

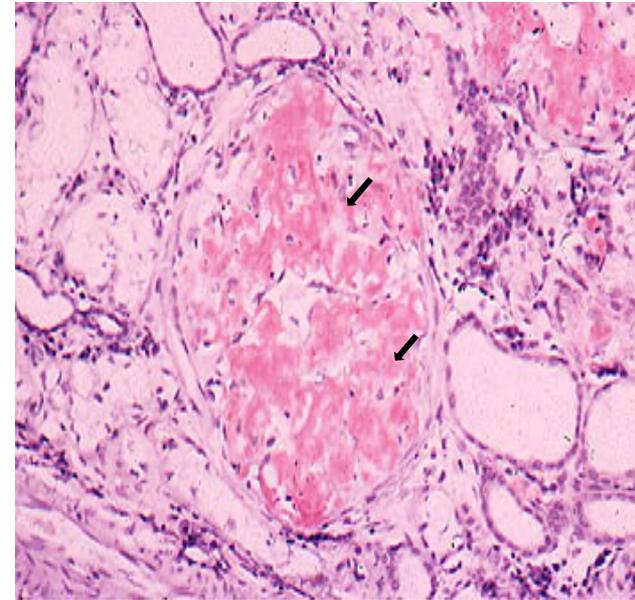
Histochemistry of Amyloid and Amino Acids

Amyloid

- Amyloidosis is a disorder of protein folding.
- The deposited proteins (amyloid) → damage the structure and function of the tissues.
- Occurs in other diseases e.g. Alzheimer's disease and type 2 diabetes mellitus.
- The diagnosis of amyloidosis requires presence of amyloid in a tissue, and the gold standard technique is Congo red histology.

Demonstration of Amyloid

- In routine H&E staining amyloid appears amorphous, eosinophilic, hyaline, extracellular substance.
 - Sometimes displays green birefringence under polarized light.
 - Collagen has same appearance under polarized light.
- Other proteins are stained pink by eosin, hence not specific.



Amyloid: homogeneous and eosinophilic (pink with H&E stain)

Congo Red

- Is a linear dye molecule. It binds to amyloid by hydrogen bonds.
- Two factors are important to the Congo red-amyloid reaction:
 - Linearity of the dye molecule and
 - The β -pleated sheet configuration
- ❖ If the spatial configuration of either is altered, even though the chemical groupings are left intact, the reaction fails.

Congo Red

Principle of Congo Red Staining Reaction

- Congo Red dye forms hydrogen bonds with amyloid and red to green birefringence occurs when viewed by polarised light due to parallel alignment of the dye molecules on the linearly arranged amyloid fibrils.

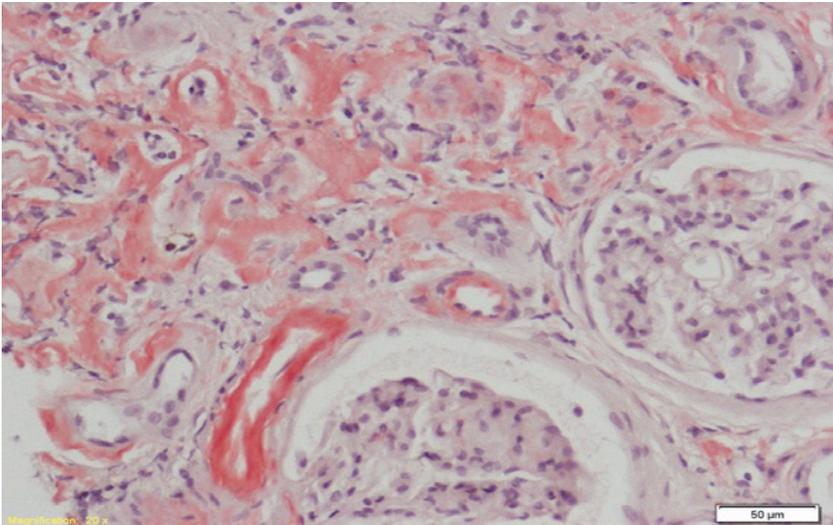
Congo Red

- Disadvantage: its not specific for amyloid.
- Any tissue component that binds Congo red in a linear way also exhibits green birefringence in polarized light.
 - E.g. collagen fibers
- The specificity can be increased by:
 - Using an alkaline Congo method
 - Alcoholic method combined with high ion strength & High pH (The high pH enhances the non-polar hydrogen bonding of congo red and amyloid)
 - Green birefringence under polarized light.

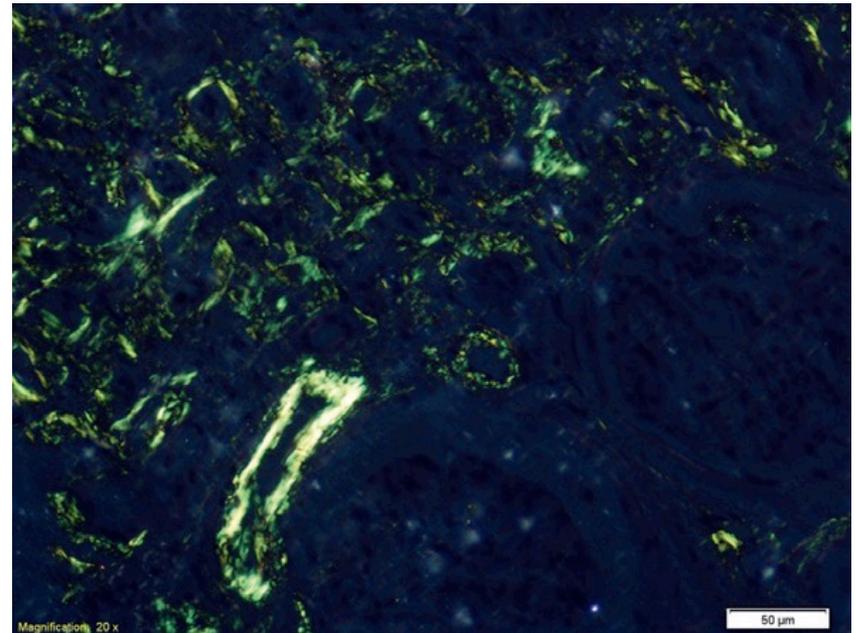
Congo Red

Results

- Amyloid → red
- Nuclei → blue



Same field viewed by polarized light (crossed polarizer and analyzer). The characteristic 'green birefringence' is clearly seen against the dark background.



Sirius Red

- Sirius red F3B (direct red 80), is similar to Congo red
 - Also gives green birefringence with polarized light.
- Gives a more intense staining reaction which is valuable for photographic purposes.

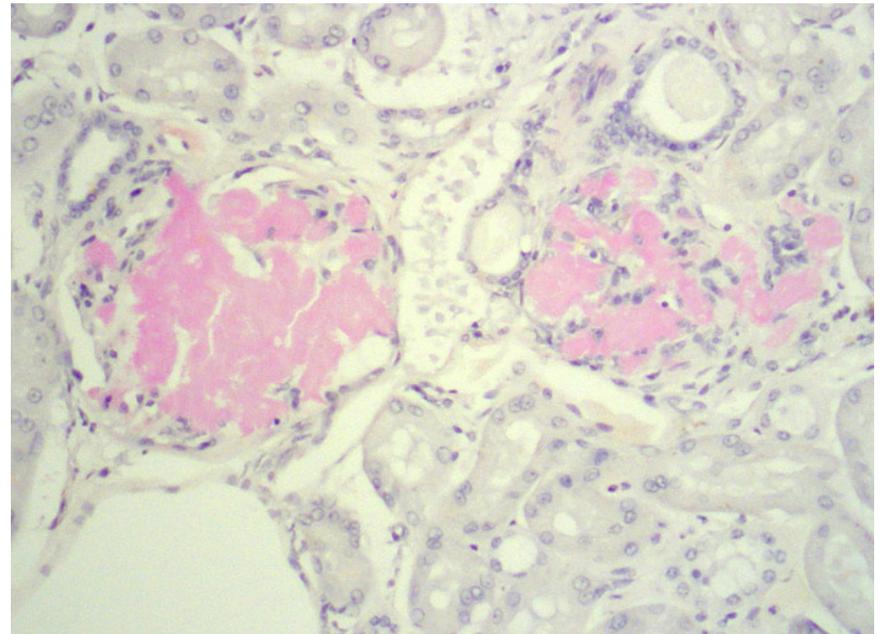
Sirius Red

Results

Amyloid.....rose red.

Nuclei.....blue.

Background...clear to
pale pink.

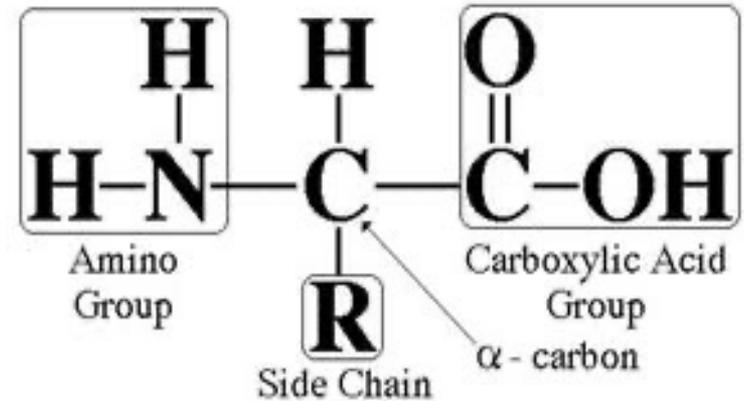


Miscellaneous Methods for Demonstrating Amyloid

- Alcian blue
- Toluidine blue
- Periodic acid Schiff's
- Methenamine silver method of Haga.

Amino acids

- Are molecules containing one amino group, one carboxyl group, one H atom, and a side-chain (R group) that is specific to each amino acid.
- R group varies in structure, size, electric charge and influence the solubility of amino acid in water.



Amino acids

- Amino acids are basic structural building blocks of protein.
- Amino acid histochemical methods can demonstrate some amino acids rather than the whole protein.
 - E.g. protein bound amino group such as lysine (Ninhydrin Schiff method)

Amino Acids Histochemical Methods

- Ninhydrin-Schiff method
- Millon`s Reaction
- Diazotization-coupling method
- Performic acid-Alcian blue method
- DMAB-Nitrite method
- The Sakaguchi Reaction

Ninhydrin-Schiff Method

Aim

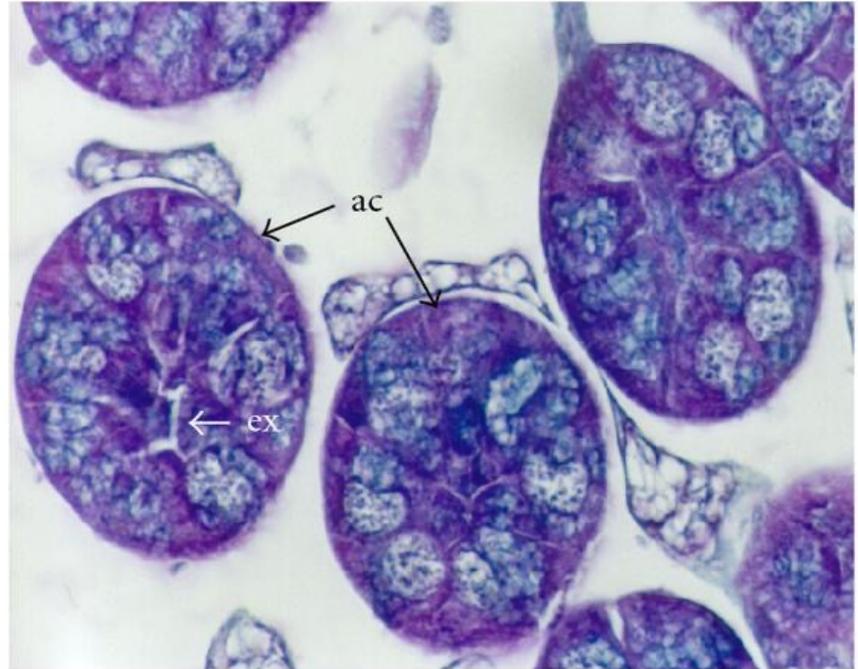
- To demonstrate Protein-bound α -amino groups.
E.g. Glycine, lysine

Principle

- At a neutral pH and 37°C, Ninhydrin reacts with α -amino groups to produce aldehydes which react with Schiff's reagent to give a pinkish- purple colour.

Ninhydrin-Schiff Method

Results:
 α -amino
groups.....Pinkish- purple



A micrograph showing the histochemical appearance of the secretory units of the complete developed gland of an *A. cerana* guard; the cytoplasm is stained pink with NHS technique

Millon`s Reaction

Aim

- To demonstrate the Phenyl groups.

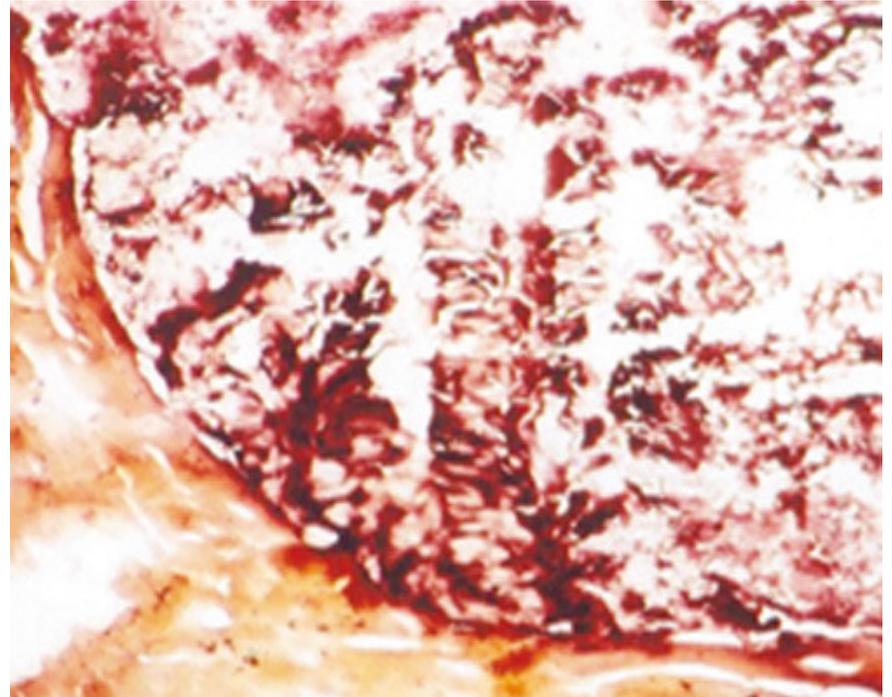
Principle

- This method is specific for Tyrosine; the only amino acid which contains the Hydroxyphenyl group in a form that can be histochemically demonstrated.
- Tissue sections are treated with hot Mercuric sulfate-sulfuric acid-sodium Nitrate mixture. The reaction produces Red-pinkish color at the sites of tyrosine containing proteins.

Millon`s Reaction

Results:

Tyrosine containing
proteins.....Red or Pink



Diazotization-Coupling Method

Aim:

- To demonstrate the Phenyl groups

Principle:

- When tissue containing Tyrosine reacts with Sodium Nitrites; Tyrosine is nitrozated to form Diazonium nitrites; which react with a coupling Amine (8-amino-1-naphthol-5-sulfonoc acid) to give purple and red colours at Tyrosine-containing sites of protein molecules.
- Both nitroization and coupling reactions are better carried out at low temperatures.

Performic Acid-Alcian Blue Method

Aim

- To demonstrate disulfide and sulfhydryl linkages.

Principle

- Disulfide and sulfhydryl linkages are found in amino acids Cysteine, Methionine and Cystine.
- Disulfide linkages occur between 2 sulfur atoms while sulfhydryl linkages occur between a sulfur atom and a Hydrogen atom; and can be produced by the reduction of a Disulfide linkage.
- Results** : Sulfhydryl, Disulfides..... Blue

DMAB-Nitrite Method

Aim:

- To demonstrate the Indole groups.

Principle:

- Indole groups are contained in amino acids Tryptophan and Tryptamine.
- These amino acids react with DMAB (ρ -dimethylaminobenzaldehyde) to produce β -carboline; which is then oxidized by the Nitrite solution to produce a deep blue pigment.

DMAB-Nitrite Method

- Nuclei are counterstained with Neutral Red.

Results:

- Tryptophan.....Dark blue
- Nuclei.....Red

The Sakaguchi Reaction

Aim: to demonstrate the guanidyl groups

Principle

- This method targets Guanidyl groups contained in amino acid Arginine.
- When Arginine reacts with α -naphthol and an alkaline hypochlorite solution; an orange-red colour develops.
- This colour fades rapidly and the section must be examined immediately after the staining procedure.
- **Results** : Arginine.....Orange-Red

References

- Bancroft JD & Stevens, **Theory & Practice of Histological Techniques**, 2nd Ed.
- Cook H C, **Manual of Histological Demonstration Techniques**.
- Culling CFA, **Handbook of Histopathological and Histochemical Techniques**, 3rd Ed.
- Sheehan D, Hrapchak B (1980), **Theory and practice of Histotechnology**, 2nd Ed, Battelle Press, Ohio, USA