HISTOPATHOLOGY

Introduction

- The word *'Pathology'* is derived from two Greek words—*pathos* (meaning suffering) and *logos* (meaning study)
- Pathology is, thus, scientific study of changes in the structure and function of the body in disease
- Pathology consists of the abnormalities in normal anatomy (including histology) and normal physiology owing to disease

History

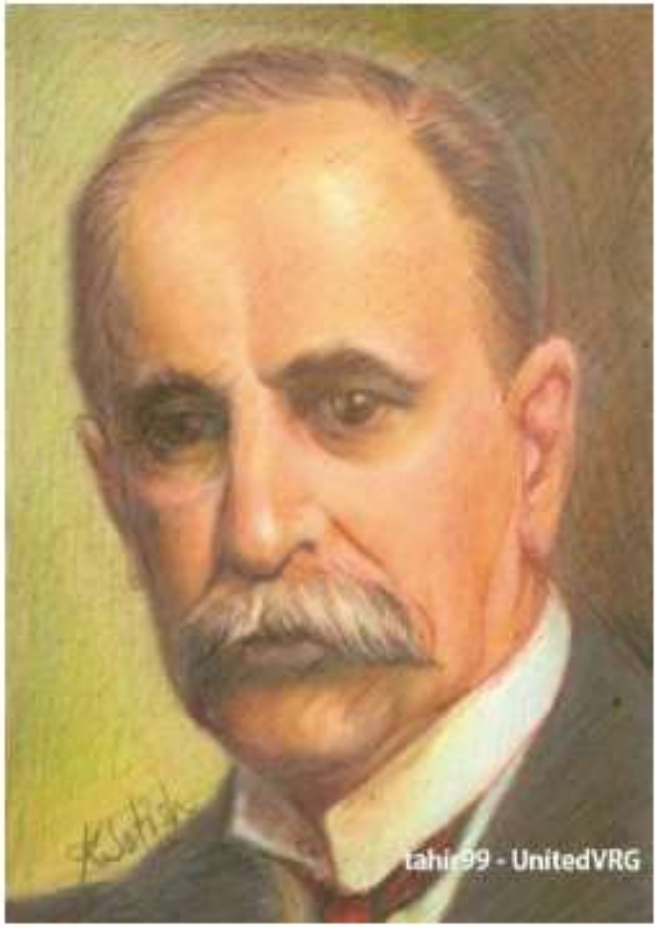


Figure 1.1 Sir William Osler (1849–1919). Canadian physician and one of the four founding Professors of Johns Hopkins Hospital, Baltimore, US, is regarded as '*Father of Modern Medicine*', Sir Osler had keen interest in pathology, was an acclaimed teacher and is also remembered for his famous quotations.

FATHER OF CPCs

Figure 1.5 Giovanni B. Morgagni (1682–1771), an Italian physician-anatomist who introduced clinicopathologic methodology in the study of disease by correlation of clinical findings with findings at postmortem examination.

FATHER OF MUSEUM IN PATHOLOGY

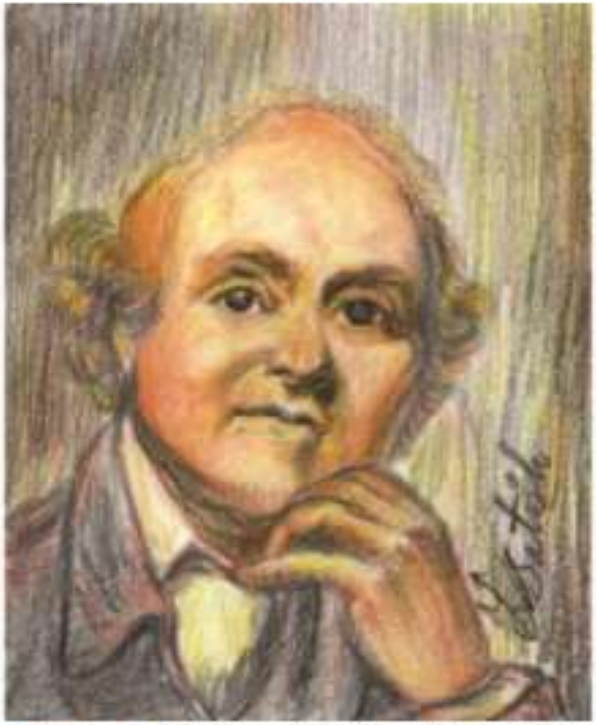


Figure 1.6 John Hunter (1728–1793). Scottish surgeon, regarded as the greatest surgeon-anatomist of all times who established first ever unique collection of pathological specimens that later resulted in the Hunterian Museum of the Royal College of Surgeons, London.

FATHER OF CLINICAL PATHOLOGY

Figure 1.7 Paul Ehrlich (1854–1915). German physician, conferred Nobel prize for his work in immunology, described Ehrlich's test for urobilinogen, staining techniques of cells and bacteria, and laid the foundations of haematology and clinical pathology.

DEFINITION OF PATHOLOGY

- "scientific study of the molecular, cellular, tissue, or organ system response to injurious agents"

- **It is the** "scientific study of disease"

DEFINITION:

Refers to the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides.

SCOPE

- Though the histopathological techniques are *labour intensive, cumbersome and time consuming*, particularly when there are *automation equipments are not available*; however, their *use in diagnosis of diseases* is unequivocal.
- **Some of the areas where histopathological diagnosis is helpful are described as follows:**
- This is useful in establishing the ***pathogenesis and pathology*** of any disease caused by *bacteria, virus, chlamydia, rickettsia, mycoplasma, parasite, toxin, poisons* etc.
- There are certain diseases in which histopathological ***examination of tissues is the only alternative to diagnose the disease. e.g. Bovine spongiform encephalopathy.*** The agent of this disease takes a *very long incubation period and very difficult to isolate and there is no immune response and inflammation in animal*. Therefore, histopathology remains the only alternative for confirmatory diagnosis.
- In some cases, the tissues from ***dead animals are only available material for laboratory diagnosis***. This may occur either due to *lack of time or due to negligence for not collecting the material for serological tests or isolation studies*. Sometimes the *transportation of material from remote areas destroys the other material and the tissues* fixed in formalin only remains for making diagnosis. In all such cases the histopathological examination has its pivotal role.

Subdivisions of pathology

- Human pathology is conventionally studied under two broad divisions:

General Pathology dealing with general principles of disease

Systemic Pathology that includes study of diseases pertaining to the specific organs and body systems

Branches of pathology

- The study of pathology includes morphological and non morphological disciplines as follows

- **Morphological branches**
  - Histopathology
  - Cytopathology
  - Haematology

- **Non Morphological branches**
  - Clinical pathology
  - Clinical Biochemistry
  - Microbiology
  - Immunology., etc

WHAT IS DISEASE??

- **It is the** "state in which an individual exhibits an anatomical, physiological, or biochemical deviation from the normal"

Disease may be defined as :

an abnormal alteration of structure or function in any part of the body.

What should we know about a Disease?

- Definition.
- Epidemiology – Where & When.
- Etiology – What is the cause?
- Pathogenesis - Evolution of dis.
- Morphology - Structural Changes
- Functional consequences

Pathology

- Management
- Prognosis
- Prevention

Pathology focuses on 4 aspects of disease:

- ***Etiology***
- ***Pathogenesis***
- ***Morphology***
- ***Functional consequence***

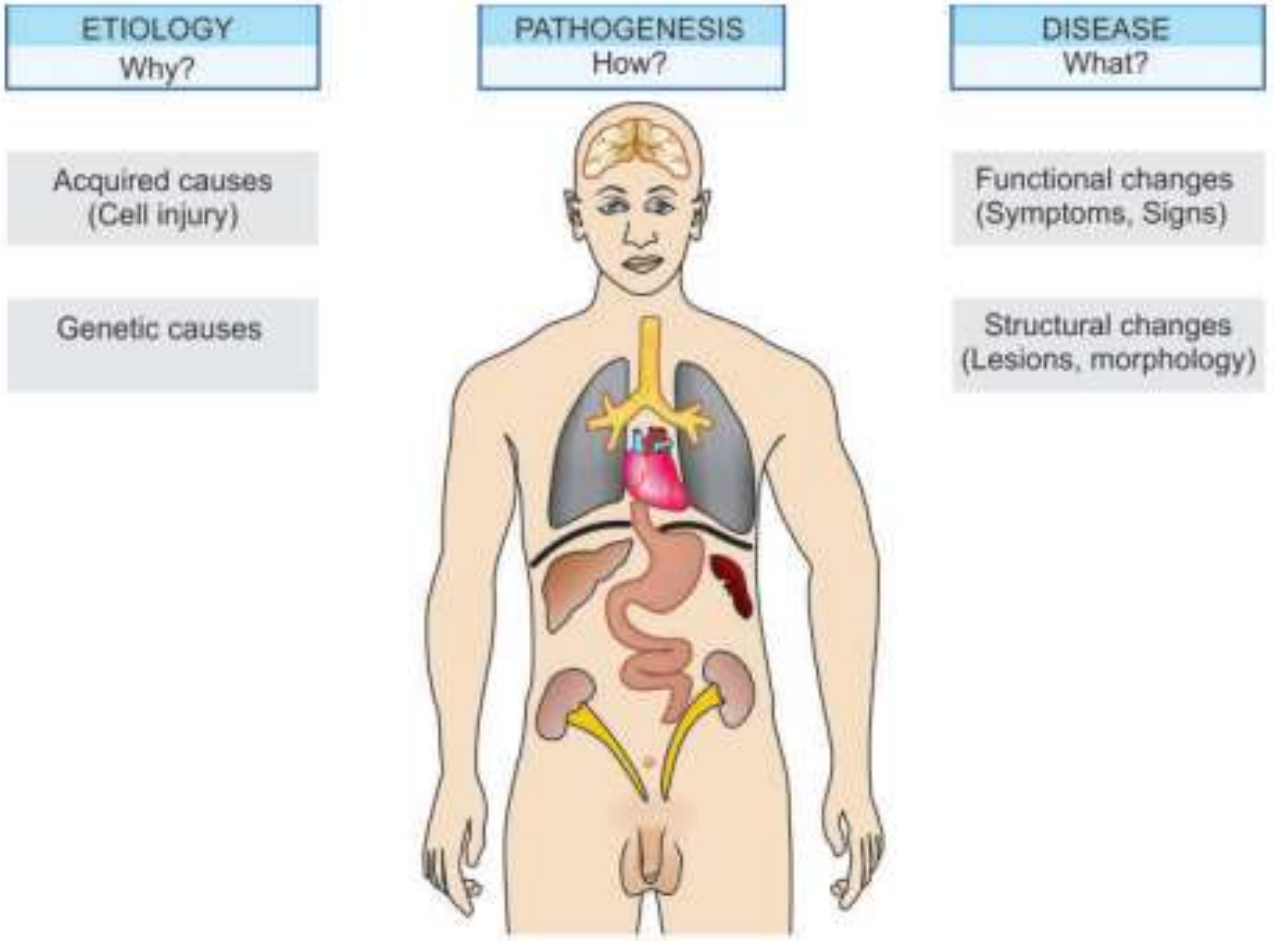
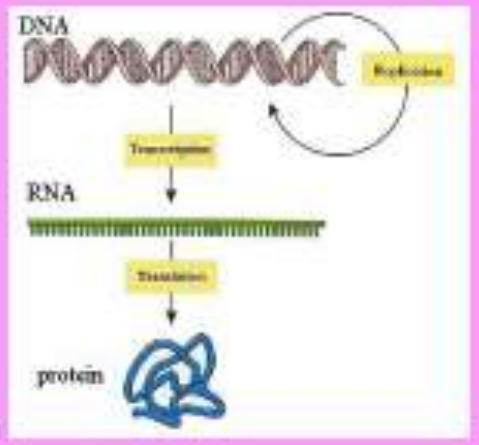


Figure 1.2 Diagrammatic depiction of disease and various terms used in pathology.

ETIOLOGY

Knowledge or discovery of the primary etiology remains the backbone on which a diagnosis can be made and a disease process can be best understood so that a treatment can be prescribed.



THE ETIOLOGICAL FACTORS ARE:

- ENVIRONMENTAL FACTORS
- GENETIC FACTORS
- IINDIRECT CAUSES

ENVIRONMENTAL FACTORS ARE:

- PHYSICAL AGENTS – radiation, trauma or mechanical injury, thermal changes, electrical, nuclear or X-rays, changes in atmospheric pressure
- CHEMICAL AGENTS – chemicals, poisons like venoms or toxins, corrosive agents like strong acids and alkalis
- NUTRITIONAL DEFICIENCES AND EXCESSES
- INFECTIONS AND INFESTATIONS
- ABNORMAL IMMUNOLOGICAL REACTIONS
- PSYCHOLOGICAL FACTORS

GENETIC FACTORS: ABNORMAL GENES

INDIRECT CAUSES: pertain to the predisposing factors like age, age, sex, environment, race, climate, state of nutrition, habits

Etiology: What is the cause?

- ***Environmental agents:***
  - **Physical**
  - **Chemical**
  - **Nutritional**
  - **Infections**
  - **Immunological**
  - **Psychological**
- ***Genetic Factors:***
  - **Age**
  - **Genes**

Multifactorial:

As Diabetes,

Hypertension

Cancer

Pathogenesis

- **The sequence events in the response of the cells or tissues to the etiologic agent, from the initial stimulus to the ultimate expression of the disease,"from the time it is initiated to its final conclusion in recovery or death"**

METHODS OF STUDYING PATHOLOGY

- GROSS EXAMINATION
- LIGHT MICROSCOPY
- IMMUNOCHEMISTRY
- ELECTRON MICROSCOPY
- MOLECULAR BIOLOGY

- **Necropsy:** Gross examination of the animal cadaver by systematic dissection in order to evaluate any abnormal changes **(lesions)** that may be present

- **Autopsy:** Synonymous to necropsy in human medicine

- ***Biopsy:*** *Removal and examination of tissue*

Collection of tissues (Histopathological examination of tissues starts with: surgery, biopsy, or autopsy.)

NOTE:

The tissue is removed from the body, and then placed in a fixative which stabilizes the tissues to prevent decay. The most common fixative is formalin (10% formaldehyde in water). The tissue is then prepared for viewing under a microscope using either chemical fixation or frozen section

BIOPSY

- is a medical test commonly performed by a surgeon, interventional radiologist, or an interventional cardiologist involving sampling of cells or tissues for examination.

- Biopsies are most commonly performed for insight into possible cancerous and inflammatory conditions.

CONDITIONS IDENTIFIED

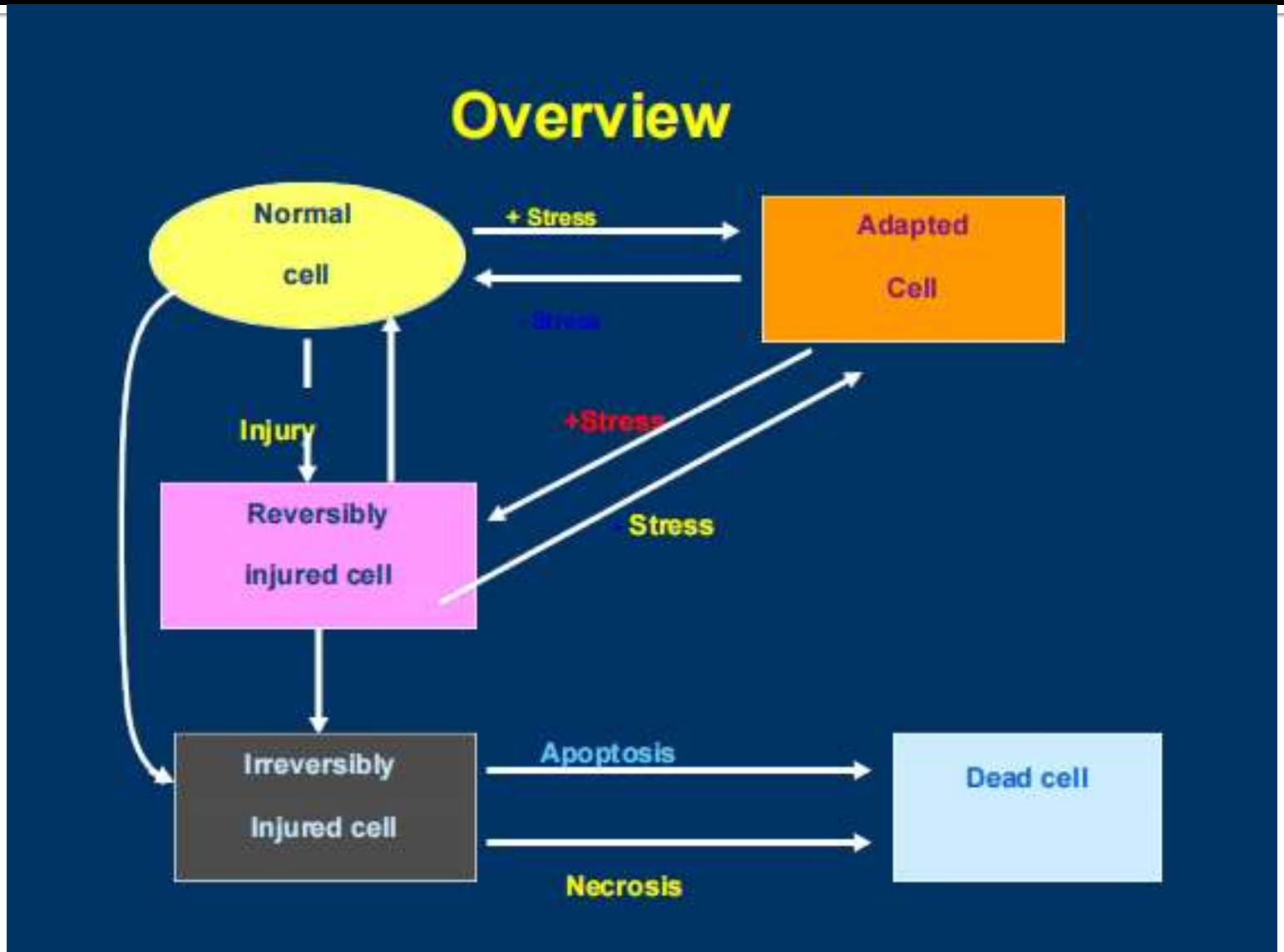
- CANCER
- PRECANCEROUS
- INFLAMMATION CONDITIONS

CELL INJURY

- *Cell injury is defined as the effect of a variety of stresses due to etiologic agents a cell encounters resulting in changes in its internal and external environment*

OR

- *Cell injury is a sequence of events that occur if the limits of adaptive capability are exceeded or no adaptive response is possible*


Overview
Normal cell
+ Stress
Adapted Cell
Injury
+Stress
Reversibly injured cell
Stress
Irreversibly Injured cell
Apoptosis
Dead cell
Necrosis

- Various forms of cellular responses to cell injury may be as follows:

1. When there is increased functional demand, the cell may adapt to the changes which are expressed morphologically, which then revert back to normal after the stress is removed *(cellular adaptations*)
2. When the stress is mild to moderate, the injured cell may recover *(reversible cell injury)*, while persistent and severe form of cell injury may cause cell death *(irreversible cell injury)*
3. The residual effects of reversible cell injury may persist in the cell as evidence of cell injury at subcellular level *(subcellular changes)*

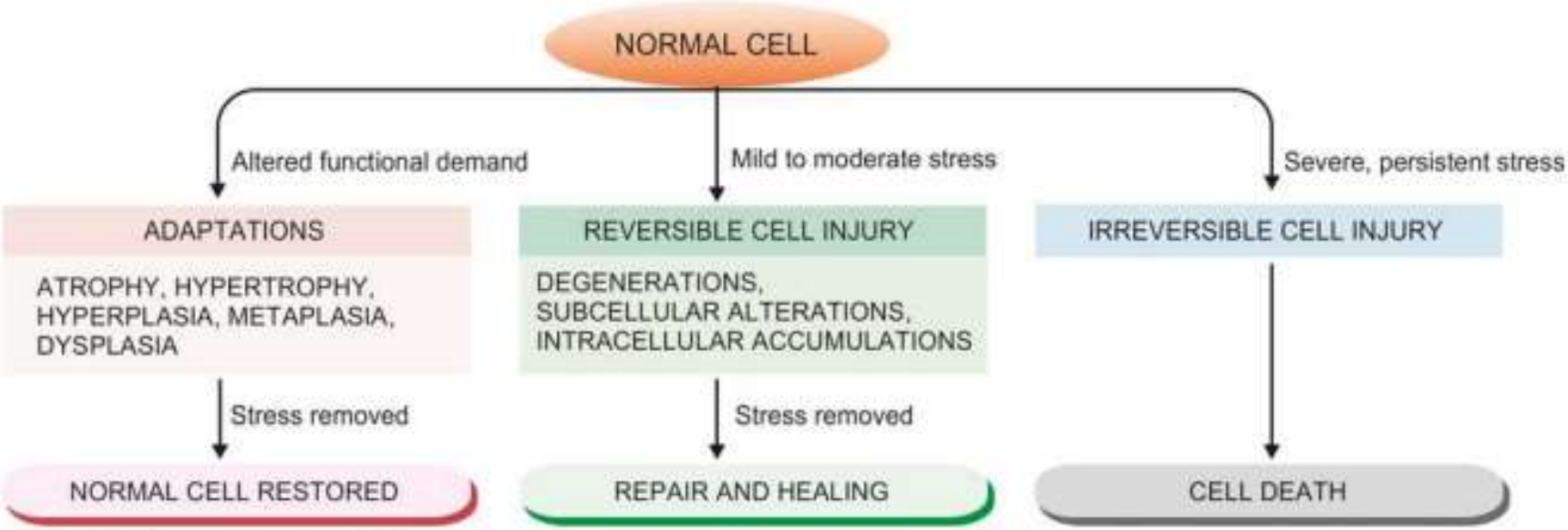


Figure 2.1 Cellular responses to cell injury.

DEGENERATION

- *Retrogressive changes/ reversible cell injury*

- Examples of morphologic forms of reversible cell injury are:

1. *Hydropic change*
2. *Hyaline change*
3. *Mucoid change*
4. *Fatty change*

Hydropic changes

- Means *accumulation of water within the cytoplasm of the cell*
- Other synonyms used are ***cloudy swelling*** (for gross appearance of the affected organ) and *vacuolar degeneration* (due to cytoplasmic vacuolation)
- Hydropic swelling is an entirely reversible change upon removal of the injurious agent

ETIOLOGY

- Commonest and earliest form of cell injury
- The common causes include acute and subacute cell injury from various etiologic agents such as bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypertonic glucose or saline etc

PATHOGENESIS

Results from impaired regulation of sodium and potassium at the level of cell membrane

↓

Intracellular accumulation of sodium and escape of potassium

↓

Accompanied with rapid flow of water into the cell to maintain *iso-osmotic* conditions

↓

cellular swelling

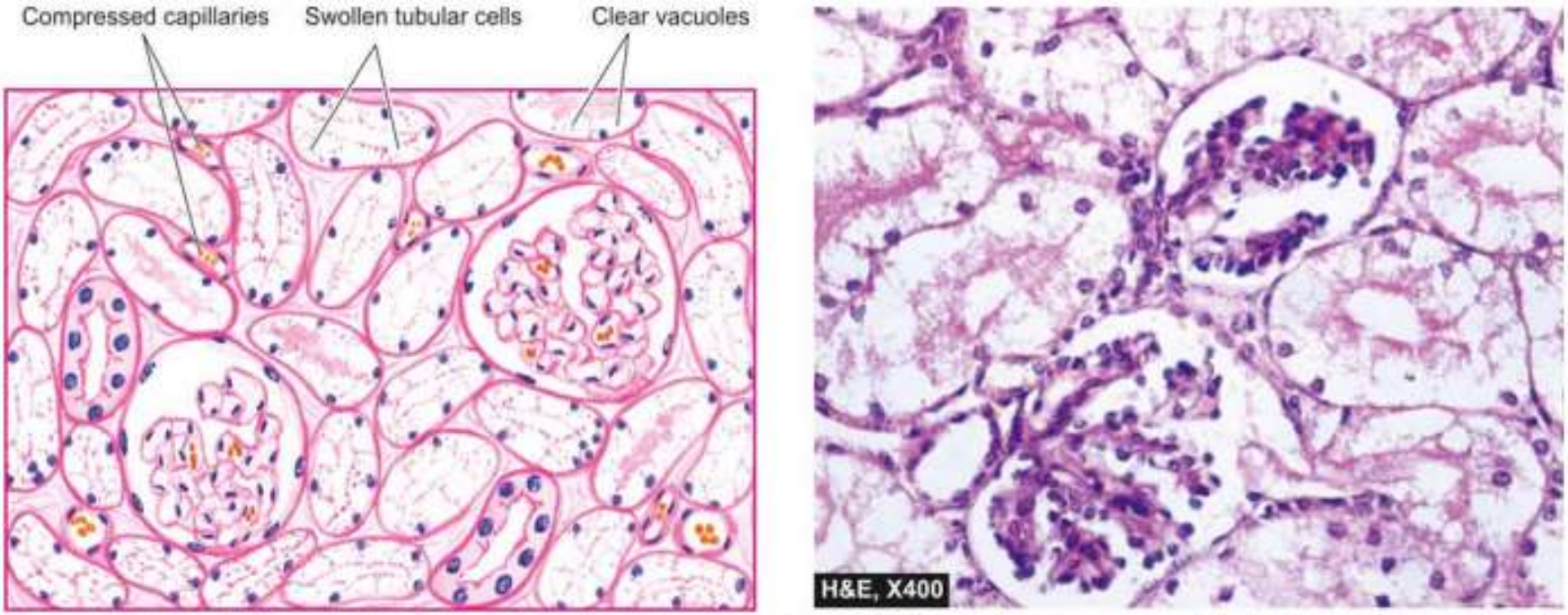


Figure 2.7 Hydropic change kidney. The tubular epithelial cells are distended with cytoplasmic vacuoles while the interstitial vasculature is compressed. The nuclei of affected tubules are pale.

HYALINE CHANGE

- The word ‘hyaline’ or ‘hyalin’ means glassy (hyalos = glass)
- Hyalinisation is a common descriptive histologic term for glassy, homogeneous, eosinophilic appearance of proteinaceous material in haematoxylin and eosin-stained sections and does not refer to any specific substance
- Hyaline change is seen in heterogeneous pathologic conditions and may be intracellular or extracellular

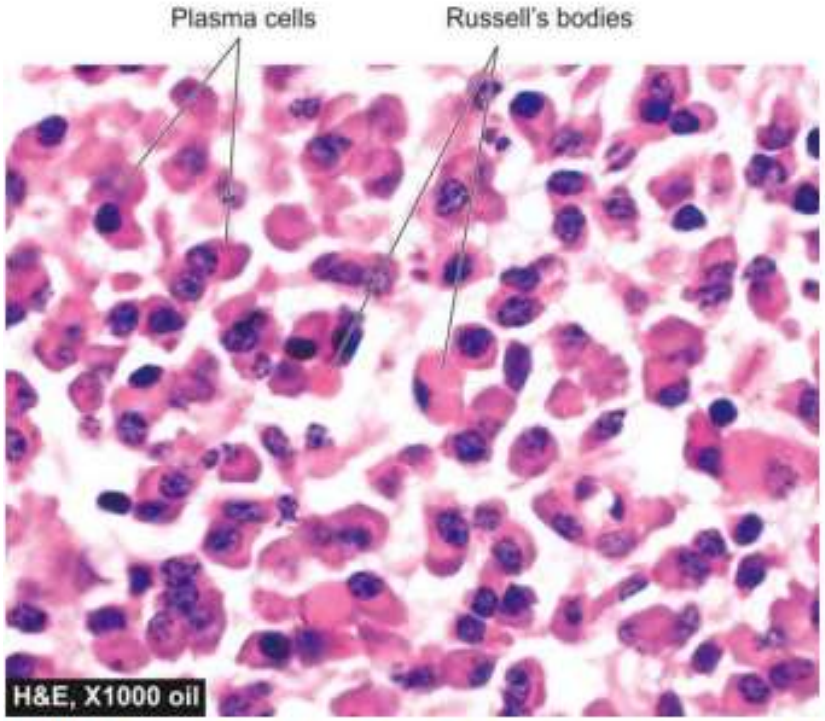


Figure 2.8 Intracellular hyaline as Russell's bodies in the plasma cells. The cytoplasm shows pink homogeneous globular material due to accumulated immunoglobulins.

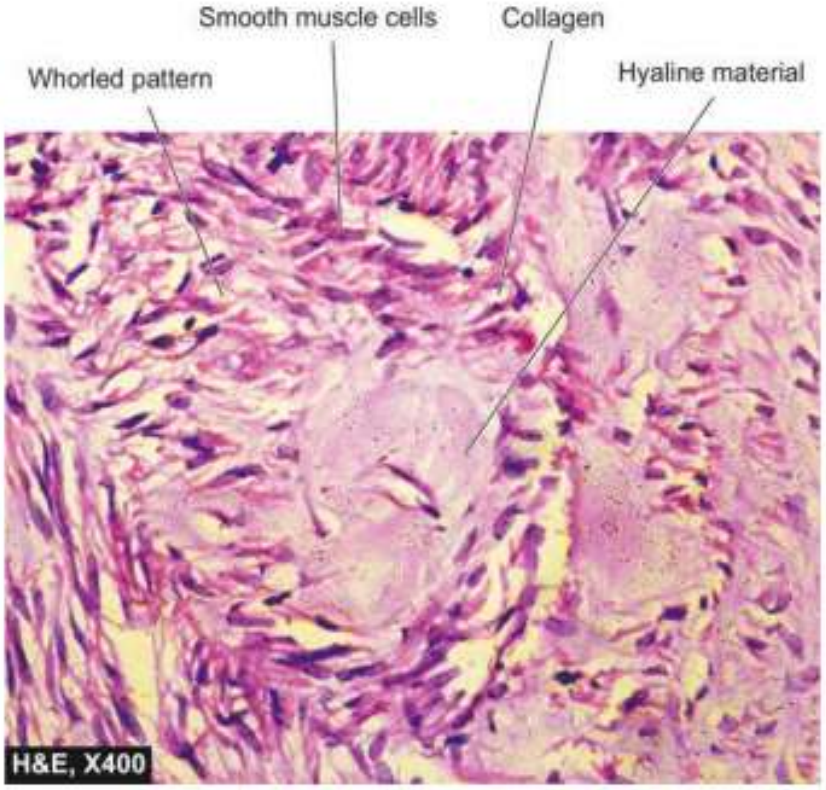


Figure 2.9 Extracellular hyaline deposit in leiomyoma uterus. The centres of whorls of smooth muscle and connective tissue show pink homogeneous hyaline material (connective tissue hyaline).

INTRACELLULAR HYALINE

- Intracellular hyaline is mainly seen in epithelial cells

- A few examples are as follows:

1. Hyaline droplets in the proximal tubular epithelial cells due to excessive reabsorption of plasma proteins in proteinuria
2. Hyaline degeneration of rectus abdominalis muscle called Zenker's degeneration, occurring in typhoid fever. The muscle loses its fibrillar staining and becomes glassy and hyaline
3. Mallory's hyaline represents aggregates of intermediate filaments in the hepatocytes in alcoholic liver cell injury
4. Nuclear or cytoplasmic hyaline inclusions seen in some viral infections
5. Russell's bodies representing excessive immunoglobulins in the rough endoplasmic reticulum of the plasma cells

EXTRACELLULAR HYALINE

- Extracellular hyaline commonly termed hyalinisation is seen in connective tissues

- A few examples of extracellular hyaline change are as under:

1. Hyaline degeneration in leiomyomas of the uterus
2. Hyalinised old scar of fibrocollagenous tissues
3. Hyaline arteriolosclerosis in renal vessels in hypertension and diabetes mellitus
4. Hyalinised glomeruli in chronic glomerulonephritis
5. Corpora amylacea seen as rounded masses of concentric hyaline laminae in the enlarged prostate in the elderly, in the brain and in the spinal cord in old age, and in old infarcts of the lung

MUCOID CHANGE

- Mucoid means mucus-like
- Mucus is the secretory product of mucous glands and is a combination of proteins complexed with mucopolysaccharides
- *Mucin, a glycoprotein, is its chief constituent*
- Connective tissue mucin is termed **myxoid**
- Both epithelial and connective tissue mucin are stained by ***alcian blue***
- Epithelial mucin stains positively with periodic acid-Schiff (PAS), while connective tissue mucin is PAS negative

EPITHELIAL MUCIN

- Following are some examples of functional excess of epithelial mucin:

1. Catarrhal inflammation of mucous membrane (eg. of respiratory tract, alimentary tract, uterus)
2. Obstruction of duct leading to mucocele in the oral cavity and gallbladder
3. Cystic fibrosis of the pancreas.
4. Mucin-secreting tumours (eg. of ovary, stomach, large bowel etc)

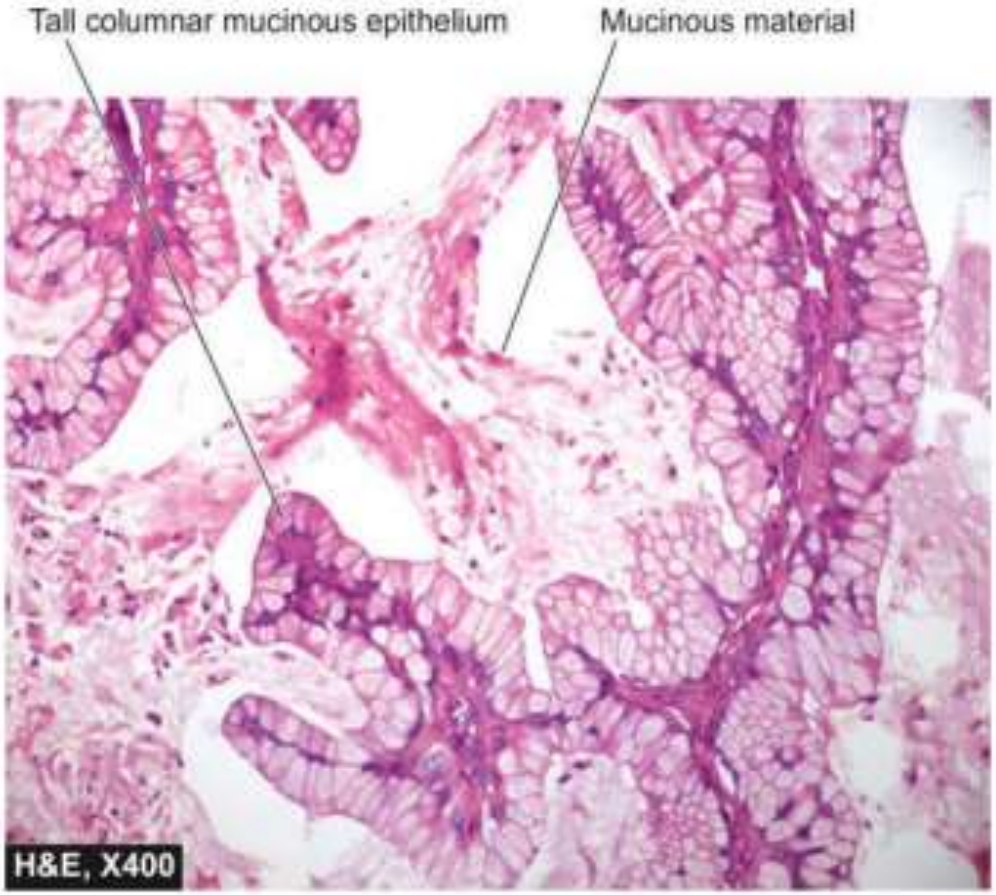


Figure 2.10 Epithelial mucin. Mucinous cystadenoma of the ovary showing intracytoplasmic mucinous material in the epithelial cells lining the cyst.

CONNECTIVE TISSUE MUCIN

- A few examples of disturbances of connective tissue mucin or myxoid change are as under:

1. Mucoid or myxoid change in some tumours eg. myxomas, neurofibromas, fibroadenoma, soft tissue sarcomas etc
2. Dissecting aneurysm of the aorta due to Erdheim's medial degeneration and Marfan's syndrome
3. Myxomatous change in the dermis in myxoedema
4. Myxoid change in the synovium in ganglion on the wrist

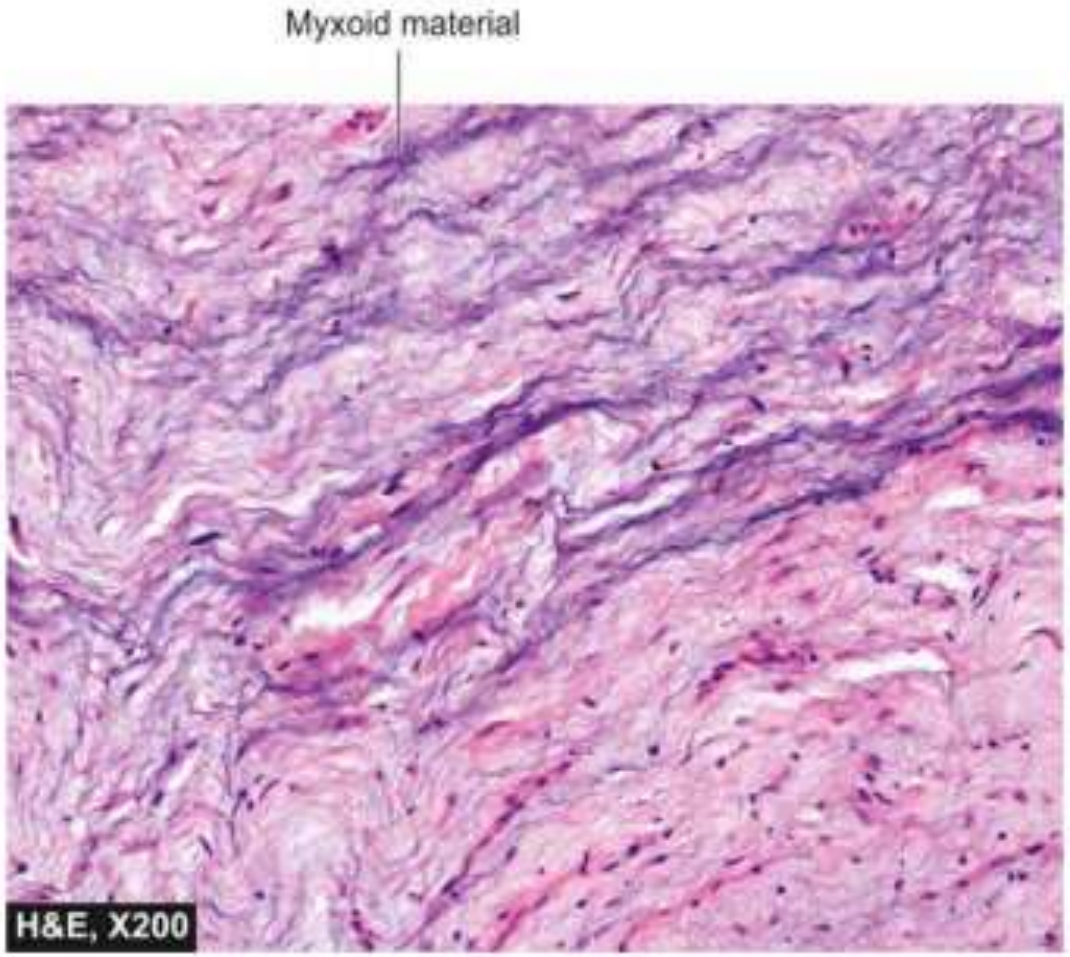


Figure 2.11 Connective tissue mucin (myxoid change) in neurofibroma.

INTRACELLULAR ACCUMULATIONS

- Intracellular accumulation of substances in abnormal amounts can occur within the cytoplasm (especially lysosomes) or nucleus of the cell
- This phenomenon was previously referred to as infiltration

INTRACELLULAR ACCUMULATIONS

- Abnormal intracellular accumulations can be divided into 3 groups:

i) *Accumulation of constituents of normal cell metabolism produced in excess* eg. accumulations of lipids (fatty change, cholesterol deposits), proteins and carbohydrates

ii) *Accumulation of abnormal substances* produced as a result of abnormal metabolism due to lack of some enzymes eg. storage diseases or inborn errors of metabolism

iii) *Accumulation of pigments* eg. endogenous pigments under special circumstances, and exogenous pigments due to lack of enzymatic mechanisms to degrade the substances or transport them to other sites

FATTY CHANGE (STEATOSIS)

- Fatty change, steatosis or fatty metamorphosis is the intracellular accumulation of neutral fat within parenchymal cells
- The deposit is in the cytosol and represents an absolute increase in the intracellular lipids
- Fatty change is particularly common in the liver but may occur in other non-fatty tissues as well eg. in the heart, skeletal muscle, kidneys (lipoid nephrosis or minimum change disease) and other organs.

Fatty Liver

- *Liver is the commonest site for accumulation of fat because it plays central role in fat metabolism*

- Depending upon the cause and amount of accumulation, fatty change may be mild and reversible, or severe producing irreversible cell injury and cell death

ETIOLOGY

- Fatty change in the liver may result from one of the two types of causes:

1. Conditions with excess fat: These are conditions in which the capacity of the liver to metabolise fat is exceeded eg.

i) Obesity

ii) Diabetes mellitus

iii) Congenital hyperlipidaemia

2. Liver cell damage: These are conditions in which fat cannot be metabolised due to liver cell injury e.g.

i) Alcoholic liver disease (most common)

ii) Starvation

iii) Protein calorie malnutrition

iv) Chronic illnesses (e.g. tuberculosis)

v) Acute fatty liver in late pregnancy

vi) Hypoxia (e.g. anaemia, cardiac failure)

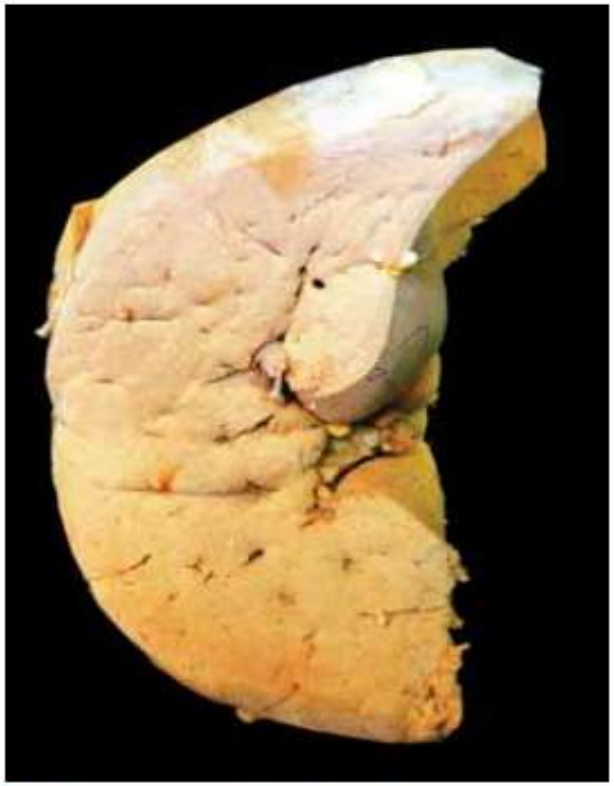
vii) Hepatotoxins (e.g. carbon tetrachloride, chloroform, ether, afl atoxins and other poisons)

viii) Drug-induced liver cell injury (e.g. administration of methotrexate, steroids, CCl4, halothane anaesthetic, tetra□cycline etc)

ix) Reye's syndrome

PATHOGENESIS

- Mechanism of fatty liver depends upon the stage at which the etiologic agent acts in the normal fat transport and metabolism
- Lipids as free fatty acids enter the liver cell from either of the following 2 sources:
- *From diet* as chylomicrons (containing triglycerides and phospholipids) and as free fatty acids
- *From adipose tissue* as free fatty acids



igure 2.13 Fatty liver. Sectioned slice of the liver shows pale yellow arenchyma with rounded borders.

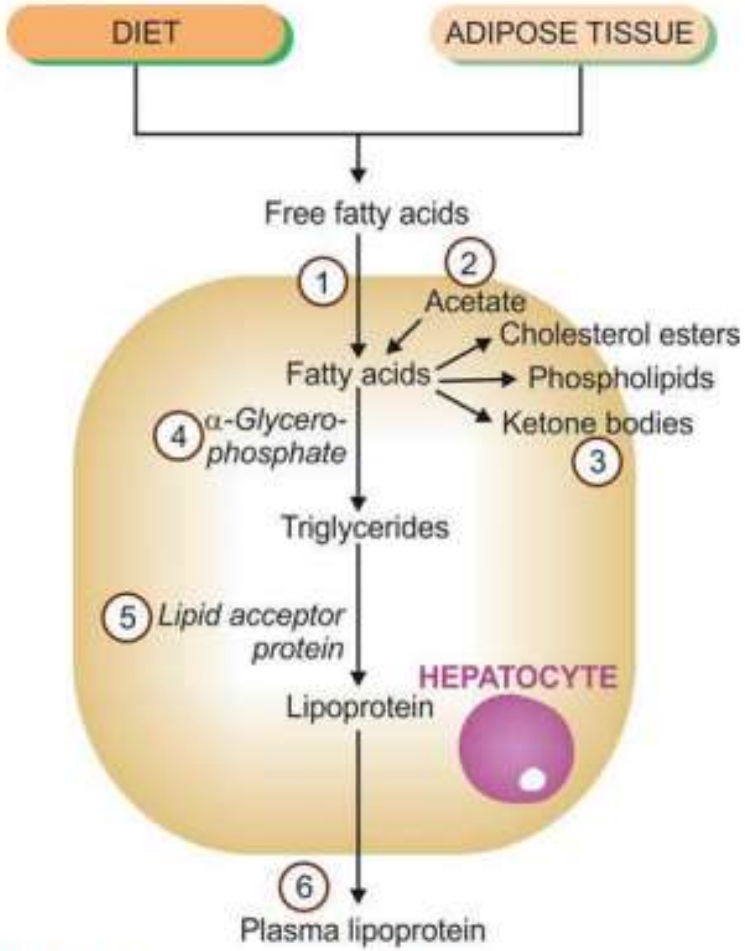


Figure 2.12 Lipid metabolism in the pathogenesis of fatty liver. Defects in any of the six numbered steps (corresponding to the description in the text) can produce fatty liver by different etiologic agents.

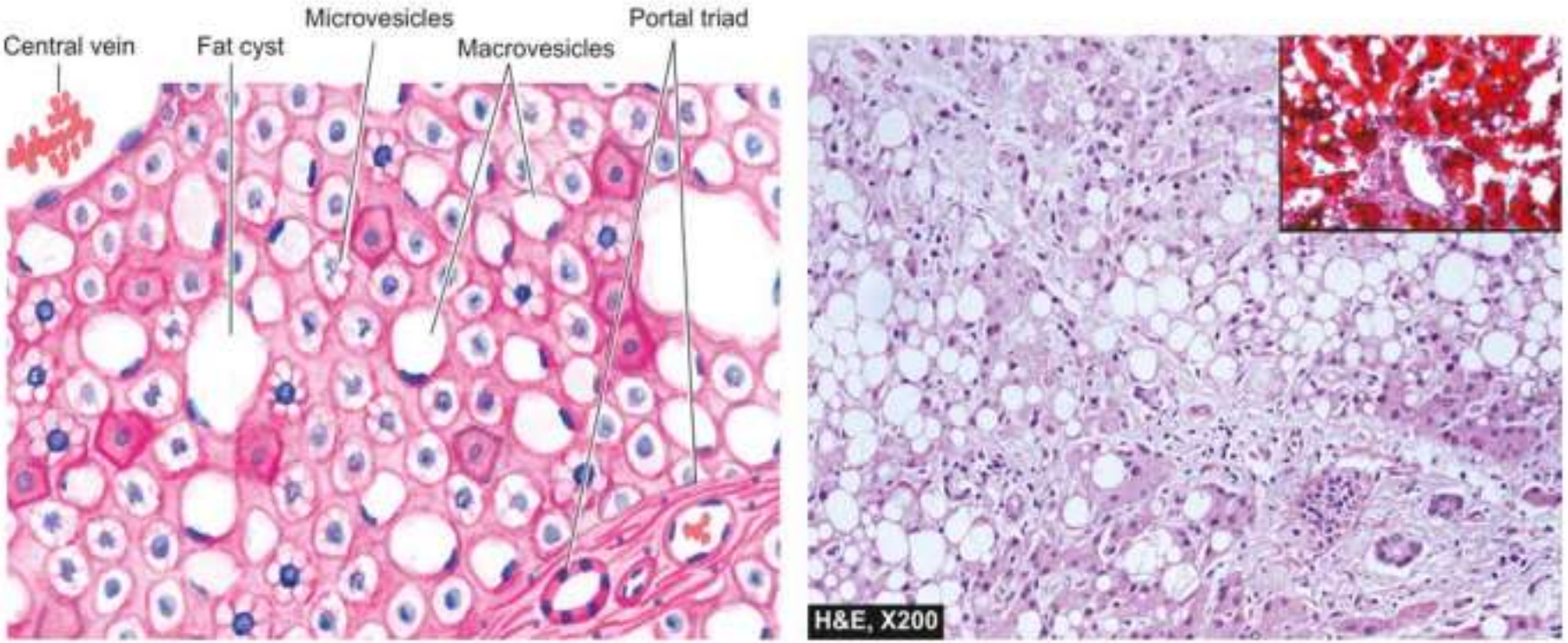


Figure 2.14 Fatty liver. Many of the hepatocytes are distended with large fat vacuoles pushing the nuclei to the periphery (macrovesicles), while others show multiple small vacuoles in the cytoplasm (microvesicles). Inbox shows red colour in the cytoplasmic fat in the hepatocytes in Oil Red O stain in frozen section.

- In fatty liver, intracellular accumulation of triglycerides occurs due to defect at one or more of the following 6 steps in the normal fat metabolism shown in :

1. Increased entry of free fatty acids into the liver
2. Increased synthesis of fatty acids by the liver
3. Decreased conversion of fatty acids into ketone bodies resulting in increased esterifi cation of fatty acids to triglycerides
4. Increased -glycerophosphate causing increased esterification of fatty acids to triglycerides
5. Decreased synthesis of 'lipid acceptor protein' resulting in decreased formation of lipoprotein from triglycerides
6. Block in the excretion of lipoprotein from the liver into plasma

- Liver cell injury from chronic alcoholism is multifactorial as follows:

i) Increased lipolysis

ii) Increased free fatty acid synthesis

iii) Decreased triglyceride utilisation

iv) Decreased fatty acid oxidation to ketone bodies

v) Block in lipoprotein excretion