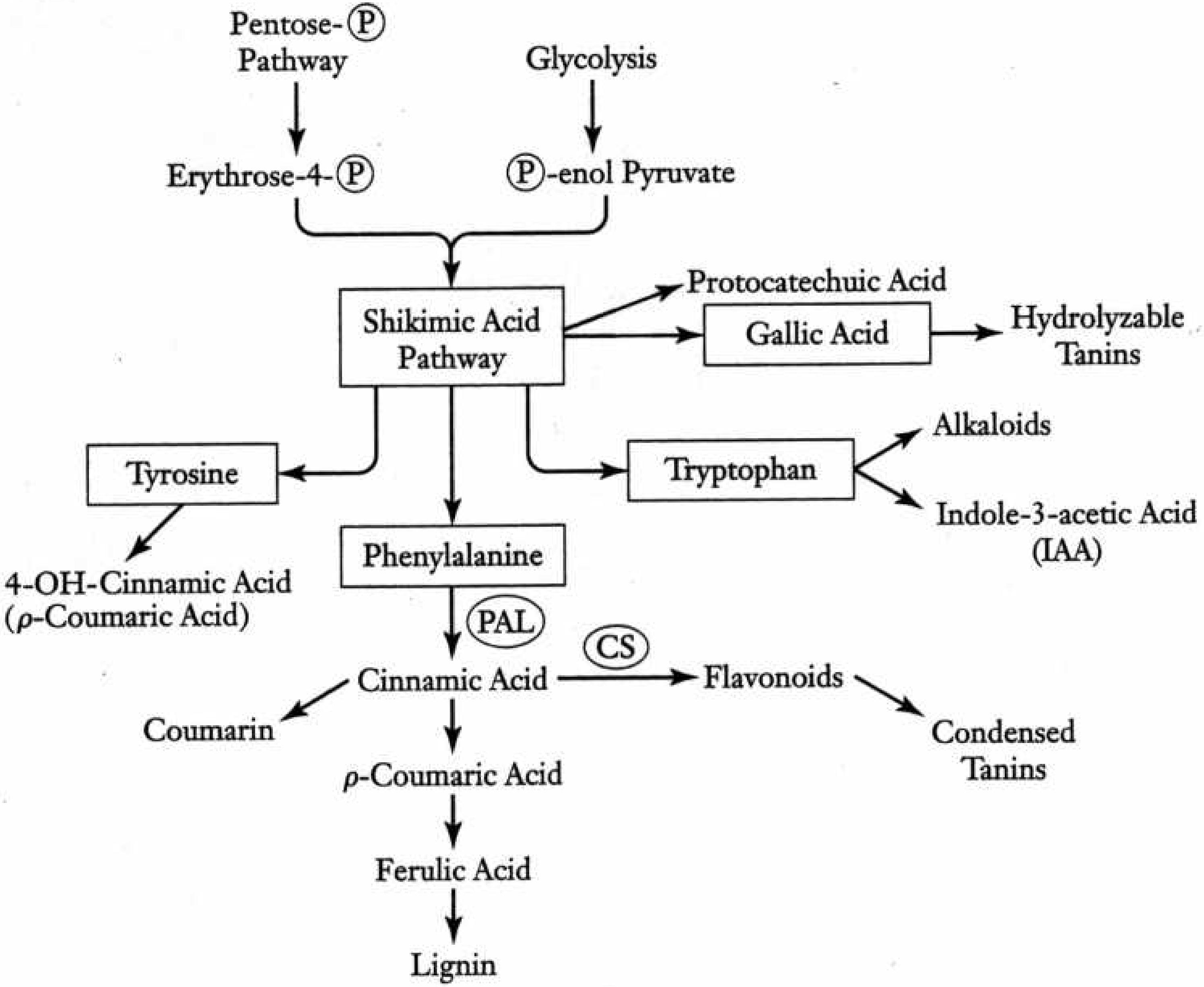


Phenolics

- Large group of diverse compounds
- Many serve as defense compounds against herbivores and pathogens
- Some function in support – primary cmpd
- Some attract pollinators
- Some absorb UV light
- Some reduce growth of competitors

Synthesis of Phenolics

- Phenolics are synthesized by a number of different pathways
- Two most important:
  - **Shikimic acid pathway** - more important in plants
  - Malonic acid pathway - more important in fungi
- One of the intermediates is shikimic acid
- Most phenolics derived from phenylanine
- Pathway occurs in plants, fungi, and bacteria but not animals



Pentose-Ⓟ Pathway

Glycolysis

Erythrose-4-Ⓟ

Ⓟ-enol Pyruvate

Shikimic Acid Pathway

Protocatechuic Acid

Gallic Acid

Hydrolyzable Tanins

Tyrosine

Tryptophan

Alkaloids

Indole-3-acetic Acid (IAA)

Phenylalanine

4-OH-Cinnamic Acid (ρ-Coumaric Acid)

PAL

CS

Cinnamic Acid

Flavonoids

Coumarin

Condensed Tanins

ρ-Coumaric Acid

Ferulic Acid

Lignin

FIGURE 14.14 The central role of the shikimic acid pathway in the synthesis of various primary and secondary metabolites. PAL = phenylalanine ammonia lyase. CS = chalcone synthase.

Number of carbon atoms	Basic skeleton	Number of phenolic cycles	Class	Examples
6	C_6	1	Simple phenols, Benzoquinones	Catechol, Hydroquinone, 2,6-Dimethoxybenzoquinone
7	C_6-C_1	1	Phenolic acids, Phenolic aldehydes	Gallic, salicylic acids
8	C_6-C_2	1	Acetophenones, Tyrosine derivatives, Phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde, Tyrosol, p-Hydroxyphenylacetic acid
9	C_6-C_3	1	Hydroxycinnamic acids, Phenylpropenes, Coumarins, Isocoumarins, Chromones	Caffeic, ferulic acids, Myristicin, Eugenol, Umbelliferone, aesculetin, Bergenon, Eugenin
10	C_6-C_4	1	Naphthoquinones	Juglone, Plumbagin
13	C_6-C_1-C_6	2	Xanthonoids	Mangiferin
14	C_6-C_2-C_6	2	Stilbenoids, Anthraquinones	Resveratrol, Emodin
15	C_6-C_3-C_6	2	Chalconoids, Flavonoids, Isoflavonoids, Neoflavonoids	Quercetin, cyanidin, Genistein
18	$(C_6$-$C_3)_2$	2	Lignans, Neolignans	Pinoresinol, Eusiderin
30	$(C_6$-C_3-$C_6)_2$	4	Biflavonoids	Amentoflavone
many	$(C_6$-$C_3)_n$, $(C_6)_n$, $(C_6$-C_3-$C_6)_n$	n > 12	Lignins, Catechol melanins, Flavolans (Condensed tannins), Polyphenolic proteins, Polyphenols	Raspberry ellagitannin, Tannic acid

Number of carbon atoms	Basic skeleton	Number of phenolic cycles	Class	Examples
6	C_6	1	Simple phenols, Benzoquinones	Catechol, Hydroquinone, 2,6-Dimethoxybenzoquinone
7	C_6-C_1	1	Phenolic acids, Phenolic aldehydes	Gallic, salicylic acids
8	C_6-C_2	1	Acetophenones, Tyrosine derivatives, Phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde, Tyrosol, p-Hydroxyphenylacetic acid
9	C_6-C_3	1	Hydroxycinnamic acids, Phenylpropenes, Coumarins, Isocoumarins, Chromones	Caffeic, ferulic acids, Myristicin, Eugenol, Umbelliferone, aesculetin, Bergenon, Eugenin
10	C_6-C_4	1	Naphthoquinones	Juglone, Plumbagin
13	C_6-C_1-C_6	2	Xanthonoids	Mangiferin
14	C_6-C_2-C_6	2	Stilbenoids, Anthraquinones	Resveratrol, Emodin
15	C_6-C_3-C_6	2	Chalconoids, Flavonoids, Isoflavonoids, Neoflavonoids	Quercetin, cyanidin, Genistein
18	$(C_6$-$C_3)_2$	2	Lignans, Neolignans	Pinoresinol, Eusiderin
30	$(C_6$-C_3-$C_6)_2$	4	Biflavonoids	Amentoflavone
many	$(C_6$-$C_3)_n$, $(C_6)_n$, $(C_6$-C_3-$C_6)_n$	n > 12	Lignins, Catechol melanins, Flavolans (Condensed tannins), Polyphenolic proteins, Polyphenols	Raspberry ellagitannin, Tannic acid

Simple Phenolics

- Allelopathic cmpds inhibits other plants
- Defense cmpds against insect herbivores and fungi
  - Certain phenolics (furanocoumarins) are phototoxic - not toxic till activated by sunlight (near UV-A)
  - Activated furanocoumarins can insert into DNA and bind to pyrimidine bases - blocks transcription and leads to cell death
  - Abundant in Apiaceae family including celery, especially under stress or diseased - can lead to skin rash in celery pickers or even handlers
  - Urushiol in poison ivy simple phenolic with side chain

Coumarins

- Two ring structure, phenol and pyrone
- 1,300 have been identified
- Responsible for odor of hay
- Prevent blood clotting, anti-inflammatory, and vasodilators
- Warfarin - well known anticoagulant and also rat poison
- Some antimicrobial, antiviral (anti-Herpes)
- Some may be contraceptive

Lignin a complex phenolic

- Primary metabolite - secondary cell wall component occurs in all vascular plants
- Structural function
- Also protective because deters herbivores due to its toughness
- Blocks growth of many pathogens because only small group of fungi can degrade

Tannins

- Tannin a general name for a large group of complex phenolic substances that are capable of tanning animal hides into leather by binding collagen
- Found in almost every plant part - abundant in unripe fruit
- Deter herbivores due to astringent properties - bind saliva and other digestive proteins
- Antimicrobial - tannins in heart wood prevent decay

Tannins

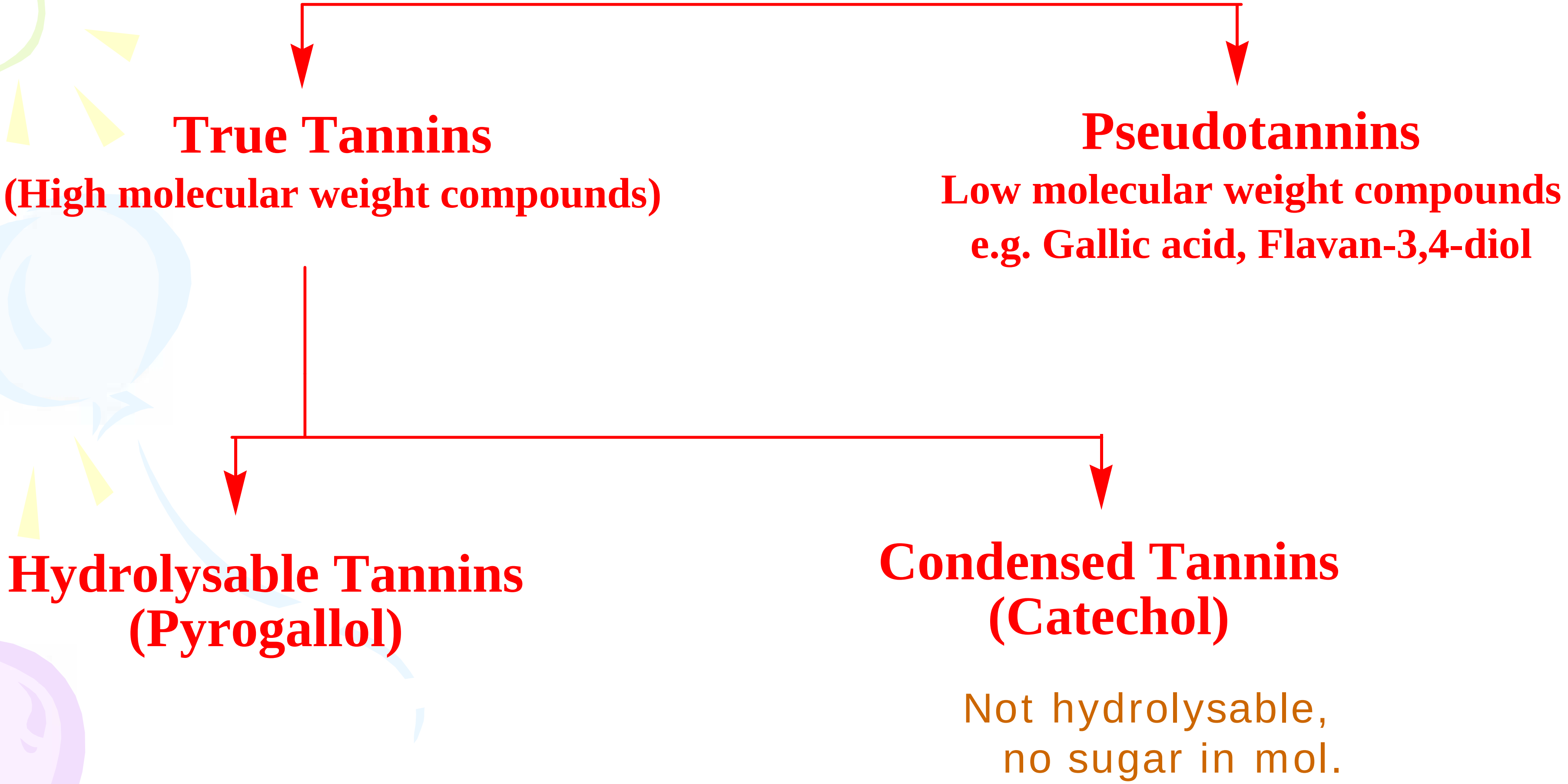
They are high molecular weight phenolic compounds capable of precipitation of animal proteins in hides and converting them into leather.

“Tanning Industry”

They are widely distributed in plants.

They are condensation products of Gallic acid or Flavan-3-ol.

Classification of Tannins:



True Tannins

(High molecular weight compounds)

Pseudotannins

Low molecular weight compounds
e.g. Gallic acid, Flavan-3,4-diol

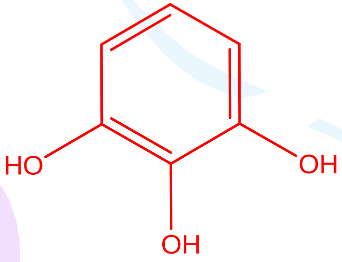
Hydrolysable Tannins
(Pyrogallol)

Condensed Tannins
(Catechol)

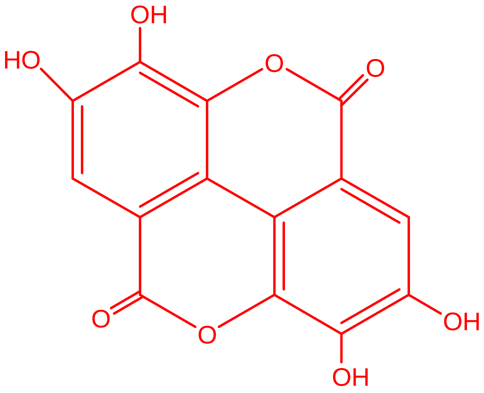
Not hydrolysable,
no sugar in mol.

1) Pyrogallol Tannins

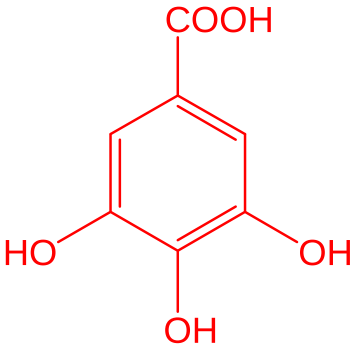
- Composed of central sugars with all its OH esterified with Phenolic acids (gallic or ellagic acid).
- They can be hydrolyzed by acids or enzymes.
- They produce sugars and:
  - Gallic acid……Gallitannins. (Clove, Hammamelis)
  - Ellagic acid…….Ellagitannins. (Geranium)
- - Produce pyrogallol on dry distillation.



pyrogallol



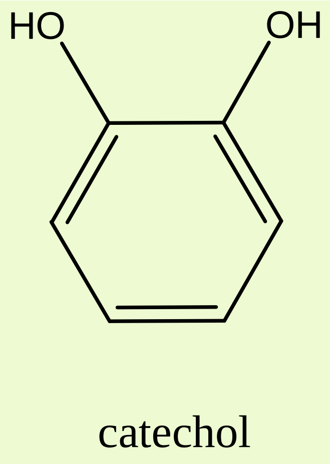
Ellagic acid



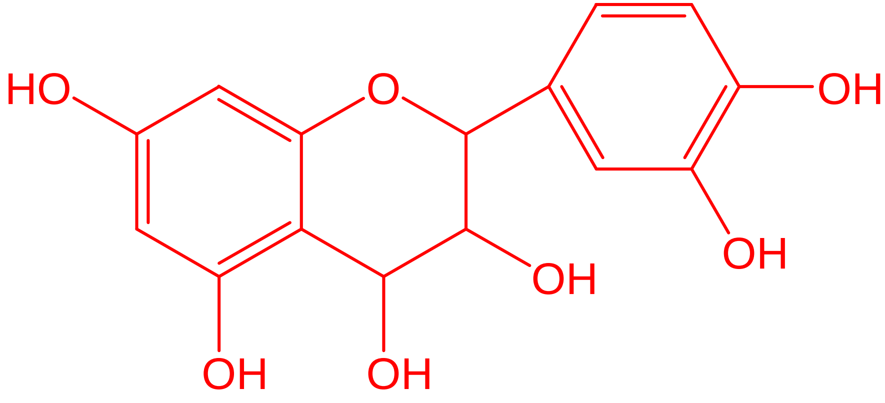
Gallic acid

2) Catechol (Condensed) Tannins

- Condensation product of Flavan-3,4-diol. They resist hydrolysis.
- Upon prolonged heating they decompose into Phlobaphene (reddish color).
- Produce catechol on dry distillation.
- Present in Cinchona, Cinnamon, Green tea.



catechol



Flavan-3,4-diol

Properties of Tannins:

-Soluble in water, polar organic solvents and Alkali solutions.

-React with $FeCl_3$ (bluish or greenish color) as they are Phenolic.

-Precipitate proteins and Gelatin.

-Form complex with alkaloids, heavy metals.

Chemical test for Identification:

$FeCl_3$:

Hydrolysable tannins → Bluish colour.

Condensed tannins → Greenish colour.

Goldbeater's Skin test (Ox-Intestine):

Soak piece of Goldbeater's skin in 2% HCl, wash with water, soak in test solution, wash with water, finally soak with $FeSO_4$ → Brown or Black colour.

Gelatin test:

Tannins + 1% Gelatin solution in NaCl → white precipitate

Uses of Tannins:

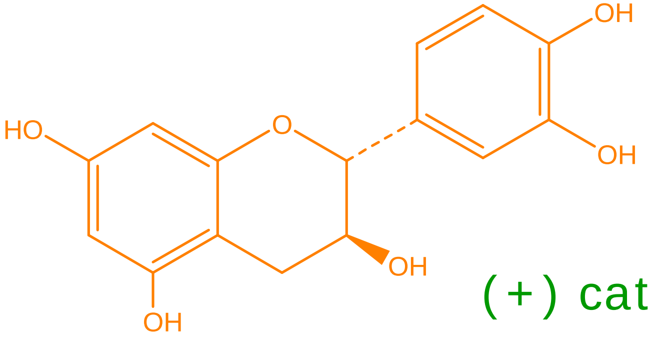
1) Antioxidant.
2) Antidiarrheal.
3) Antidote for heavy metals poisoning.
4)Treatment of burns, ulcers, inflammations
4) Astringent to stop bleeding (hemorrhage)
5)Treatment of Hemorrhoids.
6) Tanning industry.

Green and Black Tea

Green Tea:

-Prepared by rapid drying at elevated temperature to deactivate the enzymes.

-Contain Catechins with high antioxidant properties.



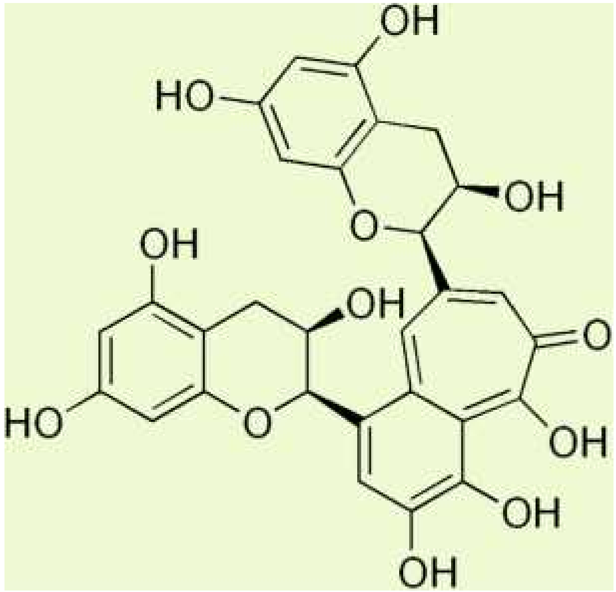
(+) catechin

Black Tea:

-Undergo slow drying (Fermentation).

-Enzyme action results in Hydrolysis and Oxidation (theaflavin).

-Antioxidant properties is greatly decreased.



theaflavin

Volatile oils

Resins and resins combination

VOLATILE OILS

- Volatile or essential oils, as their name implies, are volatile in steam.
- They differ entirely in both chemical and physical properties from fixed oils.
- They are secreted in oil cells, in secretion ducts or cavities or in glandular hairs.
- They are frequently associated with other substances such as gums and resins and themselves tend to resinify on exposure to air.

Production and uses of volatile oils

- The total annual production of volatile oils is estimated to be in the region of 45,000 tones, worth approximately US$700 million.
- There are about 100 commercially valuable **volatile oils** directly derived from plants.
- Volatile oils are used for ***their therapeutic action***, for ***flavoring*** (e.g. oil of lemon), in ***perfumery*** (e.g. oil of rose) or as ***starting materials for the synthesis of other compounds*** (e.g. oil of turpentine).

- For therapeutic purposes they are administered as ***inhalations*** (e.g. eucalyptus oil), ***orally*** (e.g. peppermint oil), as ***gargles and mouthwashes*** (e.g. thymol) and ***transdermally*** (many essential oils including those of lavender, rosemary and bergamot are employed in the practice of aromatherapy).

- Those oils with a high phenol content, e.g. clove and thyme have antiseptic properties, whereas others are used as carminatives. Oils showing antispasmodic activity, and much used in popular medicine.

Composition of volatile oils

- Volatile oils are generally mixtures of hydrocarbons and oxygenated compounds derived from these hydrocarbons.

- The odour and taste of volatile oils is mainly determined by these oxygenated constituents, which are to some extent soluble in water but more soluble in alcohol.

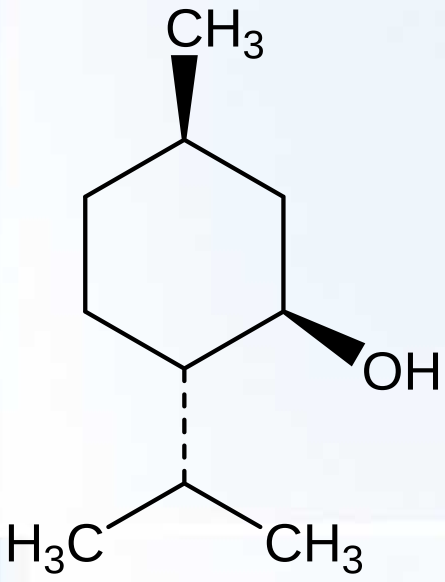
- Practically all volatile oils consist of chemical mixtures that are often quite complex; they vary widely in chemical composition.
- Almost any type of organic compound may be found in volatile oils (hydrocarbons, alcohols, ketones, aldehydes, ethers, oxides, esters, and others). ***“usually volatile oils are classified according to the type of organic compounds”.***
- It is not uncommon for a volatile oil to contain over **200** components, and often the trace constituents are essential to the odor and flavor. The absence of even one component may change the aroma.

Biosynthesis and chemical composition

- Chemical constituents of volatile oils may be divided into 2 broad classes, based on their biosynthetic origin:

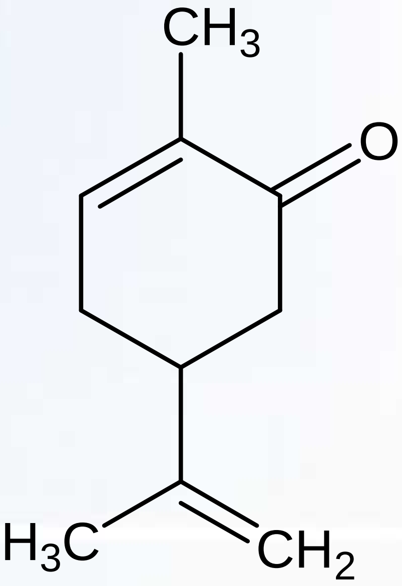
1. *Terpene derivatives* formed via the acetate-mevalonic acid pathway.
2. *Aromatic compounds* formed via the shikimic acid-phenylpropanoid route.

Terpene derivatives



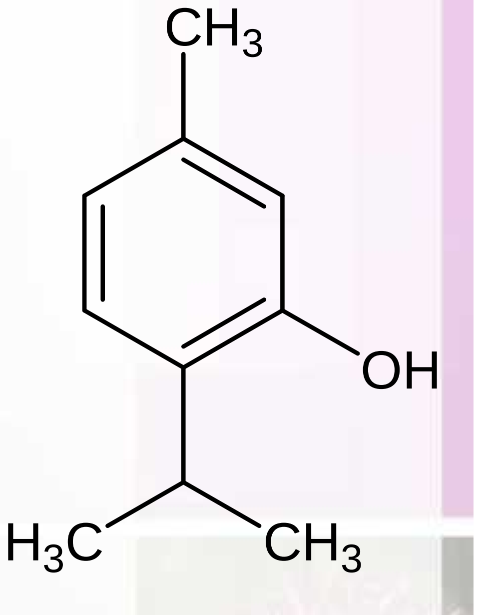
Menthol

(peppermint oil)



Carvone

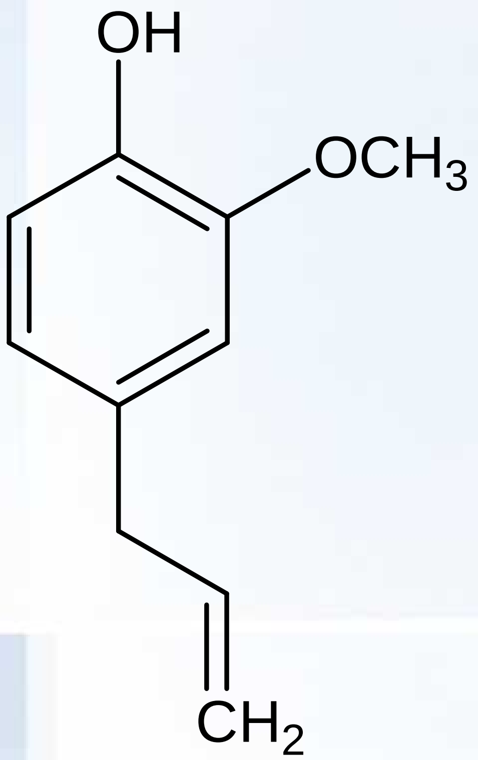
(caraway oil)



Thymol

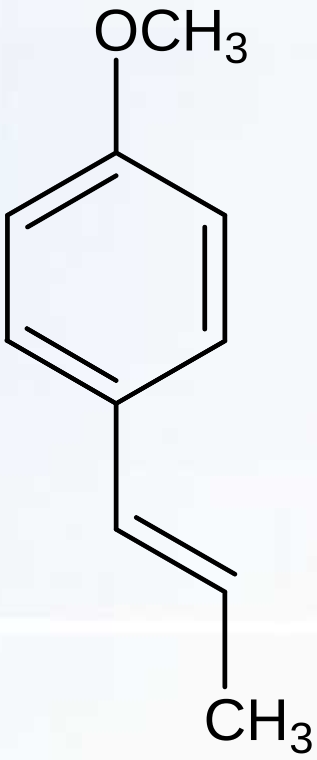
(thyme oil)

Aromatic compounds



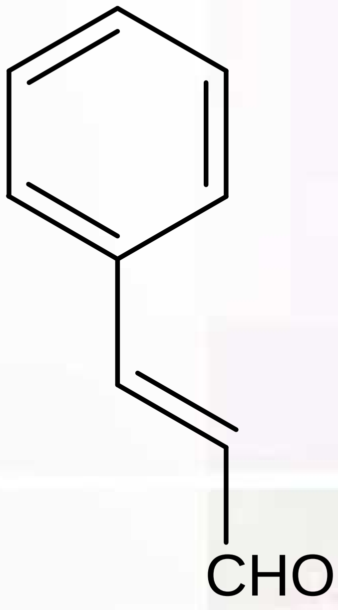
Eugenol

(clove oil)



Anethole

(anise oils)



Cinnamaldehyde

(cinnamon oil)

Physical properties

- Although volatile oils differ greatly in their chemical constitution, they have a number of physical properties in common:

1. They possess characteristic odors.
2. They are characterized by high refractive indices.
3. Most of them are optically active.
4. Their density is generally lower than that of water (the essential oils of sassafras, clove, or cinnamon are the exceptions).

- ***As a rule, volatile oils are immiscible with water, but they are sufficiently soluble to impart their odor to water. The aromatic waters are dependent on this slight solubility.***

Volatile oils ò Fixed oils

- Several points of differentiation exist between volatile oils and fixed oils.

1. ***Volatile oils can be distilled from their natural sources***.
2. ***Volatile oils do not consist of glyceryl esters of fatty acids***. Hence, they do not leave a permanent grease spot on paper and cannot be saponified with alkalies.
3. ***Volatile oils do not become rancid*** as do the fixed oils, but instead, on exposure to light and air, they oxidize and resinify.

Preparation of volatile oils

- **The principal methods used in the preparation of volatile oils from plants depend on:**

1. Distillation in water or steam.
2. Scarification and expression.
3. Extraction with solvents.
4. Enzymatic hydrolysis (***for glycosidic volatile oils e.g. mustard oil***).
5. Enfleurage (***extraction of oils used in perfumery***).

Uses of drugs containing essential oils

- ***Pharmacy***
- ***Perfumery***
- ***Food technology***
- ***Miscellaneous industries*** *(as starting materials for the synthesis of the active principles of medicines, vitamins, and fragrances).*

Resins and resins combination

Resins and resins combination

- The term ‘resin’ is applied to more or less solid, amorphous products of complex chemical nature.
- On heating they soften and finally melt.
- They are insoluble in water and usually insoluble in petroleum ether but dissolve more or less completely in alcohol, chloroform and ether.

Chemical composition

- ***Chemically***, resins are complex mixtures of resin acids, resin alcohols (resinols), resin phenols (resinotannols), esters and chemically inert compounds known as resenes.

- Resins are often associated with volatile oils (***oleoresins***), with gums (***gum-resins***) or with oil and gum (***oleo-gum-resins***).
- Resins may also be combined in a glycosidal manner with sugars.
- Resins burn with a characteristic, smoky flame.

Balsams

- **Balsams** are resinous mixtures that contain large proportions of cinnamic acid, benzoic acid or both or esters of these acids.
- The term "balsam" is often wrongly applied to **oleoresins** and should be reserved for such substances as ***balsam of Peru***, ***balsam of Tolu*** and ***storax***, which contain a high proportion of aromatic balsamic acids.

Preparation of resins

- ***Two general classes of resinous substances are recognized and this classification is based on the method used in preparing them:***

1. **Natural resins**, occur as exudates from plants, produced normally or as result of pathogenic conditions, as for example by artificial punctures e.g. mastic; or deep cuts in the wood of the plant e.g. turpentine, or by hammering and scorching, e.g. balsam of Peru.

2. **Prepared resins**; are obtained by different methods. The drug containing ***resins*** is powdered and extracted with alcohol till exhaustion. The Concentrated alcoholic extract is either evaporated, or poured into water and the precipitated resin is collected, washed and carefully dried.

   In the preparation of ***oleoresins***; ether or acetone having lower boiling point are used. The volatile oil portion is removed through distillation.

   When the resin occurs associated with gum (***gum-resins***), the resin is extracted with alcohol leaving the gum insoluble.

Classification of resins

- Resins are classified in three different ways:

1. ***Taxonomical classification***, i.e. according to botanical origin, e.g. *Berberidaceae* resins.
2. ***Classification according to predominating chemical constituent***; e.g. acid resins, resene resins, glycosidal resins; etc.
3. ***Resins may be classified according to the portion of the main constituents of the resin or resin combination***; e.g. resins, oleoresins, oleogumresins, balsams.

Examples of resins and resins combination

- **Resins**: colophony, cannabis.
- **Oleoresins**: copaiba, ginger.
- **Oleo-gum-resins**: asafoetida, myrrh.
- **Balsams**: balsam of Tolu, balsam of Peru.

Coumarin (/ˈkuːmərɪn/; 2H-chromen-2-one) is a fragrant organic chemical compound in the benzopyrone chemical class, which is a colorless crystalline substance in its standard state. It is a natural substance found in many plants.

The name comes from a French term for the tonka bean, coumarou, one of the sources from which coumarin was first isolated as a natural product in 1820. It has a sweet odor, readily recognised as the scent of new-mown hay, and has been used in perfumes since 1882. Sweet woodruff, Meadowsweet, sweet grass and sweet-clover in particular are named for their sweet (i.e., pleasant) smell, which in turn is due to their high coumarin content. When it occurs in high concentrations in forage plants, coumarin is a somewhat bitter-tasting appetite suppressant, and is presumed to be produced by plants as a defense chemical to discourage predation.

Coumarin is used in certain perfumes and fabric conditioners. Coumarin has been used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks, although in general it is banned as a flavorant food additive, due to concerns regarding its hepatotoxicity in animal models.

Coumarin was first synthesized in 1868. It is used in the pharmaceutical industry as a precursor reagent in the synthesis of a number of synthetic anticoagulant pharmaceuticals similar to dicoumarol, the notable ones being warfarin (brand name Coumadin) and some even more potent rodenticides that work by the same anticoagulant mechanism. So-called "coumarins" (modified coumarins) are a type of vitamin K antagonists. Pharmaceutical (modified) coumarins were all developed from the study of sweet clover disease; see warfarin for this history. However, unmodified coumarin itself, as it occurs in plants, has no effect on the vitamin K coagulation system, or on the action of warfarin-type drugs.

Coumarin has clinical medical value by itself, as an edema modifier. Coumarin and other benzopyrones, such as 5,6-benzopyrone, 1,2-benzopyrone, diosmin, and others, are known to stimulate macrophages to degrade extracellular albumin, allowing faster resorption of edematous fluids.[1][2] Other biological activities that may lead to other medical uses have been suggested, with varying degrees of evidence.

Coumarin is also used as a gain medium in some dye lasers,[3][4][5] and as a sensitizer in older photovoltaic technologies

Natural occurrences

Coumarin is found naturally in many plants, notably in high concentration in the tonka bean (Dipteryx odorata), vanilla grass (Anthoxanthum odoratum), sweet woodruff (Galium odoratum), mullein (Verbascum spp.), sweet grass (Hierochloe odorata), cassia cinnamon (Cinnamomum cassia) not to be confused with true cinnamon ("Ceylon cinnamon", Cinnamomum zeylanicum which contains little coumarin),[12] sweet-clover (Melilotus ssp.), deertongue (Dichanthelium clandestinum), and the leaves of many cherry blossom tree varieties (of the Prunus genus). Coumarin is also found in extracts of Justicia pectoralis.[13][14]

Related compounds are found in some but not all specimens of licorice.[15]

Derivatives

Coumarin and its derivatives are all considered phenylpropanoids.

Some naturally occurring coumarin derivatives include umbelliferone (7-hydroxycoumarin), aesculetin (6,7-dihydroxycoumarin), herniarin (7-methoxycoumarin), psoralen and imperatorin.

4-Phenylcoumarin is the backbone of the neoflavones, a type of neoflavonoids.

Medical use

Coumarins have shown some evidence of biological activity, but they are approved for few medical uses as pharmaceuticals. It is used in the treatment of asthma[18] and lymphedema.[19] Coumarin therapy is found to increase the plasma anti-thrombin levels. Both Coumarin and indandione derivatives produce a uricosuric effect, by interfering with the renal tubular reabsorption of urate.

Laser applications[edit]

Coumarin dyes are extensively used as gain media in blue-green tunable organic dye lasers.[3][4][5] Among the various coumarin laser dyes are the coumarin 480, 490, 504, 521, 504T, and 521T.[5] Coumarin tetramethyl laser dyes offer wide tunability and high laser gain,[20][21] and they are also used as active medium in coherent OLED emitters.[22]

Toxicity and use in foods, beverages, cosmetics, and tobacco[edit]

Coumarin is moderately toxic to the liver and kidneys, with a median lethal dose (LD50) of 275 mg/kg, a low toxicity compared to related compounds. Though it is only somewhat dangerous to humans, coumarin is hepatotoxic in rats, but less so in mice. Rodents metabolize it mostly to 3,4-coumarin epoxide, a toxic, unstable compound that on further differential metabolism may cause liver cancer in rats and lung tumors in mice.[23][24] Humans metabolize it mainly to 7-hydroxycoumarin, a compound of lower toxicity. The German Federal Institute for Risk Assessment has established a tolerable daily intake (TDI) of 0.1 mg coumarin per kg body weight, but also advises that higher intake for a short time is not dangerous.[25] The Occupational Safety and Health Administration (OSHA) of the United States does not classify coumarin as a carcinogen for humans.[26]

European health agencies have warned against consuming high amounts of cassia bark, one of the four main species of cinnamon, because of its coumarin content.[27][28] According to the German Federal Institute for Risk Assessment (BFR), 1 kg of (cassia) cinnamon powder contains about 2.1 to 4.4 g of coumarin.[29] Powdered cassia cinnamon weighs 0.56 g/cm3,[30] so a kilogram of cassia cinnamon powder equals 362.29 teaspoons. One teaspoon of cassia cinnamon powder therefore contains 5.8 to 12.1 mg of coumarin, which may be above the tolerable daily intake value for smaller individuals.[29] However, the BFR only cautions against high daily intake of foods containing coumarin. Its report[29] specifically states that Ceylon cinnamon (Cinnamomum verum) contains "hardly any" coumarin.

Coumarin is found naturally in many edible plants such as strawberries, black currants, apricots, and cherries.[32]

Coumarin is often found in artificial vanilla substitutes, despite having been banned as a food additive in numerous countries since the mid-20th century. Coumarin was banned as a food additive in the United States in 1954, largely because of the hepatotoxicity results in rodents.[33] Coumarin is currently listed by the Food and Drug Administration (FDA) of the United States among "Substances Generally Prohibited From Direct Addition or Use as Human Food", according to 21 CFR 189.130,[34][35] but some natural additives containing coumarin, such as the flavorant sweet woodruff are allowed "in alcoholic beverages only" (21 CFR 172.510).[36] In Europe, popular examples of such beverages are Maiwein (white wine with woodruff) and Żubrówka (vodka flavoured with bison grass).

Coumarin is subject to restrictions on its use in perfumery,[37] as some people may become sensitized to it, however the evidence that coumarin can cause an allergic reaction in humans is disputed.[38]

Minor neurological dysfunction was found in children exposed to coumarin during pregnancy. 306 children were tested at ages 7–15 years to determine subtle neurological effects from coumarin exposure. Results showed a dose response relationship between coumarin exposure and minor neurological dysfunction. Overall, a 1.9 (90%) increase in minor neurological dysfunction was observed for children exposed to coumarin. In conclusion, researchers stated, "The results suggest that coumarins have an influence on the development of the brain which can lead to mild neurologic dysfunctions in children of school age."[39]

Coumarin is still used as a legal flavorant in the tobacco industry, particularly for sweet pipe tobacco. Its presence in cigarette tobacco led former Brown & Williamson executive[40] Jeffrey Wigand to contact CBS's news show 60 Minutes in 1995, charging a “form of rat poison” was in the tobacco. He held that from a chemist’s point of view, coumarin is an “immediate precursor” to the rodenticide coumadin. Dr. Wigand later stated that coumarin itself is dangerous, pointing out that the FDA had banned its addition to human food in 1954.[41] Under his later testimony he would repeatedly classify coumarin as a "lung-specific carcinogen".[42] In Germany, coumarin is banned as additive in tobacco.

Alcoholic beverages sold in the European Union are limited to a maximum of 10 mg/l coumarin by law.[43] Cinnamon flavor is generally cassia bark steam-distilled to concentrate the cinnamaldehyde, for example, to about 93%. Clear cinnamon-flavored alcoholic beverages generally test negative for coumarin, but if whole cassia bark is used to make mulled wine, then coumarin shows up in significant levels.

Compounds derived from coumarin are also called coumarins or coumarinoids; this family includes:

brodifacoum[44][45]

bromadiolone[46]

coumafuryl[47]

difenacoum[48]

auraptene

ensaculin

phenprocoumon (Marcoumar)

Scopoletin can be isolated from the bark of Shorea pinanga[49]

warfarin (Coumadin)

Although coumarin itself has no anticoagulant properties,[citation needed] it is transformed into the natural anticoagulant dicoumarol by a number of species of fungi. This occurs as the result of the production of 4-hydroxycoumarin, then further (in the presence of naturally occurring formaldehyde) into the actual anticoagulant dicoumarol, a fermentation product and mycotoxin. Dicoumarol substance was responsible for the bleeding disease known historically as "sweet clover disease" in cattle eating moldy sweet clover silage.[50] Coumarin has anti-tumor activity[51] and anti-fungal properties.[52]

Use as pesticides[edit]

Many of the above-stated compounds (to be specific, the 4-hydroxycoumarins, sometimes loosely called coumarins) are used as anticoagulant drugs and/or as rodenticides, which work by the anticoagulant mechanism. They block the regeneration and recycling of vitamin K. These chemicals are sometimes also incorrectly referred to as "coumadins" rather than 4-hydroxycoumarins. Coumadin is a brand name for the drug warfarin.

5.Steroids

Overall Organization of the Lecture Series

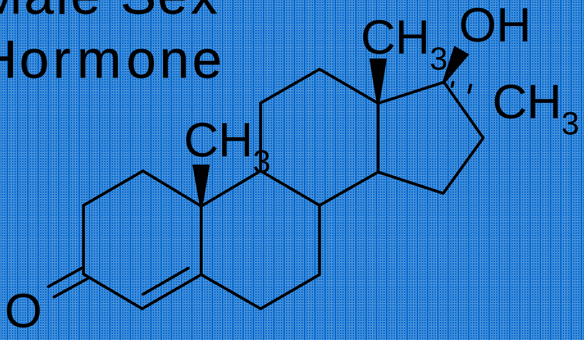
√ Structure, Nomenclature, Conformation, Configuration

√ Androgens

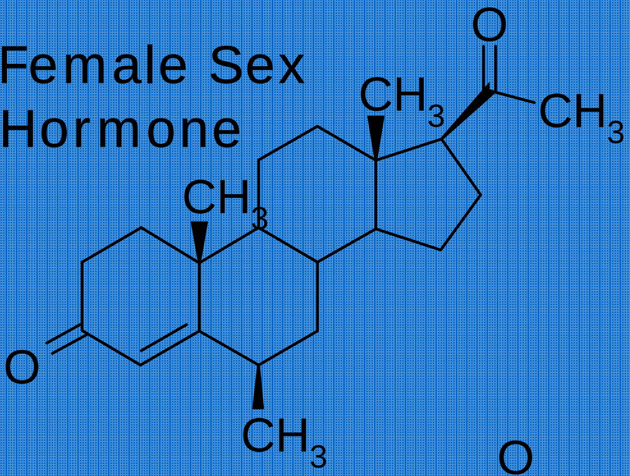
√ Estrogens and Progestins

Examples of Steroid-based Drugs in Use Today

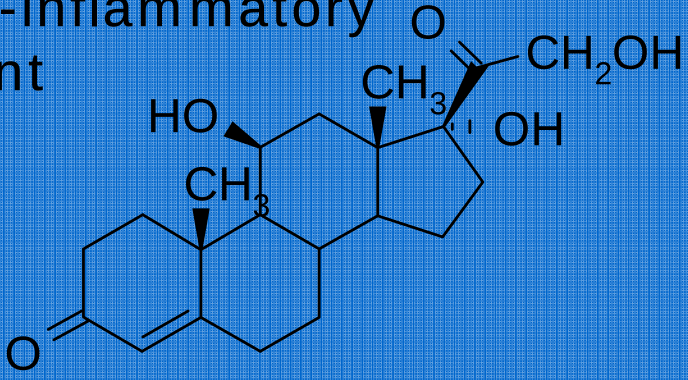
Male Sex Hormone

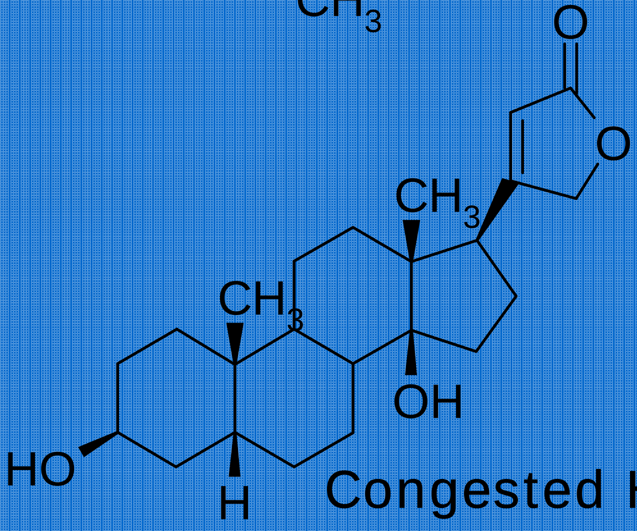


Female Sex Hormone



Anti-inflammatory Agent





Congested Heart Symptoms

Functional Classification of Steroids

√ **Anabolic Steroids**

- Interact with androgen receptor; enhance muscle mass/athlete’s performance; male sex hormones

√ **Glucocorticoids**

- regulate metabolism and immune function; anti-inflammatory activity

√ **Mineralocorticoids**

- maintain blood volume and renal excretion

√ **Progestins**

- Development of female sex organs and characteristics

√ **Phytosteroids**

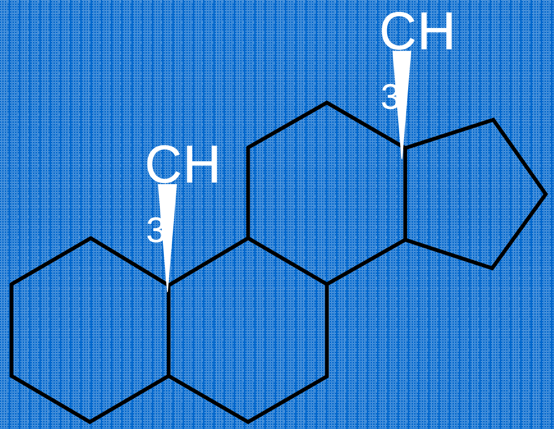
- Plant steroids

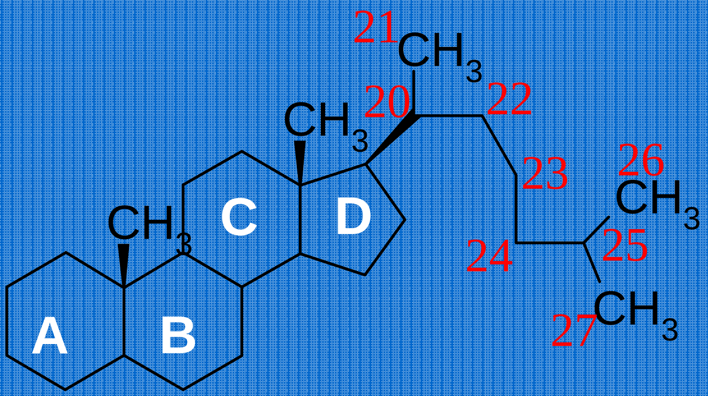
√ **Ergosteroids**

- Steroids of the fungi; vitamin D related

Structures of Steroids

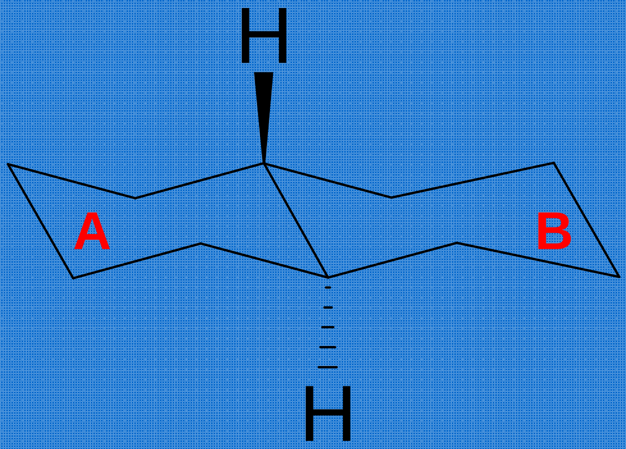
Structure and Nomenclature of the Steroid Nucleus



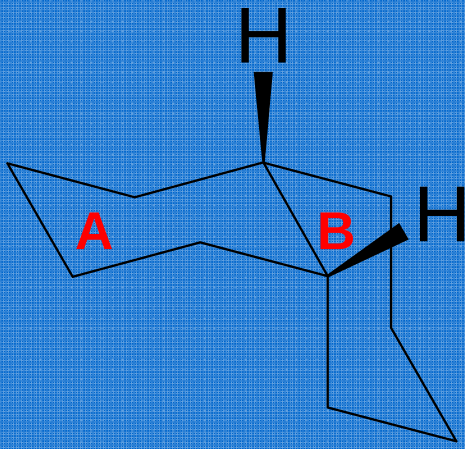


Configurational Isomers of Steroids

Fusion points between rings



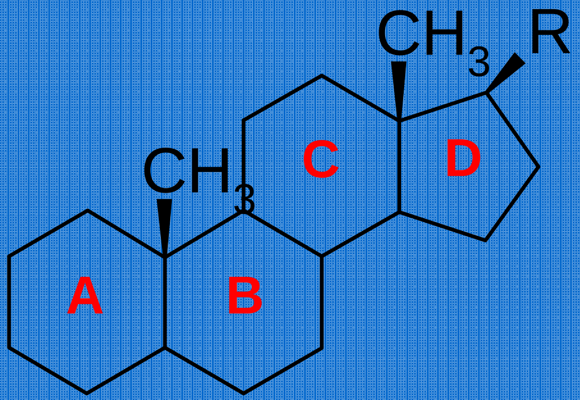
trans- configuration



cis- configuration

Configurational Isomers of Steroids

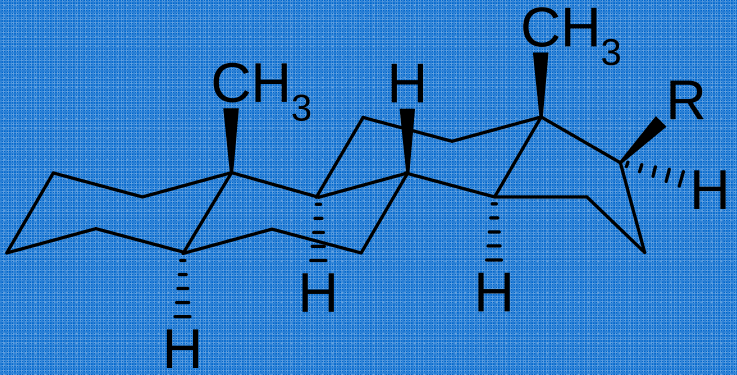
Fusion points between rings



3 fusion points → 2^3 isomers = 8

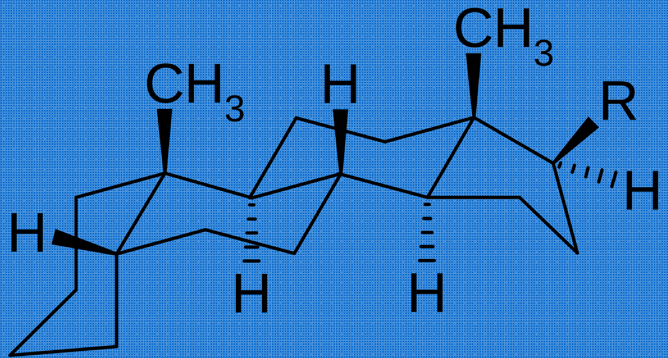
Configurational Isomers of Steroids

Three dimensional structure of three most common isomers



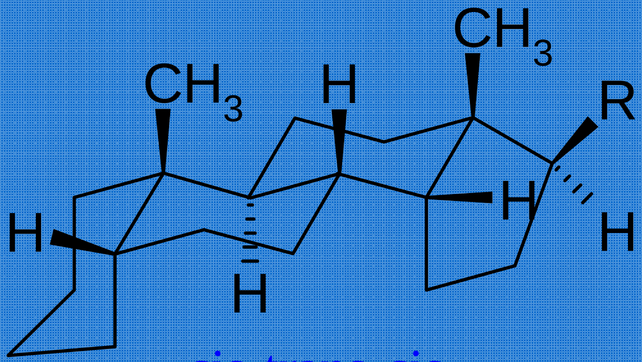
trans-trans-trans

trans-trans-trans



cis-trans-trans

cis-trans-trans

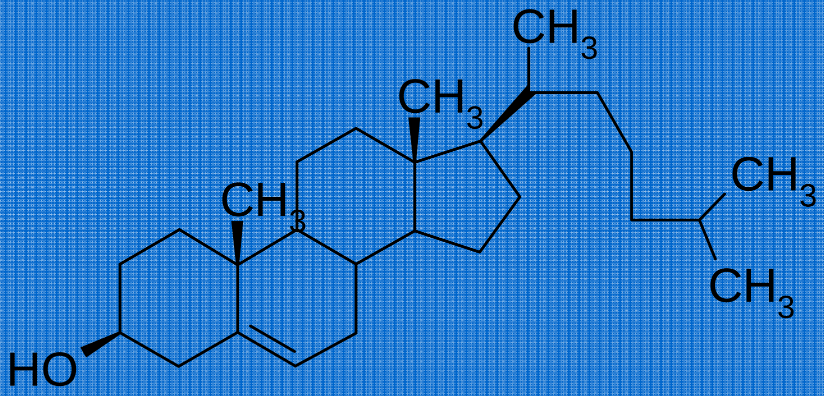


cis-trans-cis

cis-trans-cis

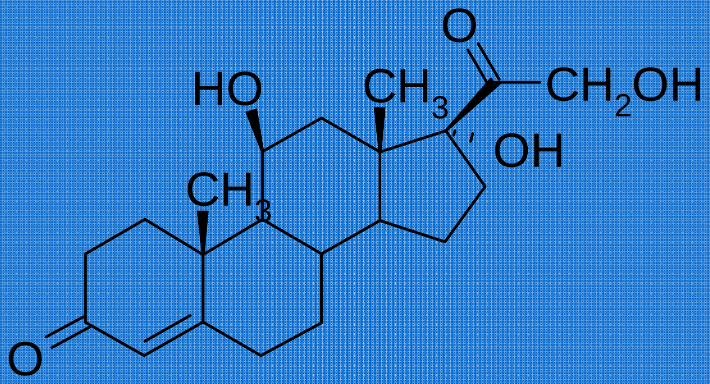
Nomenclature of Steroids

α- and β- configuration and numbering



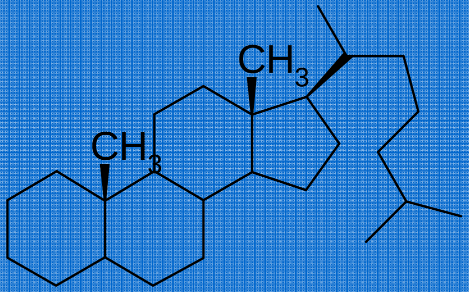
Nomenclature of Steroids

α- and β- configuration and numbering

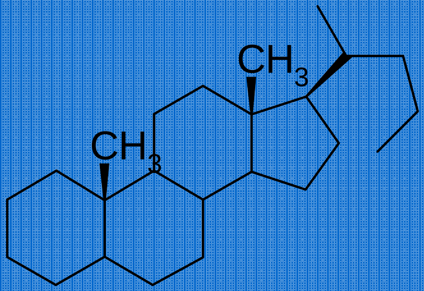


Nomenclature of Steroids

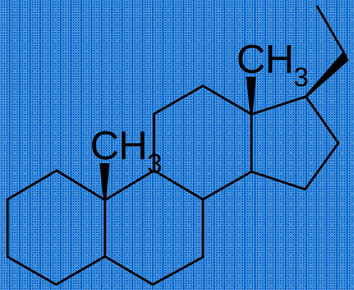
Number of Nuclear Positions and Steroid Classification



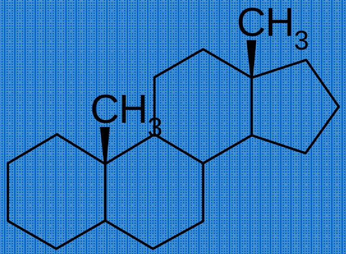
C-27 skeleton
… Cholestanes



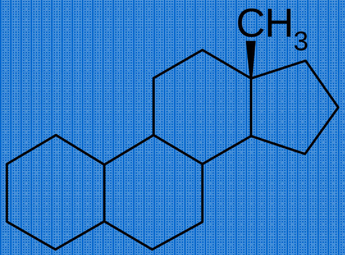
C-24 skeleton
… Cholanes



C-21 skeleton
… Pregnanes



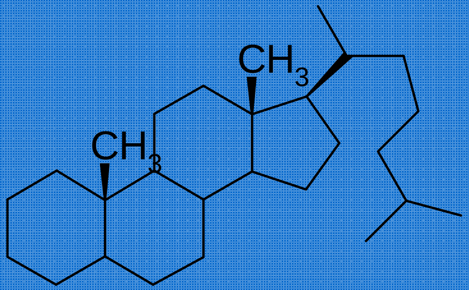
C-19 skeleton
… Androstanes



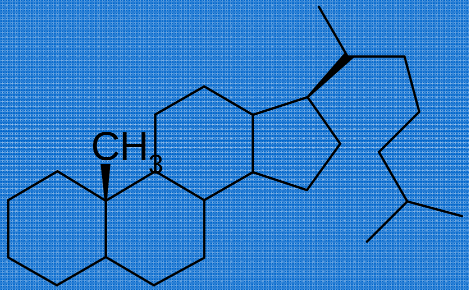
C-18 skeleton
… Estranes

Nomenclature of Steroids

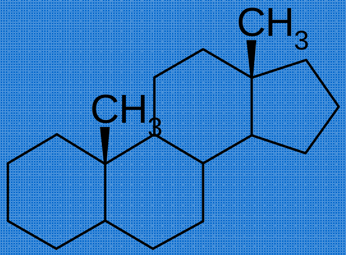
Usage of ‘Nor’ terminology



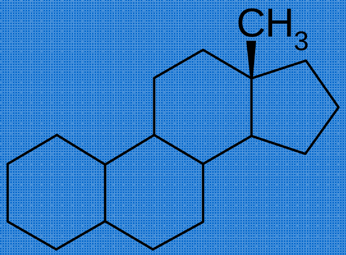
C-27 skeleton
… Cholestanes



18-Nor C-27 skeleton
… 18-nor cholestane



C-19 skeleton
… Androstanes



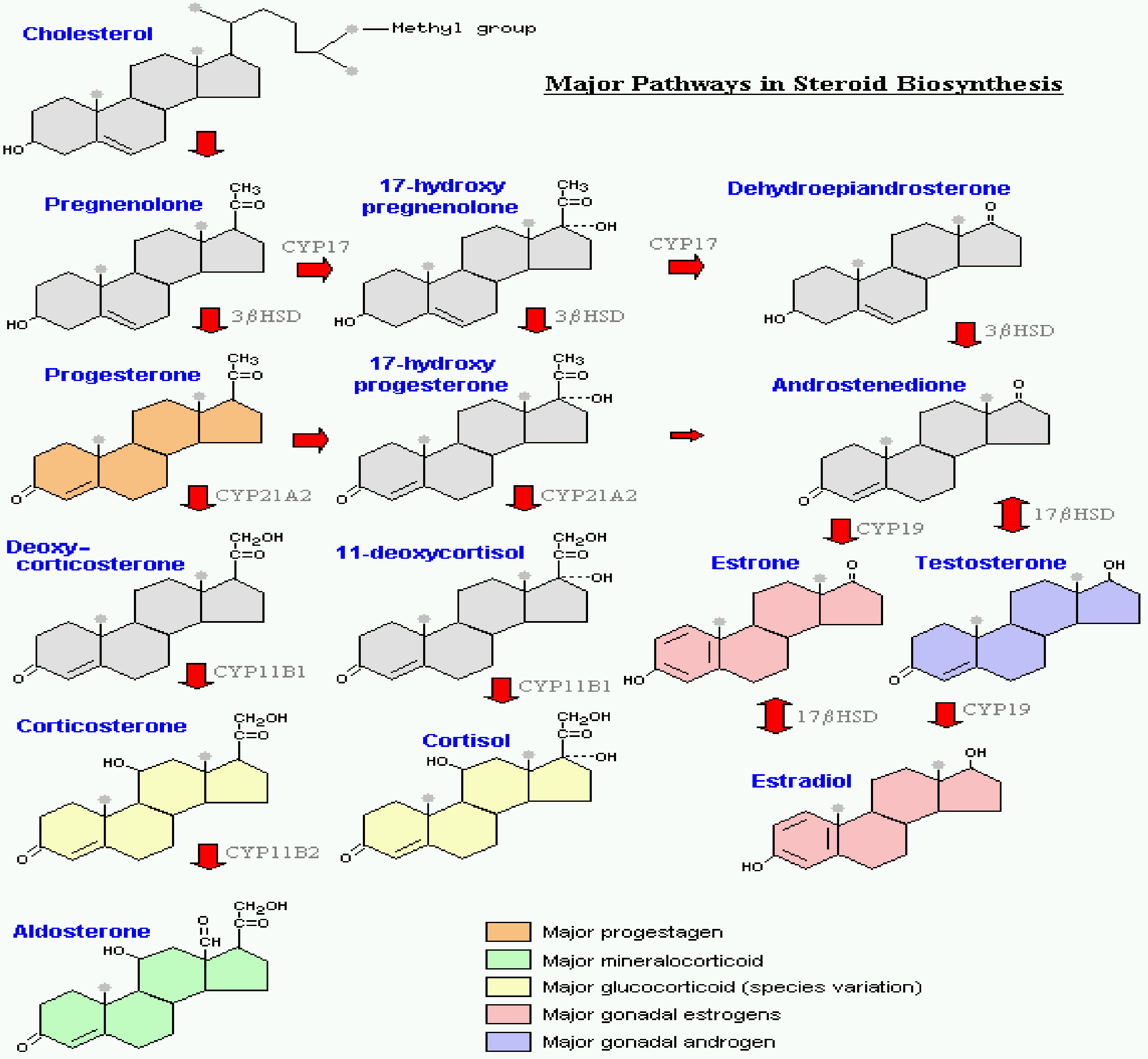
19-Nor C-19 skeleton
… 19-nor androstane

STEROIDAL HORMONES

- All steroid hormones are derived from cholesterol and differ only in the ring structure and side chains attached to it.

- All steroid hormones are lipid soluble

Major Pathways in Steroid Biosynthesis

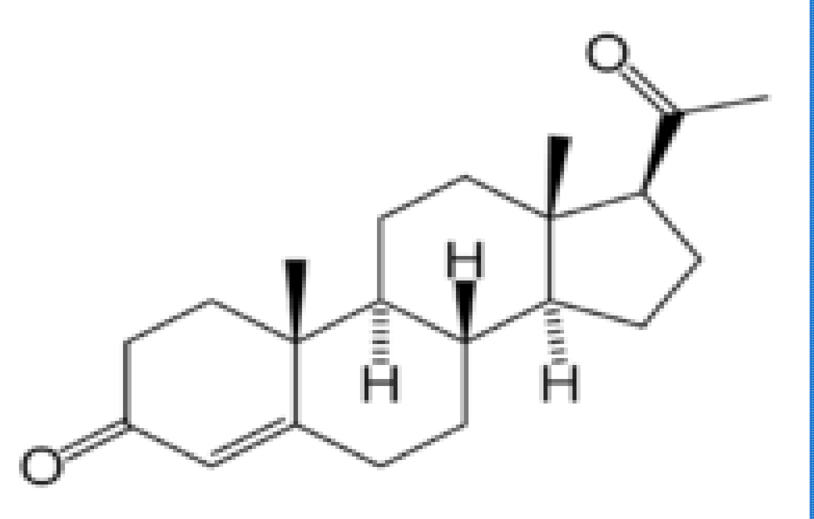


- 🟧 Major progestagen
- 🟩 Major mineralocorticoid
- 🟨 Major glucocorticoid (species variation)
- 🟥 Major gonadal estrogens
- 🟪 Major gonadal androgen

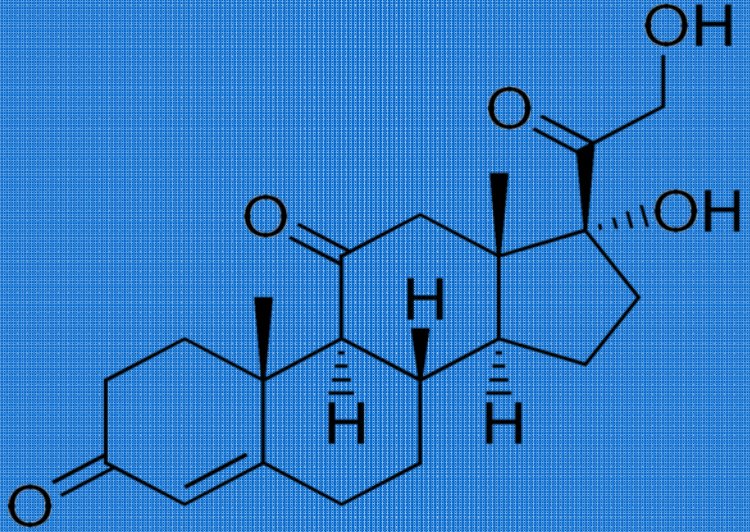
STEROID HORMONE SYNTHESIS

- All steroid hormones are derived from cholesterol.
- A series of enzymatic steps in the mitochondria and ER of steroidogenic tissues convert cholesterol into all of the other steroid hormones and intermediates.
- The rate-limiting step in this process is the transport of free cholesterol from the cytoplasm into mitochondria**. This step is carried out by the** Steroidogenic Acute Regulatory Protein (StAR)

Skeletal structures

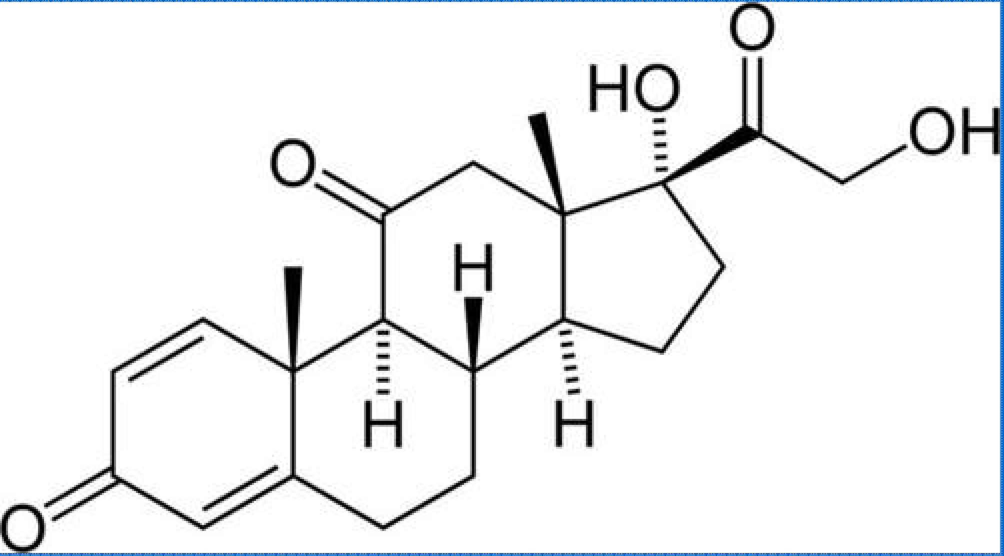


Progesterone

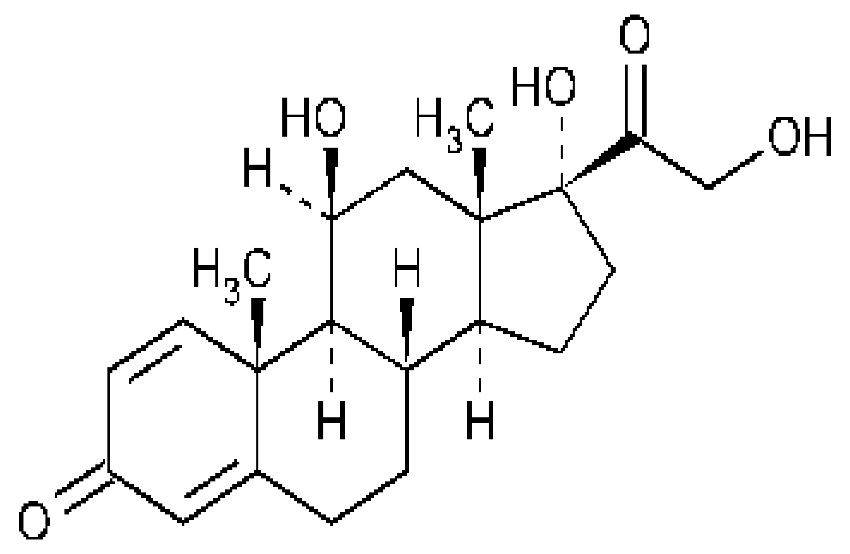


Cortisone

Skeletal structures

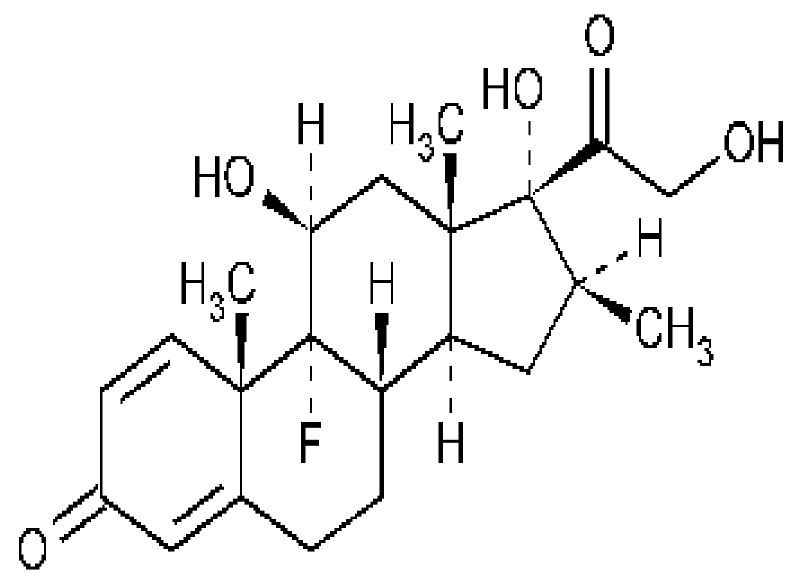


Prednisone

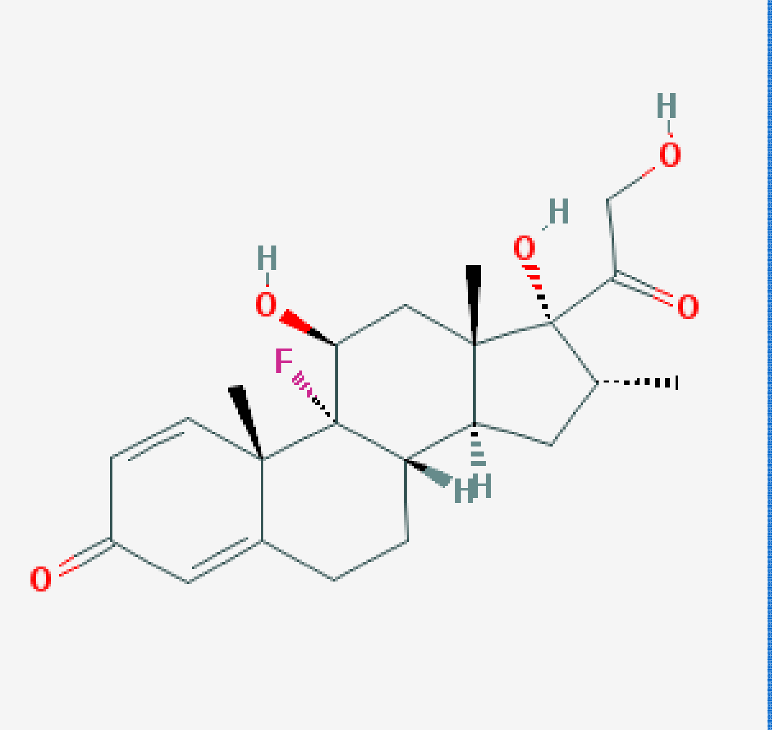


Prednisolone

Skeletal structures



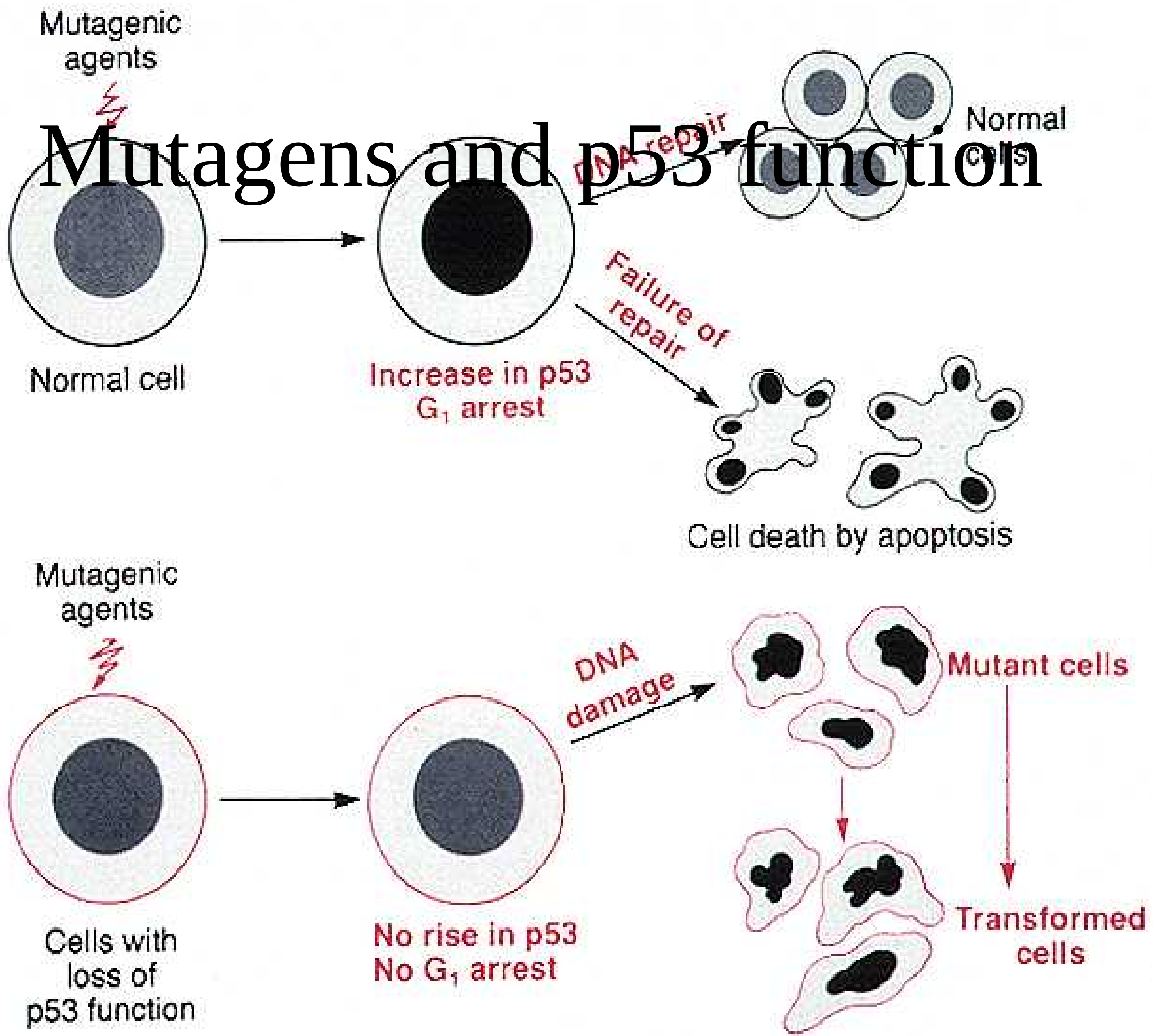
Betamethasone



Dexamethasone

Anti-Cancer Drugs

Mutagens and p53 function



Characteristics of Cancer Cells

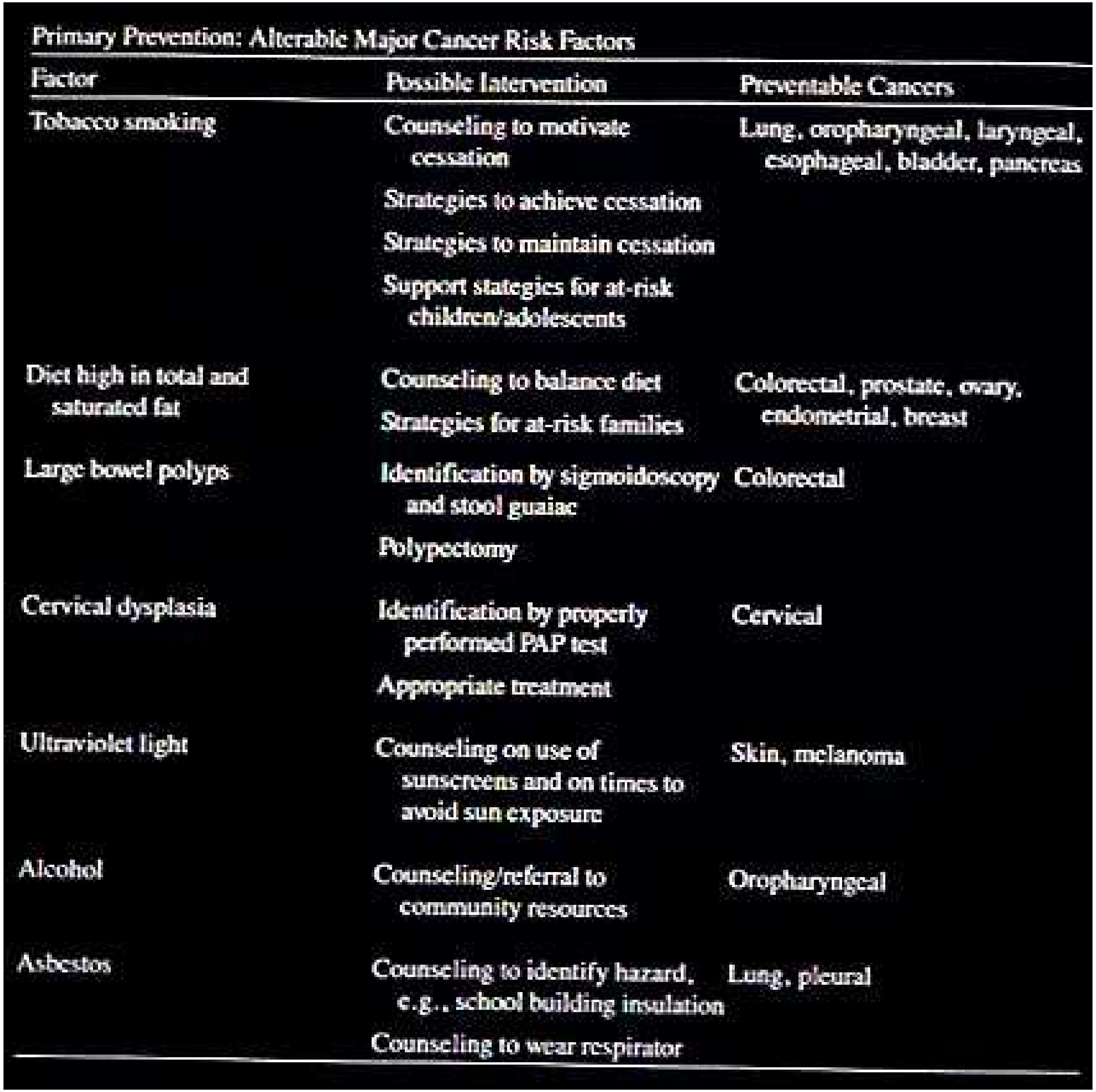
- Cancer involves the development and reproduction of abnormal cells
- Cancer cells are usually nonfunctional
- Cancer cell growth is not subject to normal body control mechanisms
- Cancer cells eventually metastasize to other organs via the circulatory and lymphatic systems

Overview of Carcinogenic Agents

- Chemical Carcinogens
- Physical Agents
- Ionizing Radiation
- Oncogenic Viruses

Impact of Environmental Carcinogens

- 80 - 90% of all cancers may be related to environmental agents including diets, lifestyles, and viruses.
- Several environmental agents often act together (co-carcinogenesis).



Primary Prevention: Alterable Major Cancer Risk Factors

Factor	Possible Intervention	Preventable Cancers
Tobacco smoking	Counseling to motivate cessation Strategies to achieve cessation Strategies to maintain cessation Support stategies for at-risk children/adolescents	Lung, oropharyngeal, laryngeal, esophageal, bladder, pancreas
Diet high in total and saturated fat	Counseling to balance diet Strategies for at-risk families	Colorectal, prostate, ovary, endometrial, breast
Large bowel polyps	Identification by sigmoidoscopy and stool guaiac Polypectomy	Colorectal
Cervical dysplasia	Identification by properly performed PAP test Appropriate treatment	Cervical
Ultraviolet light	Counseling on use of sunscreens and on times to avoid sun exposure	Skin, melanoma
Alcohol	Counseling/referral to community resources	Oropharyngeal
Asbestos	Counseling to identify hazard, e.g., school building insulation Counseling to wear respirator	Lung, pleural

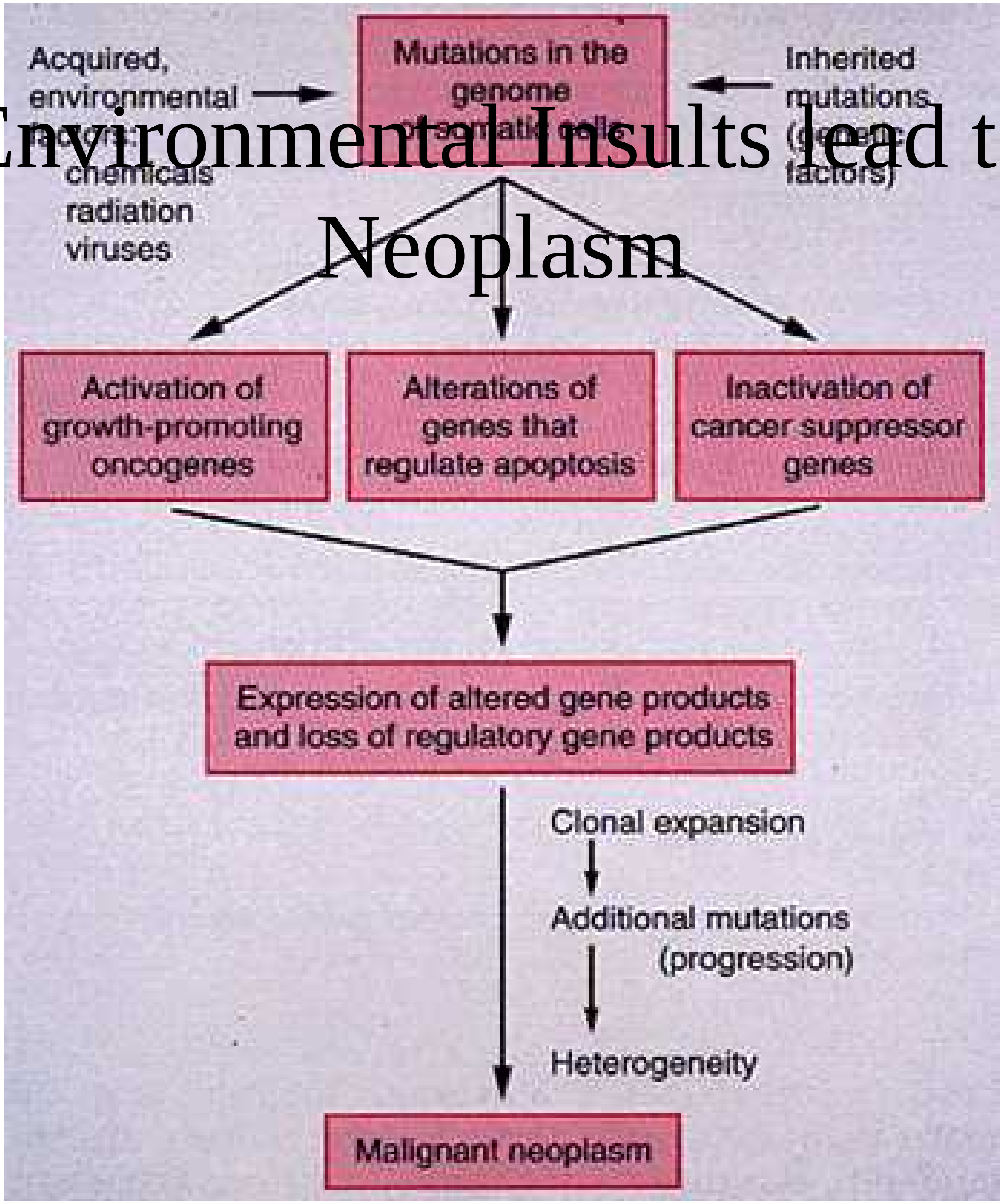
Treatment of Cancer

- Surgery to remove solid tumors
- Radiation to kill cancer cells that have spread to adjacent local or regional tissues
- Chemotherapy to kill cancer cells located throughout the body
- Antineoplastic drugs cannot differentiate between normal and cancerous cells

Principles of Carcinogenesis

- Neoplastic transformation is a progressive process involving multiple “hits” or genetic changes.
- Alterations in DNA cause changes in one or both of the following types of genes:
  - Proto-oncogenes
  - Tumor suppressor genes

Environmental Insults lead to Neoplasm



The Initiated Cell

- Unrepaired DNA damage alone cannot produce cancer.
- Cells with recently damaged DNA do not have growth autonomy or genotypic markers typical of cancer.
- Initiated cells are susceptible to promotors which induce proliferation, thus immortalizing the defect in a clone of proliferating neoplastic cells.

Ultraviolet Light

- Strong epidemiologic relationship to squamous cell, basal cell, and melano-carcinoma in fair skinned people.
- Causes formation of **pyrimidine dimers** in the DNA leading to mutations.
- Activation of T-suppressor cells facilitates emergence of tumor clones.
- Individuals with defects in the enzymes that mediate DNA excision-repair are especially susceptible.

Asbestos

- Asbestos **fiber diameter** is important
  - Thick fibers lodge in upper respiratory tract
  - Thin fibers lodge in terminal alveoli
- **Malignant mesothelioma** of the pleural and peritoneal cavities is the characteristic tumor associated with asbestos.
- Association between cancer of the lung and asbestos exposure in smokers.

Foreign Body Carcinogenesis

- Humans are highly resistant to foreign body carcinogenesis.
- Tumors associated with parasitic infections:
  - Squamous carcinoma of the bladder in persons harboring *Schistosoma Haematobium*
  - Cancer of the bile ducts following infection by the liver fluke *Clonorchis sinensis*

Ionizing Radiation

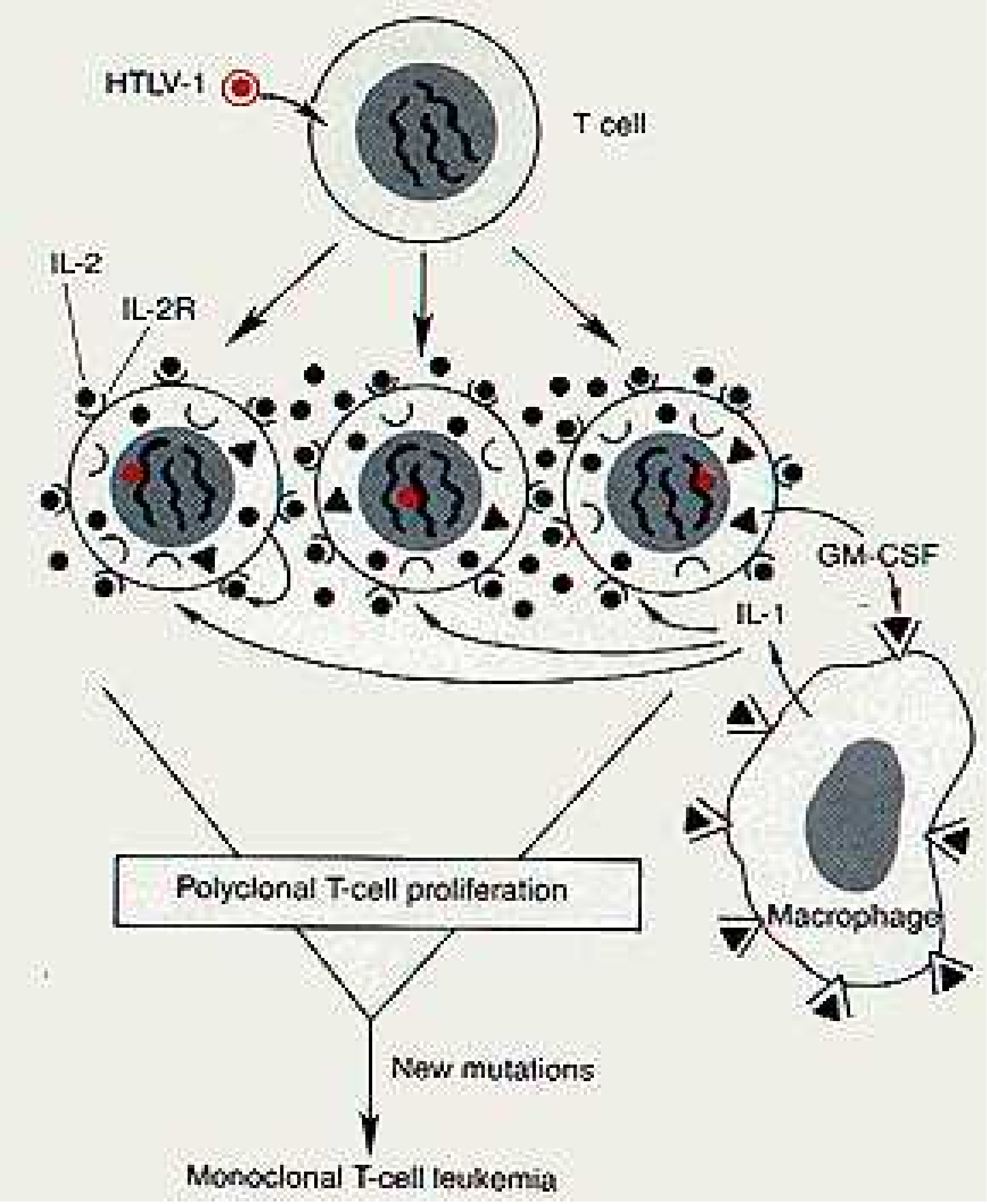
- Ionizing radiation includes: **X-rays, gamma rays**, as well as particulate radiation; **alpha, beta, positrons, protons, neutrons and primary cosmic radiation**. All forms are carcinogenic with special sensitivity in:
  - **Bone Marrow**: Acute leukemia occurs before other radiation-induced neoplasia (Seven year mean latent period in atomic bomb survivors).
  - **Thyroid**: Carcinoma occurs in 9 % of those exposed during infancy or childhood.
  - **Lung**: Increased frequency of lung cancer in miners exposed to Radon gas (an alpha particle emitter).

Mechanisms of Radiation Carcinogenesis

- Two theories: **Direct** interaction with the DNA or **indirect** damage mediated by free radicals generated from water or oxygen.
- Mutation may result by either mechanism.
- Mutagenicity of ionizing radiation correlates with:
  - Radiation quality (High linear energy transfer is most dangerous).
  - Dose
  - Dose rate
  - Efficiency of host DNA repair
  - Other host factors such as age, immune deficiency

Other Radiation and Cancer

- **Low Level Radiation**: Controversial as to whether exposure actually increases the risk of cancer.
- **Radon**: formed from the decay of uranium-radium series of elements. In the U.S.A., 4-5% of homes have 5 times background levels of radon. This may result in 16 excess lung cancer deaths/100,000 persons.
- **Other**: No firm data that microwave radiation, electromagnetic fields, and ultrasound cause cancer.


HTLV-1
T cell
IL-2
IL-2R
GM-CSF
IL-1
Macrophage
Polyclonal T-cell proliferation
New mutations
Monoclonal T-cell leukemia

Oncogenes are normal cellular genes that are involved in growth control.

Cancer results when these genes become dysregulated such that they are inappropriately activated.

Types of Normal Cellular Genes that are Homologous to Oncogenes

- Growth Factors
- Growth Factor Receptors
- G Proteins
- Kinases
- Gene Regulatory Phosphoproteins

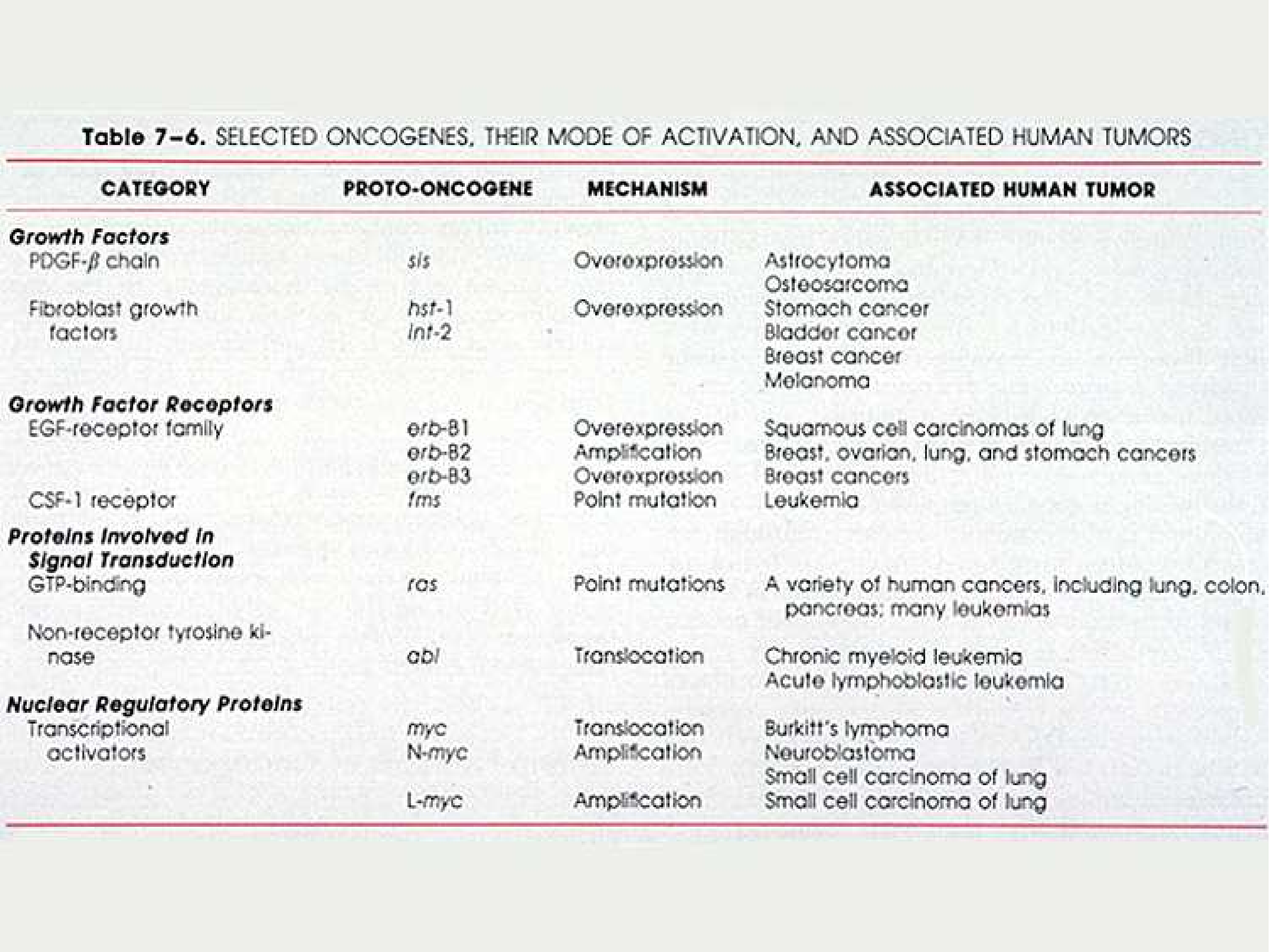
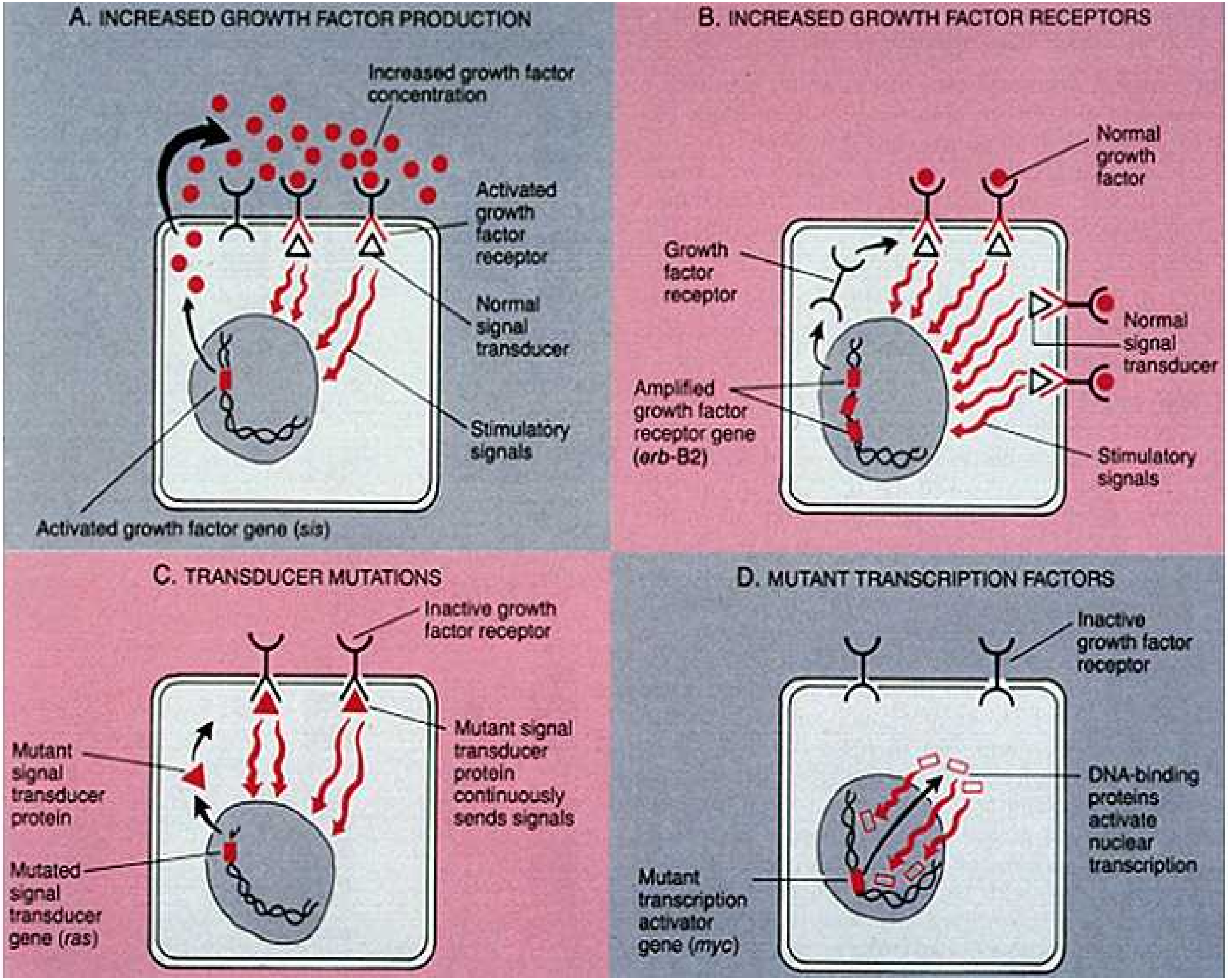


Table 7–6. SELECTED ONCOGENES, THEIR MODE OF ACTIVATION, AND ASSOCIATED HUMAN TUMORS

CATEGORY	PROTO-ONCOGENE	MECHANISM	ASSOCIATED HUMAN TUMOR
Growth Factors			
PDGF-β chain	*sis*	Overexpression	Astrocytoma Osteosarcoma
Fibroblast growth factors	*hst-*1 *int-*2	Overexpression	Stomach cancer Bladder cancer Breast cancer Melanoma
Growth Factor Receptors			
EGF-receptor family	*erb-*B1	Overexpression	Squamous cell carcinomas of lung
	*erb-*B2	Amplification	Breast, ovarian, lung, and stomach cancers
	*erb-*B3	Overexpression	Breast cancers
CSF-1 receptor	*fms*	Point mutation	Leukemia
Proteins Involved in Signal Transduction			
GTP-binding	*ras*	Point mutations	A variety of human cancers, including lung, colon, pancreas; many leukemias
Non-receptor tyrosine kinase	*abl*	Translocation	Chronic myeloid leukemia Acute lymphoblastic leukemia
Nuclear Regulatory Proteins			
Transcriptional activators	*myc*	Translocation	Burkitt's lymphoma
	N-*myc*	Amplification	Neuroblastoma Small cell carcinoma of lung
	L-*myc*	Amplification	Small cell carcinoma of lung

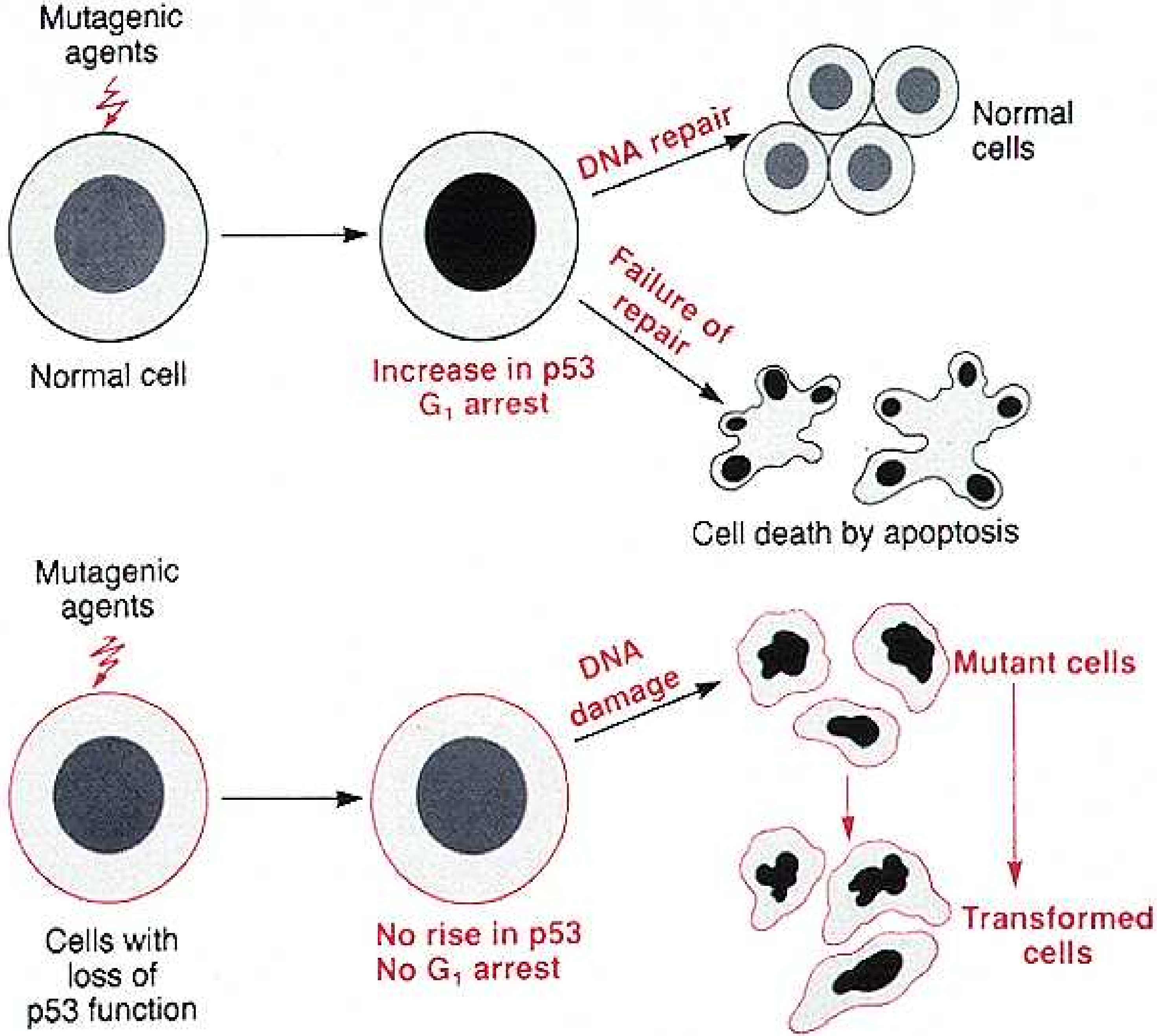

A. INCREASED GROWTH FACTOR PRODUCTION
Increased growth factor concentration
Activated growth factor receptor
Normal signal transducer
Stimulatory signals
Activated growth factor gene (sis)
B. INCREASED GROWTH FACTOR RECEPTORS
Normal growth factor
Growth factor receptor
Normal signal transducer
Amplified growth factor receptor gene (erb-B2)
Stimulatory signals
C. TRANSDUCER MUTATIONS
Inactive growth factor receptor
Mutant signal transducer protein continuously sends signals
Mutant signal transducer protein
Mutated signal transducer gene (ras)
D. MUTANT TRANSCRIPTION FACTORS
Inactive growth factor receptor
DNA-binding proteins activate nuclear transcription
Mutant transcription activator gene (myc)

Activation of Oncogenes

- **Point Mutations**
  - The ras gene is an oncogene that becomes activated by a point mutation.
- **Chromosomal Translocations**
  - Translocation of chromosome 9 and 22 in CML creating a fusion gene that produces an activated tyrosine kinase.
- **Gene Amplification**
  - Specific oncogenes such as N-myc and C-neu are amplified in neuroblastoma and breast cancer respectively.

Tumor Suppressor Genes

- A class of **genes that normally suppress cell proliferation**. Examples are p53 and Rb.
- **Mutations that inactivate the tumor suppressor gene products** can release cells from growth suppression and **lead to hyperproliferation.**
- **Both alleles** of the tumor suppressor gene **must be inactivated** by mutation for hyperproliferation to occur.


Mutagenic agents
Normal cell
Increase in p53
G_1 arrest
DNA repair
Normal cells
Failure of repair
Cell death by apoptosis
Mutagenic agents
Cells with loss of p53 function
No rise in p53
No G_1 arrest
DNA damage
Mutant cells
Transformed cells

Evidence for immune response to tumors-I

- **Immune surveillance**: a constant monitoring process aimed at eliminating emerging cancers.
- **Evidence for immune response to tumor**
  - 1) Infiltrate of lymphocytes and macrophages associated with better prognosis in many tumors.
  - 2) Peripheral blood NK activity correlates with survival.
  - 3) Peripheral blood lymphocytes counts fall as cancer overwhelms host; patients develop anergy to skin tests.

Evidence for Immune Response to Tumors-II

- 4) Non-specific vaccines can stimulate macrophages and improve prognosis. IFN-γ and IL-2 can stimulate NK cells and improve outcome.
- 5) High incidence of some tumors in immunosupressed individuals.
- 6) Spontaneous regression in some tumors.
- 7) Experimental animals cured of tumor reject rechallenge by the tumor.

Ref: Rubin & Farber
Pathology, 2nd Ed.
Lippincott, 1994

pp. 186

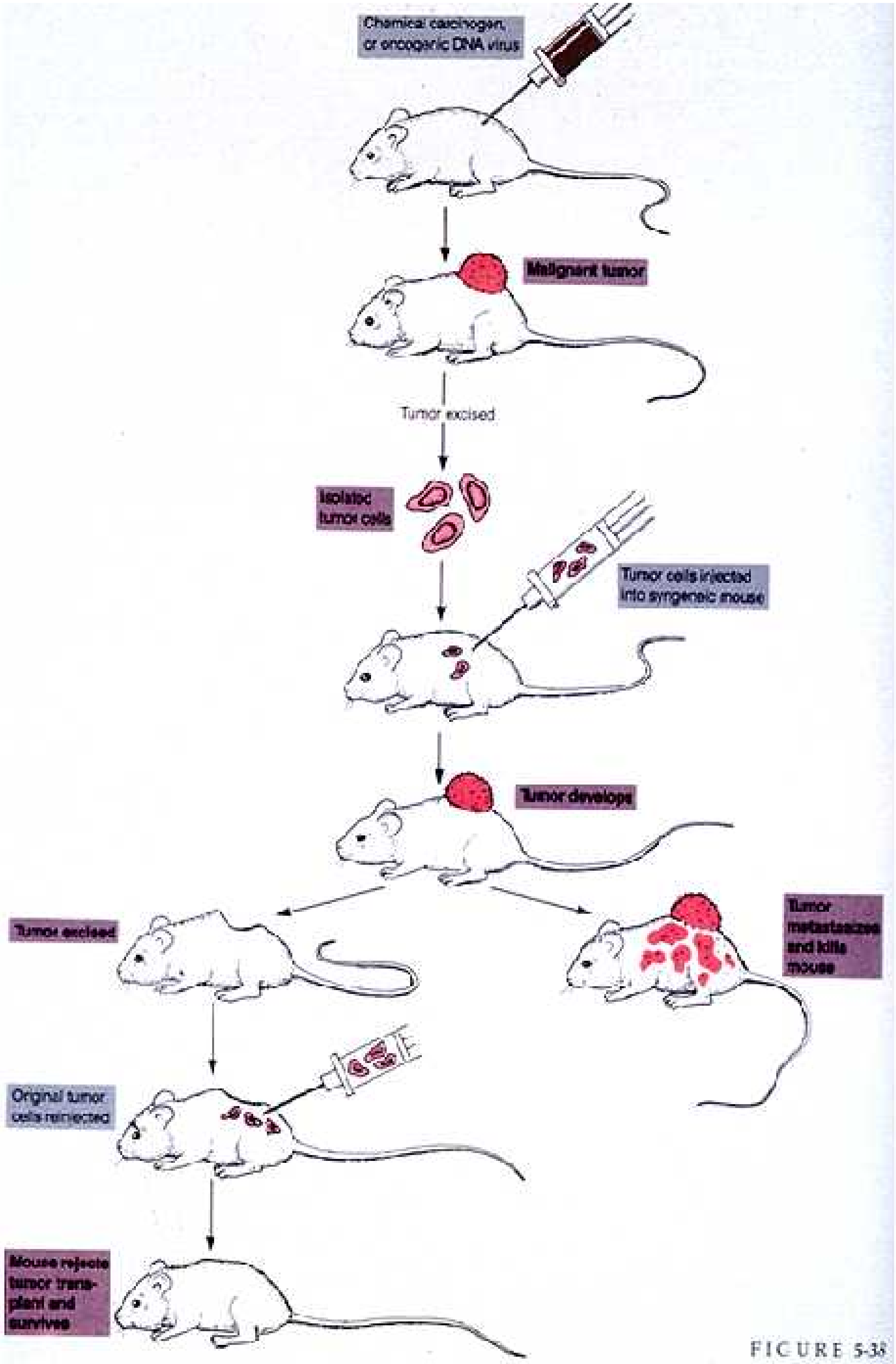


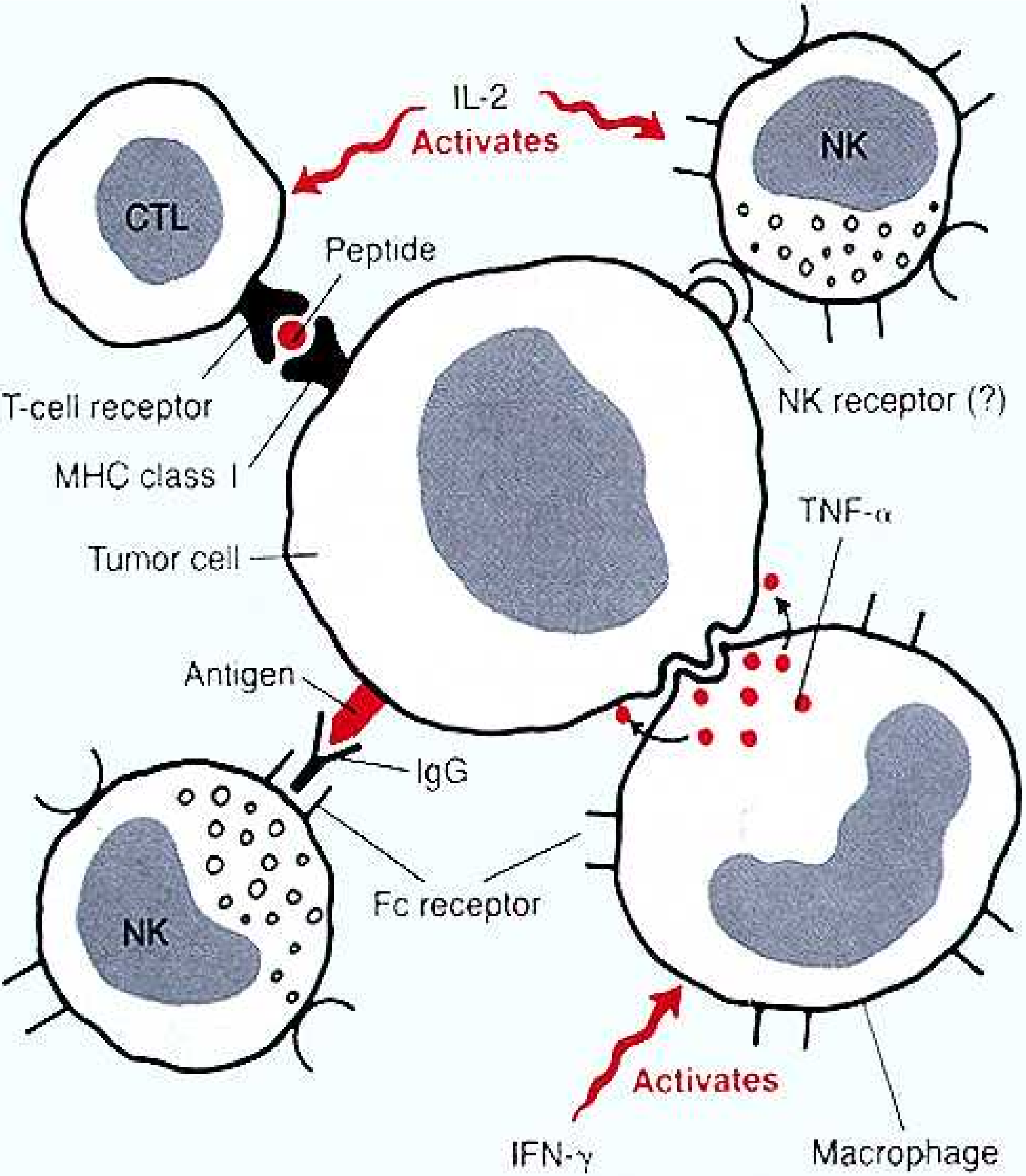
FIGURE 5-38

Mechanisms of Immunity to Tumors-I

- **Cytotoxic T lymphocytes (CTL)** - that are sensitized to TSA and perhaps other tumor antigens kill tumor cells.
- **Helper T lymphocytes** - release IL-2 and IFN-γ which stimulate CTL, macrophages, NK cells and B lymphocytes. They also produce TNF-α.
- **Natural Killer (NK) cells** - can attack tumor cells directly without antibody coating or by Antibody Dependent Cell Cytotoxicity **(ADCC)** utilizing the Fc receptor on the NK cells.

Mechanisms of Immunity to Tumors-II

- **Killer Macrophages** - activated by IFN-γ elaborated by Helper T lymphocytes. Participate in ADCC and can lyse tumor cells through release of TNF-α.
- **B lymphocytes/Plasma cells** - Produce antibody directed against tumor antigens that can kill tumor cells by complement activation.
- **Lymphokine Activated Killer (LAK) Cells** - CTL and NK cells from the tumor activated by IL-2 and IFN-γ. Tumor infiltrating lymphocytes (TIL) are CTL sensitized to the tumor that can be expanded *in vitro* and reintroduced to the patient.


IL-2
Activates
NK
CTL
Peptide
T-cell receptor
MHC class I
NK receptor (?)
TNF-α
Tumor cell
Antigen
IgG
NK
Fc receptor
Activates
IFN-γ
Macrophage

Mechanisms of Tumor Resistance to Immune Response-I

- Many human tumors are weakly antigenic
- Blocking antibodies obscure tumor associated antigens (TAA).
- Shed tumor antigens tie up receptors on ADCC mediating cells.
- Large tumor burden produces so much TAA that tolerance develops.

Mechanisms of Tumor Resistance to Immune Response-II

- Antigenic evolution occurs as tumor progresses.
- Genetic inability of host to respond to certain antigens.
- Suppressor T cells may increase and turn off cell mediated and humoral immunity.
- Protein calorie malnutrition resulting from the tumor reduces immune response.

Common Drug Toxicities

- Drugs affect cells with the fastest growth and reproductive rates, especially bone marrow and epithelial cells of the GI tract and skin
- Suppression of bone marrow and blood cell reproduction leads to anemia, infection, bleeding
- Gastrointestinal disturbances and ulcerations
- Skin ulcerations and alopecia
- Nausea and vomiting is common due to the high maximally tolerated doses that are required

Alkylator Drugs

- Irreversibly bind to DNA and interfere with cell replication and synthesis of essential cell proteins and metabolites
- Some alkylators are vesicants and will cause blistering if spilled on the skin
- Dose-limiting toxicity is bone marrow suppression and resulting susceptibility to infection
- Adverse effects also include nausea, vomiting, alopecia, and ulcerations of the GI tract and mucous membranes

Antimetabolite Drugs

- Inhibit the synthesis of folic acid, purines, and pyrimidines needed to synthesize DNA
- Antimetabolites are particularly effective in the treatment of leukemias
- Bone marrow suppression, GI ulcerations, alopecia, and nausea and vomiting are common toxicities

Drugs Derived from Plants

- Vinca alkaloids cause metaphase arrest and inhibit mitosis; neurotoxicity and leukopenia are the primary toxicities
- Etoposide and teniposide inhibit the function of DNA; adverse effects include nausea and vomiting, leukopenia, and alopecia
- Paclitaxel inhibits mitosis; peripheral neuropathy and bone marrow suppression are the most serious toxicities

Hormone Antagonists

- The female hormone estrogen is a growth factor for some types of breast cancer
- Tamoxifen is an estrogen receptor blocker that inhibits the growth of breast cancer cells
- Adverse effects include nausea, hot flashes, rash, and menstrual irregularities
- Leuprolide and goserelin inhibit the synthesis of male and female sex hormones and are used in a variety of hormonally dependent cancers

Antibiotic Drugs

- Inhibit the synthesis of DNA and other essential cell functions
- Drugs are too toxic for treatment of common bacterial infections
- The dose-limiting toxicity is usually bone marrow suppression and cardiac toxicity
- Doxorubicin is widely used to treat leukemias and a variety of solid tumors