- **Chemical Carcinogenesis: *Concepts of Initiation & Promotion***
- **Aromatic Hydrocarbons & Alcohol Toxicity**

Learning Objectives:

- Define procarcinogens, ultimate carcinogens and promoters with examples.
- Explain the steps of chemical carcinogenesis highlighting the effect of the initiators and promoters on the cell.
- Enumerate some carcinogenic agents and explain their cellular interactions.
- Describe the acute, chronic and carcinogenic effects of Benzene.
- Describe the way of exposure to polycyclic aromatic hydrocarbons (PAHs) and describe their toxicity.
- Describe the absorption, distribution and excretion of PAHs. List few examples of PAHs.
- Define the term “Alcohol Liver Disease” and describe alcohol induced liver injury.
- List some of the clinical features , lab findings and possible complications of alcohol liver disease.
- Describe the carcinogenic effect of alcohol and list some of the cancers induced by alcohol consumption.

Carcinogenesis:

- **A large number of agents cause genetic damage and induce neoplastic transformation of cells:**
  - ***Chemical carcinogens.***
  - ***Radiation energy.***
  - ***Oncogenic viruses & some other microbes.***

- **Radiation energy & some chemical carcinogens are documented causes of cancer in humans, and the evidence linking certain viruses to human cancers grows ever stronger.**

- **Each group of agents acts separately, but several may act in concert or synergies the effects of others.**

Chemical Carcinogenesis:

- **It has been over 200 years since Sir Percival Pott correctly attributed scrotal skin cancer in chimney sweeps to chronic exposure to soot.**

- **Earlier to that note, *John Hill* was the first who called attention to the association of "immoderate use of snuff" and the development of nasal polyps.**

- **As carcinogenesis, in general, is a *multistep process*; chemical carcinogenesis involves *initiation* & *promotion* phases that have been demonstrated by the classical experiment of inducing skin cancer in mice, *this experiment showed the following concepts:***

Chemical Carcinogenesis: (*see the figure in the next slide*)

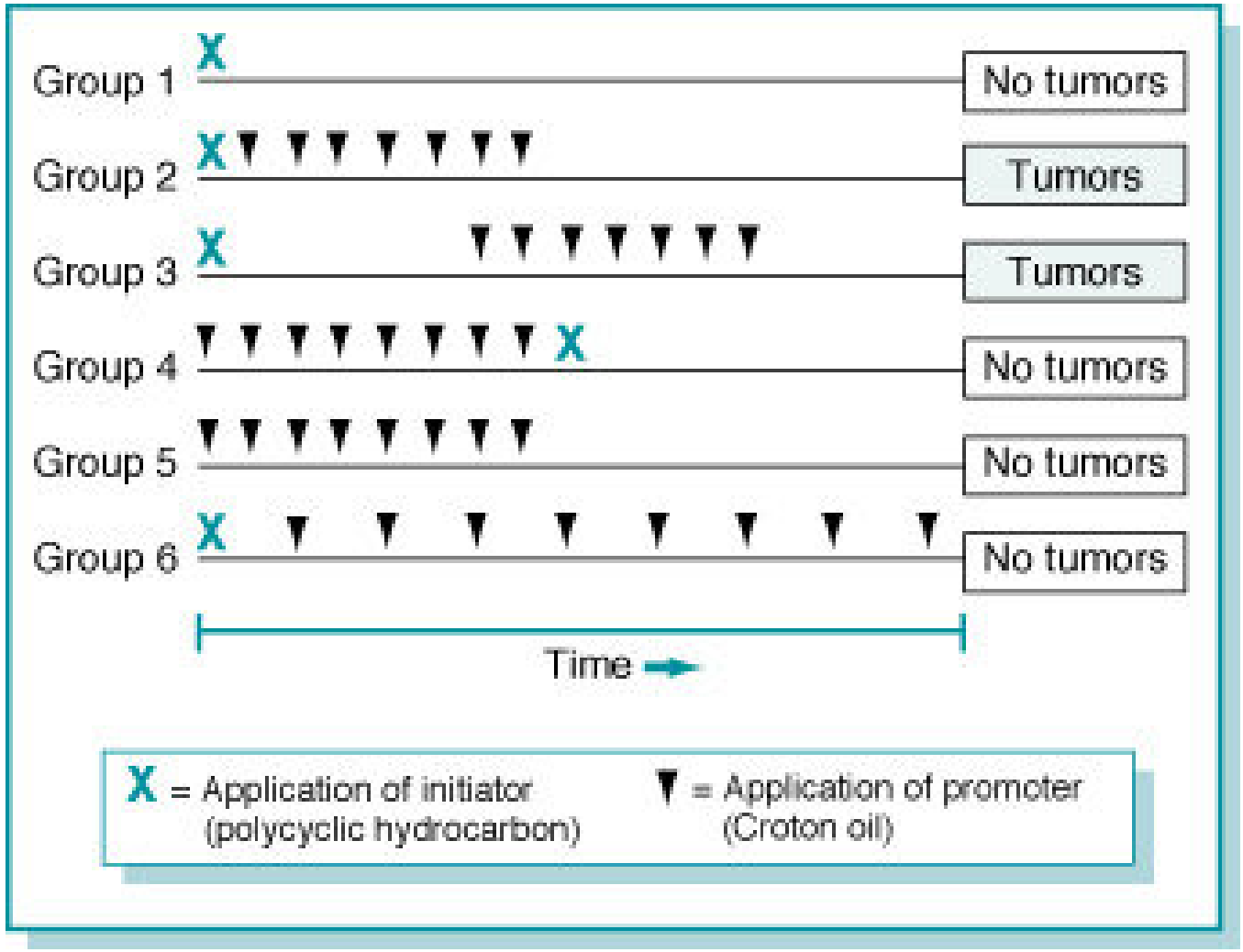
- ***Initiation results from exposure of cells to a sufficient dose of a carcinogenic agent (initiator) that alters the cell and makes it potentially capable of giving rise to tumor (groups 2&3). Initiator alone is not sufficient for tumor formation (group1).***

- ***Initiation causes permanent DNA damage (mutations). It is rapid irreversible and has a memory (group 3, in which tumor develops even if the promoter is delayed several months after a single application of the initiator).***

- ***Promoters can induce tumors in initiated cells, but they are nontummorigenic by themselves (group 5). Also, tumor does not develop when the promoter is applied before, rather than after, the initiator (group 4), this means that the promoter does not affect DNA and its effect is reversible.***

- ***Group 6 further documents the reversibility of the promoter effect, where the tumor does not develop if the time between multiple application of the promoter is extended.***

Chemical Carcinogenesis: *Experiment demonstrating phases of initiation & promotion.*



Group 2: application of promoter repeated twice weekly for several months.

Group 3: application of promoter delayed for several months and then applied twice weekly.

Group 6: promoter applied at monthly intervals.

Initiation of Chemical Carcinogenesis:

- **Chemicals that initiate carcinogenesis are extremely divers in structure and include both *natural* and *synthetic* products.**

- **Initiators can be *direct acting* compound that do not require chemical transformation for their carcinogenicity, or *indirect-acting* compounds or *procarcinogens*, which require metabolic conversion in vivo to produce *ultimate carcinogen* capable of transforming cells.**

- **Most direct-acting & ultimate carcinogens have one property in common:**
  They are highly reactive electrophiles (have electron deficient atoms) that can react with nucleophilic (electron-rich) sites in the cell.

- **These reactions are non enzymatic & result in the formation of covalent adducts (addition products) between the chemical carcinogen & a nucleotide in DNA.**

- **These elcetrophilic reactions can be *lethal* attacking DNA, RNA, and proteins, so they may sometimes *kill* the cell, and when they are *non lethal*, they will *alter DNA (primary target) and initiate* the cell for carcinogenesis.**

Metabolic Activation of Carcinogens:

- Except for the few direct-acting ***alkylating*** & ***acylating*** agents that are intrinsically elcetrophilic, most chemical carcinogens require metabolic activation for conversion into ultimate carcinogens.

- Other metabolic pathways may lead to the inactivation (*detoxification*) of the procarcinogens or its derivatives. *Thus, in addition to the inherent reactivity, the balance between metabolic activation & inactivation reactions will determine the carcinogenic potency of the chemicals.*

- Most of the known carcinogens are metabolized by *cytochrome P-450-dependant mono-oxygenases* (*the genes that encode these enzymes are quite polymorphic and vary among individuals*). These enzymes are essential for procarcinogens activation and thus regulate the susceptibility to carcinogenesis.

Molecular Targets of Chemical Carcinogens:

- **The majority of initiating chemicals are *mutageneic* that affect *oncogens, tumor suppressor genes, genes that regulate apoptosis, and genes involved in DNA repair.***

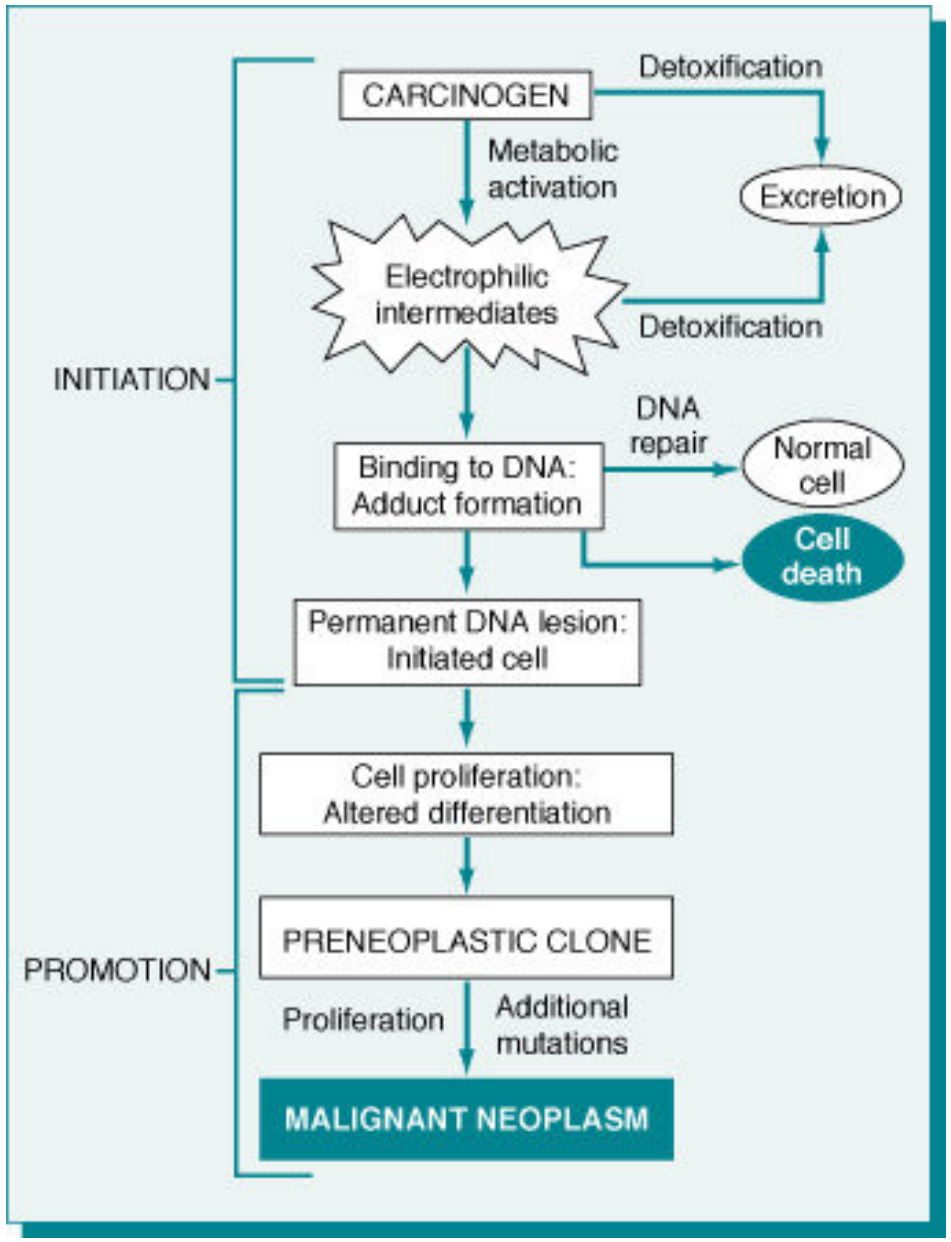
- **The mutageneic potential has been investigated using *Ames test*, that uses the ability of a chemical to induce mutations in the bacterium Salmonella typhimurium (70%-90% of known chemical carcinogens score +ve in this test).**

- **The DNA is the *primary target* for chemical carcinogens, and there is no single or unique alteration that can be associated with initiation of chemical carcinogenesis.**

- **The presence of certain types of DNA damage in human tumors can provide molecular clues to their causation. This is best exemplified by the study of mutations in the *RAS & p53* genes.**

- **Carcinogen induced DNA changes do not necessarily lead to initiation because most types of DNA damage can be *repaired by cellular enzymes.***

Chemical Carcinogenesis:



Promotion of Chemical Carcinogenesis:

- The carcinogenicity of some chemicals is augmented by subsequent administration of promoters (such as *phorbol esters, hormones, phenols & drugs*) that by themselves are non-tummorigenic. Application of promoters leads to proliferation and clonal expansion of initiated (mutated) cells.

- *Initiated cells* respond differently to promoters than do *normal cells* and hence *expand selectively*.

- Promoters force the initiated clone of cells to proliferate where it will suffer from *additional mutations*. Thus, tumor promotion includes multiple steps: *proliferation of preneoplastic cells, malignant conversion, and eventually tumor progression.*

- An important concept states that, s*ustained cell proliferation* increases the *risk of mutagenesis* and hence neoplastic transformation, this concept is applicable to human carcinogenesis (*e.g. pathologic endometrial hyperplasia increases the risk of endometrial CA*).

Carcinogenic Chemicals:

1- Direct acting carcinogens:

- **Alkylating agents** (***anticancer drugs like cyclophosphamide, chlorambucil***), usually associated with induction of ***lymphoid neoplasms, leukemia***, and ***other cancers***.
- **Acylating agents** (***e.g. 1-acetyl-imidazole & Dimethylcarbamyl chloride***).

2- Indirect acting carcinogens (procarcinogens):

- **Polycyclic & heterolcyclic aromatic hydrocarbons**(e.***g. Benz[a] anthracene & Benzo[a] pyrene***), these can cause ***skin cancer, sarcoma, and other organ CA***.
- **Aromatic amines, amides, azo dyes** (***e.g. 2-Naphthylamine & 2-Acetylaminofluorene***), they can cause ***hepatocellualr CA & bladder CA***.
- **Natural plant & microbial products** (***e.g. aflatoxin B1, Griseofulvin, & Betel nuts***)., they can cause ***hepatocellualr CA***.
- **Others** (***Nitrose amine & amides, vinyl chloride, nickel, chromium, insecticides, fungicides, Arsenic, & Asbestos***). ***Asbestos causes lung CA & mesothelioma, vinyl chloride causes liver hemangiosarcoma, nickel, chromium can cause lung CA, Arsenic can causes skin CA.***

Aromatic Hydrocarbons - *Benzene:*

- Clear, colorless to light yellow, volatile, flammable liquid with an aromatic odor. It is a high production volume chemical and a common environmental pollutant.

- Major raw material used extensively as a ***solvent*** in the chemical and pharmaceutical industries, as a starting material and intermediate in the synthesis of numerous chemicals, and as a ***gasoline additive***.

- **Exposure:** ***Is ubiquitous in the air (USA) also found in ground water.***

- ***Acute Effects:*** Acute (short-term) inhalation or dermal exposures to benzene have been observed primarily to lead to ***bone marrow damage***. Manifestations include ***anemia, leukopenia, thrombocytopenia***, etc.

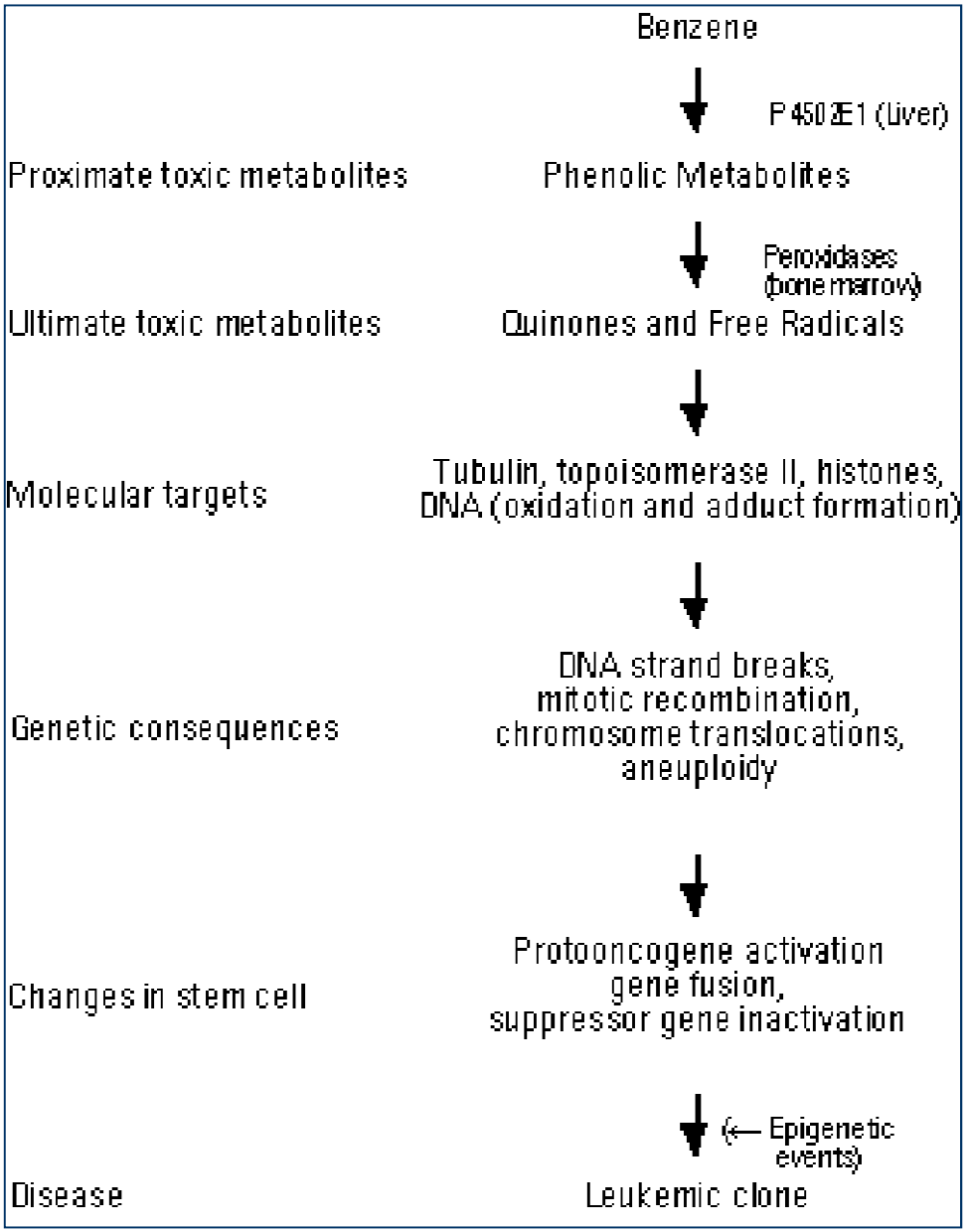
- ***Chronic Effects (Noncancer):*** Chronic depletion of bone marrow cells – ***bone marrow aplasia, pancytopenia that may lead to fatal outcome.***

Aromatic Hydrocarbons - *Benzene:*

- Benzene is ***known to be a human carcinogen*** based on sufficient evidence of carcinogenicity in humans and experimental animals.

- **Human studies**: Many case reports and case series have described the ***association of leukemia with exposure to benzene***, either alone or in combination with other chemicals. ***Most cases were acute leukemias and lymphomas.*** A series of epidemiological studies, both cohort and case-control, showed statistically significant associations between leukemia (***predominantly myelogenous***) and occupational exposure to benzene and benzene- containing solvents. These results were replicated in a number of countries and different industries. In the epidemiological studies of people exposed primarily to benzene, statistically significant excesses of leukemia were observed.

Aromatic Hydrocarbons - *Benzene:*



Aromatic Hydrocarbons – *Polycyclic Aromatic Hydrocarbons (PAHs)*

- PAHs are a group of chemicals that are formed during the ***incomplete burning of coal, oil and gas, garbage, or other organic substances***.
- PAHs can be formed through natural processes or those related to human activities.
- There are more than 100 different PAHs. Most PAHs do not occur alone in the environment. Rather they are found as mixtures of two or more PAHs.
- PAHs can occur in the air attached to organic particles, in the soil, or in the sediments as solids.
- They can also be found in substances such as crude oil, coal, creosote, and road / roofing tar.
- Evaporation into air does occur very easily. Global transport occurs.
- Examples: Acenaphthene, Anthracene, Benz[a]anthracene, Benzo[a]pyrene, Benzo[b]fluoranthene

Polycyclic Aromatic Hydrocarbons (PAHs) -Toxicity

- Although unmetabolized PAHs can have toxic effects, the major concern in animals is the ***ability of reactive metabolites to bind to proteins and DNA***.

- ***Four, five and six ring PAHs have greater carcinogenic potential than do two, three or seven ring PAHs.***

- The addition of ***alkyl groups to PAHs enhances the carcinogenic potential*** of these compounds.

- Some exposure to PAHs comes through ***inhalation:***

  - In the environment you are exposed to PAH vapors or PAHs attached to dust and other particles in the air. These can come from vehicle exhausts, coal burning, wildfires, agricultural burning, and hazardous waste sites.

  - Other inhalation exposures come from PAHs present in tobacco smoke, smoke from wood burning fireplaces, and creosote-treated wood products.

- Some exposure to PAHs by ***ingestion:***
  - Cooking meat or other foods at high temperatures that results in charring of the food increases the amount of PAHs in the food.

PAHs - Absorption, Distribution & Excretion:

- These are ***highly lipophilic compounds*** so they are absorbed quickly by all routes of exposure.

- Storage is mostly in ***kidneys, liver and fat tissue***. **PAHs do not have long half-lives**. Usually measured in days. ***Excretion is primarily by urine and feces.***

- Example of PAHs: **Benzo[a]pyrene**:

  - Benzo[a]pyrene diol epoxides show a strong preference for reaction with ***purine residues, particularly guanine***.

  - BPa is one of the most intensely studied PAHs as it is an extremely potent carcinogen.

  - Metabolic transformations catalyzed by the CYPs are mainly ***hydroxylations*** occurring at the various available sites on the aromatic rings (phase I reactions), and ***conjugations of the hydroxyl groups*** with glucuronic acid, sulphate, or glutathione (phase II reactions).

Ethyl Alcohol & Alcohol Liver Disease:

- Excessive alcohol (ethanol) consumption is the leading cause of liver disease in most Western countries.

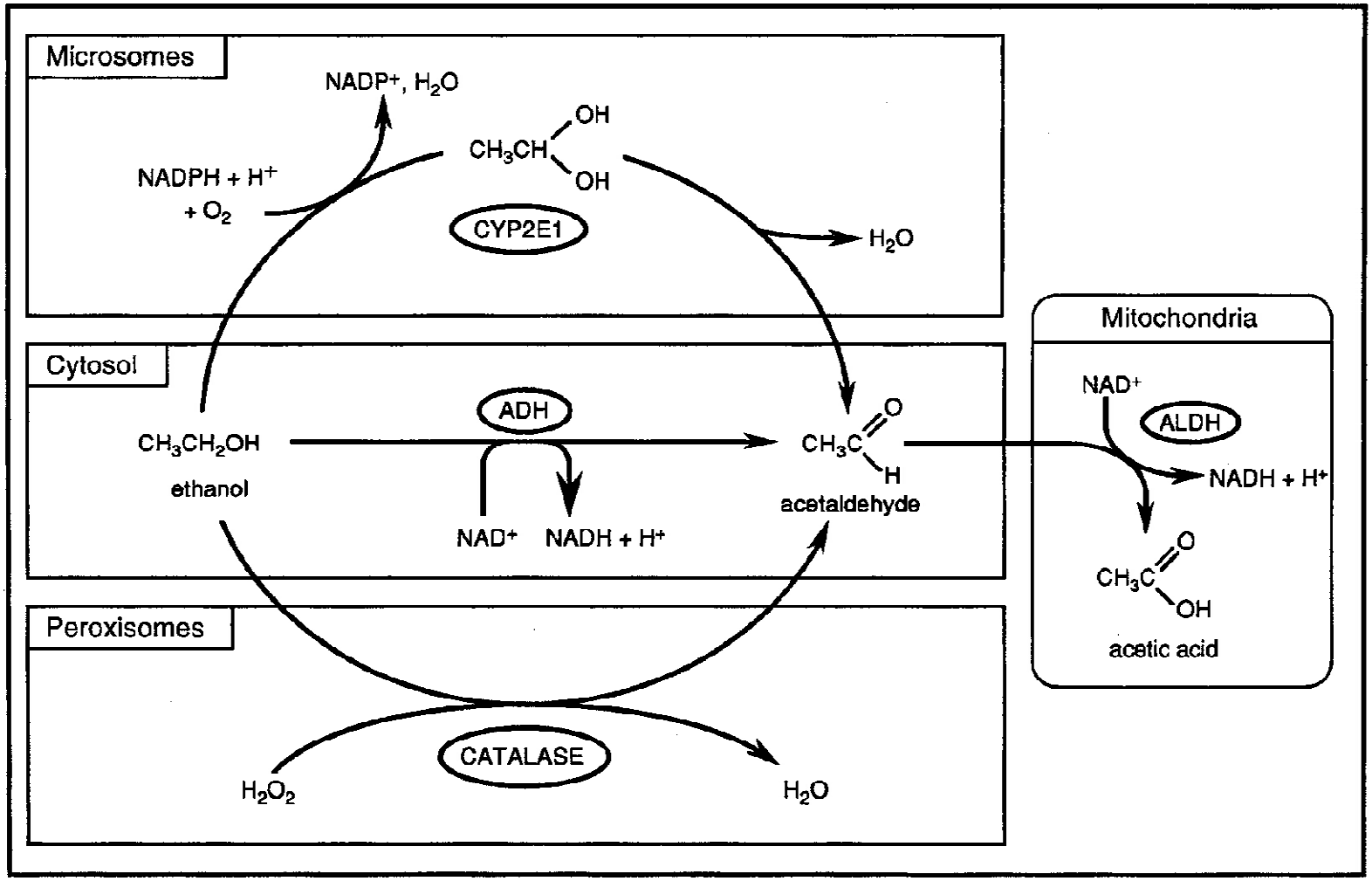
- Chronic alcohol consumption has a variety of adverse effects. Of greatest impact, are the three distinctive, albeit overlapping, forms (collectively referred to as alcoholic liver disease):

  - **Hepatic steatosis.**

  - **Alcoholic hepatitis.**

  - **Cirrhosis.**

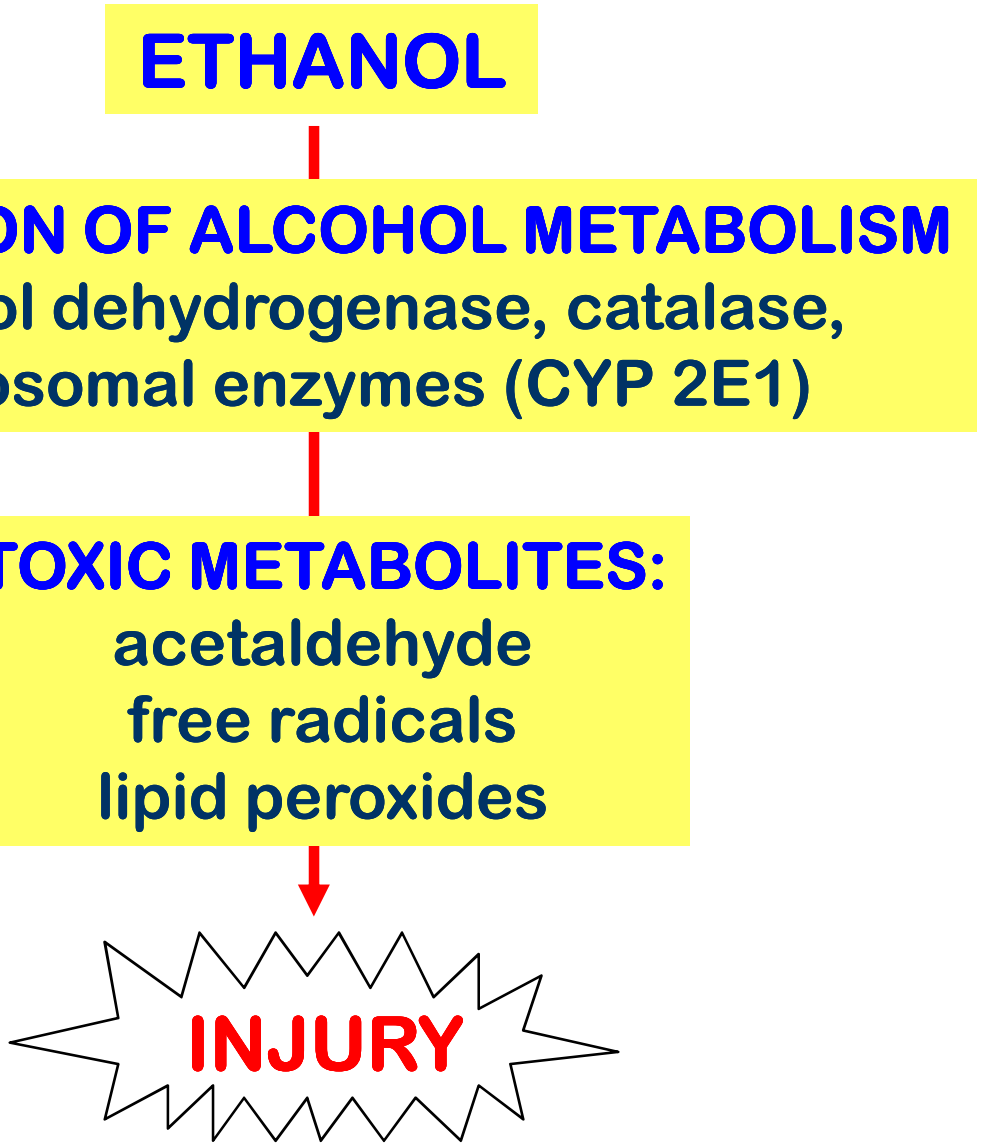
Ethanol Metabolism:

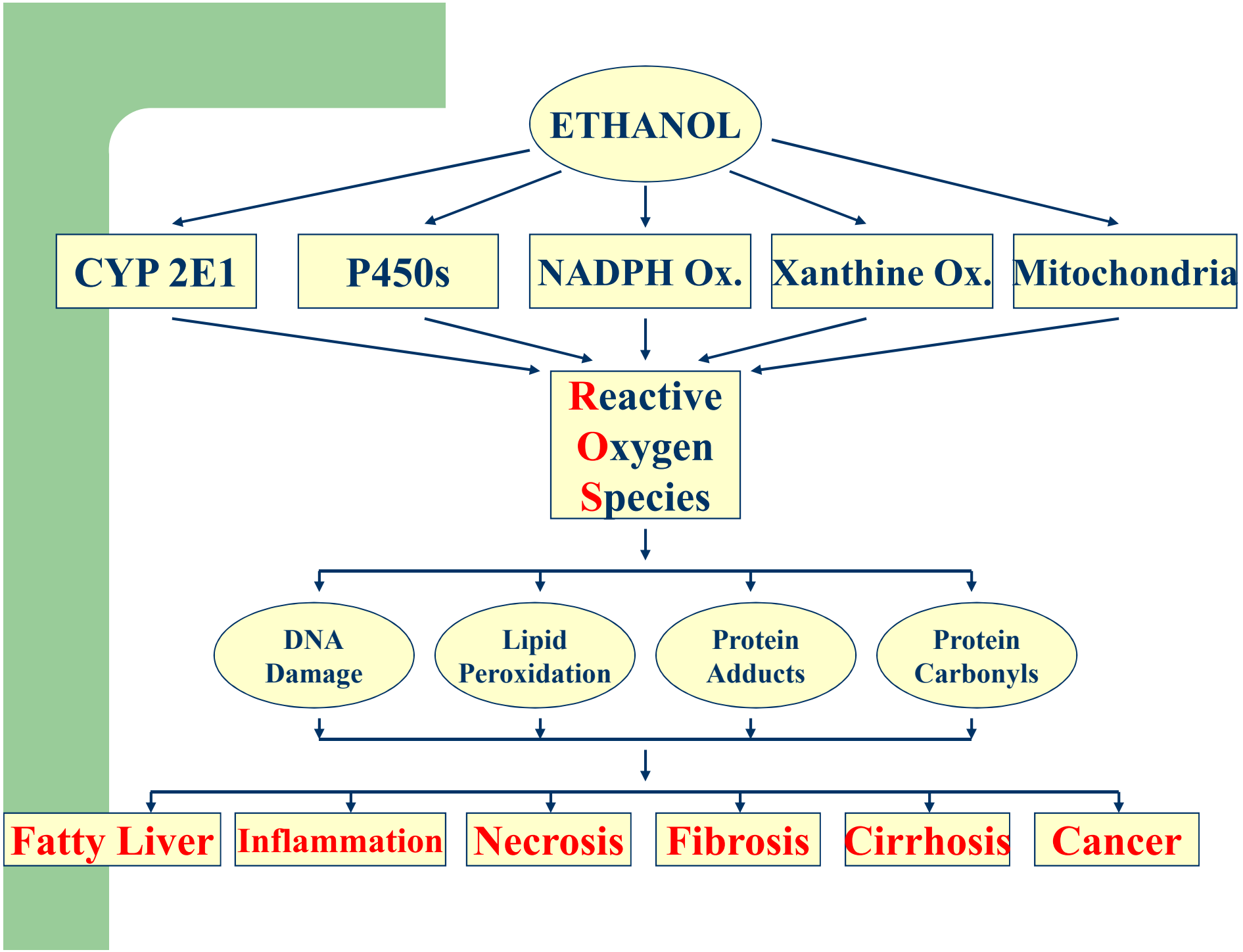


Partial $CH_3CH_2OH + O_2 \longrightarrow CH_3COOH + 3H_2O$

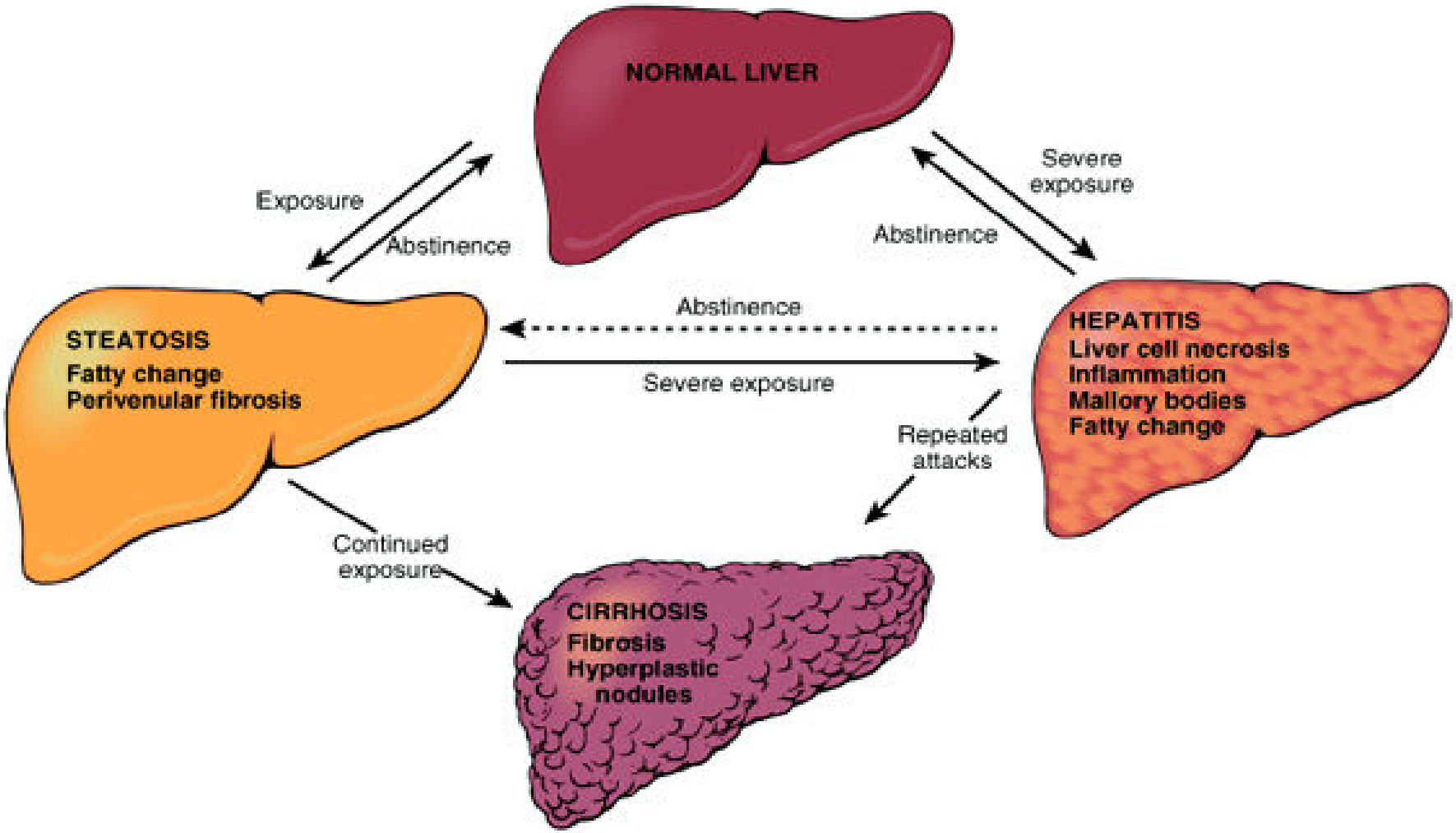
Total $CH_3CH_2OH + 3O_2 \longrightarrow 2CO_2 + 3H_2O$

Alcohol-Induced Liver Injury




ETHANOL
CYP 2E1
P450s
NADPH Ox.
Xanthine Ox.
Mitochondria
Reactive Oxygen Species
DNA Damage
Lipid Peroxidation
Protein Adducts
Protein Carbonyls
Fatty Liver
Inflammation
Necrosis
Fibrosis
Cirrhosis
Cancer

Alcoholic Liver Disease:



Alcoholic liver disease: ***The interrelationships among hepatic steatosis, hepatitis, and cirrhosis are shown, along with a depiction of key morphologic features at the morphologic level.***

Alcoholic Liver Disease – *Pathogenesis:*

- Hepatocellualr steatosis (fatty change) results from:
  - *Increased lipid biosynthesis.*
  - *Impaired assembly/secretion of lipoproteins.*
  - *Increased peripheral fat catabolism.*
- Toxic effects caused by:
  - ***P450 induction**: This produces reactive oxygen species that react with cellular proteins, damage membranes, and alter hepatocellular function.*
  - ***Free radicals** which are produced during microsomal oxidation of alcohol. Acetaldehyde also causes lipid peroxidation.*
  - ***Direct effect** on the function of cellular organelles (mitochondria).*
  - *Alcohol induced and acetaldehyde-induced changes in hepatocellualr proteins create new epitopes to which the immune system reacts, producing inflammation & immune-mediated hepatocellualr injury.*

Collagen deposition by perisinusoidal hepatic stellate cells is due to release of proinflammatory cytokines from Kupffer cell, endothelial cells and neutrophils.

- Alcohol also causes ***derangements of vascular perfusion.***

Alcoholic Liver Disease – *Clinical Features:*

- *Hepatic steatosis may become evident as hepatomegaly with mild elevation of serum bilirubin & Alkaline Phosphatase (ALP) levels. Alcohol withdrawal and the provision of an adequate diet are sufficient treatment.*

- *Alcoholic hepatitis tends to appear relatively acutely following a but of heavy drinking. Symptoms & laboratory manifestations may be minimal or those of fulminant hepatic failure. Between these extremes are the non specific symptoms of malaise, anorexia, weight loss, upper abdominal discomfort, tender hepatomegaly. Lab.findings of elevated bilirubin, elevated ALP, and a neutrophilic leukocytosis.*

- *The manifestation of alcoholic cirrhosis are similar to those of other forms of cirrhosis. Commonly, the first sign of cirrhosis relate to complications of* ***portal hypertension****, including life-threatening* ***esophageal variceal hemorrhage****.*

- *In the end stage alcoholic, the proximate causes of death are* ***(1) hepatic coma, (2) massive GIT hemorrhage, (3) an intercurrent infection, (4) hepatorenal syndrome, (5) hepatocellualr carcinoma in 3%-6% of cases.***

Alcoholic & Cancer:

- The occurrence of malignant tumors of the ***oral cavity, pharynx, larynx, esophagus, liver, female breast and colorectum*** is causally related to the consumption of alcoholic beverages.

- There is sufficient evidence for the carcinogenicity of alcoholic beverages and ethanol in alcoholic beverages in humans.

- There is sufficient evidence for the carcinogenicity of ethanol and of alcoholic beverages in experimental animals.

- **Alcoholic beverages** and ethanol in alcoholic beverages *are carcinogenic to humans.*

Mechanisms of Ethanol-induced carcinogenesis:

❖ The precise mechanism of action is unknown, but is thought to include:

- **Initiation:**
  - ✓ **Production of acetaldehyde**, the first and most toxic metabolite of ethanol and its binding to DNA.
  - ✓ **Generation of oxidants** via induced CYP2E1 and other enzymes.
  - ✓ **Increased activation environmental procarcinogens**, especially of ***nitrosamines*** by CYP2E1.
- **Promotion:**
  - ✓ **Increased cell proliferation**: *direct cytotoxicity, production of mitogenic cytokines, elevated production of eicosanoids (signaling molecules).*