Pathological calcification and Amyloidosis

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Pathologic calcification

- Def: It is the abnormal tissue depostition of calcium salts, together with smaller amounts of iron, magnesium and other mineral salts.

- Two forms: Dystrophic calcification.
  Metastatic calcification.

- **Dystrophic calcification:**

  When the depostion occurs in dying tissue, it is known as dystrophic calcification.

  It occurs despite normal normal serum calcium & in absence of derangements in calcium metabolism.

- **Metastatic calcification:**

  The depostion of calium salts in normal tissue.

  Results from hypercalcemia secondary to disturbance in calcium metabolism.

- Grossly: Appear as fine white granules or clumps felt as gritty deposits.
- Microscopically: Intracellular/extracellular
- Basophilic amorphous granular, clumped appearance.
- Heterotropic bone formation
- Psammoma bodies: lamellated configurations.

Thyroid cancers. Psammoma bodies

Asbestos in lung: Dumbell shaped

Dystrophic calcification:

- Dystrophic calcification is characterised by deposition of calcium salts in dead and degenerated tissues with normal calcium metabolism and normal serum calcium levels.

Pathogenesis:

2 Phases

1. Initiation (nucleation)
2. 2.Propagation – accumulation of Ca^{+2} phosphate salts

- Initiation (nucleation)
  - Intra-occurs in the mitochondria of necrotic cells with the creation of a microcrystal
  - Extra-Phospholipids-matrix vesicles

- Propagation is the phase in which minerals deposited in the initiation phase are propagated to form mineral crystals.

  It depends on conc. Of calcium+ phosphate& inhibitors, other proteins in the extracellular space

Etiopathogenesis

- **Calcification in dead tissues**

1.Caseous necrosis.

Tuberculous lesions, lymph nodes, lung.

2.Liquefaction necrosis in chronic abscesses may get calcified.

3.Fat necrosis following acute pancreatitis/traumatic fat necrosisin breast results in deposition of calcium soaps.

4.Infarcts may undergo dystrophic calcification.

5.Thrombi esp in veins may produce phleboliths.

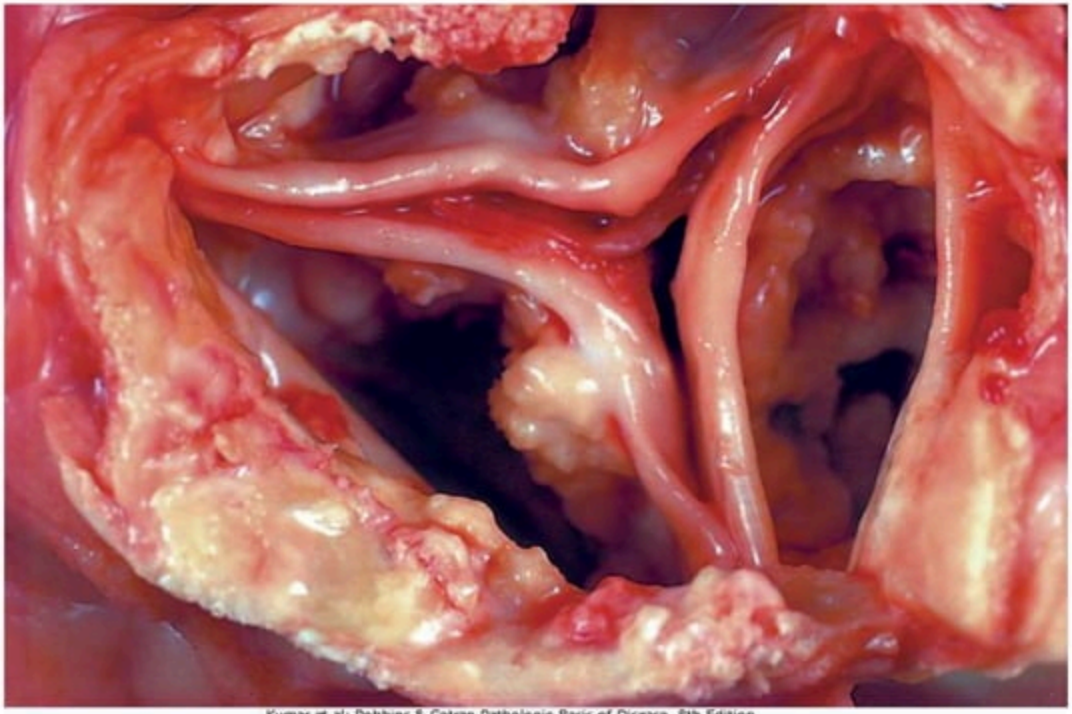
6.Haematomas in the vicinity of bones may undergo dystrophic calcification.

7.Dead parasites

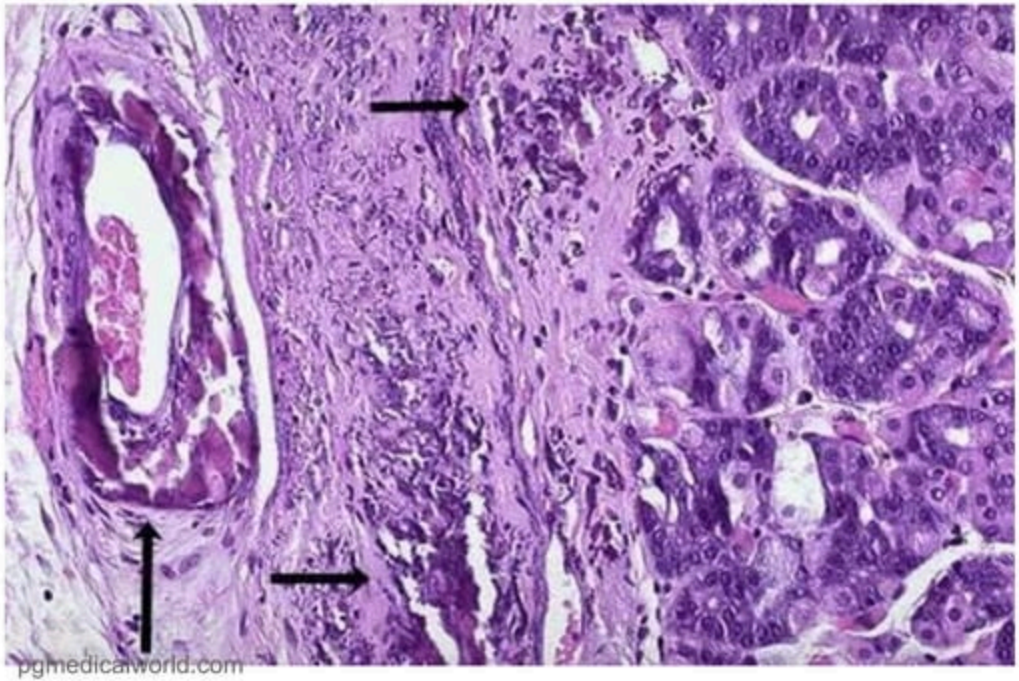
- **Calcification in degenerated tissues**

1. Dense old scars may undergo hyaline degeneration & subsequent calcification.
2. Atheromas in aorta and coronaries frequently undergo calcification.
3. Monckeberg sclerosis show calcification in tunica media of muscular arteries in elderly people.
4. Stroma of tumors such as uterine fibroids, breast ca, thyroid ca, etc PSAMMOMA BODIES(papillary carcinoma of thyroid and ovary)
5. Cysts for a long time
6. Calcinosis cutis- irregular nodules of calcium salts in skin and subcutaneous tissue.
7. Senile degenerated changes may be accompanied by dystrophic calcification.

Dystrophic calcification

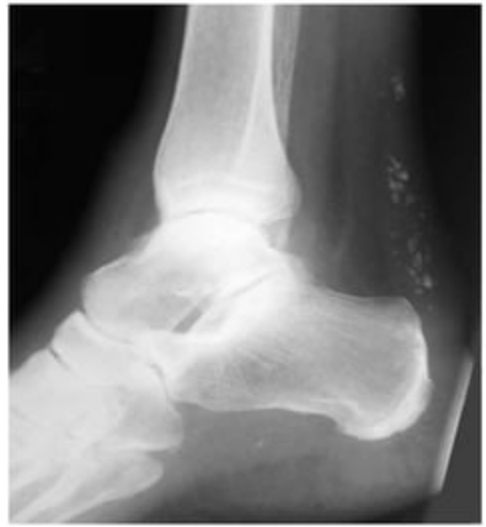


Dystrophic calcification - Stomach.

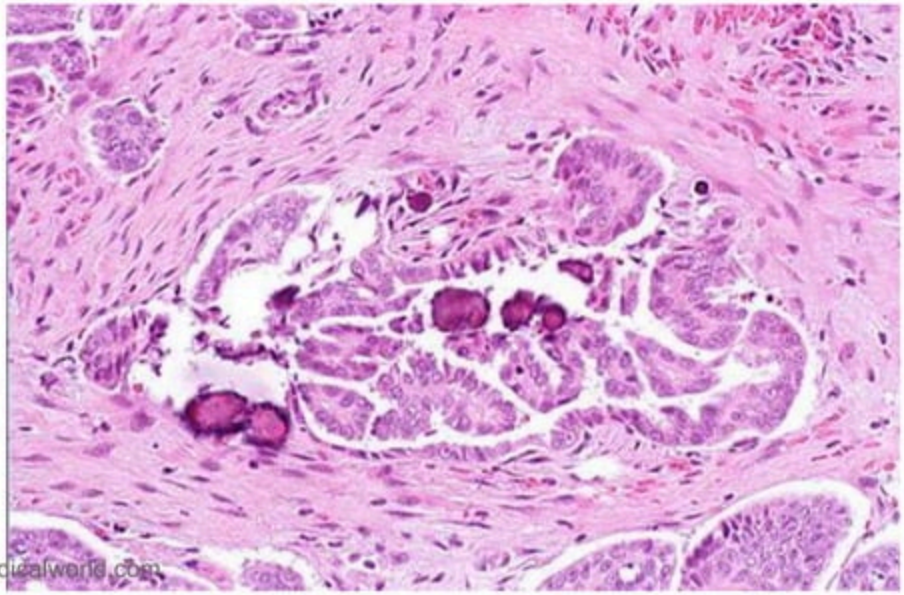


Dystrophic Calcification

Dystrophic calcification in the Achilles tendon after repeated traumatic injury.

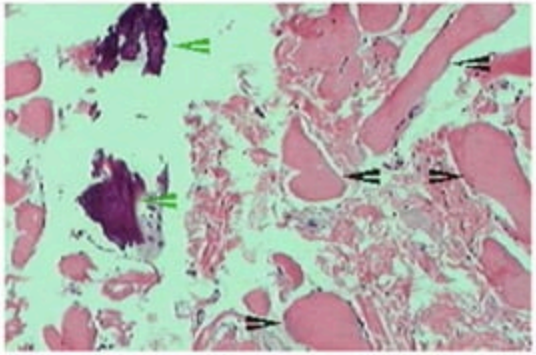


Psammoma Bodies



Dystrophic calcification in skeletal muscle

- The green areas point to two foci of dystrophic calcification in this muscle biopsy.
- Dystrophic calcification is a result of tissue necrosis.
- Calcium salts get deposited among necrotic cells, resulting in the appearance of white, chalky deposits on gross examination, and purple acellular lesions on routine histology.



Metastatic Calcification

The deposition of calcium salts in normal tissue.

Results from hypercalcemia secondary to disturbance in calcium metabolism.

- Excessive mobilisation of calcium from the bone.
- Excessive absorption of calcium from the gut.

Excessive mobilisation of calcium from the bone.

- Hyperparathyroidism secondary to parathyroid adenoma, or secondary such as from parathyroid hyperplasia, CRF.
- Bony destructive lesions such as multiple myeloma, metastatic carcinoma.
- Prolonged immobilisation of a patient results in diffuse atrophy of the bones and hypercalcemia.

Excessive absorption of calcium from the gut.

- Hypervitaminosis D
- Milk alkali syndrome
- Hypercalcemia of infancy

Metastatic calcification may occur in any normal tissue of the body but affects

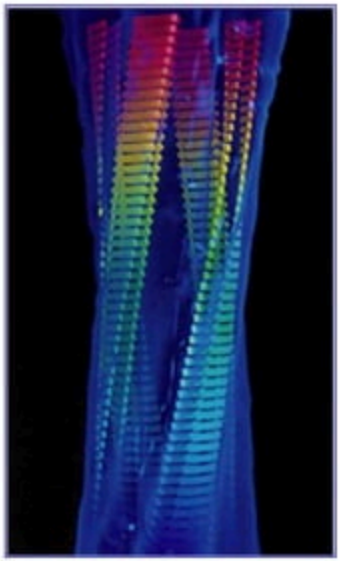
Kidney, Lungs, Stomach, Blood vessels, Cornea.

Pathogenesis: relatively high pH favours precipitation of the calcium.

Feature	Dystrophic calcification	Metastatic calcification
Definition	Deposition of calcium salts in dead & degenerated tissue.	Deposition of calcium salts in normal tissue.
Calcium metabolism	Normal	Deranged
Serum calcium levels	Normal	Hypercalcemia
Causes	Necrosis, Infarcts, thrombi, haematomas, dead parasites, old scars, atheromas, monckebrg`s sclerosis, calcinosis cutis	Hyperparathyroidism,prolonged immobilsation,hypervitaminosis D,milk alkali syndrome,hypercalcemia of infancy
Pathogenesis	Initiation and propogation	High pH at certain sited e.g lungs, stomach,blood vessels,cornea.

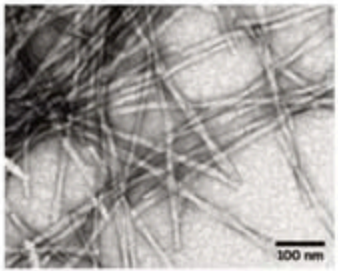
Amyloidosis

Definition



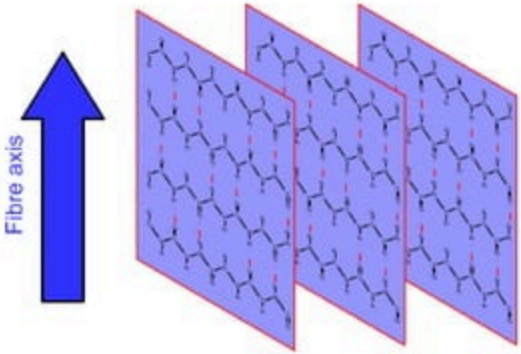
Amyloidosis is a term used for a group of diseases characterised by extracellular depostion of fibrillar proteinaceous substance called amyloid having morphological appearance, staining properties and physical structure but with variable protein or biochemical composition.

Physical Nature



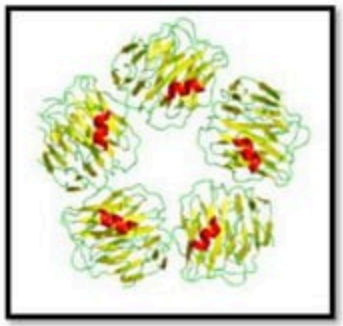
Amyloid fibril protein occurs in tissue deposits as rigid, non-branching fibrils 7-to 10 nm in dm

When analysed by X-ray diffraction, the fibrils exhibit a characteristic cross Beta diffraction pattern



Chemical Nature

- Pentagonal molecule
- 95% Protein Fibril
- 5% Glycoprotein P component



Fibril proteins

- Fibrils are delicate, randomly dispersed, non branching, each measuring 7.5 – 10 nm in diameter & indefinite length.
- Each fibril- double helix
- Xray crystallography and infra red spectroscopy- cross B pleated sheet configuration(staining properties of amyloid with congo red)
- Chemical analysis- heterogenous
- Major forms

AA(Amyloid associated) protein

AL(Amyloid light chain) protein

Other

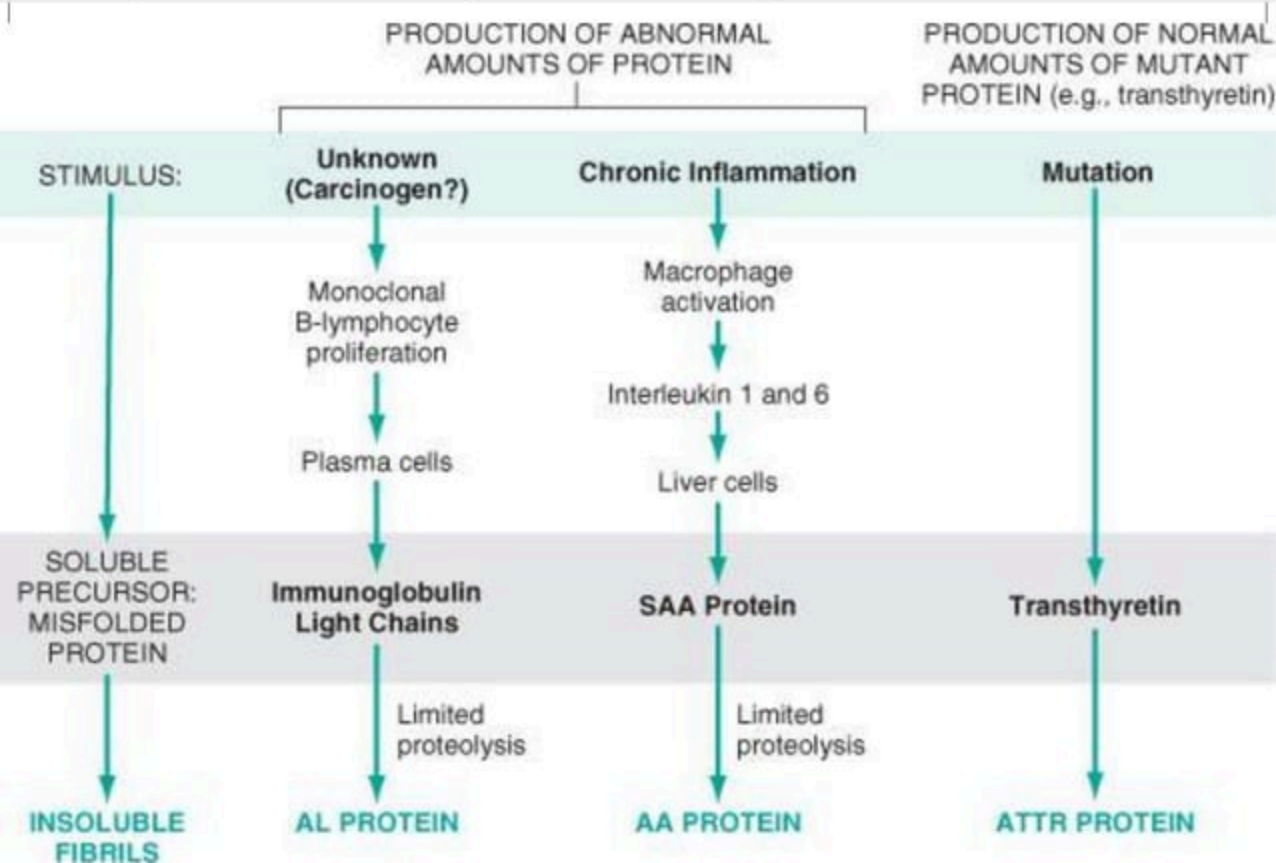
- AL- produced by immunoglobulin secreting cells & is seen in association with plasma cell dyscrasias.(Primary amyloidosis)
- AA- AA fibril protein derived from larger precursor protein SAA(secondary amyloidosis)
- Other-

Transthyretin (TTR)- mutation in structure of TTR results in a variant form of protein termed as ATTR(heredofamilial amyloidosis)

AB2 M Microglobulin- seen in long term haemodialysis

B amyloid protein(AB)- seen in cerebral plaques in Alzheimer`s disease

Pathogenesis of the major forms of amyloid fibrils



Classification

- Based on cause- primary and secondary
- Based on extent of amyloid deposition- systemic & localised

Systemic (Generalised)

Primary (AL)

Secondary(AA)

Haemodialysis associated(AB2M)

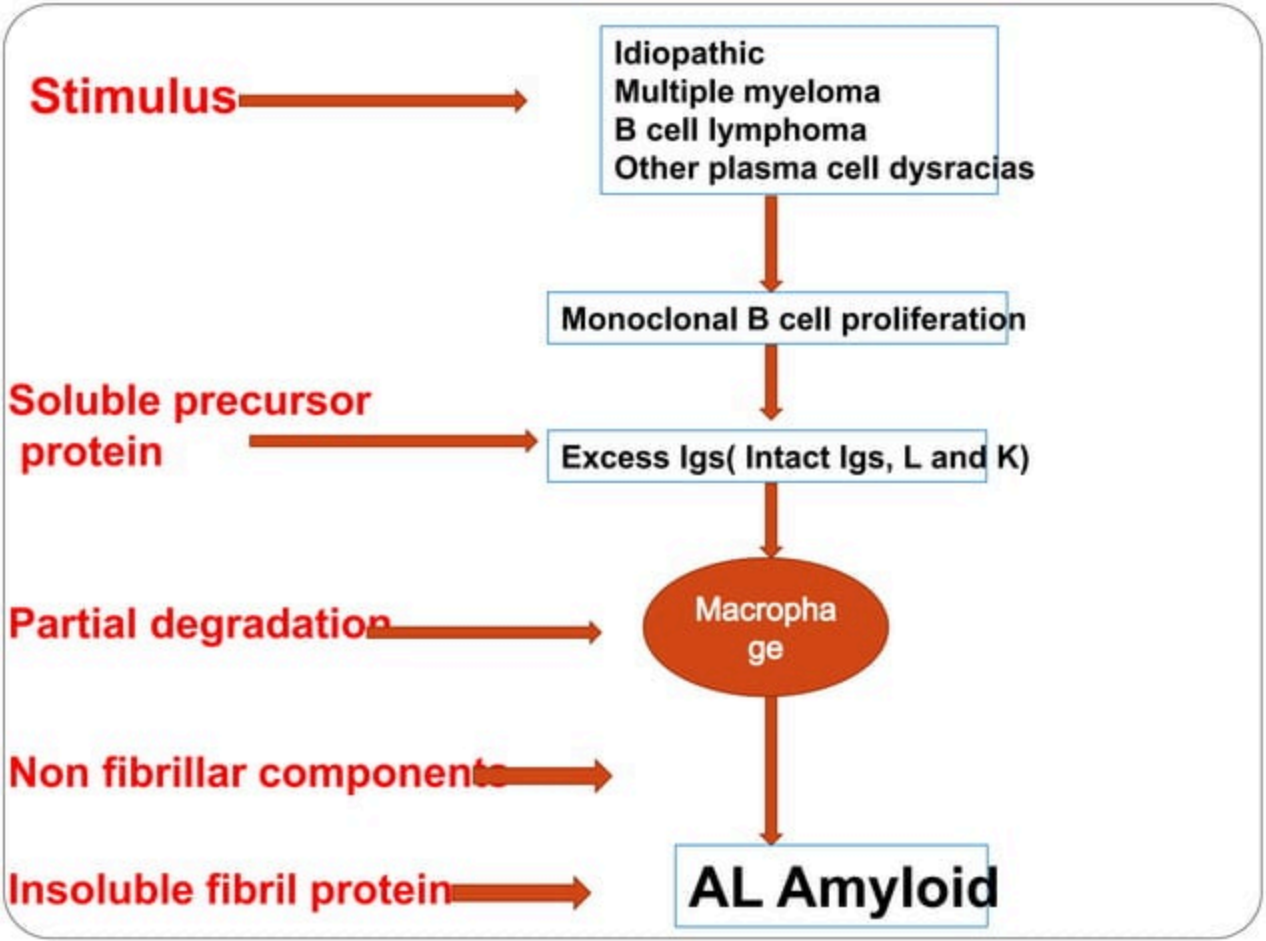
Heredofamilial (ATTR,AA,other)

Localised amyloidosis

Senile cardiac(ATTR)
Senile cerebral(Ab)
Endocrine(hormone precursors)
Tumor forming(AL)

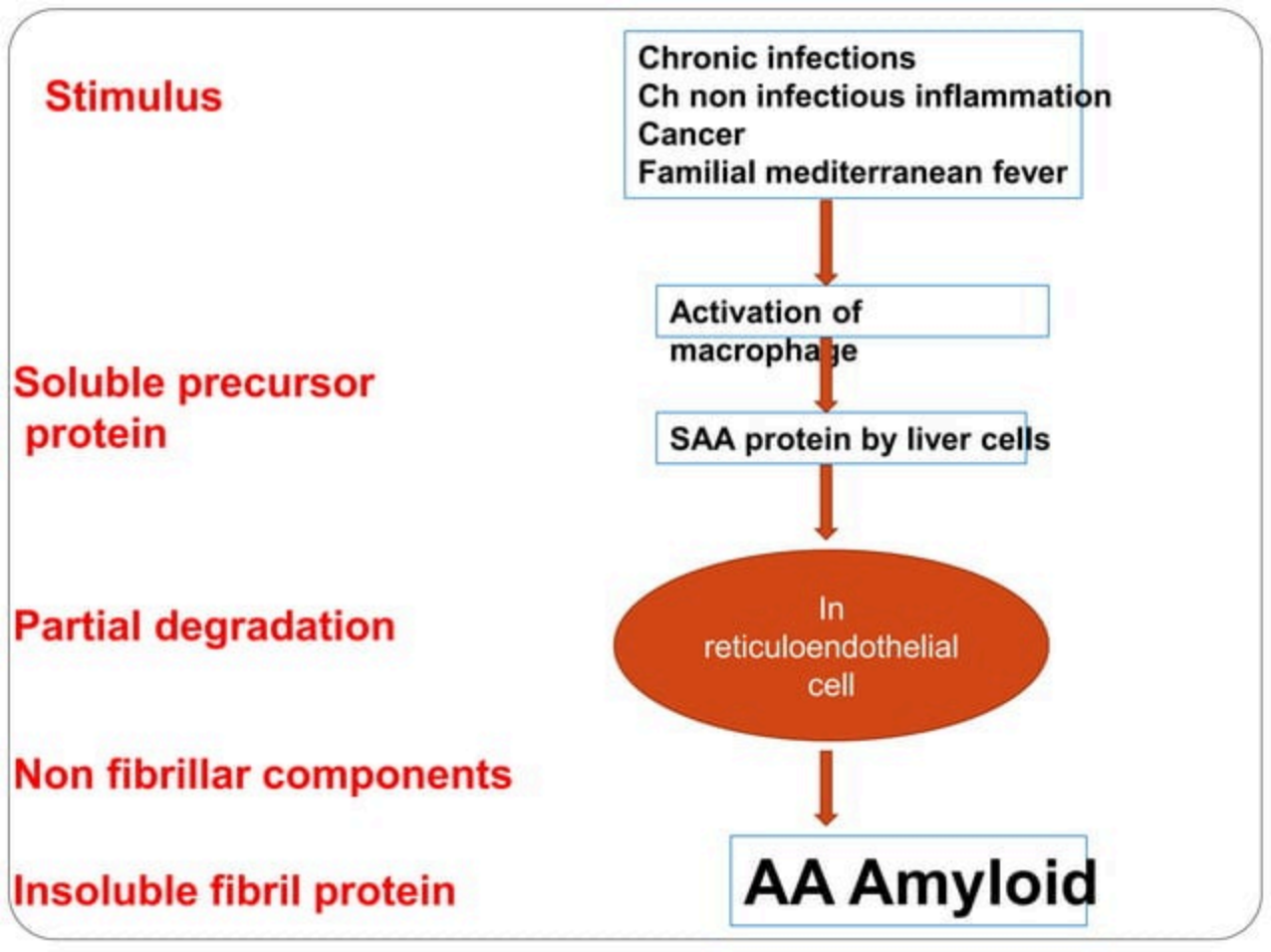
Primary amyloidosis

- AL (light chain proteins)
- Associated diseases- Plasma cell dyscrasia e.g multiple myeloma, B cell lymphoma,others
- Organ distribution- kidney,heart, bowel,nerves
- Stains to distinguish- congophilia perisists after permanganate treatment of section; specific immunostains anti L, anti K.
- Pathogenesis- Stimulus----- Monoclonal B cell proliferation----- Excess Igs & light chains----- partial degradation------Insoluble AL fibril


Stimulus
Idiopathic
Multiple myeloma
B cell lymphoma
Other plasma cell dysracias
Monoclonal B cell proliferation
Soluble precursor protein
Excess Igs(Intact Igs, L and K)
Partial degradation
Macropha ge
Non fibrillar component
Insoluble fibril protein
AL Amyloid

Secondary amyloidosis

- AA (amyloid associated protein) derived from larger precursor protein SAA.
- Associated disease chronic inflammation e.g infections(TB, leprosy, osteomyelitis, bronchiectasis), autoimmune diseases(rheumatoid arthritis, IBD), cancers (RCC, hodgkin`s disease), FMF
- Organ distribution-kidney, liver, spleen, adrenals
- Stains to distinguish- congophilia dissapears after permanganate treatment of section, specific immunostains anti AA.
- Pathogenesis- Stimulus----- chronic inflammation----activation of macrophages------cytokines(IL 1,6)----partial degradation----AEF-----Insoluble


Stimulus
Chronic infections
Ch non infectious inflammation
Cancer
Familial mediterranean fever
Activation of macrophage
Soluble precursor protein
SAA protein by liver cells
Partial degradation
In reticuloendothelial cell
Non fibrillar components
Insoluble fibril protein
AA Amyloid

Staining characteristic of amyloid

- **Stain on gross:**

Lugol`s iodine

- **H & E:**

Extracellular, homogenous, structureless, eosinophilic hyaline material

- **Metachromatic stains(rosaniline dyes)**

Methyl voilet and crystal voilet--- rose pink colour to amyloid

- **Congo red and polarised light**

Pink red colour

Apple green birfringence

- **Flourescent stains**

Thioflavin T- yellow

- **Immunohistochemistry**

Anti AP, anti AA, anti lambda, anti kappa

- **Other stains**

Standard toluidine blue

Alcian blue

PAS

Diagnosis of amyloid

Amyloidosis--- unsuspected morphological finding.

BIOPSY EXAMINATION

Biopsy of affected organ

Renal biopsy

Rectal biopsy

Subcutaneous abdominal fat

Sites of amyloid deposition

Contacts between the vascular spaces and parenchymal cells, in the extracellular matrix and within the basement membranes of blood vessels.

Gross

Affected organ usually enlarged, pale and rubbery. C/S firm, waxy and translucent, positive staining with the iodine test.

Microscopy

Deposits seen in extracellular location, walls of blood vessels

Amyloidosis of kidney

- Amyloidosis of kidney– most cases of secondary amyloidosis and in one third cases of primary amyloidosis.
- GROSS: Kidneys normal sized, enlarged or terminally contracted. Cut surface- pale,waxy and translucent.
- MICROSCOPY: Amyloid deposition occurs primarily in the glomeruli, later affects tubules and walls of small arterioles and venules.

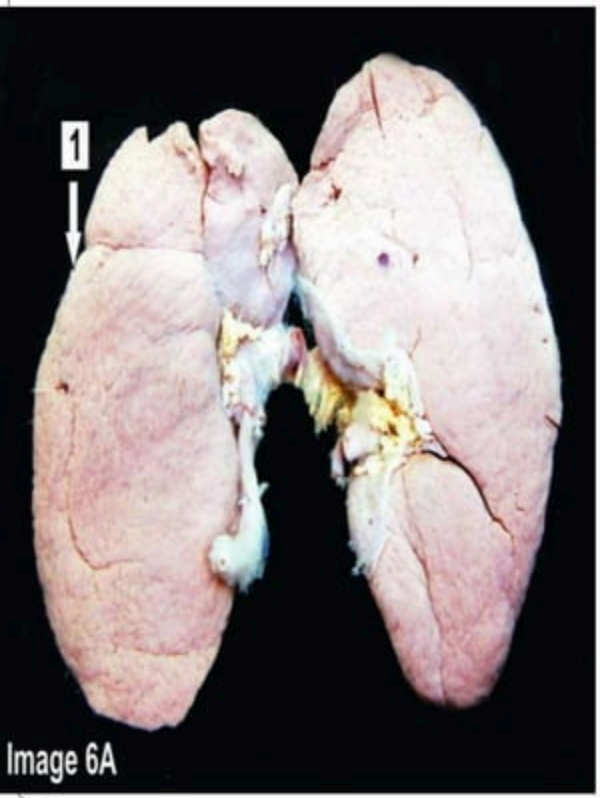


Image 6A

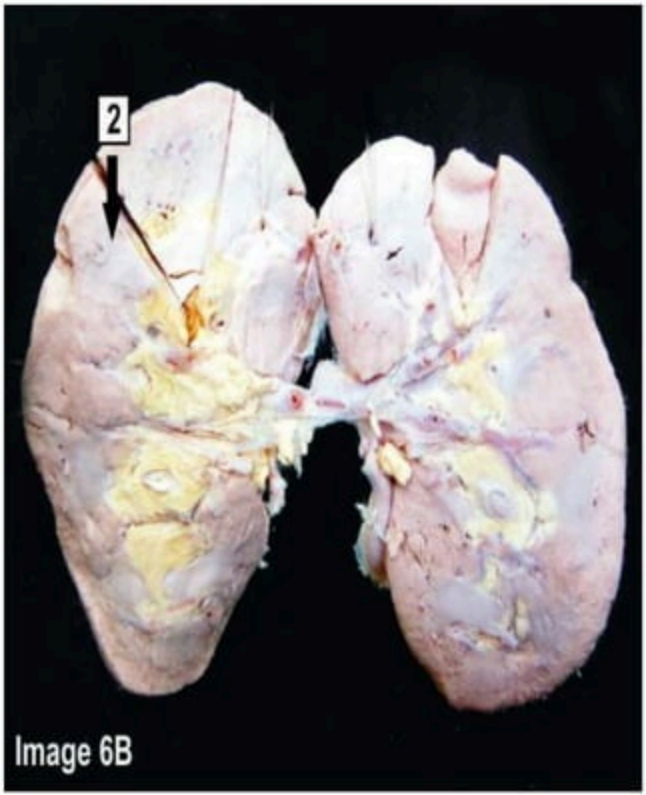
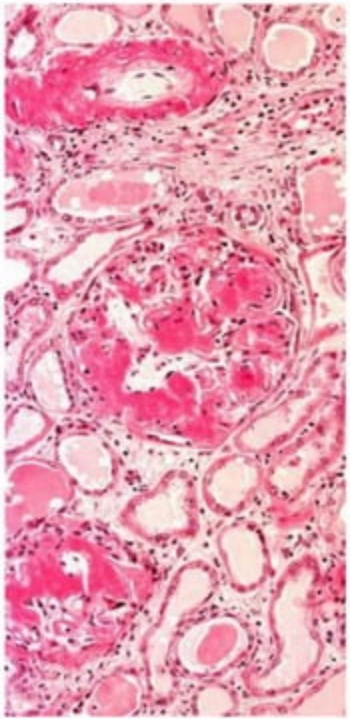


Image 6B

Microscopy of Renal Amyloidosis



- Amyloid can be found in any of the renal compartment
- The most common and the earliest site of amyloid deposition in the kidney is the glomeruli
- Glomerular amyloid formations begins in the mesangium
- Extends into capillary walls

Amyloidosis of spleen

- Sago spleen

Splenomegaly is not marked. C/S translucent, pale and waxy nodules resembling sago grain.

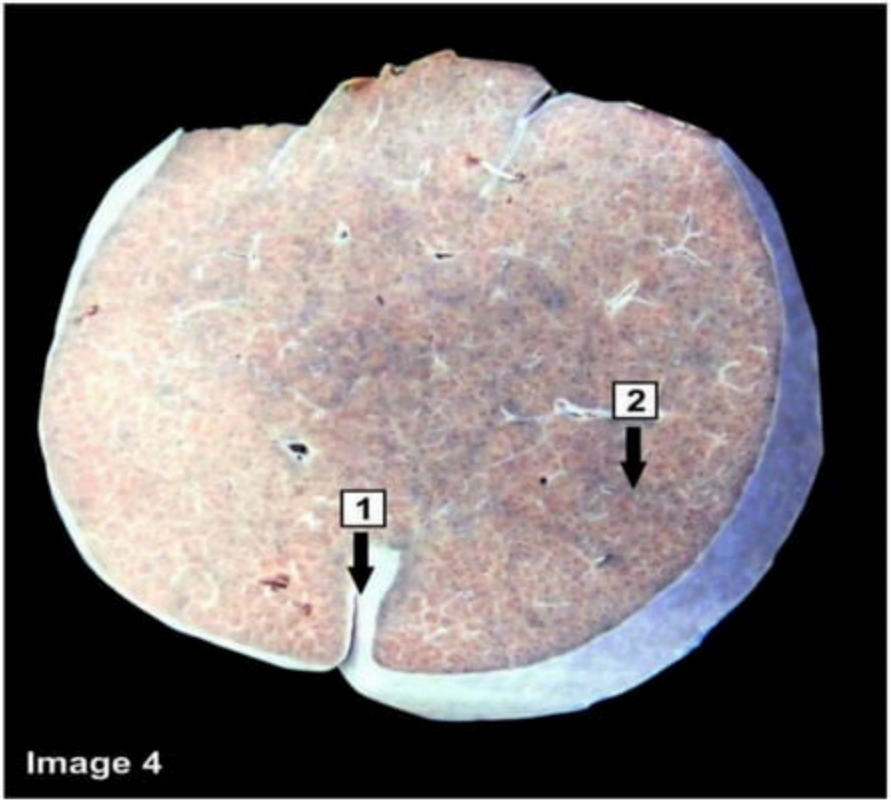
M/E- amyloid deposits involve walls of arterioles of white pulp & replace the follicles.

- Lardaceous spleen

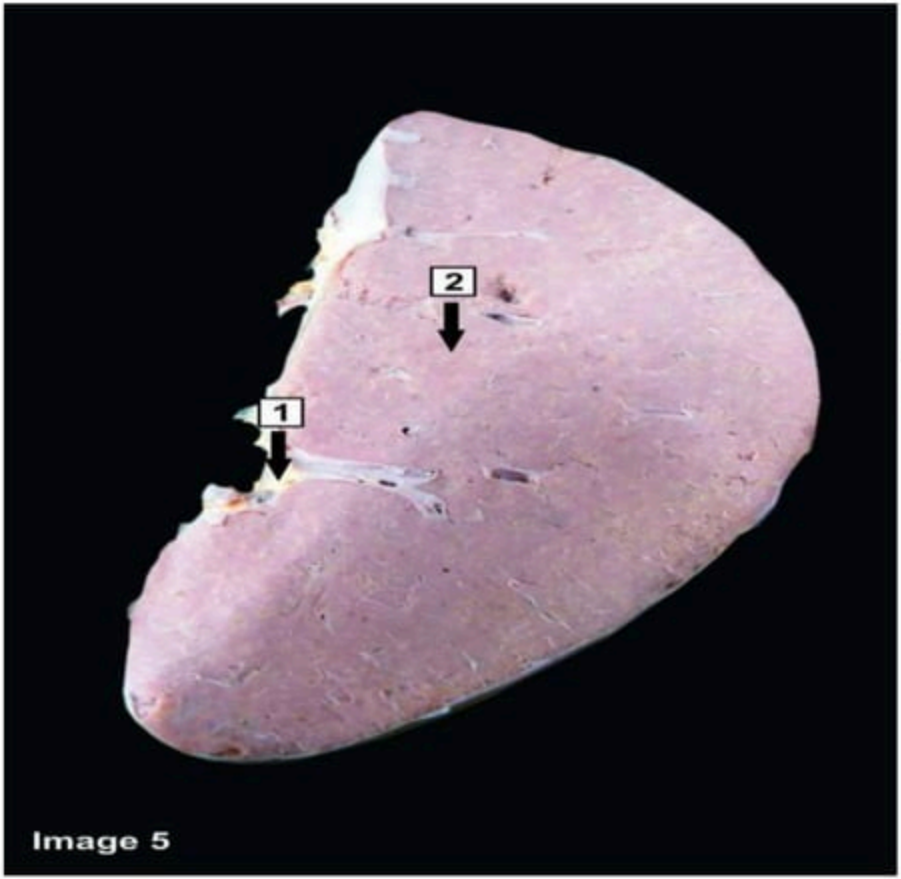
Mod to marked splenomegaly. C/S map like areas of amyloid.

M/E- deposits involve wall of splenic sinuses and small arteries & in connective tissue of red pulp.

The Spleen in Amyloidosis

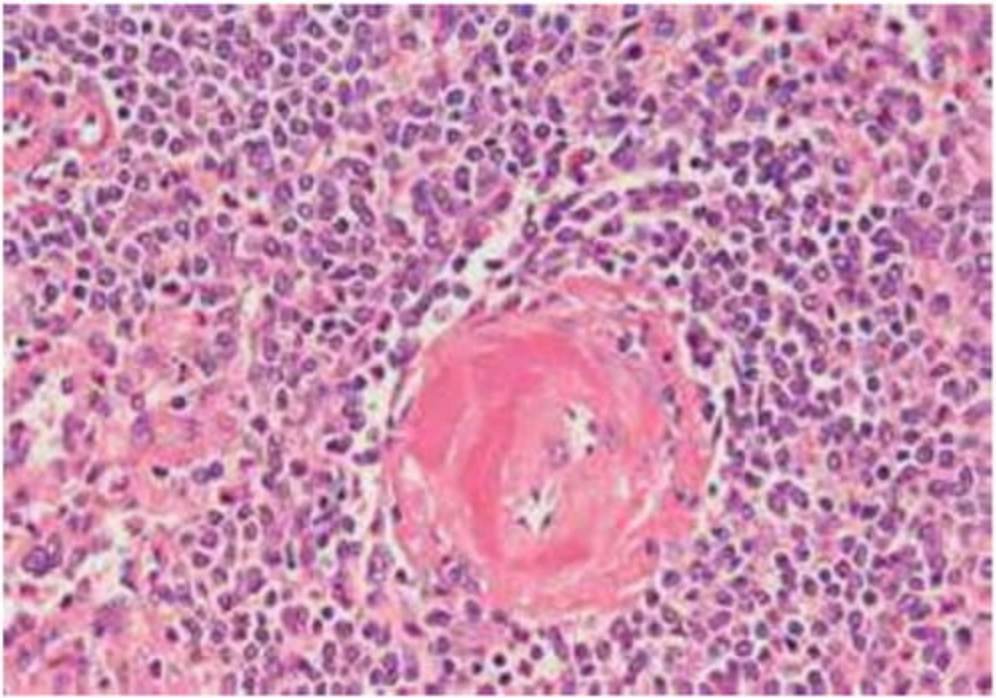


Sago splee



Lardaceous spleen

Amyloidosis of spleen



Amyloidosis of liver

- In half the cases of systemic amyloidosis, liver involvement is seen.
- GROSSLY: Liver is enlarged pale,waxy and firm
- MICROSCOPY:

Initially amyloid deposition seen in space of disse, later compresses the cords of hepatocytes---liver cells are shrunken & replaced by amyloid.

Amyloidosis of the liver

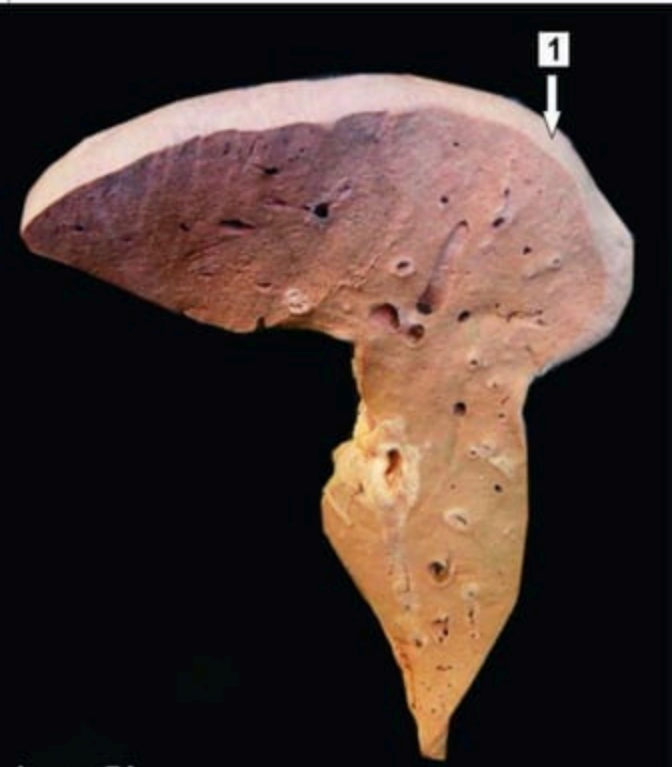


Image 7A

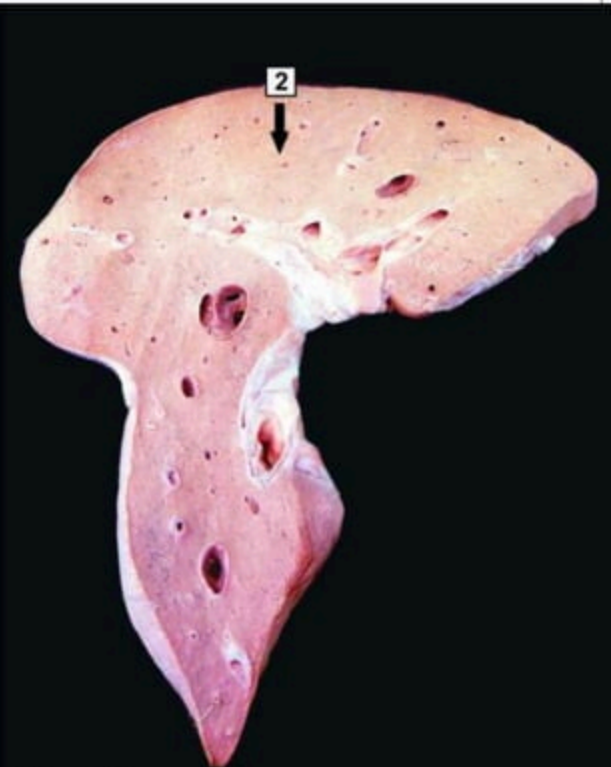
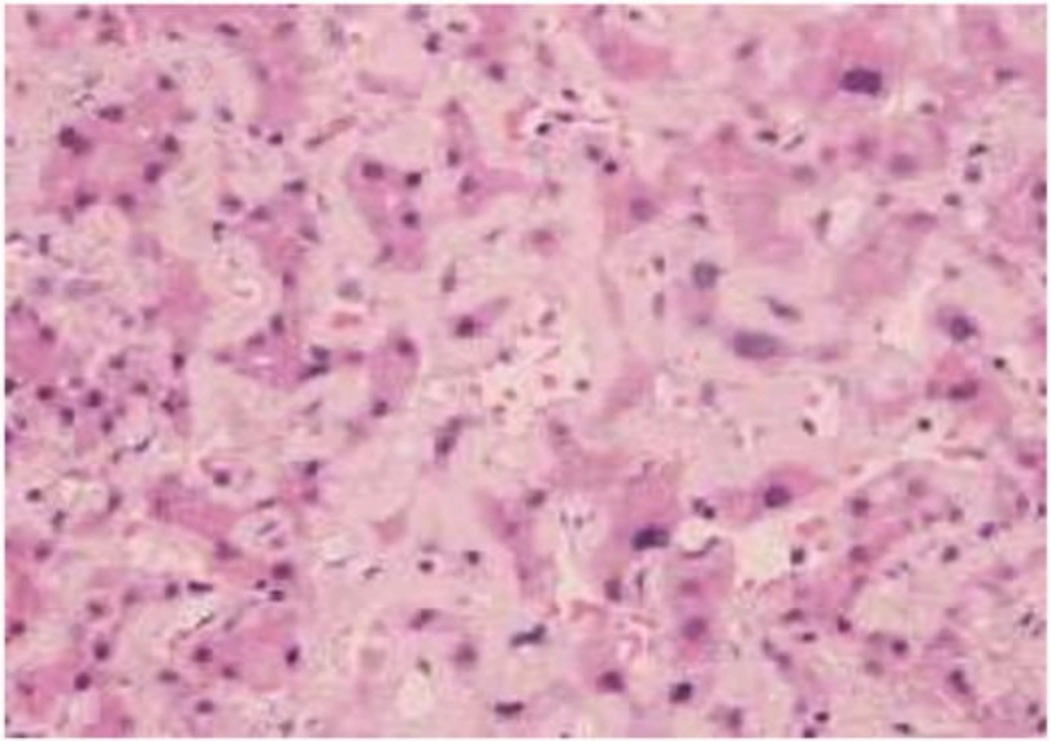


Image 7B

Liver biopsy. Hyaline material within the sinusoids is compressing the hepatocytes (hematoxylin and eosin).



Amyloidosis of heart

- Heart is involved in systemic amyloidosis.
- Localised form (Senile cardiac & AANF).
- In advanced cases---- restrictive cardiomyopathy , arrhythmias.
- GROSS: heart enlarged, ext surface pale, translucent and waxy. Epicardium,endocardium & valves show tiny nodular deposits of amyloid
- MICROSCOPY: Amyloid deposits in and around coronaries

In primary amyloidosis, deposits of AL are seen around myocardial fibres in ring like formations (ring fibres)

In localised form, deposits are seen in left atrium and interatrial septum.

Amyloidosis of Alimentary tract

- I nvolvement can occur at any level from oral cavity to anus.
- Rectal and gingival biopsy--- common sites for diagnosis of systemic amyloidosis.
- Deposits initially located around blood vessels but later involve adjacent layers of the bowel wall.
- Macroglossia- tongue site for tumor forming amyloidosis

Prognosis

- Incidental finding at autopsy or in symptomatic cases- biopsy.
- Prognosis of generalised amyloidosis is poor.
- Primary amyloidosis rapidly progressive and fatal.

Summary

Pathological calcification

Dystrophic

Metastatic

Amyloidosis

Definition

Chemical & physical structure

Pathogenesis

Classification

Stains

Diagnosis

Morphological features of organs

Prognosis

Thank you