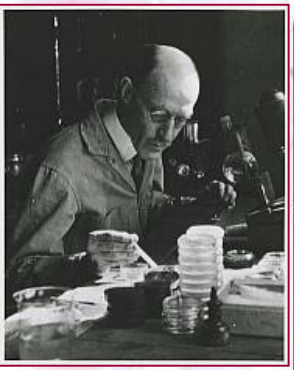
Techniques of cell and molecular biology

1. Cell fractionation
2. Autoradiography
3. X-ray crystallography
4. Histochemistry and cytochemistry
5. Immunohistochemistry
6. Hybridization techniques
7. Human brain connectivity

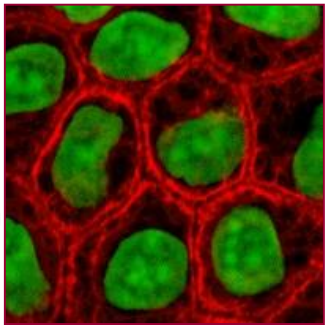


Ross Granville Harrison
(1870-1959)

Alexis Carrel
(1873-1944)
The Nobel Prize in Physiology or Medicine 1912

Vital observations

- Cell, tissue and organ cultures: *in vitro* and *in vivo*
  - ✓ primary cell cultures:
    - ➢ dissociated (cell cultures)
    - ➢ explant (tissue cultures)
  - ✓ secondary: cell lines (HeLa cells)



- Medical applications:

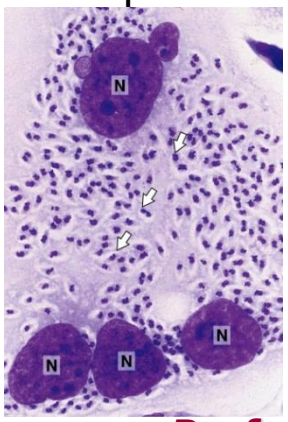
✓ Study of the metabolism of normal and cancerous cells

✓ Development of new drugs

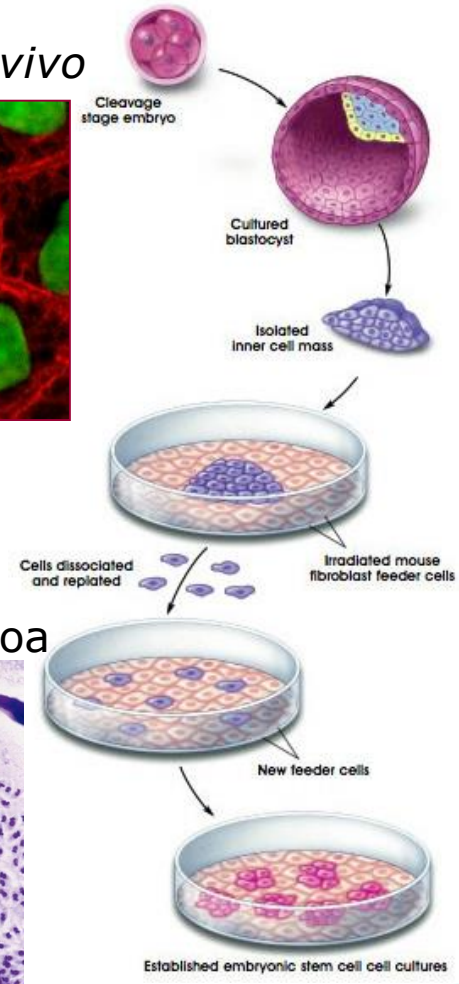
✓ Study of parasites that grow only within cells, such as viruses, mycoplasma and some protozoa

✓ Vaccine creation

✓ Cytogenetic research:
- ➢ chromosome analysis
- ➢ determination of human karyotype
- ➢ genetic disorders
- ➢ gene and cell engineering



- Supravital microscopy

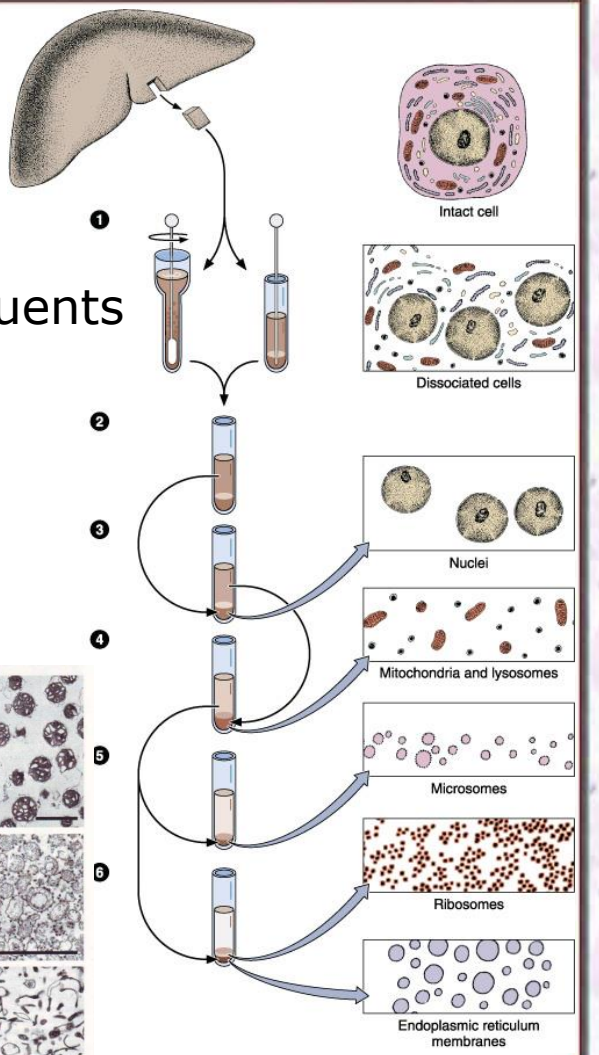


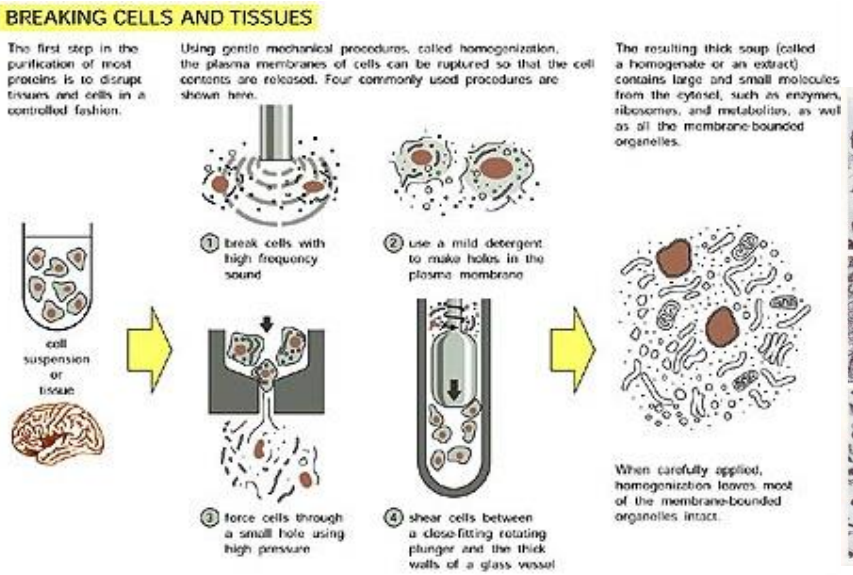
Cell fractionation

Albert Claude (1899-1983) **Nobel Prize, 1974**

Theodor Svedberg (1884-1971) **Nobel Prize, 1926**

- ultracentrifuge – **T. Svedberg**
- cell fractionation – **A. Claude**
  - ✓ allows the isolation of cell constituents by differential centrifugation
- density gradient centrifugation

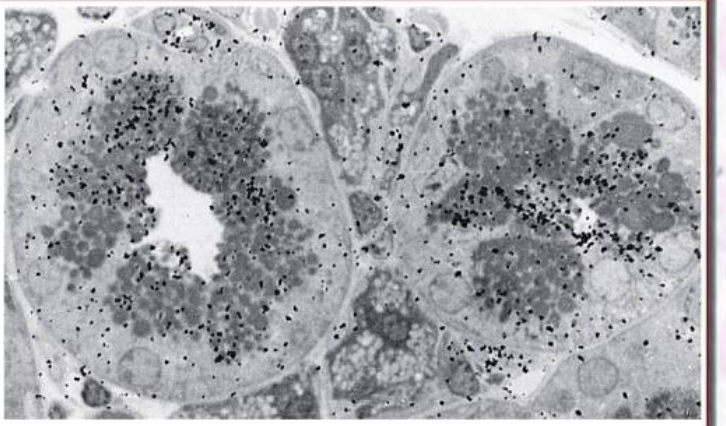


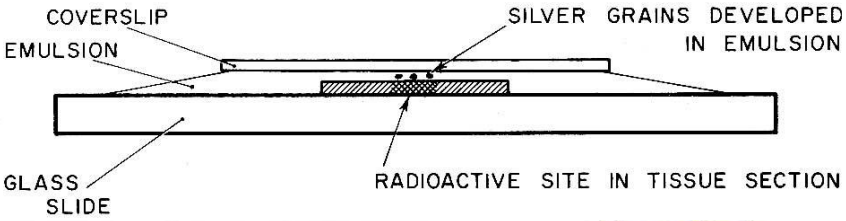


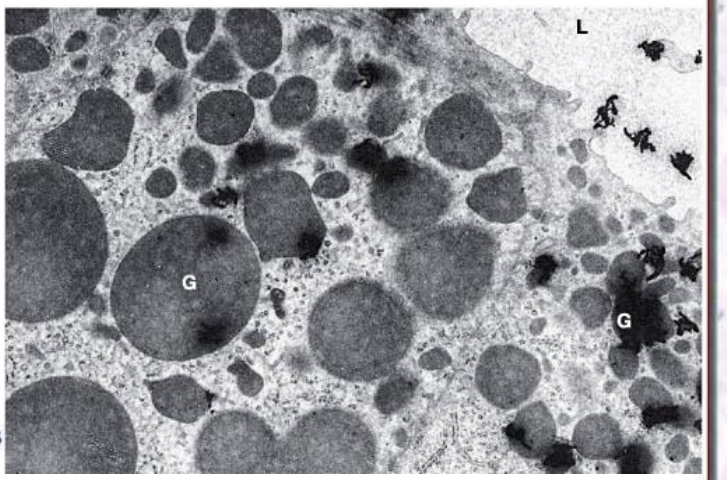
Autoradiography of tissue sections

Antoine Lacassagne (1884-1971)
1924, developed the first autoradiographic method

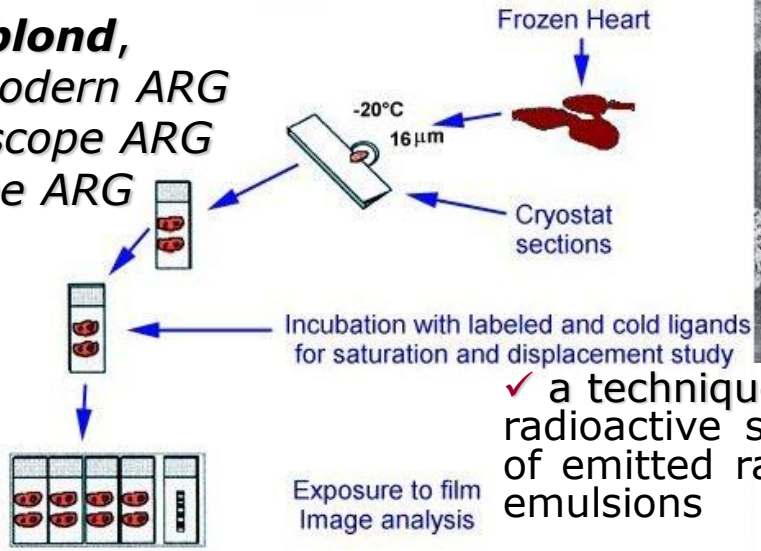
Radioautography (Autoradiography)



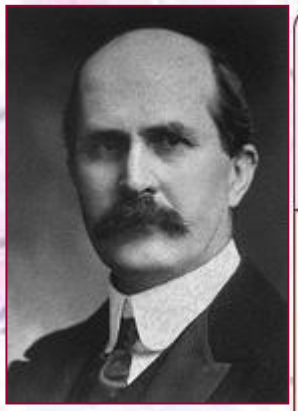




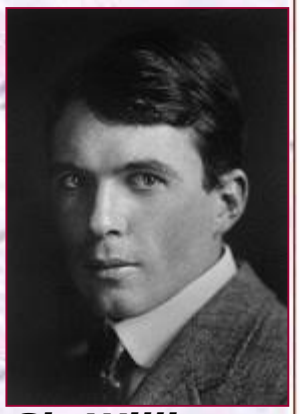
Belanger *and* ***Leblond****,*
1946 – begin of modern ARG
- ✓ *electron microscope ARG*
- ✓ *light microscope ARG*



✓ a technique that permits the localization of radioactive substances in tissues by means of emitted radiation effects on photographic emulsions



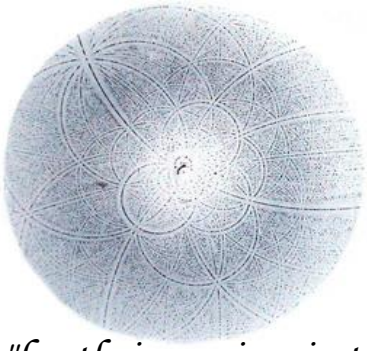
Sir William Henry Bragg *(1862-1942)*

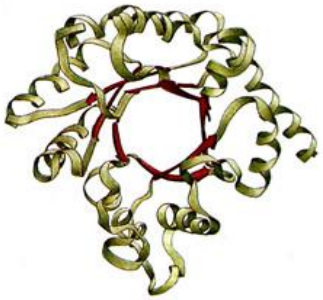


Sir William Lawrence Bragg *(1890-1971)*
Nobel Prize in Physics, 1915

X-ray crystallography

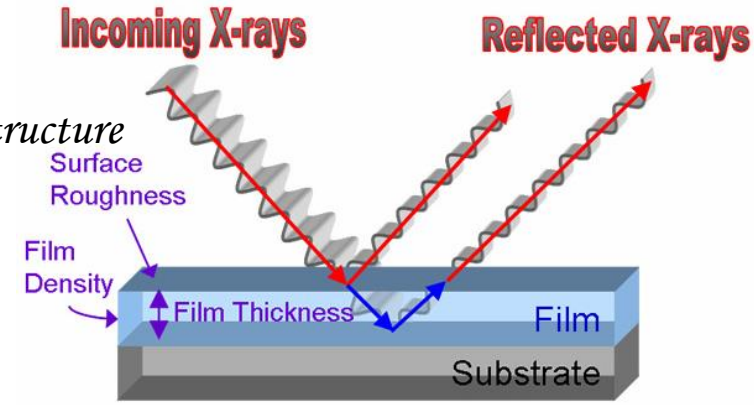
- ✓ a method of determining the arrangement of atoms within a crystal
- ✓ to solve the crystal structure of:
  - ➢ proteins
  - ➢ cholesterol and vitamin B12
  - ➢ hemoglobin and myoglobin etc.

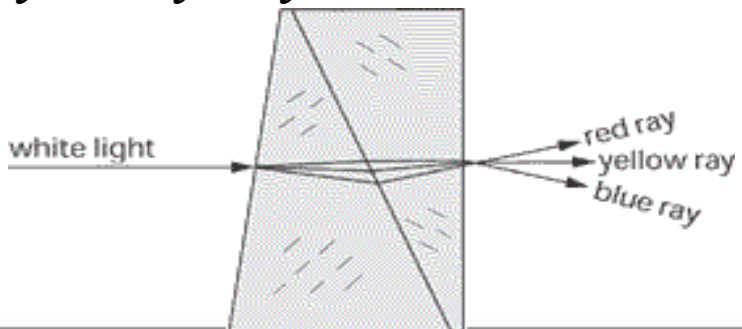




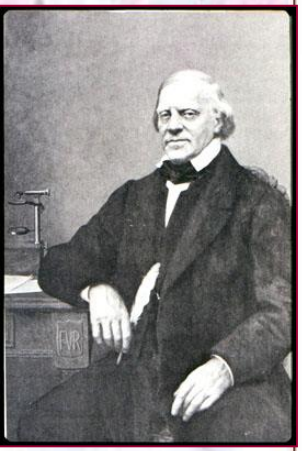
"for their services in the analysis of crystal structure by means of X-rays"

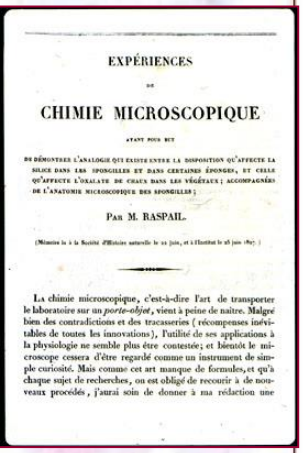
W.H. Bragg and his X-ray spectrometer at University College, London.





Histochemistry and cytochemistry





EXPÉRIENCES

DE

CHIMIE MICROSCOPIQUE

AYANT POUR BUT

DE DÉMONTRER L'ANALOGIE QUI EXISTE ENTRE LA DISPOSITION QU'AFFECTE LA SILICE DANS LES SPONGILLES ET DANS CERTAINES ÉPONGES, ET CELLE QU'AFFECTE L'OXALATE DE CHAUX DANS LES VÉGÉTAUX ; ACCOMPAGNÉES DE L'ANATOMIE MICROSCOPIQUE DES SPONGILLES ;

PAR M. RASPAIL.

(Mémoire lu à la Société d'Histoire naturelle le 22 juin, et à l'Institut le 28 juin 1830.)

La chimie microscopique, c'est-à-dire l'art de transporter le laboratoire sur un *porte-objet*, vient à peine de naître. Malgré bien des contradictions et des tracasseries (récompenses inévitables de toutes les innovations), l'utilité de ses applications à la physiologie ne semble plus être contestée; et bientôt le microscope cessera d'être regardé comme un instrument de simple curiosité. Mais comme cet art manque de formules, et qu'à chaque sujet de recherches, on est obligé de recourir à de nouveaux procédés, j'aurai soin de donner à ma rédaction une

Founder of the method: *Francois-Vincent Raspail* (1794-1878)

- ✓ Histochemistry = LM results
- ✓ Cytochemistry = EM results

Quantitative analysis: principles

- ✓ **to preserve structure** of cells and tissues
- ✓ **localizations on the original sites in the cell**: to avoid translocation
- ✓ **specificity of the reaction**: positive and negative controls

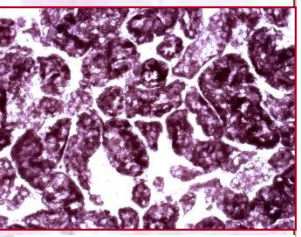
Qualitative analysis: microspectrophotometry

Enzyme histochemistry: principles and applications

- Enzyme, substrate, product
- Principles:
  - ✓ fresh, unfixed material – cryostat
  - ✓ short-term fixation at lower temperature
  - ✓ pH optimum of the detected enzyme: buffers
- Basic requirements:
  - ✓ demonstration of final product, not the enzyme
  - ✓ insoluble product: true localization in the cell
  - ✓ color product: easily visible on the background

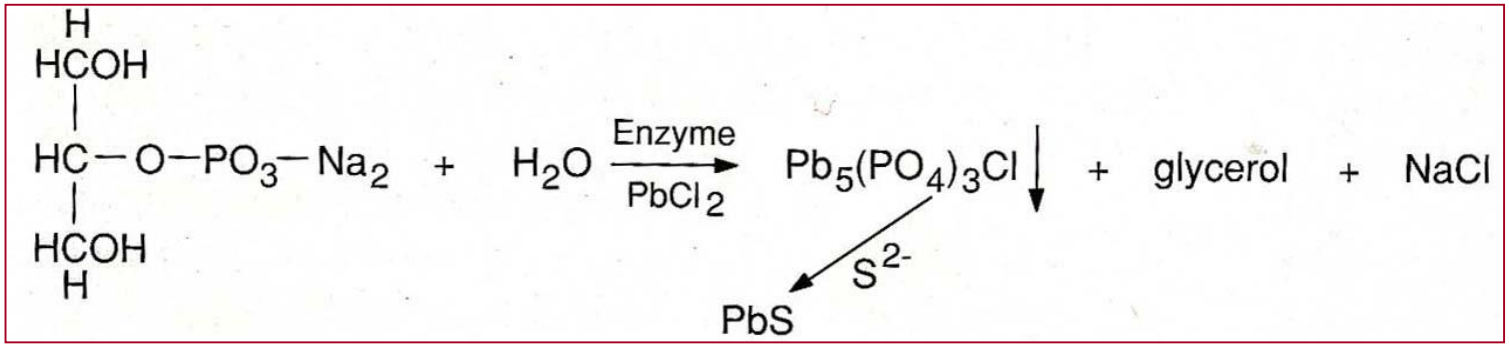
Enzyme + Substrate = unstained reaction product
Product + Dye = insoluble colored final reaction product

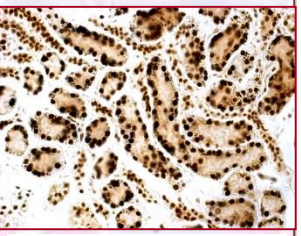
Catalytic enzyme histochemistry



Rat kidney

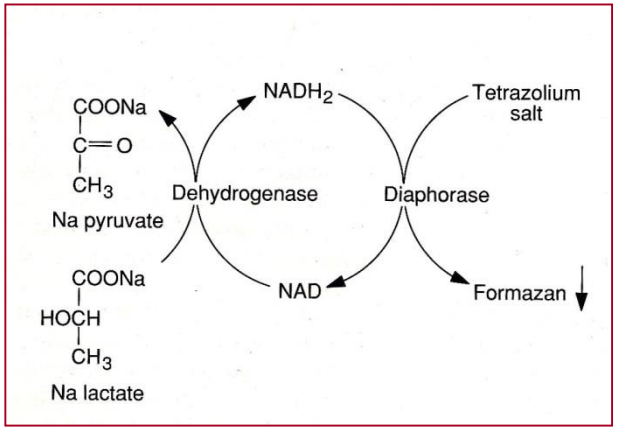
- Hydrolases: method of *Gomori* (1950, 1952)
  - ✓ acid phosphatase – lysosomes and Golgi complex





Rat kidney

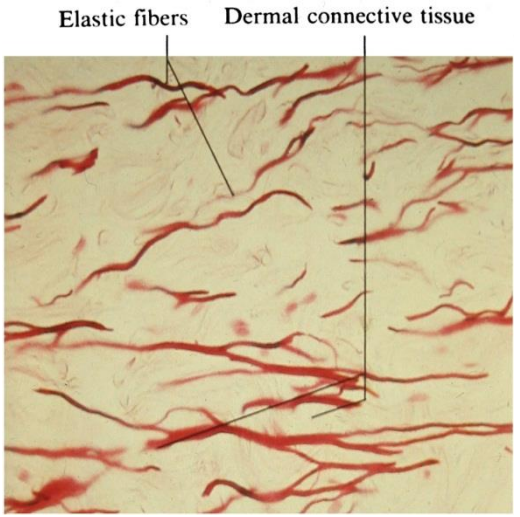
- oxidoreductases: method of *Nachlas* (1957)
  - ✓ succinate dehydrogenase – mitochondria
  - ✓ catalyzes the oxidation of succinate to fumarate



succinate + acceptor = fumarate + reducted acceptor

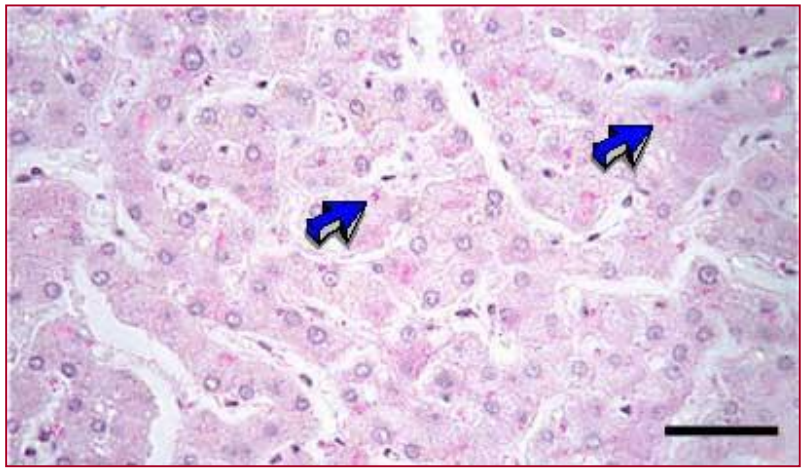
Demonstration of proteins

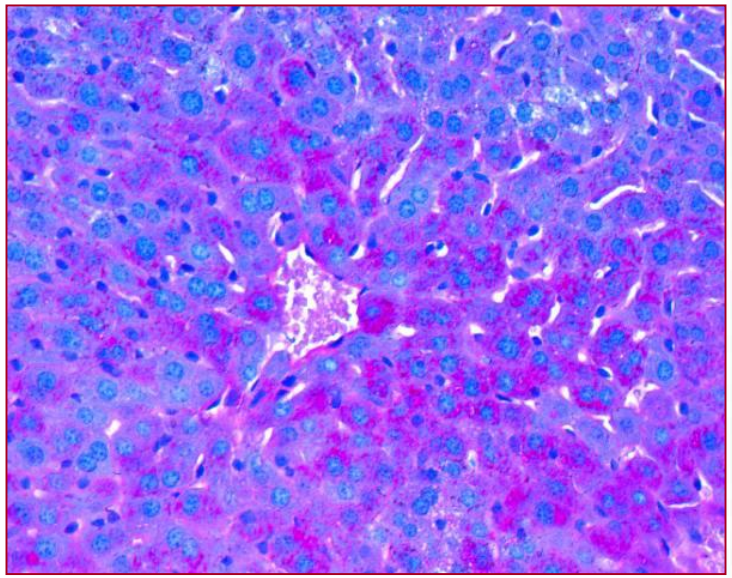
- the histochemical methods usually do not permit identification of specific proteins in cells and tissues
- elastic fibers:
  - ✓ orcein
  - ✓ *Weigert's* resorcin-fuchsin
- amino acids:
  - ✓ immunocytochemistry
- chemical groups:
  - ✓ paraldehyde-fuchsin – neurosecrete, insulin
- solubility and isoelectric point



Demonstration of oligosaccharides & polysaccharides

- ***PAS***-reaction (**P**eriodic **A**cid-**S**chiff)
  - ✓ demonstration of **glycogen** in tissues
  - ✓ demonstration of **glycoproteins**
  - ✓ demonstration of **glycosaminoglycans**





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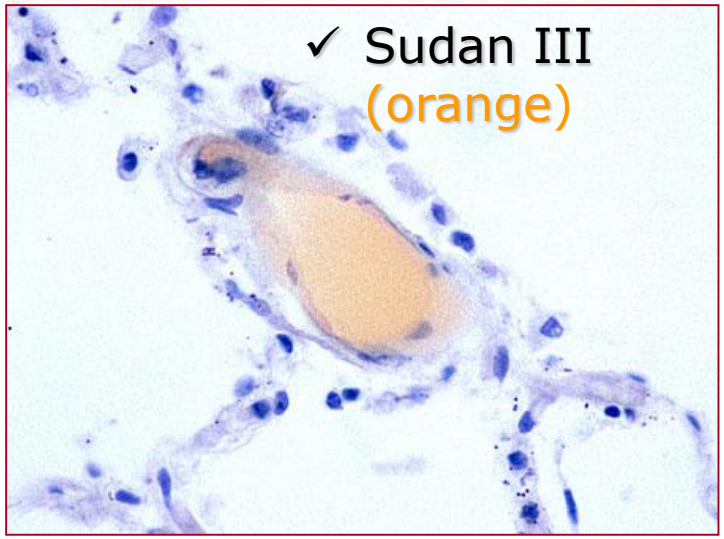
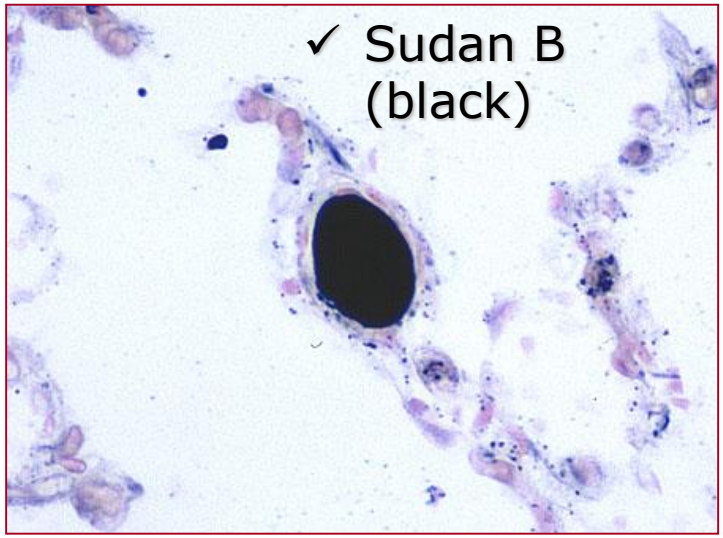
Demonstration of lipids

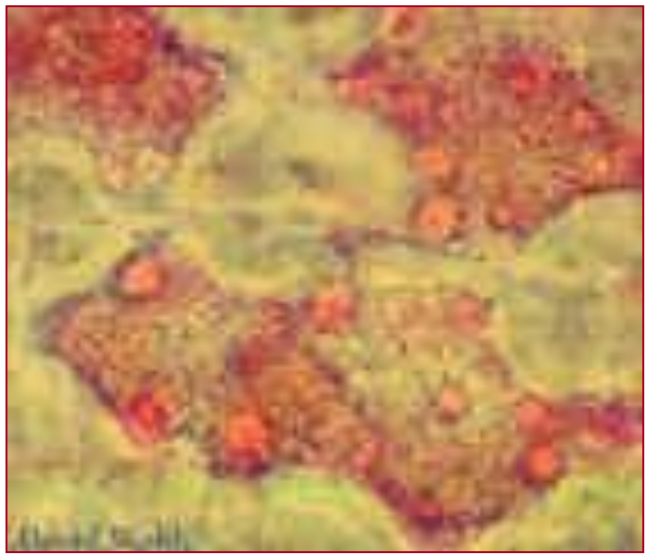


✓ Sudan III (orange)



✓ Sudan B (black)

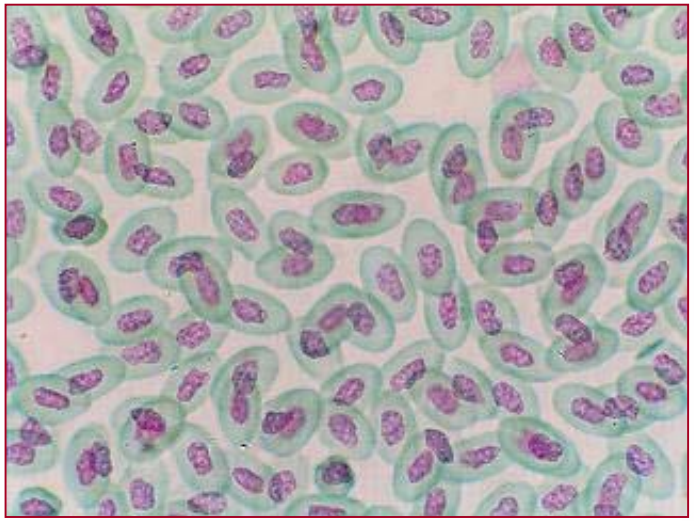
- best revealed with dyes that are soluble in lipids:



✓ Sudan IV (red)

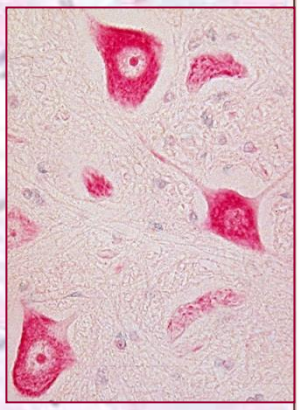
Demonstration of nucleic acids

- based on basophilia of nuclei acids



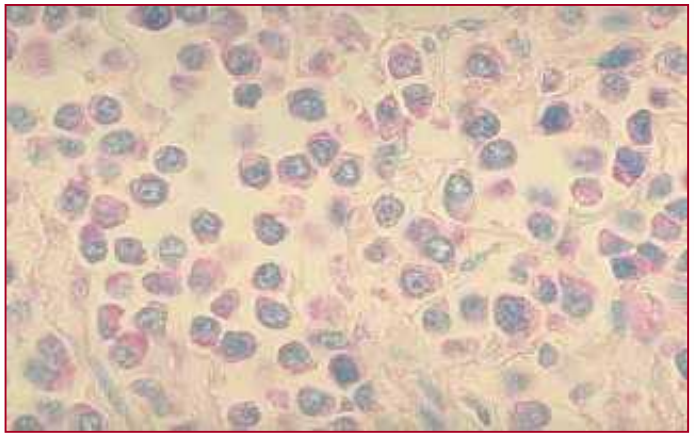
- ✓ *DNA:* method of *Feulgen* and *Rossenbeck* (1924)

  (*Feulgen* reaction)



- ✓ *RNA:* method of *Brachet* (1940-1941)

  methyl green-pyronin



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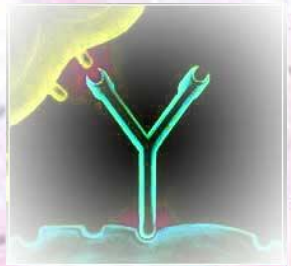


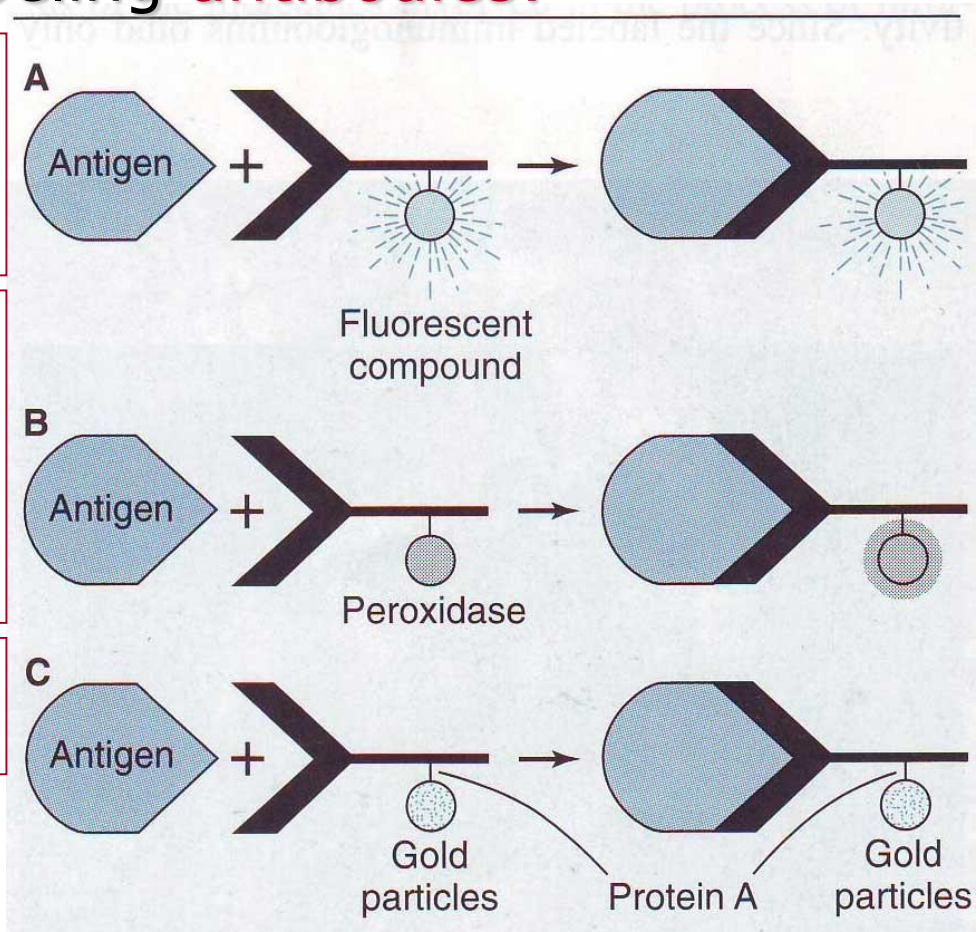
Immunohistochemistry

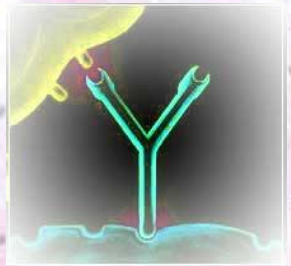
- based on an antigen-antibody reaction
- Methods of labeling antibodies:

✓ coupling with a fluorescent compound: immunofluorescence method – *Coons*, 1941

✓ coupling with an enzyme:
PAP method (*Sternberger et al.*, 1970)
ABC technique (*Hsu*, 1981)

✓ coupling with gold particles

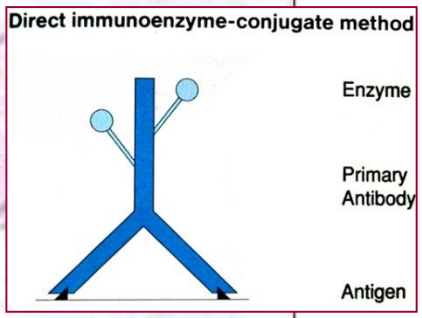


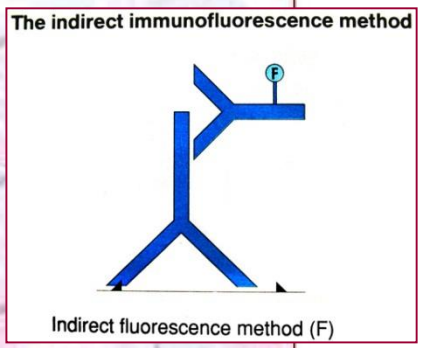


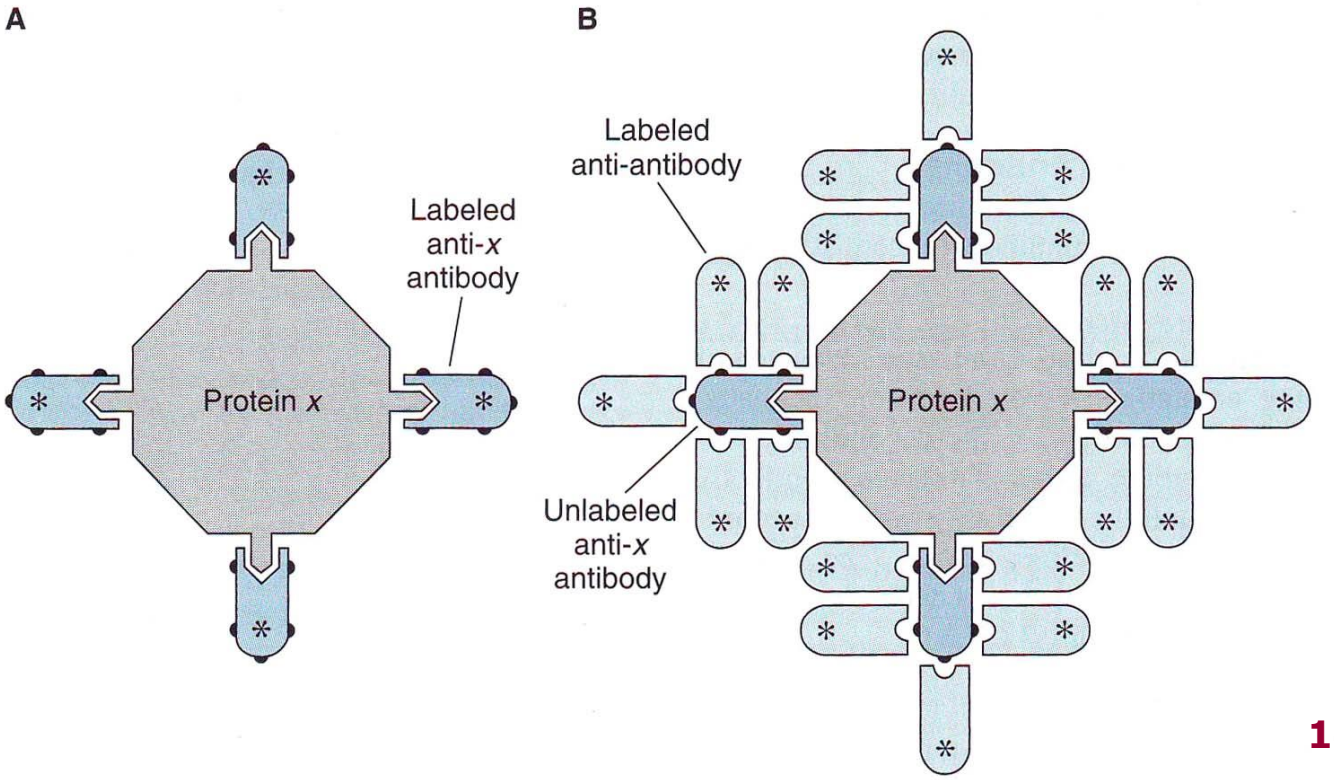
Immunohistochemistry

- based on an antigen-antibody reaction

- Methods of localizing of antigens:
  - ✓ direct method – detection with the fluorescence microscope
  - ✓ indirect method – more sensitive but requires more steps







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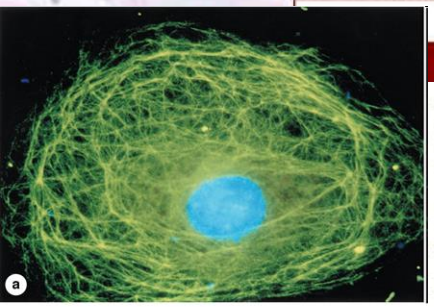
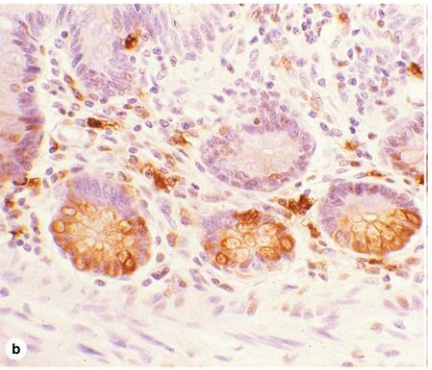
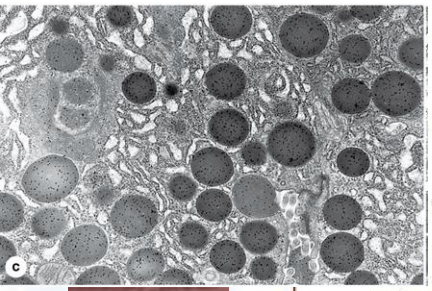
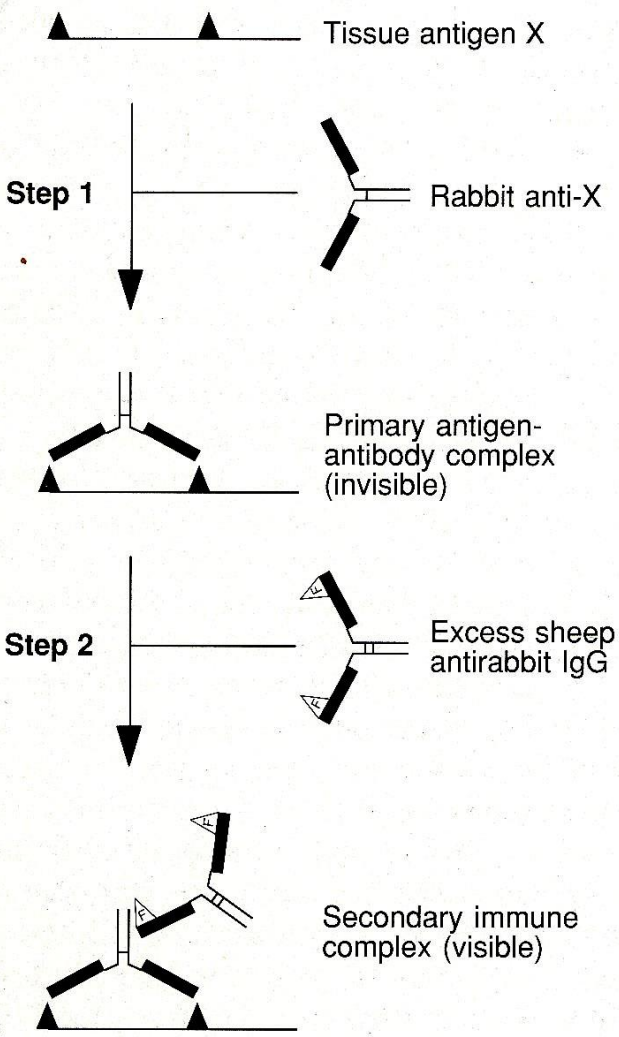
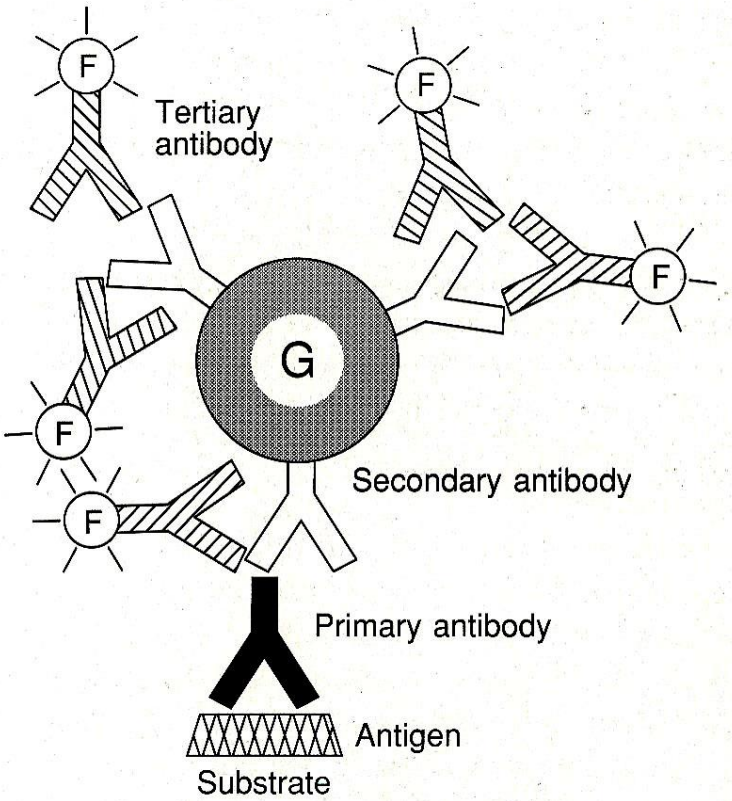
Immunohistochemical technique











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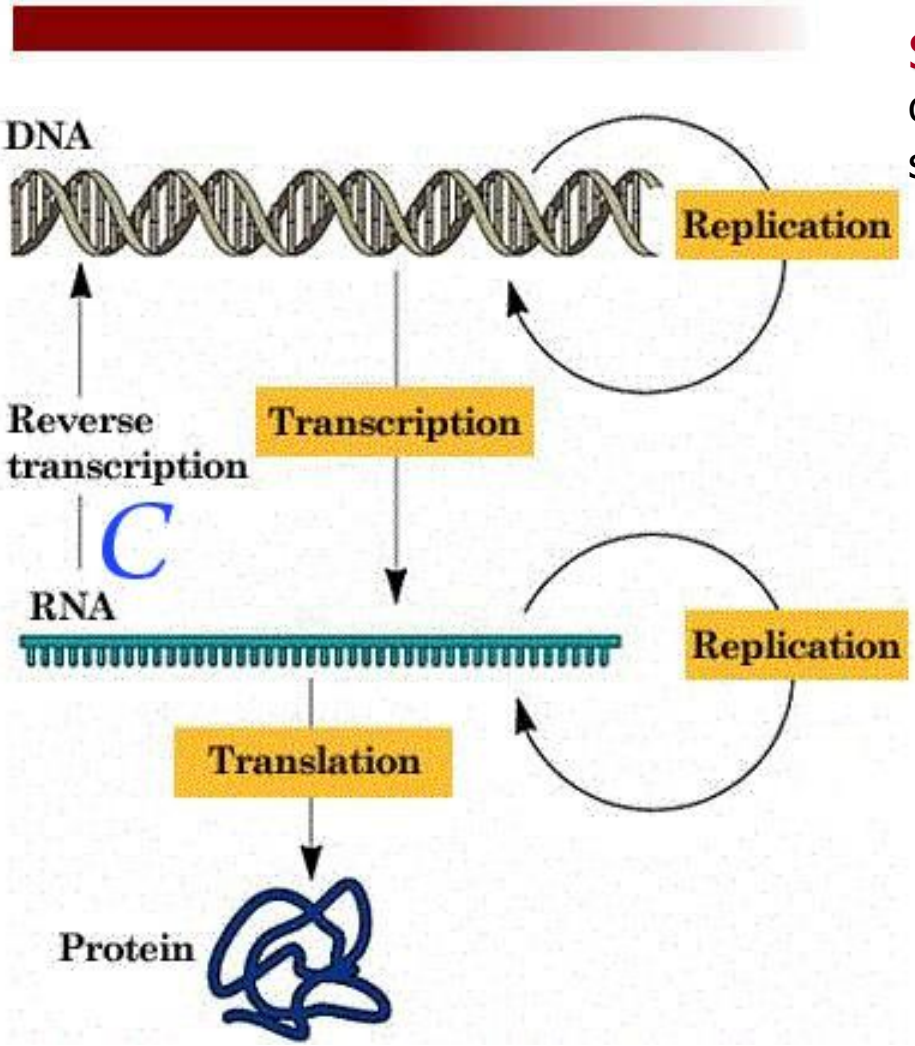
Hybridization techniques



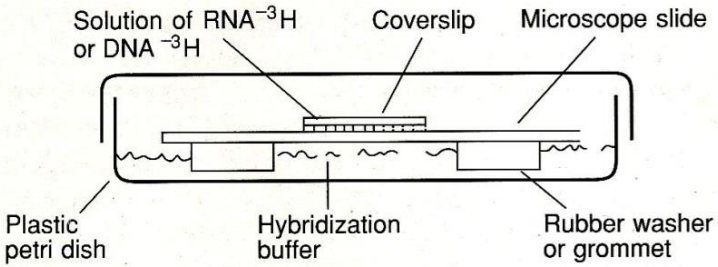
Southern blotting – detection of a specific DNA sequence, *Edwin M. Southern*

Northern blotting – detection of RNA fragments (or isolated mRNA)

Western blotting – detection of specific proteins

In situ hybridization

- Radioactive *in situ* hybridization (ISH):



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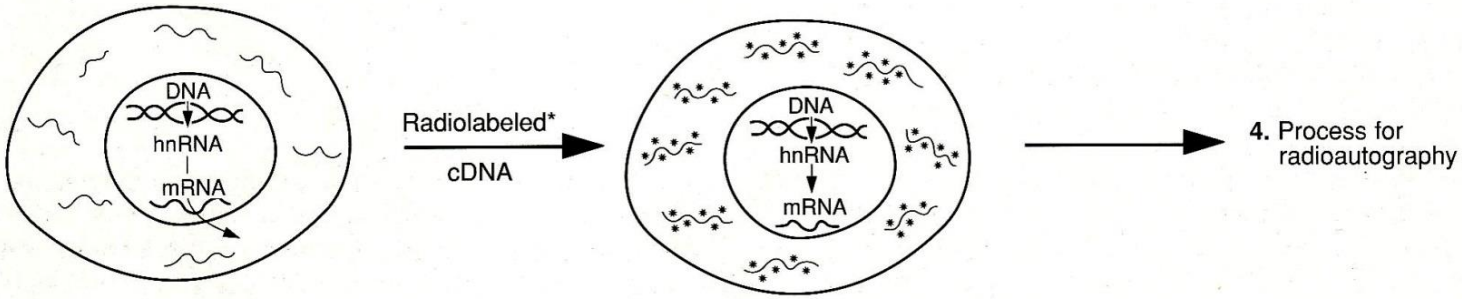


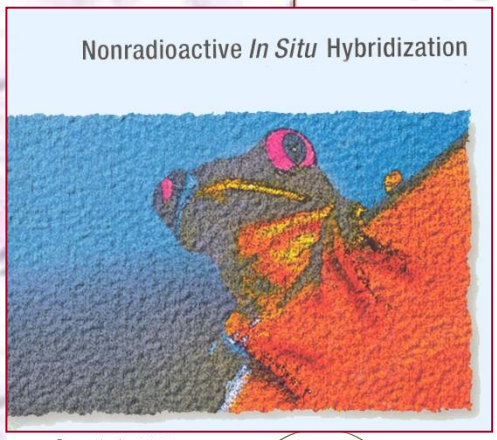
1. Tissue sections or cells cultured on slides
2. Fixation

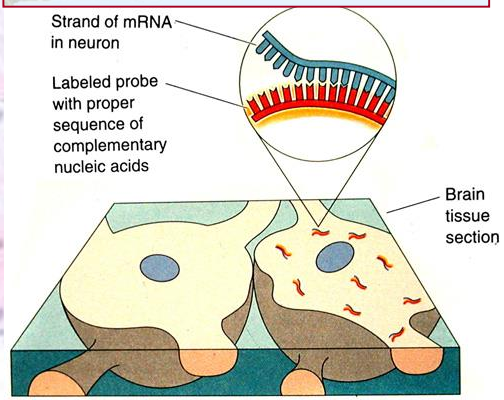
3. Hybridize radiolabeled cDNA probe with complementary mRNA molecules

b

In situ hybridization

- Nonradioactive *in situ* hybridization:
  - ✓originally developed by *Pardue* and *Gall* (1969), and (independently) by *John et al.* (1969)





Preparation of slides and fixation of material

Choice of the probe and its labeling

Denaturation of in situ target DNA (probe and target)

***In situ* hybridization**

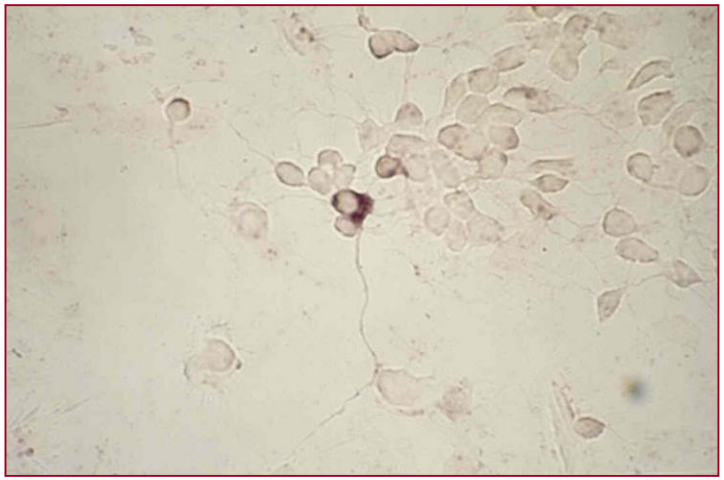
Immunocytochemical visualization

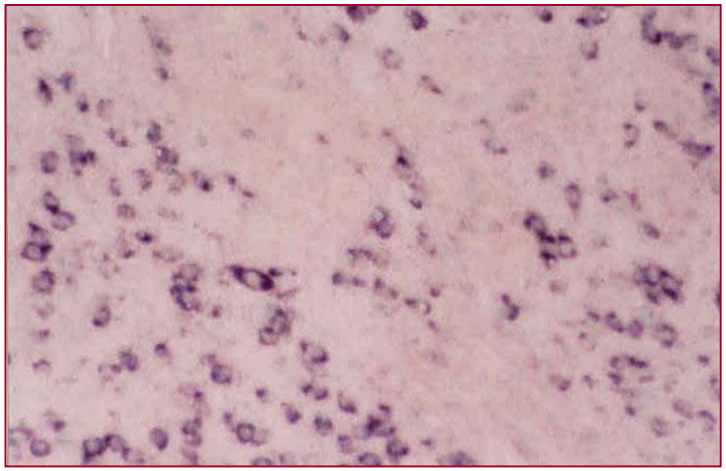
Microscopy

Flow diagram for ISH procedure

Medical applications

- In **fundamental research**:
  - ✓ gene mapping
  - ✓ localization of gene expression
  - ✓ systematization of nuclear DNA and RNA
  - ✓ replication
  - ✓ cell sorting
- In **clinical research**:
  - ✓ cytogenetics
  - ✓ prenatal diagnostics
  - ✓ gene disorders
  - ✓ diagnostics of infectious and malignant diseases
  - ✓ biological dosimetry





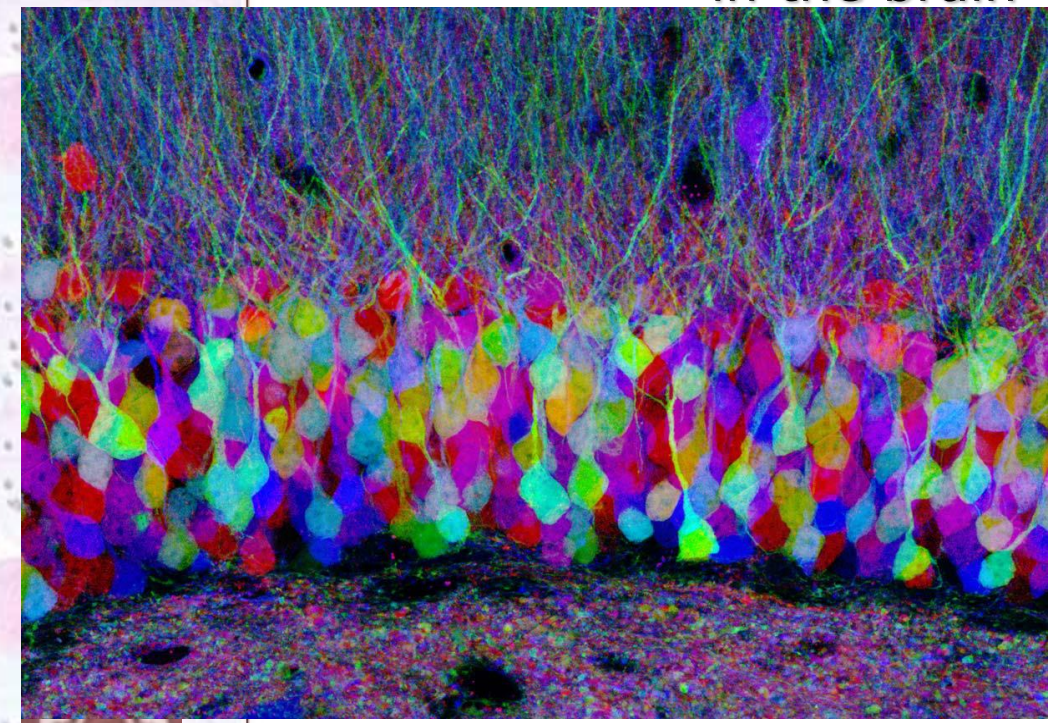
CENTER FOR BRAIN SCIENCE
NEUROIMAGING

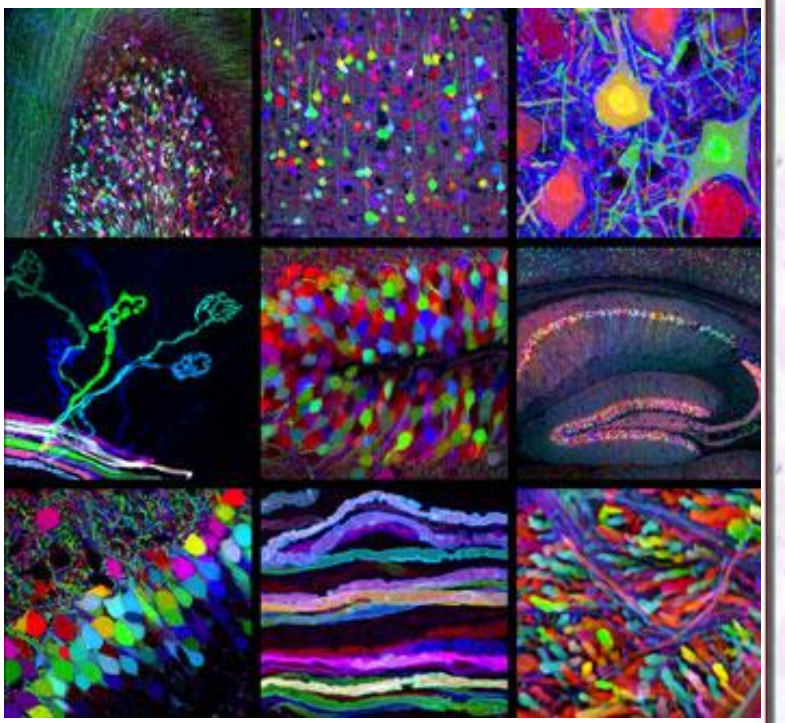
Human brain connectivity

254

LICHTMAN
LAB

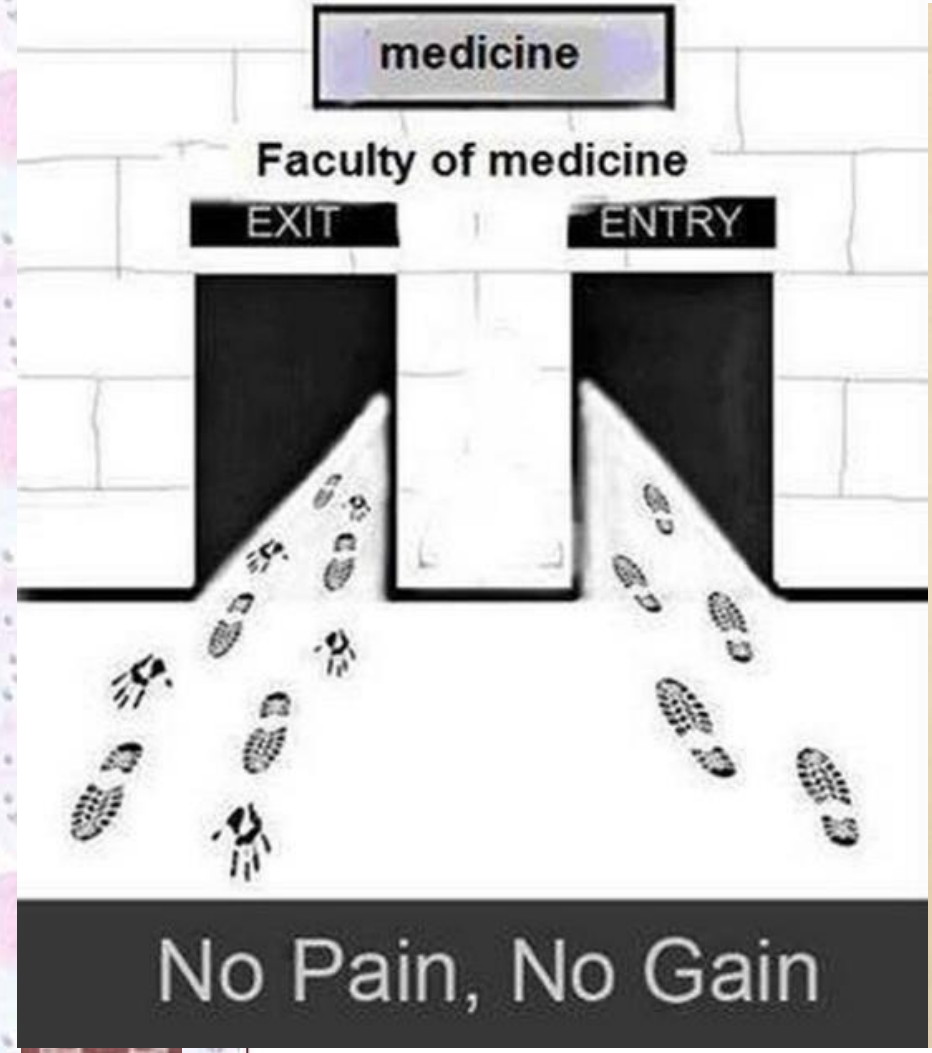
- Connectome
- Brainbow
  - ✓ transgenic painting in the brain

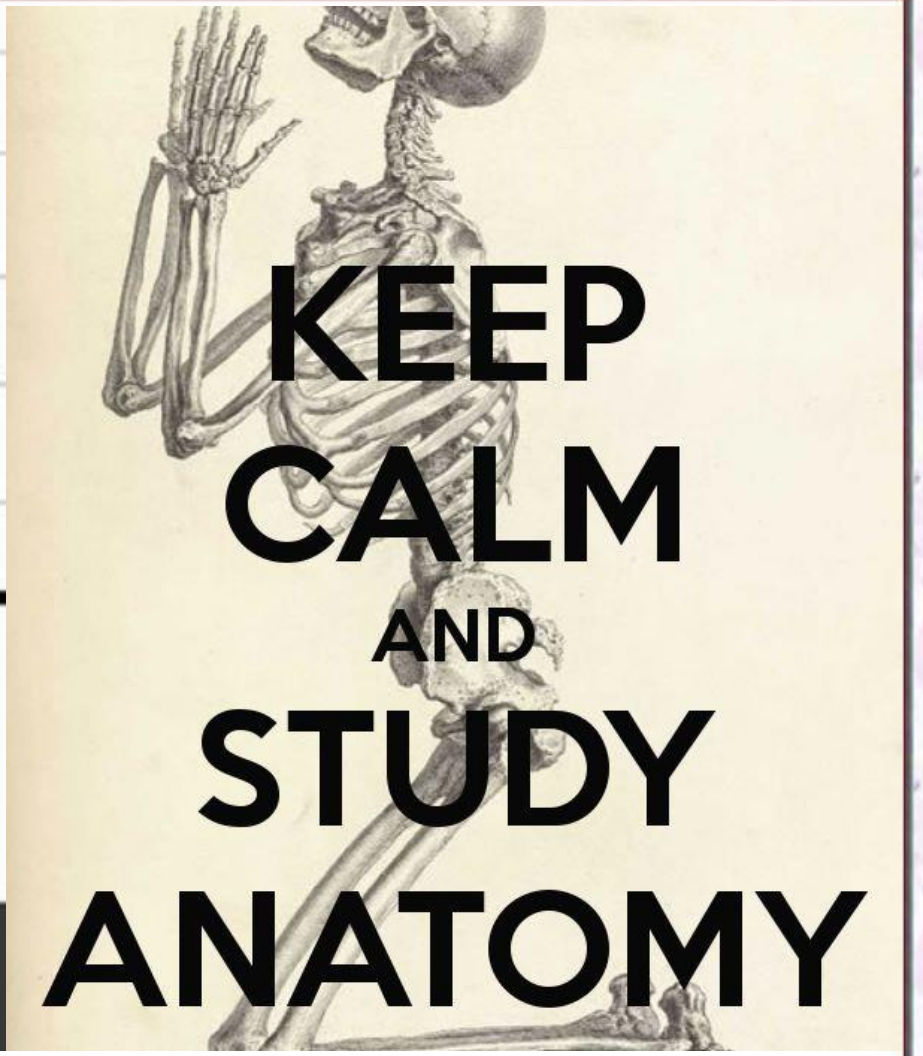




Lichtman et al.: *Nature* 2007, 450:56-62

Thank you…


medicine
Faculty of medicine
EXIT
ENTRY
No Pain, No Gain


KEEP
CALM
AND
STUDY
ANATOMY